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Literature Review and Analysis of the Chronic and Acute Toxicity of Aluminum in Aquatic Environments

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INTRODUCTION

Investigating the biological effects of aluminum has become a major focus of aquatic research. Much of this interest stems from recent work on the biological effects of acidic precipitation, because Al becomes more soluble and, hence, potentially more toxic to aquatic biota at acidic pH. Starting around 1980, it became widely accepted that Al was a major factor affecting the success of aquatic organisms and communities in acidic habitats (Cronan and Schofield 1979, Drablos and Tollan 1980, Muniz and Levistad 1980, Schofield and Trojnar 1980). Since then, research on the biological effects of acidification often has supported the contention that Al can be a major factor responsible for the demise of biotic communities (Dillon et al. 1984, Campbell and Stokes 1985, Schindler 1988, Charles 1991, Last and Watling 1991, Scheuhammer 1991). The other major concern for Al in the aquatic environment is the potential for toxicity of aluminum sulfate (alum) applications to lakes. These applications were originally designed to reduce excess phosphorus releases from sediments, thereby controlling nuisance algal blooms (Cooke et al. 1993).

The chemical properties of Al also make it interesting from a biogeochemical perspective. Aluminum is the third most abundant crustal element, yet it has little, if any, known biological function. It is generally agreed that biological systems specifically do not require Al for proper function (Wood 1984, Wood 1985, Eichenberger 1986). Wood (1984, 1985) suggested that the low solubility of Al under the circumneutral, anaerobic conditions available to primitive biota precluded the

evolution of Al-requiring metabolic pathways. Therefore, fluxes of Al into acidic habitats are likely to affect aquatic organisms largely in terms of absolute tolerance or toxicity. This is in contrast to biologically essential trace metals. For essential metals, organisms must encounter concentrations high enough to satisfy metabolic requirements, yet low enough to prevent toxicity.

The approach of this paper is to provide a literature review of the biological effects of Al, primarily with respect to the mechanisms of Al bioavailability and toxicity, and how its biological effects are best predicted. Our intent is not to duplicate the efforts of recent reviews on Al (Havas 1986, Havas and Jaworski 1986, Driscoll and Schecher 1988, Sigel and Sigel 1988, Lewis 1989, Sposito 1989, Gostomski 1990, Rosseland 1990, Scheuhammer 1991, Sparling and Lowe 1996, Sposito 1996). Rather, we will update the literature since these reviews were published, and more importantly, will examine the literature specifically with regards to mechanisms of bioavailability to a greater extent than has usually been done. A notable exception to this approach will be afforded to the freshwater phytoplankton for which a comprehensive literature review has not been prepared since that of Burrows (1977) and Gostomski (1990).

The review is organized into five main chapters. The first is a brief review of Al chemistry, with a specific focus on understanding, as well as measuring, Al chemical species of importance to aquatic biota. The second chapter comprehensively reviews Al toxicity and bioavailability to freshwater algae, with thorough analysis of the relationships between speciation and toxicity, as well as important chemical complexing agents such as P, Si, and organic carbon. The third chapter reviews the

rather sparse literature on aquatic higher plants; the fourth chapter reviews a somewhat more abundant literature of Al toxicity to freshwater invertebrates. We close with chapter five which presents an updated review of Al toxicity to fish, again with a special focus on the role of Al speciation in controlling bioavailability. Chapter 6 provides a summary of the entire review, along with some specific observations and recommendations concerning Al toxicity in light of polymerization at higher pH. References are included in the annotated bibliography enclosed with this review.

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1 ALUMINUM CHEMISTRY AND SPECIATION

1.1 Introduction

Aqueous aluminum chemistry is complex. The amount of Al in solution from normal weathering is a small proportion of the total Al in the environment, but perturbations of normal weathering can substantially increase Al mobility (Hendershot et al. 1996). Aluminum is relatively insoluble at pH 6 to 8, but its solubility is increased under more acidic and more alkaline conditions, or in the presence of complexing ligands, or at lower temperatures (Driscoll and Postek 1996). Aqueous Al is comprised of inorganic Al species (Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3^\circ$, and $Al(OH)_4^-$; waters of hydration omitted for simplicity), the proportion of which vary with pH. Aqueous Al also forms inorganic complexes with F^- and SO_4^{2-}, the formation of which varies with pH, the concentration of the inorganic ligands, ionic strength, and water temperature. Aluminum forms both weak and strong complexes with organic material such as humic and fulvic acids, which tend to keep Al in solution but make it less toxic to organisms. Finally, there is an exchangeable fraction of Al with soils, sediments, and precipitated organic material (Driscoll and Postek 1996; see Figure 1-1).

Much of this Chapter is based on a comprehensive, recent book, "The environmental chemistry of aluminum" (Sposito, 1996). Consistent with that book, and the trend in environmental chemistry today, molar units (*M*; $mol \cdot L^{-1}$) will be used for Al and other concentrations. For fast conversion, 1 μM Al = 27 $\mu g \cdot L^{-1}$ Al, 1.8-1.9 μM Al = 50 $\mu g \cdot L^{-1}$ Al, and 3.7 μM Al = 100 $\mu g \cdot L^{-1}$ Al.

1.2 Aqueous Aluminum Chemistry

1.2.1 *Mononuclear Aluminum Species*

Most of the following information comes from the review by Nordstrom and May (1996), with additional information as indicated. "Mononuclear" refers to 1:1 Al:ligand complexes. "Polynuclear" refers to 2:1, 3:1, etc., Al:ligand complexes (section 1.2.3).

Changes in speciation of inorganic Al with changes in pH are essentially instantaneous, whereas changes in Al-ligand complexes, such as Al complexed by humic or fulvic acids, may take minutes to hours to reach equilibrium. Even so, equilibrium chemical modeling of Al in natural waters is likely appropriate in most cases. Stability constants for modeling Al-inorganic complexes are reasonably well characterized. For example, the Al-F log *K* value is 7.0. This stability constant is the most important stability constant for Al-F complexes in natural waters. Sulphate binds Al much more weakly than does F^-, with a log *K* value of 3.5. Phosphate binding to Al is less well characterized, but the approximate value for $AlH_2PO_4^{2+}$ is log *K* ~3, and for $AlHPO_4^+$ is log *K* ~7 (Nordstrom and May 1996). There are no values for Al-carbonate complexes; they are very weak so can be ignored for most natural waters. Some Al can be bound with silica (Driscoll and Postek 1996).

Stability constants for modeling Al-organic complexation in natural waters are not well characterized, mainly because of the heterogeneous nature of humic and fulvic acids. A compilation of Al-organic ligand stability constants is given in Nordstrom and May (1996), which supplements that given in Morel and Hering (1993). Because carboxylic acids predominate in fulvic and humic acids, appropriate compounds to

model Al-organic ligand binding might be salicylic and phthalic acids (Nordstrom and May 1996). These Al-organic compounds have log K values of about 14 and 3, respectively, representing strong and weak binding to Al. Other ligands such as citric acid (log K ~8) may also be useful in modeling interactions of Al with natural organic compounds, but Nordstrom and May cautioned that simple model compounds may not adequately reflect Al binding to organic matter in natural waters, and that empirical approaches such as those used by Shuman (1992; log $K_{Al\text{-}FA}$~7.8) and Browne and Driscoll (1993; log $K_{Al\text{-}FA}$~7.6 at pH 5) may yield more realistic estimates of Al-organic complexation in natural waters. Schecher and Driscoll (1987, 1988) cautioned researchers and modelers not to casually select equilibrium constants from the literature, because large differences in predicted species and Al solubility can result. In addition, Lydersen (1990) showed that a decrease in temperature of about 15°C has the equivalent effect on Al species (and solubility) as a decrease in pH by one unit; temperature is therefore important to consider when calculating Al speciation.

In the scientific literature it is common to see Al^{3+} concentration or activity plotted against pH. However, calculations of Al^{3+} concentration and activity are made using measured pH, so plots of Al^{3+} *versus* pH are autocorrelated (Driscoll and Postek 1996). This autocorrelation has been pointed out in a few papers by Neal and co-workers (Neal 1988; Neal and Christophersen 1989; Neal et al. 1989; Neal et al. 1987). It is better to plot measured Al (whether total, monomeric, or inorganic) against pH, two independent measurements. Generally, the same relationships are obtained (Driscoll and Postek 1996) but without spurious correlations. However, as Hendershot et al.

(1996) pointed out, there has not been a general trend away from Al^{3+} *versus* pH plots, perhaps because the autocorrelation concern has not generally been acknowledged or fully appreciated.

1.2.2 *Mineral Phases of Aluminum*

Chemical bonds are a mix of ionic and covalent bonds. Ionic bonds are formed through electrostatic attraction that is strong enough to form a chemical bond, while covalent bonds involve distorted electron configurations between atoms which result in shared electrons. Aluminum is unusual, in that nearly all the Al-oxygen bonds that occur in minerals are ionic, with covalent bonds being of minimal importance (Hemingway and Sposito 1996). The structures of most minerals in weathering environments tend to minimize the total electrostatic energy of the crystal (e.g. the structures follow Pauling's rules).

There are many Al-bearing minerals (kaolinite, vermiculite, montmorillonite, etc.), but of most use in understanding aqueous Al chemistry is gibbsite, $Al(OH)_3$. Gibbsite has an octahedral hydroxide structure. The enthalpy (heat of formation) of gibbsite is negative (about -1293 $kJ \cdot mol^{-1}$; heat is released) and is therefore favorable, while the entropy ("disorder") of formation is unfavorable (about -0.46 $kJ \cdot (mol \cdot K)^{-1}$, going to a more ordered structure). Overall, the Gibbs energy of formation of gibbsite is favorable (about -1155 $kJ \cdot mol^{-1}$; Hemingway and Sposito 1996). Exact values for these terms are not agreed upon, because different experimental conditions can yield different solubility measurements of gibbsite, and $Al(OH)_3$ precipitating from solution does so first as

amorphous $Al(OH)_3$, not as gibbsite. These limitations of the thermodynamic data should be recognized when modeling Al precipitation in water (Hemingway and Sposito 1996).

The results of Tipping et al. (1988) suggest that Al precipitation occurs only rarely in natural waters. For example, water of low pH and high Al concentration flowing into higher pH and low Al water (e.g. a stream entering a lake) is diluted, which results in higher pH and lower Al concentrations; precipitation is not involved. This was shown by a linear correlation of Al with Ca, a conservative element and therefore an index of dilution (Tipping et al. 1988). Results of Wright and Skogheim (1983) for an acidic, Al-rich stream entering a less acidic, lower Al concentration lake, in which labile monomeric Al decreased along a pH gradient, could also be interpreted as dilution, as opposed to the Al precipitation phenomenon favored by these two authors. In contrast, precipitation of Al was convincingly shown when an Al-rich, acidic stream (115 μ*M* Al, pH 3.5) was made more basic (to pH 5.8) with sodium carbonate (Broshears et al. 1996).

Inorganic anions such as SO_4^{2-}, CO_3^{2-}, and F^- are more effective than Cl^- and NO_3^- in preventing the formation of $Al(OH)_3$ in supersaturated solutions upon aging (Jardine and Zelazny 1996; Goldberg et al. 1996). Organic anions such as citrate and malate do the same. A single ring of six Al^{3+} ions, with six pairs of OH^- ions ($Al_6(OH)_{12}(H_2O)_{12}^{6+}$) is the smallest unit of the gibbsite structure (Goldberg et al. 1996). Gibbsite will eventually form from amorphous $Al(OH)_3$, which forms first during aging. Amorphous $Al(OH)_3$ is most soluble, then bayerite (also $Al(OH)_3$), followed very closely

by similar solubilities of gibbsite and norstrandite (both $Al(OH)_3$) and boehmite and diaspore (both AlOOH; Lindsay and Walthall 1996). Jardine and Zelanzny (1996) feel that polynuclear Al species, such as Al_{13}, are unstable, transient species on the way to forming solid $Al(OH)_3$, and that polynuclear Al species are only needed to explain situations of high Al concentration or high pH.

1.2.3 *Polynuclear Aluminum Species*

For systems of low Al concentration and low pH, using the mononuclear Al species (Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3^\circ$, and $Al(OH)_4^-$) to explain Al hydrolysis seems appropriate. However, in studies for which base is added to the system or at high Al concentrations, evidence suggests the existence of polynuclear Al species. Polynuclear Al species include, for example, $Al_2(OH)_2^{4+}$, $Al_3(OH)_8^+$, $Al_6(OH)_{12}(H_2O)_{12}^{6+}$, and the tridecameric Al_{13} polynuclear species, $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ (Bertsch and Parker 1996). It is easy to envision the formation of crystalline gibbsite from the combination through aging of many hexameric rings of polynuclear $Al_6(OH)_{12}(H_2O)_{12}^{6+}$ (section 1.2.2). Unfortunately, according to Bertsch and Parker (1996), there is little direct experimental evidence for many of these polynuclear structures, but ^{27}NMR spectroscopy has yielded good evidence for the existence of the $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$ polymeric species. In contrast, recall that Jardine and Zelanzny (1996) believe that polynuclear species are transient intermediates in the formation of crystalline $Al(OH)_3$, and that at true equilibrium a solution will consist only of mononuclear Al and the stable solid phase.

Bertsch and Parker (1996) presented five situations where the formation of the Al_{13} polymer might occur in the environment. Formation of Al_{13} could occur around limestone granules used to alleviate Al toxicity in acidic water. The polymer could also form when acid water containing Al passes through carbonate bedrock. Favorable conditions for Al_{13} formation might also exist in mixing zones, where Al-rich water meets more basic water. The Al_{13} polynuclear species might form in the more alkaline region at the apex of root tips. Finally, favorable conditions for Al_{13} formation may exist at fish gills, where NH_3 is released and makes the gill micro-environment more alkaline. This last condition will be considered in more detail in Chapter 5, but it appears that Al precipitation and polymerization occur quickly enough (<4 s; Playle and Wood 1990) to be a reasonable explanation of the respiratory effects of Al (section 5.2.2).

Chemical modeling of aqueous Al is done through calculating equilibrium distributions of Al, whether or not the system is in true equilibrium. Mononuclear Al is calculated as a function of pH, with the inclusion of a few, pertinent mononuclear complexes (e.g. F^- and SO_4^{2-}). A solid phase, often gibbsite, determines the overall solubility of Al; its inclusion implies that polynuclear reactions must occur, even if the actual processes are not modeled (Bertsch and Parker 1996; see also Smith 1996). In soils, adsorption and cation exchange are added into the system, and they are also important in determining overall Al solubility. Models indicate what might happen in a system, not necessarily what does happen, and are in some respects "snapshots" of a system, especially if equilibrium is only an approximation of the system.

The presence of anions can influence the precipitation of Al from polynuclear Al species. For example, a relatively strong complexing anion such as F^- increases the pH needed for maximum precipitation, while others such as phosphate, silicate, sulphate, and nitrate increase aggregation of polynuclear species. Anions may speed precipitation by reducing repulsive forces between positively charged polynuclear forms of Al, allowing more aggregation to occur (Bertsch and Parker 1996). Organic ligands inhibit Al crystallization and precipitation in a similar manner as does F^-, in a manner consistent with their Al-binding strengths. Thus, citric acid inhibits Al crystallization more than does malic acid, which inhibits Al crystallization more than does aspartic acid. Organic ligands may keep Al_{13} from forming, or the polymer is broken down to small Al oligomers or monomers in their presence. Thus, organic ligands, including natural humic acids, have important implications for the hydrolysis of Al.

1.2.4 *Organic Complexes of Aluminum*

Algae, lichen, and fungi release complexing compounds such as citrate, oxalate, acetate, and salicylate. These compounds solubilize Al and other cations from minerals such as silicates. Once in solution, Al can be transported into lakes and streams. Thus it is Al-organic complexes, not gibbsite, that are the predominate control of Al concentrations in soils (Vance et al. 1996).

Natural organic complexing ligands in water are comprised of well defined compounds produced by micro-organisms and plants, and of poorly defined humic and

fulvic acids. The well defined compounds include the above-mentioned compounds like citric and acetic acids, as well as sugar acids and phenols. These products are degraded quickly by microbial metabolism (Vance et al. 1996). The poorly defined humic and fulvic acids are yellow to black colored substances formed by secondary synthesis reactions, and are probably the more important organic fraction in most natural waters (Vance et al. 1996). The humic acid fraction is extracted with an alkaline solution and is precipitated when acidified. The fulvic acid fraction remains in solution after the alkaline solution is acidified, and represents lower molecular weight compounds; fulvic acids are the predominate form in natural waters. A third fraction, humin, is not solubilized, and probably represents humic and fulvic acids strongly bound to clay minerals (Vance et al. 1996).

Humic substances bind Al and other cations because of their high content of oxygen containing functional groups such as carboxyl, phenolic, and alcoholic groups. High molecular weight humic acids are more likely to precipitate with Al than lower molecular weight fulvic acids, but coagulation will occur over a relatively small pH range (pH 4-7; Vance et al. 1996). At high ionic strength there is reduced binding of Al to organic ligands because of increased competition for ligand binding sites and reduced activity coefficients. Trivalent cations like Al^{3+} show greater reductions in their activity coefficients at high ionic strength than do divalent cations, which are themselves affected more than are monovalent cations.

Stability constants for Al-organic acid complexes are needed for full understanding of Al-organic acid interactions, to predict Al speciation in natural

environments. Values for soil humic acids range between about log K 4 and log K 7 (Vance et al. 1996), but geochemical modeling of natural waters requires guesswork or extensive investigation to determine an appropriate constant. Overall, dissolved organic carbon is probably a more important complexing agent of Al than is F^- (Courtijn et al. 1990). Some investigators have used a mixture model, such as a mixture of nine, low molecular weight organic acids (Sposito et al. 1982). In addition, models such as these may not reflect the many sites where Al binding occurs, or the pH and ionic strength dependence of the sites (Vance et al. 1996). For example, models may not reflect the situation of excess humic substances, where Al binds to the strongest binding sites first.

Although there are good data for inorganic binding of Al (e.g. AlF), the modeling of Al-organic binding is problematic. The amount of Al bound to dissolved organic carbon was usually well predicted by a linear logarithmic model based on Al^{3+} activity and DOC concentration (Campbell et al. 1992), except during springmelt period. During that time, DOC binds Al less well than predicted, presumably because of a nonhumic component which binds Al poorly. Simulations using "ALCHEMI" modeled a water system using two reactions with a model organic acid (Driscoll and Postek 1996). The first reaction was $Al^{3+} + A^{3-} \rightleftarrows AlA$ (log K =8.4), and the second was $Al^{3+} + H^+ + A^{3-} = AlHA^+$ (log K =13.1), with about 0.04 mol Al binding sites per mol DOC. The second reaction simulated the additional binding of Al to organic matter under very acidic conditions. Other examples of modeling attempts for Al binding by DOC are given in Driscoll and Postek (1996).

1.2.5 *Aluminum-phosphorus Complexes*

Aluminum forms an inorganic complex with P that can represent a significant portion of both dissolved and particulate Al pools under certain conditions. For example, increasing Al concentrations were well-corelated with reductions in dissolved P concentrations in natural waters (Almer et al. 1978, Dickson 1980, Hörnström et al. 1984, Nalewajko and O'Mahony 1988), yet P concentrations may not always be high enough to allow for the formation of a purely inorganic $AlPO_4$ complex (Jansson et al. 1986). Aluminum additions have also been observed to reduce dissolved P concentrations in artificial algal growth media (Gensemer 1989). These observations further suggest that Al-P complexation is pH-dependent, and appears to predominate around pH 6 (Dickson 1980, Nalewajko and O'Mahony 1988) although it may be nearly as stable near neutrality (Gensemer 1989).

Several different chemical complexes and/or processes may be responsible for dissolved P removal by Al. Two dissolved inorganic monomeric Al-P species have been reported: $AlH_2PO_4^{+2}$ (log $K \approx 3$) and $AlHPO_4^{+}$ (log $K \approx 7$), although their thermodynamic properties are difficult to characterize experimentally and may, therefore, be inexact (Nordstrom and May 1989). It is unknown whether or not these complexes would be detected as soluble molybdate-reactive P (e.g. Strickland and Parsons 1972), or as inorganic monomeric Al by common spectrophotometric assays (see section 1.4 below). Somewhat better understood is the formation of an insoluble $AlPO_4$ complex in aqueous solutions. In acidic soil solutions, variscite ($AlPO_4 \cdot 2H_2O$) is a stable mineral (Lindsay and Walthall 1989) with a log K value of -21 (Stumm and Morgan 1981).

Similar to field observations (e.g. Dickson 1980, Nalewakjo and O'Mahony 1988), variscite formation is also pH-dependent with maximal formation occurring near pH 6 (Stumm and Morgan 1981). Finally, inorganic phosphate can also adsorb onto aluminum oxides and hydroxides, but the kinetics and equilibria of these reactions are highly dependent on the type of Al solids and experimental conditions used (Davis and Hem 1989).

This process of P adsorption onto Al hydroxides has seen extensive application to both water treatment and lake management as a means of controlling excess dissolved P. For example, additions of aluminum sulfate (alum) to lakes has long been used to mitigate problems associated with nuisance algal blooms owing to excess phosphorus supply from sediments or watersheds. Alum treatments are designed to form a floc of relatively insoluble $Al(OH)_3$ in the water column or, more importantly, near sediments, which in turn adsorbs dissolved P making it less available for algal uptake and growth. However, given that rather large amounts of alum can be used (up to several hundred metric tons), concern is often raised over the potential for introducing Al toxicity to lake biota (Cooke et al. 1993).

Estimating appropriate doses of alum usually involve batch reactor tests whereby $AlSO_4$ is added to lake water samples to a maximum level whereby water pH does not fall below 6.0 (Kennedy and Cooke 1982). This dosing strategy has been used frequently to ensure that water pH does not exceed a range of 6-8, thereby minimizing Al solubility owing to control by $Al(OH)_3$ precipitation. If alum treatments are to be used in low alkalinity (<35 $mg \cdot L^{-1}$ $CaCO_3$) systems, buffers such as sodium

aluminate are typically added to keep lakewater pH in the appropriate range (Cooke et al. 1993). Continuous-flow techniques for empirically determining P reduction from field-collected sediments can also be used to set this maximum-allowable dose (Young et al. 1988). Using these doses, long-term and stable P inactivation from sediments has been achieved for as long as 5-12 years, although failures under some conditions (i.e. in reservoirs, ponds with shallow oxic sediments, etc.) have been noted (Cooke et al. 1993).

1.2.6 *Aluminum-silicate Chemical Interactions:*

Even though aluminosilicates are common components of inorganic minerals, the extent of dissolved aluminosilicate formation in surface waters is only recently being investigated. Perhaps because the chemical structure and thermodynamic properties of such complexes were at one time virtually unknown (Driscoll and Schecher 1990), their extent and importance in nature have been largely a matter of conjecture. However, both laboratory (Chappell and Birchall 1988, Exley and Birchall 1993) and field (Browne and Driscoll 1992) studies have more recently described the formation of dissolved aluminosilicates in acidic waters. Chappell and Birchall (1988) suggest that aluminosilicates could be amorphous protoimogolite percursors with variable stoichiometry (Si:Al ratio = 0.3 - 0.6) that form preferentially at pH 5.5-6.0. Exley and Birchall (1993) further described the formation mechanisms of this complex whereby aluminum hydroxide nucleation is inhibited. On the other hand, Browne and Driscoll (1992) proposed the existence of several discrete aluminosilicate complexes

for which they derived specific stoichiometries (Al:Si = 1:1, 2:1, and 2:2) and thermodynamic properties.

Regardless of which model gains further support, these important studies did much to help assess the presence and significance of dissolved aluminosilicates in natural waters. The existence of dissolved aluminosilicates (if confirmed in nature) would be of great importance not only to the accurate interpretation of dissolved Al and Si chemical speciation, but also to the interpretation of effects on organisms in the presence of each compound (especially algae, see Chapter 2). However, from the above studies (Chappell and Birchall 1988, Browne and Driscoll 1992, Exley and Birchall 1993), it appears that aluminosilicate formation may only be significant in waters containing relatively high concentrations of dissolved Si. Aluminosilicate formation in these studies was restricted to Si concentrations above ca. 100-500 $\mu mol \cdot L^{-1}$, although some evidence suggests aluminosilicate may form at concentrations as low as 40 $\mu mol \cdot L^{-1}$ (Birchall et al. 1989). Therefore, this complex may only be significant in waters with relatively high dissolved Si concentrations (e.g. streams), and be of less importance in acidic lakes which tend to be relatively low in Si (Hutchinson 1957, Driscoll and Newton 1985, Findlay and Kasian 1986, Willén 1991). In their review, Cooke et al. (1993) also mention that silicates, if sufficiently concentrated, may help control residual Al toxicity from alum treatments. However, to our knowledge no empirical evidence to support this hypothesis yet exists.

1.3 Prevalence of Aluminum in the Aquatic Environment

1.3.1 *Aqueous Geochemistry*

Surface waters contain a wide range of Al concentrations. Generally, more acidic waters contain most Al. Concentrations of Al in surface waters correspond reasonably well with the theoretical solubility of $Al(OH)_3$, but at low pH are generally undersaturated with respect to gibbsite. Aluminum undersaturation might be due to hydrologic flowpaths (e.g. less saturation under high flow conditions) and the mix of mineral and organically bound Al in the flowpath (Driscoll and Postek 1996). Chemistry changes are generally rapid, so water will have a pH and Al concentration which reflects the soil horizon through which it has flowed. Slow flows through deeper mineral soil horizons generally yield water of higher pH and lower Al and are closer to Al saturation, than are fast flows of water through porous surface soil horizons where more Al is bound to organic material, which yields low pH water and undersaturated Al conditions (Hendershot et al. 1996). Aluminum bound to organic material in soils is likely rapidly available, whereas Al in inorganic form may be kinetically constrained, but can resupply Al to the organic pool (Wesselink et al. 1996). Carbon dioxide dissolved in soil water can transport Al: once the groundwater reaches water in equilibrium with air, CO_2 degases leaving Al behind (Driscoll and Postek 1996). In the absence of acidic conditions, this mechanism transports only minimal amounts of Al.

In a large survey of lakes in the eastern United States, 203 lakes in the Adirondacks had an average of ~5.1 μ*M* total Al and ~ 1.6 μ*M* monomeric Al. The 203 lakes had a mean pH of 6.3, with a pH range of 4.2-9.4. Ten percent of the lakes had

water with pH <5.0, and thus defined as acidic. In Florida, 168 lakes had an average pH of 6.3 (range: 3.8-9.0), with 12% being classified as acidic. Overall, the Florida lakes had a mean total Al of ~3.3 μ*M* (range, 0.3-50 μ*M*) and ~0.8 μ*M* monomeric Al (range: 0-16 μ*M*; Driscoll and Postek 1996: summary of Linthurst et al. 1986). Total Al was usually much greater than monomeric Al, indicating that most Al was in the particulate form.

Clear water lakes from these surveys usually contained <1 μ*M* monomeric Al if their water pH was 6.0 or higher. The Adirondacks have a significant number of acidic, clear water lakes, of which ~14%, 12%, and 10% had >1.8, >3.7, and >5.5 μ*M* monomeric Al, respectively (50, 100, and 150 $\mu g \cdot L^{-1}$ monomeric Al). Of clear water lakes in Florida, 7% had >1.8 μ*M* monomeric Al, 4% had >3.7 μ*M* monomeric Al, and 2% had >5.5 μ*M* monomeric Al (Linthurst et al. 1986).

There is generally more inorganic and organic Al the lower the water pH, and there is generally more organic Al as the concentration of dissolved organic carbon (DOC) increases. In essence, DOC increases the solubility of Al, while decreasing Al toxicity (Chapters 2 & 5). There is more Al bound per mg C of DOC as total Al increases, likely because more total Al means that more of the lower affinity binding sites on DOC get filled.

There is a general pattern of Al concentration and speciation as water flows from high elevation to lower elevation. Upland streams are often acidic, partly because of the predominance of coniferous forests, with high DOC concentrations and therefore high total Al and high organic Al concentrations. As elevation decreases, water pH

increases, and DOC concentrations decrease as more deciduous trees are encountered, so there is more inorganic Al compared to organic Al, with a correspondingly higher organic Al:DOC ratio. As drainage area increases at lower elevation, inorganic Al and organic Al concentrations generally decrease (Driscoll and Postek 1996). In addition to Al release from stream substrates during acidic rain events, Al can be released from mosses and liverworts in the stream bed (Henriksen et al. 1988).

Lakes are net sinks for Al, with percent retention of Al varying with lake pH and Al saturation index, which are correlated. Low Al retention occurs in highly acidic lakes, or in lakes during periods of the year with high flow and acidic conditions. For example, lakes of pH <5 retain 10-50% of their Al input, whereas more neutral lakes retain ~70% of their input of both total Al and organic Al (Driscoll and Postek 1996). Aluminum speciation changes are rapid enough that water chemistry conditions in a lake or stream determine Al speciation, not the antecedent geochemical origins of the Al (Campbell et al. 1986). Precipitation of Al within a lake can remove DOC and metals in the water column through sorption, co-precipitation, or coagulation processes, which means increased lake clarity (Driscoll and Postek 1996).

1.3.2 *Sediment Geochemistry and Wetlands*

The solubility of Al in soils largely controls fluxes of dissolved Al species into aqueous ecosystem compartments such as lakes and streams (see also section 1.3.1). However, reviews of Al chemistry in aquatic systems typically do not treat aquatic

sediment Al geochemistry separately from soils; they are usually lumped together in a single category such as "soil/sediments" (Driscoll and Postek 1996). While it is doubtful that Al geochemistry in aquatic sediments differs markedly from that of terrestrial soils, enhanced knowledge of sediment geochemistry probably would benefit ecological and toxicological studies of benthic and littoral organisms. The brief discussion that follows here is based largely on soil processes reviewed by Lindsay and Walthall (1996), making the assumption that similar processes control Al solubility in sediments and well-hydrated wetland soils.

Soils and sediments contain some of the largest sources of Al in close proximity to biota, largely in the form of unavailable aluminosilicate and oxide minerals (Lindsay and Walthall 1996). Small portions of this total Al pool are solubilized during weathering, and, hence, take part in biogeochemical reactions either in soil solutions or surface waters (Driscoll and Postek 1996). Aluminum solubility is determined by many of the same reactions already discussed in this chapter, including rapid equilibria established with $Al(OH)_3$ and aluminosilicate minerals and, in the later stages of weathering, with more slowly formed secondary clay minerals (Lindsay and Walthall 1996). The actual solubility of Al thus depends on which aluminosilicate and clay minerals are being weathered, and the acidity of soil solutions in contact with these minerals. Once in aqueous solution, Al speciation is further determined by equilibria with organic carbon, mineral surfaces, and other inorganic complexes, as already described. Still, overall solubility is primarily under control of the dissolution and precipitation of solid phases, rather than these more subtle speciation or

"exchangeable" Al reactions (Driscoll and Postek 1996, Lindsay and Walthall 1996, but see Wesselink et al. 1996).

Unlike redox sensitive metals such as Fe or Mn, Al speciation in soils or aqueous solution should not depend on the redox conditions of soils or sediments. In fact, no mention of redox effects are made in any of the recent reviews of Al chemistry and speciation, thus at this point we must assume that redox (measured as Eh) is not a significant factor controlling the chemistry of Al in natural waters. In fact, the lack of redox-dependency is noted as a specific advantage to the long-term stability of alum treatments for P control in lakes (Cooke et al. 1993).

However, it is possible that minor indirect effects on speciation might occur concomitant with Eh owing to changes in the solubility of other trace metal cations such as Fe and Mn. Because these and other redox sensitive metals also associate with dissolved organic matter and inorganic complexes, they might affect exchangeable Al fractions *via* competitive binding to these same phases. Direct study of this would be prudent for understanding Al exposures in environments exposed to fluctuating Eh conditions such as seasonally hydrated wetland soils, and seasonally anoxic lake sediments, but insofar as we are aware from the literature reviewed to date, no such studies exist.

The geochemistry of Al in wetlands is largely under the control of the types and magnitudes of inputs to these systems (Driscoll and Postek 1996). For example, in bogs atmospheric deposition dominates inputs of Al even in these highly acidic systems. Once in the bogs, dissolved speciation of Al tends to be dominated by DOC

owing to the high DOC concentrations naturally present in these systems. Fens and swamps tend to have lower total Al concentrations than bogs, although significant variations exist (Driscoll and Postek 1996; their Table 6). Overall, wetlands appear to be sinks for Al from stream inputs owing to biological uptake of sulfate and nitrate along with other processes which generate acid neutralizing capacity (ANC), which ultimately neutralize acidic inputs thereby lowering Al solubility (Driscoll and Postek 1996).

1.4 Measuring Aluminum in Water

1.4.1 *Analytical Techniques*

Analytical techniques for measuring Al were thoroughly reviewed by Bloom and Erich (1996). Graphite furnace atomic absorption spectrometry is more sensitive for Al analysis than is flame AA analysis, and background correction is generally recommended. Aluminum is also commonly measured by reaction with reagents such as pyrocatechol violet, 8-hydroxyquinoline, and lumogallion, but care must be taken to buffer the analytical solutions to a pH of maximum absorbance or fluorescence, and organic matter and cations such as iron can interfere with the methods (e.g. Lewis et al. 1988; Hodges 1987; Røyset and Sullivan 1986). Some of these methods have the advantage of being relatively simple and use relatively inexpensive equipment which can be taken to the field. Colorimetric methods such as oxine or pyrocatechol violet present an additional advantage in that they can be adapted for use with automated analytical instruments such as flow injection spectrophotometers (Clarke et al. 1992, Berden et al. 1994, Berggren and Sparen 1996). Other methods for Al analysis are

also available, but for various reasons are not generally used. For example, the very sensitive neutron activation method first requires access to a nuclear reactor and then requires fast counting of the irradiated sample, because ^{28}Al has a half life of just 2.3 minutes.

When sampling, care must be taken to avoid Al contamination, and sample containers should be acid washed and rinsed with at least ASTM Type II deionized water or the equivalent. Generally, water samples are filtered through 0.1 to 0.45 μm filters, removing colloidal Al, to obtain total soluble aluminum. As with sample bottles themselves, care must be taken to avoid contaminating the samples while filtering. Acid washing filters, use of polycarbonate filters that do not need washing, or filtering and discarding some of the sample to wash the filters are ways of minimizing contamination.

Filtration has been used in many studies to separate dissolved and particulate Al. Results obtained are dependent on filter pore size, because particulate Al has a continuous size distribution, so there is no absolute distinction between the types of Al. In a similar manner, rapid extraction techniques will yield different results depending on the time allowed for the reaction (Driscoll and Postek 1996). In essence, all Al fractionation schemes are operationally defined.

1.4.2 *Techniques and Recommendations for Speciation of Aqueous Aluminum*

Speciation of Al is important because, for example, organically bound Al is less toxic than is inorganic Al. Once monomeric inorganic Al is determined, Al speciation

can be calculated using computer programs such as MINEQL$^+$ (Schecher and McAvoy 1992) and MINTEQA2 (1991). These programs estimate dissolved Al speciation based on simultaneous calculations of all important chemical reactions, assuming equilibrium conditions. Care must be taken, however, to ensure that the particular program contains the most recently determined equilibrium constants, much like those listed by Nordstrom and May (1996), Hemingway and Sposito (1996), and Lindsay and Walthall (1996). Accurate measurements of other competing ions, organic carbon complexation, and total ionic strength are also required for accurate model calculations. Note that it is important to calculate Al speciation based on the monomeric, inorganic Al component, because it is this component which can be assumed to be in true equilibrium (Boudot et al. 1996).

Separation of monomeric inorganic Al can be done using physical or chemical means. Separation methods are based on reaction rates with complexing agents (e.g. 8-hydroxyquinoline, pyrocatechol violet), dialysis, ultrafiltration, or reaction with ion-exchange resins (Bloom and Erich 1996). This last method is probably the best. An early, commonly used cation exchange method was presented by Driscoll (1984). The method determines, by subtraction, weak monomeric organic Al complexes (which pass through the column), monomeric inorganic Al (which is retained in the column), and colloidal, polymeric, and strong organic complexes which are measured only after acid digestion of a sample. Note that whatever the method used in determining inorganic Al there is generally an overestimation of soluble, monomeric inorganic Al. This overestimation occurs because whenever a chelating agent, complexing agent, or resin

is used, Al^{3+} binds, equilibria shift, and some additional Al^{3+} is released from inorganic and organic complexes of Al. Thus, more Al^{3+} becomes available to bind to the complexing agent used (Lindsay and Walthall 1996).

1.5 Measurement and Speciation of Al in Sediments and Soils

The measurement and speciation of dissolved Al in soil or sediment solutions essentially follow the same techniques and recommendations as those outlined in section 1.4. After sampling, solutions must be separated from the solid materials using either filtration or centrifugation, the latter often being preferred with soils since the separation of large amounts of solid and colloidal materials is often difficult using filtration methods (Bloom and Erich 1996).

After isolating the aqueous fraction from the soils, speciation procedures generally follow methods as described earlier except for special precautions being needed to obtain reliable estimates of Al^{+3} ion activity (Lindsay and Walthall 1996). These precautions are warranted because it is inherently more difficult to obtain clean separations of inorganic Al from soils or sediments, regardless of whether one uses complexing agents (i.e. 8-hydroxyquinoline), resin exchange, or fluoride ion activity measurements. In most of these cases, competing ligand interactions with Al^{+3} ions between the soil solution and the analytical agent often overestimate soluble monomeric Al, as was already described for aqueous solutions (section 1.4.2). Two additional methods outlined by Lindsay and Walthall (1996) may improve our ability to cleanly separate monomeric Al from soils, but they require further experimental

verification. These methods involved additions of well characterized activated carbon to soil solutions to remove organic complexes, and the addition of synthetic chelators containing both Al and a competing secondary ion which is used to back-calculate Al^{+3} activity after 3 to 7 day equilibration periods.

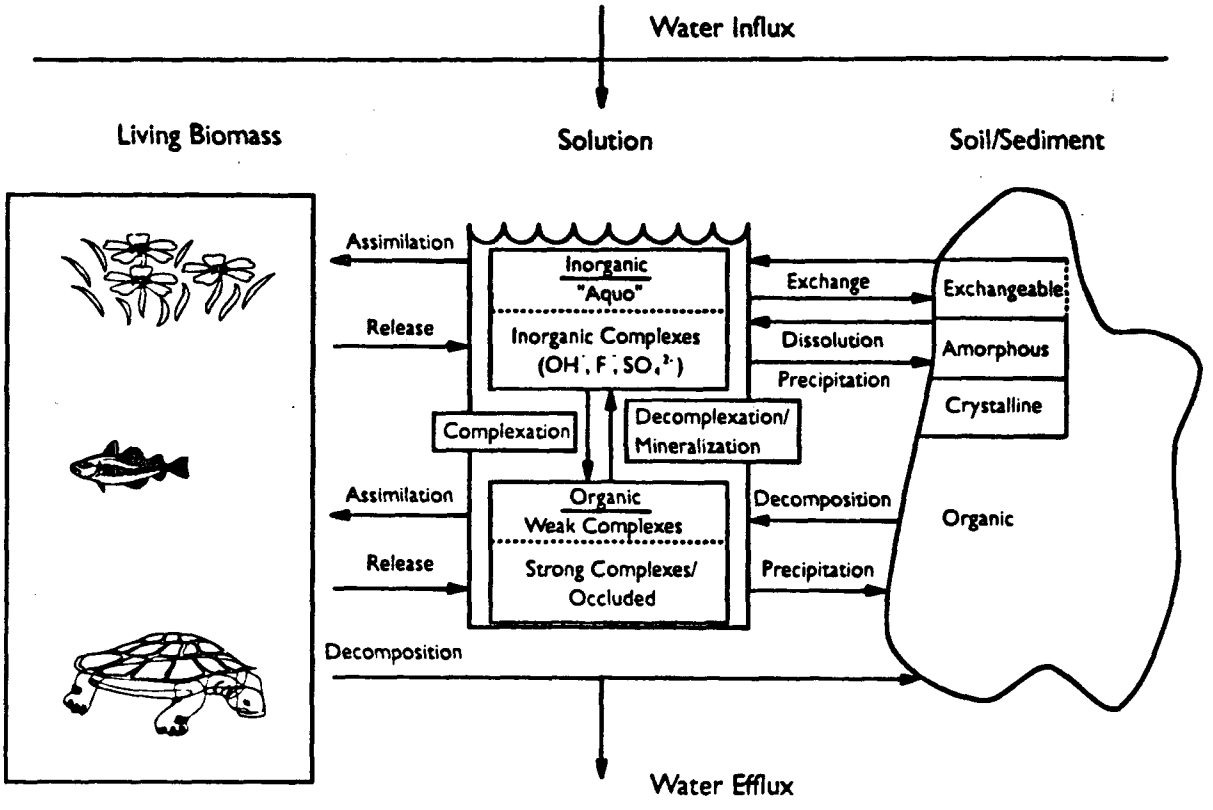


Figure 1 A schematic representation of the Al cycle. Within the aquatic environment Al can exist as pools of living biomass, solution, or nonliving particulate matter. Several transfers link individual pools and regulate Al concentrations.

Figure 1-1: A schematic representation of the Al cycle. From Driscoll and Postek (1996).

Aluminum speciation and toxicity towards freshwater plants have been most completely studied using both planktonic and benthic algae. As in much of the rest of our review, most of these studies focused on the ecological impacts of acidification, owing to significant increases in Al solubility generally occurring at acidic pH in freshwater systems (chapter 1). Only very recently have the impacts of Al at circumneutral or higher pH been under investigation. We review here the impacts of Al on freshwater algae not only in terms of single-species toxicity tests, but also with respect to its effects on cell morphology, Al-induced changes in species composition, the modifying effects of pH, anion and organic carbon complexation, and the ecological implications of interactions between Al and the essential nutrients phosphorus and silicon.

2.1 Al Toxicity to Individual Algal Taxa

Few studies have examined the effects of Al on freshwater phytoplankton until very recently, especially when compared to more intensively studied taxonomic groups such as fish. There also have been few attempts to summarize this literature since the review by Burrows (1977), and thus algal studies since that review are compiled here (Table 2-1). Even though widely different species, growth media, and culture methodologies were used, certain generalizations can be made. When total Al concentrations are used to describe biological effects, ca. 50% reductions in biological activity occur most commonly over a range of a few hundred to a few thousand $\mu g \cdot L^{-1}$

Table 2-1: Population growth and miscellaneous physiological effects of Al on phytoplankton species in isolated culture. Aluminum concentrations are given as total Al unless otherwise indicated. Only studies that measured population growth rates or biomass were included here; purely physiological studies are cited and discussed in section 2.1.

Species	Al Concentration ($\mu g \cdot L^{-1}$)	pH	Biological Response	Experiment Medium	Reference
Cyanobacteria					
Anabaena cylindrica	4680	6	50% reduction in biomass after 120 hr	Inorganic medium with 2.3 µM EDTA	Petersson et al. (1985)
	999	6	ca. 50% reduction in nitrogenase activity		
	5130	6	86% reduction in CO_2 fixation		
Nostoc linckia	16,200	7.5	ca. 50% reduction in growth rate when exposed to $AlCl_3$	modified Chu-10, with 4mM HEPES buffer (at pH 7.5 only)	Rai et al. 1996
	1,485	6.0			
	486	4.5			
	13,500	7.5	ca. 50% reduction in growth rate when exposed to AlF_4^-		
	1,080	6.0			
	324	4.5			
Chlorophyceae					
Chlorella pyrenoidosa	ca. 33[a, d]	4.8[d]	50% depression of growth rate	Synthetic Hardwater	Helliwell et al. (1983)
	25	5.1			
	15	5.4			
	7	5.6			
	< 5	6.1			
	7	6.5			
	22	6.8			
	45	6.9			
	85	7.1			

Species	Al Concentration ($\mu g \cdot L^{-1}$)	pH	Biological Response	Experiment Medium	Reference
C. pyrenoidosa	800[a]	4.3	30% growth inhibition	AAP medium, DMGA & MES pH buffers	Parent and Campbell (1994)
	660	4.6			
	410	4.8			
	90	5.0			
	63	5.2			
	53	5.5			
	7	6.0			
C. saccarophila	10,000	3	reduced growth rate	BBM, no EDTA, + 0.1% glucose	Folsom et al. (1986)
	25,000		reduced growth rate and stationary phase biomass		
	50,000		no growth		
Monoraphidium dybowskii	1110	5.0	50% reduction in growth rate	10% L-16 with MES pH buffer	Claesson and Törnqvist (1988)
	1000	5.5			
	550	6.0			
M. dybowskii	200	4.8	ca. 65%[d] growth inhibition	synthetic media (unspecified)	Hörnström et al. (1995)
	300		ca. 80% growth inhibition		
	400		ca. 100% growth inhibition		
	600	6.8	little inhibition		
	1000		ca. 40% growth inhibition		
	2000		ca. 70% growth inhibition		
M. dybowskii	400	4.8	little inhibition	synthetic media, amended with humic lakewater	Hörnström et al. (1995)
	1000		ca. 10%[d] growth inhibition		
	2000		ca. 38% growth inhibition		
	400	6.8	ca. 15% growth inhibition		
	1000		ca. 75% growth inhibition		
	2000		ca. 100% growth inhibition		

Species	Al Concentration ($\mu g \cdot L^{-1}$)	pH	Biological Response	Experiment Medium	Reference
M. dybowskii	400	4.8	little inhibition	nutrient-enriched humic lakewater	Hörnström et al. (1995)
		5.5	little inhibition		
		7	ca. 65%[d] growth inhibition		
		7.5	ca. 10% growth inhibition		
		8.5	little inhibition		
	2000	4.8	ca. 50% growth inhibition		
		5.5	ca. 85% growth inhibition		
		7	ca. 100% growth inhibition		
		7.5	ca. 80% growth inhibition		
		8.5	ca. 50% growth inhibition		
M. griffithii	100	4.8	ca. 85%[d] growth inhibition	synthetic media (unspecified)	Hörnström et al. (1995)
	200		ca. 95% growth inhibition		
	100	6.8	ca. 86% growth inhibition		
	200		ca. 94% growth inhibition		
M. griffithii	200	4.8	little inhibition	synthetic media, amended with humic lakewater	Hörnström et al. (1995)
	300		ca. 32%[d] growth inhibition		
	400		ca. 50% growth inhibition		
	200	6.8	little inhibition		
	300		ca. 50% growth inhibition		
	400		ca. 60% growth inhibition		
M. griffithii	200	4.8	ca. 75%[d] growth inhibition	nutrient-enriched humic lakewater	Hörnström et al. (1995)
		5.5	ca. 100% growth inhibition		
		7	little inhibition		
		7.5	little inhibition		
		8.5	ca. 10% growth inhibition		
	300	4.8	ca. 75% growth inhibition		
		5.5	ca. 100% growth inhibition		
		7	ca. 50% growth inhibition		
		7.5	ca. 15% growth inhibition		
		8.5	ca. 62% growth inhibition		
Mougeotia sp.	0 - 3600	4.1	no effect on chlorophyll *a* concentrations	SD-11	Graham et al. (1996)

Species	Al Concentration ($\mu g \cdot L^{-1}$)	pH	Biological Response	Experiment Medium	Reference
Selenastrum capricornutum	990-1,320	7.0	Reduced cell counts and dry weight	unknown	Peterson et al. (1974), as cited in Gostomski (1990)
Selenastrum capricornutum	570	7.6	EC50 (biomass)	unknown	Call 1984, as cited in Gostomski (1990)
Selenastrum capricornutum	460	8.2	EC50 (biomass)	unknown	Call 1984, as cited in Gostomski (1990)
Selenastrum capricornutum	900 > 1000	7.1	50% growth inhibition 50% inhibition of chlorophyll *a* fluorescence induction parameters	ASTM with MES pH buffer	Marsh and Gensemer (unpublished)
Stichococcus sp.	520 220 ca. 200	5.0 5.5 6.0	50% reduction in growth rate	10% L-16 with MES pH buffer	Claesson and Törnqvist (1988)
Dunaliella acidophila	>> 270,000 135,000 > 270,000	1 7 1	Photosynthesis reduced 50% Growth rate reduced 50%	Defined inorganic	Gimmler et al. (1991)
Dunaliella parva	27,000 1,620	7 5.5	Photosynthesis reduced 50% Growth rate reduced 50%	Defined inorganic	Gimmler et al. (1991)
Bacillariophyceae					
Asterionella ralfsii var. *americana* Körn.	[b]440.4 / 5.3 605.7 / 8.6	5.0 6.0	ca. 50% reduction in growth rate[c]	FRAQUIL with MES pH buffer and 0.5 µM EDTA	Gensemer (1991)
Asterionella ralfsii var. *americana* Körn.	[b]522.7 / 5.24 800 / 8.52 (max. exposure)	5 6	ca. 50% reduction in growth rate[c] growth reduced to 89% of control	FRAQUIL with MES pH buffer and 0.5 µM EDTA	Riseng et al. (1991)

Species	Al Concentration ($\mu g \cdot L^{-1}$)	pH	Biological Response	Experiment Medium	Reference
Asterionella formosa Hass.	[b]600 / 8.52	6	ca. 50% reduction in growth rate[c]	FRAQUIL with MES pH buffer and 0.5 µM EDTA	Riseng et al. (1991)
	800 / 11.52 (max. exposure)	7	No overall reduction in growth		
Cyclotella meneghiniana	810	7.9	Inhibited growth	unknown	Rao and Subramanian (1982) as cited in Gostomski (1990)
	3,240		algistatic		
	6,480		algicidal		

[a] Labile monomeric (inorganic) Al concentrations as measured by reaction with pyrocatechol violet
[b] Results shown as total Al / pAl^{+3}
[c] Results estimated by fitting linear regressions to relationships between Al and growth rate where possible, then solving for concentration at which growth was reduced to 50% of estimated control growth rates.
[d] Percent inhibition values estimated from manuscript figures

(with the exceptions of extreme acidophiles - discussed below). Toxicity at such high concentrations is surprising considering that only small sub-fractions of that which is measured as "total" Al are probably biologically relevant, particularly at pH 6 where Al solubility is lowest (see also pH discussion below). Toxicological data compiled by the U.S. Environmental Protection Agency (reviewed in Gostomski 1990) also agree that total Al concentrations ranging from 460 $\mu g \cdot L^{-1}$ (50% reduction in biomass using *Selenastrum capricornutum*) to 6,480 $\mu g \cdot L^{-1}$ (algicidal effect on *Cyclotella meneghiniana*) were toxic to freshwater algae. It should be noted, however, that virtually all of these studies use total Al as the analytical metric to describe toxicity, whereas dissolved monomeric Al concentrations or Al^{+3} ion activities may be functionally more appropriate descriptors (see sections 2.4.1, and 2.5 below).

Given that the studies presented in Table 2-1 used such disparate methods, it is difficult to conclude whether sensitivity to Al differs between taxonomic groups of cultured phytoplankton species; no obvious differences between different algal divisions with respect to Al sensitivity were observed in this data set (Table 2-1). In contrast, the only single study to use cultures from several algal divisions concluded that desmids and diatoms were particularly sensitive, whereas chrysophytes along with some (but not all) Chlorophyceae could tolerate Al exposures up to 400 $\mu g \cdot L-1$ at pH 5.5 (Hörnström et al. 1984). More detailed observations on species-specific differences in Al toxicity using whole phytoplankton community observations and/or experiments will be discussed in section 2.3.

In contrast, where individual algal species within a single division were directly compared in a single study, marked differences in sensitivity to Al were usually observed. In one example, *Monoraphidium dybowskii* was clearly more Al tolerant than *M. griffithii* (Hörnström et al. 1984, Hörnström et al. 1995). Using two different chlorophytes, Lindemann et al. (1990) concluded that a *Chlorella* culture isolated from a well-buffered stream was more sensitive to Al toxicity in semi-continuous culture than a *Scenedesmus* culture isolated from a more acidic site (Table 2-1). Similarly, the more acidophilic diatom *Asterionella ralfsii* var. *americana* was slightly more tolerant of Al at similar pH (= 6.0) than its circumneutral congener *A. formosa* (Gensemer 1991).

Thus, it seems reasonable to conclude that species living in more acidic, Al rich waters will be inherently more adapted to and, hence, tolerant of Al exposure. Indeed, the extreme acidophiles *Chlorella saccarophila* (Folsom et al. 1986) and *Dunaliella acidophila* (Gimmler et al. 1991) are roughly ten times more tolerant of Al than the other taxa (Table 2-1). This result could be a consequence of reduced toxicity in the presence of high H^+ activities (Campbell and Stokes 1985, see also pH effects discussion below), but high tolerance was also maintained at circumneutral pH (Gimmler et al. 1991). This later study pointed to potential fundamental differences in ionic membrane potentials for acidophiles *vs.* more circumneutral taxa.

Comparing different bioassay endpoints within individual studies drew widely different conclusions as to the effects of Al on algae. Pettersson et al. (1985a) concluded that CO_2 fixation rates were the least sensitive to Al, with biomass production and N-fixation rates being progressively more sensitive (Table 2-1). This

result agrees with comparisons made by Gimmler et al. (1991) where far more Al was required to reduce photosynthetic rates as compared to growth rates by 50% for both species tested. Similarly, the quantum efficiency of photosynthesis in *Selenastrum capricornutum* (as detected by chlorophyll *a* fluorescence induction) was inhibited less by Al than was population growth rate at pH 7.1 (Marsh and Gensemer, unpublished data). Taken together, these data suggest that growth rates are more strongly inhibited by Al than photosynthesis *per se*.

The older literature (as reviewed in Burrows 1977) regarding freshwater algae showed toxic concentrations of Al to be much higher than in more recent studies (Table 2-1). In these earlier studies, concentrations ranging from tens to thousands of $mg \cdot L^{-1}$ were required to completely kill several species of algae. These results, however, were largely compiled from cell physiological studies (Böhm-Tüchy 1960, as cited in Burrows 1977) that were not designed to assess specific toxic levels of Al. Therefore, most of the concentrations reported in Burrows' table 8 are probably considerably higher than actually required to impair algal growth. Given also that pH was not controlled in most studies, it is especially difficult to judge whether such high Al concentrations needed to elicit toxicity are realistic in comparison to more recent work.

2.2 Effects of Al on Algal Cell Size and Ultrastructure

Changes in cell size or cellular ultrastructure have also been observed in freshwater algae as a response to chronic exposures of Al. Hörnström et al. (1984) noted that *Monoraphidium griffithii* cells became 'enlarged' at Al concentrations less

than those needed to inhibit growth, although no quantitative length measurements were given. Higher Al exposures in this same study resulted in cells with significant morphological aberrations, including bifurcations in a normally lanceolate cell, and other contortions in cell shape. Similarly, mean population cell sizes in *Scenedesmus obtusiusculus* increased slightly (by less than 1 µm diameter) after 24hr exposure to 220 $\mu g \cdot L^{-1}$ Al (Greger et al. 1992). Increases in cell size (as cell volume) were also observed in the presence of 560 - 1000 $\mu g \cdot L^{-1}$Al for the green algae *M. dybowskii* and *Stichococcus sp.* (Törnqvist and Claesson 1987), but the magnitude of these effects differed for each taxon with respect to which growth cycle phase (i.e. lag, logarithmic, stationary) was preferentially affected by Al. They postulated that Al may have been disrupting specific phases of cell division, thereby preventing the asexual formation of two normal-sized daughter cells from some of the larger parent cells.

In contrast to green algae, diatom cell lengths usually decrease in the presence of Al, but perhaps only when dissolved Si supplies are relatively scarce. Frustule deformities (bubbles) were noted in *Synedra cf nana* populations exposed to at least 100 $\mu g \cdot L^{-1}$ Al, although it was not noted whether cell lengths changed appreciably (Hörnström et al. 1984). Experiments using the acidophilic diatom *Asterionella ralfsii* var. *americana* demonstrated that relatively low Al additions (76 and 168 $\mu g \cdot L^{-1}$; pH = 6.0) decreased mean cell length, frustule surface area, and biovolume by 40-50% when populations were grown in Si-limited semicontinuous cultures (Gensemer 1990). These cell-length reductions were a result of an increase in frequency of a single size class of cells (15-20 µm length) that were substantially smaller than normal (ca. 50

μm), rather than a gradual, unimodal, decrease in overall population mean lengths. Further experiments using Si-limited continuous cultures of *A. ralfsii* suggested that cell length reductions occur during a single cell division, as indicated by increased cell length variability within intact colonies (Gensemer et al. 1993a). However, pH reduction from 6 to 5 also induced the decrease in cell length, rather than additions of 540 $\mu g \cdot L^{-1}$ of Al which, at the lower pH, was completely toxic. Single-generation decreases in diatom cell length may be more widespread since rapid cell length reductions were also observed in natural populations of Si-limited *A. formosa* (Kling 1993). Thus, an as yet undetermined combination of nutrient, pH, or metal-related stressors may induce such responses in pennate diatoms.

Because cell length reductions in diatoms may be linked to environmental stressors such as pH or Al, this may help diagnose paleoecological changes in these factors in diatom assemblages preserved in lake sediment geologic records. Diatoms are important bioindicators of environmental change in freshwaters, and have been used extensively to assess the onset and extent of lake acidification (Davis 1987, Dixit et al. 1992). *A. ralfsii* is often abundant in the plankton of acid lakes, and as such is a useful indicator of changes in lake pH (Schindler et al. 1985, Duthie 1989, Charles et al. 1990, Gensemer et al. 1993b). In Adirondack lakes *A. ralfsii* var. *americana* is operationally subdivided into two morphological forms: long (>45 μm) and short (<45 μm); their relative distributions are thought to relate to changes in pH and/or dissolved organic carbon concentrations (Charles et al. 1990). Lakes in Kejimkujik Park, Nova Scotia, also contain both length varieties of *A. ralfsii*, and their relative

distributions vary seasonally or as a function of changing environmental conditions (McIntyre & Duthie 1993). Taken together with the culture studies described above (Gensemer 1990, Gensemer et al. 1993a), different length 'forms' of this taxon may be environmentally-induced morphotypes of the same taxon, rather than distinct species or sub-species. Whether Al, pH, or other as yet undetermined environmental factors are primarily responsible for these changes in nature remains to be determined.

2.3 Planktonic and Benthic Algal Communities

Many have suggested that Al can induce significant changes in the community structure of freshwater phytoplankton and benthic algae. Much of this evidence is somewhat circumstantial, however, because many studies focused on acidification as a stressor, where increased Al toxicity is usually assumed to be a major factor responsible for such changes in most systems. More importantly, the few experimental Al manipulations of whole algal assemblages provide clear evidence that chronic Al toxicity can manifest itself in significant changes in community structure and function.

2.3.1 *Inferred Al Effects from Natural surveys - Phytoplankton*

Much of the evidence linking Al exposure to changes in phytoplankton community structure is drawn from synoptic studies of lake acidification. As for many other biological groups, profound changes in pelagic community structure usually are observed when lake pH is low enough for Al to be relatively soluble (i.e. below pH 5-6). In north temperate zones remarkably similar trends in phytoplankton community

structure exist when such acidic lakes are compared to (or acidified from) circumneutral sites: species diversity decreases, along with shifts in dominant taxonomic groups. Typically, dinoflagellates (especially *Peridinium* or *Gymnodinium* spp.) dominate planktonic flora in acidic lakes (Dillon et al. 1984, Hörnström et al. 1984, Geelen and Leuven 1986, Stokes 1986), with cyanobacteria (Kwiatkowski and Roff 1976) or occasionally the acidophilic diatom *Asterionella ralfsii* (Schindler et al. 1985, Charles 1990) dominating in some sites. These taxa usually replace the more diverse planktonic assemblage in circumneutral lakes which includes chrysophytes, diatoms (which are virtually absent below pH 5.8; Charles 1984), green algae, etc. Dillon et al. (1984) and Brezonik et al. (1984) mentioned that lakes in Florida respond to acidification somewhat differently than most north-temperate lakes with green algae dominating at low pH as opposed to the dominance of blue-greens on the Trail Ridge. Interestingly, changes in phytoplankton species composition in experimental lake acidification experiments were strikingly similar to those observed in synoptic surveys of acidic lakes (Schindler et al. 1985, Ravera 1986, Havens and DeCosta 1987a, Baker and Christensen 1991, Schindler et al. 1991).

Given the synoptic nature of most of these studies, it is difficult to determine whether or not Al toxicity contributes directly to these changes in community structure, as opposed to other important acidification-related variables (i.e. changes in pH, zooplankton grazing pressure, nutrient supplies, etc.). Studies reviewed by Havas (1986) more clearly link the presence of certain algal species to habitats enriched in Al (Havas' Table 3-5; also cited in Table 2 of Sparling and Lowe 1996). However, it is

unclear from this simple list what other environmental factors may or may not have been responsible for the persistence of these particular taxa at high Al concentrations and, presumably, low pH.

Others have attempted to more quantitatively associate increasing Al concentrations with changes in phytoplankton community structure. Havens (1993) correlated pelagic food web models with declining pH in softwater lakes in the Adirondacks, finding that declining pH was associated with decreasing planktonic species diversity, food web complexity, food web links, etc. While some of these food web parameters (low predator/prey ratios and high proportions of basal species) were also associated with acid lakes containing high Al concentrations, these relationships were not statistically significant. Using phytoplankton assemblages alone, Nicholls et al. (1992) found clear statistically significant relationships between Al and both algal biovolume and numbers of taxa for phytoplankton in Sudbury, Ontario lakes. In their study, the biovolume of Cryptophyceae, Chlorophyceae, and Bacillariophyceae were all negatively correlated with lake Al concentrations; decreasing numbers of taxa within these groups plus the Cyanobacteria and Chrysophyceae were also significantly related to Al. Particularly Al-tolerant taxa in this data set included *Tabellaria, Chrysolykos, Mallomonas, Cryptomonas, Chrysochromulina, Rhabdoderma, Paramastix, Chrysosphaerella, Navicula, Asterionella,* and *Chrysococcus* (Nicolls et al. 1992).

2.3.2 *Mesocosm and Whole-lake Experiments - Phytoplankton*

Only a few experiments have directly examined the comparative role of acidity and Al toxicity in controlling phytoplankton species succession in acidified lakes. The first was a set of limnocorral experiments in Lake O'Woods, West Virginia, where circumneutral lake waters were either acidified to pH 4.8, or acidified and exposed to 300 $\mu g \cdot L^{-1}$ Al (Havens and Decosta 1987b). Generally, similar species of phytoplankton and zooplankton typical of those present in acidified waters dominated in both acidified treatments, regardless of whether or not Al was present. This led the authors to conclude that most acid-tolerant species are also Al-tolerant. This conclusion agrees with single-species tests of Al and acid tolerances already described in section 2.1. The only additional stresses imposed upon the plankton community by Al was a short-term decrease in chlorophyll *a* concentrations, along with a longer-term drop in zooplankton abundances. The decrease in chlorophyll *a* occurred before the pH dropped from 7 to 4.8 (which took 14 days), therefore the authors concluded that Al additions to circumneutral waters helped remove algal biomass by coprecipitating either P or algal cells or both along with the $Al(OH)_3$ which forms at circumneutral pH. Similar declines in algal biomass have been observed when acidic, Al-rich waters are neutralized with lime (Bukaveckas 1989).

Havens and Heath (1990) performed a similar enclosure experiment in East Twin Lake, Ohio, but the acidification to pH 4.5 (with and without 200 $\mu g \cdot L^{-1}$ Al) was initiated more gradually to examine species shifts during acidification itself. Perhaps owing to this experimental design difference, or the more calcareous water in East

Twin Lake, more pronounced differences in algal community structure and succession occurred during acidification when Al was present. Even though filamentous cyanobacteria, diatoms and chrysophytes eventually were lost from both acidified treatments, their losses occurred earlier in the acid+Al treatments. In fact, diatoms still persisted in small numbers in the acid treatment but not in the acid+Al treatment. Dinoflagellates such as *Peridinium inconspicuum* dominated both acidified treatments, yet they were more numerous when Al was present. Similarly, *Mougeotia* and other filamentous chlorophytes dominated acidified enclosures more rapidly in the acid+Al treatments. The results of Havens and Heath (1990) lend strong support to the hypothesis that Al alone can alter community structure and rates of succession in freshwater phytoplankton assemblages during lake acidification.

Aluminum has the potential to change phytoplankton community structure even under non-acidified conditions. Support for this hypothesis comes from semi-continuous culture experiments performed using natural phytoplankton communities from Franklin Lake, WI (Pillsbury and Kingston 1990). They compared higher pH treatments (5.7) with the addition of 0, 50, 100, and 200 $\mu g \cdot L^{-1}$ Al to acidified treatments (pH 4.7) with no Al added. *Asterionella ralfsii* var. *americana*, and several species of *Arthrodesmus* and *Staurastrum* responded with no particular pH preference over this range, but exhibited low tolerance to even 50 $\mu g \cdot L^{-1}$ Al. In contrast, taxa which tolerated these Al additions included *Dinobryon bavaricum, Peridinium limbatum, Stenokalyx monilifera, Elaktothrix* sp., and *Oedogonium* sp.

2.3.3 *Benthic Algae and Paleolimnological Studies*

One of the dominant structural changes in the littoral zone of north temperate lakes undergoing acidification is the extensive development of benthic algal populations. These populations usually consist of benthic or epiphytic mats or 'clouds' of filamentous green or blue-green algae (Dillon et al. 1984, Stokes 1986). Chlorophytes from the Zygnemetales are particularly important in this regard, with assemblages often becoming dominated by *Mougeotia, Zygnema,* or *Spirogyra.* Although Al would clearly be more abundant under the acidic conditions associated with the development of these benthic communities, no specific studies have yet, to our knowledge, implicated Al as a direct causal factor for their development. Hypotheses usually put forward for the acidification-induced development of these communities include: 1) low pH preference of the dominant taxa, 2) reduced herbivore grazing as pH drops to pH 6 and below, 3) reduced microbial decomposition allowing for more biomass accumulation, and 4) reduced competition from other algae for light or nutrients under acidic conditions (Stokes 1986).

Substantially more evidence exists suggesting that Al can affect benthic algal community structure when one examines the paleolimnological literature. This evidence largely stems from intensive efforts to use the fossilized remains of two important algal groups, the diatoms and chrysophytes, as paleolimnological indicators of changes in lake water chemistry owing to acidification. Diatoms and Chrysophytes have achieved wide use in paleolimnological studies because diatom frustules and chrysophyte scales preserve well in lake sediments, and their taxonomic identification

requires examination of only these preserved structures (Smol et al. 1991, Dixit et al. 1992a). It has long been known that diatom community structure is a sensitive indicator of changes in surface water pH (Hustedt 1939, as cited in Charles et al. 1989). The assumption is also often made that changes in diatom community structure also correlate well with changes in pH-related chemical variables such as Al concentration.

Although this assumption is rarely tested experimentally (see Gensemer et al. 1993), numerous data sets demonstrate strong relationships between lakewater Al concentrations and diatom and chrysophyte community structure. Such data sets are usually constructed as an initial step in paleolimnological reconstructions, whereby statistical models are constructed which correlate community structure with environmental variables (Dixit et al. 1992a). Such models include canonical correspondence analysis (Charles et al . 1989, Dixit et al. 1991) and weighted averaging regression (Birks et al. 1990, Kingston and Birks 1990). For diatoms, community structure correlated with monomeric Al concentrations with a high precision second only to pH itself (Huttunen and Turkia 1990, Dixit et al. 1991, Dixit et al. 1993). In fact, regressions explained substantial amounts of variance between measured and diatom-inferred Al concentrations for lakes in both Sudbury, Ontario ($r^2 = 0.54$, Dixit et al. 1991) and in particular the Adirondacks, NY ($r^2 = 0.83$, Dixit et al. 1993). Specific taxa appear to be particularly good indicators of Al concentration, with 16 such species being listed in the Adirondack data set (Dixit et al. 1993) including *Fragillaria acidobiontica* which is often found in habitats containing relatively high Al

concentrations (Charles 1986). Scaled Chrysophytes are also often used in paleolimnological studies, and are potentially even more sensitive to Al than diatoms. In the data set of Cumming et al. (1992), monomeric Al explained a greater proportion of variance in Chrysophyte community structure than does pH or other pH-related variables in surface sediment samples in the Adirondacks. Among the best indicators of Al in these lakes were *Mallomonas hindonii, M. crassisquama, M. pseudocoronata, Synura petersenii,* and *S. spinosa.*

These strong statistical relationships between diatom and chrysophyte community structure and Al has enabled paleolimnologists to reconstruct past Al histories of north temperate surface waters. Such reconstructions have been made for acidified and metal-polluted lakes near Sudbury, Ontario (Dixit et al. 1991, Dixit et al. 1992a, Dixit et al. 1992b), in Adirondack Park, NY (Cumming et al. 1992, Kingston et al. 1992), northern Michigan (Cook et al. 1990), and other regions of North America covered by the Paleolimnological Investigation of Recent Lake Acidification (PIRLA) investigations (Kingston and Birks 1990). Similar techniques have also been used to correlate diatom-inferred pH in Wildsee, Germany, with Al extracted from core sediments (Steinberg and Tayarani-Dastmalian 1993). Although these highly precise Al reconstructions have seen wide use in helping reconstruct geochemical events that occur during lake acidification, including even fisheries declines (Kingston et al. 1992), they are still purely statistical correlations of community structure with many environmental variables that co-vary with pH. Whether or not these represent true

physiological or ecological response to changes in Al concentrations *per se* has yet to be confirmed experimentally using whole assemblages.

2.3.4 *Stream Periphyton*

Studies which examined acidification-induced changes in stream periphyton community structure often implicated Al as a factor responsible for these changes, although little is known compared to the more abundant phytoplankton or paleolimnological literature. Green algae and, in particular, diatoms replaced chrysophytes and blue-green algae in acidic streams (pH < 5) in the Great Smoky Mountains National Park, with biomass and productivity being greatest at the most acidic sites (Mulholland et al. 1986). In a study of the Grosse Öhe system in Germany, the redevelopment of benthic diatom communities in acidic streams following spring floods was clearly pH-dependent, and Al was implicated as being partially responsible (Steinberg and Putz 1991).

Experimental acidification of stream channels by Maurice et al. (1987) agreed with Mulholland et al. (1986) insofar as blue-green algae were lost as the stream was acidified to pH 4, but the blue-green algae were replaced with chlorophytes such as *Chlorella, Microthamnion,* and *Ulothrix*. Maurice et al. (1987) also observed a nine-fold increase in total Al concentrations, and claimed that this increase in Al could partially have been responsible for the observed changes in community structure. When the pH of Norris Brook (Hall et al. 1980) was experimentally decreased to pH 4 (accompanied by increases of Al by 181%), periphyton biomass increased at the same time that algal

productivity decreased. These workers attributed the increased periphyton biomass to either decreased grazing pressure, reduced microbial decomposition rates, or a successional shift to more acid and/or metal tolerant species. In contrast to Hall et al. (1980), however, Maurice et al. (1987) did not observe an increase in periphyton standing crop as pH dropped and Al increased to 181 $\mu g \cdot L^{-1}$.

Three sets of experimental Al manipulations of stream assemblages more directly emphasized the potential importance of Al in shaping periphyton community structure and function. In the Mullholland et al. (1986) study already described above, experimental additions of 250 $\mu g \cdot L^{-1}$ Al significantly decreased chlorophyll-specific productivity at one of the two most acidic sampling sites on Walker Camp Brook (Tennessee). However, they still concluded that differential grazing pressure was mostly responsible for differential biomass accumulation at acidic sites, rather than Al toxicity *per se*. Similar conclusions were reached in an acidification of dystrophic experimental streams in Québec from pH 6.7 to 4.5, both with and without the addition of 400 $\mu g \cdot L^{-1}$ Al (Planas et al. 1989). Algal densities in both acidified treatments were 30-70 times higher than in non-acidified controls, and both the species composition and vertical structure of the assemblages changed significantly. However, Al alone exerted little impact in addition to that of pH, with decreased grazing pressure being the most likely explanation for acidification-induced increases in periphyton biomass.

In contrast, Al additions to acidified stream water exerted more significant impacts than acidification alone in two studies using water from Foot Branch, Vermont, with periphyton biomass generally decreasing at low pH (Genter and Amyot 1994,

Genter 1995), which is in general agreement with results from Maurice et al. (1987). In the first study (Genter and Amyot 1994), Al additions ranging from 50 - 500 $\mu g \cdot L^{-1}$ were coupled with daily acidification of streamwater in experimental channels from pH 7.5-8 down to 4.8. After 28 days diatoms, green and blue-green algal populations were more significantly depressed in the acid+Al treatments than in the acid-only treatments. A more balanced design was used in Genter (1995) where 500 $\mu g \cdot L^{-1}$ Al was added both with and without the periodic additions of sulfuric acid sufficient to depress short-term pH values to 4.8. Interestingly, taxonomic responses (both increases or decreases in population abundance) were similar for both acidified treatments regardless of the presence of Al, some were similar for Al regardless of whether pH was lowered, and some taxonomic responses indicated interactions between Al additions and acidification. In the latter case, *Mougeotia, Schizothrix* and *Anacystis* were more sensitive to the combined stresses of low pH and Al than to acidified waters alone. Total community biomass suggested that decreases in biomass were more significant in the presence of both Al and acid as compared to acid-only treatments.

Curiously, results from these studies (Maurice et al. 1987, Genter and Amyot 1994, Genter 1995) contradict those of Mullholland et al. (1986) and Planas et al. (1989) with respect to Al-induced changes in periphyton biomass. Because only the latter two studies used experimental designs that included either intact or nearly intact invertebrate grazing communities, the enhanced sensitivity of periphyton biomass to low pH and Al may have been an artifact of their exclusion of invertebrate grazers. Thus, while the Maurice et al. (1987) and studies by Genter and Amyot (1994) and

Genter (1995) examined changes in periphyton communities owing to physiological tolerance (or algal interspecific interactions) alone, responses to Al in acidified streams differ markedly when invertebrate grazers are included in the experimental design.

2.4 Factors Influencing Al Bioavailability to Freshwater Algae

A wide variety of exogenous chemical factors are known to influence Al toxicity in algae. Chief among these are pH, Ca, dissolved organic carbon, and phosphorus complex formation. The first three will be discussed in detail below, with issues concerning Al-P complex formation and its implications for Al toxicity and algal nutrition being treated on their own (section 2.6).

2.4.1 *pH*

Easily the most important environmental factor controlling the toxicity of Al is the pH of the exposure medium. The solubility and speciation of Al are under direct control by pH, so at the very least, total nominal exposures of dissolved Al will change as a function of pH. Although most algal studies of Al examine pH-dependent toxicity in some fashion, drawing general conclusions is difficult given the wide diversity of experimental approaches, particularly with regards to Al speciation. Despite its relative logistic ease, surprisingly few studies actually measure dissolved monomeric Al using colorimetric reactions such as pyrocatechol violet which clearly and empirically measure these fractions (see chapter 1). The discussion to follow is largely based on

single species toxicity tests in which controlled laboratory conditions allow for direct comparisons among studies to be made.

pH-dependent Toxicity with respect to Total Al: When one examines relationships between toxicity using *total* Al additions as a function of pH, it is extremely difficult to reach a general conclusion as to what pH represents maximum toxicity to algae. Intuitively, Al should be more toxic at low pH given a fixed exposure to a total Al pool (Al_T), because the presumably most toxic soluble forms of Al become increasingly more abundant as pH values drop below 5.5. However, when one examines relationships between toxicity and pH from data presented in Table 2-1, no such relationship is apparent. In fact, when all the studies from which reliable EC50 values could be obtained are examined as a group, no simple monotonic or linear relationship between pH and algal EC50s (as a function of *total* Al) could be observed (Fig. 2-1). Paradoxically, the lowest toxicities observed were at the lowest pH values tests (5 and 5.5). However, variance among these data at low pH is extremely high, with the highest toxicity values (near 200 $\mu g \cdot L^{-1}$ total Al) occurring at pH 5.5 to 6. Toxicity values at basic pH ranged from 460 to 900 $\mu g \cdot L^{-1}$ total Al, which were still substantially more toxic that the lowest values observed at acidic pH.

Results within individual studies more clearly demonstrated pH-dependent toxicity as a function of total Al concentrations, yet they did not always agree with one another. In one group of studies, algal toxicity increased as pH became more acidic (relative to pH 6 to 7), presumably owing to the increasing solubility and, hence, exposure concentration of Al. For example, increasing toxicity (considered using total

Al) with decreasing pH was observed for the circumneutral *Asterionella formosa* (Riseng et al. 1991) and its acidophilic congener *A. ralfsii* var. *americana* (Gensemer 1991, Riseng et al. 1991). The cyanobacterium *Nostoc linckia* also exhibited this kind of pH-dependent Al toxicity with respect to growth rate (Rai et al. 1996), as well as with ATP synthesis, nutrient uptake, and photosynthetic rates between pH 4.5 and 7.5 (Husaini and Rai 1992). Similar trends were observed for *Monoraphidium dybowskii* when grown in synthetic media, even though the reverse was true when this medium was amended with humic lakewater (Hörnström et al. 1995). However, in the same study *M. griffithii* exhibited virtually the opposite behavior by exhibiting maximal toxicity at acidic pH (vs. circumneutral) in media containing humic lakewater. Closely related congeners also showed opposite behavior with respect to pH-dependent toxicity using *Dunaliella* (Gimmler et al. 1991). *D. parva* exhibited maximal toxicity at pH 5.5 relative to 7, whereas the extreme acidophile *D. acidophila* exhibited substantially less toxicity at pH 1 relative to 7.

In other studies, the opposite general trend was observed with toxicity as measured relative to total Al was highest at pH 6, and lower at more acidic pH. Using whole algal assemblages, for example, Nalwajko and Paul (1985) demonstrated that phosphate uptake and photosynthetic rates were more inhibited by 50 $\mu g \cdot L^{-1}$ Al at pH 5.2-6.9 than at pH 4.5. Single species tests using both *Stichococcus* sp. and *Monoraphidium dybowskii* also suggested Al toxicity was diminished at low pH. In this case, approximately two-fold more total Al was required to induce 50% growth inhibition at pH 5 vs. 6 (Claesson and Törnqvist 1988). However, a more recent study

using *M. dybowskii* found that toxicity was far greater at pH 4.8 relative to 6.8 (Hörnström et al. 1995). Why these studies contradict so directly using the same taxon grown in artificial media is difficult to discern. However, as previously mentioned, Hörnström et al. (1995) also found that Al toxicity to *M. dybowskii* decreased at acidic relative to circumneutral pH, but only when media contained humic lakewater.

Perhaps owing the abundance of Al work pertaining to acidification concerns, only a very few studies have examined pH-dependent Al toxicity at circumneutral pH and higher. Again using total Al as the analytical metric, Hörnström et al. (1995) found Al to be less toxic to *M. dybowskii* at pH 7.5-8.5 relative to pH 7.0 when grown in nutrient-enriched humic lakewater. As with other aspects of their study, the congener *M. griffithii* showed the opposite trend with Al being slightly more toxic at pH 8.5 than at pH 7 or 7.5, although the differences were not large. Enhanced toxicity at high and low pH relative to circumneutral conditions was also observed when *Selenastrum capricornutum* was exposed to alum sludge extracts for rivers in North America (George et al. 1995).

Clearly, an examination of pH-dependent Al toxicity in algae using total Al as the analytical metric makes it extremely difficult to draw consistent and generalized conclusions on the effects of pH. This is likely a result of use by various investigators of widely diverse growth media and growing conditions, thereby inducing large changes in Al speciation that were not usually considered in the studies, yielding results which cannot be compared using total Al data alone. Also in many cases, pH buffering was not consistently achieved, thereby compromising the utility of many

results. As the discussion below will show, more frequent use of analytical measurements of dissolved monomeric Al, coupled with an assessment of inorganic speciation, would greatly improve our understanding of pH-dependent toxicity. Species (and even clonal) differences among test organisms, as well as the presence of dissolved humic materials, also certainly contribute to the inconsistences among these studies and need to be resolved experimentally.

pH-dependent Toxicity with respect to Dissolved Monomeric Al: In stark contrast to the situation with total Al, Al toxicity studies in algae exhibit far more consistent trends relative to pH when dissolved monomeric Al is used as the analytical metric of exposure. Dissolved monomeric Al includes the free aquo ion, simple Al hydroxides sulfates and fluorides, as well as low molecular weight humic and fulvic acid complexes (Chapter 1). For the purposes of this discussion, we will examine only inorganic complexes, with humic and fulvic complexes being considered separately below.

While only three such studies currently exist whereby toxicity (presented as either EC50 or EC30 estimates: Helliwell et al. 1983, Gensemer 1991, Parent and Campbell 1994) can be compared directly using inorganic monomeric Al, a remarkably consistent trend between pH and toxicity is observed, regardless of the fact that two different taxa and three different exposure media were used (Fig. 2-2). This plot suggests a curvilinear relationship whereby toxicity is maximal at pH 6 with monotonic decreases in toxicity as pH approaches both 4 and 7. This may reflect, as pointed out in Helliwell et al. (1983), that $Al(OH)_2^+$ is the most toxic Al species because it is most

abundant at this pH relative to other simple dissolved Al hydroxides. However, as pointed out in the discussions of free ion and polymeric Al hydroxides as predictors of toxicity below (see section 2.5), the situation is not likely to be this simple.

pH-dependent Toxicity with respect to free Al^{+3} ion activity: Even more striking is the apparent relationship between free Al^{+3} and H^{+} ion activity. Only three studies (Gensemer 1991, Riseng et al. 1991, Parent and Campbell 1994) cited in table 2-1 estimated Al^{+3} ion activity from monomeric Al measurements, and when EC30 or EC50 values (using the -log of Al^{+3} activity, or pAl) were plotted as a function of pH, a strong ($r^2 = 0.85$) linear relationship was observed between pH and Al toxicity (Fig. 2-3). Although these data are limited only to an acidic pH range (<6), this strong relationship suggests that free Al^{+3} ion activity is an excellent predictor of Al toxicity in algae. It should still be noted, however, that at least part of this relationship may be autocorrelated given that Al^{+3} ion activity is, in part, estimated using pH values (see section 1.2.1 for further discussion).

This is consistent with the free ion model (FIAM) of metal toxicity (Morel 1983, Campbell and Stokes 1985, Campbell 1995) whereby even if the free aquo ion is not the actual ion responsible for inducing toxicity biochemically, it is often the best predictor of toxicity. This has also been discussed at length for Al and algae by Gensemer (1991), and Parent and Campbell (1994). In both of these studies, as well as in Fig. 2-3, toxicity is diminished (with low pAl values representing highest Al^{+3} ion activities) as acidity increases. According to the FIAM, this results from the more abundant H^{+} ions displacing Al^{+3} ions from the cell surface at low pH, thereby

diminishing toxicity. Section 2.5 below will more fully address the implications of this relationship for predicting toxicity.

2.4.2 *Base Cation Amelioration*

Similar to the protective role H^+ ions play in the FIAM, other cations, chiefly base cations, may also protect against Al toxicity in algae. The mechanism presumably would be similar in that increased concentrations of base cations bound to cell surface ligands would prevent or displace Al cations from binding to these same ligands. Although there are several demonstrations of this type of amelioration in higher plants (Kinraide and Parker 1987), as well as in fish and invertebrate toxicity tests (see chapters 4 and 5), curiously few such demonstrations exist in the algal literature. Only a single study was found that experimentally verified that Ca (along with other base cations including Na, Mg, and K) protected against Al toxicity in the acid-tolerant *Chlorella saccarophila* (Folsom et al. 1986).

Somewhat more is known about the reverse process whereby Al can interfere with Ca uptake and metabolism. Foy and Gerloff (1972) found that a strain of *C. pyrenoidosa* with no Ca requirement was much more tolerant of Al than other plants with known Ca requirements. Regardless of differential sensitivity, Al may also directly affect Ca uptake and metabolism. Studies using *Scenedesmus obtiusculus* suggested that cell surface-bound Al enhanced membrane permeability to base cations, thereby enhancing cellular quotas of Ca (Greger et al. 1992). However, this contrasts with the prevailing view in higher plants that Al actually inhibits Ca uptake, transport, and

metabolism (Haug 1984, Taylor 1988, Delhaize and Ryan 1995, Barcelo et al. 1996). Clearly more studies are required to verify the interactive influences between Ca exposure, Al toxicity, and their subsequent influence on Ca metabolism in algae.

2.4.3 *Anionic Complexation*

Besides H^+ or other cations, dissolved anions have the potential to affect Al toxicity by changing Al speciation, particularly with regards to the activity of the free Al^{+3} ion. Disregarding solid or colloidal phase complexes, these might include complexes with fluoride, sulphate, phosphate, nitrate, or silicates (chapter 1). Thermodynamics predict that if conditions warranted the formation of such complexes, they would decrease Al^{+3} free ion activity and, hence, toxicity. For algae, the impacts of Al-fluoride complexes were examined using the cyanobacterium *Nostoc linckia* (Husaini et al. 1996, Rai et al. 1996). However, these studies did not support the notion that Al-F complexation affected Al toxicity simply by changes in Al^{3+} activity alone, although Al speciation was not calculated. At pH 6.0 and 4.5, they confirmed the formation of an AlF_4^- complex concomitant with decreases in algal growth, photosynthesis, nutrient uptake and ATPase activity which were more severe than equivalent concentrations of $AlCl_3$. They postulated that the fluoroaluminate complex interfered with phosphate metabolism specific to ATP biochemistry. Thus the toxicity of the fluoroaluminate may be an important factor in acidic systems containing significant quantities of dissolved F, but this has yet to be confirmed for other algal taxa or in natural systems.

2.4.4 *Organic Carbon Complexation*

As reviewed in chapter 1, Al forms complexes with many forms of both synthetic and natural dissolved organic carbon (DOC) including humic and fulvic acids. Because it is generally thought that organic-bound Al is not available for algal uptake, the presence of organic C should in most cases ameliorate algal toxicity. From a theoretical viewpoint, this would be consistent with the FIAM in that given a fixed pool of dissolved Al, DOC additions would decrease the free ion activity of Al^{+3}, thereby diminishing toxicity. For metals other than Al, experiments using synthetic organic ligands generally conform to these FIAM predictions, whereas results are more mixed for the few experiments in which natural DOC was used (Campbell 1995).

For Al toxicity to algae, only a very few experiments constitute appropriate tests of the FIAM specifically with regards to the influence of DOC complexation. Using the synthetic organic chelator EDTA, Riseng et al. (1991) found that increasing EDTA concentrations indeed diminished toxicity consistent with decreases in Al^{+3} activity. EDTA (and pH) manipulations in this experiment also affected free ion activities of other metals know to both limit and inhibit growth (i.e. Fe, Mn, Cu, Zn, Co). Because speciation models predicted that Al bound more strongly to the EDTA, Al additions presumably enhanced the activity of these ions, thereby either ameliorating limitation or inducing toxicity. A similar EDTA-mediated interaction was observed when Al toxicity was attributed to indirect increases in Cu^{+2} activity using *Scenedesmus quadricauda* (Reuter et al. 1987). However, this is not always the case under all conditions as indicated by similar studies using *Asterionella ralfsii* where Al^{+3} activities alone can

explain Al-induced changes in growth rather than indirect interactions with either Cu or Fe (Gensemer 1991).

A far more definitive test of the FIAM in the face of DOC complexation was recently run using additions of a soil fulvic acid to cultures of *Chlorella pyrenoidosa* (Parent et al. 1996). Their elegant experimental design specifically held Al^{+3} ion activities constant even in the face of increasing DOC concentrations by holding pH constant, and increasing total Al concentrations in concert with DOC. Thus, if the DOC simply functioned to mediate Al toxicity via control of free ion activity (in accordance with FIAM predictions), no differences in algal growth should have been observed between the various DOC/Al treatment combinations. Surprisingly, growth rates increased as the concentrations of organic bound Al increased in concert with DOC additions, meaning that more than Al^{+3} activity alone controlled algal growth under these conditions. The authors suggested several interesting hypotheses explaining this apparent contradiction to the FIAM, the most likely among them being organic phosphorus contained in the fulvic acid was bioavailable to the algae, thereby diminishing phosphorus-limitation in their cultures. They confirmed phosphorus stress using enhancement of alkaline phosphatase enzyme activity as an index of P-starvation. This is an important conclusion challenging the widely held assumption that control of trace metal toxicity or limitation is one of the primary means by which DOC influences algal growth in natural waters.

Aside from experimental tests of DOC's role in the FIAM, evidence from acidification studies further support the notion that DOC complexation not only

decreases dissolved Al concentrations, but that this may result in decreased algal toxicity. That inverse chemical relationships exist between increasing DOC and decreasing dissolved Al concentrations in acidic lakes is widely known (Schindler et al. 1992). Thus, because DOC additions diminish dissolved Al concentrations (and vice versa), they should also diminish phytoplankton toxicity. Data from Adirondack food web studies support this hypothesis in that lakes high in monomeric Al and low in DOC tended to possess simpler plankton communities than those in lakes with higher DOC concentrations and, hence, lower monomeric Al (Havens 1993). Havens implies that these simpler assemblages are typical of those in which chemical toxicity is largely responsible for determining community structure. Paleolimnological evidence using diatom or chrysophyte community structure also points to an inverse relationship between DOC and monomeric Al levels (Huttunen and Turkia 1990, Kingston and Birks 1990, Dixit et al. 1992), indirectly implying that algal community structure responds to the chemical interaction between them in some fashion.

2.5 Identification of Al Species that Best Predict Toxicity Towards Algae

Which one or more Al chemical species is most responsible for inducing or predicting toxicity in algae is only recently becoming possible to identify. This is because to make such determinations, careful attention to empirical measurements of monomeric Al coupled with estimates of dissolved ion speciation (i.e. using MINTEQ modeling) must be considered in the study. As we have already discussed above, only a very few algal studies have used such methods.

The practical implication of identifying a most toxic species is that such an understanding allows for more accurate, mechanistically meaningful, predictions of algal toxicity based on ambient water chemistry. As was seen above in the discussion of pH-dependent toxicity using total Al measurements, it is virtually impossible to predict Al toxicity without at least some attempt to measure or estimate chemical speciation of the exposure waters. Only by using speciation-based techniques can toxic species be identified, and thus be used in making quantitative predictions of Al toxicity under particular exposure conditions either in the lab or in the field. However, one should also be aware that the most appropriate and practical goal in not necessarily to identify "the most toxic" Al species, but rather to best predict and understand Al toxicity in the face of changing chemical conditions.

2.5.1 *Free Al^{+3} ion vs. Dissolved Monomeric Al Hydroxides*

We have already established that pH is the dominant controlling factor in Al toxicity to algae. Thus, the discussion that follows begins with studies of pH-dependent Al toxicity. One of the first studies to use speciation methods and, thus, propose a most toxic (or most predictive) Al species was the study by Helliwell et al. (1983) in their frequently cited experiments using *Chlorella pyrenoidosa*. They observed that algal EC50s were lowest at pH 6, with toxicities decreasing both at pH values lower and higher than 6. They argued that because the dominant Al species in solution at pH 6 was $Al(OH)_2^+$, and that this was the species most likely to be responsible for the pH-dependent toxicity observed in their experiments. However, it appears that this

argument was based mostly on the *relative* abundance of the different species in solution, not on absolute analytical concentrations of monomeric Al *per se*.

An alternative hypothesis was posed by Gensemer (1991) such that, similar to predictions of the FIAM, free Al^{+3} ion activity was not only the best predictor of Al toxicity, but also helped explain pH-dependent toxicity more clearly. In this experiment using the acidophilic diatom *Asterionella ralfsii* var. *americana*, algal growth rates were analyzed using total Al, dissolved monomeric Al, and modeled predictions of Al^{+3} ion activity. Conclusions reached using monomeric Al and free ion activity were similar in that Al toxicity diminished at pH 5 relative to pH 6. pH-dependent responses using free ion activity further suggested that H^+ ions were indeed protecting against Al toxicity at low pH, also in agreement with the FIAM.

Unlike Helliwell et al. (1983), Gensemer's (1991) results did not support the hypothesis that an Al hydroxide could be the best predictor of toxicity because monomeric Al concentrations were highest even in the face of diminished toxicity at low pH. Additional benefits to using speciation modeling is that it allows one to test whether indirect chemical interactions between Al and other potentially toxic (or limiting) trace elements could be confounding results presumed to occur owing to Al ions alone (Rueter et al. 1987, Gensemer 1991, Riseng et al. 1991).

Further support for the hypothesis that the free Al^{+3} ion is a strong predictor of algal toxicity comes from work with *Chlorella pyrenoidosa* (Parent and Campbell 1994). These workers found that predictions of the FIAM, namely that the greater abundance of H^+ ions at low pH ameliorate Al toxicity, were consistent with decreased algal toxicity

at low pH using solutions free of potential interference by polymeric Al (see section 2.6.2). Their work also provided direct empirical support to FIAM by demonstrating that less Al adsorbed onto algal cell surfaces at the most acidic pHs tested. But even though the FIAM was the most straightforward for interpreting their results, not all aspects of their results were perfectly consistent with the FIAM. Several alternatives were proposed to explain these residual pH effects, including pH-dependent changes in membrane permeability, changes in Al-cell ligand equilibrium constants with decreasing pH, or even chemical or conformational changes in the algal surface ligands with decreasing pH.

2.5.2 *Polymeric Al*

Many Al toxicity experiments are run at concentrations and pH values that also favor the formation of polymeric Al hydroxides (chapter 1). Because they form most favorably near pH 6, the production of polymeric Al potentially confounds interpretations of maximal Al toxicity also often observed at this pH. Thus, even though empirical results and predictions of the FIAM might predict high toxicity at pH 6 relative to more acidic pH owing to dissolved Al species alone, polymeric Al is also present at its highest relative *and* absolute abundances at pH 6. Thus, it is difficult to separate experimentally whether the dissolved or polymeric Al species can best predict or understand pH-dependent algal toxicity. Given that polymeric Al has recently been shown to be even more toxic than dissolved Al species to terrestrial plants (Parker et

al. 1989, Kinraide 1991), it is critical that we improve our understanding of its relative potency in aquatic species.

That polymeric Al can be at least equally toxic as monomeric species at pH 6 was borne out by an elegant set of experiments in Parent and Campbell (1994). These workers added various doses of polymeric Al to a medium otherwise containing only monomeric Al, using base neutralization of an acidic $AlCl_3$ stock solution in such a fashion as to maximize the formation of the Al_{13} polymeric cation. Under these conditions, toxicity owing to the Al polymer was approximately additive to that of monomeric Al species (Parent and Campbell 1994). In contrast, both Helliwell et al. (1983) and Gensemer (1991) rejected the importance of polymeric Al in their experiments, but neither study empirically manipulated polymeric species.

Thus, maximal Al toxicity at pH 6 may also result from the presence of these highly toxic polymeric cations and colloids in addition to relaxed competition from H^+ ions relative to lower pH values. Actively photosynthesizing algae can also setup an alkaline microenvironment around the cell owing to photosynthetic CO_2 uptake shifting the external equilibrium between HCO_3^- and CO_3^{2-} toward the latter (Reynolds 1984). Such physiologically-induced shifts toward higher pH could thus induce rapid formation of Al polymers during the day could conceivably induce Al toxicity in similar fashion to that demonstrated at the fish gill (chapter 5). However, this process has never been tested experimentally. Although our understanding is certainly improving, additional studies will be required to confirm and quantify the relative importance of polymeric vs.

monomeric Al in predicting or understanding pH-dependent toxicity across a wider range of exposure conditions.

2.6 Effects of Al on Algal Phosphorus Dynamics

It has long been suggested that Al may induce some level of phosphorus limitation or stress in freshwater phytoplankton populations. This could occur owing to an indirect chemical interaction of P with Al which may reduce dissolved P supplies to the point that algal population growth rates and/or biomass could become limited by this resource (Dickson 1980, Driscoll 1989). In addition, Al may directly affect physiological properties of the phytoplankton themselves such that growth or productivity could be compromised. In either case, Al clearly has the potential to alter P dynamics in freshwater ecosystems, but the evidence for this is far from unequivocal.

2.6.1 *Direct Effects of Aluminum on Phosphorus Physiology:*

Al may induce P stress and/or limitation by inhibiting the function of physiological processes for P acquisition and utilization by freshwater phytoplankton. The sparse literature on P uptake shows Al can either inhibit P uptake rates (Nalewajko and Paul 1985), or that P uptake rates area unaffected by Al (Pettersson et al. 1988). In their experiments using whole plankton communities from Plastic and St. Nora Lakes (Ontario, Canada), Nalewajko and Paul (1985) found P uptake rates to be significantly reduced after only modest Al additions (50 $\mu g \cdot L^{-1}$ Al). They concluded that complexation with Al made some of the ambient P unavailable for enzymatic transport,

thereby reducing uptake rates. This effect also was more pronounced at pH values 5.2-6.9 as opposed to a lower pH of 4.5, which is consistent with maximal formation of the $AlPO_4$ complex near pH 6 (Stumm and Morgan 1981; see also discussion above).

In a related study examining the effects of short-term pH shocks on algal P uptake kinetics, Nalewajko and O'Mahony (1988) reasoned that $FePO_4$ complex formation may, in fact, be more important in controlling the availability of dissolved P, at least at the low ambient Al concentrations in these waters. However, even after taking into account Al-P complexation, P uptake rates were also reduced as a result of direct 'toxic' inhibition of P uptake velocity by Al (Nalewajko and Paul 1985). In contrast, Pettersson et al. (1988) found that Al additions of 243 $\mu g \cdot L^{-1}$ Al (pH = 6.0) did not significantly inhibit P uptake velocity in *Anabaena cylindrica* cultures, even after accounting for inorganic Al-P complexation. As the authors correctly pointed out, these contradictory results may have been a function of less Al-P complex formation (intentionally set to only 10% of the total inorganic P added), or that this single species was more tolerant of Al than the natural populations assayed by Nalewajko and Paul (1985).

In acidic stream waters which contained relatively higher Al concentrations than in more neutral waters, PO_4 turnover rates were actually lower than at the neutral sites, which suggests that Al (and/or low pH) could have reduced P uptake rates (Mulholland et al. 1986). However, P uptake rates were not specifically corrected for Al-P complexation, so the authors pointed out that their result could simply be a function of

Al-induced reductions in ambient P supply at the higher pH's (near 6) where $AlPO_4$ is most likely to form (see Chapter 1).

Some evidence also exists that Al can affect the acquisition of organic phosphorus via acid phosphatase enzyme hydrolysis by algae. Acid phosphatases presumably function similarly to alkaline phosphatases in that they are phosphomono-esterase enzymes which help supply algae with inorganic P (Healey and Hendzel 1979). Phosphatase activity commonly increases in the presence of low inorganic P concentrations, therefore high biomass-specific phosphatase activity is considered to indicate P-limitation in algal populations (Healey 1973, Healey and Hendzel 1979, Wetzel 1983). The effects of Al on acid phosphatase activity, however, are poorly understood and contradictory. Jansson (1981) found that Al significantly increased acid phosphatase activity in Lake Gårdsjön (Sweden) by competitively binding with the PO_4 groups on the organic P substrate, thereby making it more resistant to enzymatic hydrolysis. As a result, algae would be forced to produce more phosphatase enzymes to compensate for the reduced activity. In contrast, stream periphyton communities were found to exhibit reduced phosphatase activities in more acidic waters containing higher Al concentrations (Mulholland et al. 1986). These authors attributed their contradiction with Jansson (1981) to the relatively 'low' (< 300 $\mu g \cdot L^{-1}$) Al concentrations present in these waters, but did not specifically test whether Al directly affected enzyme activity or not. Furthermore, Pettersson et al. (1988) demonstrated that Al (972 $\mu g \cdot L^{-1}$) additions to cultures of *Anabaena cylindrica* reduced intracellular acid phosphatase activity. As with their P uptake results, this particular species may have different

responses than the natural populations of Lake Gårdsjön (their assays were also done under more natural conditions). This discrepancy may also be a pH-related phenomenon because Jansson's studies were performed at lower pH (4.6) than those of Pettersson's (pH = 6.0).

Evidence also exists that Al often becomes associated with internal P storage pools in algae, and may affect the amount or biochemical utilization of these pools. In *Anabaena cylindrica*, Al was found to accumulate in internal polyphosphate bodies, particularly when cells were grown in P-rich media which increased the amount of P stored in these structures (Pettersson et al. 1985b). However, Al uptake was found not to require energy, and was unaffected by the P status and, hence, the extent of polyphosphate bodies in the cells (Pettersson et al. 1986). These authors concluded that polyphosphate binding may serve to detoxify Al, but no direct toxicological evidence was presented to support this conclusion. More recent work on *A. cylindrica* suggested that Al may also affect both the size and utilization of internal polyphosphate bodies even though net P uptake rates were unaffected by Al (Pettersson et al. 1988). In this study, 972 $\mu g \cdot L^{-1}$ Al reduced the breakdown of polyphosphate granules over time, suggesting that Al inhibits the biochemical use of this P storage pool, most probably by reducing internal acid phosphatase activity. Similarly, Al was found to increase the internal P content of *Scenedesmus obtusiusculus* cells, unless external P supplies were reduced by $AlPO_4$ precipitation at high ambient P concentrations (Greger et al. 1992a). However, their medium was not buffered and so pH decreased with increasing Al additions, and the only experimental controls used were pH reductions in

the absence of Al. Therefore, Al speciation could not have been constant among the different Al additions, thereby limiting the generality of their conclusions.

Other physiological processes involved in P metabolism may be affected by Al. For example, ATP pool size and activity may be affected by Al. Continuing their work with *Anabaena cylindrica*, Pettersson and Bergman (1989) found that additions of 97 - 972 $\mu g \cdot L^{-1}$ Al increased intracellular ATP pools by 20-40%, and this could be reversed by adding excess P to the growth medium. They hypothesized that Al may be competing with Mg^{2+} ions thereby rendering ATP unavailable for cellular metabolism. Cells would thus have to compensate for reduced ATP availability by producing excess amounts of ATP. This compensation would affect general energy production by the algae, with the potential for affecting virtually any metabolic process.

2.6.2 *Indirect Effects of Aluminum on P-limitation - the "Oligotrophication Hypothesis":*

A potential consequence of Al-induced P-limitation in natural phytoplankton communities is that lakes may become less productive and, hence, more oligotrophic during acidification. This, of course, is the rationale for using alum treatments to control lake and reservoir eutrophication (see section 2.6.3). Natural P-reduction by Al has been a significant concern since the term "oligotrophication" was first applied to acidifying lakes in Scandanavia (Grahn et al. 1974). Little specific reference was made by Grahn et al. (1974) to the role of Al, but the formation of Al-P complexation soon was considered to be a *potentially* important mechanism for reducing algal productivity (Almer et al. 1978, Dickson 1980). Since these papers were published, many

researchers focused on testing this "hypothesis" by examining whether P became more limiting in acidified lakes as a consequence of, in part, chemical complexation with Al.

Only limited support for the role of Al in acidified lake oligotrophication was ever found. The most significant study demonstrating a link between Al-P complexation and reduced algal productivity and/or biomass was that on Lake Gårdsjön, Sweden. In this case, however, Al-P complexation did not occur to any significant extent in the lakewater itself, but rather in the B-horizon of catchment soils (Persson and Broberg 1985, Jansson et al. 1986). Complexation by Al in the B-horizon lowered P concentrations in the lake by reducing P-loading from the catchment, rather than by $AlPO_4$ formation in the surface waters themselves. Even with reduced P inputs to Lake Gårdsjön, however, overall algal biomass and productivity were still not atypical of other acidified lakes in this region (Lydén and Grahn 1985). Circumstantial evidence also exists in favor of the oligotrophication hypothesis in a study of P sorption to sediments from acidic lakes in Florida, U.S.A. (Ogburn and Brezonik 1986). Soluble P sorbed to sediments maximally at pH values *near* where $AlPO_4$ occurs (pH 4-5.5 in this study), but Al was not specifically manipulated.

Evidence rejecting the oligotrophication hypothesis and, more specifically, the role of Al in this process is more abundant and convincing. The dominant study in this regard was the experimental acidification of Lake 223 in the Experimental Lakes Area in Ontario, Canada (see Schindler et al. 1985 for summary of acidification results). In this example, artificial acidification to a pH of 5.0 did not decrease algal biomass or productivity, but rather there was a slight, but significant increase in these parameters

as compared to reference lakes (Schindler 1980, Schindler et al. 1985, Findlay and Kasian 1986, Shearer et al. 1987, Schindler et al. 1991). A similar study on Little Rock Lake showed qualitatively similar results (Schindler et al. 1991).

In both cases, the generality of these results are somewhat questionable because strong acids were added directly to surface waters, thereby reducing the amount of Al that could have reached the lake owing to catchment soil reactions (Shearer et al. 1987). However, considering that virtually all aspects of phytoplankton responses to acidification in the experimental studies were nearly identical to those found in synoptic surveys (Dillon et al. 1984, Ravera 1986, Geelen and Leuven 1986, Muniz 1991, Schindler et al. 1991), it is doubtful that differences in acidification method biased their conclusions. Excluding acid-induced Al leaching from catchment soils thus may not have affected their conclusions because 1) Al-P complexation *in situ* may, in fact, not be a significant process by which P-limitation can be exacerbated in acid lakes, or 2) P concentrations were never high enough to exceed the solubility product of $AlPO_4$ regardless of Al concentrations or fluxes from the watershed.

2.6.3 *Alum Treatment and Phosphorus-Inactivation for Algal Control*

As mentioned in Chapter 1, aluminum sulfate (alum) has frequently been used to control nuisance algal blooms in lakes owing to the formation of insoluble Al hydroxides which adsorb dissolved P thereby diminishing algal P supplies from sediments (reviewed in Cooke et al. 1993). Dosing strategies typically target alum additions at the highest concentrations possible without dropping water pH below 6.0 (Kennedy and

Cooke 1982). Phosphorus is removed to some extent from the water column directly, whereas the primary goal is to form a floc of Al hydroxides on the sediment surface to control P release from sediment sources (Cooke et al. 1993).

Using these dosing strategies, successful and long-term reductions in P supply and algal growth often have been noted, particularly in well-stratified lakes with alkalinities exceeding 75 $mg \cdot L^{-1}$ $CaCO_3$, and high levels of Si, Ca, SO_4 and DOC (Cooke et al. 1993). More recent observations further support the effectiveness of alum treatments for P control (Lund and Chester 1991, DeGasperi et al. 1993, Romo and Bécares 1994).

However, alum additions to acidic or low alkalinity lakes have proven more problematic, even though additional buffers (i.e. sodium aluminate) are usually added to maintain lakewater pH between 6 and 8 (Cooke et al. 1993). Under these conditions, the authors suggested that maximal alum additions may not be the most appropriate, and non-Al buffers (i.e. lime) might also be used to help control pH and prevent solubilization of Al from the alum floc. Cooke et al. (1993) also pointed out that alum is less effective in controlling P release from oxic sediments in shallow lakes, but may still minimize nuisance cyanobacterial populations by inhibiting their sediment-to-water migration (Welch and Schrieve 1994).

Given the widespread use of alum to control algal blooms, concern is often raised over whether these rather large Al additions (sometimes up to hundreds of metric tons) could be toxic to aquatic biota. Relatively few instances of direct toxicity to invertebrates and fish have been noted following alum treatments, and these occur

usually under marginally acidic conditions (i.e. when lakewater pH nears 6), or shortly after alum additions when $Al(OH)_3$ has not yet had sufficient time to fully age into an insoluble floc (Cooke et al. 1993). Alum sludges for use in water treatment plants are also rarely toxic to algae, and then only when pH values approach 5 (George et al. 1996).

However, considering more recent knowledge of Al speciation and bioavailability reviewed in this chapter, certain conditions following alum treatment could conceivably introduce conditions leading to Al toxicity. Target alum doses are supposed to keep dissolved Al concentrations below 50 $\mu g \cdot L^{-1}$ (Kennedy and Cooke 1982), but from this study we can not determine whether the fraction they consider to be "dissolved" actually represents inorganic monomeric Al as defined in chapter 1 of this review. This is important biologically since for algae, inorganic monomeric Al measurements are one of the most meaningful predictors of pH-dependent Al toxicity (Section 2.4.1). Furthermore, systems designed to treat stormwater runoff using alum injection (Livingston et al. 1994) typically keep dissolved Al concentrations (measured from acidified solutions *following* 0.45 µm-filtration) in the receiving water body below the 4-day average EPA Ambient Water Quality Criteria of 87 $\mu g \cdot L^{-1}$ (EPA 1988).

Although these concentrations are below that which are typically toxic to algae (Table 2-1), inorganic monomeric or Al^{+3} ion activity estimates should be used to determine more reliable, mechanistically meaningful, target doses. This suggestion is motivated in part because of various investigators use different operational definitions of the term "dissolved", whereas inorganic monomeric Al measurements typically utilize

more highly selective colorimetric and/or ion exchange separations (chapter 1). Furthermore, some potentially toxic colloids or polymers might pass through filters commonly used to separate dissolved from particulate fractions (i.e. 0.45 µm filters). For example, Al concentrations used to set EPA water quality criteria are not true dissolved monomeric Al concentrations, but rather "acid-soluble" aluminum which is derived from a non-filtered water sample that is acidified to pH 1.5-2.0, *prior to* filtration through a 0.45 µm filter (EPA 1988). Given that filtration is performed following acidification, most forms of colloidal and polymeric Al would be included in these measurements (chapter 1). Thus, any Al measurements considered to be "dissolved" by these EPA standards, might not be the most appropriate descriptors of toxicity, particularly under acid/base mixing conditions that favor the formation of the Al_{13} cationic polymer.

Another risk in the currently accepted alum dosing strategies is that the maximum Al dose is designed to achieve lakewater pH values as low as 6.0 (Kennedy and Cooke 1982). Given our discussion of pH-dependent toxicity to algae above (Section 2.4.1, Figs. 2-2, and 2-3), pH 6 represents maximal toxicity to algae in several studies, particularly those using dissolved monomeric Al as the metric for Al exposure. This could be a significant concern for algal populations exposed to maximal alum doses, but this has yet to be tested under field conditions.

In addition, the potential for formation of polymeric Al cations of high toxicity at pH 6 (Chapter 1; see also Parent and Campbell 1994) also raises concern for using pH 6 as a lower limit in setting maximal alum doses. While the formation of these

polymeric cations are highly dependent on transient chemical conditions such as whether Al is being added to a base or vice versa (Bertsch and Parker 1996), such transient conditions may exist during the initial stages of alum formation (Cooke et al. 1993). The presence of these polymers may help explain Al toxicity under some circumstances following alum treatment, but both the formation and toxicity of these cationic polymers need further study under field conditions to better evaluate this hypothesis.

Given the potential risks of currently accepted alum dosing levels, we recommend that careful analysis of Al speciation (using methods outlined in Chapter 1) be performed following alum treatment to ensure that dissolved monomeric Al levels and pH do not exceed levels known to be toxic to algae or other aquatic biota (Chapters 4-5). Such measurements are logistically simple, and allow direct estimation of freely available Al, particularly under variable conditions of pH, or in the presence of other complexing agents (i.e. DOC, silicates, etc.).

2.7 Effects of Al on Algal Silica Dynamics

Aluminum also may affect the bioavailability and biogeochemistry of another important algal nutrient, silicon. To affect bioavailability, dissolved Al would have to form soluble aluminosilicate complexes with dissolved Si (Chapter 1). The formation of such complexes would be of great importance to algae which require Si (Bacillariophyceae, and Chrysophyceae), yet compared to phosphorus, little is yet known about the effects of Al on Si supply or utilization. Aluminum can also affect

frustule dissolution rates in diatoms (Lewin 1961, Van Benekom 1981, Van Bennekom et al. 1989, Van Bennekom et al. 1991), so the presence of Al could have significant effects on biogenic Si fluxes and cycling in both marine and freshwaters.

2.7.1 *Impacts of Silica on Biological Effects of Al:*

Regardless of the extent of dissolved aluminosilicate formation in natural waters, the complex can reduce the bioavailability and, hence, toxicity of Al to fish in controlled experimental exposures (Birchall et al. 1989). The same research group (Exley et al. 1993) also suggested that Si could reduce Al toxicity in both a diatom (*Navicula pelliculosa*) and a green alga (*Chlorella vulgaris*). They invoked an indirect mechanism by which Si reduces Al-P complexation thereby preventing growth limitation by phosphorus, rather than a direct amelioration of Al toxicity by aluminosilicate formation. This is an interesting possibility, but Exley et al. (1993) did not provide direct evidence of Al-induced P-limitation in their algal cultures, nor that Si additions ameliorated this nutrient stress. They only demonstrated a correlation between reduced P concentrations and reduced algal growth rates, which is not by itself sufficient evidence of nutrient limitation *per se*. Reductions in P concentrations by Al must be considered relative to the physiological requirements of any given species to determine whether or not specific P concentrations may be indicative of P-limitation (Tilman 1982, Tilman et al. 1982).

Limited evidence also exists that aluminum can affect Si uptake and utilization in freshwater diatoms. Given the importance of diatoms in understanding the ecology and

past history of acid lakes (Davis 1987, Schindler 1988, Charles et al. 1990, Dixit et al. 1993), understanding the effects of Al on nutrient-limited growth and physiology in diatoms could be important in correctly interpreting changes in diatom species composition in these systems. This is particularly important because Si concentrations may also be low in acid lakes (Hutchinson 1957, Driscoll and Newton 1985, Findlay and Kasian 1986, Willén 1991). If Al were to affect Si physiology in some taxa, it potentially could affect the competitive ability for and/or utilization of these scarce Si supplies, which could have subsequent impacts on algal community structure (Tilman 1982, Tilman et al. 1982).

Experiments using the acidophilic diatom *Asterionella ralfsii* var. *americana* Körn examined Si-limited growth and uptake physiology when grown at an acidic pH of 6.0 in the presence of Al (Gensemer 1991, Gensemer 1992, Gensemer et al. 1993). This taxon often is a dominant member of acid lake floras at acidic pHs where Al concentrations would also be high (Charles 1985, Davis 1987, Charles et al. 1990). Silica uptake rates changed when 20 $\mu mol \cdot L^{-1}$ Al was added to Si-limited continuous cultures at pH 6, but the effect was to stimulate maximum rates (Vm) by ca. 50% (Gensemer et al. 1993). This is unusual for the effect of a trace metal on algal nutrient uptake, and deserves further investigation. At the same time, evidence for an effect of Al on Si cell quota (an index of Si utilization by an alga, and often predictive of cellular nutrient status; Paasche 1980, Morel 1987) is more equivocal. In the same experiments, cell quotas were unaffected by Al (Gensemer et al. 1993), yet earlier experiments using the same taxon (but using more moderate Al additions of 5.56

$\mu mol \cdot L^{-1}$) suggested that Al may have increased cell quotas at low growth rates (Gensemer 1990).

Assuming that uptake rates are indeed stimulated by Al at pH 6, depending on how one interprets the cell quota results, two different predictions can be made with respect to the effect of Al on Si competitive ability. First, if uptake rates increase and if cell quotas (and maximum growth rates) are unaffected (e.g. Gensemer et al. 1993), one would predict that Al could reduce the half-saturation constant for nutrient limited growth (Kilham 1978, Morel 1987). Reducing growth half-saturation constants subsequently corresponds to reduced competitive ability for limiting nutrients (Tilman 1982, Tilman et al. 1982). Alternatively, if *both* Si uptake rates and cell quotas increase in the presence of Al (e.g. Gensemer 1990), their effects would be compensatory, thereby predicting that growth half-saturation constants and, hence, competitive ability, would remain unaffected by Al. In effect, increased uptake velocity could offset the increased Si requirements of each cell, thereby maintaining the same overall population growth rate. Interestingly, this agrees with results from Si-monod experiments run using *A. ralfsii* which showed that Si growth half-saturation constants were unaffected by moderate Al additions (5.56 $\mu mol \cdot L^{-1}$, Gensemer 1991).

Regardless of the effect of Al on Si growth kinetics in *A. ralfsii*, the experiments described above suggest that this taxon is particularly efficient at growth when Si supplies are scarce. This taxon maintains extremely low Si requirements compared to other *Asterionella* species (Gensemer 1991, Gensemer et al. 1993) and to most other freshwater planktonic diatoms (Paasche 1980, Tilman et al. 1982, Willén 1991).

Coupled with its ability to tolerate low pH and moderately high Al concentrations, *A. ralfsii* appears to be uniquely well-adapted for conditions commonly encountered in acidified lakes. In fact, *A. ralfsii* is one of the few planktonic diatoms to dominate planktonic assemblages at pH values below 6 (Findlay and Kasian 1986, Davis 1987, Charles et al. 1990, Findlay and Shearer 1992), which could be linked to a superior competitive ability for nutrients under conditions of pH and/or metal stress (Stokes 1986). The interacting influences of Al toxicity, pH tolerance, and Si-limitation must now be investigated in other diatom species commonly found in acidic lakes.

Finally, Al distributions in nature may be linked to Si biogeochemical cycles (indirectly via interactions with diatoms), although evidence only exists for marine waters. Diatoms have been linked directly to Al spatial distributions in open waters (van Beusekom 1988) and in experimental marine mesocosms (Moran and Moore 1988). Furthermore, diatoms are known to take up Al at realistic concentrations when either live or dead (van Bennekom 1981, van Beusekom 1988). This phenomenon also was observed in the mesocosm studies of Moran and Moore (1988) which directly linked diatom production to particulate Al fluxes in their water columns.

Once taken up or adsorbed by the diatom, Al is also known to delay frustule dissolution rates and/or solubility in marine diatoms (Lewin 1961, Van Bennekom 1981, Van Bennekom et al. 1989, Van Bennekom 1991). This effect of Al may help explain why dissolution of diatom frustules is relatively low in some marine sediments, even though the overlying waters are significantly undersaturated with respect to solid phase silicates (Van Bennekom et al. 1989, Van Bennekom 1991). Both Al uptake, and

the retardation of frustule dissolution by Al also could be important in freshwaters, yet to our knowledge no such measurements exist. This could be a serious oversight because freshwaters also posses generally undersaturated dissolved Si concentrations, yet frustules are generally well-preserved in most sediments. Furthermore, Al concentrations are often much higher in freshwaters than in seawater (at least in acidic waters; Driscoll and Newton 1985, Driscoll and Schecher 1990), thus Al has a high probability of retarding frustule dissolution in freshwater species.

FIGURE LEGENDS

Figure 2-1: Relationship between Al toxicity to algae, expressed as EC50, for those experiments that used total Al as the analytical metric for Al exposure. Data are from Table 2-1.

Figure 2-2: Relationship between Al toxicity to algae, expressed as EC50 or EC30, for those experiments that used labile (i.e. inorganic) monomeric Al as the analytical metric for Al exposure. Data are from Table 2-1, with specific studies noted with different symbols.

Figure 2-3: Relationship between Al toxicity to algae, expressed as EC50 or EC30, for those experiments that used pAl (-log(Al^{+3} ion activity)) as the analytical metric for Al exposure. Data are from Table 2-1, with specific studies noted with different symbols.

Total Al-dependent Toxicity

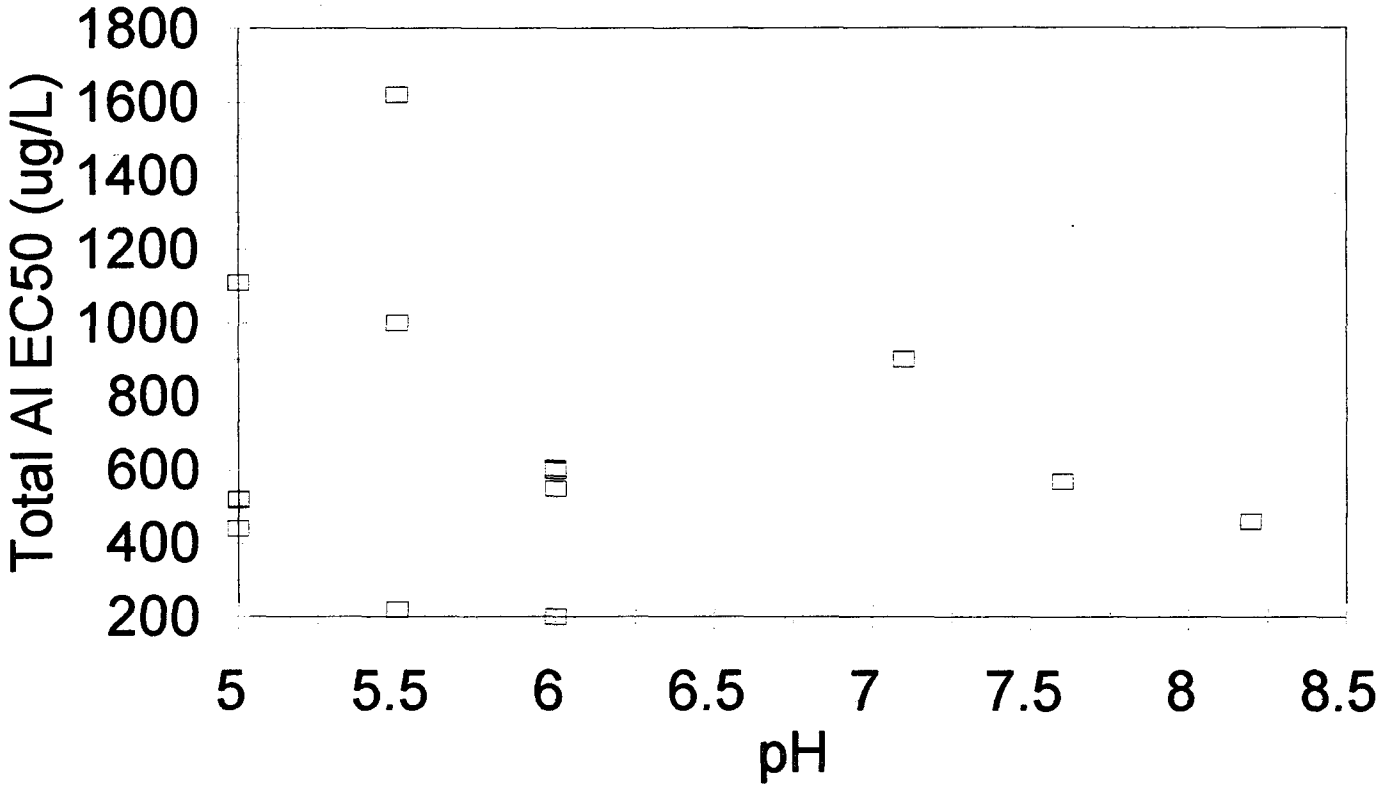


Fig. 2-1

Labile Al-dependent Toxicity

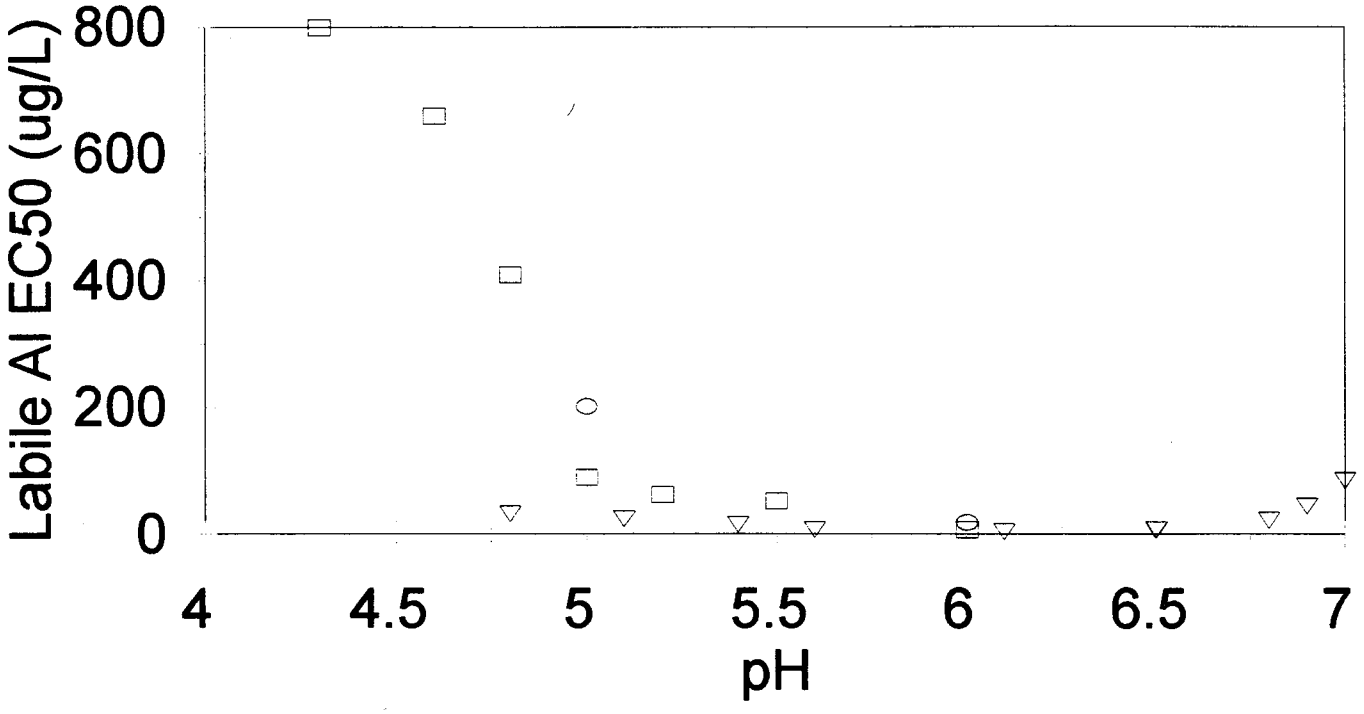


Fig. 2-2

pAl-dependent Toxicity

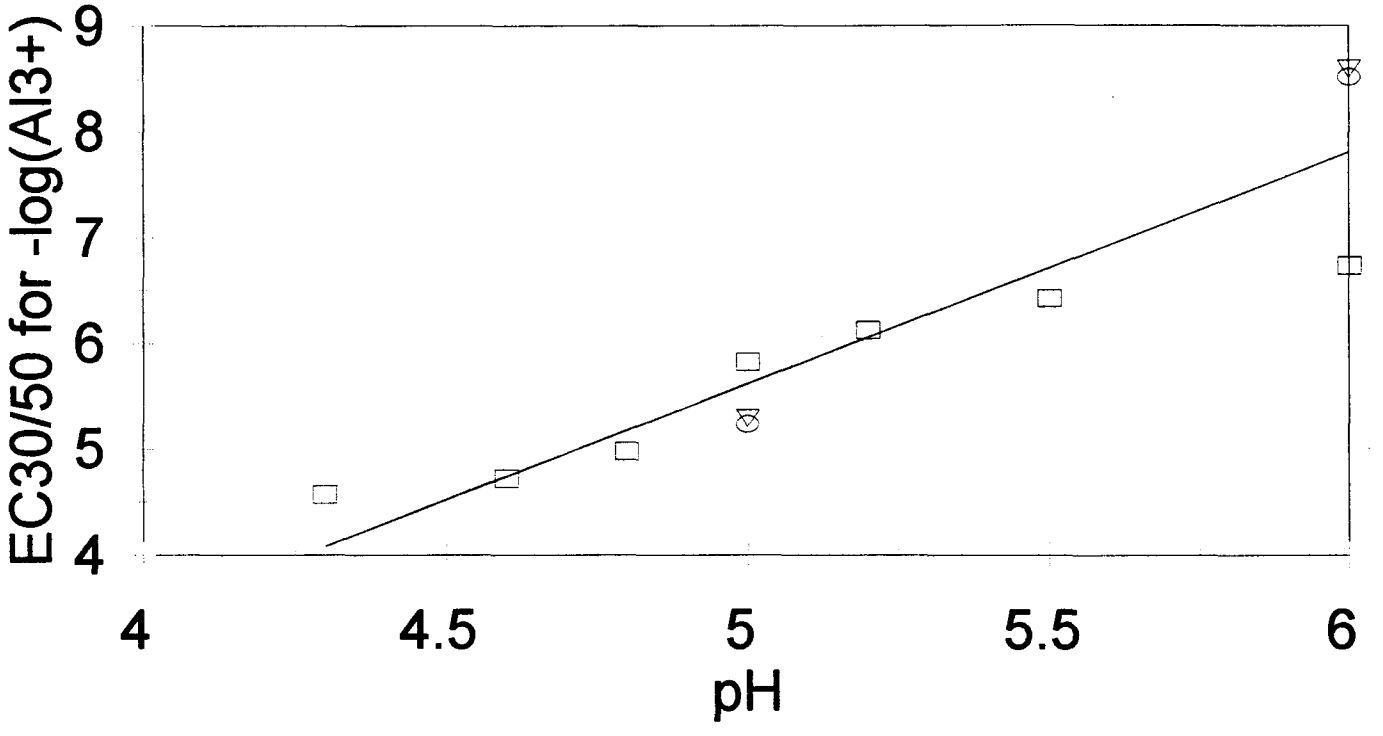


Fig. 2-3

3 HIGHER AQUATIC PLANTS

Compared to virtually all taxonomic groups discussed in this review, relationships between Al exposure and either the growth or community structure of freshwater macrophytes are very poorly understood. The work reviewed below consists almost entirely of field surveys and bioaccumulation studies, with virtually no direct experimental work with Al being done on macrophytes. Given the proximity of submerged, emergent, and floating-leaved macrophytes to Al inputs from watersheds, as well as their potentially direct exposure to both monomeric and polymeric Al in sediments (see chapter 1), this is a critical shortcoming in our understanding of the impacts of Al in freshwater systems.

Although not considered explicitly in this review, there is by contrast a vast and detailed literature on the effects of Al on terrestrial plants. This knowledge base reflects widespread concern over Al toxicity in acidic soils, which affects many commercially important agricultural and silvicultural plant species. Many detailed and thorough reviews are available that discuss symptoms of Al toxicity, speciation and control of bioavailability, and physiological/biochemical mechanisms of toxicity (Haug 1984, Haug and Caldwell 1985, Andersson 1988, Delhaize and Ryan 1995, Kochian 1995). However, none of these reviews treat studies of aquatic or wetland plants, so will not be discussed further in detail here.

3.1 Direct Toxicity or Tolerance of Al to Aquatic Plants

Only a very few, mostly older, studies have examined the direct effects of Al on the growth or physiology of individual macrophyte species. As reviewed by Burrows

(1977), relatively high exposures of 2500 $\mu g \cdot L^{-1}$ total Al (pH not given) inhibited root growth of *Myriophyllum spicatum*, whereas much less (50 - 200 $\mu g \cdot L^{-1}$) Al inhibited the growth of rice seedlings at acidic (3.5 - 5.0) pH. Similarly, only 50 $\mu g \cdot L^{-1}$ Al diminished shoot dry weight of black crowberry shrub (*Empetrum nigrum*) seedlings at pH 4, even though seedlings exposed to as much as 5000 $\mu g \cdot L^{-1}$ Al survived, albeit poorly, as long as 15 weeks (as reviewed in Burrows 1977). The only data available for plants grown at higher pH demonstrated that Al never inhibited *Lemna gibba* growth less than 50% at basic pH (7.6 & 8.2) when exposed to 45,700 $\mu g \cdot L^{-1}$ Al or less (as reviewed in Gostomski 1990).

The only recent study we found described exposures of field collected specimens of *Ranunculus peltatus, R. ololeucos, Myriophyllum alterniflorum, Potamogeton gramineus,* and *Luronium natans* for 10 weeks to 2700 $\mu g \cdot L^{-1}$ Al at pH 4 concomitant with additions of $CaCl_2$ ranging from 0 to 1000 $\mu mol \cdot L^{-1}$ (Maessen et al. 1992). Although most of the plants exhibited relative decreases in biomass of nearly 100% over the ten week experiment in the controls, Al exposed plants fared no worse, nor did Ca manipulations exert any demonstrable effects. These results lead the authors to conclude that Al had no visible effect on vitality, but the validity of this conclusion is compromised by the extreme losses of biomass even in control exposures. In contrast, separate experiments which manipulated only pH demonstrated that plant growth was inhibited at pH 3 relative to either 4 or 5. Maessen et al. (1992) thus contended that pH was a much more important factor than Al in restricting growth of these macrophytes, but further experimentation with plants

performing better under control conditions would more strongly support or refute this conclusion.

The above mentioned studies make no reference as to whether toxicity is more likely to be expressed in the root vs. shoot tissues of aquatic plants. However, as outlined in Section 3.3, sediment uptake is likely to be the primary site of Al uptake in aquatic plants, so roots may also represent at least the initial site of action in submersed macrophytes and wetland plants. This is also likely given that root uptake is the dominant route of Al entry into terrestrial plants, and that root growth inhibition is a significant mechanism explaining Al toxicity for crop plants in acidic soils (Haug and Caldwell 1985, Andersson 1988, Delhaize and Ryan 1995, Kochian 1995).

Given the importance of macrophytes to the structure and function of the littoral zone of freshwater systems, it is surprising that so few experimental exposures of Al to macrophytes exist. This unfortunately mirrors similar situations for many contaminants (Wang 1991, Lewis 1995) for which aquatic higher plants are only rarely used in toxicity testing. Part of this tendency may reflect the relative paucity of standardized methods for aquatic plant toxicity testing other than for the duckweed *Lemna sp.* However, without detailed experimental exposures, our understanding of the physiological and ecological effects of Al on macrophytes is extremely limited.

3.2 Relationship of Macrophyte Community Structure to Al Exposure

Although many trace metals have been shown to affect macrophyte community structure in freshwater systems (Guilizzoni 1991, Catallo 1993), relatively little is known

about the potential for Al to affect macrophyte community structure. As with the algal studies reviewed in chapter 2, most of the evidence that Al may affect macrophyte community structure emanates from studies of lake acidification. In most cases, trends were found correlating pH directly with changes in community structure, but the only suggestions that Al contributed to these changes was from observations of pH-dependent changes in Al solubility which were not always directly measured.

3.2.1 *Trends Associated with Lake Acidification*

As already discussed in chapter 2 for benthic algae, extensive littoral plant development typically occurs in response to lake acidification. As part of the process originally termed "oligotrophication", acidified lakes become more transparent (owing to decreases in phytoplankton biomass and DOC/Al complexation; see chapter 2), accompanied by significant increases in the biomass and areal coverage of both submerged macrophytes and epiphytic algae. In oligotrophic Scandinavian lakes, typical *Lobelia - Isoetid* assemblages are replaced by extensive development of *Sphagnum* mosses as pH values fall below 6 (Grahn et al. 1974, Hendrey et al. 1976, Almer et al. 1978). These plants are uniquely adapted to acidic conditions owing to their ability to use CO_2 or H_2CO_3 directly as a photosynthetic carbon source, coupled with an ability to remove Ca^{2+} ions from solution, thereby making Ca^{2+} less available for other plants. Aluminum was never mentioned in these studies as a factor potentially responsible for *Sphagnum*'s dominance, although Al concentrations are usually higher in the most acidic systems.

Similar development of *Sphagnum*-dominated communities also have been observed in the Adirondacks, Ontario, and Nova Scotia (Dillon et al. 1984). However, Canadian studies reviewed in Dillon et al. (1984) mentioned that most macrophyte taxa, excluding perhaps *Vallisneria americana* and *Nitella flexis*, could also persist in acidic, metal-contaminated lakes, albeit often at significantly higher overall densities than in circumneutral sites. A more recent survey in the Adirondacks agrees that similar macrophyte taxa can dominate both acidic and circumneutral oligotrophic lakes, even though overall species diversity dropped at the most acidic sites (Roberts et al. 1985). In this study, taxa similar to those found in Scandinavia dominated all of their oligotrophic lakes including: *Utricularia spp., Lobelia dortmanna, Eriocaulon septangulare, Myriophyllum tenellum*, with *Potamogeton confervoides, Scirpus subterminalis, Sphagnum spp.*, and *Utricularia miniscapa* being exclusively present in the most acidic sites.

3.2.2 *Potential Role of Al in Floristic Changes*

Although Al concentrations are clearly higher in the acidified lakes already mentioned above, little direct evidence exists that directly implicates Al as being even partly responsible for changes in macrophyte community structure. This is in stark contrast to the situation reviewed in chapter 2 where Al commonly was found to correlate strongly with changes in algal species composition. Most macrophyte surveys that involved multivariate analysis comparing community structure with

chemical variables found pH to be the single, or nearly so, dominant factor (Catling et al. 1986, Jackson and Charles 1988, Arts et al. 1990, Weiher and Boylen 1994). In fact, only Catling et al. (1986) and Jackson and Charles (1988) presented correlations with Al. In the former case, only Ca, pH, and HCO_3 (and not Al; p= ca. 0.50) were significant explanatory variables, and in the later case, Al was only included with their group of pH-related factors.

The only other suggestion that Al could have species-specific impacts on macrophyte assemblages comes from the review of Sparling and Lowe (1996). They concluded that acid-tolerance is linked to Al tolerance, and tabulated a list of potentially Al-tolerant aquatic plants (their table 3). However, their criteria for such rankings was not made explicit, and without experimental verification, these qualitative categories need to be viewed with caution.

3.3 Sediment Biogeochemistry and Bioavailability of Al to Aquatic Plants

Perhaps part of our inability to identify Al as a significant factor controlling macrophyte community structure in freshwater systems relates to paths of metal uptake in these benthic plants. Recent reviews suggest that rooted submergent and emergent macrophytes take up trace metal cations most readily from sediments via root uptake as opposed to direct transport through the leaves from overlying waters (Guilizzoni 1991, Catallo 1993). Thus, correlations with aqueous Al in overlying waters are much less likely to be statistically, as well as mechanistically, significant (but see Lehtonen 1989; discussed in 3.3.1 below). It also follows that correlations with sediment-bound

Al would be more significant, and this is reflected in studies of metal bioaccumulation by macrophytes and wetland plants. As before, many of these studies focus on lake acidification, with no work being done on systems with pH exceeding 7.

3.3.1 *Evidence for Al Bioaccumulation*

Havas (1986) tabulated Al bioaccumulation data for U.S. and Canadian lakes, and macrophytes accumulated from less than 40 to 32,000 μg $Al \cdot g^{-1}$ dry weight, with differences being observed as a function of season, location, portion of plant analyzed, and species tested. Tissue contents ranging from several hundred to several thousand μg $Al \cdot g^{-1}$ dry weight were also observed in macrophytes sampled in acidic lakes in northwestern New Jersey (Sprenger and McIntosh 1989), southern Finland (Lehtonen 1989), constructed wetlands at the Pautuxent Wildlife Research Center in Maryland (Albers and Camardese 1993), and an acid-mine drainage impacted watershed in Pennsylvania (Engleman and McDiffett 1996).

Plant tissue contents tend to be best correlated with sediment Al concentrations (Jackson and Kalff 1993, Jackson et al. 1993), although this relationship was shown to be less important for macrophytes that gain most of their mineral nutrition from water via their leaves such as *Nuphar lutea* (Lehtonen 1989). Within single plants, Al tends to accumulate preferentially in submergent tissue vs. emergent tissue in monocots (as reviewed by Havas 1986), and ultimately becomes associated with cell walls (Havas 1986, Denton and Oughton 1993).

Given that so few studies have been done, it is as yet difficult to conclude whether or not individual plant species are more or less likely to accumulate Al and thus be considered appropriate Al bioindicators. However, Sprenger and McIntosh (1989) found that submerged-rooted species (i.e. *Isoetes, Potamogeton, Myriophyllum, Eriocaulon*) tended to have higher Al contents by almost an order of magnitude than did floating-leafed taxa (i.e. *Nuphar, Brasenia, Nymphaea, Pontederia, Orontium*). Similarly, Jackson and Kalff (1993) derived different sediment-plant correlation models depending on macrophyte growth form, with understory species (i.e. *Elodea canadensis, Potamogeton robbinsii,* and *Vallisneria americana*) tending to posses higher tissue metal levels (including Al) for a given sediment content than did canopy-forming taxa. They attributed accumulation this to differential accumulation of fine-grained sediments amongst different macrophyte canopy types.

3.3.2 *Environmental Factors Controlling Al Bioaccumulation*

Most bioaccumulation studies point to pH as the environmental factor that best explains variations in Al content in macrophyte tissues (Lehtonen 1989, Sprenger and McIntosh 1989, Jackson et al. 1993, Engleman and McDiffett 1996). In all of these cases, enhanced Al contents were measured in plant tissues collected from the most acidic sites, pointing to a process of simple acid solubility explaining the enhanced partitioning of Al from either sediments or water into plant tissues. This trend also refutes the notion, as supported by algal toxicity experiments (chapter 2), that increasing H^+ ion activity would displace Al^{+3} ions in accordance with the FIAM

(Campbell and Stokes 1985, Campbell 1995), thereby actually decreasing Al bioaccumulation at low pH. However, without direct experimental manipulation or measurement of dissolved Al speciation, it is difficult to fully assess the role of H^+ ions in controlling Al transport and accumulation in aquatic plants.

Few other environmental factors have been linked to differential Al bioaccumulation by freshwater macrophytes. Other factors include hardness and humic acid concentrations (Lehtonen 1989), and the presence of Ca^{+2} ions (Maessen et al. 1992). Redox potential was also examined for Al and other metals in a survey of macrophytes from Québec lakes (Jackson et al. 1993), but at least among relatively oxic redox potentials ranging from 88-305mV, no relationship was found for Al and plant tissue contents. In this study, only tissue contents of the redox-dependent Fe and Mn were significantly related to redox potential. Since Al has only a single oxidation state (+III), the lack of relationship is not surprising. However, as already mentioned for toxicity tests and pH-dependent bioaccumulation, these conclusions are only based on field survey data, and have yet to be confirmed experimentally.

4 ALUMINUM AND AQUATIC INVERTEBRATES

4.1 Introduction

Aluminum toxicity to aquatic invertebrates has been less well studied compared to fish, but in general aquatic invertebrates are less sensitive to Al (Ormerod et al. 1987; Wren and Stephenson 1991; Sparling and Lowe 1996). As with fish (Chapter 5), most studies have dealt with acidic conditions and Al toxicity, so little is known about the effects of Al on invertebrates in alkaline water. Aluminum is usually seen as an additive stress to the ionoregulatory disturbances produced by H^+ alone, and reports of respiratory effects of Al are much less common than for fish. Unlike fish, aquatic invertebrates do not release ammonia at their gills, so the postulated mechanism of respiratory effects due to Al precipitation or polymerization in the more alkaline gill micro-environment (Chapter 5) does not usually apply to invertebrates. In this review we will concentrate on more recent research that contributes to our knowledge of the mechanisms of Al toxicity to invertebrates. Some good recent reviews of Al and aquatic invertebrates are by Wren and Stephenson (1991), Locke (1991), and Sparling and Lowe (1996).

4.2 Ionoregulatory Effects of Aluminum

Havas (1985) found that Al was toxic to the cladoceran *Daphnia magna* (Straus) at 320 and 1020 $\mu g \cdot L^{-1}$ Al at pH 6.5 (62 μM Ca), but this amount of Al was not toxic with added Ca (310 μM; 48 h tests). Aluminum only marginally increased the toxicity due to

H^+ alone at pH 5.0, and 1,020 $\mu g \cdot L^{-1}$ Al temporarily slowed H^+ toxicity at pH 4.5. Losses of Na and Cl were the causes of death. Aluminum toxicity at pH 6.5 was likely due to Al oversaturation, possibly causing some respiratory distress, and the temporary amelioration of H^+ toxicity by Al was likely due to Al^{3+} competition with H^+ (Havas 1985). Havas and Likens (1985) also showed that *Daphnia catawba*, *Holopedium gibberum* (Cladocera), *Chaoborus punctipennis* (Diptera), and *Chironomus anthrocinus* (Diptera) were not very sensitive to Al (20, 320, 1,020 $\mu g \cdot L^{-1}$ Al; pH 3.5-6.5; 60 μM Ca). Loss of any of these organisms from acidic water, especially the acid-sensitive *D. catawba*, would most likely be due to direct effects of H^+ itself on osmoregulation, or to indirect effects of H^+ or Al on their predators or food sources (see section 4.5).

France and Stokes (1987) also showed that H^+ toxicity was of primary importance for *Hyalella azteca* (Amphipoda), with a significant increase in toxicity at pH 4.8 with 400 $\mu g \cdot L^{-1}$ Al (8-d exposures, 50 μM Ca). They felt that mortality of *Hyalella* during springmelt pulses would be primarily due to H^+, and only secondarily due to Al. They found no protective effect of 50 to 200 μM Ca on H^+ or Al toxicity (pH 4.0-5.3, 250-700 $\mu g \cdot L^{-1}$ Al), but the range of Ca concentrations used may have been too narrow to demonstrate a protective effect. Aluminum (80 to 600 $\mu g \cdot L^{-1}$) added to the impairment by H^+ of egg hatching of *Cloeon triangulifer* (Ephemeroptera), but Al decreased slightly the 96-h toxicity of H^+ to nymphs of *Cloeon* (pH 4.0, 5.0, ~28 μM Ca; Tabak and Gibbs 1991). Calcium (~300, 2,600 μM) was protective against H^+ and Al effects in *Cloeon* eggs. Again, Al had little toxicity independent of H^+ ions themselves, and in nymphs the toxic mechanism of both H^+ and Al was thought to be Na and Cl

losses, as was found by Havas (1985). Storey et al. (1992) examined the relative susceptibility of *Cyclops viridis* (Copepoda) and *Gammarus pulex* (Amphipoda) to acidity and Al. Both organisms showed little or no mortality at pH 4.5 to 6.9 over 7 d, but had 70 to 100% mortality at pH 4.0, an effect exacerbated by 270 to 27,000 (nominal) $\mu g \cdot L^{-1}$ Al (pond water chemistry not given). *G. pulex* was somewhat more susceptible to acid and Al compared to *C. viridis* (Storey et al. 1992).

Whole-body Na content and survival of the acid-sensitive cladoceran *Daphnia galeata mendotae* decreased during Al exposures at pH 7.5 and 6.0 (0, 100, or 200 $\mu g \cdot L^{-1}$ nominal Al; 12 and 24 h exposures; 60 μM Ca; Havens, 1992). The highest concentration of Al protected against the effects of H^+ alone at pH 4.5. However, *Bosmina longirostris*, an acid-tolerant cladoceran, was not affected by Al in these exposure regimes. Aluminum had earlier been shown to bind to the osmoregulatory maxillary glands of *D. galeata mendotae* better than to those of *B. longirostris* (Havens 1990). Under very acidic conditions (pH ~3), high concentrations of Al (~15 $mg \cdot L^{-1}$) protected the stoneflies *Acroneuria carolinensis* and *Paragnetina media* against Na losses (Whipple and Dunson 1993). Acid and Al tolerance appear to be linked, and H^+ ions and Al^{3+} may work by affecting the same osmoregulatory mechanism.

In experiments using the littoral macroinvertebrates *H. azteca*, *Gyraulus* sp. (Gastropoda), *Paratanytarsus* sp. and *Zavrelimyia* sp. (Diptera), Havens (1993) demonstrated faster mortality at pH 4.5 with the addition of 200 $\mu g \cdot L^{-1}$ Al than was seen with pH 4.5 alone (~60 μM Ca, 48-h exposures). Aluminum was slightly protective against H^+ toxicity for *Caenis* sp. (Ephemeropter) and *Enallagma* sp. (Odonata), and pH

4.5 conditions with or without Al had no effect on survival of *Arrenurus* sp. (Hydracarina). Havens (1993) speculated that the presence of gills and caudal lamellae contributed to the high acid sensitivity of some of the organisms (*H. azteca*, *Enallagma* sp., and *Caenis* sp.) because of their high surface area; conversely, lack of these structures was thought to contribute to the acid tolerance of *Arrenurus* sp. and the chironomids (Diptera).

In another study, exposure to 200 $\mu g \cdot L^{-1}$ Al at pH 6.0 caused Na losses and decreased survival of *Skistodiaptomus oregonensis* (Copepoda), but slightly reduced the effects of H^+ (Na losses and animal death) at pH 4.5 (60 μM Ca, 24 h exposures; Havens 1993b). The author thought that Al could contribute to extinction of this freshwater copepod from a body of water at higher pH, but that Al acted antagonistically towards the osmoregulatory effects of H^+ at low pH. Laboratory bioassay (pH 4.5-7.0, 60 μM Ca, 48 h LC50s) and field survey data from 305 acid sensitive Ontario lakes agreed in the order of susceptibility of crustacean zooplankton to acidification (Havens et al. 1993). From most to least sensitive they were *Daphia galeata mendotae* > *Daphnia retrocurva* = *Skistodiaptomus oregonensis* > *Diaphanosoma birgei* > *Mesocyclops edax* > *Bosmina longirostris* (Havens et al. 1993). The authors concluded that lakewater pH is the only factor that is needed to explain composition of zooplankton populations in acid sensitive waters: that is, pH is the most important controlling factor (e.g. master variable) and that Al, for example, is of lesser importance.

In addition to effects on Na and Cl uptake, Al has a small additive effect to that of H^+ on Ca uptake by postmolt crayfish. Calcium is needed for hardening of the new exoskeleton after molting. Uptake of ^{45}Ca by *Orconectes virilis* (Hagen) at pH 5.5 was just 30% of that in circumneutral water, and 180 to 1,300 $\mu g \cdot L^{-1}$ Al at pH 5.5 reduced ^{45}Ca uptake further to 20% of control (12-h experiments, ~70 μM Ca; Malley and Chang 1985). Aluminum could therefore act as an additional stress and be important in determining crayfish survival in the pH range 5.0 to 6.0. In more acidic conditions, acidity alone is so toxic to crayfish that Al has no additional effect on survival of *Orconectes propinquus*, *Orconectes rusticus*, and *Cambarus robustus* (15-d exposures to pH 4.5-5.0, 60 μM Ca, ~1,000-2,000 $\mu g \cdot L^{-1}$ Al; Berrill et al. 1985). Liming a reach of an experimentally acidified stream (pH ~4.5, ~700 $\mu g \cdot L^{-1}$ Al, ~60 μM Ca, 24 h) appeared to alleviate some of the hemolymph Na loss in the crayfish *Austropotamobius pallipes* (Weatherley et al. 1989).

Aluminum was shown to have an effect on the activity of the freshwater snail *Lymnaea stagnalis*. At pH 6.6 to 7.2, exposure to 100, 200, 500, and 1,000 $\mu g \cdot L^{-1}$ Al decreased snail activity, as judged by distance they moved during 19 h (Truscott et al. 1995). Depressed activity could have been a way of minimizing the uptake and toxicity of Al. Juvenile snails appeared more sensitive than adults, which the authors thought might have been because of the greater surface area to volume ratio or higher metabolic rate in juvenile snails. Snails reared for one year in 0, 100, and 500 $\mu g \cdot L^{-1}$ Al showed identical activity, indicating that the snails had become tolerant to those concentrations of Al (Truscott et al. 1995).

4.3 Localization of Al on Aquatic Invertebrates

Aquatic invertebrates can accumulate Al over the whole body, particularly on ionoregulatory and perhaps respiratory surfaces. Havas (1986) used a hematoxylin stain to localize Al on and in aquatic animals and plants that were exposed to 1 $mg \cdot L^{-1}$ total Al for 24 h (pH 5, 62 μ*M* Ca). Chloride cells stained for Al in the crustaceans *Branchinecta paludosa*, *Daphnia magna*, and *Holopedium* sp., as did the hind gut and penis tip of *Branchinecta*. *Chaoborus* sp. stained for Al on the anal papillae, where ion uptake occurs. Unfortunately, mayfly and dragonfly nymphs were not examined so we do not know the pattern of Al depositon on these insects. Also using hematoxylin staining, Havens (1990) demonstrated that two acid- and Al-sensitive cladocerans, *D. galeata mendotae* and *Daphnia retrocurva*, accumulated Al on ion uptake sites of the maxillary glands (24 h, pH 5.0, 200 $\mu g \cdot L^{-1}$ Al). *B. longirostris*, an acid- and Al-tolerant cladoceran, did not accumulate Al on the maxillary glands. Havens speculated that acid-sensitivity was related to Al binding to ion-exchange sites, interfering with osmoregulation.

The mayflies *Baetis rhodani* and *Ecdyonurus venosus* were exposed to pH ~5.0 and 350 $\mu g \cdot L^{-1}$ Al for 24 h in a stream and were examined for Al and mucus accumulation (~75 μ*M* Ca; McCahon et al. 1987). No mucus was seen on mayfly gills, but the gills did stain for Al (using solochrome azurine), as did all parts of the body. Strongest staining was in the gut, in and on gill plates, and on the abdominal surface. In contrast, gills of fish in the stream showed accumulation of both Al and mucus on the gills (McCahon et al. 1987).

Aluminum was localized in chloride cells of nymphs of *Perla marginata*, an acid sensitive stonefly. *P. marginata* were held for 21 d in synthetic, acidic soft water (pH 5.2, 20 μ*M* Ca) with 600 $\mu g \cdot L^{-1}$ added Al, or for 21 d in synthetic soft water with no added Al (pH 6.8, 80 μ*M* Ca, 30 $\mu g \cdot L^{-1}$ Al), then were examined for Al using laser microprobe mass spectrometry and histochemical staining (aluminon) of histological sections (Gurerold et al. 1995). Aluminum was localized in the gut contents, cuticle, and chloride cells on the gills and thorax of the stoneflies exposed to Al. Localization of Al on the chloride cells suggested again that the effect of Al on aquatic invertebrates is on ion regulation (Guerold et al. 1995).

Vuori (1996) used darkening of the anal papillae ion-regulatory organs of larval Trichoptera as an index for the effective concentration of Al (EC50). The 96 h EC50 concentrations at pH 5.0 were ~1000 $\mu g \cdot L^{-1}$ total Al for *Arctopsyche ladogensis*, ~1300 $\mu g \cdot L^{-1}$ total Al for *Hydropsyche siltalai*, and ~2,400 $\mu g \cdot L^{-1}$ total Al for *Hydropsyche angustipennis* (water Ca concentrations were not given). Vuori (1996) did not see any consistent effect of Al on the gills of these caddis larvae, indicating that the toxic effect of Al was through impairment of osmoregulation from damage to the anal papillae ion-regulatory organs, not through a respiratory effect of Al.

4.4 Respiratory Effects of Aluminum

Ionoregulatory effects of Al on acid-sensitive aquatic invertebrates have been well established (section 4.2), and may be a result of increased membrane permeability (Locke 1991). Are there respiratory effects of Al at environmentally-relevant Al

concentrations, as there are for fish? (see Chapter 5) Correa et al. (1985) showed that oxygen consumption by dragonfly nymphs (*Somatochlora cingulata*) decreased at low pH (pH 4.2 and 3.6), and at pH 4.2 was lower still in the presence of high concentrations of Al (10 to 30 $mg \cdot L^{-1}$ Al). Ammonia excretion also decreased as pH decreased and as Al increased, possibly indicating a switch away from protein catabolism by the nymphs. The effect of high concentrations of Al was seen as additive to that of H^+.

At more reasonable Al concentrations, caddisfly larvae (*Limnephilus* sp.) without their cases showed no changes in oxygen consumption when exposed to pH 4.0 in the presence or absence of 300 $\mu g \cdot L^{-1}$ Al (Correa et al. 1986). In these larvae, NH_3 excretion increased slightly (but significantly) in the pH 4.0 exposure, but there was no change in NH_3 excretion in the pH 4.0 plus 300 $\mu g \cdot L^{-1}$ Al exposure. The authors thought that perhaps Al was mitigating some of the effects of H^+ alone; in any case, there was no change in oxygen consumption in response to environmentally realistic Al concentrations in these insects, even with their gills exposed.

Herrmann and Andersson (1986) showed that nymphs of the mayflies *Ephemera danica*, *Heptagenia fuscogrisea*, and *Heptagenia sulphurea* had elevated respiration when exposed to high Al concentrations (500 and 2,000 $\mu g \cdot L^{-1}$ Al) at pH 4.0 or 4.8 for 10 d (70 μM Ca), compared to respiration at pH 4.0 and 4.8 in the absence of Al. They suggested that increased respiration was caused either by impaired osmoregulation and ion transport (a "chemical impact route"), or by a mechanical route of Al precipitation and mucus accumulation on the gills, impairing respiration (a "mechanical

impact route"). They favored the chemical impact route, of increased respiration needed to supply the energy required to compensate the ionoregulatory disturbances associated with Al. However, their exposures would likely have created supersaturated conditions, especially at pH 4.8 with 2,000 $\mu g \cdot L^{-1}$ Al, so the physical route can not be discounted.

Decreases in oxygen consumption were seen in the dragonfly nymph *Libellula julia* in response to H^+ ions (pH 4, 45 μM Ca); 300 $\mu g \cdot L^{-1}$ Al did not significantly alter this response, but higher Al concentrations (3 and 30 $mg \cdot L^{-1}$ Al) decreased oxygen consumption further (96-h exposures; Rockwood et al. 1990). Again, high Al concentrations were needed to affect oxygen consumption. These workers speculated that reduced oxygen consumption was due to blockage of dragonfly gills by mucus or $Al(OH)_3$ precipitation, and the confined space in dragonfly respiratory chambers may not have allowed for clearance of material occluding the gills. Rockwood et al. (1990) suggested that decreased oxygen consumption is the more likely response to Al than is increased oxygen consumption if respiratory surfaces are being affected.

It appears that aquatic invertebrates are, in general, relatively resistant to the ionoregulatory effects of H^+ ions, and that Al usually adds to those ionoregulatory effects that occur. Respiratory effects are secondary, and only occur at high Al concentrations (>300 $\mu g \cdot L^{-1}$ Al), unlike in fish where respiratory effects can occur at lower, environmentally realistic Al concentrations. Why are respiratory effects at the gills of aquatic insects so unimportant in Al toxicity? A likely explanation is found in the construction of insect gills and in the patterns of gas transfer at their gills.

Carbon dioxide is released at tracheal gills of aquatic insect nymphs, and its release can acidify soft water adjacent to the gills. Nymphs of *Stenacron* sp. and *Stenonema vicarium* (Ephemeroptera) placed in basic solutions of bromothymol blue indicator dye (blue) produced a green-yellow color (pH <7) in water around and behind their gills (Fig. 4A; Playle, unpublished), as water was drawn past their abdomens (where cuticular gas transfer also occurs; Eriksen and Mœur, 1990). Acidification of basic, soft water as it passes over the gills of aquatic insects, presumably a consequence of CO_2 released at the gills, is similar to what occurs at gills of fish (see Chapter 5 of this review, and Fig. 5A).

However, unlike in fish, release of basic NH_3 at tracheal gills does not occur. Instead, NH_3 is intermittently released in high concentrations in rectal fluid (e.g. Staddon 1964). *Stenacron* sp. and *S. vicarium* placed in acidic bromocresol green solution (amber) released basic "puffs" of rectal fluid (blue); no colour change due to base release at the gills was observed (Fig. 4B; Playle, unpublished). Gas-filled insect gills act as a barrier to highly water- and hemolymph-soluble ammonia, because NH_3 has to diffuse first from hemolymph into tracheal air (NH_3 is much less soluble in air than in water), then into ambient water, whereas in fish NH_3 readily diffuses across gill membranes from blood into water. Thus, acidic, Al-rich water passing over respiratory surfaces of aquatic insects is not made more basic, as occurs at fish gills, so Al does not precipitate from solution (see Fig. 5C for fish). Respiratory effects in insects will therefore only occur at very high Al concentrations when Al precipitation from oversaturated solutions is likely. An exception may be dragonfly nymphs, which have

gills enclosed in respiratory chambers where oxygen uptake and NH_3 release occur together in a confined space. The "fish-like" response of *L. julia* in Al-rich soft water (Rockwood et al. 1990) may in fact be due to Al precipitating onto gills in the more basic environment of the respiratory chamber.

4.5 Field Studies

In general, field studies have demonstrated the relative hardiness of aquatic invertebrates to H^+ and Al, and have usually not been able to separate their toxicities. Aluminum addition to a stream (pH ~5, >280 $\mu g \cdot L^{-1}$ Al) caused more drift of *Epeorus* sp. (Ephemeroptera), *Prosimulium* sp. (Diptera), and Orthocladiinae chironomids compared to the addition of H^+ alone (pH ~5, ~120 $\mu g \cdot L^{-1}$ Al, 50-100 μM Ca; Hall et al. 1987). Another stream acidification study showed little added effect of Al on invertebrates compared to the effects of H^+ alone. *Chironomus riparius*, *Hydropsyche angustipennis* (Trichoptera), and *Dinocras cephalotes* (Plecoptera) showed no effects of Al or H^+, and toxicity to *Ecdyonurus venosus*, *Baetis rhodani* (Ephemeroptera), and *Gammarus pulex* was similar at ~20% in acidified (pH ~4.3, 50 $\mu g \cdot L^{-1}$ Al) and acid plus Al (pH 5.0, 350 $\mu g \cdot L^{-1}$ Al) reaches of the stream (60-90 μM Ca; Ormerod et al. 1987). In contrast, brown trout and Atlantic salmon showed significantly increased mortalities in the acid plus Al zone (50-87%) compared to the acid-only reach (7-10%).

Addition of acid to a stream in the Experimental Lakes Area, northwestern Ontario, resulted in decreased benthic density and increased drift of invertebrates such as *Similium* sp. and *Ectemnia* sp. (Diptera), the chironomids *Tanytarsus* sp.,

Eukiefferiella sp., and *Procladius* sp., and *Baetis* sp. (Ephemeroptera) when stream pH was lowered for 4 d from pH ~6.8 to pH 5.0, 4.5, and 4.0 (~100 µ*M* Ca; Hall 1994). Increases in stream Al content in the pH 4.0 and 4.5 experiments were too small (from ~1 to ~11 $\mu g \cdot L^{-1}$ inorganic monomeric Al; from ~40 to ~50 $\mu g \cdot L^{-1}$ total Al) to be a significant additive effect to that of decreased pH. The author concluded that the observed effects at even the relatively moderate pH 5.0 conditions in the absence of an increase in stream Al concentration, compared to those observed in other pH acidification experiments at lower pH, were a result of the high sensitivity of aquatic organisms to acidification when they inhabit relatively pristine habitats (Hall 1994).

Of the aquatic invertebrates *Plectrocnemia conspersa*, *Hydropsyche instabilis*, and *Rhyacophila dorsalis* (Trichoptera), *Rhithrogena semicolorata*, *Baetis rhodani*, and *Leptophlebia marginata* (Ephemeroptera), *Amphinemura sulcicollis*, *Isoperla grammatica*, and *Dinocras cephalotes* (Plecoptera), and *Gammarus pulex* (Amphipoda), only *Gammarus* showed significant mortality in an acid stream dosed with Al for 24 h (~700 $\mu g \cdot L^{-1}$ Al, pH ~4.7, ~60 µ*M* Ca; McCahon et al. 1989). *Gammarus* infected with an acanthocephalan parasite, *Pomphorhynchus laevis* (Müller), were more affected than were uninfected animals. *Gammarus* and *Isoperla* showed a linear accumulation of Al with time in a reach of the stream treated with lime, presumably due to Al precipitating from solution or absorbing to particles of lime, which then attached to the invertebrates (McCahon et al. 1989). Liming of the River Audna (Norway) was successful in allowing recolonization by acid-sensitive species of mayflies, stoneflies, and caddis flies (Fjellheim and Raddum 1992). In another study,

Baetis rhodani and *Rhithrogena semicolorata* (Ephemeroptera) were adversely affected during dosing of streams with Al and H^+ (pH ~5, 360-840 $\mu g \cdot L^{-1}$ Al), but *Amphinemura sulcicollis* (Plecoptera) and *Hydropsyche instabilis* (Trichoptera) were relatively unaffected (Merrett et al. 1991). Longer dosing episodes (18 or 24 h) caused more mortalities in *Baetis* and *Rhithrogena* than did 6 or 12 h episodes, and there were cumulative effects of multiple exposures.

An effort was made to determine appropriate taxa indicating low pH in softwater lakes and to separate the effects of water pH from factors such as Ca and Al concentrations, conductivity, and colour, which often co-vary with water pH. From 72 benthic taxa, an eight taxon empirical acidification model was derived, which explained 86% of the variation in water pH (Lonergan and Rasmussen 1996). For example, Gastropoda generally were not a good indicator of acidification in softwater lakes, because they usually do not occur in Ca-poor lakes susceptible to acidification. The single best indicator species was *Hyallela azteca*, which explained 49% of the variation in lakewater pH. The taxa remaining in the model were the Coleoptera, the Ephemeroptera family Heptageniidae, *Agrypnia improba* (Trichoptera), *Boyeria* sp. and *Gomphus* sp. (Odonata), the Gastropoda family Sphaeriidae, the Oligochaeta, and *H. azteca*. The model worked well, with the occasional outlier such as a lake with a high dissolved organic carbon concentration which would mitigate the effects of Al, and another lake with lower than expected concentrations of Al given its pH (Lonergan and Rasmussen 1996). The authors felt that a multispecies index is preferable to a single species index because it includes multiple pH tolerance thresholds and yields some

redundancy of information, which together create a more sensitive index over a broad range of conditions.

The influence of predators on aquatic invertebrates was demonstrated by Bendell and McNicol (1987), who showed that the presence or absence of fish was an important factor in determining aquatic insect assemblages, with larger Notonectidae, Corixidae, Dytiscidae, and Chaoboridae abundant in fishless lakes. Thus, if fish populations were affected by H^+ or Al (Chapter 5), then aquatic insect populations would likely be affected indirectly. This principal was also demonstrated by Bukaveckas and Shaw (1997). Calcite (lime) additions to Woods Lake in the Adirondack Mountains, New York, shifted the zooplankton community from small, acid tolerant organisms such as *Diaptomus minutus*, *Keratella taurocephela*, and *Bosmina longirostris* to a more diverse assemblage of larger zooplankton, including *D. catawba* (Bukaveckas and Shaw 1997). Zooplankton densities decreased when planktivorous brook trout (*Salvelinus fontinalis*) were introduced into the lake, and the decrease in zooplankton density resulted in increased phytoplankton abundance in the lake. These authors thought that the introduced trout, not greater availability of P to phytoplankton, were responsible for the increased phytoplankton abundance (e.g. zooplanktivory by fish, not the "oligotrophication hypothesis", section 2.6.2).

Protective effects of dissolved organic carbon (DOC) and the complexing agent citrate against H^+ and Al toxicity to aquatic invertebrates have been demonstrated in field studies. In 28-d experiments using *Asellus* (=*Caecidotea*) *intermedius* (Isopoda), *Pycnopsyche guttifer* and *Lepidostoma liba* (Trichoptera), *Nemoura* sp. (Plecoptera),

and *Physella heterostropha* (Gastropoda), 42 to 47 $mg \cdot L^{-1}$ DOC and citrate (amount not specified) both protected against mortality caused by 500 $\mu g \cdot L^{-1}$ Al (pH 4.0-4.2, low alkalinity water; Burton and Allan 1986). In another, 2 d experimental stream manipulation, only *Baetis rhodani* showed increased drift in response to H^+ and Al (pH 4.9, ~230-270 $\mu g \cdot L^{-1}$ Al; Weatherley et al. 1988). The immediate increase in *Baetis* drift due to H^+ and Al was reduced by the addition of 300 µM citrate, presumably by complexing Al and reducing labile Al to below detection. In a survey of six soft water lakes, Bendell Young and Harvey (1991) found that the amount of Al in chironomid larvae had a negative correlation with organic matter in sediments, presumably because organic matter reduced the availability of Al to the invertebrates.

In seawater, the likely toxic agent in bauxite sludge from an aluminum factory was Hg, although there were also high concentrations of Al, Fe, and Cr in the sludge (His et al. 1996). The oyster embryo-larval bioassay was used, and the lowest effect level of the sludge contained ~8.8 $mg \cdot L^{-1}$ Al, about the concentration which had previously been shown not to inhibit oyster embryogenesis. Mercury, on the other hand, was present at 34 $\mu g \cdot L^{-1}$ Hg, which was higher than LC50 values reported for oyster embryogenesis, so the likely toxic agent in the bauxite sludge was Hg (His et al. 1996).

4.6 Bioaccumulation of Al in Aquatic Invertebrates

Wren and Stephenson (1991) summarized data regarding Al in aquatic invertebrates, and found no evidence of biomagnification of Al in aquatic systems. Otto and Svensson (1983) showed that larval *Potamophylax cingulatus* (Trichoptera) do not accumulate Al and, in fact, lost Al if they were transferred from an alkaline stream (pH 7.7) to more acidic streams of pH 4.8 and 6.8. *Prosimulium fuscum/mixtum* (Diptera) and *Leptophlebia cupida* (Ephemeroptera) transplanted from a stream of pH ~6 to more acidic streams (pH 4.5-5.8; 50-100 μ*M* Ca) for 4 or 10 d also lost body Al, as well as body Ca (Hall et al. 1988). In these studies, Al likely desorbed from the insects into the acidic water.

In the Little Rock Lake acidification study, there was no evidence of Al biomagnification in the food chain: the rank of Al burden was perch < zooplankton < phytoplankton (King et al. 1993). Finally, Herrmann and Frick (1995) found no evidence of accumulation of Al up the food chain. In a survey of Swedish streams of different acidities and Al concentrations, predators such as *Isoperla grammatica* (Plecoptera) consistently had about one-third the concentrations of Al as did shredders and deposit feeders upon which they fed. These authors concluded that elevated levels of Al in fish and birds is not likely a result of feeding upon benthic organisms from acidic, Al-rich conditions (Herrmann and Frick 1995).

Adverse effects of Al on animals eating invertebrates from water with high Al concentrations are considered unlikely, due to lack of Al bioaccumulation by aquatic invertebrates, unless Al uptake is accompanied by low Ca and P availability in the diet

(reviewed by Scheuhammer 1991; Miles et al. 1993). For example, dippers (*Cinclus cinclus*) near acidic streams showed blood chemistry reflecting calcium scarcity (Tyler and Ormerod 1992); insufficient dietary Ca can affect eggshell production in birds. On the other hand, birds may alter their foraging behavior to compensate for lower calcium food sources (Mahony et al. 1997). St. Louis and Barlow (1993) demonstrated reduced hatching success of tree swallows (*Tachycineta bicolor*) nesting near acidified lakes at the Experimental Lakes Area, northwestern Ontario. Acidified waters are often deficient in taxa rich in Ca such as Gastropoda, Pelecypoda, and Crustacea (Blancher and McNicol 1991; Scheuhammer 1991; Økland 1992; Albers and Camardese 1993). Thus, any effects of Al on terrestrial animals are likely indirect, working in concert with H^+ to eliminate Ca-rich invertebrate food sources.

FIGURE LEGENDS

Figure 4. A. Composite sketch of acidification of water near the gills of the mayfly nymphs *Stenonema vicarium* and *Stenacron* sp. Dark background was blue (pH 7-8, 0.02% bromothymol blue in synthetic soft water, ~20 μ*M* Ca). Lighter area around the abdomen was green-yellow (pH 6-7). Colour changes were discernible within about 15 s, and were presumably due to CO_2 released at the gills.

B. The converse experiment, with mayfly nymphs placed in acidic, 0.02% bromocresol green in synthetic soft water (pH ~4.5; amber = light background). Intermittent release of basic rectal fluid (blue, pH >5) appears dark, but no base (e.g. NH_3) release at the gills was observed. *S. vicarium*; lines drawn on the filter paper upon which the nymph was sitting were 5 mm apart.

A

B

5 ALUMINUM AND FISH

5.1 Introduction

In his extensive review of the Al literature, Burrows (1977) indicated that Al toxicity towards fish at pH <7 had not been studied, that turbidity decreased Al toxicity (presumably because Al was absorbed on colloids in suspension), Al was not very toxic to fish eggs, and that the toxic action at fish gills was due to gill damage such as hyperplasia, leading to anoxia. Burrows (1977) identified four problems with most Al toxicity studies, which were: experiments that were too short, lack of pH control or even pH measurement, unreported water characteristics, and no standardization of fish used. One of the better Al toxicity papers included in the review concluded by stating that Al in neutral to basic water should not exceed 100 $\mu g \cdot L^{-1}$ Al if trout are to grow and survive normally (Freeman and Everhart 1971). Just a few years after the review of Burrows (1977), the connection between acid precipitation and Al toxicity was firmly established, and the survey paper by Muniz and Leivestad (1980) described two mechanisms of Al toxicity to fish. They were 1) ionoregulatory, involving losses of plasma chloride, and 2) respiratory effects, shown by lowered plasma oxygen tension, due to clogging of gills by mucus at high Al concentrations (150-900 $\mu g \cdot L^{-1}$ Al).

In the review by Howells et al. (1983) the importance of Ca in reducing the toxicity of acidity and Al was stressed. These reviewers suggested that lakes of pH 4.5 and 20 μM Ca would be fishless, and that 250 $\mu g \cdot L^{-1}$ Al would be toxic. Spry and Wiener (1991) covered the geochemistry of Al, and modifying effects of Ca, pH,

dissolved organic carbon, pre-exposure to Al, and life stage on Al toxicity to fish in acidic waters. Rosseland et al. (1990) and Rosseland and Staurnes (1994) are other reviews we recommend, both with a more European viewpoint. Rosseland and Staurnes (1994) stressed that declines in fish populations caused by acidic precipitation are due to the sum of the effects of elevated H^+ and Al concentrations and low Ca concentrations, affecting critical life processes during critical life stages of fish. They felt that the main effect of precipitated Al on fish gills is osmoregulatory failure; different water chemistry in the gill micro-environment compared to the bulk (acidic) water can lead to Al precipitation on the gills (Rosseland and Staurnes 1994, citing Exley et al. 1991). The review by Sparling and Lowe (1996) has a good summary of experimental conditions (Al and Ca concentrations, water pH) and effects (survival, plasma ions, blood parameters) of Al exposures on various fish species.

The reviews cited above are all mainly concerned with Al toxicity in acidic conditions, which reflects the general concern of Al toxicity to fish in acidic waters. The review by Sparling and Lowe (1996) is a good indication of the paucity of Al experiments run in alkaline conditions, where only two of the forty-seven cited studies involved exposing fish to Al at pH >8.

In the present section we will concentrate on recent literature (in approximately chronological order to emphasize the development of the understanding of Al toxicity), will re-examine some studies covered in previous reviews, and most importantly will concentrate on mechanisms and physiological aspects of Al toxicity. In brief, Al is a gill toxicant to fish (dietary Al has no significant adverse or beneficial effects; Poston 1991,

Handy 1993), and its toxic action at the gills is ionoregulatory, respiratory, or a mixture of both. It is the interplay between Al concentration, H^+ concentration (pH), and Ca concentration which determines which toxic mechanism predominates at the gill. In hard water, for example, Ca protects the gills against ionoregulatory effects of Al so that respiratory effects (smothering) occur at high Al concentrations. Good summaries of Al toxicity to fish under different water chemistry conditions are given in Exley et al. (1991), Poléo (1995), and Sparling and Lowe (1996). Effects of Al are more subtle in soft waters susceptible to acidification, and it is for these conditions on which our review will concentrate. For all studies discussed we will try to report exposure duration and pH, fish species and life stage, water Ca concentrations (in μ*M*), and Al concentrations (in $\mu g \cdot L^{-1}$ or in μ*M*, depending on the units used in the original papers).

5.2 Physiological Effects of Al Towards Fish

5.2.1 *Ionoregulatory and respiratory disturbances*

The influences and interactions of Ca, Al, and H^+ on 16-d survival of brown trout (*Salmo trutta*) yolk sac fry were studied by Brown (1983), who found that Ca (25, 50 μ*M versus* 6, 12 μM) ameliorated Al toxicity (250, 500 $\mu g \cdot L^{-1}$). The order of Al toxicity with respect to pH was pH 5.4 >5.1 >4.8 >4.5. Brown (1983) suggested that Ca protected against ionoregulatory effects of Al. Staurnes et al. (1984) found that 30-60 g rainbow trout (*Salmo gairdneri* = *Oncorhynchus mykiss*) and salmon (*S. salar*) exposed to 200 $\mu g \cdot L^{-1}$ Al at pH 5.0 for 4-7 d had reduced Na,K-ATPase and carbonic anhydrase activities in their gills. Both these enzymes are important for ion regulation across the

gills; the 23-39% reduction in their activities was accompanied by 8-39% declines in plasma Na and Cl concentrations. It was assumed that these effects were due to Al alone, because brown trout exposed to pH 5.0 for 7 d (10 μ*M* Ca) had normal plasma Cl concentrations (Muniz and Leivestad 1980). In a later experiment, Staurnes et al. (1993) showed that decreased plasma Na concentrations in 1 yr old Atlantic salmon were linearly related to decreases in gill Na,K-ATPase activity when the fish were exposed to acidic conditions (pH 5) and to 50 $\mu g \cdot L^{-1}$ Al at pH 5 (25-40 μ*M* Ca; 24 d exposures). In addition, carbonic anhydrase activity decreased in the Al plus acid exposures.

Witters (1986) showed that adult rainbow trout exposed 3.5 h to 350 $\mu g \cdot L^{-1}$ Al in very acidic conditions (pH 4.1, 20 or 95 μ*M* Ca) lost ions through the gills at about twice the rate as at pH 4.1 in the absence of Al. The higher concentration of Ca did not reduce ion losses, possibly because of the severity of the acid and Al exposures. Battram (1988) showed that the most severe effects of 175 $\mu g \cdot L^{-1}$ Al on Cl fluxes in juvenile brown trout were at pH 5.5 (pH 4.0, 5.5, 7.0, 2-h experiments; 150 μ*M* Ca). Aluminum also interferes with Ca balance: brown trout fry exposed to 40 to ~160 or 490 $\mu g \cdot L^{-1}$ Al at pH 5.8-6.6 (20 μ*M* Ca, 72 h exposures) showed increased net efflux of Ca, and, at the higher Al concentrations, reduced Ca influx (Sayer et al. 1991a).

Christine Neville was one of the early investigators to systematically show the dual respiratory and ionoregulatory effects of Al at fish gills. In a complex set of experiments, cannulated juvenile rainbow trout primarily showed hypoxia at pH 6.1 when exposed for 6 to 11 d to 75 $\mu g \cdot L^{-1}$ Al in soft water (50 to 140 μ*M* Ca), suffered

electrolyte loss at pH 4.5, and showed a combination of the two responses to Al at pH 5.5 and 5.0 (Neville 1985). Aluminum provided some protection against the effects of H^+ at pH 4.0. All fish had acidified blood, either from CO_2 accumulation (respiratory acidosis), lactic acid accumulation due to anaerobic respiration (metabolic acidosis), or a mixture of the two. In an effort to explain some of her results, Neville suggested that gill surface pH may be more basic than ambient (acidic) pH due to acid uptake or base release at the gills (Neville 1985). A later, re-interpretation of these results re-emphasized the dual ionoregulatory and respiratory nature of Al toxicity, and Neville and Campbell (1988) stated that Al speciation theories of Al toxicity may not be useful because rapid equilibria between chemical species makes any species of Al a source of free Al to interact with binding sites on gill membranes. Precipitation phenomena, a "physical" process at the gill surface, was considered a likely explanation of the respiratory effects of Al at pH 6.1, especially if gill surfaces were more basic than the acidic bulk water, and the protective effect of Al^{3+} against H^+ (pH 4.0) was seen as competition for binding sites on the gills (Neville and Campbell 1988).

In adult brook trout (*Salvelinus fontinalis*), exposure to 110 to 1000 $\mu g \cdot L^{-1}$ Al (pH 4.4 to 5.2) for up to 11 d reduced Na influx as well as stimulated its efflux, the latter presumably due to displacement of Ca from gill membranes by Al, weakening tight junctions (Booth et al. 1988). Calcium (200 *versus* 12 μM) was partially protective against Na losses, and reduced ion efflux was part of an incomplete recovery phase. Booth et al. (1988) suggested either Al precipitation or Al speciation changes at more alkaline gills as the cause of greater Al toxicity at higher pH and higher Al

concentrations. In a companion paper, respiratory effects of 330 $\mu g \cdot L^{-1}$ Al at pH 4.8 were elucidated by Wood et al. (1988a) using cannulated brook trout. Gill inflammation, mucus production, and thickening of the blood-water barrier was suggested as the mechanism of the respiratory effects of Al, which were manifested as low arterial O_2 tension (PO_2), high arterial CO_2 tension (PCO_2), and blood acidosis.

Respiratory effects of Al were clearly demonstrated by Malte and Weber (1988), who exposed cannulated adult rainbow trout to high Al concentrations (860 $\mu g \cdot L^{-1}$) at pH 5.0 in soft water (50 μM Ca). These workers eliminated the ionoregulatory effects of Al by adding 150 mM NaCl to the water (~same concentration as fish plasma) but hyperventilation, low arterial PO_2 and high arterial PCO_2, acidosis, and high blood lactate concentrations still occurred within 24 h. These results confirmed for soft water some earlier work by Malte (1986) and Jensen and Weber (1987; tench, *Tinca tinca*) in which very high Al concentrations (2 $mg \cdot L^{-1}$) were used in very hard water (~3,500 μM Ca; 4-6 d exposures, pH 5). Under these extreme conditions, the cannulated fish showed large respiratory disturbances due to Al precipitation and mucus clogging of the gills.

Dietrich and Schlatter (1989a) also demonstrated the dual ionoregulatory (lower pH, lower Al conditions) and respiratory nature (higher pH, higher Al conditions) of Al toxicity in adult rainbow trout, shown by mortality, plasma ion concentrations, and gill pathology (pH 5.2 to 5.6, 0 to 530 $\mu g \cdot L^{-1}$ Al, 370 μM Ca, 96-h). These workers thought that Al precipitating onto the gills, enhanced by higher pH near the gills due to ammonia release, was the likely toxic mechanism in most of their exposures. In

addition, they measured plasma Al concentrations using graphite furnace atomic absorption spectrometry (AAS), and showed significant Al entry into the blood, from about 31-56 $ng \cdot ml^{-1}$ Al (controls) to 50-105 $ng \cdot ml^{-1}$ Al (exposed to 140-530 $\mu g \cdot L^{-1}$ Al for 46-96 h). Dietrich and Schlatter (1989a) emphasized that Al entry into fish blood was not the toxic mechanism of Al: instead, Al toxicity was due to effects of Al directly on the gills. To our knowledge their paper is the only demonstration of Al entry into fish blood: Handy and Eddy (1989) did not see Al entry into blood of adult rainbow trout (950 $\mu g \cdot L^{-1}$ Al, pH ~5.4, ~10 μM Ca), but their 1 h exposure time may have been too short. The 1 h exposure was, however, long enough to stimulate mucus production at the gills.

Continuing where Neville (1985) stopped, Playle et al. (1989) again showed the dual ionoregulatory and respiratory effects of Al on cannulated adult rainbow trout (105 $\mu g \cdot L^{-1}$ Al; pH 4.4 to 5.2; 22 or 205 μM Ca; 66 h exposures). Ionoregulatory toxicity of Al, possibly due to Al^{3+}, was seen as additional to the ionoregulatory effects of acidity alone, but the respiratory effects (low arterial PO_2, high arterial PCO_2) were seen as being unique to Al, possibly due to Al precipitation on the gills. Blood acidification was a combination of respiratory and metabolic acidosis, and Ca reduced the ionoregulatory disturbances of Al at lower pH, but did not reduce the respiratory effects of Al at higher pH (Playle et al. 1989).

Witters et al. (1990a) showed that erythrocytes were released into the blood when adult rainbow trout were exposed to 60 and 200 $\mu g \cdot L^{-1}$ Al for 3 d, after 14 d exposures to low pH alone (pH 5.0, 25 μM Ca), which added to the increased

hematocrit due to the osmotic effects of Na and Cl losses from the blood. They proposed, on the basis of spleen appearance, that the spleen released more red blood cells into the blood in response to hypoxia caused by Al exposure, as was found in other studies. However, their spleen weights and spleen hemoglobin contents did not change significantly.

Adult rainbow trout exposed to 60 $\mu g \cdot L^{-1}$ Al at pH 5 in synthetic soft water (27 μM Ca) for 2.5 d showed, in response to Al, the usual decreases in plasma Na, blood PO_2, and blood pH, increased hematocrit, plus increased plasma concentrations of epinephrine, norepinephrine, and cortisol (Witters et al. 1991). Increased plasma catecholamine concentrations might have been to maintain erythrocyte O_2 carrying capacity. The cortisol results of Witters et al. (1991) agree with those of Goss and Wood (1988), who found that plasma cortisol, lactate, and glucose concentrations increased by 24 to 30 h in cannulated rainbow trout exposed to pH 4.8 plus 110 $\mu g \cdot L^{-1}$ Al (25 μM Ca) in comparison to fish exposed to pH 4.8 alone, presumably due to the combined respiratory and ionoregulatory effects of Al. Brown and Whitehead (1995) showed that brown trout exposed to pH 5 and about 200 to 300 $\mu g \cdot L^{-1}$ total Al showed elevated plasma glucose, cortisol, noradrenaline, and adrenaline concentrations during 36 h exposures (50 μM Ca).

Sayer et al. (1991b) measured plasma Na and hematocrit of dying juvenile brown trout (320 $\mu g \cdot L^{-1}$ Al, pH 4.5, 20 μM Ca) and once again showed hemoconcentration due to ionoregulatory failure as the primary cause of death, although blood gases were not measured. By now, the hemoconcentration results of

Milligan and Wood (1982) have been confirmed for Al and need no longer be addressed specifically. Sensitive monitoring of Ca and Na influx and efflux using ^{45}Ca and ^{24}Na indicated a dose dependent inhibition of Ca influx by Al, and stimulation of both Ca and Na efflux at higher Al concentrations (30-440 $\mu g \cdot L^{-1}$ Al, 780 μM Ca, pH 5.2, 1-4 h exposures, carp *Cyprinus carpio*; Verbost et al. 1992). Stimulation of Ca and Na efflux by Al may have been due to displacement of Ca from tight junctions on the gill epithelium, but inhibition of Ca influx was harder to explain. The authors speculated that Al may inhibit Ca influx by interfering with the second messengers involved in keeping open Ca channels on the apical membrane (Verbost et al. 1992).

In a study using early life stages of golden trout (*Oncorhynchus aguabonita aguabonita*), DeLonay et al. (1993) found that swim-up larvae were most sensitive to Al, with significant, additional mortality due to Al accurring at pH 5.5, 100 $\mu g \cdot L^{-1}$ Al and pH 5.0, 100 and 300 $\mu g \cdot L^{-1}$ Al (~34 μM Ca, 7 d exposures). Whole-body Na concentrations decreased, and locomotary and feeding impairment due to Al was an even more sensitive indication of Al toxicity than was mortality. Cannulated brown trout exposed to ~12, 24, and 60 $\mu g \cdot L^{-1}$ Al in synthetic soft water showed moderate to severe respiratory effects of Al (e.g. decreased arterial blood oxygen content) but few ionoregulatory effects (20 μM Ca, pH 5.0; Waring and Brown 1995). Brown trout were extremely sensitive to Al in this study, with mortalities occuring at 24 $\mu g \cdot L^{-1}$ Al (67% in 5 d) and at 60 $\mu g \cdot L^{-1}$ Al (100% in 2 d). Plasma cortisol and thyroxine (T_4) increased in these Al-exposed trout, and there was some suggestion that triiodothyronine (T_3) may

be involved in aiding Na and Cl homeostasis in these trout (Waring et al. 1996). There was no clear association of plasma prolactin with ionoregulatory status.

Laitinen and Valtonen (1995) studied the effects of 280 and 450 $\mu g \cdot L^{-1}$ Al on adult brown trout in cold (1-3°C) humic river water (pH 4.7, ~90 μM Ca, 7 d exposures). Heart and ventilation rates were monitored by a non-contact bioelectical monitoring system. Heart and ventilation rates increased in all treatments, with greatest increases in the pH 4.7 plus 450 $\mu g \cdot L^{-1}$ Al treatments, with greater effects on ventilation rate and ventilation in the Al-exposed fish. Blood samples at the end of the experiment showed a few hematological disturbances in the acid and acid plus Al exposures, the most notable and consistent effect being increases in blood glucose concentration (Laitinen and Valtonen 1995). In general, these fish showed respiratory effects of Al consistent with the relatively high Al concentrations and the pH of the exposures, and only minimal ionoregulatory effects. In addition, the effects of Al were relatively minor compared to experiments run in synthetic soft water, because of the humic nature of the river water used.

Atlantic salmon (50 g, ~1 yr old) in soft water (~60 μM Ca) showed mortality in response to 24 $\mu g \cdot L^{-1}$ labile Al (76 $\mu g \cdot L^{-1}$ total monomeric Al; pH 6.0; LT50 =36 h), 31 $\mu g \cdot L^{-1}$ labile Al (68 $\mu g \cdot L^{-1}$ total Al; pH 5.6; LT50 =60 h), and 46 $\mu g \cdot L^{-1}$ labile Al (91 $\mu g \cdot L^{-1}$ total Al; pH 5.6; LT50 =29 h; Berntssen et al. 1997). Speciation of Al was done using the cation exchange column method of Driscoll (1984). Plasma chloride concentrations decreased significantly by 24 h in fish from each of these treatments, and the fish showed blood hemoconcentration. No mortalities were seen in fish exposed for 80 h to

$\leq$18 $\mu g \cdot L^{-1}$ labile Al (<72 $\mu g \cdot L^{-1}$ total monomeric Al) at pH 6.2, 6.0, and in control (pH 6.8) conditions, and plasma chloride concentrations did not decrease significantly in these treatments. Fish showing Al toxicity had rapid decreases in skin mucous cell numbers and developed a layer of mucus on the skin and edges of the opercula, and mucous cell number and plasma chloride concentrations correlated extremely well. Berntssen et al. (1997) attributed these effects to labile Al, not Al polymerization in their experimental setup. The decrease in skin mucous cell number was probably a result of exhaustion of the cells through high rates of mucus secretion in response to Al, and a relatively slow rate of formation of new mucous cells. The authors presented the argument that high mucus secretion rates were to help slough Al from the gills, not to prevent ions from diffusing from within the fish to the water. They also documented a shift to secretion of more negatively charged mucus, which would tend to bind labile Al better. The authors suggested that excessive mucus secretion with Al deposition could have caused respiratory problems in their fish, as reported in other studies, but they did not measure any respiratory blood parameters.

5.2.2 *Respiratory disturbances in fish: precipitation and polymerization of Al*

The dual nature of Al toxicity to fish in soft water has been well established, with its ionoregulatory effects predominating at lower pH, and respiratory effects predominating at more moderate - but still acidic - pH. The effect of Al at lower pH is commonly viewed as the same mechanism as H^+ toxicity alone, displacement of Ca from tight junctions of the gill membrane (e.g. Freda et al. 1991) or interference with

Na,K-ATPase (e.g. Staurnes et al. 1993), leading to ion losses. In this manner Al^{3+} and H^+ can have additive or antagonistic effects at fish gills. Mechanisms of the respiratory effects of Al have been slower to be elucidated, and consensus has not necessarily been reached.

Playle and Wood (1989a), Lin and Randall (1990), and Playle et al. (1992) showed that soft, acidic water (~pH<6) is made more basic as it passes over the gills of rainbow trout and fathead minnows (*Pimephales promelas*), primarily due to NH_3 release at the gills. Conversely, basic water is made more acidic as it passes over the gills, due to CO_2 release at the gills. The partial neutralization of acidic water by NH_3 is particularly important with respect to acidic, poorly buffered soft water containing Al. In theory, the various species of Al in acidic water passing over fish gills would shift from mostly Al^{3+} in favour of Al-hydroxides, and Al would be less soluble in the more basic water in the gill micro-environment (Neville and Campbell 1988; Playle and Wood 1989b). From the data of Playle and Wood (1989b), soft water at pH 5.2 can hold ~150 $\mu g \cdot L^{-1}$ Al, and its composition is about 23% Al^{3+}, 40% $AlOH^{2+}$, 25% $Al(OH)_2^+$, 10% $Al(OH)_3^\circ$, and only about 2% $Al(OH)_4^-$. As this water passes over the gills, its pH increases to pH ~5.5, which can only hold about 30 $\mu g \cdot L^{-1}$ Al, and the Al composition is now about 8% Al^{3+}, 30% $AlOH^{2+}$, 40% $Al(OH)_2^+$, 15% $Al(OH)_3^\circ$, and 8% $Al(OH)_4^-$.

Playle and Wood (1989b) favoured the interpretation that soft water at pH 5.0 to 5.5 containing 100 to 200 $\mu g \cdot L^{-1}$ Al would become oversaturated with Al in the more basic gill micro-environment, with the precipitated Al being responsible for the respiratory effects of Al. These workers used a filtration apparatus to show that Al can

polymerize and precipitate from solution in about 1-4 s after base (NH_3) addition, likely fast enough for Al accumulation on fish gills to occur through precipitation (Playle and Wood 1990). There was ~10% of 160 $\mu g \cdot L^{-1}$ total Al which did not pass through 0.2 µm filters at pH 5.3; 1-4 s after NH_3 was added to raise the pH to 5.7, ~35% of the Al was retained by the filters. Lydersen et al. (1991) found that formation of Al polymers occurred in <5 min when 300 to 800 $\mu g \cdot L^{-1}$ inorganic, monomeric Al solutions (pH 4.5) were raised to pH 5.5 or 6.0. The 300 $\mu g \cdot L^{-1}$ inorganic, monomeric Al solution decreased to about 250 $\mu g \cdot L^{-1}$ inorganic, monomeric Al within 5 min, and to about 200 $\mu g \cdot L^{-1}$ in 24 h. These workers suggested that extraction times used for Al analysis must be reduced, or another method used, if instantaneous Al polymer formation was to be measured; using a filtration method, Playle and Wood (1990) achieved the fastest measurement of Al precipitation or polymerization to date.

It is possible that Al precipitation *per se* is not the toxic mechanism at fish gills, and that the Al species formed in the gill micro-environment is the Al_{13} polymer (Bertsch 1987; Bertsch and Parker 1996). Parker and Bertsch (1992a) have been able to measure the Al_{13} tridecameric polycation using a ferron method. Their method allows measurement of Al_{13} in partially neutralized solutions at 10-100 times lower concentrations than was possible with ^{27}Al-NMR (nuclear magnetic resonance spectroscopy), which brings Al_{13} measurements into the range (~270 $\mu g \cdot L^{-1}$ Al) more useful for water. In addition, it may be possible to improve Al_{13} detection to about 30 $\mu g \cdot L^{-1}$ by increasing the pathlength in the spectrophotometic cell (Parker and Bertsch 1992a). Could the Al_{13} polycation form as acidic water containing Al passes into the

more basic gill micro-environment? Parker and Bertsch (1992b) showed that, after 24 d, a 2,700 $\mu g \cdot L^{-1}$ Al solution (pH ~2) brought to pH 4.7 with NH_3 contained about 75% mononuclear Al, about 14% Al_{13}, and about 11% larger polynuclear Al or solid-phase $Al(OH)_3$. A 540 $\mu g \cdot L^{-1}$ Al solution (pH ~2) brought to pH 5.3 with $NaHCO_3$ yielded about 4% monomeric Al, 53% Al_{13}, and about 42% larger polynuclear Al or $Al(OH)_3$, after storage overnight before ferron analysis. Parker and Bertsch (1992b) designed their experiments mainly to simulate liming of acidic waters so did not use shorter time periods or lower Al concentrations.

It appears that Al_{13} formation may indeed be possible in the gill micro-environment, but proof is not yet available, and there may still be considerable $Al(OH)_3$ or large polymers forming (~equal to Al_{13}). Formation of $Al(OH)_3$ or large polymers was in fact favoured (at the expense of Al_{13} formation) at lower total Al concentrations and as m (the base/Al molar ratio) increased above 2.5 (Parker and Bertsch 1992b), so the conclusions of Playle and Wood (1990) that Al polymerization and precipitation at fish gills can explain respiratory effects of Al may, in the end, be correct. At fish gills, m may be as high as 3 (base =20 μM, Al =6 μM; Playle and Wood 1989a). In a similar manner, increasing ligand:Al molar ratios favours ligand Al formation at the expense of Al_{13} formation. A 1:1 ratio of lactate:Al produced about 50% Al_{13}, but a 3:1 ratio produced no Al_{13}; a 0.5% salicylate ratio produced ~30% Al_{13}, whereas a 1:1 salicylate ratio produced no Al_{13} (Al=0.02 *M*, pH 5-6; Thomas et al. 1993). A problem in resolving this issue is the lag time for Al_{13} analysis by ferron, which takes about 2 to 4 min

(Parker and Bertsch 1992a), which is much longer than the 1-4 s lag time with the filtration system used by Playle and Wood (1990).

Poléo (1995) presented a nice compilation of relevant fish experiments in a discussion of the possibility of Al polymerization being the mechanism of acute toxicity of Al to fish, especially in cases of increased Al toxicity after an increase in water pH (e.g. through liming). Still, throughout his discussion, it is difficult to separate the respiratory effects theoretically due to Al polymerization at the gills from the respiratory effects theoretically due to Al precipitation at the gills, since they both result from an increase in pH of acidic, Al-rich solutions. Poléo et al. (1995) showed that anoxia-tolerant crucian carp (*Carassius carassius*) survived a 25 d Al exposure (290 $\mu g \cdot L^{-1}$ inorganic Al) at pH 5.2, in spite of decreased plasma chloride concentrations in the fish and Al accumulation on the gills of the fish (~9 g, ~75 μM Ca). These workers assumed that Al polymerization processes were occurring but that the anoxia-tolerant fish were able to survive the respiratory effects of Al, whereas fish such as salmon and trout that are not hypoxia-tolerant cannot survive these conditions. The authors realized their conclusions were only indirect (they did not measure blood PO_2, for example) and concluded their paper by saying that more detailed studies on crucian carp and Al, including respiratory physiology, were under way.

The toxicity of Al in mixing zones of neutral and acidic, Al-rich water was studied by Verbost et al. (1995), Witters et al. (1996), and Exley et al. (1996). Acidic (pH 5.1, 345 $\mu g \cdot L^{-1}$ total Al, 20 μM Ca) and neutral lakewaters (pH 7.0, 73 $\mu g \cdot L^{-1}$ Al, 160 μM Ca) were mixed in an artificial channel, resulting in water of pH 6.4 with 245 $\mu g \cdot L^{-1}$ Al

(Verbost et al. 1995). The water at the top of the mixing channel (just 12 s after mixing) was much more damaging to gills of 10 g brown trout, as judged by chloride cell damage, than was water at the bottom of the channel (~340 s, nearly 6 min, after mixing). The mixing zone was more damaging than further down the channel, even though the Al concentrations were the same at the top and bottom of the channel. 245 $\mu g \cdot L^{-1}$ Al at pH 6.4 is clearly an oversaturated Al solution, so either Al precipitation or Al polymerization mechanisms could have been responsible for the gill damage seen, and either process would be more severe right after mixing.

Witters et al. (1996) set out to simulate in more controlled laboratory conditions the mixing situations studied by Rosseland et al. (1992) and Verbost et al. (1995). Brown trout (~60 g) were exposed to a pH 6.4, 2.8 μM Al solution (75 $\mu g \cdot L^{-1}$ Al) produced 90 s (1.5 min) or 390 s (6.5 min) after an acidic, Al-rich solution (pH 4.0, 200 $\mu g \cdot L^{-1}$ Al) was mixed with pH 6.7 soft water containing no Al (Witters et al. 1996). Fish mortality was higher in the freshly mixed Al solution compared to the 5 min older mixture (98% *versus* 14% mortality in 48 h), even though water chemistry was nearly identical in each treatment (e.g. total Al and Al >10 kD, below). Ventilation frequency increased most and plasma Na and Cl concentrations decreased most in fish exposed to the freshly mixed Al solution. Changes in mucous cell number and type also agreed with Al toxicity, with a faster depletion of mucous cells and a faster production of acid glycoprotein-containing mucous cells in fish exposed to the 1.5 min mixed solutions than in fish exposed to the 6.5 min solutions (Witters et al. 1996).

In each treatment, about 80% of the total Al (~2.8 μ*M*) was in forms >10 kD, that is, high molecular weight forms of Al, thought by the authors to represent Al polymers such as $Al_6(OH)_{12}^{6+}$ or $Al_{13}(OH)_{30}^{9+}$ (Witters et al. 1996). Note, however, that 2.8 μ*M* Al at pH 6.4 represents oversaturated Al conditions. Witters et al. (1996) hypothesized that different patterns of Al polymerization explain the higher toxicity in the 1.5 min mixed solution compared to the 6.5 min solution, with the low molecular weight Al polymers predominating at 1.5 min being more toxic than higher molecular weight Al polymers in the older solution. Note that Neville (1985) also reported acute toxicity of Al to fish at pH ~6.1 and similar Al concentrations, so the study of Witters et al. (1996) was not the first time Al was shown to be toxic near pH 6.0, and Neville's explanation of mixed ionoregulatory and respiratory effects of Al was similar, although less detailed, especially regarding the possible Al precipitation and polymerization phenomenon to explain respiratory effects. The novelty and importance of the Witters et al. (1996) study is the illustration of differential toxicity of Al in mixing zones.

The importance of time after mixing of an Al solution was also illustrated by Exley et al. (1996). A pH 6.5, 11 μ*M* Al solution (300 $\mu g \cdot L^{-1}$ Al) prepared from a pH 4.0, 23 μ*M* stock Al solution was toxic to rainbow trout exposed to the un-aged solution (~25% mortality in 24 h), whereas aging of the solution for either 8 or 15 min reduced the toxicity to about 4% in 24 h (rainbow trout ~1.4 g, ~50 μ*M* Ca; Exley et al. 1996). Clearly, these solutions were also supersaturated with respect to Al, with only about 1.2 μ*M* Al in small Al species (<10 kD) in both fresh and aged solutions, as shown by ultrafiltration. As in the Witters et al. (1996) study, and admitted by Exley et al. (1996),

their fractionation scheme was too insensitive to pick up changes in colloidal chemistry which presumably would explain the different solution toxicities. In its simplest interpretation, fresh Al solutions may be undergoing rapid polymerization and precipitation phenomena in solution and in water passing by the gills, causing highly toxic conditions to fish, whereas in solutions aged for just a few minutes these reactions have already occurred in solution so that water passing over the gills contains less toxic Al in higher molecular weight polymers. Recall that Playle and Wood (1990) showed that Al precipitation or polymerization happens in less than 4 s.

As well as considering solution aging, Exley et al. (1996) demonstrated that toxicity was related to the state of the Al stock solution used in an experiment. In the experiment outlined above, a 23 μ*M* stock Al solution was prepared at pH 4.0, left for 96 h, then diluted to 11 μ*M* Al at pH 6.5, and ~25% fish mortality occurred in 24 h. If 23 μ*M* stock Al solutions were prepared at pH 5.0, 6.5, or 10.2, then left for 96 h, the final pH 6.5, 11 μ*M* Al solution was only minimally toxic when prepared from the pH 5.0 stock (~5% toxicity in 24 h) and was not toxic in 24 h if the 11 μ*M* Al solution was prepared from either the pH 6.5 or 10.2 stock Al solutions. The authors measured just 0.2 μ*M* Al <10 kD in the stock Al solution prepared at pH 6.5, indicating the existence of large colloids or polymers in the supersaturated stock solution, so that the Al in the final test solution was already in a relatively non-toxic form and little Al was measured on the fish gills, and there was no toxicity to the fish. Exley et al. (1996) explained their results through precipitation of Al at the gill surface and resultant respiratory effects of Al. Small, newly formed Al polymers could infiltrate the mucus layer covering fish gills,

then form aggregates within and with mucus (a "tanning" effect), increasing the resistance to diffusion of oxygen and carbon dioxide. Larger (older) Al polymers and precipitates would not infiltrate the mucus and bind to the mucus to the same degree as smaller Al species, so would be less toxic. An intriguing aspect of their work which the authors did not discuss is that the Al stock solution prepared at pH 10.2 had about 90% of its Al in forms <10 kD (e.g. no large polymers), and Al was presumably in the $Al(OH)_4^+$ form in solution (*versus* all in the Al^{3+} form in the pH 4.0 stock solution). When brought to pH 6.5 both solutions would theoretically form the $Al(OH)_3^\circ$ monomer then polymerize, but presumably this polymerization occurred faster from the $Al(OH)_4^+$ state than from the Al^{3+} state (perhaps because a loss of only one OH^- group was involved, not the addition of three OH^- groups), so there was no toxicity in the 11 μ*M* Al solution made from the pH 10.2 stock. Clearly, as the authors stated in the title of their paper, kinetic constraints are important in acute aluminum toxicity to fish.

5.2.3 *Model representing Al interactions at fish gills*

A model of the effects of Al at fish gills must integrate mechanisms of Al accumulation with the observed respiratory and ionoregulatory effects of Al. In the model presented in Figure 5.1, respiratory effects of Al are assumed to be a result of Al polymerization and precipitation on the gills and to associated gill responses. Ionoregulatory disturbances are assumed to be caused by interactions of positively charged Al species with negatively charged branchial surfaces.

For fish in circumneutral soft water, active uptake of Na and Cl across the branchial epithelium compensates for ion losses from blood to water (Fig. 5.1A). Oxygen diffuses across the gill membrane into the blood, and CO_2 diffuses out. Carbon dioxide acidifies water in the gill micro-environment (catalyzed by carbonic anhydrase). Ammonia also diffuses through the branchial membrane, tending to make water next to the gills more alkaline. The extent of net acidification or alkalization depends on inspired water pH and its buffer capacity. In acidic water (Fig. 5.1B), H^+ ions reduce the active uptake of Na and Cl, and increase their passive effluxes, possibly by displacing Ca from tight junctions (McDonald 1983; Freda et al. 1991). Transfers of O_2 and CO_2 are unaffected by acidity alone. Release of CO_2 no longer acidifies water near the gills (the p*K* of the reaction is ~6.3), so partial neutralization due to NH_3 transfer predominates.

In the presence of 100 to 200 $\mu g \cdot L^{-1}$ total Al in moderately acidic soft water, the pH increase in the gill micro-environment (due to NH_3) causes Al accumulation on the gill surface (Fig. 5.1C). This deposition is due to decreased Al solubility leading to polymerization and precipitation (simplest explanation), Al_{13} polycation formation, or to changes in Al chemistry to Al species which better react with branchial surfaces (difficult to prove), or to a combination of the three mechanisms. In the model, respiratory effects of Al are a result of Al accumulation itself, of excess mucus production stimulated by Al accumulation, or of gill swelling, inflammation, and damage caused by Al. These effects would all increase diffusion distance for blood gases, resulting in decreased blood PO_2 and increased blood PCO_2, which, in conjunction with

ionoregulatory effects of Al, could kill the fish. Smaller, more recently formed Al polymers are more toxic than older, larger Al polymers, because they can insinuate themselves into the layer of mucus at the gills and polymerize there.

Sloughing of mucus continually removes Al precipitating on gill surfaces (see below), but may not remove positively charged Al species bound to negative charges on the branchial surface itself. In the model Al intimately bound to the gills is the portion of Al accumulation that is responsible for the ionoregulatory effects of Al (Fig. 5.1C). Bertsch and Parker (1996) suggested that the tightly bound Al may be the Al_{13} polynuclear species. Once bound on the branchial surfaces, Al reduces active ion uptake, by reducing Na-K-ATPase activity, through damage to chloride cells, or perhaps by interfering with ion channels. Increased ion efflux is possibly by the same action as H^+ ions in more acidic water, the displacement of Ca from gill surfaces by positively-charged Al species.

In the presence of 100 to 200 $\mu g \cdot L^{-1}$ Al in very acidic water, accumulation of Al on the gill surface is low (Fig. 5.1D). This situation prevails because, even though the solubility of Al is reduced in the more alkaline gill micro-environment, its solubility is not exceeded. As there is no precipitation of Al (or the formation of Al_{13}) on branchial surfaces, respiratory toxicity of Al does not develop. Any Al accumulating on the gills is likely to be positively-charged Al species binding to negative charges on the gills, but the small amount of bound Al may not add appreciably to the ionoregulatory disturbances already caused by H^+ ions. In extremely acidic conditions, Al^{3+} may compete with H^+ for binding sites, reducing the toxic effects caused by H^+ ions alone.

Presumably, Ca^{++} reduces the effects of both Al^{3+} and H^{+} ions through similar competitive interactions.

Initial, slow accumulation of Al onto gills of Atlantic salmon (Fig. 5.1B), and subsequent, rapid loss (depuration) to water once Al exposure is halted has been mimicked by incorporating mucus production and sloughing into a computer model (Wilkinson and Campbell 1993). In their model, Al initially binds to mucus from where it is passed to gill tissue, and total gill Al increases linearly then reaches a plateau when mucus production and mucus sloughing are equal. Rapid depuration of gill Al occurs as mucus production decreases when the Al stress is removed, but mucus (and therefore mucus-bound Al) sloughing continues. The results of Lacroix et al. (1993) agree with those of Wilkinson and Campbell (1993), in that Al depuration was rapid and related to the physical process of sloughing from the gills. The addition of citrate to exposure water decreased the amount of Al binding to the gills, but did not accelerate its removal (0.3 g Atlantic salmon, pH ~5.5, ~90 μM Ca, 15 d, ~270 $\mu g \cdot L^{-1}$ Al; Lacroix et al. 1993). X-ray microanalysis located Al on the the surface of gills, presumably associated with mucus. Although Al content of the gills fell to background in 2 to 4 d, gill damage persisted, as shown by light and scanning electron microscopy, indicating that sloughing of Al is faster than gill tissue repair. Rapid Al depuration from gill epithelia, but slower repair mechanisms, would contribute to observations of intermittent exposure to high concentrations of Al (e.g. pulses during storm events) being more toxic than longer exposures to lower concentrations of Al (e.g. review by Handy, 1994), because damage due to Al precipitation or polymerization (at high Al

concentrations) in the gill micro-environment may take time to be repaired even after the Al exposure ends.

The model presented in Fig. 5.1 - of more basic conditions next to the gill, and associated Al interactions at the gill - is similar to that proposed by Exley et al. (1991). Their model adds the point that Al bound to ligands such as DOC in water may still be toxic if bound Al, in equilibrium with free Al, is passed to negatively charged sites on gills which bind free Al more strongly. If all Al species are in equilibrium (Neville and Campbell 1988), then attempts made to determine which individual Al species is responsible for Al toxicity, such as those made by Sadler and Lynam (1987), Palmer et al. (1989), and Playle and Wood (1991), are of questionable value. Perhaps this is why the Al precipitation and polymerization theory at fish gills is so attractive. It is not necessary to know which specific Al precipitate or polymer is produced at the gills, nor is it necessary to know which specific positively-charged Al species binds to the negatively charged gill surfaces when Al precipitation or polymerization does not occur: it is enough to know that binding and precipitation of Al can occur at fish gills. Ionoregulatory effects of Al may be due to positively charged Al species binding to the gills and displacing Ca from intercellular tight junctions, and respiratory effects may be due to Al accumulation on the general gill surface. Ionoregulatory effects of many metals and H^+ at the gills is a common response when they are at low concentrations, and at high metal and H^+ concentrations respiratory effects can occur due to gill damage (Wilson and Taylor 1993). What makes the respiratory effects of Al unique is

their occurrence at environmentally relevant Al concentrations in acidic water, due to the higher water pH in the gill micro-environment.

5.3 Modifying factors of Al toxicity towards fish

5.3.1 *Dissolved organic carbon*

Besides Ca concentration, dissolved organic carbon (DOC) affords protection against the physiological and toxicological effects of Al, by rendering Al in solution less available to interact at fish gills. Neville (1985) demonstrated that ~10 $mg \cdot L^{-1}$ DOC protected against the effects of Al: approximately five times higher total Al concentration was needed to saturate the organic acids to leave ~2.8 µ*M* inorganic Al in solution, which then had similar effects as 2.8 µ*M* inorganic Al alone. Dissolved organic carbon was protective against Al toxicity to Atlantic salmon alevins as long as inorganic Al not complexed by natural DOC was less than 50 $\mu g \cdot L^{-1}$ (pH 4.8-5.8, ~20 µ*M* Ca, 30 d exposures; Peterson et al. 1989). Aluminum accumulation by the fish (and hence toxicity) was related to the amount of free Al. Twenty-one day survival of brook trout fry was consistently enhanced by $\geq$3.1 mg $C \cdot L^{-1}$ DOC (Aldrich humic acid) during exposures to $\leq$340 $\mu g \cdot L^{-1}$ total Al at pH 5.1 and 5.6 (55 µ*M* Ca; Parkhurst et al. 1990). The protective effect of DOC was less at pH 4.8 and was virtually eliminated at pH 4.4 and high Al concentrations (~1,200 $\mu g \cdot L^{-1}$ Al). Dissolved organic carbon was able to bind Al best at higher pH (as shown by low inorganic, monomeric Al concentrations); presumably DOC did not bind Al well at lower pH because of H^+ competition for binding sites on DOC (=protonation of binding sites), because of Al^{3+} predominance at lower

pH, and higher Al solubility at lower pH. Monomeric Al (=available Al) best predicted Al toxicity to fish (Parkhurst et al. 1990).

In a field survey involving juvenile Atlantic salmon and brook trout gill histology, >5 mg $C \cdot L^{-1}$ DOC appeared to mitigate the effects of up to ~310 $\mu g \cdot L^{-1}$ total Al, even though gill Al concentrations increased with increasing exchangeable Al in water (<40 $\mu g \cdot L^{-1}$ exchangeable Al in all streams surveyed, $\leq$32 μM Ca; Lacroix et al. 1990). Witters et al. (1990b) found that 10 mg $C \cdot L^{-1}$ humic material (soil and bog sources) virtually eliminated the toxicity of 180 $\mu g \cdot L^{-1}$ total Al (pH 4.7, 25 μM Ca, 10 d exposure, cannulated rainbow trout), and about ten times less Al accumulated on trout gills in the presence of humic material than in its absence. Presence of lake trout (*Salvelinus namaycush*) in Sudbury area lakes has been predicted by a combination of pH, alkalinity, and conductivity: concentration of Al in water also correlates with these variables (Conlon et al. 1992). Lake trout were not usually found in lakes of pH $\leq$5.4 or in lakes which contained $\geq$160 $\mu g \cdot L^{-1}$ Al.

In a field study involving blacknose dace (*Rhinichthys astratulus*) and brook trout placed in four Adirondack headwater streams, high water flows associated with snowmelt or rain were toxic to the fish, with water pH (pH 4.4-6.8), inorganic Al concentration (20-350 $\mu g \cdot L^{-1}$ Al), episode duration (to ~2 wk), and DOC (3-9 mg $C \cdot L^{-1}$) all being important in determining fish mortality (~50-150 μM Ca; Simonin et al. 1993). Blacknose dace were consistently more sensitive than brook trout to the acidic water episodes, and showed good survival if exposed to inorganic Al concentrations of $\leq$100 $\mu g \cdot L^{-1}$.

In a study in the Catskill Mountains, New York, caged brook trout were exposed to natural, fluctuating stream pH, Al concentration, and other stream conditions. In essence, fish mortality correlated best with high inorganic monomeric Al concentrations, followed by low water pH (which generally controlled Al concentrations), and DOC, Ca, and Cl concentrations (Baldigo and Murdoch 1997). Dissolved organic carbon complexes Al, Ca protects against some toxic effects of Al, and Cl may (along with other ions in the water, e.g. Na) help reduce the osmotic gradient from inside to outside the fish. Exposure of brook trout to $\geq$225 $\mu g \cdot L^{-1}$ inorganic monomeric Al for two days resulted in >20% mortality in the fish (Baldigo and Murdoch 1997). In a survey of 584 Norwegian lakes, Bulger et al. (1993) found that lakewater pH, monomeric inorganic aluminum concentration, and alkalinity (with Ca and SO_4 as components) best predicted brown trout populations. Lakes with 133 $\mu g \cdot L^{-1}$ Al, pH 4.8, and an alkalinity of -34 $\mu eq \cdot L^{-1}$ had extinct brown trout populations (39% of the 584 lakes), whereas 11 $\mu g \cdot L^{-1}$ Al, pH 6.0, and +27 $\mu eq \cdot L^{-1}$ alkalinity indicated healthy brown trout populations. Organically bound Al was not important in determining brook trout population status, as expected if organically bound Al is not toxic to fish (Bulger et al. 1993).

Modifying effects of DOC and water hardness against Al toxicity to juvenile rainbow trout were studied by Gundersen et al. (1994). Calcium concentrations of about 830 and 1,190 µM Ca increased survival of trout exposed to approximately 4 and 9 $mg \cdot L^{-1}$ Al at pH 8.0 to 8.6 for 96 h, compared to Ca concentrations of <380 µ*M*. Survival under similar conditions but at pH ~7.6 was always 100%, indicating that high

concentrations of Al under slightly basic conditions are more toxic to trout than at near-neutral conditions, indicating that $Al(OH)_4^-$ was more toxic than insoluble Al species. Sixteen day experiments showed similar results. In a follow up study, Gunderson and Curtis (1995) showed no effects of Al on osmotic permeability and Ca binding to gills when *in vitro* experiments were run using isolated gill arches (100 or 1,000 μ*M* Ca; 3.7 to 37 μ*M* Al; pH 8.3 to 8.5). These workers suggested that the absence of the acidic gill micro-environment (i.e. no CO_2 release at the isolated gills) meant that the $Al(OH)_4^-$ species was not converted to species of Al (e.g. $Al(OH)_3°$) that more readily react with the gills. That is, negatively charged Al species are unlikely to bind to negatively charged gills. Humic acid (Aldrich Chemical Co.) at $\geq$ 3 mg $C \cdot L^{-1}$ showed protective effects against Al toxicity of up to ~5 mg $\cdot L^{-1}$ Al, but not to ~9 mg $\cdot L^{-1}$ Al (Gundersen et al. 1994). Five mg $C \cdot L^{-1}$ humic acid protected against toxic effects of 0.5 to 2 mg $\cdot L^{-1}$ Al over 16 d. These authors warned that extrapolation of their humic acid results to natural conditions may be difficult, because of the high metal binding of commercial, terrestrial humic acids compared to aquatic humic acids.

Using isolated cells from gills of largemouth bass, Wilkinson et al. (1993) determined the electrophoretic mobility of suspended gill cells in the presence and absence of Al. As up to 50 μ*M* Al (1,350 μg $\cdot L^{-1}$ Al) was added to isolated gill cells (solution pH = 4.5), the negatively charged cells became progressively more positive as Al^{3+} bound to negative sites. Soil fulvic acid added to the isolated cell suspensions (0-20 mg $C \cdot L^{-1}$) was able to reduce the amount of Al binding to the isolated gill cells, as indicated by the greater negative charge of the cells as the fulvic acid concentration

was increased (Wilkinson et al. 1993). These workers interpreted their data as Al-DOC complexes binding to the gills, but the DOC was also likely able to keep Al off the gills through complexation. Previous estimates of Al-dissolved organic matter binding strengths are log $K_{Al\text{-}FA}$=7.8 (Shuman 1992), log $K_{Al\text{-}FA}$=6.4 at pH 4.5 (Browne and Driscoll 1993), and log $K_{Al\text{-}DOC}$=6.2 (Urban et al. 1990), so DOC should be able to bind Al well enough to keep Al off fish gills (log $K_{Al\text{-}gill}$~6.5; Wilkinson et al. 1990). The model of Shuman (1992) indicated that ~75% of 1 µ*M* Al (=27 µg·L^{-1}) would be bound by 10 mg C·L^{-1} DOC at pH 5.5. Shuman suggested that the normal manner in which Al reacts with ligands such as fulvic acid is through the disjunctive reaction, of Al dissociating then re-assembling with another ligand, as opposed to adjunctive "attack" of an Al-ligand complex by another ligand. Wilkinson et al. (1993) suggested that fulvic acid itself bound to isolated gill cells, thus making the cells more negative, but an alternative scenario is that the negatively charged fulvic acid did not bind to the negatively charged cells, but stripped positive charges such as Ca^{++} off the cells.

Roy and Campbell (1997) demonstrated that the protective effect of a natural organic matter against Al toxicity to juvenile Atlantic salmon was greater than expected due to just the reduction of available Al to interact at the gills. In essence, it took more inorganic Al in the presence of 2 to 3 mg C·L^{-1} fulvic acid to be toxic to salmon (96 h LC50 = 6.1-8.0 µ*M* inorganic Al) than in the absence of fulvic acid (96 h LC50 = 2.9-4.0 µ*M* inorganic Al; ~45 µ*M* Ca, pH 5.0). Inorganic Al was measured using a cation-exchange resin and through dialysis. Pre-exposure of fish to fulvic acid did not impart any protecion against Al toxicity in soft water containing no fulvic acid.

Roy and Campbell (1997) interpreted the better than expected protective effect of fulvic acid against Al toxicity as some type of additional protective effect of DOC on top of its ability to complex Al, possibly DOC itself interacting at fish gills. Their experiments were run at pH 5.0, which could protonate the DOC enough that DOC might indeed bind to fish gills. However, a possibility the authors did not consider is that the presence of DOC would prevent the precipitation and polymerization of inorganic Al in the more alkaline gill micro-environment (e.g. Fig. 5.1C; see also section 1.2.3 and Bertsch and Parker 1996). This interpretation is also in agreement with the fact that pre-exposure of juvenile salmon to fulvic acid did not protect the fish against Al toxicity in fulvic acid free water (Roy and Campbell 1997). Thus, the protective effect of DOC against Al toxicity in their experiments might involve both binding of Al so less inorganic Al is available to bind at the gill, and an additional effect of DOC in reducing Al precipitation or polymerization on the gill surfaces.

5.3.2 *Fluoride*

Although the above-mentioned studies indicated decreased Al toxicity if Al was complexed by, for example, DOC, complexed Al may not always be rendered as non-toxic as might be thought. For example, Wilkinson et al. (1990) found that 9 μ*M* Al in the presence of 1 to 10 μ*M* F^- was indeed less toxic to juvenile Atlantic salmon than was Al alone, but was more toxic than predicted by calculated Al^{3+} concentrations (pH 4.5, 4.9; 45 μ*M* Ca; 7 d exposures). Similar results were found for Al accumulation on the gills in the presence of F^- (Wilkinson and Campbell 1993) and in the presence of

citrate (Lacroix et al. 1993). Wilkinson et al. (1990; see also Campbell 1995) interpreted their data as Al accumulation and toxicity due to an Al-F complex itself, but another interpretation is that fish gills can out-compete the Al-F complex for Al. Thus, there would be more Al to interact at the gills than predicted simply by the calculated concentration of free ion, Al^{3+}. Estimates of conditional stability constants of Al^{3+} made by Wilkinson et al. (1990) are log $K_{Al\text{-}gill}$=6.5, and log $K_{Al\text{-}F}$=7.0: the Al^{3+}-F complex is only three times stronger than the Al^{3+}-gill complex. That is, the strength of the Al-ligand complex relative to the Al-gill complex is important in considering Al available to interact at the gills, whether the ligand is F^- or citrate. For recent papers on this approach to modelling metal-gill interactions using conditional stability constants, see Janes and Playle (1995) and Richards and Playle (1998).

As mentioned above (section 5.3.1), suspended gill cells of largemouth bass became progressively more positive as Al^{3+} bound to negative gill sites (Wilkinson et al. 1993). For 10 μ*M* Al, the addition of up to 10 μ*M* F^- kept some Al off the gills, as judged by electrophoretic mobility of negatively charged isolated cells (suspension pH =4.5), but not by as much as predicted by the decrease in free Al^{3+} through complexation by F^-. These workers interpreted their results as Al-F complexes binding to the gills, as well as binding of Al^{3+}, but an alternative explanation, as discussed above, is that the gill cells outcompete F^- for Al^{3+}. At very high Al-F concentrations (10 m*M*) these workers were able to show some Al-F_n deposition on fish gill cells.

5.3.3 *Silicon*

Silicon can also reduce Al toxicity to fish. At pH 5, ~50 µ*M* Ca, 170 to 190 µg·L^{-1} Al (~7 µ*M*), a 13:1 molar ratio of Si:Al eliminated Al toxicity to 1 g Atlantic salmon fry (96-h exposures; Birchall et al. 1989). Silicon concentrations of ~90 µ*M* have been reported for streams (Brown and Driscoll 1992). Birchall et al. (1989) suggested that the formation of hydroxy-aluminosilicate species, enhanced by more alkaline conditions at the gills, blocks the binding (or precipitation) of Al-OH species at the gills. Recall that we suggested the Al and fulvic acid results of Roy and Campbell (1997) could perhaps be explained through a reduction in Al precipitation and polymerixation of Al in the more basic gill micro-environment (section 5.3.1). Birchall et al. (1989) emphasized that Si has been neglected in Al toxicity studies, and could be used to ameliorate Al toxicity. However, recent work by Exley and Birchall (in press) suggests that silicon concentrations must be greater than 500 µ*M* to significantly reduce Al-OH formation (4 µ*M* total Al), as shown by filtration through 0.04 µm membranes.

5.3.4 *Temperature*

The effect of temperature on Al toxicity to Atlantic salmon fingerlings was studied by Poléo et al. (1991). They found that toxicity of a 270 or 400 µg·L^{-1} Al, pH 5.0, 25 µ*M* Ca solution increased as temperature increased (1 to 19°C; 12 d exposures). They interpreted their toxicity data as being due to Al polymerization, which is faster at higher temperature, but these workers did not dismiss the possibility of greater Al toxicity at higher temperature being due to higher metabolic rate of fish at higher temperature (e.g. Roch and Maly 1979 for Cd; Q_{10}) or to heat stress of the fish (they

were all acclimated to 6°C). Parkhurst et al. (1990) also showed a tendency of decreased fish survival as temperature increased (5, 7, 12°C; ~90 $\mu g \cdot L^{-1}$ Al; pH 5.2, 5.6).

Temperature does affect Al speciation and Al solubility: Lyderson (1990) showed that a decrease in temperature of about 15°C has the equivalent effect on Al species as a decrease in pH by one unit. Temperature effects on metal speciation can be shown using computer programs such as MINTEQA2 (1991) and MINEQL+ (Schecher and McAvoy, 1992; see Fig. 6.3). The value of the work of Poléo et al. (1991) is to bring to attention the complicating factors of Al speciation and solubility changes into studies of temperature effects on Al toxicity, but purely metabolic effects of temperature on Al toxicity have not been adequately addressed. Although not studied for Al, seasonal changes can affect the toxicity of various pollutants. Lemly (1996) has coined the term "winter stress syndrome", which is a general metabolic response to stress which occurs if fish reduce activity and feeding in winter and therefore reduce their energy stores. A pollutant must increase a fish's metabolic activity for the fish to show winter stress syndrome, that is, the fish must show reduced survival when exposed to the stress in the cold compared to exposure in warmer conditions.

5.3.4 *Metal mixtures*

It is certainly worth remembering that Al is not the only potentially toxic metal to be mobilized in water, and that there may be synergistic effects between metals. Alone, 95 $\mu g \cdot L^{-1}$ Al was the threshold of acute toxicity to flagfish (*Jordanella floridae*) held in soft water (~60 μM Ca, pH 5.8), whereas in the presence of 5 $\mu g \cdot L^{-1}$ Zn (0.08 μM) and 2 $\mu g \cdot L^{-1}$ Cu (0.03 μM), only 29 $\mu g \cdot L^{-1}$ Al was needed to reach the toxicity threshold (Hutchinson and Sprague 1986). Other metals at low concentrations (Mn, Fe, Ni, and Pb) did not have additional effects to those of Al, Zn, and Cu on flagfish reproduction. As with single metal toxicity, toxicity of metal mixtures was reduced by humic substances (Hutchinson and Sprague 1987). In contrast, laboratory work with trace metal mixtures (Al, Mn, Fe, Ni, Zn, Cu, and Pb) in soft water showed that Al, Zn, and especially Cu were responsible for all toxicity of the full metal mixture, and that Al was alone responsible for observed toxicity at pH 4.9 (60 μM Ca, alevin rainbow trout and larval fathead minnow; Hickie et al. 1993). Thus, toxicities of combinations of metals are not necessarily intractable. Sayer et al. (1991c) found that Cu (5 $\mu g \cdot L^{-1}$; 0.08 μM), Pb (10 $\mu g \cdot L^{-1}$; 0.05 μM), and Al (160 $\mu g \cdot L^{-1}$; 5.9 μM) caused brown trout fry mortalities and impaired skeletal calcification in synthetic soft water, but Zn (20 $\mu g \cdot L^{-1}$; 0.31 μM) did not (pH ~5.6, 20 μM Ca).

The effect of a six metal solution on whole body ion loss was investigated in the laboratory, to test the hypothesis that whole body ions could be used as a biomarker for mine-polluted water (Grippo and Dunson 1996a). Their experimental conditions were meant to mimic conditions in a hardwater, mine-affected stream, and included Al

at 4.4 and 5.8 mg·L^{-1} (163 and 213 µ*M* Al, pH 3.3, ~1.1 m*M* Ca) or Al at 49 and 77 µ*M* at pH 3.9 (~300 µ*M* Ca; 3 g brook char, *Salvelinus fontinalis*). In essence, low pH alone (pH 3.3) caused ion losses in fish, and the metals did not add to or ameliorate the toxic effects of extremely low pH itself. At pH 3.9, some metals (Mn, Zn, Ni) reduced the toxicity due to low pH itself, but Al (49 or 77 µ*M*), Pb, and Fe did not. Use of the body Na loss assay as a biomarker was assessed in the field in four streams polluted by mine effluents. In the polluted streams (pH 3.2 to 4.8, 32 to 376 µ*M* Al, 140 to 970 µ*M* Ca) caged brook trout (1-32 g) had large body Na and Ca losses in 4 h compared to fish in a control stream (pH 6.7, 0.6 µ*M* Al, 110 µ*M* Ca; Grippo and Dunson 1996b). Sodium flux was the best ion to monitor, and multiple regression analysis explaining Na losses included the metals Al, Mn, Zn, Mg, and Fe.

Over a continuous exposure to 40 d post-hatching, Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) showed signifcantly decreased whole body Na concentrations and decreased survival at 50 µg·L^{-1} total Al and pH 6.0, 5.5, 5.0, and 4.5 (~40 µ*M* Ca; Farag et al. 1993). At pH 6.0, total monomeric Al was about 33 µg·L^{-1}, and was ~50 µg·L^{-1} at pH 5.5 and below. In contrast, the fish survived these concentrations with only minor effects on whole body Na concentrations during a 7 d exposure, but not at pH 5.5, 100 µg·L^{-1} Al.

5.4 Acclimation to Al

There is good evidence that fish can acclimate to Al. Orr et al. (1986) found that juvenile rainbow trout doubled their tolerance to Al when exposed for 2-3 weeks to ~90 and 150 $\mu g \cdot L^{-1}$ Al (pH 5.1-5.3, 72 μM Ca), but did not speculate as to what the acclimation process was. Adult brook trout exposed for 10 wk to pH 5.2, 12 or 200 μM Ca, and 75 or 150 $\mu g \cdot L^{-1}$ Al were better able to tolerate a 48 h pulse exposure to pH 4.8, 330 $\mu g \cdot L^{-1}$ Al than fish held at pH 5.2 or 6.5 in the absence of Al (Wood et al. 1988b,c), owing to a faster recovery phase during the acid and Al pulse. The mechanism of acclimation to Al was unclear, but in a concurrent study adult brook trout held in 150 $\mu g \cdot L^{-1}$ Al at pH 5.2 for 10 wk showed significantly higher Na transport activity than fish held at pH 5.2 or 6.5 with no Al (12 or 200 μM Ca), which suggests that the fish could at least partially acclimate to Al through increased synthesis of gill Na,K-ATPase (McDonald and Milligan 1988). Brook trout fry pre-exposed for up to 18 d to 50 $\mu g \cdot L^{-1}$ Al at pH 5.2 (64 μM Ca) showed better survival during a 6 d, 330 $\mu g \cdot L^{-1}$ Al, pH 4.6, 1.6 μM Ca pulse as the pre-exposure period lengthened (Mount et al. 1990). As these authors realized, their results were complicated by growth of the fry during the pre-exposure period, which may have added to the apparent acclimation effect.

McDonald et al. (1991) were able to follow the acclimation process of brook trout to Al. There was an initial damage (shock) phase, in which juvenile brook trout exposed for up to 42 d to 75-150 $\mu g \cdot L^{-1}$ Al (pH 5.2, 15 μM Ca) accumulated Al on their gills, showed a reduction in a gill mucus indicator (sialic acid), and showed ionoregulatory and respiratory effects of Al. After 10 d there was a recovery phase,

shown by less Al on the gills, normal gill sialic acid content, and decreased ionoregulatory and respiratory effects. Gill damage and repair followed this general time course (Mueller et al. 1991). After 4 wk acclimation, fish resistance to a 2 wk exposure to 100 or 300 $\mu g \cdot L^{-1}$ Al was enhanced (pH 4.8 or 5.2) as the recovery and acclimation process continued, with less mortality, less ionoregulatory disturbance, and only about half the Al accumulation on gills as Al-naïve fish (McDonald et al. 1991).

Changes in binding properties of Al to the gill surface during the acclimation process were studied by Reid et al. (1991). A 21 d exposure of juvenile rainbow trout to 30 $\mu g \cdot L^{-1}$ Al (pH 5.2, 15 μM Ca) reduced the physiological effects and mortality when the fish were later exposed to 120 $\mu g \cdot L^{-1}$ Al. During this time the binding affinity of the gill surface to Al decreased, and that of Ca increased, so that the ability of Al to displace Ca at the gills, causing the ionoregulatory effects of Al, would be reduced. In total, results of the studies mentioned above suggest that, for example, springmelt conditions experienced in the field might not be as devastating as predicted in the laboratory using naïve fish, because of acclimation to high Al and low Ca conditions. However, there is likely a metabolic cost associated with acclimation.

Wilson and Wood (1992) studied the metabolic costs of acclimation to Al over 22 d (<3 or 31 $\mu g \cdot L^{-1}$ Al, pH 5.2, 13 μM Ca) by measuring critical swimming speed in juvenile rainbow trout. Acclimation to Al (as shown by reduced mortality when exposed to 210 $\mu g \cdot L^{-1}$ Al, pH 5.2) developed by day 17, and was reflected in recovery of whole body Na^+ and Cl^- (lost in the first 7 d, at ~double the rate of fish exposed to pH 5.2, no Al) to normal by 22 d. Critical swimming speed (U_{crit}), however, did not recover in the

Al-exposed fish, and was always significantly lower than U_{crit} for pH 5.2, no Al-exposed fish, which in turn was always significantly less than for control fish (pH 6.5, no Al), although some recovery did occur in both groups after day 7. Gill Al content was elevated and approximately constant after 2 d in the Al-exposed fish, with a temporary decrease at 7 d, and growth was reduced in this group. Their study indicates that fish can acclimate to sublethal concentrations of Al (ion recovery, greater tolerance to Al), but at some cost to overall fitness (U_{crit}, decreased growth). Gill damage and repair, plus mucus production and thicker gill lamellae, could reduce ion losses in Al-exposed fish, but would also reduce gas transfer at the gills and would consume energy that could otherwise be expended on growth. Alternatively, reduced appetite in the Al-exposed fish could have resulted in decreased growth (Wilson and Wood 1992), and decreased food energy ultimately could also explain the persistently-reduced U_{crit} at 22 d, but probably not earlier.

In a continuation of their earlier work, Wilson et al. again showed the costs to fish of acclimation to Al (e.g. reduced feeding and growth; Wilson et al. 1994a), but were also able to show that acclimation to Al was specific to Al, and did not protect fish against acute Cu toxicity (Wilson et al. 1994b). In these studies, juvenile rainbow trout were exposed for 34 d to soft water at pH 5.2 containing 38 $\mu g \cdot L^{-1}$ Al (for Al acclimation), pH 5.2 water with background Al (3 $\mu g \cdot L^{-1}$), or to pH 6.5 water, 4 $\mu g \cdot L^{-1}$ Al (control; all ~15 μM Ca). Acclimation to Al developed in 5 d, as shown by fish mortalities during exposures to 162 $\mu g \cdot L^{-1}$ Al at pH 5.2, but there was no concurrent, protective effect against a 32 $\mu g \cdot L^{-1}$ Cu challenge (also at pH 5.2; Wilson et al. 1994a).

Aluminum acclimated fish had about 22% smaller gill surface area (shorter lamellae and thicker filaments) and increased mucous cell density (Wilson et al. 1994b), which perhaps helped protect against respiratory effects and some of the ionoregulatory effects of the 162 $\mu g \cdot L^{-1}$ Al challenge, but did not protect against toxicity (presumably ionoregulatory effects) caused by the 32 $\mu g \cdot L^{-1}$ Cu challenge. Reduced gill surface area resulted in decreased U_{crit}, decreased maximum rate of oxygen uptake, and decreased aerobic scope in the Al-acclimated fish (Wilson et al. 1994b), again indicating that acclimation to Al has metabolic and physiological costs to a fish.

Juvenile rainbow trout (~13 g) had ~30% cummulative mortality when exposed to 30 $\mu g \cdot L^{-1}$ Al over 32 d (pH 5.2, ~15 μM Ca), and the survivors showed increased resistance to a lethal Al challenge to 200 $\mu g \cdot L^{-1}$ (Wilson et al. 1996). Feeding rate fell initially in the fish exposed to 30 $\mu g \cdot L^{-1}$, recovered within a week, but final fish weights were still below control fish (pH 6.5, 0 Al) by 32 d despite an increase in food conversion efficiency in the Al-exposed fish. Increased food conversion may have been related to reduced routine activity in the Al-exposed fish. There initially was increased protein synthesis and degradation in gills and liver of the Al-exposed fish, presumably related to the damage and repair stage of Al exposure. The authors cautioned that the surviving Al-exposed fish did have a metabolic cost to acclimation (damage repair at the gills, compensated by increased food conversion efficiency due to reduced activity) that in the wild may have been of more consequence because of lower availability of food that needs to be caught and the necessity of actively avoiding predators (Wilson et al. 1996).

Rainbow trout chronically exposed to Al in a fish farm in Scotland had elevated Al in the brain, liver, gonads, and heart, but there were no overt signs of toxicity (Exley, 1996). Water flowing into the farm had high concentrations of Al (~8.4 μ*M*; ~225 $\mu g \cdot L^{-1}$ Al) at low pH (pH 6.0), so was limed for years to raise water pH to 6.7, with about 8.9 μ*M* Al and about 100 μ*M* Ca. Compared to control fish never exposed to >2.0 μ*M* Al, the farmed fish had about ten times more Al in all organis studied. Histochemical analysis showed a small proportion of degeneration of brain neurons in the chronically exposed fish, which had some resemblance to pathological conditions seen in humans suffering from Alzheimer's disease. Exley (1996) proposed that fish could act as potential model animals for studies of the effects of chronic Al exposures in vertebrates, but it should be noted again that there were no discernable effects of the ten times higher organ Al concentration in these fish.

5.5 Gill Morphology and Al Accumulation: Laboratory and Field Studies

Laboratory and *in situ* exposures of fish to Al have usually shown morphological changes which support gill effects as the primary toxic action of Al. Youson and Neville (1987) exposed juvenile rainbow trout for 11 d to 75 $\mu g \cdot L^{-1}$ Al at pH 5.5, 5.0, and 4.5 (50 to 125 μ*M* Ca), and found Al in and on gill epithelial cells (transmission electron microscopy, TEM). No obvious hyperplasia was seen at this relatively low Al concentration. Adult rainbow trout exposed for 3 d to 200 $\mu g \cdot L^{-1}$ Al at pH 5 (~28 μ*M* Ca) had fused lamellae, disrupted chloride cells, and separation of epithelial cells from basement tissue (TEM; Goossenaerts et al. 1988). Aluminum was localized mostly on

the surface of lamellae, with some Al in epithelial cells. Gills of 40-d old cutthroat trout (*Oncorhynchus clarki*) exposed to 50 $\mu g \cdot L^{-1}$ Al at pH 6.0 and 5.5 (7-d exposures as alevins, 35 μM Ca) showed mucous cell proliferation and noticeable mucus production (light microscopy (LM); Woodward et al. 1989). Gills of juvenile rainbow trout exposed to 950 $\mu g \cdot L^{-1}$ Al at pH 5.4 (10 μM Ca) accumulated significant amounts of Al in 30 min, and skin mucus accumulated significant amounts of Al in 5 min (Handy and Eddy 1989). Gills of fathead minnows reared at pH 6.0, 5.5, and 5.2 in the presence (30-60 $\mu g \cdot L^{-1}$ Al) or absence (15 $\mu g \cdot L^{-1}$ Al) of added Al had major histopathological changes at the gills compared to pH 7.5 with or without added Al (200 μM Ca; Leino et al. 1990). Aluminum ameliorated slightly the gill damage seen at pH 5.2 without added Al. Fathead minnows are highly sensitive to H^+ ions, which is associated with the high sensitivity of the minnow to low Ca concentrations (Leino et al. 1990).

Gills of one-year old brown trout exposed to 50-500 $\mu g \cdot L^{-1}$ Al, pH ~5 in soft water (<250 μM Ca) for a minimum of 3 wk in either farmed or experimental conditions were studied by Karlsson-Norrgren et al. (1989a, b; scanning EM and TEM). Gill damage included swelling of gill lamellae due to an increased number of chloride cells, resulting in increased diffusion distance from water to blood. More Al accumulated on the gills at pH 5.5, with or without added humus, than at pH 7. Aluminum was localized on and in epithelial cells (Karlsson-Norrgren et al. 1989b). Similar effects on gills of adult brook trout were seen after ~5 mo exposure to pH 4.4 plus 390 $\mu g \cdot L^{-1}$ Al (~40 μM Ca; LM; Tietge et al. 1988). Norrgren et al. (1991) showed gross deformities of minnow gills (*Phoxinus phoxinus* L.) after 12 to 48 d exposures to 160-170 $\mu g \cdot L^{-1}$ Al at pH 5 and 6

(90 μ*M* Ca). Aluminum on the gills was localized mainly in mucus between gill lamellae, and there were changes in the morphology of cilia in the olfactory mucosa (LM, SEM). After 48 d there was some additional Al in liver and kidney if fish were exposed to pH 6 plus Al, but mortality at pH 5 and 6 in the presence of Al was likely due to gill effects (Norrgren et al. 1991).

Under overwintering, laboratory conditions of ~4°C, largemouth bass (*Micropterus salmoides*) showed thickened respiratory epithelia, presumably a mechanism to decrease ion losses at the gills during torpor (LM, TEM; Leino and McCormick 1993). Thickening of the gill epithelium was exacerbated under acidic conditions, as were decreases in blood osmolality (pH 4.5 or 5.0, 38 or 335 μ*M* Ca), and both were affected more in the presence of ~30 $\mu g \cdot L^{-1}$ monomeric Al (50 $\mu g \cdot L^{-1}$ total Al). In addition, Al interfered with Ca resorption from fish scales during overwintering conditions, perhaps by stabilizing Ca crystals in the scales, further reducing the ability of bass to replace Ca lost at the gills. Lacroix et al. (1993) also showed gill hyperplasia, lamellar fusion, and increased blood-water distance in Atlantic salmon fry exposed to variable amounts of Al (270 to 800 $\mu g \cdot L^{-1}$) in the presence and absence of citrate (SEM, LM; ~90 μ*M* Ca, pH ~5.5, 15 d exposures). Fish rapidly cleared their gills of Al but the gills still showed some damage after 5 d of depuration, indicating that gill tissue repair was still occurring.

Hamilton and Haines (1995) showed that gill damage due to Al in Atlantic salmon alevins was worse at pH 5.5 than at pH 6.0 (measured Al ~3 μ*M* in each; ~70 μ*M* Ca). Approximately 6 μ*M* F^- reduced the gill damage by complexing Al to some

degree (see section 5.3.2). Paradoxically, higher concentrations of F^- (~13 μ*M*) increased gill damage, possibly by keeping more Al in solution which was then available to interact at the gills.

The expensive ^{26}Al isotope has been used to trace Al accumulation on fish gills (Playle 1987, redbelly dace *Chrosomus eos*; Bjørnstad et al. 1992, Oughton et al. 1992, *S. salar*). The usefulness of these laboratory studies suffered from lack of replication, due mainly to the extremely high cost of ^{26}Al. Except perhaps for very particular experiments, graphite furnace AAS is as good a method for Al localization and is certainly cheaper. However, localization of ^{26}Al in brain tissue of rats after ^{26}Al uptake across the gut was successfully measured using accelerator mass spectrometry (Walton et al. 1995); this method of ^{26}Al measurement may be of use in aquatic studies. Still, this study was also limited to just eight ^{26}Al-dosed rats because of the expense of the isotope and expense of its measurement.

In field manipulations fish often show morphological changes at the gills which can be associated with exposure to Al. Atlantic salmon and brown trout (0^+ year) both showed Al deposition and mucus production on gills when exposed for 24 h in a stream to ~350 $\mu g \cdot L^{-1}$ Al at pH 5 (McCahon et al. 1987). Adult brown trout exposed *in situ* to a Swiss alpine lake of pH 5.4, 120 $\mu g \cdot L^{-1}$ Al, and 12 μ*M* Ca had gill damage, electrolyte losses, mucus clogging of the gills, high hematrocrit values, and high mortalities (Dietrich and Schlatter 1989b), all presumably caused by Al in the lake. These authors commented on the discrepancy between the then-generally accepted view that waters of Al content <200 $\mu g \cdot L^{-1}$ should not be toxic to brown trout at pH ~5.4; however, the

soft water, moderately-high Al concentration, and moderately acidic pH would be ideal conditions for Al precipitation and polymerization in the more alkaline gill micro-environment, as discussed earlier (section 5.2.2).

Accumulation of Al on gills of caged Atlantic salmon and sea trout (*S. trutta*) smolts, placed in the mixing zone of acid water meeting water limed with $CaCO_3$, was thought to be due to polymerization of Al in the mixing zone (Rosseland et al. 1992). The mixing zone was more toxic than the acid water alone (containing about 250 $\mu g \cdot L^{-1}$ monomeric Al, pH ~4.8, ~32 μM Ca) or the limed water (~90 $\mu g \cdot L^{-1}$ monomeric Al, pH ~7.0, ~92 μM Ca). Lime additions to lakes reduce acid and Al toxicity to fish (Gunn et al. 1990, lake trout, *Salvelinus namaycush*; Lacroix 1992, 1996, Atlantic salmon, brook trout), but care must be taken in its application. For example, if water pH rises too high, the $Al(OH)_4^-$ anion could become toxic (Skogheim et al. 1987; Atlantic salmon smolts in enclosure experiments). Liming of acid streams can also be effective in reducing the effects of Al on fish, although toxicity might recur downstream of an acidic tributary (Weatherley et al. 1991), which indicates the complexity of combating acidic conditions by liming streams. Liming of watersheds may be of more use as an ameliorative measure than liming the water itself.

The difficulty in attributing toxicity observed in the field to either H^+ or Al is nicely illustrated by a study in which Atlantic salmon parr were held in floating pens for 54 d in four acidic streams during natural acidification in November and December (Lacroix and Townsend 1987). All fish died in the two streams where pH decreased below pH 4.7; none died in two streams where pH did not decrease below pH 4.9. Total Al (200-

350 $\mu g \cdot L^{-1}$) and inorganic Al (10-50 $\mu g \cdot L^{-1}$) were similar in all four brownwater streams (DOC 12-18 $mg \cdot L^{-1}$); acute toxicity was attributed to Na and Cl losses due primarily to H^+, not to accumulation of Al on the gills.

Another problem in field studies is the variability in Al concentration in lakes and streams. For example, Ormerod et al. (1989) showed that upland Welsh streams containing <100 $\mu g \cdot L^{-1}$ filterable Al often had episodes of high Al concentrations of ~100-500 $\mu g \cdot L^{-1}$ filterable Al, with exceptional episodes up to 800 $\mu g \cdot L^{-1}$. These workers suggested that 80 $\mu g \cdot L^{-1}$ filterable Al is toxic to salmonids. In a 60 d study starting with eyed Atlantic salmon, Buckler et al. (1995) showed that feeding and swimming activity were most sensitive to Al (33 $\mu g \cdot L^{-1}$ Al), significant mortality occurred at 71 $\mu g \cdot L^{-1}$ Al, and egg hatching was not affected by 264 $\mu g \cdot L^{-1}$ Al (pH 5.5, 75 μM Ca).

A study of imperfect bilateral symmetry in perch (*Perca fluviatilis*) from acidic to neutral Norwegian lakes (pH 4.6 to 7.0, 25 to 140 μM Ca) containing 0 to 350 $\mu g \cdot L^{-1}$ inorganic monomeric Al concluded that the method is, at present, not a useful tool for evaluating stresses on natural fish populations (Østbye et al. 1997). In a study of four softwater, Finnish lakes, most gill anomolies (e.g. chloride cell proliferation, lamellar fusion) were systemic and with no apparent cause, but roach (*Rutilus rutilus*) had more gill anomolies than did perch (Haaparanta et al. 1997). There were also seasonal changes in anomoly frequencies, with more anomolies in the fall in perch, and more in fall and spring in roach. Clearly, season may be important in any assessment of gill damage caused by pollutants, in this case likely pulp mill effluents.

Free-ranging fish in the field, unlike laboratory or caged fish, can sometimes avoid acid and Al pulses. During an autumn rainstorm, caged brook trout adults suffered ~80% mortality over 20 d (lowest pH ~5.0, highest total dissolved Al ~50 $\mu g \cdot L^{-1}$, ~80 μM Ca), whereas free-ranging brook trout fitted with radio transmitters showed only 33% mortality (number of fish implanted not stated; Carline et al. 1992). Surviving free-ranging fish moved downstream or into refuges of groundwater entering the stream. In a similar manner, two strains of brook trout fry within long enclosures survived spring snowmelt by moving into deeper, less acidic, lower Al, and higher Ca water (pH 6.3, ~480 $\mu g \cdot L^{-1}$ Al, ~160 μM Ca at >2 m depth; pH 4.8, ~1,200 $\mu g \cdot L^{-1}$ Al, ~60 μM Ca at 0.7 m; Van Offelen et al. 1994). In the laboratory, Atlantic salmon (5 to 7 cm) were able to detect and avoid low pH water (pH 4.0 and 4.5, but not at pH 5.0) but did not avoid 70 and 150 $\mu g \cdot L^{-1}$ Al at pH 5.0, although it appeared there was some detection of the 150 $\mu g \cdot L^{-1}$ Al (Åtland and Barlaup 1996; ~50 μM Ca). In essence, fish may not be able to sense environmentally relevant Al concentrations and seek refuge unless high Al concentrations are accompanied by very low water pH.

Episodic acidification of coastal watersheds by sea salt blown onto shore during heavy storms has been reported to kill fish (Barlaup and Åtland 1996). Sea salts release H^+ and Al ions from acidic soils through ion exchange of Na. After winter storms in 1993, for 2 to 4 days runoff water in the coastal watersheds was acidic (pH 4.5 to 5.1) with high labile Al concentrations (200-350 $\mu g \cdot L^{-1}$ Al; 20-40 μM Ca). Dead brown trout adults were found, many with obvious mucus on their gills, and some survivors had low plasma Cl concentrations. Some fish were observed to avoid the

acidic episode by seeking refuge in less acidic parts of the rivers involved (Barlaup and Åtland 1996).

Morphological changes of gills from fish sampled from waters of low pH and high Al concentrations are generally harder to attribute to Al alone, compared to laboratory or field manipulations. Chevalier et al. (1985) collected wild adult brook trout from three acidified lakes (pH 5.2 to 5.5, 200-300 $\mu g \cdot L^{-1}$ Al, ~35 μM Ca). The trout showed gill damage and hyperplasia, and accumulation of Al on the gills, compared to fish from three circumneutral lakes (pH 6.8-7.0, <50 $\mu g \cdot L^{-1}$ Al, 55-88 μM Ca). Gill damage, especially to chloride cells, was attributed to sublethal H^+ and Al stress in combination with low environmental Ca concentrations. Adult perch from four Finnish lakes had more Al deposits (as indicated by hematoxylin staining) in their gills as water Al content increased (to 250 $\mu g \cdot L^{-1}$ Al) and water pH decreased (down to pH 4.3; $\leq$80 μM Ca; Vuorinen et al. 1992). Gills of perch in all lakes otherwise appeared healthy. In what was likely a conservative estimate of Adirondack lakes losing one or more fish populations due to acidic precipitation (16-19%), it was found that fish loss was correlated with lower water pH, higher concentrations of ionorganic Al, and higher elevation (Baker et al. 1993).

Laboratory toxicity studies and field studies of H^+ and Al usually agree, but do not necessarily duplicate one another. For example, during the Little Rock Lake acidification project, in which half the lake was acidified to pH ~4.8 and the other half was left at pH ~6.0, toxicity studies were also run in the laboratory (pH 4.5, 5.0; ~38 μM Ca; 10-90 $\mu g \cdot L^{-1}$ Al; Eaton et al. 1992). Test fish were rock bass (*Ambloplites*

rupestris), largemouth bass, black crappie (*Pomoxis nigromaculatus*; most sensitive), and yellow perch (*Perca flavescens*; least sensitive). Although field data and *in situ* and laboratory toxicity tests generally agreed, especially for adult fish, the authors suggested that laboratory tests should be validated in the field whenever possible. Further, they believe that whole-ecosystem experiments provide unique information which more than compensates for their cost and lack of replication. A later review paper of the Little Rock Lake project indicated that rock bass was most sensitive to lower pH, followed by largemouth bass, black crappie, and perch (least sensitive; Brezonik et al. 1993). In general, juvenile stages of fish were most susceptible to lake acidification: only rock bass (most sensitive fish) were affected as adults. During the study dissolved Al increased from about 5 $\mu g \cdot L^{-1}$ (pre-acidification) to about 40 $\mu g \cdot L^{-1}$ at pH 4.7 (Brezonik et al. 1993).

DeWalle et al. (1995) used a combination of stream flow data, total dissolved Al concentrations, and field and laboratory toxicity data to determine the duration of elevated Al concentrations which would be toxic to brook trout. For example, their data indicated that total dissolved Al concentrations greater than 200 $\mu g \cdot L^{-1}$ would be lethal to brook trout if the exposures lasted 24 to 48 h, and 100 to 200 $\mu g \cdot L^{-1}$ Al would be lethal after 48 to 400 h. Stream acidity was not considered in their analysis; it must be assumed that acidic conditions played some part in the observed toxicity to Al. In the same streams, radio-tagged brook trout showed considerable, probably passive movement downstream during severe acidic episodes (pH <5.0, >200 $\mu g \cdot L^{-1}$ Al), and one third of the fish died during the severe episodes (Gagen et al. 1994). These

authors suggested that the ability of fish to exploit refuge areas of higher pH and lower Al concentrations during acidic episodes is limited.

FIGURE LEGENDS

Fig. 5.1. Model of Al interactions at fish gills, modified from Wood and McDonald (1987). The branchial surface is represented, with soft water on the left and blood on the right. RBC = red blood cell. Width of arrows depicts relative transfers across the gill.

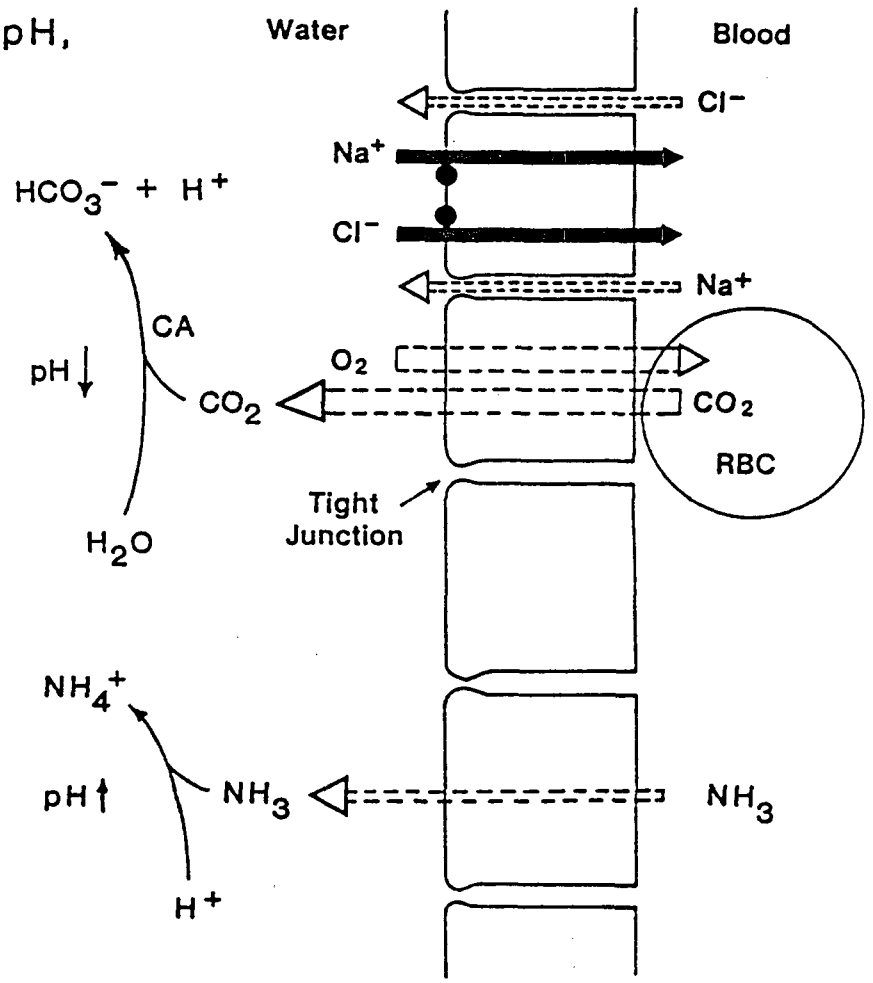
A. Circumneutral conditions in the absence of Al. Active ion uptake balances ion effluxes. O_2, CO_2, and ammonia diffuse through the gill, with a net acidification of the gill micro-environment due to CO_2 dissociation. Excretion of NH_4^+ is ignored in the model, because it does not affect water pH. CA = carbonic anhydrase.

B. Acidic conditions in the absence of Al. Active ion uptake is reduced, and ion effluxes increase, possibly due to Ca displacement at tight junctions by H^+. There is no interference with gas transfer, but water near the gill now becomes more basic because CO_2 no longer dissociates to HCO_3^- and H^+.

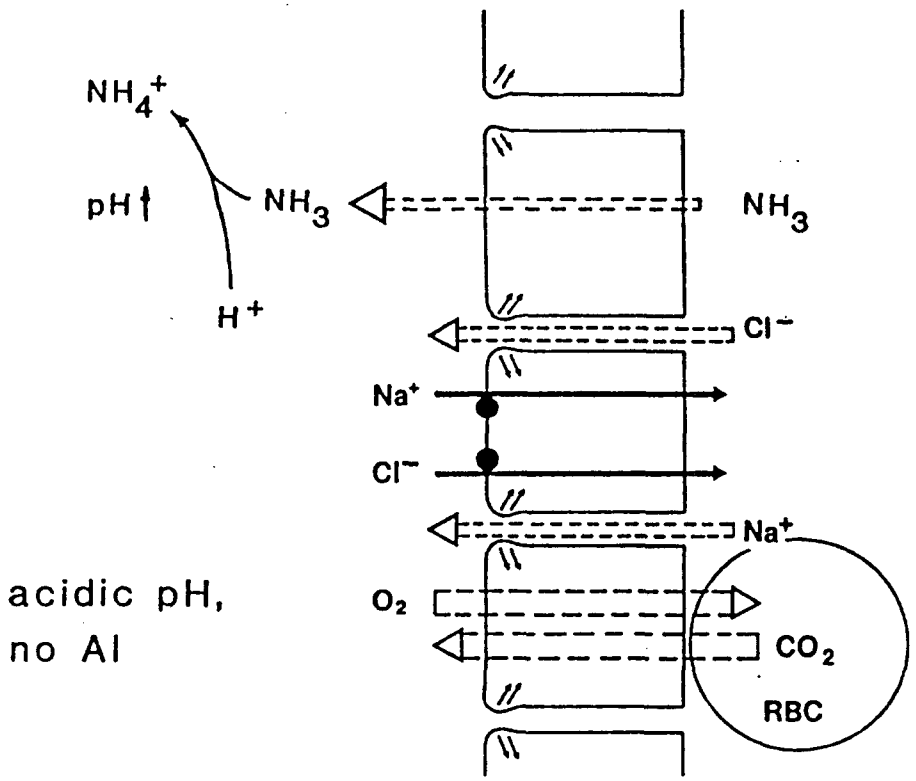
C. Moderate acidity (pH 5-6) in the presence of Al. More basic conditions in the gill micro-environment cause changes in Al species and decreased Al solubility leading to precipitation and polymerization, resulting in Al deposition on the gill surface. Mucus production, cell swelling, reduced ion uptake, and increased ion effluxes result. Gas transfers are reduced at the gills because of increased diffusion distance, resulting in decreased arterial O_2 tension and increased arterial CO_2 tension.

D. Very acidic conditions (pH <5) in the presence of Al. Although the gill micro-environment is made more basic, Al deposition does not occur because Al solubility is not exceeded, so there are no changes in gas transfer at the gills. The small amount of Al binding to branchial surfaces does not add to the ionoregulatory disturbances caused by acidity alone (B), and may even reduce the toxic effects of H^+ ions through competition for negatively charged binding sites on the gills.

circumneutral pH,
no Al



B



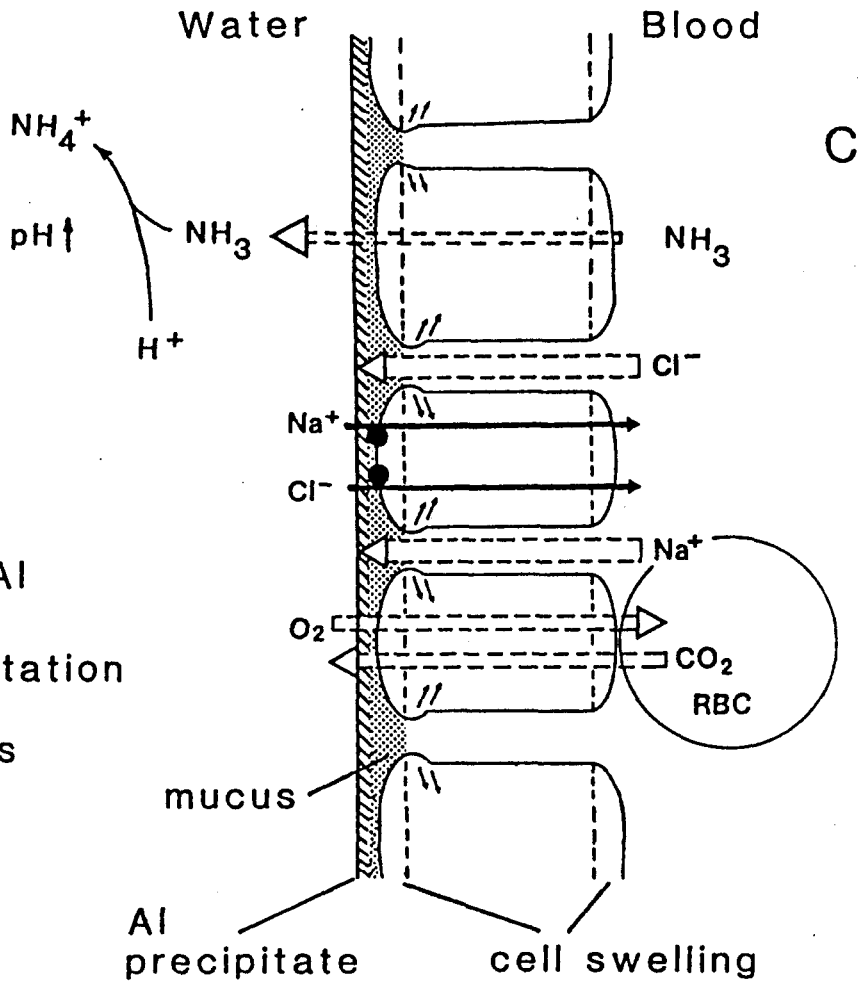
acidic pH,
no Al

C

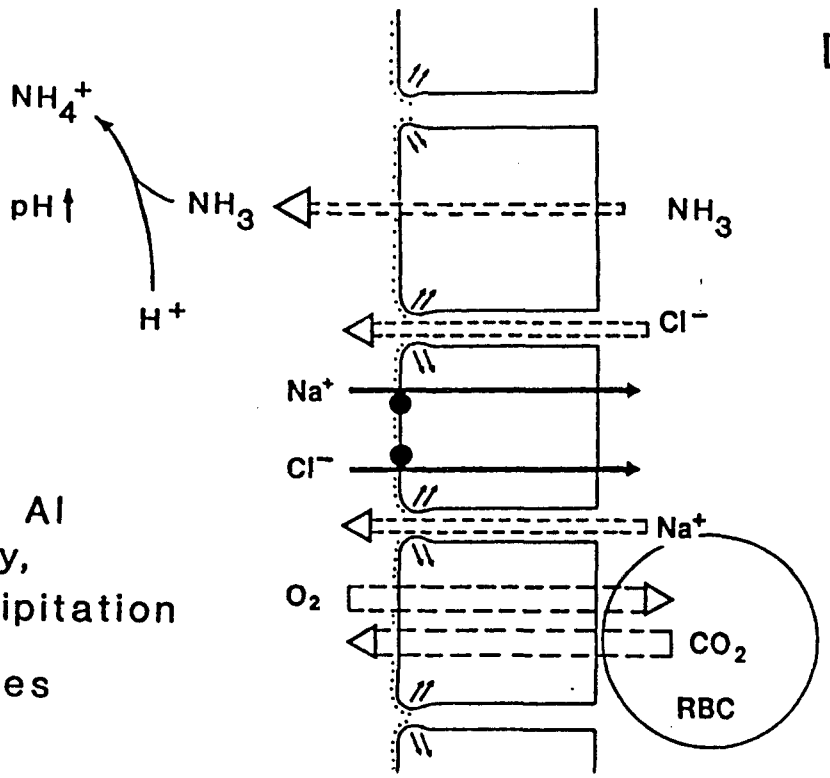
moderate acidity,
Al

pH↑ = reduced Al solubility → precipitation

= Al species changes



D



acidic pH,
Al

pH↑ = reduced Al solubility, no precipitation

= Al species changes

6 SUMMARY AND RECOMMENDATIONS

Appended below is a brief summary from each chapter of the review (sections 6.1 - 6.5). We also include a section discussing and modeling the polymerization and, hence, toxicity of Al as it is influenced by pH, temperature, other inorganic and organic compounds, etc. Finally, we close with a brief set of recommendations concerning water quality criteria benchmarks, and thoughts for future research to better understand Al toxicity under more basic pH conditions.

6.1 Aluminum Chemistry and Speciation (Chapter 1)

Aluminum chemistry is complex, with Al existing as inorganic, monomeric species (Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3^\circ$, and $Al(OH)_4^-$, from acidic to basic conditions), as amorphous $Al(OH)_3$ leading to gibbsite formation and precipitation, and as polynuclear species such as the tridecameric Al_{13} polynuclear species ($Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$). Aluminum can be bound to F and DOC, which reduces the amount of inorganic Al available to interact at biological membranes, and also slows the precipitation and polymerization of Al, which may reduce the biological effects of Al (e.g. at fish gills). Dissolved organic carbon is probably a more important complexing agent than is F or Si.

There is usually more inorganic and organic Al at lower water pH, and usually more organic Al as DOC concentration increases. Dissolved organic carbon increases the solubility of Al but decreases Al toxicity. Lakes are net sinks for Al, and it is

important to remember that dilution processes may be just as reasonable an explanation of Al loss from the water column as is precipitation of Al.

Graphite furnace atomic absorption spectrometry with background correction is recommended for Al measurements in water, although colorimetric methods such as pyrocatechol violet are also reliable. Separation of total monomeric inorganic Al (e.g. through ion-exchange resins) allows Al speciation to be calculated using geochemical computer programs. Inorganic monomeric Al measurements are probably the most meaningful with respect to predicting the biological effects of Al.

6.2 Freshwater Algae (Chapter 2)

The effects of Al on freshwater algae have not been studied extensively, and most work has been done at acidic pH. Desmids and diatoms are sensitive to Al, whereas chrysophytes and some Chlorophyceae are relatively tolerant of Al, but there are species sensitivity differences within these divisions. Generally, growth rate is reduced more by Al than is photosynthesis, so is a better indicator of algal susceptibility to Al. Although the effects of low pH and the effects of Al are often difficult to separate, some mesocosm work has demonstrated that Al alone can alter algal community structure. Paleolimnological work based on diatom frustules and chrysophyte scales in sediments can therefore be used to infer past Al chemistry of lakes as well as their past pH.

Aluminum toxicity to algae is influenced by water pH and by concentrations of Ca, DOC, and phosphorus. Generally, Al is more toxic to algae in slightly acidic

conditions (pH ~6.0), but interpretation of most experimental results is hampered by the lack of analytical measurements of inorganic monomeric Al followed by speciation calculations. Calcium (and H^+) likely protect against Al toxicity through cation competition for surface binding sites on algae. Complexation of Al^{3+} by DOC and by other dissolved anions (e.g. F^-, SO_4^{2-}) decreases Al toxicity by reducing available Al. Overall, there is reasonable evidence that Al^{3+} or inorganic monomeric Al are the best predictors of Al toxicity to algae, although polymeric Al species present near pH 6 may also contribute to Al toxicity.

The effects of Al on algal phosphorus dynamics are far from clear. Aluminum likely reduces P uptake rate in algae, perhaps by hydrolysis of the acid phosphatase enzyme. There is only limited support for indirect effects of Al on P limitation to algae in acidic lakes, through binding of P by Al then precipitation of the complex (e.g. the "oligotrophication hypothesis"). In contrast, alum treatment to control nuisance algal blooms in more circumneutral lakes can produce long term reductions in P supply and algal growth, especially in well stratified, alkaline lakes. However, the formation and potential toxicity of polymeric Al cations near pH 6.0 needs further study. Silica uptake and utilization by diatoms may be affected by Al, but the ecological significance of this effect is not well understood.

6.3 Aquatic Higher Plants (Chapter 3)

Studies of the effects of Al on freshwater macrophytes are particularly scarce, especially when compared to the large amount of information available on the effects of

Al on terrestrial plants. The likely uptake site of Al by submerged plants is through the roots, although leaf uptake is also possible in some circumstances. Overall, there is little evidence that Al itself has any influence on macrophyte community structure, even in acidified lakes with higher concentrations of Al, and Al-tolerant plants are also likely H^+-tolerant plants, with pH as the master variable.

6.4 Aluminum and Aquatic Invertebrates (Chapter 4)

In general, aquatic invertebrates are not very sensitive to Al compared to fish. The toxic effects of Al are usually considered to be ionoregulatory, in the same manner as are H^+ ions. Respiratory effects of Al for aquatic invertebrates are uncommon, likely because ammonia is not released by tracheal gills of aquatic insects, so precipitation and polymerization of Al does not usually occur around their gills. Possible exceptions are dragonfly nymphs, which have gills in respiratory chambers which may be a more basic micro-environment as ammonia is released into the chambers. Field studies have generally demonstrated the hardiness of aquatic invertebrates to Al, and aquatic insect populations may be affected indirectly if their predators (e.g. fish) are influenced by Al.

6.5 Aluminum and Fish (Chapter 5)

Aluminum is a gill toxicant to adult fish, causing both ionoregulatory and respiratory effects, depending on water pH and on Al concentration. Ionoregulatory effects of Al predominate at low pH, and are commonly viewed as the same mechanism

as H^+ toxicity alone, displacement of Ca from tight junctions of the gill membrane and interference with gill Na,K-ATPase activity. Calcium reduces Al toxicity by competing with Al^{3+} binding to negatively charged fish gills, and by reducing passive efflux of Na and Cl from the fish to the water by keeping tight junctions intact.

Respiratory effects of Al predominate in moderately acidic water (pH ~5.0-6.0). A plausible mechanism of the respiratory effects of Al is the precipitation or polymerization of Al as acidic, Al-rich water passes into the gill micro-environment, which is made more basic by virtue of ammonia released at fish gills. Precipitation or polymerization of Al is fast enough (<4 s) to be the cause of the respiratory effects of Al in fish. Basic water passing over fish gills is made more acidic, as CO_2 is released at the gills, but the influence of pH decreases at the gills on Al toxicity in basic conditions (e.g. $Al(OH)_4^-$ transformation to $Al(OH)_3^\circ$ at the gills) is not known.

Mixing zones where acidic, Al-rich water is neutralized are most toxic to fish, whereas the toxicity to fish is much reduced even six minutes after mixing has occurred, although the concentration and speciation of Al are essentially the same for both conditions. The explanation here, although tentative, is that immediately after neutralization an Al solution may be undergoing rapid precipitation and polymerization phenomena, both in the bulk water and near the gills, whereas in Al solutions aged for just a few minutes these reactions have already occurred in the bulk water so that larger, less toxic Al polymers and precipitates reach the gills and do not easily pass into and through the mucus layer of the gills.

Dissolved organic carbon reduces the toxicity of Al to fish, by complexing Al and reducing the amount of Al in solution that can bind to fish gills. Dissolved organic carbon apparently has an additional protective effect against Al toxicity to fish, which could be related to a DOC-caused reduction of Al precipitation and polymerization in the gill micro-environment. Dissolved organic carbon is likely more important in protecting against Al toxicity than is F or Si. Finally, fish can acclimate to Al, but at a metabolic cost.

6.6 Equilibrium modeling of gibbsite formation in water

Aquatic chemistry computer programs such as MINTEQA2 and MINEQL$^+$ calculate water chemistry using equilibrium binding constants and thermodynamic data. A MINEQL$^+$ simulation using five aqueous species of Al (Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3^\circ$, and $Al(OH)_4^-$), plus gibbsite to represent Al precipitation, yields the typical Al speciation and solubility diagram as pH is varied (Figure 6.1). The Al^{3+} species dominates at pH <5, gibbsite precipitation is maximal between pH 6 and 8, and the $Al(OH)_4^-$ anion dominates at pH >9. Note that these are equilibrium calculations, so kinetic aspects of gibbsite formation, for example, are not included in the simulation.

The concentration of Al influences the formation of gibbsite. At any pH, if the concentration of Al is less than the theoretical solubility limit of gibbsite then no gibbsite forms, and if the concentration exceeds the solubility limit then gibbsite will eventually precipitate from solution. Recall the amphoteric nature of Al, which is soluble in acidic and basic conditions but has a minimal solubility between about pH 6 and 8 (Fig. 6.1,

for 4 μ*M* total Al). A MINEQL$^+$ simulation run at pH 8.0 at 15°C while varying Al concentration from 0.1 to 10 μ*M* (3 to 270 μg·L^{-1} Al) simulated no gibbsite formation at about 0.1 μ*M* total Al, about 50% gibbsite formation at about 0.4 μ*M* total Al, and about 90% gibbsite formation at >4 μ*M* total Al (Figure 6.2).

There are many other factors besides pH and Al concentration which influence the production of gibbsite. One factor is temperature. The simulations depicted in Figures 6.1 and 6.2 were run at 15°C. If one pH condition is chosen, the effects of water temperature on gibbsite formation can be predicted. The simulation depicted in Figure 6.3 was run at pH 8.0, where gibbsite comprises ~90% of the 4 μ*M* total Al. Generally speaking, intermediate temperatures (10 to 20°C) favor gibbsite formation, and there is some decrease in its formation (at equilibrium) near 30°C, as more $Al(OH)_4^-$ stays in solution. Temperatures <10°C reduce the production of gibbsite, with the aqueous $Al(OH)_3°$ species accounting for an increasing percentage of total Al. Note, however, that kinetic aspects of gibbsite are not modeled by chemical equilibrium programs: higher temperatures have more favorable reaction kinetics for gibbsite formation, so gibbsite will form faster at higher temperature, even though the absolute amount formed at equilibrium may be lower (e.g. at 30°C).

Anions can disrupt Al precipitation. This phenomenon can also be demonstrated using computer simulations and a model anionic ligand such as citrate. The equilibrium binding constant for citrate and Al (log *K* =8.2) is a bit higher than for Al binding to fulvic acids (log *K* ~7.6-7.8), but is useful for demonstration purposes. As the concentration of citrate is increased from 1 m*M* (~0 in Figure 6.4) to 1 *M* there is a

decrease in the amount of gibbsite formed (from ~90% to 80% of 4 μ*M* total Al at pH 8.0, 15°C). However, the effect of citrate on gibbsite formation in an equilibrium simulation of this type is small, because only a portion of the effect of an anionic ligand is mimicked. For example, it is thought that the mechanism by which organic ligands decrease Al precipitation is through the disruption of aluminum-hydroxyl bridges and therefore the disruption of crystalline Al-hydroxides (Jardine and Zelazny 1996), but this mechanism is not simulated in chemical equilibrium programs such as MINEQL$^+$. Thus, organic ligands such as humic and fulvic acids would prevent gibbsite formation better than the citrate simulation in Figure 6.4 suggests, in essence having the effect of keeping more Al in solution. Of course, the toxicity of the Al held in solution by organic ligands would be lower than if the Al was in an inorganic, monomeric form.

Theoretically, higher concentrations of cations such as Ca and Mg, and higher concentrations of anions such as Cl, NO_3, phosphate, and sulphate, should all decrease Al precipitation. However, MINEQL$^+$ simulations in which Ca, Na, Cl, or NO_3 were varied from 1 m*M* to 1 *M* produced no changes in gibbsite formation (4 μ*M* total Al, pH 8.0, 15°C). Again, the lack of effect of varying these ions within an equilibrium program is because kinetic effects are not considered in these programs. In addition, using either a system open to the atmosphere (log K_{CO2}=21.7) or closed to the atmosphere (log K_{CO2}=18.2) did not alter gibbsite formation, because its formation is independent of the formation of bicarbonate (HCO_3^-) or carbonate (CO_3^{2-}) from dissolved carbon dioxide.

6.7 Recommendations

The acute U.S. National Ambient Water Quality Criteria for the Protection of Aquatic Life (NAWQC) for Al is 750 $\mu g \cdot L^{-1}$ Al (28 μM), which is intended to result in less than 50% mortality in 5% of the exposed population in a short exposure (Suter 1996). The chronic value, intended to protect against significant toxicity in most chronic exposures, is 87 $\mu g \cdot L^{-1}$ Al (3.2 μM). These values match those derived for the EPA Ambient Water Quality Criteria for Al (EPA 1988). However, these values are probably too high to protect aquatic organisms from Al toxicity under some circumstances. In addition, the paucity of toxicity studies under circumneutral pH conditions makes the application of these values to many surface waters potentially subject to significant errors.

Chronic values of about 50 $\mu g \cdot L^{-1}$ total Al (1.8 to 1.9 μM) may be more appropriate, based on the amount of Al that has been shown to be toxic to fish and to decrease P uptake in algae. Particular attention must be made to mixing zones, where acidic or basic water containing high Al concentrations meet more neutral water and oversaturated Al conditions result. These oversaturated solutions are more toxic just after mixing, and even six minutes after mixing the Al solutions are less toxic to fish. The antecedent condition of an Al solution before mixing may also need to be considered, because final Al solutions made from Al-rich acidic solutions may be more toxic than final Al solutions made from Al-rich basic solutions. The pH of the gill micro-environment of fish also needs to be considered, particularly in poorly buffered water where the pH changes at the gills will be maximal. In essence, acidic water is made

more basic in the gill micro-environment, and basic water is made more acidic, both of which could lead to oversaturated Al conditions leading to precipitation and polymerization of Al on fish gills. Ultimately, of course, chronic tests using live animals and plants, either caged in the wild or with test water brought into the laboratory, need to be run to assess the toxicity of Al at a particular site. The organisms most likely to be affected and, hence, most appropriate to monitor would be freshwater algae and fish, with higher plants and aquatic invertebrates being less critical for monitoring of acute Al toxicity. Monitoring for acute toxicity would be particularly important in or near Al mixing zones to ensure that Al polymerization is complete enough to minimize toxicity to organisms.

FIGURE LEGENDS

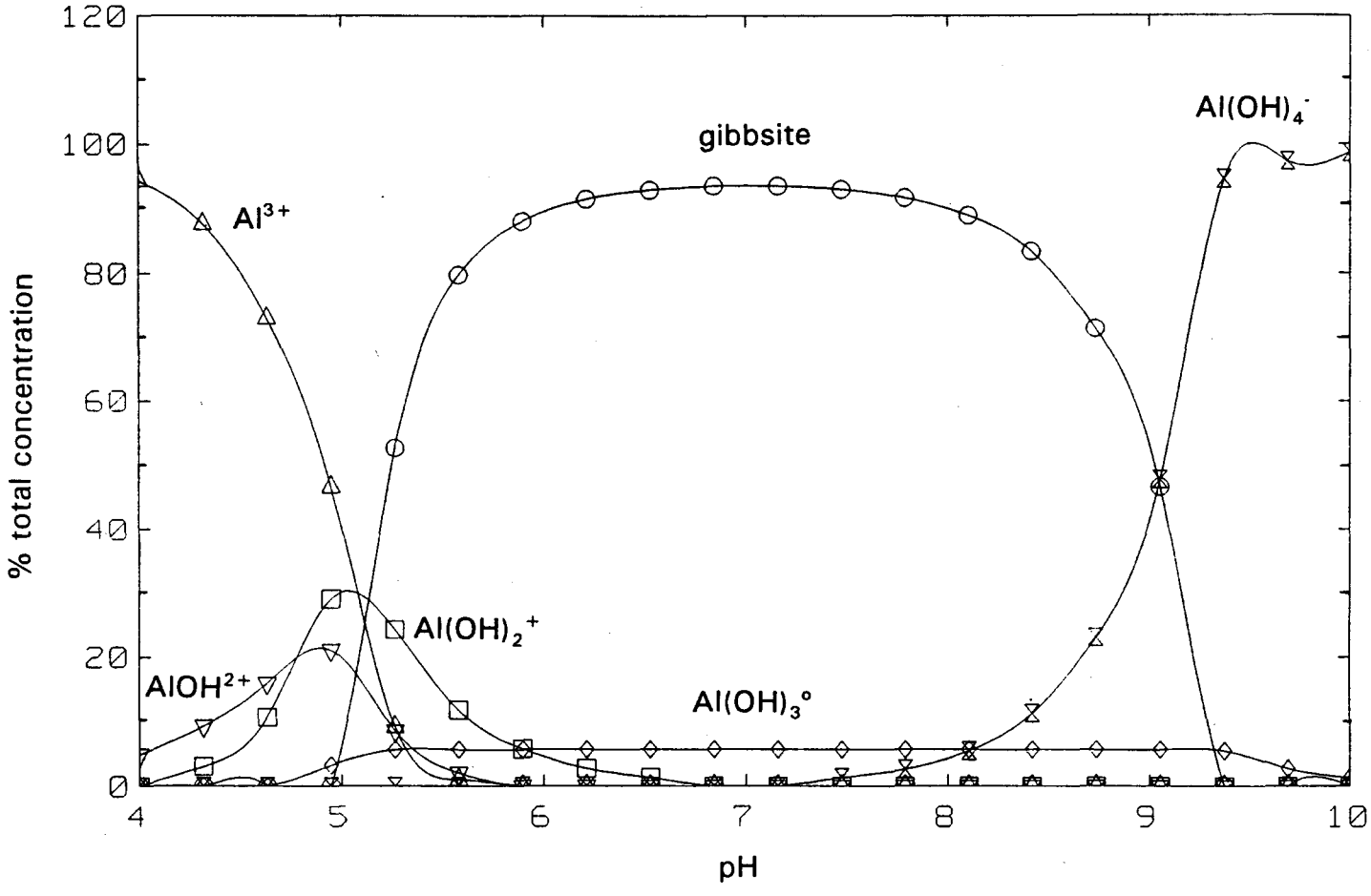
Figure 6.1 The results of a MINEQL$^+$ simulation using five aquatic species of Al plus gibbsite, and varying water pH from pH 4 to 10. The simulation was run using with 4 μ*M* total Al (=100%) and 15°C. In the simulation we used 1 m*M* concentrations for Ca, Cl, Na, and NO_3, with the system open to the atmosphere. These conditions were also used in the other simulations.

Figure 6.2 The results of a MINEQL$^+$ simulation illustrate the influence of total Al concentration (0.1 to 10 μ*M*) on gibbsite formation (at pH 8.0 and 15°C). As Al concentration increases, the solubility of Al is exceeded and gibbsite forms, at the expense of aqueous $Al(OH)_3^\circ$ and $Al(OH)_4^-$. At pH 8.0 the species Al^{3+}, $AlOH^{2+}$, and $Al(OH)_2^+$ are <1% of total Al.

Figure 6.3 The results of a MINEQL$^+$ simulation using five aquatic Al species plus gibbsite formation, at pH 8.0, and varying water temperature from 0 to 30°C. Gibbsite production at equilibrium is high between 10 and 25°C. The effect of lower temperature is particularly dramatic in reducing the formation of gibbsite. The Al species Al^{3+}, $AlOH^{2+}$, and $Al(OH)_2^+$ are <1% of total Al (4 μ*M*) for this simulation at pH 8.0.

Figure 6.4 Increasing concentrations of citrate (from 1 m*M* to 1 *M*) decreased the formation of gibbsite, as modeled using MINEQL$^+$ (pH 8.0, 15°C). The $Al(OH)_3^\circ$ and $Al(OH)_4^-$ species were about 5% of total Al at all citrate concentrations; the other Al species were <1% of 4 μ*M* total Al.

6.1


120
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% total concentration
4
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pH
Al3+
gibbsite
Al(OH)4-
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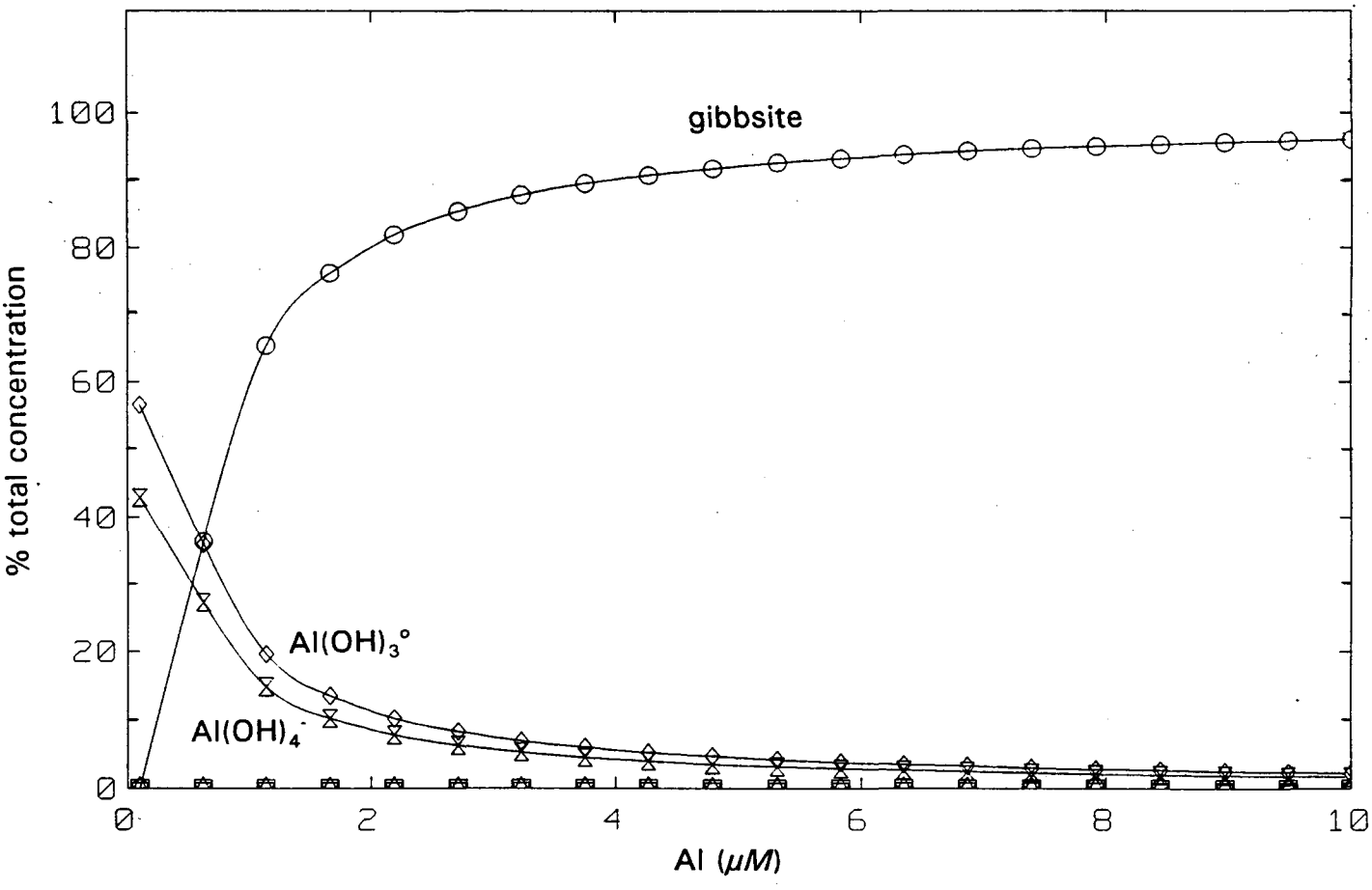
Al(OH)2+
Al(OH)3°


gibbsite
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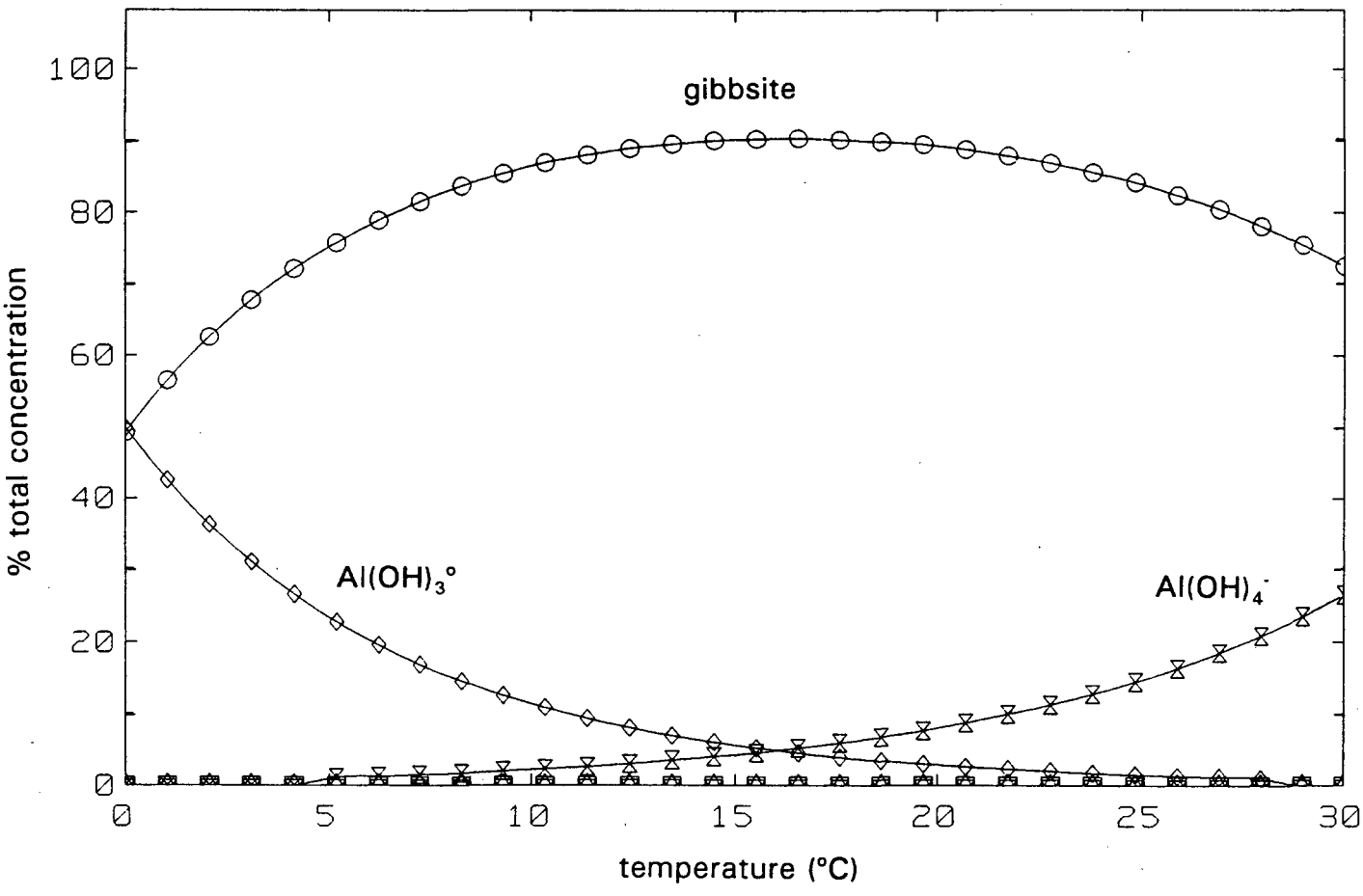
gibbsite
Al(OH)3°
Al(OH)4-
% total concentration
temperature (°C)
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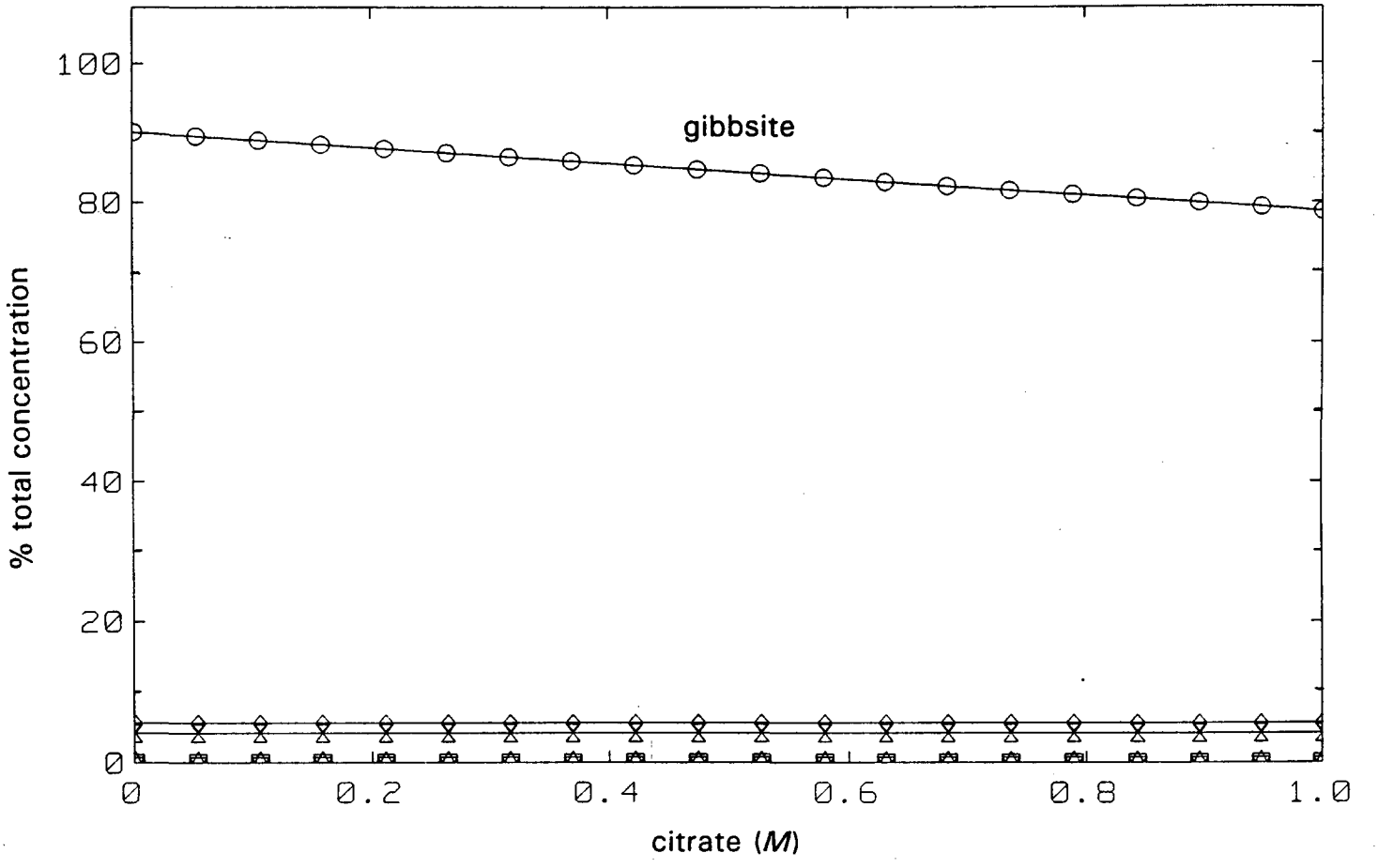
gibbsite
% total concentration
100
80
60
40
20
0
0
0.2
0.4
0.6
0.8
1.0
citrate (M)

Annotated Bibliography

Literature Review and Analysis of Chronic and Acute Toxicity of Aluminum in Aquatic Environments

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Annotated Bibliography

Search Methods and Sources

The following is an updated version of the annotated bibliography already submitted to the District as milestone #3. It has been extensively updated to include papers referenced in the Literature Review and Analysis, but we have left in any extra references that were not specifically cited in the text. The organization has also been updated to better correspond to the review paper itself.

This bibliography primarily represents reprints from our own private reference collections, which were established for our own research and publishing activity from the early 1980's to the present date. They were established from personal library searches, library database searches (see details below), and Science Citation Index searches. Any further information regarding the availability or accuracy of these references can be directed to myself or Dr. Playle. This collection has now been amended with database searches using Biological Abstracts, WAT-TOX (an aquatic toxicology reference database maintained by Dr. L.S. McCarty, Oakville, Ontario) Aquatic Plant Information Retrieval Service (APIRS), and other commercially available library databases including UNCOVER, ACQUIRE, etc.

Annotations

Annotations are provided for most, but not all of the references included below, and immediately follow each appropriate reference or group of references (the appropriate group will be so noted). References that we chose to annotate were particularly noteworthy or familiar to us at the present time. We kept them fairly brief, and should largely serve to help identify key references of particular interest to the reader.

TABLE OF CONTENTS

1 Introduction and General Reviews (Chapter 1):

Bertsch, P.M., 1990. The hydrolytic products of aluminum and their biological significance. Environ. Geochem. Health 12:7-14.
Excellent treatment and review of Al hydroxides, their formation, and environmental significance. Good examination of polymeric Al hydroxides, and their potential toxicity.

Burrows, W.D., 1977. Aquatic aluminum: Chemistry, toxicology, and environmental prevalence. CRC Crit. Rev. Environ. Control 7:167-216.
Most complete and recent comprehensive review of Al chemistry and toxicity to aquatic organisms, even though it is 20 years old. Much useful background, but needs updating.

Campbell, P.G.C., and P.M. Stokes. 1985. Acidification and toxicity of metals to aquatic biota. Can. J. Fish. Aquat. Sci. 42:2034-2049.
Excellent review of metal toxicity in acidic waters.

Charles, D.F. (ed.). 1991. Acidic deposition and aquatic ecosystems: Regional case studies. Springer-Verlag, New York, N.Y.. 747 p.
Excellent book that includes several review chapters for water chemistry, biological effects, etc, as well as regional case studies for all major projects in the National Surface Water Survey (NSWS).

Connery, J., 1990. Summary report workshop on aluminium and health, Oslo, May 2-5, 1988. Environ. Geochem. Health 12:179.

Cooke, G.D., E.B. Welch, S.A. Peterson, and P.R. Newroth. 1993. Restoration and management of lakes and reservoirs (2nd. edition). Lewis Publishers, Boca Raton, FL. 560 p.
Thorough examination of recent trends in lake and reservoir management with regards to control of cultural eutrophication. Includes chapter on P inactivation methods, including alum treatment.

Dillon, P.J., N.D. Yan, and H.H. Harvey. 1984. Acidic deposition: Effects on aquatic ecosystems. CRC Crit. Rev. Environ. Control 13:167-194.
Excellent review of effects of acid rain on chemistry, physics, and biota - even if a bit dated by now.

Drablos, D., and A. Tollan (eds.). 1980. Ecological impact of acid precipitation. Proceedings of an international conference, Sandfjord, Norway, March 11-14, 1980. SNSF Project, Oslo, Norway. 383 p.

Driscoll, C.T., and W.D. Schecher, 1988. Aluminum in the environment. In: Sigel, H., and A. Sigel, Metal ions in biological systems, Vol. 24: Aluminum and its role in biology. Marcel Dekker, Inc., New York. pp. 59-122.
Driscoll is probably the world's foremost authority on the aquatic chemistry and geochemistry of Al. Excellent review.

Eichenberger, E. 1986. The interrelation between essentiality and toxicity of metals in the aquatic ecosystem. In: H. Sigel (ed.), Metal ions in biological systems.

Volume 20: Concepts on metal ion toxicity. Marcel Dekker, Inc., New York, NY. pp. 67-100.

Gostomski, F., 1990. The toxicity of aluminum to aquatic species in the US. Environ. Geochem. Health 12:51-54.
A compilation of acute and chronic Al toxicity test results for aquatic organisms.

Havas, M. 1986. Effects of aluminum on aquatic biota. In: M. Havas, and J.F. Jaworski (eds.), Aluminum in the Canadian Environment. National Research Council of Canada, publication No. 24759, Ottawa, Ontario. pp. 79-127.
Very thourough review of information regarding Al toxicity and content in all groups of aquatic biota. Contains many useful tables and citations, including unpulished information.

Havas, M., and J.F. Jaworski, 1986. Aluminum in the Canadian environment. National Research Council of Canada publication No. 24759. National Research Council of Canada, Ottawa, Ontario. 331 p.
Excellent "grey literature" review of aluminum chemistry and toxicology that was prepared to assist in developing water quality standards for Canadian waters.

Hutchinson, G.E., 1943. The biogeochemistry of Al and of certain related elements. Quart. Rev. Biol. 18:331-363.
Although extremely dated, Hutchinson as always provides an interesting and scholarly review of Al biogeochemistry that still has much to say.

Last, F.T., and R. Watling (eds.). 1991. Acidic deposition: Its nature and impacts. Proceedings of the International Symposium, Glasgow, Scotland, 16-21 September 1990.. The Royal Society of Edinburgh, Section B (Biological Sciences), Edinburgh, Scotland. 343 p.
Volume containing the main invited lectures from the Glasgow conference in September 1990. Contains also site locations of major acidifcation studies worldwide, and a conference summary.

Lewis, T.E. (ed.). 1989. Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. 344 p.
Book with several good chapters on chemistry and toxicology of Al.

Muniz, I.P., and H. Leivstad. 1980. Acidification - effects on freshwater fish. In: D. Drablos, and A. Tollan (eds.), Ecological impact of acid precipitation: Proceedings of an international conference, Sandfjord, Norway, March 11-14, 1980. SNSF Project, Oslo, Norway. pp. 84-92.

Parker, D.R., L.W. Zelazny, T.B. Kinraide, 1989. Chemical speciation and plant toxicity of aqueous aluminum. In: T.E. Lewis (ed.), Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. pp. 117-145.
Thorough review of Al chemistry and toxicology with a specific focus on terrestrial plants.

Rosseland, B.O., T.D. Eldhuset, and M. Staurnes, 1990. Environmental effects of aluminium. Environ. Geochem. Health 12:17-27.
Good complement to Driscoll's review above.

Scheuhammer, A.M., 1991. Acidification-related changes in the biogeochemistry and ecotoxicology of mercury, cadmium, lead and aluminum: Overview. Environ. Pollut. 71:87-90.
Good review of metal biogeochemistry (including Al) with a specific focus on how acidification induces changes in element cycles.

Schindler, D.W. 1988. Experimental studies of chemical stressors on whole lake ecosystems. Verh. internat. Verein. limnol. 23:11-41.
Baldi lecture at New Zealand S.I.L. meeting. General discussion and review of the utility of whole-lake ecosystem manipulations in understanding anthropogenic stress on lakes.

Sigel, H., and A. Sigel. 1988. Metal ions in biological systems, Vol. 24: Aluminum and its role in biology. Marcel Dekker, Inc., New York. 424 p.
Collection of papers regarding Al. Mostly human toxicity, but one good chapter on chemistry, another Driscoll chapter, and 2 good chapters on higher plant toxicity.

Sparling, D.W. and Lowe, T.P. 1996. Environmental hazards of aluminum to plants, invertebrates, fish, and wildlife. Rev. Environ. Contam. Toxicol. 145, 1-127.

Sposito, G. (ed.). 1989. The environmental chemistry of aluminum. CRC Press, Inc., Boca Raton, FL. 317 p.
Great looking collection of papers regarding Al chemistry, speciation, geochmistry, etc. for both soils and surface waters.

Sposito, G. (Ed.). 1996. The environmental chemistry of aluminum. 2nd Edition. Lewis Publishers, Boca Raton, FL. 464 p.
Excellent review that updates the 1989 book with regards to all important aspects of Al chemistry, speciation, measurements, and geochemistry.

Suter, G.W. II. 1996. Toxicological benchmarks for screening contaminants of potential concern for effects on freshwater biota. Environ. Toxicol. Chem. 15, 1232-1241.
This is the published version of the database used by Oak Ridge National Lab risk assessors for aiding in screening ecological risk assessments. Contains summaries of acute and chronic toxicity data for many organic and inorganic contaminants, including Al.

Wood, J.M. 1984. Microbial strategies in resistance to metal ion toxicity. In: H. Sigel, (ed.), Metal ions in biological systems, vol. 18: Circulation of metals in the environment. Marcel Dekker, Inc., New York. pp. 333-351.

Wood, J.M. 1985. Effects of acidification on the mobility of metals and metalloids: An overview. Environ. Health Perspect. 63:115-119.

2 Aluminum Chemistry and Speciation (Chapter 2)

Achilli, M., Ciceri, G., Ferraroli, R., Culivicchi, G, and Pieri, S. 1991. Aluminum speciation in aqueous solutions. Water Air Soil Pollut. 57-58, 139-148.
Examines and compares a couple of important Al fractionation methods: specifically those incorporating pyrocatechol violet, and 8-hydroxyquinoline.

Alberts, J.J., and J.P Giesy, 1983. Conditional stability constants of trace metals and naturally occurring humic materials: application in equilibrium models and verification with field data. In: R.F. Christman, and E.T. Gjessing (eds.), Aquatic and terrestrial humic materials. Ann Arbor Science, Ann Arbor, Michigan. pp. 333-348.
Theoretical and experimental treatment of trace metal binding constants with natural humic materials.

Alfassi, Z.B. and B. Rietz. 1994. Determination of aluminium by instrumental neutron activation analysis in biological samples with special reference to NBS SRM 1577 bovine liver. Analyst 119:2407-2410.

Almer, B., W. Dickson, C. Ekström, and E. Hörnström, 1978. Sulfur pollution and the aquatic ecosystem. In: Nriagu, J.O., Sulphur in the environment. Part II: Ecological impacts. John Wiley and Sons, New York. pp. 271-311.
Al chemistry also discussed in this general review of ecological effects of acidification.

Alvarez, E., A. Perez, and R. Calvo. 1993. Aluminium speciation in surface waters and soil solutions in areas of sulphide mineralization in Galacia (N.W. Spain). Sci. Total Environ. 133:17-37.
Examines Al speciation in soil solutions, with a particular focus on mineral control of solubility.

Anderson, A.R., J.K. Adamson, D.G.,Pyatt, and M. Hornung. 1993. pH, aluminium and calcium concentrations of streams draining acidic soils on calcareous till at Kershope, Cumbria. J. Hydrology 149:27-37.

Anonymous, 1981. Aluminum; Chemistry, analysis, and biology. Environmental geochemistry report No. 1981/2. McMaster University, Department of Biology, Hamilton, Ontario. 174 p.

Arent, L.J., and T.E. Lewis. 1989. The stability of aluminum species in a natural audit sample: possible application as a quality control solution. In: T.E. Lewis (ed.), Environmental chemistry and toxicology of aluminum. Lewis Publishers, Chelsea, MI. pp. 41-57.
Good treatment of methods for controlling Al solubility in quality control samples in order to maintain adequate accuracy and precision.

Arp, P.A. and Ouimet, R. 1986. Uptake of Al, Ca, and P in black spruce seedlings: effect of organic versus inorganic Al in nutrient solutions. Water Air Soil Pollut. 31, 367-375.

Arp, P.A., and R. Ouimet, 1986. Aluminum speciation in soil solutions: equilibrium calculations. Water Air Soil Pollut. 31:359-366.
Both studies by Arp focus on modeling and measurement of Al solubility and speciation in soil solutions.

Arruda, M.A.Z., M.J. Quintela, M. Gallego, and M. Valcárcel. 1994. Direct analysis of milk for aluminium using electrothermal atomic absorption spectrometry. Analyst 119:1695-1699.

Ashley, J.T.F. 1996. Adsorption of Cu(II) and Zn(II) by estuarine, riverine and terrestrial humic acids. Chemosphere 33: 2175-2187.
Artificial seawater matrix and humic acids extracted from estuary and river sediments. K_{ads} agreed with Irving-Williams series (Cu>Zn). Al not considered, but this paper could be useful.

Backes, C.A., and E. Tipping, 1987. Aluminium complexation by an aquatic humic fraction under acidic conditions. Water Res. 21:211-216.
One of many papers by these authors concerning the modeling of Al binding to humic acids in acidic waters.

Baes, C.F., and R.E. Mesmer, 1976. The hydrolysis of cations. John Wiley and Sons, New York. 489 p.
Theoretical treatment of metal hydrolysis reactions, including good collection of stability constants.

Baird, S.F., D.C. Buso, and J.W. Hornbeck. 1987. Acid pulsed from snowmelt at acidic cone pond, New Hampshire. Water Air Soil Pollut. 34: 325-338.
In lakes, acid meltwater tends to be confined to surface waters near inlet streams, as colder meltwater stays above warmer lake water, and may only contribute a small amount of Al to the lake. Therefore, deeper areas in a lake can provide refuge for mobile organisms, and fall storms may alter water chemistry more than does springmelt.

Berden, M., N. Clarke, L.-G. Danielsson and A. Sparen. 1994. Aluminium speciation: variations caused by the choice of analytical method and by sample storage. Water Air Soil Pollut. 72:213-233.
Good companion to Arent and Lewis (1989) in that it examines QA/QC aspects of Al quantitation and sample storage.

Bergman, H.L., and E.J. Dorward-King [eds]. 1997. Reassessment of metals criteria for aquatic life protection: priorities for research and implementation. SETAC Pellston Workshop on Reassessment of Metals Criteria for Aquatic Life Protection; 1996 Feb. 10-14; Pensacola FL. Pensacola, FL: SETAC Pr. 114p.
Although this book has only a few direct references to Al, it is important to incorporate it into the review. See especially Chapters 3 to 6, on regulatory practices for metals, environmental toxicology of metals (Playle was on that panel), chemical speciation and metal toxicity in surface freshwaters, and environmental fate and transport. There is also an Appendix in which the discussion paper by Playle is given, in which the gill modeling approach is

presented. That approach is discussed and even attacked in some of the other chapters.

Berggren, D. and S. Sparén. 1996. A modified FIA system for the determination of high levels of quickly reacting aluminium in aqueous solutions. Intern. J. Environ. Anal. Chem. 62:115-128.

NOTE: The following papers by Bertsch et al. are probably the first and best presentations of the formation of the Al_{13} polymeric cation, along with some of its biological significance. Formation of this polymer under natural conditions is uncertain, but is highly toxic. Therefore, this is an important new area of research currently underway.

Bertsch, P.M. 1987. Conditions for Al_{13} polymer formation in partially neutralized aluminum solutions. Soil Sci. Soc. Am. J. 51: 825-828.
It is not yet clear whether the Al_{13} polymer develops for solutions containing less than $mg \cdot L^{-1}$ concentrations of Al, and the formation of Al_{13} or colloidal or precipitated Al is dependent on the method of base addition. - need fast base addition (essential!); need OH:Al ratio $\geq$0.4 or so (lots made if 2.0-2.5x more OH than Al. eg: if 108 $\mu g \cdot L^{-1}$ Al (~4 µM), 160 $\mu g \cdot L^{-1}$ Al (~6 µM), need ~3 µM NH_3. ~10-20 µM base added at gills (Playle and Wood 1989a), therefore theoretically possible at fish gills. Whether Al_{13} forms as acidic water containing Al passes over the gills, and the pH is raised by NH_3 release, is currently under investigation by P. Campbell and K. Wilkinson (INRS-Eau, P.Q., personal communication). See also: Parker and Bertsch 1992b.

Bertsch, P.M. 1989. Aqueous polynuclear aluminum species. *In* The environmental chemistry of aluminum. Sposito, G. [ed] CRC Press, Inc., Boca Raton, Florida, 87-115.
Polymerization of Al may involve the rapid, irreversible formation of the Al_{13} polynuclear species, possibly from an $Al(OH)_4^-$ precursor. Reaction rates of precipitation reactions are influenced by the method and rate of base addition and the temperature of the reaction, and would only occur in very specific conditions in nature (this last point is from Bertsch 1987, above).

Bertsch, P.M., 1990. The hydrolytic products of aluminum and their biological significance. Environ. Geochem. Health 12:7-14.

Bertsch, P.M., and M.A. Anderson, 1989. Speciation of aluminum in aqueous solutions using ion chromatography. Anal. Chem. 61:535-539.

Bertsch, P.M., W.J. Layton, and R.I. Barnhisel. 1986. Speciation of hydroxy-aluminum solutions by wet chemical and aluminum-27 NMR methods. Soil Sci. Soc. Am. J. 50:1449-1454.

Bertsch, P.M., and D.R. Parker. 1996. Aqueous polynuclear aluminum species. In: Sposito, G., The environmental chemistry of aluminum. Lewis Publishers, New York. pp. 117-168.

Bi, S.P., J.S. Tang, and Y.Z. Wang. 1996. Determination of μg levels of aluminum in water by potentiometric titration in mixed organic medium using F/Ise. Abstracts of papers of the American Chem. Soc. 212:124 Envr.

Birchall, J.D., 1990. The role of silicon in biology. Chemistry in Britain February. pp 141-144.

Birchall, J.D., and A.W. Espie, 1986. Biological implications of the interaction (via silanol groups) of silicon with metal ions. In: Silicon biochemistry (Ciba Foundation Symposium 121). Wiley, Chichester, U.K.. pp. 140-159.

Birchall, J.D., and J.S. Chappell, 1987 . The solution chemistry of aluminium and silicon and its biological significance. In: Thornton, I., Proceedings of the second international symposium on geochemistry and health (Monograph series: Geochemistry and health). Science Reviews, Ltd., Middlesex, U.K.. pp. 1-15.

Birchall, J.D., and J.S. Chappell, 1988. Aluminium, chemical physiology, and Alzheimer's disease. The Lancet II:1008-1010.

Birchall, J.D., and J.S. Chappell, 1989. Aluminium, water chemistry, and Alzheimer's disease. The Lancet I:953.

Birchall, J.D., and J.S. Chappell. 1989. The chemistry of aluminium and silicon within the biological environment. In: R. Massey, and D. Taylor (eds.), Aluminium in food and the environment. Royal Society of Chemistry, London. pp. 40-51.

Birchall, J.D., C. Exley, J.S. Chappell, and M.J. Phillips. 1989. Acute toxicity of aluminium to fish eliminated in silicon-rich acid waters. Nature 338:146-148.
The preceding papers by Birchall et al. detail chemical interactions between Si and Al, with respect to the potential role Si plays in ameliorating Al toxicity.

Bjørnstad, H.E., D.H. Oughton, and B. Salbu. 1992. Determination of aluminium-26 using a low-level liquid scintillation spectrometer. Analyst 117:435-438.
Rare look at the radioactive Al-26 isotope. Interesting, although little used in biological studies.

Bloom, P.R., and M.S. Erich. 1989. The quantitation of aqueous aluminum. In: G. Sposito (ed.), The environmental chemistry of aluminum. CRC Press, Inc., Boca Raton, FL. pp. 1-27.
Excellent review of Al measurement methods, and methods for speciation and fractionation.

Bloom, P.R., and M.S. Erich. 1996. The quantitation of aqueous aluminum. In: Sposito, G., The environmental chemistry of aluminum (2nd Ed.). Lewis Publishers, New York. pp. 1-38.

Boudot, J.-P., O. Maitat, D. Merlet, and J. Rouiller. 1996. Occurrence of non-monomeric species of aluminium in undersatruated soil and surface waters: consequences for the determination of mineral saturation indices. J. Hydrol. 177: 47-63.
An interesting paper. They found polymeric or colloidal Al formation even when their solutions were undersaturated with respect to amorphous $Al(OH)_3$, gibbsite, etc., which indicates some unreliability with the traditional determination of saturation indices.

Broshears, R.E., R.L. Runkel, B.A. Kimball, D.M. McKnight, and K.E. Bencala. 1996. Reactive solute transport in an acidic stream: experimental pH increase and simulation of controls on pH, aluminum, and iron. Environ. Sci. Technol. 30: 3016-3024.
An acid mine drainage system. Carbonate chemistry, solid phase formation, and buffering by the stream bed were all important. Al formed microcrystalline gibbsite. Limitations of the assumption of reactions controlled by equilibrium chemistry were revealed. That is, kinetic constraints to modeling.

Browne, B.A., and C.T. Driscoll. 1992. Soluble aluminum silicates: Stoichiometry, stability, and implications for environmental geochemistry. Science 256:1667-1670.
Similar to Birchall's studies, they examine the conditions for Al silicate formation.

Browne, B.A., and C.T. Driscoll. 1993. pH-dependent binding of aluminum by fulvic acid. Environ. Sci. & Technol. 27:915-922.

Browne, B.A., C.T. Driscoll, and J.G. McColl, 1990. Aluminum speciation using morin: II. Principles and procedures. J. Environ. Qual. 19:73-82.

Browne, B.A., J.G. McColl, and C.T. Driscoll. 1990. Aluminum speciation using morin: I. Morin and its complexes with aluminum. J. Environ. Qual. 19:65-72.
These two papers detail Al measurement methods using Morin as a colorimetric agent.

Burrows, W.D.. 1977. Aquatic aluminum: Chemistry, toxicology, and environmental prevalence. CRC Crit. Rev. Environ. Control 7:167-216.
Although dated, this provides an excellent review of Al chemistry and speciation.

Cabaniss, S.E. 1992. Synchronous fluorescence spectra of metal-fulvic acid complexes. Environ. Sci. & Technol. 26:1133-1139.
Looks at individual and paired complexation of metals with fulvic acid at both high and low pH. Also shows that Al competitively displaces Cu and Mg from the fulvic acid.

NOTE: P.G.C. Campbell and his associates (references below) have done some of the best and most thorough examinations of Al chemistry, geochemistry, and toxicity.

Campbell, P.G.C. 1995. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. *p.* 45-102 *In:* Metal speciation and bioavailability in aquatic systems, A. Tessier, and D.R. Turner (eds). IUPAC, John Wiley & Sons Ltd.
An excellent review and theoretical discussion of the free ion activity model which predicts that metal toxicity is ameliorated at low pH owing to competitive displacement by H^+ ions. See also Campbell and Stokes (1985). Aluminum is considered specifically on page 67+, which is a re-capitulation of their Al-F work. See also pg. 91+ for specific examples of violations of the free-ion activity model. A lot of the examples are based on "less than expected" protection of a given ligand, which could be due to fish gills (for example) out-competing the

ligand for Al or another metal (useful for fish section). Competition is considered, too.

Campbell, P.G.C., and A. Tessier. 1987. Metal speciation in natural waters: Influence of environmental acidification. In: R.A. Hites, and S.J. Eisenreich (eds.), Sources and fates of aquatic pollutants. Advances in chemistry series No. 216. American Chemical Society, Washington, D.C.. pp. 185-207.
Another good review of metal speciation as affected by acidification.

Campbell, P.G.C., and P.M. Stokes, 1985. Acidification and toxicity of metals to aquatic biota. Can. J. Fish. Aquat. Sci. 42:2034-2049.

Campbell, P.G.C., M. Bisson, R. Bougie, A. Tessier, and J.-P. Villeneuve. 1983. Speciation of aluminum in acidic freshwaters. Anal. Chem. 55:2246-2252.

Campbell, P.G.C., D. Tomassin, and A. Tessier, 1986. Aluminum speciation in running waters on the Canadian pre-Cambrian shield: kinetic aspects. Water Air Soil Pollut. 30:1023-1032.
Al speciation and reaction kinetics for acidic streams in Quebec. Aluminum speciation changes are rapid enough that water chemistry conditions in a lake or stream determine Al speciation, not the antecedent geochemical origins of the Al.

Campbell, P.G.C., H.J. Hansen, B. Dubreuil, and W.O. Nelson. 1992. Geochemistry of Quebec North Shore salmon rivers during snowmelt: organic acid pulse and aluminum mobilization. Can. J. Fish. Aquat. Sci. 49:1938-1952.
Examines geochemistry of Al in Quebec rivers during snowmelt. Constructs models to predict monomeric Al concentrations involving pH, and DOC. The amount of Al bound to dissolved organic carbon was usually well predicted by a linear logarithmic model based on Al^{3+} activity and DOC concentration, except during springmelt period. During that time, DOC binds Al less well than predicted, presumably because of a nonhumic component which binds Al poorly.

Campbell, P.G.C., R. Bougie, A. Tessier, and J. Villeneuve. 1984. Aluminum speciation in surface waters on the Canadian pre-Cambrian shield. Verh. Internat. Verein. Limnol. 22:371-375.

Cathalifaud, G., J. Ayele, and M. Mazet. 1997. Aluminium ions/organic molecules complexation: formation constants and stoichiometry. Application to drinking water production. Wat. Res. 31: 689-698.
Coagulation-flocculation work with Al and activated carbon. They used phenol, tannic acid, etc., as simple models for humic matter. Some complexation of the Al occurred, and some formation constants are calculated. This paper is in French, so will take some time to go through carefully again.

Chappell, J.S., and J.D. Birchall, 1988. Aspects of the interaction of silicic acid with aluminium in dilute solution and its biological significance. Inorg. Chim. Acta 153:1-4.
Dissolved silicon (silicic acid, $Si(OH)_4$) may be important in reducing the availability of Al in solution.

Clarke, N. and Danielsson, L.-G. 1995. The simultaneous speciation of aluminium and iron in a flow-injection system. Anal. Chim. Acta 306, 5-20.

Clarke, N., L.-G. Danielsson, and A. Sparén, 1992. The determination of quickly reacting aluminium in natural waters by kinetic discrimination in a flow system. Intern. J. Environ. Anal. Chem. 48:77-100.
Studies by Clarke et al. describe an automated method for Al chemistry and fractionation using pyrocatechol violet.

Coleman, N.T. and G.W. Thomas, 1964. Buffer curves of acid clays as affected by the presence of ferric iron and aluminum. Proc. Soil Science Society 28:187-190.

Colombo, C. and Violante, A. 1996. Effect of time and temperature on the chemical composition and crystallization of mixed iron and aluminum species. Clays and Clay Minerals 44, 113-120.

Connery, J., 1990. Summary report workshop on aluminium and health, Oslo, May 2-5, 1988. Environ. Geochem. Health 12:179.
Workshop report summary from work group 1: Analytical methods and environmental aspects of Al.

Cooke, G.D., E.B. Welch, S.A. Peterson, and P.R. Newroth. 1993. Restoration and management of lakes and reservoirs (2nd. edition). Lewis Publishers, Boca Raton, FL. 560 p.
Excellent review of lake and reservoir management. Contains a chapter on chemical phosphorus abatement techniques, including alum treatment. Good selection of chemical background and case studies.

Cooke, G.D., E.B. Welch, A.B. Martin, D.G. Fulmer, J.B. Hyde, and G.D. Schrieve. 1993. Effectiveness of Al, Ca, and Fe salts for control of internal phosphorus loading in shallow and deep lakes. Hydrobiologia 253:323-335.
This focusses specifically on the use of cation salts (including Al) for controlling internal P loading in lakes. Contains useful case studies.

Corain, B., G.G. Bombi, A. Tapparo, M. Perazzolo, and P. Zatta. 1996. Aluminum toxicity and metal speciation: established data and open questions. Coordin. Chem. Rev. 149:11-22.

Courtijn, E., C. Vandecasteele, and R. Dams. 1990. Speciation of aluminum in surface water. Sci. Total Environ. 90:191-202.
Uses both neutron activation analysis and ICP-emission spectroscopy to examine Al speciation. They suggest that dissolved organic carbon is probably a more important complexing agent of Al than is F.

Craig, D. and L.M Johnston. 1988. Acidification of shallow groundwaters during the spring melt period. Nordic Hydrol. 19: 89-98.
Examined acidification-induced mobilization of Al, therefore could get low pH, high Al, low Ca (versus alum addition, usually higher [Ca]). eg. in lakes, chronic, low (but elevated) Al, and in streams, acute (eg. 1 wk), high [Al], low cations, low pH. Neutralization of acidic rain by carbonate weathering in thin soils is limited by slow reaction rates, so that during heavy, acidic rains carbonate cannot buffer

all the incoming acidity, alumino-silicate minerals are weathered, and acidic, Al-rich groundwater is released into lakes and streams.

Cronan, C.S., and C.L. Schofield. 1990. Relationships between aqueous aluminum and acidic deposition in forested watersheds of North America and Northern Europe. Environ. Sci. & Technol. 24:1100-1105.
Large-scale biogeochemical treatment of acidification-induced changes in Al chemistry.

David, M.B., and C.T. Driscoll. 1984. Aluminum speciation and equilibria in soil solutions of a haplorthod in the Adirondack mountains (New York, U.S.A.). Geoderma 33:297-318.

Davis, J.A., and J.D. Hem. 1989. The surface chemistry of aluminum oxides and hydroxides. In: G. Sposito (ed.), The environmental chemistry of aluminum. CRC Press, Inc., Boca Raton, FL. pp. 185-219.
Detailed review of surface reactions with Al oxides and hydroxides.

Denning, A.S., J. Baron, M.A. Mast, and M. Arthur. 1991. Hydrologic pathways and chemical composition of runoff during snowmelt in Loch Vale watershed, Rocky Mountain National Park, Colorado, USA. Water Air Soil Pollut. 59: 107-123.
During springmelt, as pH and ANC decreased in Colorado subalpine streams, both DOC and Al increased. Soil solutions flushed by melting snow were thought to be the source of the DOC and Al.

Dickson, W. 1978. Some effects of the acidification of Swedish lakes. Verh. Internat. Verein. Limnol. 20:851-856.
Interesting early report of acidification-induced changes in Al chemistry.

Dickson, W. 1980. Properties of acidified waters. In: D. Drablos, and A. Tollan (eds.), Proceedings of the international conference on ecological impacts of acid precipitation. SNSF Project, Oslo, Norway. pp. 75-83.

Dillon, P.J., and B.D. LaZerte. 1992. Response of the Plastic Lake catchment, Ontario, to reduced sulphur deposition. Environ. Pollut. 77:211-217.
Changes in water chemistry of Plastic Lake, Ontario, owing to reductions in atmospheric deposition of acids. This site has seen many years of solid Al geochemistry work done by LaZerte's group.

Dillon, P.J., N.D. Yan, and H.H. Harvey. 1984. Acidic deposition: Effects on aquatic ecosystems. CRC Crit. Rev. Environ. Control 13:167-194.
Good Al chemistry discussion in this excellent general review.

Dobbs, A.J., P. French, A.M. Gunn, D.T.E. Hunt, and D.A. Winnard. 1989. Aluminum speciation and toxicity in upland waters. In: T.E. Lewis (ed.), Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. pp. 209-228.
Uses both empirical and modeling methods for measurement of Al speciation in natural waters subject to differential DOC complexation. Uses MICROTOX test to examine toxicity of fractions.

Dougan, W.K., and A.L. Wilson. 1974. The absorptiometric determination of aluminum in water. A comparison of some chromogenic reagents and the development of an improved method. Analyst 99:413-430.
The first detailed description of the pyrocatechol violet technique for spectroscopically quantitating Al in freshwaters. Basis for virtually all related method development since the mid 1970s.

Drablos, D., and A. Tollan (eds.), 1980. Ecological impact of acid precipitation. Proceedings of an international conference, Sandfjord, Norway, March 11-14, 1980. SNSF Project, Oslo, Norway. 383 p.

NOTE: C.T. Driscoll is probably the world's foremost authority on Al chemistry and geochemistry (along with P.G.C. Campbell). Many of the papers by Driscoll and his associates that follow below are either general reviews of Al chemistry and speciation, or field studies of Al geochemistry in the Adirondacks.

Driscoll, C.T. 1984. A procedure for the fractionation of aqueous aluminum in dilute acidic waters. Int. J. Environ. Anal. Chem. 16:267-283.
This is the classic paper detailing his techniques for fractionating Al into dissolved, inorganic, and organic forms. Involves 8-hydroxyquinoline in MIBK; cation exchange resin used to fractionate inorganic from organic forms (near pH of sample).

Driscoll, C.T. 1985. Aluminum in acidic surface waters: Chemistry, transport, and effects. Environ. Health Perspect. 63:93-104.

Driscoll, C.T. 1989. The chemistry of aluminum in surface waters. In: G. Sposito (ed.), The environmental chemistry of aluminum. CRC Press, Inc., Boca Raton, FL. pp. 241-277.

Driscoll, C.T., J.P. Baker, J.J. Bisogni, and C.L. Schofield. 1980. Effect of aluminum speciation on fish in dilute acidified waters. Nature 284:161-164.
Classic early demonstration of the importance of Al speciation in understanding its toxicity to fish.

Driscoll, C.T., J.P. Baker, J.J. Bisogni, and C.L. Schofield. 1984. Aluminum speciation and equilibria in dilute acidic surface waters of the Adirondack Region of New York State. In: O.P. Bricker (Ed.), Acid precipitation: Geological aspects. Ann Arbor Science, Ann Arbor, Michigan. pp. 55-75.

Driscoll, C.T., and R.M. Newton. 1985. Chemical characteristics of Adirondack lakes. Environ. Sci. & Technol. 19:1018-1024.

Driscoll, C.T., and K.M. Postek. 1996. The chemistry of aluminum in surface waters. In: Sposito, G., The environmental chemistry of aluminum (2nd Ed.). Lewis Publishers, New York. pp. 363-418.

Driscoll, C.T., N. van Breemen, and J. Mulder. 1985. Aluminum chemistry in a forested spodosol. J. Soil Sci. Soc. Am. 49:437-444.

Driscoll, C.T., and W.D. Schecher. 1988. Aluminum in the environment. In: Sigel, H., and A. Sigel, Metal ions in biological systems, Vol. 24: Aluminum and its role in biology. Marcel Dekker, Inc., New York. pp. 59-122.

Driscoll, C.T., W.A. Ayling, G.F. Fordham, and L.M. Oliver. 1989. Chemical response of lakes treated with $CaCO_3$ to reacidification. Can. J. Fish. Aquat. Sci. 46:258-267.

Driscoll, C.T., and W.D. Schecher. 1989. Aqueous chemistry of aluminum. In: H.J. Gitelman (ed.), Aluminum and health, a critical review. Marcel Dekker, New York. pp. 27-65.

Driscoll, C.T., B.J. Wyskowski, P. DeStaffan, and R.M. Newton. 1989. Chemistry and transfer of aluminum in a forested watershed in the Adirondack region of New York, USA. In: T.E. Lewis (ed.), Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. pp. 83-105.

Driscoll, C.T., G.F. Fordham, W.A. Ayling, and L.M. Oliver. 1989. Short-term changes in the chemistry of trace metals following calcium carbonate treatment of acidic lakes. Can. J. Fish. Aquat. Sci. 46:249-257.

Driscoll, C.T., and W.D. Schecher. 1990. The chemistry of aluminum in the environment. Environ. Geochem. Health 12:28-49.
One of the best of his many reviews on Al chemistry.

Duffy, S.J. and G.W. vanLoon. 1994. Characterization of amorphous aluminum hydroxide by the ferron method. Environ. Sci. Technol. 28:1950-1956.
A detailed examination of how the ferron spectroscopic method of Al analysis can be used to characterize Al hydroxides under conditions of water and wastewater treatment.

Egeberg, P.K. 1996. Acidification signals in estuarine sediment cores. Limnol. Oceanogr. 40:1438-1446.
Sediment cores could contain signals which could be used to reconstruct changes in dissolved Al and riverine humic matter during acidification (in Norway). Dated cores analyzed for riverine and marine humic matter through C:N ratios. They did see a doubling in Al and 50% reduction in humic matter (around 1900), which coincided with poorer salmon catches and with increased acid rain.

Exley, C. and Birchall, J.D. In press. A mechanism of hydroxyaluminosilicate formation. Polyhedron

Exley, C., and J.D. Birchall, 1990 . Aluminum reactions with silicic acid in dilute solution. Manuscript submitted (or accepted) to Hydrobiologia.

Exley, C., and J.D. Birchall, 1992. Hydroxyaluminosilicate formation in solutions of low total aluminium concentration. Polyhedron 11:1901-1907.

Exley, C., and J.D. Birchall, 1992?. The cellular toxicity of aluminium. J. Theor. Biol. In press:

Exley, C., J.S. Chappell, and J.D. Birchall, 1991. A mechanism for acute aluminium toxicity in fish. J. Theor. Biol. 151:417-428.
Along with Birchall, Exley has done much to examine the role of Si in controlling Al toxicity to freshwater organisms.

Fairman, B., A. Sanz-Medel, M. Gallego, M.J. Quintela, P. Jones, and R. Benson. 1994. Method comparison for the determination of labile aluminium species in natural waters. Anal. Chim. Acta 286:401-409.
Interesting comparison of various methods for measuring labile (usually dissolved inorganic or organic) Al.

Findlay, D.L., and S.E.M. Kasian. 1986. Phytoplankton community responses to acidification of Lake 223, Experimental Lakes Area, northwestern Ontario. Water Air Soil Pollut. 30:719-726.

Franken, G., M. Pijpers, and E. Matzner. 1995. Aluminum chemistry of the soil solution in an acid forest soil as influenced by percolation rate and soil-structure. European J. Soil Sci. 46:613-619.

Furrer, G., B. Trusch, and C. Muller, 1992. The formation of polynuclear Al_{13} under simulated natural conditions. Geochim. Cosmochim. Acta 56:3831-3838.

Gensemer, R.W. 1989. Influence of aluminum and pH on the physiological ecology and cellular morphology of the acidophilic diatom *Asterionella ralfsii* var. *americana*. Ph.D. Dissertation. University of Michigan, Ann Arbor, Michigan. 159 p.
First chapter examines use of pyrocatechol violet as a means of measuring EDTA-bound Al, and its implications for speciation modelling.

Goldberg, S., J.A. Davis, and J.D. Hem. 1996. The surface chemistry of aluminum oxides and hydroxides. In: Sposito, G., The environmental chemistry of aluminum (2nd Ed.). Lewis Publishers, New York. pp. 271-331.

Gjessing, E.T., G. Riise, R.C. Petersen, and E. Andruchow. 1989. Bioavailability of aluminium in the presence of humic substances at low and moderate pH. Sci. Total Environ. 81-82:683-690.

Hamilton-Taylor, J., and M. Wills. 1990. A quantitative assessment of the sources and general dynamics of trace metals in a soft-water lake. Limnol. Oceanogr. 35:840-851.
Study of trace metal biogeochemistry in Lake Windermere.

Hamilton-Taylor, J., Davison, W., and Morfett, K. 1996. The biogeochemical cycling of Zn, Cu, Fe, Mn, and dissolved organic C in a seasonally anoxic lake. Limnol. Oceanogr. 41:408-418.

Haug, A., 1984. Molecular aspects of aluminum toxicity. CRC Crit. Rev. Plant Sci. 1:345-373.

Havas, M., 1986. Aluminum chemistry of inland waters. In: M. Havas, and J.F. Jaworski (eds.), Aluminum in the Canadian Environment. National Research Council of Canada, publication No. 24759, Ottawa, Ontario. pp. 51-77.

Hemingway, B.S., and G. Sposito. 1996. Inorganic aluminum-bearing solid phases. In: Sposito, G., The environmental chemistry of aluminum (2nd Ed.). Lewis Publishers, New York. pp. 81-116.

Hemstra, T., J.C.M. De Wit, and W.H. Van Riemsdijk. 1989. Multisite proton adsorption modelling at the solid/solution interface of (Hydr)oxides: a new approach. J. Colloid Interface Sci. 133:105-117.

Hendershot, W.H., F. Courchesne, and D.S. Jeffries. 1996. Aluminum geochemistry at the catchment scale in watersheds influenced by acidic precipitation. In: Sposito, G., The environmental chemistry of aluminum (2nd Ed.). Lewis Publishers, New York. pp. 419-449.

Hendershot, W.H., H. Lalonde, and A. Dufresne. 1984. Aluminum speciation and movement in three small watersheds in the southern laurentians. Water Pollut. Res. J. Can. 19: 11-26.
Hydrology is important in determining pH and Al content of stream water after storm events.

Henriksen, A., B.M. Wathne, E.J.S. Røgeberg, S.A. Norton, and D.F. Brakke. 1988. The role of stream substrates in aluminium mobility and acid neutralization. Wat. Res. 22, 1069-1073.
In addition to Al release from stream substrates during acidic rain events, Al can be released from mosses and liverworts in the stream bed.

Henshaw, J.M., T.E. Lewis, and E.M. Heithmar. 1988. A semi-automated colorimetric method for the determination of monomeric aluminum species in natural waters by flow injection analysis. Intern. J. Environ. Anal. Chem. 34:119-135.
Another look at the flow injection PCV method for fractionation and quantitation.

Hindar, A., F. Kroglund, E. Lydersen, A. Skiple, and R. Hrgberget. 1996. Liming of wetlands in the acidified Lake Røynelandsvatn catchment in southern Norway: effects on stream water chemistry. Can. J. Fish. Aquat. Sci. 53:985-993.

Hodges, S.C. 1987. Aluminum speciation: a comparison of five methods. Soil Sci. Soc. Am. J. 51: 57-64.
Five methods for Al speciation were compared. All five (8-hydroxyquinoline, ferron, cation exchange column, chelating resin, and fluoride electrode) had their advantages and disadvantages, susceptibility to interference by dissolved organic carbon, and ability to fractionate Al.

Hooper, R.P. and C.A. Shoemaker. 1985. Aluminum mobilization in an acidic headwater stream: temporal variation and mineral dissolution disequilibria. Science 229: 463-465.
They suggested that the labile pool of Al in soils is only slowly forming, then snowmelt (or rain) events will contribute differing "pulses" of Al to a stream depending on the nature of the event. For example, a low volume of snowmelt (eg. midwinter thaw) or rain would move most of this pool (resulting in high Al concentrations), whereas high volume events will move the Al but in more dilute form.

Huang, W.H., and W.D. Keller. 1972. Geochemical mechanics for the dissolution, transport, and deposition of aluminum in the zone of weathering. Clays and Clay Minerals 20:69-74.

Hunter, D., and D.S. Ross. 1991. Evidence for a phytotoxic hydroxy-aluminum polymer in organic soil horizons. Science 251:1065-1058.
Using NMR spectra, they identify a significant abundance of an Al_{13} polymer in soil solutions.

Hutchinson, G.E. 1957. A treatise on limnology. Volume 1, part 2 (Chemistry of Lakes). Wiley and Sons, New York. 1015 p.

Jackson, M.L. 1963. Aluminum bonding in soils: a unifying principle in soil science. Proc. Soil Science Society 27:1-9.

Jansson, M., G. Persson, and O. Broberg. 1986. Phosphorus in acidified lakes: The example of Lake Gårdsjön, Sweden. Hydrobiologia 139:81-96.
Very interesting treatment of Al-P interactions in Swedish acidified lakes. Claimed that Al reduced P input to the lake by ppt. in soils, productivity still limited by P, yet algal P utilization impared by Al..probably due to interaction with organic P enzyme recycling. See also Jansson 1981 Arch. Hydrobiol. 93:32 and 92:377.

Jardine, P.M., and L.W. Zelazny. 1989. A speciation method for partitioning mononuclear and polynuclear aluminum using ferron. In: T.E. Lewis (ed.), Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. pp. 20-40.
Good description of ferron spectrophotometric method for Al fractionation.

Jardine, P.M., and L.W. Zelazny. 1996. Surface reactions of aqueous aluminum species. In: Sposito, G., The environmental chemistry of aluminum (2nd Ed.). Lewis Publishers, New York. pp. 221-270.

Jaworski, J.F., 1986. The determination of aluminum in environmental samples. In: M. Havas, and J.F. Jaworski (eds.), Aluminum in the Canadian Environment. National Research Council of Canada, publication No. 24759, Ottawa, Ontario. pp. 181-209.

Jeffries, D.S., and W.H. Hendershot. 1989. Aluminum geochemistry at the catchment scale in watersheds influenced by acidic precipitation. In: G. Sposito (ed.), The environmental chemistry of aluminum. CRC Press, Inc., Boca Raton, FL. pp. 279-301.
Comparative geochemistry of Al in acidified catchments in Ontario and Quebec. Mean aluminum concentration in lakes and streams in an acidified catchment area (Turkey Lakes watershed) are from about 30 to 200 $\mu g \cdot L^{-1}$ total Al, with values up to 420 $\mu g \cdot L^{-1}$ total Al (370 monomeric inorganic Al) during springmelt (Jeffries and Hendershot 1989). eg: to give an idea of <u>appropriate</u> [Al] to use in expt's; ~1 to 10 µM (~30 to 400 $\mu g \cdot L^{-1}$). Springmelt: 30 to 50% (or more) of total annual runoff; heavy rainfall also (pg. 288d).

Johnson, M.G. 1991. Temporal trends in metal concentrations in rivers, coastal-zone waters, and sediments of northern and eastern Georgian Bay. J. Great Lakes Res. 17:241-254.
Interesting look at trace metal concentrations between seasons in eastern and northern Georgian Bay waters and tributaries. Shows excellent trends with pH and point pollution sources.

Johnson, N.M., C.T. Driscoll, J.S. Eaton, G.E. Likens, and W.H. McDowell, 1981. 'Acid rain', dissolved aluminum and chemical weathering at the Hubbard Brook

Experimental Forest, New Hampshire. Geochim. Cosmochim. Acta. 45:1421-1437.

Karbassi, A.R., and Sh. Nadjafpour. 1996. Flocculation of dissolved Pb, Cu, Zn, and Mn during estuarine mixing of river water with the Caspian sea. Environ. Pollut. 93: 257-260.
A study of river water mixing into the largest lake in the world (the Caspian Sea). All these metals showed non-conservative behavior during estuarine mixing (e.g. they were flocculating out). Pb and Mn flocculation were controlled by salinity. May have some relevance to Al.

Keller, W., J.R. Pitblado, and J. Carbone. 1992. Chemical responses of acidic lakes in the Sudbury, Ontario, area to reduced smelter emissions, 1981-89. Can. J. Fish. Aquat. Sci. 49(Suppl. 1): 25-32.
Release of Al into Swan Lake, near Sudbury, Ontario, was observed in a wet year (1988) which followed two very dry years (1986-7).

Kennedy, R.H. and G.D. Cooke. 1982. Control of lake phosphorus with aluminum sulfate: dose determination and application techniques. Water Resources Bull. 18: 389-395.
The use of aluminum sulphate for phosphorus control through floc formation in eutrophic lakes is outlined. They suggested a safe upper limit of 50 $\mu g \cdot L^{-1}$ dissolved Al after treatment, which would be obtained, regardless of dose, if final lakewater pH was 5.5 to 9.0. This reference looks good for the review.

Kennedy, V.C., G.W. Zellweger, and B.F. Jones. 1974. Filter pore-size effects on the analysis of Al, Fe, Mn, and Ti in water. Water Resour. Res. 10: 785-790.

Kerekes, J., S. Beauchamp, R. Tordon, and T. Pollock. 1986. Sources of sulphate and acidity in wetlands and lakes in Nova Scotia. Water Air Soil Pollut. 31: 207-214.

Kerekes, J., S. Beauchamp, R. Tordon, C. Tremblay, and T. Pollock. 1986. Organic versus anthropogenic acidity in tributaries of the Kejimkujik watersheds in Western Nova Scotia. Water Air Soil Pollut. 31: 165-173.

King, S.O., C.E. Mach, and P.L. Brezonik. 1992. Changes in trace metal concentrations in lake water and biota during experimental acidification of Little Rock Lake, Wisconsin, USA. Environ. Pollut. 78: 9-18.

Kinraide, T.B. 1990. Assessing the rhizotoxicity of the aluminate ion, $Al(OH)_4^-$. Plant Physiol. 94: 1620-1625.

Kinraide, T.B., and D.R. Parker. 1987. Cation amelioration of aluminum toxicity in wheat. Plant Physiol. 83: 546-551.

Kirchner, J.W., P.J. Dillon, and B.D. LaZerte. 1992. Predicted response of stream chemistry to acid loading tested in Canadian catchments. Nature 358: 478-482.

Kozuh, N., R. Milacic, and B. Gorence. 1996. Comparison of two methods for speciation of aluminum in soil extracts. Annali di Chimica 86: 99-113.

Kramer, J.R., 1986. Aluminum geochemistry. *In* M. Havas, and J.F. Jaworski [eds.], Aluminum in the Canadian Environment. National Research Council of Canada, publication No. 24759, Ottawa, Ontario. pp. 25-49.

Kramer, J.R., J. Gleed, and K. Gracey. 1994. Aluminum-pyrocatechol violet reactivity with various complexing agents. Anal. Chimi. Acta 284: 599-604.

Kuma, K., J. Nishioka, and K. Matsunaga. 1996. Controls on iron(III) hydroxide solubility in seawater: the influence of pH and natural organic chelators. Limnol. Oceanogr. 41: 396-407.
Solubility of Fe was about 10X lower in the ocean than in coastal waters. They thought their results indicated natural chelators which control dissolved iron concentrations in seawaters. (Might similar controls for Al exist?)

Lawrence, G.B. and C.T. Driscoll. 1988. Aluminum chemistry downstream of a whole-tree-harvested watershed. Environ. Sci. & Technol. 22: 1293-1299.
Some of the water chemistry showed that Al concentrations were controlled by dilution, rather than by hydrolysis/precipitation following mixing. This would agree with other work, e.g. Neal. Compared pre- and post-cut water chemistry.

Lawrence, G.B., C.T. Driscoll, and R.D. Fuller. 1988. Hydrologic control of aluminum chemistry in an acidic headwater stream. Water Resources Res. 24: 659-669.
Low flow and high flow rain events will differ in their hydrologic flow paths and therefore in the resultant surface water chemistry, and high or low elevation sites in a watershed also behave differently.

LaZerte, B.D. 1984. Forms of aqueous aluminum in acidified catchments of Central Ontario: A methodological analysis. Can. J. Fish. Aquat. Sci. 41: 766-776.
LaZerte determined that a short oxine (8-hydroxyquinoline-MIBK) extraction used to separate inorganic monomeric and organic Al from polymeric and amorphous Al, coupled with total organic carbon measurements, adequately modeled inorganic monomeric Al. This method eliminated his dialysis step of Al speciation.

LaZerte, B.D. 1989. Aluminum speciation and organic carbon in waters of Central Ontario. *In* T.E. Lewis [ed.], Environmental chemistry and toxicology of aluminum. A.C.S. Symposium, New Orleans, LA. Lewis Publishers, Chelsea, MI. pp. 195-207.

LaZerte, B.D. 1991. Metal transport at retention: The role of dissolved organic carbon. Finnish Humus News 3: 71-82.

Lee, Y.H. 1985. Aluminium speciation in different water types. Ecol. Bull. (Stockholm) 37: 109-119.

Lewis, T.E. [ed.]. 1989. Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. 344 p.
A lot of this book (at least nine chapters) is related to humans (e.g. encephalopathies). Does consider speciation, etc., of Al in natural water.

Lewis, T.E., D.E. Dobb, J.M. Henshaw, S.J. Simon, and E.M. Heithmar. 1988. Apparent monomeric aluminum concentrations in the presence of humic and fulvic acid and other ligands: an intermethod comparison study. Intern. J. Environ. Anal. Chem. 34: 69-87.
Organic complexes were found to interfere, to varying degrees depending on the ligand, with the 8-hydroxyquinoline/MIBK, pyrocatechol violet, and the fluoride

complexation kinetic methods. Nice chemical diagrams of Al complexing agents (p. 74). Carboxyl groups are important in complexing Al. Kinetics. Good paper.

Lindemann, J., E. Holtkamp, and R. Herrmann: 1990. The impact of aluminium on green algae isolated from two hydrochemically different headwater streams, Bavaria, Germany. Environ. Pollut. 67: 61-77.

Lindsay, W.L., and P.M. Walthall. 1989. The solubility of aluminum in soils. *In* G. Sposito [ed.], The environmental chemistry of aluminum. CRC Press, Inc., Boca Raton, FL. pp. 221-239.

Lindsay, W.L., and P.M. Walthall. 1996. In: Sposito, G., The environmental chemistry of aluminum (2nd Ed.). Lewis Publishers, New York. pp. 333-361.

Linthurst, R.A., D.H. Landers, J.M. Eilers, D.F. Brakke, W.S. Overton, E.P. Meier, and R.E. Crowe. 1986. Characteristics of lakes in the eastern United States. Vol. 1, Population descriptions and physico-chemical relationships, EPA/600/4-86/007a, U.S. EPA, Washington, D.C.

Lydersen, E. 1990. The solubility and hydrolysis of aqueous aluminium hydroxides in dilute fresh waters at different temperatures. Nordic Hydrol. 21: 195-204.
Lydersen showed that a decrease in temperature of about 15°C has the equivalent effect on Al species as a decrease in pH by one unit; temperature is therefore important to consider when calculating Al speciation.

Lydersen, E., A.B.S. Polèo, D.H. Oughton, and B. Salbu. 1993. Addition of sulphuric acid to high organic carbon lake water: effects on macro-chemistry, aluminium, and iron. Water Air Soil Pollut. 66: 349-363.
This is a laboratory experiment. As more acid was added, more Al was displaced from the DOC. There were estimates of the *p*K of Al and DOC (about 4.5).

Lydersen, E., B. Salbu, A.B.S. Polèo, and I.P. Muniz. 1990. The influences of temperature on aqueous aluminium chemistry. Water Air Soil Pollut. 51: 203-215.
Formation of Al polymers occurs more quickly at higher temperature.

Lydersen, E., B. Salbu, A.B.S. Polèo, and I.P. Muniz. 1991. Formation and dissolution kinetics of $Al(OH)_3$ (s) in synthetic freshwater solutions. Water Resour. Res. 27: 351-357.
Formation of Al polymers occurred in <5 min when 300 to 800 $\mu g \cdot L^{-1}$ inorganic, monomeric Al solutions (pH 4.5) were raised to pH 5.5 or 6.0. The 300 $\mu g \cdot L^{-1}$ inorganic, monomeric Al solution decreased to about 250 $\mu g \cdot L^{-1}$ inorganic, monomeric Al within 5 min, and to about 200 $\mu g \cdot L^{-1}$ in 24 h. These workers suggested that extraction times used for Al analysis must be reduced, or another method used, if instantaneous Al polymer formation was to be measured. (Playle and Wood 1990 did that.)

Mackin, J.E. and R.C. Aller. 1984. Dissolved Al in sediments and waters of the East China Sea: Implications for authigenic mineral formation. Geochim. Cosmochim. Acta 48: 281-297.

Mackin, J.E. 1986. Control of dissolved Al distributions in marine sediments by clay reconstruction reactions: experimental evidence leading to a unified theory. Geochem. Cosmochim. Acta. 50: 207-214.

Martell, A.E., and R.J. Motekaitis. 1989. Coordination chemistry and speciation of Al(III) in aqueous solution. In: T.E. Lewis (ed.), Environmental chemistry and toxicology of aluminum. Lewis Publishers, Chelsea, MI. pp. 3-17.
A detailed review of Al equilibrium constants and speciation, with a special emphasis on catechol and hydroxy carboxylate ligands.

Martin, R.B. 1986. Bioinorganic chemistry of metal ion toxicity. *In* H. Sigel (ed.), Metal ions in biological systems. Volume 20: Concepts on metal ion toxicity. Marcel Dekker, Inc., New York, NY. pp. 21-65.

Martin, R.B. 1988. Bioinorganic chemistry of aluminum. *In* Sigel, H., and A. Sigel, Metal ions in biological systems, Vol. 24: Aluminum and its role in biology. Marcel Dekker, Inc., New York. pp. 1-57.

Martín-Esteban, A., P. Fernández, C. Pérez-Conde, A. Gutiérrez, and C. Cámara. 1995. On-line preconcentration of aluminium with immobilized chromotrope 2B for the determination by flame atomic absorption spectrometry and inductively coupled plasma mass spectrometry. Anal. Chim. Acta 304: 121-126.

May, H.M., P.A. Helmke, and M.L. Jackson. 1979. Gibbsite solubility and thermodynamic properties of hydroxy-aluminum ions in aqueous solution at 25°C. Geochim. Cosmochim. Acta 43: 861-868.

McAvoy, D.C. 1989. Episodic response of aluminum chemistry in an acid-sensitive Massachusetts catchment. Wat. Resources Res. 25: 233-240.
A fall study of streams in Massachusetts. Ion exchange processes, not mineral dissolution, controlled stream water Al concentrations during rainfall events (e.g. fast reactions). Upland streamwater could be toxic to fish, whereas the wetland water showed minimal (theoretical) toxicity to fish.

McKnight, D.M., K.E. Bencala, G.W. Zellweger, G.R. Aiken, G.L. Feder, and K.A. Thorn. 1992. Sorption of dissolved organic carbon by hydrous aluminum and iron hydroxides occurring at the confluence of Deer Creek with the Snake River, Summit County, Colorado. Environ. Sci. & Technol. 26: 1388-1396.
About 40% of the DOC entering another stream was removed from solution by aluminum and iron oxides. A multi-year study. Lots of detail, if wish to get into what specific moieties Al binds to on DOC (e.g. carboxyl groups, amino acid residues).

MINTEQA2, 1991. A geochemical assessment model for environmental systems: version 3.0. U.S. Environ. Prot. Agen. Center for Exposure Assessment Modelling, College Station Road, Athens, GA 30613-0801.

Mitrovic, B., R. Milacic, and B. Pihlar. 1996. Speciation of aluminum in soil extracts by employing cation-exchange fast protein liquid chromatography inductively coupled plasma atomic emission spectrometry. Analyst 121: 627-634.

Merino, A., and E. García-Rodeja. 1997. Heavy metal and aluminium mobilization in soils from Galicia (NW Spain) as a consequence of experimental acidification. Appl. Geochem. 12: 225-228.
Laboratory studies of forest soils acidified to release their metals. Modifying factors of metal mobilization are outlined, but there are no real revelations here.

Méranger, J.C. 1989. How aluminum levels in subsurface drinking water supplies in Canada can be used to predict possible impact by acidic deposition. *In* T.E. Lewis [ed.], Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. pp. 107-116.

Molot, L.A., P.J. Dillon, and B.D. LaZerte. 1989. Factors affecting alkalinity concentrations of streamwater during snowmelt in central Ontario. Can. J. Fish. Aquat. Sci. 46: 1658-1666.
During spring melt, the major cause of depressed alkalinity is dilution of base cations by melt water, although the contribution of dilution to decreased alkalinity is less in acidic (and $SO_4^=$ or NO_3^- increases more important) than in circumneutral streams. - later, heavy rains important (other refs)

Molot, L.A., P.J. Dillon, and G.M. Booth. 1990. Whole-lake and nearshore water chemistry in Bowland Lake, before and after treatment with $CaCO_3$. Can. J. Fish. Aquat. Sci. 47: 412-421.
Calcium carbonate added to the lake raised its pH from 4.9 to about 6.7. Aluminum decreased gradually from about 130 to 30 $\mu g \cdot L^{-1}$, and Mn decreased from about 80 to 30 $\mu g \cdot L^{-1}$. An acidic layer still formed <1 m thick under ice in winter (e.g. colder Al rich melt water was less dense than deeper water, so stayed just under the ice). Like Baird's results.

Moran, S.B., and R.M. Moore. 1988. Evidence from mesocosm studies for biological removal of dissolved aluminum from sea water. Nature 335: 706-708.

Moran, S.B., and R.M. Moore. 1989. The distribution of colloidal aluminum and organic carbon in coastal and open ocean waters off Nova Scotia. Geochim. Cosmochim. Acta 53: 2519-2527.

Morel, F.M.M., and J.G. Hering. 1993. Principles and applications of aquatic chemistry. John Wiley & Sons, Inc. New York. 588 pp.

Morrison, B., J.P. Dempster, and W.J. Manning [eds.]. 1992. Effects of acidic pollutants on freshwater plants and animals. Special issue from Glasgow Acid Precipitation conference (1990). Environ. Pollut. Vol. 78.

Morrison, G.M. 1990. Relevance of the reaction of aluminium with pyrocatechol violet to speciation and complexation capacity analysis. Analyst 115:1371-1373.
Another good look at the pyrocatechol violet analytical technique for Al speciation and quantitation.

Motkosky, N. and B. Kratochvil. 1993. Characterization of trace amounts of aluminium in biological reference materials by electrothermal atomic absorption spectrometry. Analyst 118: 1313-1316.

Nalewajko, C., and M.A. O'Mahony. 1988. Effects of acid pH shock on phosphate concentrations and microbial uptake in an acidifying and a circumneutral lake. Can. J. Fish. Aquat. Sci. 45:254-260.

Neal, C. 1988. Aluminium solubility relationships in acid waters - a practical example of the need for a radical reappraisal. J. Hydrol. 104: 141-159.
See Neal et al. (1987) for the argument for the need for this "practical reappraisal". Again, the need for independent axes to avoid spurious correlations. He has a point.

Neal, C. and N. Christophersen. 1989. Inorganic aluminium-hydrogen ion relationships for acidified streams: the role of water mixing processes. Sci. Tot. Environ. 80: 195-203.
This paper analyses the contribution of stormflow water (through upper, acidic, Al rich zones) and regular flow water (from deeper, where inorganic sources of Al predominate). This paper would go well with others talking about source of Al in streams. Neal also emphasizes linear plots, instead of log-log or semilog plots (e.g. not pH but H^+) to make relationships clearer.

Neal, C., T. Musgrove, and P.G. Whitehead. 1989. Predicting the long-term variations in stream and lake inorganic aluminum concentrations for acidic and acid sensitive catchments. Sci. Tot. Environ. 80: 205-211.
The one box "MAGIC" model of stream and lake acidification, in which there is no consideration of $Al(OH)_3$ solubility controls. That is, assume that Al is never oversaturated. This approach seems reasonable, given some of the results of Tipping et al. Note that Colin uses total Al, not calculated Al^{3+}, when comparing Al concentrations with pH (see next ref. of his).

Neal, C., R.A. Skeffington, R. Williams, and D.J. Roberts. 1987. Aluminium solubility controls in acid waters: the need for a reappraisal. Earth Planet. Sci. Lett. 86: 105-112.
This is an interesting criticism of gibbsite (etc.) solubility curves. In essence, it has to do with "spurious correlations", in which anything *calculated* using pH or H^+ will give a good correlation when plotted against pH or H^+. So, don't plot calculated concentrations of Al^{3+} against pH, plot total Al instead. That is, you need independent axes.

Negrín, M.A., M. Espino-Mesa, and J.M. Hernández-Moreno. 1996. Effect of water:soil ratio on phosphate release: P, aluminium and fulvic acid associations in water extracts from Andisols and Andic soils. Eur. J. Soil Sci. 47: 385-393.
DOC-Al-fulvic acid interactions, but in soils. Probably not very relevant, unless we consider Al dissolution from sediments.

Nelson, W.O., and P.C.G. Campbell. 1991. The effects of acidification on the geochemistry of Al, Cd, Pb and Hg in freshwater environments: A literature review. Environ. Pollut. 71: 91-130.

Nicholls, K.H., L. Nakamoto, and W. Keller. 1992. Phytoplankton of Sudbury area lakes (Ontario) and relationships with acidification status. Can. J. Fish. Aquat. Sci. 49(Suppl. 1): 40-51.

Noble, A.D., M.E. Sumner, and A.K. Alva. 1988. Comparison of aluminon and 8-hydroxyquinoline methods in the presence of fluoride for assaying phytotoxic aluminum. Soil Sci. Soc. Am. J. 52: 1059-1063.
In essence, Al toxicity (to soybeans) agrees best with any method that measures available Al, and not too much F-Al. So, if use 8-hydroxyquinoline, react it for just 15 s, which will measure phytotoxic Al but not too much Al that is bound by F and is therefore less toxic.

Noller, B.N., P.J. Cusbert, N.A. Currey, P.H. Bradley, and M. Tuor. 1985. Analytical scheme for speciation of aluminium in natural waters. Environ. Technol. Lett. 6: 381-390.

Nordstrom, D.K. and J.W. Ball. 1986. The geochemical behavior of aluminum in acidified surface waters. Science 232: 54-56.

Nordstrom, D.K., and H.M. May. 1989. Aqueous equilibrium data for mononuclear aluminum species. *In* G. Sposito [ed.], The environmental chemistry of aluminum. CRC Press, Inc., Boca Raton, FL. pp. 29-53.

Nordstrom, D.K, and H.M. May. 1996. Aqueous equilibrium data for mononuclear aluminum species. pp. 39-80. *In:* Sposito, G., The environmental chemistry of aluminum (2nd Ed.). Lewis Publishers, New York.

Norrström, A.-C. 1993. Retention and chemistry of aluminium in groundwater discharge areas. Environ. Pollut. 82:269-275.

Öhman, L.-O., and S. Sjöberg. 1996. The experimental determination of thermodynamic properties for aqueous aluminium complexes. Coordination Chem. Rev. 149: 33-57.
A chemical modelling paper. Predictive modelling. Considers ionic effects, too. Mostly from the acidic, soft water angle. ^{27}NMR work. Some ambiguous results are presented; ultimately, I'm not sure this is much use for the review, except to illustrate some pitfalls of determining Al stability constants.

Öhman, L., and S. Sjoberg. 1988. Thermodynamic calculations with special reference to the aqueous aluminum system. In: J.R. Kramer, and H.E. Allen (eds.), Metal speciation: theory, analysis and application. Lewis Publishers, Inc., Chelsea, MI. pp. 1-40.

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

Oughton, D.H., B. Salbu, H.E. BjNrnstad, and J.P. Day. 1992. Use of an aluminium-26 tracer to study the deposition of aluminium species on fish gills following mixing of limed and acidic waters. Analyst 117: 619-621.
See reference in Playle (1987), below.

Parker, D.R., and P.M. Bertsch. 1992a. Formation of the "Al_{13}" tridecameric polycation under diverse synthesis conditions. Environ. Sci. & Technol. 26: 914-921.
Gives more information on the formation of the Al_{13} polymer at more reasonable (~540 $\mu g \cdot L^{-1}$ Al) concentrations of Al. Al_{13} can form with the fast addition of base, including NH_3, so this reaction could theoretically occur at the gills of fish

in acidic water. Parker and Bertsch discuss the possible formation of Al_{13} at fish gills, and quote some good studies of Al toxicity to fish.

Parker, D.R., and P.M. Bertsch. 1992b. Identification and quantification of the "Al_{13}" tridecameric polycation using ferron. Environ. Sci. & Technol. 26: 908-914.
The use of ferron instead of ^{27}NMR allows the determination of the Al_{13} polymer at much lower Al concentrations, to about 270 $\mu g \cdot L^{-1}$, which brings the determination of Al_{13} into the range that is needed for environmental relevance.

Parker, D.R., L.W. Zelazny, and T.B. Kinraide. 1988. Comparison of three spectrophotometric methods for differentiating mono- and polynuclear hydroxy-aluminum complexes. Soil Sci. Soc. Am. J. 52: 67-75.

Parker, D.R., L.W. Zelazny, and T.B. Kinraide. 1989. Chemical speciation and plant toxicity of aqueous aluminum. *In* T.E. Lewis [ed.], Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. pp. 117-145.

Parker, D.R., T.B. Kinraide, and L.W. Zelazny. 1989. On the phytotoxicity of polynuclear hydroxy-aluminum complexes. Soil Sci. Soc. Am. J. 53: 789-796.

Parkhurst, B.R., H.L. Bergman, J. Fernandez, D.D. Gulley, J.R. Hockett, and D.A. Sanchez. 1990. Inorganic monomeric aluminum and pH as predictors of acidic water toxicity to brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 47: 1631-1640.

Parthasarathy, N. and J. Buffle. 1985. Study of polymeric aluminium(III) hydroxide solutions for application in waste water treatment. Properties of the polymer and optimal conditions of preparations. Water Res. 19: 25-36.
A good paper on the theory of flocculation with alum. On p. 31 they summarize their results regarding the nature of the Al_{13} polymer: net charge is +0.5, 10-20 Å. Aging effects are also considered.

Peeters, K., P. Grobet, and E.F. Vansant. 1996. Intercalation ability of hydrolyzed aluminum species in N-alkylmouoamine alpha-zirconium phosphate compounds. J. Materials Chem. 6: 239-246.

Percival, H.J., K.M. Giddens, R. Lee, and J.S. Whitton. 1996. Relationships between soil solution aluminum and extractable aluminum in some moderately acid New-Zealand soils. Austr. J. Soil Res. 34: 769-779.

Persson, G., and O. Broberg. 1985. Nutrient concentrations in the acidified Lake Gårdsjøn: The role of transport and retention of phosphorus, nitrogen and DOC in watershed and lake. Ecol. Bull. (Stockholm) 37: 158-175.

Plankey, B.J. and H.H. Patterson. 1987. Kinetics of aluminum-fulvic acid complexation in acidic waters. Environ. Sci. Technol. 21: 595-601.
Aluminum reactions with fulvic acid: $t_{1/2}$=35 to 230 s (~4 min), to salicylic acid type Al binding sites.

Plankey, B.J. and H.H. Patterson. 1988. Effect of fulvic acid on the kinetics of aluminum fluoride complexation in acidic waters. Environ. Sci. Technol. 22: 1454-1459.
Contrary to expectations, Al-F interactions are increased in the presence of fulvic acid. Fulvic acid-Al interactions are slowed less at low temperature than

are F-Al interactions. They also say that any substance which increases $AlOH^{2+}$ compared to Al^{3+} will increase the rate of complexation by other ligands. Chlorides and sulphates, however, will not affect complexation rates (see p. 1459).

Plankey, B.J., H.H. Patterson, and C.S. Cronan. 1986. Kinetics of aluminum fluoride complexation in acidic waters. Environ. Sci. Technol. 20: 160-165.
F-Al interactions in pH range 2.9-4.9. As ionic strength is decreased, the complexation rate will increase. However, reaction rates may be very slow at, for example, 0°C, where it may take several hours to reach completion. These rates (and pH) are more relevant for soils; reactions in water will be faster (e.g 20 min at pH 3.4 and 16 s at pH 4.8). That is, it may be adequate to use equilibrium models for Al-F interactions in water. But, during springmelt, have lower temperatures and lower pH, both of which would slow complexation.

Plankey, B.J., H.H. Patterson, and C.S. Cronan. 1995. Kinetic analysis of aluminum complex formation with different soil fulvic acids. Anal. Chim. Acta 300: 227-236.

Playle, R.C. 1987a. Chemical effects of spring and summer alum additions to a small, northwestern Ontario lake. Water Air Soil Pollut. 34: 207-225.

Playle, R.C. 1987b. Methods and feasibility of using aluminum-26 as a biological tracer in low pH waters. Can. J. Fish. Aquat. Sci. 44(Suppl. 1): 260-263.
The ^{26}Al isotope has been used to measure Al accumulation by redbelly dace gills and bodies (Playle 1987; liquid scintillation counting) and in salmon par fish gills (Oughton et al. 1992; Cerenkov counting). The high cost of ^{26}Al and its limited to non-existent advantages over graphite furnace AAS make the isotope of little biological use, except for extremely specific, small scale experimentation.

Playle, R.C., and C.M. Wood. 1990. Is precipitation of aluminum fast enough to explain aluminum deposition on fish gills?. Can. J. Fish. Aquat. Sci. 47: 1558-1561.
Playle and Wood used a filtration method. They were able to determine that Al polymer formation (as shown by Al filtered from solution) occurs in <1.4 s. For example, a 160 $\mu g \cdot L^{-1}$ Al solution at pH 5.3 had about 16 $\mu g \cdot L^{-1}$ Al which did not pass through a 0.2 µm filter; 1.4 s after NH_4OH was added to raise the pH to 5.7, about 56 $\mu g \cdot L^{-1}$ Al did not pass through the filter. (could be Al_{13} formation!)

Postek, K.M., C.T. Driscoll, J.S. Kahl, and S.A. Norton. 1995. Changes in the concentrations and speciation of aluminum in response to an experimental addition of ammonium sulfate to the Bear Brook watershed, Maine, USA. Wat. Air Soil Pollut. 85: 1733-1738.
A paired watershed study (East and West Bear). $(NH_4)_2SO_4$ treatment. Monomeric Al concentrations increased, mainly as inorganic Al. pH down, too. Al was mobilized from the top soil horizons during rain events. Nothing terribly new here.

Quintela, M.J., M. Gallego, and M. Valcárcel. 1993. Flow injection spectrophotometric method for the speciation of aluminum in river and tap waters. Analyst 118: 1199-1203.
Comparison of automated (flow-injection) and manual pyrocatechol violet methods for Al speciation and quantitation. Uses river and tap water samples for this comparison.

Ramamoorthy, S. 1988. Effect of pH on speciation and toxicity of aluminum to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 45: 634-642.

Riseng, C.M., R.W. Gensemer, and S.S. Kilham. 1991. The effect of pH, aluminum, and chelator manipulations on the growth of acidic and circumneutral species of *Asterionella*. Water Air Soil Pollut. 60: 249-261.

Rϕyset, O., and T.J. Sullivan. 1986. Effect of dissolved humic compounds on the determination of aqueous aluminum by three spectrophotometric methods. Int. J. Environ. Anal. Chem. 27: 305-314.
A flow injection PCV method is included. Interference of high concentrations of humic acids on three spectrophotometric methods for Al determination were investigated. At the more usual DOC concentrations of <10 mg $C \cdot L^{-1}$ there were no interferences, but interference at >20 mg $C \cdot L^{-1}$ occurred for the eriochrome cyanine R/CTA and pyrocatechol violet methods, but not for 8-hydroxyquinoline/MIBK extraction.

Rosseland, B.O. 1980. Physiological responses to acid water in fish. 2. Effects of acid water on metabolism and gill ventilation in brown trout, (*Salmo trutta*) L., and brook trout, (*Salvelinus fontinalis*) Mitchill. *In* D. Drablos, and A. Tollan [eds.], Ecological impact of acid precipitation: Proceedings of an international conference, Sandfjord, Norway, March 11-14, 1980. SNSF Project, Oslo, Norway. pp. 348-349.

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

Rϕgeberg, E.J.S. and Ar. Henriksen. 1985. An automatic method for fractionation and determination of aluminum species in fresh-waters. Vatten 41: 48-53.
Automated catechol violet method of Dougan and Wilson (1974), with some of Driscoll (1980). Three fractions separated. Can probably find a more recent version of this method.

Salbu, B. 1990. Analytical techniques in studies of aluminium-species in aqueous solutions. Environ. Geochem. Health 12: 3-6.

Schafran, G.C., and C.T. Driscoll. 1987. Spatial and temporal variations in aluminum chemistry of a dilute, acidic lake. Biogeochemistry (Dordr) 3: 105-119.

Schecher, W.D. and C.T. Driscoll. 1995. ALCHEMI: A chemical equilibrium model to assess the acid-base chemistry and speciation of aluminum in dilute solutions. *In* Chemical Equilibrium and Reaction Models, SSSA Special Publication 42, p. 325-356.

Schecher, W.D. and C.T. Driscoll. 1988. An evaluation of the equilibrium calculations within acidification models: the effect of uncertainty in measured chemical components. Water Resources Res. 24: 533-540.
Here is there ALCHEMI model. Most variability was due to imprecise measurements of total F and of pH, and, after some modification, with SO_4^{2-}. Ionic strength was not very important in the model.

Schecher, W.D., and C.T. Driscoll. 1987. An evaluation of uncertainty associated with aluminum equilibrium calculations. Water Resources Res. 23: 525-534.
Schecher and Driscoll cautioned researchers and modelers not to casually select equilibrium constants from the literature, because large differences in predicted species and Al solubility can result.

Schecher, W.D., and D.C. McAvoy. 1992. MINEQL$^+$: a software environment for chemical equilibrium modeling. Computers, Environment and Urban Systems 16: 65-76.

Schultz, A.M., M.H. Begemann, D.A. Schmidt and K.C. Weathers. 1993. Longitudinal trends in pH and aluminum chemistry of the coxing kill, Ulster County, New York. Water Air Soil Pollut. 69:113-125.

Sculley, N.M., D.J. McQueen, D.R.S. Lean, and W.J. Cooper. 1996. Hydrogen peroxide formation: the interaction of ultraviolet radiation and dissolved organic carbon in lake waters along a 43-75°N gradient. Limnol. Oceanogr. 41: 540-548.

Seip, H.M., D.O. Andersen, N. Christophersen, T.J. Sullivan, and R.D. Vogt. 1989. Variations in concentrations of aqueous aluminium and other chemical species during hydrological episodes at Birkenes, southernmost Norway. J. Hydrology 108: 387-405.
For a small, forested catchment in Norway, both H^+ and Al^{3+} concentrations in streamwater increased with low discharge rate, but at higher discharge the increase in H^+ was less, and was erratic for Al^{3+}. Hydrological conditions therefore help determine streamwater chemistry. (These guys talk about Al ppt'n vs. speciation as toxic mechanisms to fish.) They argued that neither gibbsite, kaolinite, or jurbanite control Al concentrations in soil and stream water. Different controlling mechanisms may operate in different soil layers.

Seip, H.M., L. Müller, and A. Naas. 1984. Aluminium speciation: comparison of two spectrophotometric analytical methods and observed concentrations in some acidic aquatic systems in southern Norway. Water Air Soil Pollut. 23: 81-95.
Seip et al. compared the pyrocatechol violet method of fractionating Al with the ferron method of Driscoll (1984). They found that a reading at four min was adequate to determine monomeric Al, and that the method agreed well with the ferron method.

Severino, A., A. Esculcas, J. Rocha, J. Vital, and L.S. Lobo. 1996. Effect of extra-lattice aluminum species on the activity, selectivity and stability of acid zeolites in the liquid-phase isomerizetion of alpha-pinene. Applied Catalysis A - General 142: 255-278.

Shultz, A.M, M.H. Begemann, D.A. Schmidt, and K.C. Weathers. 1993. Longitudinal trends in pH and aluminum chemistry of the coxing kill, Ulster County, New York. Water Air Soil Pollut. 69: 113-125.

Shuman, L.M., D.O. Wilson, and E.L. Ramseur. 1991. Testing aluminum-chelate equilibria models using sorghum root growth as a bioassay for aluminum. Water Air Soil Pollut. 57-58: 149-158.
Aluminum complexed by NTA did not reduce sorghum root growth, whereas un-complexed Al did. Agrees with no free Al, as calculated by MINTEQA2. The sorghum root growth assay validated the complexation and detoxification of Al by NTA. In other words, they were considering equilibrium models as a basis to predict Al toxicity. (Similar to efforts to model metal interactions at fish gills!) They were using mM concentrations of Al and NTA. They used MINTEQA2. This could be a very important paper for the review.

Shuman, M.S. 1992. Dissociation pathways and species distribution of aluminum bound to an aquatic fulvic acid. Environ. Sci. & Technol. 26: 593-598.
Shuman determined a conditional binding constant for Al-fulvic acid complexes of log $K_{Al\text{-}FA}$~7.8. His model indicated that ~75% of 1 µM Al would be bound by 10 mg $C \cdot L^{-1}$ at pH 5.5. He suggested that the normal manner in which Al reacts (binds) with (to) ligands such as fulvic acid is through the disjunctive reaction, of Al dissociating then re-assembling to another ligand, as opposed to "attack" of an Al-ligand complex by another ligand (adjunctive reaction).

Sigel, H., and A. Sigel. 1988. Metal ions in biological systems, Vol. 24: Aluminum and its role in biology. Marcel Dekker, Inc., New York. 424 p.

Sikora, F.J., and M.B. McBride. 1989. Aluminum complexation by catechol as determined by ultraviolet spectrophotometry. Environ. Sci. & Technol. 23: 349-356.

Soltero, R.A., D.G. Nichols, A.F. Gasperino, and M.A. Beckwith. 1981. Lake restoration: Medical Lake, Washington. J. Freshwat. Ecol. 1: 155-165.
Aluminum sulfate (alum) was added to the lake to remove phosphorus, which was contributing to excessive algal production in the lake. "Floc" formation and co-precipitation of algae with P is described. Algal production was reduced considerably as P was reduced. No indication of Al concentrations in the lake are given.

Smith, M.A. 1990. The ecophysiology of epilithic diatom communities of acid lakes in Galloway, southwest Scotland. Phil. Trans. R. Soc. Lond. B 327: 251-256.

Smith, R.W. 1996. Kinetic aspects of aqueous aluminum chemistry - environmental implications. Coordination Chem. Rev. 149: 81-93.
The fate and transport of Al once mobilized from soils is considered. Al(III) is considered the toxic form of Al, but the further the water has travelled after falling as acidic rain the less Al(III) it will contain, unless the water is still very acidic (e.g. pH<5). The Al will react with organic substances, etc. Some very nice chemistry; worth incorporating into the paper.

Sposito, G. [ed.]. 1989. The environmental chemistry of aluminum. CRC Press, Inc., Boca Raton, FL. 317 p.

Sposito, G. 1996. The environmental chemistry of aluminum. Lewis Publishers, Boca Raton, FL. 464 p.
This is an excellent new (apparently updated from 1989 version above) text reviewing recent developments in Al chemistry and speciation. Includes chapters on equilibrium constants, analytical quantitation, solubility considerations, polynuclear species, organic complexes, surface reactions, soil and water chemistry of Al, and watershed-level geochemistry.

Sposito, G., F.T. Bingham, S.S. Yadav, and C.A. Inouye. 1982. Trace metal complexation by fulvic acid extracted from sewage sludge. II. Development of chemical models. Soil. Sci. Soc. Am. J. 46: 51.

Stapanian, M.A., T.E. Lewis, and D.C. Hillman. 1988. Water chemistry methods in acid deposition research: a comparative study of analyses from Canada, Norway, and the United States. Intern. J. Environ. Anal. Chem. 34: 299-314.
In essence, there were significant differences between these labs. However, there were different methods used, and Al analysis was held up for 2 weeks in the Norwegian lab, so no wonder there were differences. There was a call for more standardized techniques.

Steinberg, C. and W. Kuhnel. 1987. Influence of cation acids on dissolved humic substances under acidified conditions. Water Res. 21: 95-98.

Steinberg, C.E.W. and Th. Tayarani-Dastmalian. 1993. Changes in metal speciation in a sediment core from an acidified black forest lake Wildsee, Germany. Water Air Soil Pollut. 68:525-537.
Interesting paleolimnological study that examines past metal speciation (including Al) as a function of acidification history. Uses sequential extraction techniques to separate metal fractions from the sediments at various depths.

Steinnes, E. and A. Henriksen. 1993. Metals in small Norwegian lakes: relation to atmospheric deposition of pollutants. Water Air Soil Pollut. 71: 167-174.

Stevenson, F.J., and G.F. Vance. 1989. Naturally occurring aluminum-organic complexes. *In* G. Sposito [ed.], The environmental chemistry of aluminum. CRC Press, Inc., Boca Raton, FL. p. 117-145.

Sullivan, T.J., D.F. Charles, J.P. Smol, B.F. Cumming, A.R. Selle, D.R. Thomas, J.A. Bernert, and S.S. Dixit. 1990. Quantification of changes in lakewater chemistry in response to acidic deposition. Nature 345: 54-58.

Sullivan, T.J., C.T. Driscoll, S.A. Gherini, R.K. Munson, R.B. Cook, D.F. Charles, and C.P. Yatsko. 1989. Influence of aqueous aluminium and organic acids on measurement of acid neutralizing capacity in surface waters. Nature 338: 408-410.
Measured and calculated values of ANC generally agree, except for low ANC waters. Aluminum and DOC cause errors, which can be minimized for Al through calculations and assuming a mean charge on Al of 2+. Titration to pH 3.0 or so reduces the error associated with DOC, because it takes care of

curvature of the Gran plot analysis. Interesting, but really only of value when dealing with very unbuffered lakes and streams.

Sullivan, T.J., H.M. Seip, and I.P. Muniz. 1986. A comparison of frequently used methods for the determination of aqueous aluminum. Int. J. Environ. Anal. Chem. 26: 61-75.

Sutheimer, S.H. and S.E. Cabaniss. 1995. Determination of trace aluminum in natural waters by flow-injection analysis with fluorescent detection of the lumogallion complex. Anal. Chim. Acta 303: 211-221.

Taylor, T.A. and H.H. Patterson. 1993. Determination of aqueous aluminum with the fluorescent chelating ligand, 2-hydroxy-1-carbazole carboxylate. Part I. A model for speciation and stability constants. Anal. Chim. Acta 278: 249-257.

Taylor, T.A., H.H. Patterson, C.S. Cronan, and C.L. Schofield. 1993. Determination of aqueous aluminum with the fluorescent chelating ligand, 2-hydroxy-1-carbazole carboxylate. Part II. Application of ratio fluorescence spectroscopy. Anal. Chim. Acta 278: 259-268.

Thomas, F., A. Maison, J.Y. Bottero, J. Rouiller, F. Genévrier, and D. Boudot. 1991. Aluminum(III) speciation with acetate and oxalate. A potentiometric and 27Al NMR study. Environ. Sci. & Technol. 25: 1553-1559.
Along with other Al species, these workers deal with Al_{13} formation. In their introduction they give a nice overview of different Al speciation methods. Worth looking at again; however, their work was done at extremely high Al concentrations (~1-5 mM, or 27-135 $mg \cdot L^{-1}$).

Thomas, F., A. Mansion, J.V. Bottero, J. Rouller, F. Montigny and F. Genévrier. 1993. Aluminum (III) speciation with hydroxy carboxylic acids. 27Al NMR study. Environ. Sci. Technol. 27:2511-2516.

Tipping, E., and C.A. Backes. 1988. Organic complexation of Al in acid waters: model-testing by titration of a streamwater sample. Water Res. 22: 593-595.

Tipping, E., C. Woof, and M.A. Hurley. 1991. Humic substances in acid surface waters; modelling aluminium binding, contribution to ionic charge-balance, and control of pH. Water Res. 25: 425-435.

Tipping, E., C. Woof, C.A. Backes, and M. Ohnstad. 1988. Aluminium speciation in acidic natural waters: testing of a model for Al-humic complexation. Water Res. 22: 321-326.

Tipping, E., C. Woof, P.B. Walters, and M. Ohnstad. 1988. Conditions required for the precipitation of aluminum in acidic natural waters. Water Res. 22: 585-592.
The results of Tipping et al. suggest that Al precipitation occurs only rarely in natural waters. For example, low pH, high Al water flowing into higher pH, lower Al water (e.g. a stream entering a lake) is diluted, which results in higher pH, lower Al concentrations; precipitation is not involved. This was shown by a linear correlation with Ca concentration, an index of dilution (e.g. Ca is a conservative element). 1988).

Tipping, E., C.A. Backes, and M.A. Hurley. 1989. Modelling the interactions of Al species, protons and Ca2+ with humic substances in acid waters and soils. *In*

T.E. Lewis [ed.], Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. p. 59-82.

Tipping, E., M. Ohnstad, and C. Woof. 1989. Adsorption of aluminium by stream particulates. Environ. Pollut. 57: 85-96.
An empirical equation for adsorption of Al to particulates was developed, which gave a correlation coefficient of 0.99. From their Table 2, I calculate a correlation coefficient of r=0.958 (d.f.=6), for $P<0.001$ (very good). The relationship took into account Al^{3+} activity, H^+ activity, and particle concentration. The strong negative relationship between H^+ activity and Al^{3+} adsorption implies that the two cations compete for the same binding sites. Rapid reversibility can only be assumed at $pH \leq 5.5$. Particulate Al may constitute 10 to 50% of total, depending on the conditions.

Uehara, N., M. Kanbayashi, H. Hoshino, and T. Yotsuyanagi. 1989. An ion-pair reversed-phase HPLC-fluorimetric system for ultratrace determination of aluminium with salycylaldehydebenzoylhydrazone. Talanta 36: 1031-1035.
For routine quality control measurements (e.g. for Al in tap water). Detection limit: ~40 $\mu g \cdot L^{-1}$.

Urban, N.R., E. Gorham, J.K. Underwood, F.B. Martin, and J.G. Ogden III. 1990. Geochemical processes controlling concentrations of Al, Fe, and Mn in Nova Scotia lakes. Limnol. Oceanogr. 35: 1516-1534.

Van Benschoten, J.E. and J.K. Edzwald. 1990a. Chemical aspects of coagulation using aluminum salts - I. Hydrolytic reactions of alum and polyaluminum chloride. Wat. Res. 24: 1519-1526.
Lab experiments involving alum addition to deionized water. There was little evidence of polymer formation, and Al solubility was adequately described by amorphous $Al(OH)_3$ formation. Temperature effects are also covered.

Van Benschoten, J.E. and J.K. Edzwald. 1990b. Chemical aspects of coagulation using aluminum salts - II. Coagulation of fulvic acid using alum and polyaluminum chloride. Wat. Res. 24: 1527-1535.
This study goes along with the other. Read again if we're going to get heavily into the alum treatment aspect.

Vance, G.F., F.J. Stevenson, and F.J. Sikora. 1996. Environmental chemistry of aluminum-organic complexes. In: Sposito, G., The environmental chemistry of aluminum (2nd Ed.). Lewis Publishers, New York. pp. 169-220.

Vilchez, J.L., A. Navalón, R. Avidad, T. Garcia-López, and L.F. Capitán-Vallvey. 1993. Determination of trace amounts of aluminium in natural waters by solid-phase spectrofluorimetry. Analyst 118: 303-307.
This method seems very sensitive in natural waters: 0.2 to 14 $\mu g \cdot L^{-1}$, with a standard deviation of 1% (in relative terms) and a detection limit of 0.02 $\mu g \cdot L^{-1}$. The method involves Al reaction with salicylidene-*o*-aminophenol. This complex was adsorb onto a cation exchange gel, and the fluorescence of the gel was measured.

Vitt, D.H., S.E. Bayley, and T.-L. Jin. 1995. Seasonal variation in water chemistry over a bog-rich fen gradient in continental Western Canada. Can. J. Fish. Aquat. Sci. 52: 587-606.

Warfvinge, P. and H. Sverdrup. 1988. Soil liming as a measure to mitigate acid runoff. Water Resources Res. 24: 701-712.
Liming the watershed works well (as opposed to liming the actual stream or lake). Finer limestone does a better job than coarse limestone, but is more expensive, so there may be a tradeoff.

Watanabe, H. and S. Iwata. 1996. Theoretical studies of boron-water cluster ions $B^+(H_2O)_n$ and aluminum-water cluster ions $Al^+(H_2O)_n$: isomers and intracluster reactions. J. Phys. Chem. 100: 3377-3386.
Far too theoretical and technical to be useful here. Involves reactions of small clusters.

Weatherley, N.S., G.P. Rutt, S.P. Thomas, and S.J. Omerod, S.J. 1991. Liming acid streams: aluminium toxicity to fish in mixing zones. Wat. Air Soil Pollut. 55: 345-353.

Welch, E.B., C.L. DeGasperi, and D.E. Spyridakis. 1986. Effectiveness of alum in a weedy, shallow lake. Water Resources Bull. 22: 921-926.
The alum treatment in Long Lake, Washington State, in 1980 work well for four years. The fifth summer after treatment the lake returned to its high P state. It looks as if the alum floc got buried by sediment, so was no longer available to scavenge P.

Welch, E.B., J.P. Michaud, and M.A. Perkins. 1982. Alum control of internal phosphorus loading in a shallow lake. Water Resources Bull. 18: 929-936.
Long Lake, Washington State. A soft water lake, with a mean depth of 2 m. pH 8 to 10 in the summer. Total P decreased from 40 $\mu g \cdot L^{-1}$ to <10 $\mu g \cdot L^{-1}$ after alum treatment, so chlorophyl *a* also dropped (also, as a physical coagulation process, e.g. floc formation). The introduction and discussion both cover pros and cons, and successes and failures, of alum application to control P.

Wesselink, L.G., N. Van Breemen, J. Mulder, and P.H. Janssen. 1996. A simple model of soil organic matter complexation to predict the solubility of aluminium in acid forest soils. Europ. J. Soil Sci. 47: 373-384.
This is an equilibrium model for competitive binding of Al to soil organic matter. The model involves fast reactions with complexes, in which Al can be re-supplied by the slow, inorganic, mineral Al reactions. A nice model is presented on page 382.

Willén, E. 1991. Planktonic Diatoms - an ecological review. Algol. Stud. 62:69-106.
Comprehensive review of diatom ecology containing good selection of useful citations.

Williams, T.I. 1993. Aluminum: latecomer to the metal industry. Endeavour 17: 89-93.

Wong, H.K.T., J.O. Nriagu, and K.J. McCabe. 1989. Aluminum species in porewaters of Kejimkujik and Mountain Lakes, Nova Scotia. Water Air Soil Pollut. 46: 155-164.

Wood, J.M. 1985. Effects of acidification on the mobility of metals and metalloids: An overview. Environ. Health Perspect. 63: 115-119.

Wood, J.M. 1988. Transport, bioaccumulation, and toxicity of metals and metalloids in microorganisms under environmental stress. *In* J.R. Kramer, and H.E. Allen [eds.], Metal speciation: theory, analysis and application. Lewis Publishers, Inc., Chelsea, MI. p. 295-314.

Wren, C.D., and G.L. Stephenson. 1991. The effect of acidification on the accumulation and toxicity of metals to freshwater invertebrates. Environ. Pollut. 71: 205-241.

Wright, R.F. 1985. Chemistry of Lake Hovvatn, Norway, following liming and reacidification. Can. J. Fish. Aquat. Sci. 42: 1103-1113.
Added limestone onto ice on the lake. It worked fine in raising lake pH and lowering Al concentrations in the lake. However, lakes in that part of Norway need to be limed every year or every other year, because of their higher runoff rates (compared to the situation in Sweden and Ontario, where the water retention time is longer, e.g. 2-5 yr).

Wright, R.F. and O.K. Skogheim, O.K. 1983. Aluminium speciation at the interface of an acid stream and a limed lake. Vatten 39: 301-304.
Results of Wright and Skogheim for an acidic, Al-rich stream entering a less acidic, lower Al concentration lake, in which labile monomeric Al decreased along a pH gradient, could also be interpreted as dilution, as opposed to the Al precipitation phenomenon favoured by these two authors. (e.g. see Tipping 1988).

Wright, R.F., and E.T. Gjessing. 1976. Changes in the chemical composition of lakes. Ambio 5: 219-223.

Young, T.C., A.G. Collins and R.A. Armstrong. 1988. A pilot-scale evaluation of alum treatments to reduce lake sediment phosphorus release. J. Environ. Qual. 17:673-676.

Zernichow, L. and W. Lund. 1995. Size exclusion chromatography of aluminium species in natural waters. Anal. Chim. Acta 300: 167-171.

3 Aquatic Plants

3.1 Algae / Phytoplankton (Chapter 2)

Almer, B., W. Dickson, C. Ekström, and E. Hörnström, 1978. Sulfur pollution and the aquatic ecosystem. In: Nriagu, J.O., Sulphur in the environment. Part II: Ecological impacts. John Wiley and Sons, New York. pp. 271-311.
Excellent early review of general aspects of lake acidification, and its biological effects. Most examples from Sweden.

Anderson, D.S., R.B. Davis, and M.S. (Jesse) Ford, 1993. Relationships of sedimented diatom species (Bacillariophyceae) to environmental gradients in dilute northern New England lakes. J. Phycol. 29:264-277.
One of many paleolimnological studies that use diatom community structure (as preserved in lake sediments) to predict past environmental conditions in acidifying lakes. This study also includes Al predictions.

Anonymous, 1981. Aluminum: Chemistry, analysis, and biology. Environmental geochemistry report No. 1981/2. McMaster University, Department of Biology, Hamilton, Ontario. 174 p.
Internal review document, including a review of Al toxicity to phytoplankton.

Baker, J.P., and S.W. Christensen, 1991. Effects of acidification on biological communities in aquatic ecosystems. In: Charles, D.F. (ed.), Acidic deposition and aquatic ecosystems: Regional case studies. Springer-Verlag, New York, NY. pp. 83-106.
Results from experimental acidification at ELA, including phytoplankton responses, and consideration of Al influences.

Barcelo, J., Ch. Poschenrieder, M.D. Vazquez and B. Gunse. 1996. Aluminium phytotoxicity. A challenge for plant scientists. Fertilizer Res. 43:217-223.

Birchall, J.D., C. Exley, J.S. Chappell, and M.J. Phillips. 1989. Acute toxicity of aluminium to fish eliminated in silicon-rich acid waters. Nature 338:146-148.
Very interesting paper showing that silicic acid can ameliorate Al toxicity in fish. Also discusses chemical interactions of Al and Si, and Al:Si ratios and their biological effects.

Birks, H.J.B., J.M. Line, S. Juggins, A.C. Stevenson, and C.J.F. terBraak. 1990. Diatoms and pH reconstruction. Philos. Trans. R. Soc. Lond. B Biol. Sci. 327:263-278.
Thorough treatment of the construction, assumptions, and application of pH calibration and reconstruction models used in the SWAP program. Includes excellent discussion of weighted average and maximum likelihood models for pH reconstruction.

Bukaveckas, P.A. 1989. Effects of calcite treatment on primary producers in acidified Adirondack lakes. II. Short-term response by phytoplankton communities. Can. J. Fish. Aquat. Sci. 46:352-359.

Burrows, W.D., 1977. Aquatic aluminum: Chemistry, toxicology, and environmental prevalence. CRC Crit. Rev. Environ. Control 7:167-216.
This is a dated, but very comprehensive review of earlier Al toxicity studies using phytoplankton.

Campbell, P.G.C. 1995. Interactions between trace metals and aquatic organisms: A critique of the free ion activity model. In: A. Tessier, and D.R. Turner (eds.), Metal speciation and bioavailability in aquatic systems. Chapter 2. John Wiley and Sons. pp. 45-102.
Classic review and critique of the free ion activity model of metal speciation and bioavailability.

Campbell, P.G.C., and P.M. Stokes, 1985. Acidification and toxicity of metals to aquatic biota. Can. J. Fish. Aquat. Sci. 42:2034-2049.
This is the classic paper outlining arguments in favor of the importance of speciation in predicting the pH-dependent toxicity of metals to aquatic organisms. Al included.

Charles, D.F. 1984. Recent pH history of Big Moose Lake (Adirondack Mountains, New York, U.S.A.) inferred from sediment diatom assemblages. Verh. Internat. Verein. Limnol. 22:559-566.

Charles, D.F. 1985. Relationship between surface sediment diatom assemblages and lakewater characteristics in Adirondack lakes. Ecology 66:994-1011.
Charles' thesis - compares among different pH predictive models.

Charles, D.F. 1986. A new diatoms species, (*Fragillaria acidobiontica*), from acidic lakes in northeastern North America. In: J.P. Smol, R.W. Battarbee, R.B. Davis, and J. Meriläinen (eds.), Diatoms and lake acidity. Dr W. Junk Publishers, Dordrecht. pp. 35-44.

Charles, D.F. 1990. Effects of acidic deposition on North American lakes: palaeolimnological evidence from diatoms and chrysophytes. Phil. Trans. R. Soc. Lond. B 237:403-412.

Charles, D.F., R.W. Battarbee, I. Renberg, H. van Dam, and J.P. Smol. 1989. Paleoecological analysis of lake acidification trends in North America and Europe using diatoms and chrysophytes. In: S.A. Norton, S.E. Lindberg, and A.L. Page (eds.), Acid Precipitation. Vol. 4: Soils, aquatic processes, and lake acidification. Springer-Verlag, New York, NY. pp. 207-276.
Excellent review of paleoecology in acidification studies; good background of methods.

Claesson, A., and L. Törnqvist, 1988. The toxicity of aluminum to two acido-tolerant green algae. Wat. Res. 22:977-983.
Good work on pH-dependent Al toxicity, but speciation not directly considered. Useful morphological observations.

Cook, R.B., R.G. Kreis Jr., J.C. Kingston, K.E. Camburn, S.A. Norton, M.J. Mitchell, B. Fry, and L.C.K. Shane, 1990. Paleolimnology of McNearney Lake: an acidic lake in northern Michigan. J. Paleolimnology 3:13-34.

Cooke, G.D., E.B. Welch, S.A. Peterson, and P.R. Newroth. 1993. Restoration and management of lakes and reservoirs (2nd. edition). Lewis Publishers, Boca Raton, FL. 560 p.
Thorough examination of recent trends in lake and reservoir management with regards to control of cultural eutrophication. Includes chapter on P inactivation methods, including alum treatment.

Crist, R.H., K. Oberholser, J. McGarrity, D.R. Crist, J.K. Johnson, and J.M. Brittsan, 1992. Interactions of metals and protons with algae. 3. Marine algae, with emphasis on lead and aluminum. Environ. Sci. & Technol. 26:496-502.
This, and other studies by Crist, examine mechanisms of interactions between H ions and trace metals for algal cell surface binding sites.

Cumming, B.F., J.P. Smol, and H.J.B. Birks, 1992. Scaled chrysophytes (Chrysophyceae and Synurophyceae) from Adirondack drainage lakes and their relationship to environmental variables. J. Phycol. 28:162-178.

Cumming, B.F., J.P. Smol, J.C. Kingston, D.F. Charles, H.J.B. Birks, K.E. Camburn, S.S. Dixit, A.J. Uutala, and A.R. Selle, 1992. How much acidification has occurred in Adirondack region lakes (New York, USA) since preindustrial times?. Can. J. Fish. Aquat. Sci. 49:128-141.
These last two studies by Cumming et al. use both diatom and chrysophyte remains to examine past lake acidification patterns, including the role of Al. See also papers by Dixit et al. immediately below.

Davis, R.B. 1987. Paleolimnological diatom studies of acidification of lakes by acid rain: an application of quaternary science. Quat. Sci. Rev. 6:147-163.
Good overall review of diatom-inferred pH methods along with several examples of application to acidification.

DeGasperi, C.L., D.E. Spyridakis and E.B. Welch. 1993. Alum and nitrate as controls of short-term anaerobic sediment phosphorus release: an *In vitro* comparison. Lake and Reservoir Management 8:49-59.

Delhaize, E. and P.R. Ryan. 1995. Aluminum toxicity and tolerance in plants. Plant Physiol. 107:315-321.

Dickson, W. 1980. Properties of acidified waters. In: D. Drablos, and A. Tollan (eds.), Proceedings of the international conference on ecological impacts of acid precipitation. SNSF Project, Oslo, Norway. pp. 75-83.

Dillon, P.J., N.D. Yan, and H.H. Harvey, 1984. Acidic deposition: Effects on aquatic ecosystems. CRC Crit. Rev. Environ. Control 13:167-194.
Excellent review of Al toxicity in the context of freshwater acidification for several groups of aquatic organisms, including phytoplankton.

Dixit, S.S., B.F. Cumming, J.C. Kingston, J.P. Smol, H.J.B. Birks, A.J. Uutala, D.F. Charles, and K.E. Camburn. 1993. Diatom assemblages from Adirondack Lakes (New York, U.S.A.) and the development of predictive models for retrospective environmental assessment. J. Paleolimnol. 8:

Contains CCA, WA, and WACALIB models and reconstructions for adirondack lakes. Determines environmental optima for all taxa (including all Asterionella sps.) and determines indicator species for pH and Al.

Dixit, A.S., S.S. Dixit, and J.P. Smol, 1992. Algal microfossils provide high temporal resolution of environmental trends. Water Air Soil Pollut. 62:75-87.

Dixit, A.S., S.S. Dixit, and J.P. Smol, 1992. Long-term trends in lake water pH and metal concentrations inferred from diatoms and chrysophytes in three lakes near Sudbury, Ontario. Can. J. Fish. Aquat. Sci. 49(Suppl. 1):17-24.

Dixit, S.S., A.S. Dixit, and J.P. Smol, 1991. Multivariable environmental inferences based on diatom assemblages from Sudbury (Canada) lakes. Freshwater Biol. 26:251-266.

Dixit, S.S., A.S. Dixit, and J.P. Smol, 1992. Assessment of changes in lake water chemistry in Sudbury area lakes since preindustrial times. Can. J. Fish. Aquat. Sci. 49(Suppl. 1):8-16.

Dixit, S.S., A.S. Dixit, and R.D. Evans, 1989. Paleolimnological evidince for trace-metal sensitivity in scaled chrysophytes. Environ. Sci. & Technol. 23:110-115.

Dixit, S.S., J.P. Smol, J.C. Kingston, and D.F. Charles, 1992. Diatoms: Powerful indicators of environmental change. Environ. Sci. & Technol. 26:22-33.

Drablos, D., and A. Tollan (eds.), 1980. Ecological impact of acid precipitation. Proceedings of an international conference, Sandfjord, Norway, March 11-14, 1980. SNSF Project, Oslo, Norway. 383 p.
Early review from an excellent collection of papers from the SNSF project in Norway. Discusses Al toxicity.

Driscoll, C.T. 1989. The chemistry of aluminum in surface waters. In: G. Sposito (ed.), The environmental chemistry of aluminum. CRC Press, Inc., Boca Raton, FL. pp. 241-277.
Compilation/combination of his other recent reviews (Driscoll and Schecher 1988 and 1990) - much of which is copied verbatim, and using the same figures.

Driscoll, C.T., and R.M. Newton. 1985. Chemical characteristics of Adirondack lakes. Environ. Sci. & Technol. 19:1018-1024.
Excellent review of chemistry of acidified Adirondack lakes, and a comparison of seepage vs. drainage lake characteristics.

Driscoll, C.T., and W.D. Schecher. 1990. The chemistry of aluminum in the environment. Environ. Geochem. Health 12:28-49.
Similar to previous review by same authors (in Sigel and Sigel 1988), but with more emphasis on geochemistry of Al and weathering reactions.

Duthie, H.C. 1989. Diatom-inferred pH history of Kejimkujik Lake, Nova Scotia: a reinterpretation. Water Air Soil Pollut. 46:317-322.

EPA. 1988. Ambient water quality criteria for aluminum - 1988. U.S. Environmental Protection Agency, EPA 440/5-86-008. Washington, D.C. 47 p.

Exley, C., A. Tollervey, G. Gray, S. Roberts, and J.D. Birchall. 1993. Silicon, aluminium, and the biological availability of phosphorus in algae. Proc. R. Soc. Lond. B 253:93-99.

Suggests that Si plays a biological 'role' for algae that protects them against Al toxicity in the presence of P. However, all this really is is a competitive chemical interaction between Al and both Si and P, thereby affecting P availability and, subsequently, Al availability.

Findlay, D.L., and J.A. Shearer. 1992. Relationships between sedimentary diatom assemblages and lakewater pH values in the Experimental Lakes Area. J. Paleolimnol. 7:145-156.
Relates current sediment assemblages to water chemistry parameters of 36 ELA lakes, generating Hustedt category correlations. Also discusses importance of A. ralfsii v. americana in L. 223, and also discusses its *cell size*, potentially as a function of pH and/or Si concentrations. Also mentions Kling's manuscript on this account.

Findlay, D.L., and S.E.M. Kasian. 1986. Phytoplankton community responses to acidification of Lake 223, Experimental Lakes Area, northwestern Ontario. Water Air Soil Pollut. 30:719-726.

Folsom, B.R., N.A. Popescue, and J.M. Wood, 1986. Comparative study of aluminum and copper transport and toxicity in an acid-tolerant freshwater green alga. Environ. Sci. & Technol. 20:616-620.
Using highly acid tolerant alga, they clearly demonstrated amelioration of metal toxicity by base cations.

Foy, C.D., and G.C. Gerloff, 1972. Response of *Chlorella pyrenoidosa* to aluminum and low pH. J. Phycol. 8:268-271.
Linked Al tolerance to Ca requirements in *Chlorella*. Interesting, but did not consider Al or other chemical speciation.

Gardea-Torresdey, J.L., M.K. Becker-Hapak, J.M. Hosea, and D.W. Darnall, 1990. Effect of chemical modification of algal carboxyl groups on metal ion binding. Environ. Sci. & Technol. 24:1372-1378.

Geelen, J.F.M., and R.S.E.W. Leuven. 1986. Impact of acidification on phytoplankton and zooplankton communities. Experientia 42:486-494.
Good general review of effects of acidification on phytoplankton and zooplankton. Covers many geographic areas worldwide.

Gensemer, R.W., 1989. Influence of aluminum and pH on the physiological ecology and cellular morphology of the acidophilic diatom *Asterionella ralfsii* var. *americana*. Ph.D. Dissertation. University of Michigan, Ann Arbor, MI. 159 p.
Examines Al fractionation and speciation in algal media, pH dependence of Al toxicity, and morphological changes induced by Al under Si-limitation.

Gensemer, R.W. 1990. Role of aluminum and growth rate on changes in cell size and silica content of silica-limited populations of *Asterionella ralfsii* var. *americana* (Bacillariphyceae). J. Phycol. 26:250-258.
Demonstrated that under Si-limitation, that cell length could be reduced in the presence of Al.

Gensemer, R.W. 1991a. The effects of aluminum on phosphorus and silica-limted growth in *Asterionella ralfsii* var. *americana*. Verh. Internat. Verein. Limnol. 24:2635-2639.
Examines influence of Al on P and Si resource limitation in this acidophilic diatom.

Gensemer, R.W. 1991b. The effects of pH and aluminum on the growth of the acidophilic diatom *Asterionella ralfsii* var. *americana*. Limnol. Oceanogr. 36:123-131.
Study of pH-dependent Al toxicity in this acidophilic diatom, and specifically the protective effects of H ions at low pH.

Gensemer, R.W., R.E.H. Smith, and H.C. Duthie. 1993a. Comparative effects of pH and aluminum on silica-limited growth and nutrient uptake in *Asterionella ralfsii* var. *americana* (Bacillariophyceae). J. Phycol. 29, 36-44.
More detailed examination of the influence of Al on Si uptake physiology and utilization.

Gensemer, R.W., R.E.H. Smith, H.C. Duthie, and S.L. Schiff. 1993b. pH tolerance and metal toxicity in populations of the planktonic diatom *Asterionella*: influences of synthetic and natural dissolved organic carbon. Can. J. Fish. Aquat. Sci. 50, 121-132.
Using both the acidophilic *A. ralfsii,* and its circumneutral congener, *A formosa*, we examined how manipulating metal exposures (including Al) and DOC levels affect comparative pH tolerance.

Gensemer, R.W., R.E.H. Smith, and H.C. Duthie. 1995. Interactions of pH and aluminum on cell length reduction in *Asterionella ralfsii* var. *americana* Körn. Proceedings of the 13th International Diatom Symposium. pp. 39-46.
Followup to Gensemer (1990). Examined pH-dependence of cell length reduction owing to Al under Si-limitation, along with a discussion of cell length variability within a single colony.

Genter, R.B. 1995. Benthic algal populations respond to aluminum, acid, and aluminum-acid mixtures in artificial streams. Hydrobiologia 306:7-19.

Genter, R.B., and D.J. Amyot, 1994. Freshwater benthic algal population and community changes due to acidity and aluminum-acid mixtures in artificial streams. Environ. Toxicol. Chem. 13:369-380.
Study of changes in benthic algal community structure in response to changes in pH and Al additions. Not a well-balanced experimental design, but interesting.

George, D.B., S.G. Berk, V.D. Adams, R.S. Ting, R.O. Roberts, L.H. Parks and R.C. Lott. 1995. Toxicity of alum sludge extracts to a freshwater alga, protozoan, fish, and marine bacterium. Arch. Environ. Contam. Toxicol. 29:149-158.

Gimmler, H., B. Treffny, M. Kowalski, and U. Zimmermann, 1991. The resistance of *Dunaliella acidophila* against heavy metals: The importance of the zeta potential. J. Plant Physiol. 138:708-716.
Interesting mechanistic explanation of metal resistance in this highly acidophilic alga.

Gostomski, F., 1990. The toxicity of aluminum to aquatic species in the US. Environ. Geochem. Health 12:51-54.
A compilation of actute and chronic Al toxicity test results for aqautic organisms. including algae.

Grahn, O., H. Hultberg, and L. Landner. 1974. Oligotrophication - a self-accelerating process in lakes subjected to excessive supply of acid substances. Ambio 3:93-94.
Describes general biological impoverishment of Swedish lakes subject to acidification. Original reference to the 'oligotrophication hypothesis' describing how acid lakes become more oligotrophic.

Greger, M., J.-E. TIllberg, and M. Johansson, 1992. Aluminium effects on *Scenedesmus obtusiusculus* with different phosphorus status. I. Mineral uptake. Physiol. Plant. 84:193-201.

Greger, M., J.-E. Tillberg, and M. Johansson, 1992. Aluminium effects on *Scenedesmus obtusiusculus* with different phosphorus status. II. Growth, photosynthesis, and pH. Physiol. Plant. 84:202-208.
These two studies by Greger et al. represent some of the few direct studies of the interaction between Al toxicity, and P nutrient status and physiology.

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

Haug, A. 1984. Molecular aspects of aluminum toxicity. CRC Crit. Rev. Plant Sci. 1:345-373.
Extensive review of molecular aspects of Al toxicity to crop and aother terrestrial plants. Contains a great deal of information regarding Al binding to biomolecules of all sorts, membrane interactions, and a discussion of Al's interaction with Ca metabolism via calmodulin.

Havas, M. 1986. Effects of aluminum on aquatic biota. In: M. Havas, and J.F. Jaworski (eds.), Aluminum in the Canadian Environment. National Research Council of Canada, publication No. 24759, Ottawa, Ontario. pp. 79-127.
Very thourough review of information regarding Al toxicity and content in all groups of aquatic biota. Contains many useful tables and citations, including unpulished information.

Havens, K.E. 1993. Pelagic food web structure in Adirondack Mountain, USA, Lakes of varying acidity. Can. J. Fish. Aquat. Sci. 50:149-155.
Uses large composite data set to examine effects of acidification on food web structure in Adirondack lakes.

Havens, K.E. and R.T. Heath. 1990. Phytoplankton succession during acidification with and without increasing aluminum levels. Environ. Pollut. 68:129-145.
Describes phytoplankton results for enclosures where treatments included long-term reduction in pH both with and without Al additions (200 µg/L). Found similar species succession as other studies, but also showed pronounced

differences between acid and acid+Al treatments. Lower pH limits were different between acid and acid+Al treatments.

Havens, K.E., and J. DeCosta. 1987a. Freshwater plankton community succession during experimental acidification. Arch. Hydrobiol. 111:37-65.
Experimental acidification of a circumeutral, but relatively low ANC lake. Showed sps. shifts resembled those in naturally acidified lakes.

Havens, K.E., and J. DeCosta. 1987b. The role of aluminum contamination in determining phytoplankton and zooplankton responses to acidification. Water Air Soil Pollut. 33:277-293.
Interesting set of bag encloures in which both pH (from neutral to 4.7) and Al (300 µg/L) were manipulated. Acid+Al treatment reduced biomass, but did not change community structure (zooplankton or algae) as compared to acid treatment alone. Authors suggest that this indicates some linkage between acid and metal tolerance.

Havens, K.E., and R.T. Heath. 1990. Phytoplankton succession during acidification with and without increasing aluminum levels. Environ. Pollut. 68:129-145.
Describes phytoplankton results for enclosures where treatments included long-term reduction in pH both with and without Al additions (200 µg/L). Found similar species succession as other studies, but also showed pronounced differences between acid and acid+Al treatments. Lower pH limits were different between acid and acid+Al treatments.

Healey, F.P. 1973. Characteristics of phosphorus deficiency in *Anabaena*. J. Phycol. 9:383-394.
One of the original demonstrations that Pase activity increases when algae are P-limited.

Healey, F.P. and L.L. Hendzel. 1979. Fluorometric measurement of alkaline phosphatase activity in algae. Freshwater Biol. 9:429-439.
One of the 'original' demonstrations that phosphatase activity increases in response to inorganic P-limitation

Healey, F.P., and L.L. Hendzel. 1988. Competition for phosphorus between desmids. J. Phycol. 24:287-292.

Helliwell, S., G.E. Batley, T.M. Florence, and B.G. Lumsden, 1983. Speciation and toxicity of aluminum in a model freshwater. Environ. Technol. Lett. 4:141-144.
Frequently-cited paper that considers pH-dependent Al speciation and its effect on Al toxicity to *Chlorella*. Claimed that Al(OH)2 was the toxic species, but the direct evidence from their model presentation is weak.

Hörnström, E., C. Ekström, and J. Ek, 1993. Plankton and chemical-physical development in six Swedish West Coast lakes under acidic and limed conditions. Can. J. Fish. Aquat. Sci. 50:688-702.
Examined responses of 6 acid lakes in Sweden to liming; both biological and chemical effects discussed, as well as Al and DOC changes.

Hörnström, E., C. Ekström, and M.O. Duraini, 1984. Effects of pH and different levels of aluminum on lake plankton in the Swedish West Coast area. In: L. Nyman, and

B. Ericsson (Eds.), Institute of Freshwater Research, Report no. 61. National Swedish Board of Fisheries, Drottingholm, Sweden. pp. 115-127.
Extremely interesting paper with a great deal of useful observations on Al and plankton communities. Both experimental and descriptive.

Hörnström, E., A. Harbom, F. Edberg and C. Andrén. 1995. The influence of pH on aluminium toxicity in the phytoplankton species *Monoraphidium dybowskii* and *M. griffithii*. Water Air Soil Pollut. 85:817-822.
Comparative study of pH-dependent toxicity of Al to two different green algae. Claims that even small amounts of monomeric Al at circumneutral pH can be toxic, and that the toxicity is species-specific.

Husaini, Y., and L.C. Rai. 1992. pH dependent aluminium toxicity to *Nostoc linckia*: Studies on phosphate uptake, alkaline and acid phosphatase activity, ATP content, and photosynthesis and carbon fixation. J. Plant Physiol. 139:703-707.

Husaini, Y., L.C. Rai, and N. Mallick. 1996. Impact of aluminum, fluoride and fluoraluminate complex on ATPase activity of *Nostoc linckia* and *Chlorella vulgaris*. Biometals 9:277-283.

Hustedt, F. 1939. Systematische und ökologische untersuchungen uber die diatomeen-flora von Java, Bali, und Sumatra nach dem material der Deutschen Limnologischen Sunda-Expedition III. Die ökologischen factorin und ihr einfluss auf die diatomeenflora. Arch. Hydrobiol. Suppl. 16:274-394.
Contains original description of the 5 "Hustedt" pH categories.

Hutchinson, G.E. 1957. A treatise on limnology. Volume 1, part 2 (Chemistry of Lakes). Wiley and Sons, New York. 1015 p.

Huttunen, P. and J. Turkia, 1990. Surface sediment diatom assemblages and lake acidity. In: P. Kauppi, P. Anttila, and K. Kenttamies (eds.), Acidification in Finland. Springer-Verlag, Berlin. pp. 995-1008.

Jansson, M. 1981. Induction of high phosphatase activity by aluminum in acid lakes. Arch. Hydrobiol. 93:32-44.

Jansson, M., G. Persson, and O. Broberg, 1986. Phosphorus in acidified lakes: The example of Lake Gårdsjön, Sweden. Hydrobiologia 139:81-96.
Very interesting treatment of Al-P interactions in Swedish acidified lakes. Claimed that Al reduced P input to the lake by ppt. in soils, productivity still limited by P, yet algal P utilization impaired by Al...probably due to interaction with organic P enzyme recycling. See also Jansson 1981 Arch. Hydrobiol. 93:32 and 92:377.

Kingston, J.C. and H.J.B. Birks, 1990. Dissolved organic carbon reconstructions from diatom assemblages in PIRLA project lakes, North America. Phil. Trans. Roy. Soc. London B 327:279-288.

Kingston, J.C., H.J.B. Birks, A.J. Uutala, B.F. Cumming, and J.P. Smol, 1992. Assessing trends in fishery resources and lake water aluminum from paleolimnological analyses of siliceous algae. Can. J. Fish. Aquat. Sci. 49:116-127.

Kinraide, T.B. 1990. Assessing the rhizotoxicity of the aluminate ion, $Al(OH)_4^-$. Plant Physiol. 94:1620-1625.

Kinraide, T.B., and D.R. Parker. 1987. Cation amelioration of aluminum toxicity in wheat. Plant Physiol. 83:546-551.

Lee, L.H., B. Lustigman, I-Y. Chu, and H.-L. Jou, 1991. Effect of aluminum and pH on the growth of *Anacystis nidulans*. Bull. Environ. Contam. Toxicol. 46:720-726.
Study of effects of Al and pH on *Anacystis nidulans*. Used very poor pH buffering, no speciation, and limited discussion. Some interesting patterns, though.

Lewin, J.C., 1961. The dissolution of silica from diatom walls. Geochim. Cosmochim. Acta 21:182-198.

Lindemann, J., E. Holtkamp, and R. Herrmann, 1990. The impact of aluminium on green algae isolated from two hydrochemically different headwater streams, Bavaria, Germany. Environ. Pollut. 67:61-77.
Examination of Al toxicity in two natural isolates, using semicontinuous cultures for exposures.

Livingston, E.H., H.H. Harper, J.L. Herr. 1994. The use of alum injection to treat stormwater, pp. 31-53 *In:* E.H. Livingston and H.H. Harper (Eds.), Proceedings of the 2nd Annual Conference on Soil and Water Management for Urban Development. Sydney, N.S.W.

Lund, M.A., and E.T. Chester, 1991. The use of aluminium sulphate to control algal blooms and chironomids in Jackadder Lake, Western Australia. Verh. Internat. Verein. Limnol. 24:1129-1133.
Study of alum treatment and its ability to control algal blooms.

Lydén, A., and O. Grahn. 1985. Phytoplankton species composition, biomass and production in Lake Gårdsjön - an acidified cleawater lake in SW Sweden. Ecol. Bull. (Stockholm) 37:195-202.
More evidence that acidified lakes show reduced species diversity, but not reduced biomass or productivity as compared to more circumneutral lakes.

Maurice, C.G., R.L. Lowe, T.M. Burton, and R.M. Stanford, 1987. Biomass and compositional changes in the periphytic community of an artificial stream in response to lowered pH. Water Air Soil Pollut. 33:165-177.

Moran, S.B., and R.M. Moore, 1988. Evidence from mesocosm studies for biological removal of dissolved aluminum from sea water. Nature 335:706-708.

Moran, S.B., and R.M. Moore, 1992. Kinetics of the removal of dissolved aluminum by diatoms in seawater: A comparison with thorium. Geochim. Cosmochim. Acta 56:3365-3374.
Both Moran and Moore papers examine how marine diatoms scavenge Al from seawater. Interesting link between diatoms and Al biogeochemistry.

Morel, F.M.M. 1983. Principles of aquatic chemistry. John Wiley and Sons, New York. 446 p.
Excellent water chemistry textbook,particularly with regards to metal complexation and speciation methods, models, and bioavailability.

Morel, F.M.M. 1987. Kinetics of nutrient uptake and growth in phytoplankton. J. Phycol. 23:137-150.
Extremely thorough treatment of relationship between steady-state and transient models for nutrient uptake, growth, and cell quota.

Morrison, B., J.P. Dempster, and W.J. Manning (eds.), 1992. Effects of acidic pollutants on freshwater plants and animals. Special issue from Glasgow Acid Precipitation conference (1990). Environ. Pollut., Vol. 78.
Good recent review of general acidification effects on freshwater plants, with an emphasis on the role of chemical pollutants (e.g. Al).

Mulholland, P.J., J.W. Elwood, A.V. Palumbo, and R.J. Stevenson, 1986. Effect of stream acidification on periphyton composition, chlorophyll, and productivity. Can. J. Fish. Aquat. Sci. 43:1846-1858.
Examines periphyton communities, biomass, and productivity along gradients of Al and pH in streams. Discusses role of Al-PO_4 complexation and P limitation of productivity.

Muniz, I.P. 1991. Freshwater acidification: Its effects on species and communities of freshwater microbes, plants and animals. Proceedings of the Royal Society of Edinburgh, Section B (Biological 97:227-254.

Nalewajko, C., and B. Paul, 1985. Effects of manipulations of aluminum concentrations and pH on phosphorus uptake and photosynthesis of planktonic communities in two Precambrian Shield lakes. Can J. Fish. Aquat. Sci. 42:1946-1953.
Interesting treatment of pH dependent effect of Al on PO_4 uptake in two Canadian Shield lakes.

Nicholls, K.H., L. Nakamoto, and W. Keller, 1992. Phytoplankton of Sudbury area lakes (Ontario) and relationships with acidification status. Can. J. Fish. Aquat. Sci. 49(Suppl. 1):40-51.
Examines temporal change (1970's - 1980's) in phytoplankton composition in Sudbury, Ontario, lakes relative to acidification-related changes in surface water chemistry (including Al).

Ogburn, R.W. III, and P.L. Brezonik, 1986. Examination of the oligotrophication hypothesis: phosphorus cycling in an acidic Florida lake. Water Air Soil Pollut. 30:1001-1006.
Examines oligotrophication hypothesis for a Florida Lake (McCloud). Maximal P sorption by sediments at pH 4-5.5 lends some support for hypothesis, although macrophyte and phytoplankton uptake and release studies showed no relationship between P dynamics and pH. No mention of Al, although it might be a factor worth examining more closely.

Paasche, E. 1980. Silicon content of five marine plankton diatom species measured with a rapid filter method. Limnol. Oceanogr. 25:474-480.
Paper which describes biogenic silica digestion method using a soda hydrolysis. More gentle that NaOH boiling method for total particulate Si described by Paasche 1973 (Mar. Biol. 19:117-126).

Parent, L., and P.G.C. Campbell. 1994. Aluminum bioavailability to the green alga *Chlorella pyrenoidosa* in acidified synthetic soft water. Environ. Toxicol. Chem. 13(4):587-598.
Very interesting and thorough study of pH-dependence of Al toxicity in algae. Does an excellent job of examining the free ion activity model and H^+ competition for cell surface binding 'protection' of toxicity. Also examines toxicity of Al_{13} polymeric cation.

Parent, L., M.R. Twiss, and P.G.C. Campbell. 1996. Influences of natural dissolved organic matter on the interaction of aluminum with the microalga *Chlorella*: A test of the free-ion model of trace metal toxicity. Environ. Sci. Technol. 30:1713-1720.
Tremendously interesting and revealing study of influence of organically-bound Al. Found that at constant Al activities, that organically bound Al actually has a benefitial effect on growth. Discusses several interesting hypotheses to explain their observations.

Parker, D.R., L.W. Zelazny, T.B. Kinraide. 1989. Chemical speciation and plant toxicity of aqueous aluminum. In: T.E. Lewis (ed.), Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. pp. 117-145.

Perrin, C.J., B. Wilkes, and J.S. Richardson, 1992. Stream periphyton and benthic insect responses to additions of treated acid mine drainage in a continuous-flow on-site mesocosm. Environ. Toxicol. Chem. 11:1513-1525.

Persson, G., and Broberg, O. 1985. Nutrient concentrations in the acidified Lake Gårdsjön: The role of transport and retention of phosphorus, nitrogen and DOC in watershed and lake. Ecol. Bull. (Stockholm) 37:158-175.
Examines whole-catchment geochemistry to assess why Lake Gårdsjön is so oligotropic. Suggests that in-lake P cycling not different from more neutral lakes, but that P loading from catchment soils is reduced, primarily owing to complexation with Al in surface soils.

Pettersson, A., and B. Bergman, 1989. Effects of aluminium on ATP pools and utilization in the cyanobacterium *Anabaena cylindrica*: a model for the in vivo toxicity. Physiol. Plant. 76:527-534.

Pettersson, A., L. Hällbom, and B. Bergman, 1985. Physiological and structural responses of the cyanobacterium *Anabaena cylindrica* to aluminium. Physiol. Plant. 63:153-158.

Pettersson, A., L. Hällbom, and B. Bergman. 1986. Aluminium uptake by *Anabaena cylindrica*. J. Gen. Microbiol. 132:1771-1774.
Not particularly strong paper, but does consider some mechanisms of Al toxicity relative to P uptake and nutrition.

Pettersson, A., L. Hällbom, and B. Bergman. 1988. Aluminium effects on uptake and metabolism of phosphorus by the cyanobacterium *Anabaena cylindrica*. Plant Physiol. 86:112-116.
A fine paper that examines how Al affects P metabolism in *Anabaena*. They did *not* observe reductions in P uptake, but did get enhanced polyphosphate

accumulation, and reduced acid phosphatase activity. Did a nice job of handling $AlPO_4$ precipitation as it affected other assays/results. They suggest that P-starvation is induced by Al.

Pettersson, A., L. Kunst, B. Bergman, and G.M. Roomans, 1985. Accumulation of aluminium by *Anabaena cylindrica* into polyphosphate granules and cell walls: an x-ray energy-dispersive microanalysis study. J. Gen. Microbiol. 131:2545-2548.
Three physiological studies by Pettersson et al. which examine Al impacts on *Anabaena*, much of which involving some aspect of P metabolism or storage.

Pillsbury, R.W. and J.C. Kingston, 1990. The pH-independent effect of aluminum on cultures of phytoplankton from an acidic Wisconsin lake. Hydrobiologia 194:225-233.
Natural community experiment showing species-specific responses of phytoplankton community to Al and pH manipulations. Includes also some interesting observations on ecology of *Asterionella ralfsii*.

Planas, D., L. Lapierre, G. Moreau, and M. Allard, 1989. Structural organization and species composition of a lotic periphyton community in response to experimental acidification. Can. J. Fish. Aquat. Sci. 46:827-835.
Examines periphyton community responses to experimental acidification. Also considers role of Al toxicity.

Rai, L.C., Y. Husaini, and N. Mallick. 1996. Physiological and biochemical responses of *Nostoc linckia* to combined effects of aluminium, fluoride and acidification. Environ. Exp. Bot. 36:1-12.

Ravera, O., 1986. Effects of experimental acidification on freshwater environments. Experientia 42:507-516.
Literature review of freshwater experimental acidification studies. Includes algal responses, and mentions role of Al.

Reynolds, C.S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press. Cambridge, England. 384 p.

Riseng, C.M., R.W. Gensemer, and S.S. Kilham, 1991. The effect of pH, aluminum, and chelator manipulations on the growth of acidic and circumneutral species of *Asterionella*. Water Air Soil Pollut. 60:249-261.
Comparative study of pH tolerance in both *A. ralfsii* and *A. formosa* as influenced by Al toxicity and EDTA manipulation.

Romo, S. and E. Becares. 1994. Water management of two shallow urban eutrophic lakes. Water Sci. Technol. 30:299-302.

Rueter, J.G. Jr., K.T. O'Reilly, and R.R. Petersen. 1987. Indirect aluminum toxicity to the green alga *Scenedesmus* through increased cupric ion activity. Environ. Sci. & Technol. 21:435-438.
Important paper in understanding the effects of Al^{+3} ions on toxicity in algae. Demonstrates that Al indirectly induced Cu toxicity. See also Gensemer (1991) for the opposite demonstration.

Schindler, D.W. 1980. Experimental acidification of a whole lake: A test of the oligotrophication hypothesis. In: D. Drablos, and A. Tollan (eds.), Ecological impacts of acid precipitation: Proceedings of an international conference, Sandefjord, Norway, March 11-14, 1980. SNSF project, Oslo, Norway. pp. 370-374.
Initial results from L. 223 (ELA) acidification show little support for oligotrophication hypothesis. Little mention of Al, although their acidification method would underestimate its importance.

Schindler, D.W. 1988. Experimental studies of chemical stressors on whole lake ecosystems. Verh. internat. Verein. limnol. 23:11-41.
Baldi lecture at New Zealand S.I.L. meeting. General discussion and review of the utility of whole-lake ecosystem manipulations in understanding anthropogenic stress on lakes.

Schindler, D.W., S.E. Bayley, P.J. Curtis, B.R. Parker, M.P. Stainton, and C.A. Kelly, 1992. Natural and man-caused factors affecting the abundance and cycling of dissolved organic substances in precambrian shield lakes. Hydrobiologia 229:1-21.
Among other things, discusses role of Al in the reduction of DOC concentrations in freshwaters. Impact on phytoplankton also mentioned.

Schindler, D.W., T.M. Frost, K.H. Mills, P.S.S. Chang, I.J. Davies, L. Findlay, D.F. Malley, J.A. Shearer, M.A. Turner, P.J. Garrison, C.J. Watras, K. Webster, J.M. Gunn, P.L. Brezonik, and W.A. Swenson. 1991. Comparisons between experimentally- and atmospherically-acidified lakes druing stress and recovery. Proceedings of the Royal Society of Edinburgh, Section B (Biological 97:193-226.

Schindler, D.W., K.H. Mills, D.F. Malley, D.L. Findlay, J.A. Shearer, I.J. Davies, M.A. Turner, G.A. Linsley, and D.R. Cruikshank. 1985. Long-term ecosystem stress: the effects of years of experimental acidifcation on a small lake. Science 228:1395-1401.
Excellent treatment of main results from ELA acidification of lake 223. Demonstrates that species responses were more sensitive than community metabolism measurements. Also described reduced fish recruitment as reason for decline in fish populations.

Shearer, J.A., E.J. Fee, E.R. DeBruyn, and D.R. DeClercq, 1987. Phytoplankton primary production and light attenuation responses to the experimental acidification of a small Canadian Shield lake. Can. J. Fish. Aquat. Sci. 44:83-90.
Although phytoplankton production increased as L. 223 (ELA) acidified, this increase was the same as observed at control sites. This must be the main evidence presented in Schindler's big 1985 Science paper. Transparency did increase, though, along with DOC decrease (owing to Al?). More evidence to reject the oligotrophication hypothesis, at least for this system. Al concentrations low, however, owing to acid application directly to the lake.

Smith, M.A., 1990. The ecophysiology of epilithic diatom communities of acid lakes in Galloway, southwest Scotland. Phil. Trans. R. Soc. Lond. B 327:251-256.
Has some interesting data concerning interactions between phosphatase, Al, and pH as they affect periphyton communities in acid lakes.

Smol, J.P., I.R. Walker, and P.R. Leavitt. 1991. Paleolimnology and hindcasting climatic trends. Verh. Internat. Verein. Limnol. 24:1240-1246.

Sparling, D.W., and T.P. Lowe. 1996. Environmental hazards of aluminum to plants, invertebrates, fish, and wildlife. Rev. Environ. Contamin. Toxicol. 145:1-127.
Thorough recent review of Al toxicity and hazards. Not very detailed, nor is the algae section very good. But has nice tables of some toxicity data, and lots of bioaccumulation data.

Steinberg, C., and R. Putz, 1991. Epilithic diatoms as bioindicators of stream acidification. Verh. Internat. Verein. Limnol. 24:1877-1880.

Steinberg, C.E.W. and Th. Tayarani-Dastmalian. 1993. Changes in metal speciation in a sediment core from an acidified black forest lake Wildsee, Germany. Water Air Soil Pollu. 68:525-537.

Stokes, P.M. 1986. Ecological effects of acidification on primary producers in aquatic ecosystems. Water Air Soil Pollut. 30:421-438.

Sullivan, T.J., D.F. Charles, J.P. Smol, B.F. Cumming, A.R. Selle, D.R. Thomas, J.A. Bernert, and S.S. Dixit, 1990. Quantification of changes in lakewater chemistry in response to acidic deposition. Nature 345:54-58.
Suggests that acidification is regionally important in the Adirondacks, but has happened to less of an extent than previously thought. Their arguments are based both on DI-inferred pH and alkalinity reconstructions, and how these interact with current geochemical models. Also discusses role of Al.

Taylor, G.J. 1988. The physiology of aluminum phytotoxicity. In: Sigel, H., and A. Sigel, Metal ions in biological systems, Vol. 24: Aluminum and its role in biology. Marcel Dekker, Inc., New York. pp. 123-163.

Tilman, D. 1982. Resource competition and community structure. MPB #17. Princeton University Press, Princeton, New Jersey. 296 p.
The classic book outlining his mechanistic resource competition theory, with most examples pertaining to phytoplankton communities.

Tilman, D., S.S. Kilham, and P. Kilham. 1982. Phytoplankton community ecology: the role of limiting nutrients. Ann. Rev. Ecol. Syst. 13:349-372.

Törnqvist, L., and A. Claesson, 1987. The influence of aluminum on the cell-size distribution of two green algae. Environ. Exper. Bot. 27:481-488.
Interesting study of morphological changes induced by Al in two species of green algae.

Van Bennekom, A.J., 1981. On the role of aluminium in the dissolution kinetics of diatom frustules. In: R. Ross (ed.), Proceedings of the sixth symposium on recent and fossil diatoms. Otto Koeltz, Koenigstein. pp. 445-456.

Van Bennekom, A.J., A.G.J. Buma, and R.F. Nolting, 1991. Dissolved aluminium in the Weddell-Scotia confluence and effect of Al on the dissolution kinetics of biogenic silica. Mar. Chem. 35:423-434.

Van Bennekom, A.J., J.H.F. Jansen, S.J. van der Gaast, J.M. van Iperen, and J. Pieters, 1989. Aluminium-rich opal: An intermediate in the preservation of biogenic silica in the Zaire (Congo) deep-sea fan. Deep-Sea Res. 36:173-190. van Bennekom's studies examine various aspects of how Al impacts diatom frustule dissolution and formation of biogenic silica deposits.

Van Beusekom, J.E.E., 1988. Distribution of dissolved aluminium in surface waters of the North Sea: Influence of biological processes. Mitt. Geol. Palaont. Inst. 65:1-20.

Welch, E.B. and G.D. Schrieve. 1994. Alum treatment effectiveness and longevity in shallow lakes. Hydrobiologia 275-276:423-431.

Willén, E. 1991. Planktonic Diatoms - an ecological review. Algol. Stud. 62:69-106. Comprehensive review of diatom ecology containing good selection of useful citations.

Zimnik, P.R., and J. Sneddon, 1988. Binding and removal of aluminum ions in waters by an algal biomass. Analytical Letters 21:1383-1396.

3.2 **Aquatic Macrophytes (Chapter 3)**

Albers, P.H. and M.B. Camardese. 1993. Effects of acidification on metal accumulation by aquatic plants and invertebrates. 1. Constructed wetlands. Environ. Toxicol. Chem. 12:959-967.

Almer, B., W. Dickson, C. Ekström, and E. Hörnström, 1978. Sulfur pollution and the aquatic ecosystem. In: Nriagu, J.O., Sulphur in the environment. Part II: Ecological impacts. John Wiley and Sons, New York. pp. 271-311.
Excellent early review of acidification impacts on freshwater systems; includes macrophyte impacts.

Andersson, M. 1988. Toxicity and tolerance of aluminum in vascular plants. Water Air Soil Pollut. 39:439-462.

Burrows, W.D. 1977. Aquatic aluminum: Chemistry, toxicology, and environmental prevalence. CRC Crit. Rev. Environ. Control 7:167-216.
Excellent, thourough review of Al chemistry and toxicology, although its a bit dated.

Catallo, W.J. 1993. Ecotoxicology and wetland ecosystems: current understanding and future needs. Environ. Toxicol. Chem. 12:2209-2224.

Crowder, A., 1991. Acidification, metals and macrophytes. Environ. Pollut. 71:171-203.
Good recent review of acidification and metal toxicity, specifically with respect to macrophyte communities.

Delhaize, E. and P.R. Ryan. 1995. Aluminum toxicity and tolerance in plants. Plant Physiol. 107:315-321.

Denton, J., and D.H. Oughton. 1993. The use of an acid solochrome azurine stain to detect and assess the distribution of aluminium in Sphagnum moss. Ambio 22:19-21.
Describes a staining technique for localizing Al deposits in Sphagnum moss, that was originally used for bone samples.

Dillon, P.J., N.D. Yan, and H.H. Harvey, 1984. Acidic deposition: Effects on aquatic ecosystems. CRC Crit. Rev. Environ. Control 13:167-194.

Engleman, C.J., Jr. and W.F. McDiffett. 1996. Accumulation of aluminum and iron by bryophytes in streams affected by acid-mine drainage. Environ. Pollut. 94:67-74.

Grahn, O., H. Hultberg, and L. Landner. 1974. Oligotrophication - a self-accelerating process in lakes subjected to excessive supply of acid substances. Ambio 3:93-94.
Describes general biological impoverishment of Swedish lakes subject to acidification. Original reference to the 'oligotrophication hypothesis' describing how acid lakes become more oligotrophic.

Gostomski, F., 1990. The toxicity of aluminum to aquatic species in the US. Environ. Geochem. Health 12:51-54.

Guilizzoni, P. 1991. The role of heavy metals and toxic materials in the physiological ecology of submeersed macrophytes. Aquat. Bot. 41:87-109.

Haug, A. 1984. Molecular aspects of aluminum toxicity. CRC Crit. Rev. Plant Sci. 1:345-373.
Extensive review of molecular aspects of Al toxicity to crop and aother terrestrial plants. Contains a great deal of information regarding Al binding to biomolecules of all sorts, membrane interactions, and a discussion of Al's interaction with Ca metabolism via calmodulin.

Haug, A.R., and C.R. Caldwell. 1985. Aluminum toxicity in plants: The role of the root plasma membrane and calmodulin. In: J.B. St. John, E. Berlin, and P.C. Jackson (eds.), Frontiers of membrane research in agriculture. Rowan and Allanheld, Ottawa. pp. 359-381.

Havas, M., 1986. Effects of aluminum on aquatic biota. In: M. Havas, and J.F. Jaworski (eds.), Aluminum in the Canadian Environment. National Research Council of Canada, publication No. 24759, Ottawa, Ontario. pp. 79-127.
Both of these two papers are good general reviews of acidification impacts that include macrophyte information.

Hendrey, G.R., K. Baalsrud, T.S. Traaen, M. Laake, and G. Raddum. 1976. Acid Precipitation: some hydrological changes. Ambio 5:224-227.
Basic review of early literature on this subject.

Jackson, L.J. and J. Kalff. 1993. Patterns in metal content of submerged aquatic macrophytes: the role of plant growth form. Freshwater Biol. 29:351-359.
Examines relationships between sediment metal content (including Al) and bioaccumulation in littoral macrophytes in southern Quebec lakes. Sediment geochemistry differences was less important than the physical growth form of the submersed vegetation beds in controlling this relationship.

Jackson, L.J., J. Kalff and J.B. Rasmussen. 1993. Sediment pH and redox potential affect the bioavailability of Al, Cu, Fe, Mn and Zn to rooted aquatic macrophytes. Can. J. Fish. Aquat. Sci. 50:143-148.
Examined role of sediment pH, organic matter, and redox conditions in controlling metal bioaccumulation in aquatic macrophytes.

Kochian, L.V. 1995. Cellular mechanisms of aluminum toxicity and resistance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46:237-260.

Lehtonen, J. 1989. Effects of acidification on the metal levels in aquatic macrophytes in Espoo, S. Finland. Ann. Bot. Fennici 26:39-50.

Maessen, M., J.G.M. Roelofs, M.J.S. Bellemakers, and G.M. Verheggen, 1992. The effects of aluminium, aluminium/calcium ratios and pH on aquatic plants from poorly buffered environments. Aquat. Bot. 43:115-127.
Study of pH tolerance and Al toxicity in aquatic plants. Claims that Al had no effect on low pH tolerance - but the Al chemistry was not well done and, hence, somewhat inconclusive.

Ogburn, R.W. III, and P.L. Brezonik, 1986. Examination of the oligotrophication hypothesis: phosphorus cycling in an acidic Florida lake. Water Air Soil Pollut. 30:1001-1006.

Roberts, D.A., R. Singer, and C.W. Boylen. 1985. The submersed macrophyte communities of Adirondack Lakes (New York, U.S.A.) of varying degrees of acidity. Aquat. Bot. 21:219-235.
Compares macrophyte community structure in 9 Adirondack lakes to water pH-related chemistry variables.

Sparling, D.W., and T.P. Lowe. 1996. Environmental hazards of aluminum to plants, invertebrates, fish, and wildlife. Rev. Environ. Contamin. Toxicol. 145:1-127.
Thorough recent review of Al toxicity and hazards. Not very detailed, nor is the algae section very good. But has nice tables of some toxicity data, and lots of bioaccumulation data.

Sprenger, M., and A. McIntosh. 1989. Relationship between concentrations of aluminum, cadmium, lead, and zinc in water, sediments and aquatic macrophytes in six acidic lakes. Arch. Environ. Contam. Toxicol. 18:225-231.

3.3 **Other higher plants**

Andersson, M., 1988. Toxicity and tolerance of aluminum in vascular plants. Water Air Soil Pollut. 39:439-462.

Anonymous, 1981. Aluminum: Chemistry, analysis, and biology. Environmental geochemistry report No. 1981/2. McMaster University, Department of Biology, Hamilton, Ontario. 174 p.

Baker, J.P., and S.W. Christensen, 1991. Effects of acidification on biological communities in aquatic ecosystems. In: Charles, D.F. (ed.), Acidic deposition and aquatic ecosystems: Regional case studies. Springer-Verlag, New York, NY. pp. 83-106.

Bi, S.P. and Correll, D.L. 1996. A review of the toxicity of aluminum on plants. Abstracts of papers of the American Chem. Soc. 212:43.

Birchall, J.D., 1990. The role of silicon in biology. Chemistry in Britain, February, pp. 141-144.
A review which includes Birchall's interesting contention that part of the importance of Si in biological systems is to help control against Al toxicity.

Burrows, W.D., 1977. Aquatic aluminum: Chemistry, toxicology, and environmental prevalence. CRC Crit. Rev. Environ. Control 7:167-216.

Cronan, C.S., R. April, R.J. Bartlett, P.R. Bloom, C.T. Driscoll, S.A. Gherini, G.S. Henderson, J.D. Joslin, J.M Kelly, R.M. Newton, R.A. Parnell, H.H. Patterson, D.J. Raynal, M. Schaedle, C.L. Schofield, E.I. Sucoff, H.B. Tepper, and F.C. Thorton, 1989. Aluminum toxicity in forests exposed to acidic deposition: the ALBIOS results. Water Air Soil Pollut. 48:181-192.

Crowder, A., 1991. Acidification, metals and macrophytes. Environ. Pollut. 71:171-203.
A review of the effects of acidification and metals (including Al) on freshwater macrophytes.

Dallagnol, M., J.H. Bouton, and W.A. Parrott. 1996. Screening methods to develop alfalfa germplasms tolerant of acid, aluminum toxic soils. Crop Sci. 36:64-70.

Deandrade, L.R.M., M. Ikeda, and J. Ishizuka. 1996. Effect of nitrogen sources on aluminum toxicity in wheat varieties differing in tolerance to aluminum. Soil Sci. Plant Nutrition 42:651-657.

DeCarvalho, M.M., D.G. Edwards, C.J. Asher and C.S. Andrew. 1982. Effects of aluminium on nodulation of two *Stylosanthes* species grown in nutrient solution. Plant and Soil 64:141-152.

Drablos, D., and A. Tollan (eds.), 1980. Ecological impact of acid precipitation. Proceedings of an international conference, Sandfjord, Norway, March 11-14, 1980. SNSF Project, Oslo, Norway. 383 p.

Foy, C.D., A.L. Fleming and G.C. Gerloff. 1972. Differential aluminum tolerance in two snapbean varieties. Agronomy 61:815-818.

Foy, C.D., A.L. Fleming and J.W. Schwartz. 1973. Opposite aluminum and manganese tolerances of two wheat varieties. Agronomy 65:123-126.

Foy, C.D., A.L. Fleming and W.H. Armiger. 1969. Aluminum tolerance of soybean varieties in relation to calcium nutrition. Agronomy Journal 61:505-511.
The previous three papers represent a subset of several studies by Foy et al. that examine impacts of Al on crop plants.

Franco, A.A. and D.N. Munns. 1982. Acidity and aluminum restraints on nodulation, nitrogen fixation, and growth of *Phaseolus vulgaris* in solution culture. Soil Sci. Soc. Am. J. 46:296-301.
One of several studies examining impacts of Al on nodulation and N fixation in soybeans.

Gostomski, F. 1990. The toxicity of aluminum to aquatic species in the US. Environ. Geochem. Health 12:51-54.

Haug, A., 1984. Molecular aspects of aluminum toxicity. CRC Crit. Rev. Plant Sci. 1:345-373.
Extensive review of molecular aspects of Al toxicity to crop and other terrestrial plants. Contains a great deal of information regarding Al binding to biomolecules of all sorts, membrane interactions, and a discussion of Al's interaction with Ca metabolism via calmodulin.

Havas, M., 1986. Effects of aluminum on aquatic biota. In: M. Havas, and J.F. Jaworski (eds.), Aluminum in the Canadian Environment. National Research Council of Canada, publication No. 24759, Ottawa, Ontario. pp. 79-127.

Hoyt, P.B. and M. Nyborg. 1971. Toxic metals in acid soil: 1. Estimation of plant-available aluminum. Soil Sci. Soc. Amer. Proc. 35:236-240.
Older treatment of Al bioavailability in soils.

Hunter, D., and D.S. Ross, 1991. Evidence for a phytotoxic hydroxy-aluminum polymer in organic soil horizons. Science 251:1065-1058.
Using NMR spectra, they identify a significant abundance of an Al_{13} polymer in soil solutions. They claim it could have a significant impact on Al toxicity to plants, but present no biological data of their own.

Hutchinson, G.E., 1943. The biogeochemistry of Al and of certain related elements. Quart. Rev. Biol. 18:331-363.

Kinraide, T.B., and D.R. Parker, 1987. Cation amelioration of aluminum toxicity in wheat. Plant Physiol. 83:546-551.

Lawrence, G.B. and M.B. David. 1997. Response of aluminum solubility to elevated nitrification in soil of a red spruce stand in Eastern Maine. Environ. Sci. Technol. 31:825-830.
Interesting examination of how Al solubility in soils can impact one aspect of N cycling in boreal forests.

Long, F.L. and C.D. Foy. 1970. Plant varieties as indicators of aluminum toxicity in the A2 horizon of a Norfolk soil. Agronomy Journal 62:679-681.

Maessen, M., J.G.M. Roelofs, M.J.S. Bellemakers, and G.M. Verheggen, 1992. The effects of aluminium, aluminium/calcium ratios and pH on aquatic plants from poorly buffered environments. Aquat. Bot. 43:115-127.

Morimura, S. and H. Matsumoto. 1978. Effect of aluminium on some properties and template activity of purified pea DNA. Plant Cell Physiol. 19:429-436.

Morrison, B., J.P. Dempster, and W.J. Manning (eds.), 1992. Effects of acidic pollutants on freshwater plants and animals. Special issue from Glasgow Acid Precipitation conference (1990). Environ. Pollut., Vol. 78.

Mugwira, L.M., V.T. Sapra, S.U. Patel and M.A. Choudry. 1981. Aluminum tolerance of triticale and wheat cultivars developed in different regions. Agronomy Journal 73:470-474.

Naidoo, G., J. McD. Stewart and R.J. Lewis. 1978. Accumulation sites of Al in snapbean and cotton roots. Agronomy Journal 70:489-492.

Orellana, R.G., C.D. Foy and A.L. Fleming. 1975. Effect of soluble aluminum on growth and pathogenicity of *Verticillium albo-atrum* and *Whetzelinia sclerotiorum* from sunflower. Phytopathology vol?:202-205.

Parker, D.R., L.W. Zelazny, T.B. Kinraide, 1989. Chemical speciation and plant toxicity of aqueous aluminum. In: T.E. Lewis (ed.), Environmental chemistry and toxicology of aluminum. Lewis Publishers, Inc., Chelsea, MI. pp. 117-145. Excellent review of Al toxicity to plants, and the importance of chemical speciation. Along with next study below, discusses the formation and potential importance of the presumably toxic Al_{13} polymeric cation.

Parker, D.R., T.B. Kinraide, and L.W. Zelazny, 1989. On the phytotoxicity of polynuclear hydroxy-aluminum complexes. Soil Sci. Soc. Am. J. 53:789-796.

Pintro, J., J. Barloy, and P. Fallavier. 1996. Aluminum effects on the growth and mineral composition of corn plants cultivated in nutrient solution at low aluminum activity. J. Plant Nutr. 19:729-741.

Rasmussen, H.P. 1968. The mode of entry and distribution of aluminum in *Zea mays*. Electron microprobe X-ray analysis. Planta 81:28-37.

Sartain, J.B. and E.J. Kamprath. 1975. Effect of liming a highly Al-saturated soil on the top and root growth and soybean nodulation. Agronomy 67:507-510.

Shuman, L.M., D.O. Wilson, and E.L. Ramseur. 1991. Testing aluminum-chelate equilibria models using sorghum root growth as a bioassay for aluminum. Water Air Soil Pollut. 57-58:149-158.

Sigel, H., and A. Sigel, 1988. Metal ions in biological systems, Vol. 24: Aluminum and its role in biology. Marcel Dekker, Inc., New York. 424 p.
Collection of papers regarding Al. Mostly deals with human toxicity, but contains one good chapter on chemistry, another Driscoll chapter, and 2 good chapters on higher plant toxicity.

Strid, H. 1996. Effects of root-zone temperature on aluminum toxicity in two cultivars of spring wheat with different resistance to aluminum. Physiol. Plant. 97:5-12.

Taylor, G.J., 1988. The physiology of aluminum phytotoxicity. In: Sigel, H., and A. Sigel, Metal ions in biological systems, Vol. 24: Aluminum and its role in biology. Marcel Dekker, Inc., New York. pp. 123-163.

Taylor, G.J., 1988. The physiology of aluminum tolerance. In: Sigel, H., and A. Sigel, Metal ions in biological systems, Vol. 24: Aluminum and its role in biology. Marcel Dekker, Inc., New York. pp. 165-198.
Both of these papers (above) by Taylor review Al toxicity and tolerance in higher plants.

Taylor, G.J., 1989. Multiple metal stress in *Triticum aestivum*. Differentiation between additive, multiplicative, antagonistic, and synergistic effects. Can. J. Bot. 67:2272-2276.
Examines interaction between Ni and Al uptake in wheat, and discusses whether or not effects are additive, synergistic, or antagonistic.

Taylor, G.J., K.J. Stadt, and M.R.T. Dale, 1991. Modeling the phytotoxicity of aluminum, cadmium, copper, manganese, nickel, and zinc using the Weibull frequency distribution. Can. J. Bot. 69:359-367.

Unkovich, M.J., P. Sanford, and J.S. Pate. 1996. Nodulation and nitrogen-fixation by subterranean clover in acid soils as influenced by lime application, toxic aluminum, soil mineral N, and competition from annual ryegrass. Soil Biol. Biochem. 28:639-648.

Waters, B.M. and D.G. Blevins. 1996. Effects of aluminum toxicity on components of root apex redox systems. Plant Phys. 111:132-142.

Wood, J.M., 1985. Effects of acidification on the mobility of metals and metalloids: An overview. Environ. Health Perspect. 63:115-119.
Review of acidification-induced metal exposures, whether or not various metals are essential biologically, and what impact this has on their toxicity. Raises interesting issues for Al considering it is not a biologically essential metal.

Žel, J. and L. Bevc. 1993. Effects of aluminum on mineral content of mycorrhizal fungi in vitro. Water Air Soil Pollut. 71:271-279.

Žel, J., M. Schara, J. Svetek, M. Nemec and N. Gogala. 1993. Influence of aluminum on the membranes of mycorrhizal fungi. Water Air Soil Pollut. 71:101-109.
Studies by Zel et al. examine influence of Al on micorrhizal fungi associated with terrestrial plant roots.

4 Aquatic Animals

4.1 Invertebrates (Chapter 4)

Albers, P.H., and M.B. Camardese. 1993. Effects of acidification on metal accumulation by aquatic plants and invertebrates. 2. Wetlands, ponds, and small lakes. Environ. Toxicol. Chem. 12: 969-976.
Acidified waters are often deficient in taxa rich in Ca such as Gastropoda, Pelecypoda, and Crustacea. Thus, any effects of Al on terrestrial animals are likely indirect, working in concert with H^+ to eliminate Ca-rich invertebrate food sources.

Almer, B., W. Dickson, C. Ekström, and E. Hörnström. 1978. Sulfur pollution and the aquatic ecosystem, p. 271-311. *In* J.O. Nriagu [ed.], Sulphur in the environment. Part II: Ecological Impacts. John Wiley and Sons, New York.

Anonymous, 1981. Aluminum: Chemistry, analysis, and biology. Environmental geochemistry report No. 1981/2. McMaster University, Department of Biology, Hamilton, Ontario. 174 p.
An early review of Al. R. Playle was one of the "anonymous" authors. Nearly of historical interest more than anything.

Baker, J.P., and S.W. Christensen. 1991. Effects of acidification on biological communities in aquatic ecosystems, p.83-106. *In* D.F. Charles [ed.], Acidic deposition and aquatic ecosystems: Regional case studies. Springer-Verlag, New York, NY.

Bendell Young, L., and H.H. Harvey. 1991. Metal concentrations in chironomids in relation to the geochemical characteristics of surficial sediments. Arch. Environ. Contam. Toxicol. 21: 202-211.
A survey of six soft water lakes. They found that the amount of Al in chironomid larvae had a negative correlation with organic matter in sediments, presumably because organic matter reduced the availability of Al to the invertebrates.

Bendell, B.E., and D.K. McNicol. 1987. Fish predation, lake acidity, and the composition of aquatic insect assemblages. Hydrobiol. 150: 193-202.
The influence of predators on aquatic invertebrates was demonstrated. They showed that the presence or absence of fish was an important factor in determining aquatic insect assemblages, with larger Notonectidae, Corixidae, Dytiscidae, and Chaoboridae abundant in fishless lakes.

Berrill, M., L. Hollett, A. Margosian, and J. Hudson. 1985. Variation in tolerance to low environmental pH by the crayfish *Orconectes rusticus*, *O. propinquus*, and *Cambarus rubustus*. Can. J. Zool. 63: 2586-2589.
At acidic pH, acidity alone is so toxic to crayfish that Al has no additional effect on survival of *Orconectes propinquus*, *Orconectes rusticus*, and *Cambarus robustus* (15-d exposures to pH 4.5-5.0, 60 µM Ca, Al ~1,000-2,000 $\mu g \cdot L^{-1}$).

Bukavackas, P.A. and W. Shaw. 1997. Effects of base addition and brook trout (*Salvelinus fontinalis*) introduction on the plankton community of an acidic Adirondack lake. Can. J. Fish. Aq. Sci. 54:1367-1376.

Burton, T.M., and J.W. Allan. 1986. Influence of pH, aluminum, and organic matter on stream invertebrates. Can. J. Fish. Aquat. Sci. 43: 1285-1289.
In 28-d experiments using *Asellus* (=*Caecidotea*) *intermedius* (isopoda), *Pycnopsyche guttifer* and *Lepidostoma liba* (caddis flies), *Nemoura* sp. (stonefly), and *Physella heterostropha* (gastropoda), DOC (42-47 $mg \cdot L^{-1}$) and citrate protected against mortality caused by 500 $\mu g \cdot L^{-1}$ Al (pH 4.0-4.2, low alkalinity water).

Correa, M., R. Coler, C.-M. Yin, and E. Kaufman. 1986. Oxygen consumption and ammonia excretion in the detritivore caddisfly *Limnephillus* sp. exposed to low pH and aluminum. Hydrobiol. 140: 237-241.
At fairly reasonable Al concentrations, caddisfly larvae (*Limnephilus* sp.) without their cases showed no changes in oxygen consumption when exposed to pH 4.0 in the presence or absence of 300 $\mu g \cdot L^{-1}$ Al. In these larvae, NH_3 excretion increased slightly (but significantly) in the pH 4.0 exposure, but there was no change in NH_3 excretion in the pH 4.0 plus 300 $\mu g \cdot L^{-1}$ Al exposure. The authors thought that perhaps Al was mitigating some of the effects of H^+ alone; in any case, there was no change in oxygen consumption in response to environmentally realistic Al concentrations in these insects with their gills exposed.

Correa, M., R.A. Coler, and C.-M. Yin. 1985. Changes in oxygen consumption and nitrogen metabolism in the dragonfly *Somatochlora cingulata* exposed to aluminum in acid waters. Hydrobiol. 121: 151-156.
Correa et al. showed that oxygen consumption by dragonfly nymphs (*Somatochlora cingulata*) decreased at low pH (pH 4.2 and 3.6), and at pH 4.2 was lower still in the presence of high concentrations of Al (10 to 30 $mg \cdot L^{-1}$). Ammonia excretion also decreased as pH decreased and as Al increased, possibly indicating a switch away from protein catabolism by the nymphs. The effect of Al at high concentrations was seen as additive to that of H^+.

Dillon, P.J., N.D. Yan, and H.H. Harvey. 1984. Acidic deposition: Effects on aquatic ecosystems. CRC Crit. Rev. Environ. Control 13: 167-194.
A good review, but somewhat dated, and not that much on Al and invertebrates.

Drablos, D., and A. Tollan [eds.]. 1980. Ecological impact of acid precipitation. Proceedings of an international conference, Sandfjord, Norway, March 11-14, 1980. SNSF Project, Oslo, Norway. 383 p.
Of historical interest, but not a lot of information useful for this section.

Eriksen, C.H., and J.E. Mœur. 1990. Respiratory functions of motile tracheal gills in ephemeroptera nymphs, as exemplified by *Siphlonurus occidentalis* Eaton p. 109-118. *In* Mayflies and stoneflies, life histories and biology. (Campbell, I.C., Ed.) Kluwer Academic Publishers.

Cuticular gas transfer also occurs at the abdomen of these insects. e.g. not just at the gills.

Fjellheim, A., and G.G. Raddum. 1992. Recovery of acid-sensitive species of Ephemeroptera, Plecoptera, and Trichoptera in River Audna after liming. Environ. Pollut. 78: 173-178.
Liming of the River Audna (Norway) was successful in allowing recolonization by acid-sensitive species of mayflies, stoneflies, and caddis flies.

France, R.L., and P.M. Stokes. 1987. Influence of manganese, calcium, and aluminum on hydrogen ion toxicity to the amphipod *Hyalella azteca*. Can. J. Zool. 65: 3071-3078.
France and Stokes showed for *Hyalella azteca* (amphipoda) that H^+ toxicity was of primary importance, with a significant ($P<0.05$) increase in toxicity at pH 4.8 with 400 $\mu g \cdot L^{-1}$ Al (8-d exposures, Ca =50 µM). They felt that mortality of *Hyalella* during springmelt pulses would be primarily due to H^+, and only secondarily due to Al. They found no protective effect of 50-200 µM Ca on H^+ or Al toxicity (pH 4.0-5.3, Al =250-700 $\mu g \cdot L^{-1}$), but the range of Ca concentrations used may have been too narrow.

Gherhardt, A. 1993. Review of impact of heavy metals on stream invertebrates with special emphasis on acid conditions. Water Air Soil Pollut. 66: 289-314.
Check this review again, for other insights into Al and for other references.

Gostomski, F. 1990. The toxicity of aluminum to aquatic species in the US. Environ. Geochem. Health 12: 51-54.

Guerold, F., L. Giamberini, J.-L. Tourmann, J.-C. Pihan, and R. Kaufmann. 1995. Occurrence of aluminium in chloride cells of *Perla marginata* (Plecoptera) after exposure to low pH and elevated aluminum concentration. Bull. Environ. Contam. Toxicol. 54: 620-625.
Aluminum was localized in chloride cells of nymphs of *Perla marginata*, an acid sensitive stonefly. *P. marginata* were held for 21 d in synthetic, acidic soft water (pH 5.2, Ca =20 µM) with Al (600 $\mu g \cdot L^{-1}$) or for 21 d in synthetic soft water (pH 6.8, Ca =80 µM) with no added Al (30 $\mu g \cdot L^{-1}$ Al), then were examined for Al using laser microprobe mass spectrometry and histochemical staining (aluminon) of histological sections. Aluminum was localized in Al-exposed stoneflies in the gut contents, cuticle, and chloride cells on the gills and thorax. Localization of Al on the chloride cells suggests that an effect of Al on aquatic invertebrates is on ion regulation.

Hörnström, E., C. Ekström, and J. Ek. 1993. Plankton and chemical-physical development in six Swedish West Coast lakes under acidic and limed conditions. Can. J. Fish. Aquat. Sci. 50: 688-702.
For plankton section.

Hörnström, E., C. Ekström, and M.O. Duraini. 1984. Effects of pH and different levels of aluminum on lake plankton in the Swedish West Coast area, p.115-127. *In* L. Nyman, and B. Ericsson [eds.], Institute of Freshwater Research, Report no. 61. National Swedish Board of Fisheries, Drottingholm, Sweden.

Hall, R.J. 1994. Responses of benthic communities to episodic acid disturbances in a lake outflow stream at the Experimental Lakes Area, Ontario. Can. J. Fish. Aquat. Sci. 51: 1877-1892.
Addition of acid to a stream in the Experimental Lakes Area, northwestern Ontario, resulted in decreased benthic density and increased drift of invertebrates such as *Similium* sp. and *Ectemnia* sp. (blackflies), *Tanytarsus* sp., *Eukiefferiella* sp., *Procladius* sp. (chironomids), and *Baetis* sp. (mayfly) when stream pH was lowered for 4 d from pH ~6.8 to pH 5.0, 4.5, and 4.0 (Ca ~100 µM). Increases in stream Al content in the pH 4.0 and 4.5 experiments were too small (from ~1 to ~11 $\mu g \cdot L^{-1}$ inorganic monomeric Al; from ~40 to ~50 $\mu g \cdot L^{-1}$ total Al) to be a significant additive effect to that of decreased pH. The author concluded that the observed effects at relatively high pH (5.0) in the absence of a rise in stream Al concentration, compared to those observed in other pH acidification experiments at lower pH, were a result of relative sensitivity of aquatic organisms to acidification when they inhabit more pristine habitats.

Hall, R.J., R.C. Bailey, and J. Findeis. 1988. Factors affecting survival and cation concentration in the blackflies *Prosimulium fuscum/mixtum* and the mayfly *Leptophlebia cupida* during spring snowmelt. Can. J. Fish. Aquat. Sci. 45: 2123-2132.
Prosimulium fuscum/mixtum and *Leptophlebia cupida* transplanted from a stream of pH ~6 to more acidic streams (pH 4.5 to 5.8; Ca =50-100 µM) for 4 or 10 d also lost body Al, as well as body Ca. In these studies, Al likely desorbed from the insects into the acidic water.

Hall, R.J., C.T. Driscoll, and G.E. Likens. 1987. Importance of hydrogen ions and aluminium in regulating the structure and function of stream ecosystems: an experimental test. Fresh. Biol. 18: 17-43.
Aluminum addition to a stream (pH ~5, >280 $\mu g \cdot L^{-1}$ Al) caused more drift of *Epeorus* sp. (mayfly), *Prosimulium* sp. (blackfly), and Orthocladiinae (chironmids) compared to H^+ addition alone (pH ~5, Al ~120 $\mu g \cdot L^{-1}$, Ca 50-100 µM).

Havas, M. 1986. A hematoxylin staining technique to locate sites of aluminum binding in aquatic plants and animals. Wat. Air Soil Pollut. 30: 735-741.
Havas used a hematoxylin stain to localize Al on and in aquatic animals and plants that were exposed to 1 $mg \cdot L^{-1}$ total Al for 24 h (pH 5, Ca =62 µM). In crustaceans (*Branchinecta paludosa, Daphnia magna, Holopedium* sp.) chloride cells stained for Al, as did the hind gut and penis tip of *Branchinecta*. *Chaoborus* sp. stained for Al on the anal papillae, where ion uptake occurs. Unfortunately, mayfly and dragonfly nymphs were not examined so we do not know Al depositon on these insects.

Havas, M., 1985. Aluminum bioaccumulation and toxicity to *Daphnia magna* in soft water at low pH. Can. J. Fish. Aquat. Sci. 42: 1741-1748.
Havas found that Al was toxic to the cladoceran *Daphnia magna* (Straus) at 320 and 1020 $\mu g \cdot L^{-1}$ at pH 6.5 (Ca =62 µM), but was not toxic with added Ca (310

μM; 48-h tests). Aluminum only marginally increased toxicity due to H^+ alone at pH 5.0, and it temporarily slowed H^+ toxicity at pH 4.5 (1,020 $\mu g \cdot L^{-1}$ Al); losses of Na and Cl were causes of death. Aluminum toxicity at pH 6.5 was likely due to Al oversaturation, possibly causing some respiratory distress, and the temporary amelioration of H^+ toxicity was likely due to Al^{3+} competition with H^+.

Havas, M., 1986. Effects of aluminum on aquatic biota, p. 79-127. *In* M. Havas, and J.F. Jaworski [eds.], Aluminum in the Canadian Environment. National Research Council of Canada, publication No. 24759, Ottawa, Ontario.
There's not much in here that is not contained in the other Havas publications.

Havas, M., and G.E. Likens, 1985. Toxicity of aluminum and hydrogen ions to *Daphnia catawba*, *Holopedium gibberum*, *Chaoborus punctipennis*, and *Chironomus anthrocinus* from Mirror Lake, New Hampshire. Can. J. Zool. 63: 1114-1119.
Havas and Likens showed that *Daphnia catawba*, *Holopedium gibberum* (cladocera), *Chaoborus punctipennis* (diptera), and *Chironomus anthrocinus* (diptera) were not very sensitive to Al (20, 320, 1,020 $\mu g \cdot L^{-1}$ Al; pH 3.5-6.5; Ca =60 μM). Loss of any of these organisms from acidic water, especially the acid-sensitive *D. catawba*, would most likely be due to direct effects of H^+ itself on osmoregulation, or to indirect effects of H^+ or Al on their predators or food sources.

Havas, M., and J.F. Jaworski. 1986. Aluminum in the Canadian environment. National Research Council of Canada publication No. 24759. National Research Council of Canada, Ottawa, Ontario. 331 p.

Havens, K. 1992. Acid and aluminum effects on sodium homeostasis and survival of acid-sensitive and acid-tolerant cladocera. Can. J. Fish. Aquat. Sci. 49: 2392-2398.
Whole-body Na content and survival of the acid-sensitive cladoceran *Daphnia galeata mendotae* decreased during Al exposures at pH 7.5 and 6.0 (0, 100, or 200 $\mu g \cdot L^{-1}$ nominal Al; 12 and 24 h exposures; Ca =60 μM). The highest concentration of Al protected against the effects of H^+ alone at pH 4.5. However, *Bosmina longirostris*, an acid-tolerant cladoceran, was not affected by Al in these exposure regimes. Aluminum had earlier been shown to bind to the osmoregulatory maxillary glands of *D. galeata mandotae* better than to those of *B. longirostris* (see Havens 1990).

Havens, K.E. 1990. Aluminum binding to ion exchange sites in acid-sensitive versus acid-tolerant cladocerans. Environ. Pollut. 64: 133-141.
Also used hematoxylin staining. Havens demonstrated that two acid- and Al-sensitive cladocerans, *D. galeata mendotae* and *Daphnia retrocurva*, accumulated Al on ion uptake sites of the maxillary glands (24 h, pH 5.0, 200 $\mu g \cdot L^{-1}$ Al). *B. longirostris*, an acid- and Al-tolerant cladoceran, did not accumulate Al on the maxillary glands. Havens speculated that acid-sensitivity was related to Al binding to ion-exchange sites, interfering with osmoregulation.

Havens, K.E. 1993. Acid and aluminum effects on osmoregulation and survival of the freshwater copepod *Skistodiaptomus oregonensis*. J. Plankton Res. 15: 683-691.
Exposure to 200 $\mu g \cdot L^{-1}$ Al at pH 6.0 caused Na losses and decreased survival of *Skistodiaptomus oregonensis* (copepoda), but slightly reduced the effects of H^+ (Na losses and animal death) at pH 4.5 (Ca =61 µM, 24 h exposures). Havens thought that Al could contribute to extinction of this freshwater copepod from a body of water at higher pH, but had antagonistic effects fo the osmoregulatory effects of H^+ at low pH.

Havens, K.E. 1993. Acid and aluminum effects on the survival of littoral macro-invertebrates during acute bioassays. Environ. Pollut. 80: 95-100.
Experiments using littoral macroinvertebrates. Havens demonstrated accelerated mortality at 200 $\mu g \cdot L^{-1}$ Al (pH 4.5, Ca ~60 µM, 48-h exposures), above that due to pH 4.5 alone, for *H. azteca*, *Gyraulus* sp. (gastropoda), *Paratanytarsus* sp. and *Zavrelimyia* sp. (diptera). Aluminum was slightly protective against H^+ toxicity for *Caenis* sp. (mayfly) and *Enallagma* sp. (damselfly), and pH 4.5 with or without Al had no effect on survival of *Arrenurus* sp. (water mite). Havens speculated that the presence of gills and caudal lamellae contributed to the high acid sensitivity of some of the organisms (*H. azteca*, *Enallagma* sp., and *Caenis* sp.) because of their high surface area, and lack of these structures contributed to the acid tolerance of *Arrenurus* sp. and the chironomids.

Havens, K.E. 1993. Pelagic food web structure in Adirondack Mountain, USA, Lakes of varying acidity. Can. J. Fish. Aquat. Sci. 50: 149-155.

Havens, K.E., and J. DeCosta. 1987. Freshwater plankton community succession during experimental acidification. Arch. Hydrobiol. 111: 37-65.

Havens, K.E., and J. DeCosta. 1987. The role of aluminum contamination in determining phytoplankton and zooplankton responses to acidification. Water Air Soil Pollut. 33: 277-293.
Could be useful for the algae section. Aluminum addition caused reduced chlorophyll *a* concentration (through P removal, I assume) and zooplankton abundance.

Havens, K.E., and R.T. Heath. 1989. Acid and aluminum effects on freshwater zooplankton: an *in situ* mesocosm study. Environ. Pollut. 62: 195-211.
East Twin Lake, Ohio. Acid sensitive zooplankton were removed as pH of an enclosure was reduced from pH 8.8 to pH 4.5 over 3 wks. Aluminum was also added in another treatment, to reach 180 $\mu g \cdot L^{-1}$ (also at pH 4.5). *Bosmina longirostris* and *Chydorus sphaericus*, both cladocerans, were most acid and Al tolerant. Aluminum did not play a role above that of H^+, because acid tolerant species were also Al tolerant (e.g. not an additive effect; more of less same effect).

Havens, K.E., N.D. Yan, and W. Keller. 1993. Lake acidification: effects on crustacean zooplankton populations. Environ. Sci. Technol. 27: 1621-1624.
Laboratory bioassay (pH 4.5-7.0, Ca =60 µM, 48 h LC50s; from Havens et al. 1993) and field survey data (305 acid sensitive Ontario lakes) agreed in the order of susceptibility of crustacean zooplankton to acidification. From most to least sensitive they were *Daphia galeata mendotae* > *Daphnia retrocurva* = *Skistodiaptomus oregonensis* > *Diaphanosoma birgei* > *Mesocyclops edax* > *Bosmina longirostris*. The authors concluded that lakewater pH is the only factor that is needed to explain composition of zooplankton populations in acid sensitive waters: that is, pH is the most important controlling factor (e.g. master variable) and that Al, for example, is of lesser importance.

Herrmann, J., and K. Frick. 1995. Do stream invertebrates accumulate aluminium at low pH conditions? Water Air Soil Pollut. 85: 407-412.
Yes, they do, but Al does not accumulate or biomagnify up the food chain to birds or fish. Stream invertebrates may more-or-less passively get extra Al from the water (cf. Exley, 1996, for fish). Of course, if Al or H^+ kill various invertebrates (like snails) then there could be food chain effects, but not directly due to Al in the food.

Herrmann, J., and K.G. Andersson. 1986. Aluminum impact on respiration of lotic mayflies at low pH. Wat. Air Soil Pollut. 30: 703-709.
Herrmann and Andersson showed that nymphs of the mayflies *Ephemera danica*, *Heptagenia fuscogrisea*, and *Heptagenia sulphurea* had elevated respiration when exposed to high Al concentrations (500 and 2,000 $\mu g \cdot L^{-1}$ Al) at pH 4.0 or 4.8 for 10 d (Ca =70 µM), compared to respiration at pH 4.0 and 4.8 in the absence of Al. They suggested that increased respiration was caused by impaired osmoregulation and ion transport (a "chemical impact route"), or by a mechanical route of Al precipitation and mucus accumulation on the gills, impairing respiration ("mechanical impact route"). They favoured the chemical impact route, of increased respiration needed to supply the energy required to compensate the ionoregulatory disturbances associated with Al. However, their exposures, especially at 2,000 $\mu g \cdot L^{-1}$ Al, pH 4.8, would likely have created supersaturated conditions, so the physical route can not be discounted.

His, E., R. Beiras, M.N. Seaman, G. Pagano, and N.M. Trieff. 1996. Sublethal and lethal toxicity of aluminum industry effluents to early developmental stages of the *Crassostrea gigas* Oyster. Arch. Environ. Contam. Toxicol. 30: 335-339.
The effluent's effects appeared mostly due to Hg, not Al, but Al, Fe, and Cr could be having synergistic effects. In the toxic effluent (0.1 g/L) there was 8.8 mg Al/L. Previous work (Calabrese et al. 1973) showed that oysters were unaffected at 7.5 mg Al/L. Trieff (1995) showed that sea urchins were a bit less sensitive to the same effluent tested here.

Locke, A. 1991. Zooplankton responses to acidification: A review of laboratory bioassays. Water Air Soil Pollut. 60: 135-148.
A good, relatively recent review of Al and aquatic invertebrates.

Lonergan, S.P., and J.B. Rasmussen. 1996. A multi-taxonomic indicator of acidification: isolating the effects of pH from other water-chemistry variables. Can. J. Fish. Aquat. Sci. 53: 1778-1787.
A survey of 45 Canadian lakes. Overall mean Al conc. was 85 $\mu g \cdot L^{-1}$, and mean pH was 6.56. Multifactorial analysis. Considered snails, leeches, crayfish, and mayflies. *Hyallela azteca* alone explained 49% of the pH variation in the lakes.

MacIsaac, H.J., T.C. Hutchinson, and W. Keller. 1987. Analysis of planktonic rotifer assemblages from Sudbury, Ontario, area lakes of varying chemical composition. Can. J. Fish. Aquat. Sci. 44: 1692-1701.

Mahony, N., E. Nol, and T. Hutchinson. 1997. Food-chain chemistry, reproductive success, and foraging behaviour of songbirds in acidified maple forests of central Ontario. Can. J. Zool. 75: 509-517.

Malley, D.F., and P.S.S. Chang. 1985. Effects of aluminum and acid on calcium uptake by the crayfish *Orconectes virilis*. Arch. Environ. Contam. Toxicol. 14: 739-747.
Uptake of ^{45}Ca by *Orconectes virilis* (Hagen) at pH 5.5 was just 30% of that in circumneutral water, and 180 to 1,300 $\mu g \cdot L^{-1}$ Al at pH 5.5 reduced ^{45}Ca uptake further to 20% of control (12-h experiments, Ca ~70 µM). Aluminum could therefore act as an additional stress and be important in determining crayfish survival in the pH range 5.0-6.0. See Berrill et al. 1985, too.

Merrett, W.J., G.P. Rutt, N.S. Weatherley, S.P. Thomas, and S.J. Ormerod. 1991. The response of macroinvertebrates to low pH and increased aluminium concentrations in Welsh streams: multiple episodes and chronic exposure. Arch. Hydrobiol. 121: 115-125.
Baetis rhodani and *Rhithrogena semicolorata* were adversely affected during dosing of streams with Al and H^+ (pH ~5, 360-840 $\mu g \cdot L^{-1}$ Al), but *Amphinemura sulcicollis* (stonefly) and *Hydropsyche instabilis* (caddis fly) were relatively unaffected. Longer dosing episodes (18 or 24 h) caused more mortalities in *Baetis* and *Rhithrogena* than did 6 or 12 h episodes, and there were cumulative effects of multiple exposures.

Ormerod, S.J., P. Boole, C.P. McCahon, N.S. Weatherley, D. Pascoe, and R.W. Edwards. 1987. Short-term experimental acidification of a Welsh stream: comparing the biological effects of hydrogen ions and aluminium. Fresh. Biol. 17: 341-356.
A stream acidification study which showed little added effect of Al on invertebrates compared to the effects of H^+ alone. *Chironomus riparius*, *Hydropsyche angustipennis* (caddis fly), and *Dinocras cephalotes* (stonefly) showed no effects of Al or H^+, and toxicity to *Ecdyonurus venosus*, *Baetis rhodani* (mayflies), and *Gammarus pulex* was similar at ~20% in acidified (pH ~4.3, 50 $\mu g \cdot L^{-1}$ Al) and acid plus Al (pH 5.0, 350 $\mu g \cdot L^{-1}$ Al) reaches of the stream (Ca =60-90 µM). In contrast, brown trout and Atlantic salmon showed significantly increased mortalities in the acid plus Al zone (50-87%) compared to the acid-only reach (7-10%).

Ormerod, S.J., A.P. Donald, and Brown, S.J. 1989. The influence of plantation forestry on the pH and aluminium concentration of upland Welsh streams: a re-examination. Environ. Pollut. 62: 47-62.

Økland, J. 1992. Effects of acidic water on freshwater snails: results from a study of 1000 lakes throughout Norway. Environ. Pollut. 78: 127-130.
Acidic waters won't have many snails (Al - Ca antagonism).

Ravera, O. 1986. Effects of experimental acidification on freshwater environments. Experientia 42: 507-516.

Rockwood, J.P., D.S. Jones, and R.A. Coler. 1990. The effect of aluminum in soft water at low pH on oxygen consumption by the dragonfly *Libellula julia* Uhler. Hydrobiol. 190: 55-59.
Decreases in oxygen consumption were seen in the dragonfly nymph *Libellula julia* in response to H^+ (pH 4, Ca =45 µM); 300 $\mu g \cdot L^{-1}$ Al did not significantly alter this response, but higher Al concentrations (3 and 30 $mg \cdot L^{-1}$) decreased oxygen consumption further (96-h exposures). High Al concentrations were needed to affect oxygen consumption. These workers speculated that reduced oxygen consumption was due to blockage of dragonfly gills by mucus or $Al(OH)_3$ precipitation, and the confined space in dragonfly respiratory chambers may not have allowed for clearance of material occluding the gills. They suggested that decreased oxygen consumption is the more likely response to Al than is increased oxygen consumption if respiratory surfaces are being affected.

Schindler, D.W. 1980. Experimental acidification of a whole lake: A test of the oligotrophication hypothesis, p. 370-374. *In* D. Drablos, and A. Tollan [eds.], Ecological impacts of acid precipitation: Proceedings of an international conference, Sandefjord, Norway, March 11-14, 1980. SNSF project, Oslo, Norway.
Of historical interest, mainly. Not much on Al here.

Schumaker, R.J., W.H. Funk and B.C. Moore. 1993. Zooplankton responses to aluminum sulfate treatment of Newman Lake, Washington. J. Freshwater Ecol. 8:375-387.
Examines effect of alum treatments on zooplankton communities.

Staddon, B.W. 1964. Water balance in *Corixa Dentipes* (Thoms.) (Hemiptera, Heteroptera). J. Exp. Biol. 41: 609-619.
Unlike fish, release of NH_3 (base) at tracheal gills of aquatic insects does not occur. Instead, NH_3 is intermittently released in high concentrations in rectal fluid.

Storey, D.M., F.B. Pyatt, and L. Broadley. 1992. An appraisal of some effects of simulated acid rain and aluminium ions on *Cyclops viridis* (Crustacea, Copepoda) and *Gammarus pulex* (Crustacea, Amphipoda). Intern. J. Environ. Studies 42: 169-176.
Storey et al. examined the relative susceptibility of *Cyclops viridis* (copepoda) and *Gammarus pulex* (amphipoda) to acidity (pH 4.0-6.9) and Al. Both organisms showed little or no mortality at pH $\geq$4.5 over 7 d, but 70-100%

mortality at pH 4.0, an effect exacerbated by 270 to 27,000 (nominal) $\mu g \cdot L^{-1}$ Al (pond water chemistry not given). *G. pulex* was more susceptible to acid and Al compared to *C. viridis*.

Tabak, L.M., and K.E. Gibbs. 1991. Effects of aluminum, calcium and low pH on egg hatching and nymphal survival of *Cloeon triangulifer* McDunnough (Ephemeroptera: Baetidae). Hydrobiol. 218: 157-166.
Aluminum (80 to 600 $\mu g \cdot L^{-1}$) added to the impairment by H^+ of egg hatching of *Cloeon triangulifer* (mayfly), but Al decreased slightly the 96-h toxicity of H^+ (pH 4.0, 5.0) to nymphs of *Cloeon* (Ca ~28 µM). Calcium (~300, 2,600 µM) was protective against H^+ and Al effects in eggs. Aluminum had little independent toxicity, and in nymphs the toxic mechanism of both H^+ and Al was thought to be Na and Cl losses.

Truscott, R., C.R. McCrohan, S.E.R. Bailey, and K.N. White. 1995. Effect of aluminium and lead on activity in the freshwater pond snail *Lymnaea stagnalis*. Can. J. Fish. Aquat. Sci. 52: 1623-1629.
Short (19 h) and long term (1 yr) exposure to Al or Pb. Neutral pH, static, hard water conditions. Aluminum (100 to 1,000 $\mu g \cdot L^{-1}$) normally depressed activity over 19 h. Snails became tolerant in the 1 yr exposure. The effect of Al was surprising, considering Al is not very "available" at neutral pH.

Van Hattum, B., N.M. van Styraalen, and H.A.J. Govers. 1996. Trace metals in populations of freshwater isopods: influence of biotic and abiotic variables. Arch. Environ. Contam. Toxicol. 31: 303-318.
Twenty-eight water systems in the Netherlands. Looked at Cd, Cu, Pb, and Zn. Did not consider Al. Used a predictive model (Ca, Cl, DOC) to explain 42-63% of the metal burdens. *Asellus aquaticus*, *Proasellus meridianus*, and *Proasellus coxalis*.

Vijverberg, J., D.F. Kalf, and M. Boersma. 1996. Decrease in *Daphnia* egg viability at elevated pH. Limnol. Oceanogr. 41: 789-794.
D. galeata eggs at pH 9.0 to 10.5 did fine; threshold effect at pH 10.5 and 11.5. In fact, some effect at pH 10.0, which is common in eutrophic lakes, so high ph could affect *Daphnia* more than was previously thought.

Vuori, K.-M. 1996. Acid-induced acute toxicity of aluminium to three species of filter feeding caddis larvae (Trichoptera, Arctopsychidae and Hydropsychidae). Freshw. Biol. 35: 179-188.
As an index of Al toxicity in the lab, they looked at morphological abnormalities in the anal papillae of the larvae (e.g. darkening and reduction of anal papillae). Looks as if the toxic mechanism of Al is impairment of normal osmoregulation through damage to ion-regulation organs. Very little, if any, Al precipitation was observed on the tracheal gills. This is a nice paper which will likely tie together the insect-Al part of the review.

Weatherley, N.S., S.J. Ormerod, S.P Thomas, and R.W. Edwards. 1988. The response of macroinvertebrates to experimental episodes of low pH with different forms of aluminium, during a natural spate. Hydrobiol. 169: 225-232.

In a 2 d experimental stream manipulation, only *Baetis rhodani* showed increased drift in response to H^+ (pH 4.9) and Al (~230-270 $\mu g \cdot L^{-1}$). The immediate increase in *Baetis* drift due to H^+ and Al was reduced by the addition of 300 µM citrate, presumably by complexing Al and reducing labile Al to below detection.

Weatherley, N.S., S.P. Thomas, and S.J. Omerod. 1989. Chemical and biological effects of acid, aluminium and lime additions to a Welsh hill-stream. Environ. Pollut. 56: 283-297.
Liming a reach of an experimentally acidified stream (pH ~4.5, Al ~700 $\mu g \cdot L^{-1}$, Ca ~60 µM, 24 h) appeared to alleviate some of the hemolymph Na loss in the crayfish *Austropotamobius pallipes*.

Whipple, A.V., and W.A. Dunson. 1993. Amelioration of the toxicity of H^+ to larval stoneflies by metals found in coal mine effluent. Arch. Environ. Contam. Toxicol. 24: 194-200.
Under very acidic conditions (pH ~3), high concentrations of Al (~15 $mg \cdot L^{-1}$) protected the stoneflies *Acroneuria carolinensis* and *Paragnetina media* against Na losses. Acid and Al tolerance appear to be linked, and may work by affecting the same osmoregulatory mechanism.

Wren, C.D., and G.L. Stephenson. 1991. The effect of acidification on the accumulation and toxicity of metals to freshwater invertebrates. Environ. Pollut. 71: 205-241.
They summarized data regarding Al in aquatic invertebrates, and found no evidence of biomagnification of Al in aquatic systems. A nice review.

Yan, N.D., G.L. Mackie, and D. Boomer. 1989. Chemical and biological correlates of metal levels in crustacean zooplankton from Canadian Shield lakes: a multivariate analysis. Sci. Total Environ. 87/88: 419-438.

Yan, N.D., P.G. Welsh, H. Lin, D.J. Taylor, and J.-M. Filion, J.-M. 1996. Demographic and genetic evidence of the long-term recovery of *Daphnia galeata mendotae* (Crustacea: Daphniidae) in Sudbury lakes following additions of base: the role of metal toxicity. Can. J. Fish. Aquat. Sci. 53: 1328-1344.
This study deals with Cu, Ni, and Cd. Aluminum was only 7 to 16 $\mu g \cdot L^{-1}$. They felt that excluding Al from the analysis was justified, because Al was always <100 $\mu g \cdot L^{-1}$, which was shown by Havens (1992) to have no effect on *D. g. mendotae*.

4.2 Fish (Chapter 5)

Åltand, Å., and B.T. Barlaup. 1996. Avoidance behavior of Atlantic salmon (*Salmo salar* L.) fry in waters of low pH and elevated aluminum concentration: laboratory experiments. Can. J. Fish. Aquat. Sci. 53: 1827-1834.
Fish avoided pH 4.0 and 4.5 water. 70 and 150 $\mu g \cdot L^{-1}$ Al did not increase avoidance, but did increase disrupted entries. e.g. behavior can be affected by Al, but only when accompanied with low pH. They make the point that natural avoidance of Al in the field has not been studied.

Baldigo, B.P., and P.S. Murdoch. 1997. Effect of stream acidification and inorganic aluminum on mortality of brook trout (*Salvelinus fontinalis*) in the Catskill Mountains, New York. Can. J. Fish. Aquat. Sci. 54: 603-615.
Brook trout exposed in cages in streams. Mortality increased during acidic episodes in a poorly buffered stream, as inorganic monomeric Al increased. DOC modified the mortality, but pH, Ca, and Cl concentrations were also important. Need 225 $\mu g \cdot L^{-1}$ Al for 2 d or more to be toxic to brook trout.

Balm, P.H.M., and T.G. Pottinger. 1993. Acclimation of rainbow trout (*Oncorhynchus mykiss*) to low environmental pH does not involve an activation of the pituitary-interrenal axis, but evokes adjustments in branchial ultrastructure. Can. J. Fish. Aquat. Sci. 50: 2532-2541.

Barlaup, B.T. and Å. Åltand. 1996. Episodic mortality of brown trout (*Salmo trutta* L.) caused by sea-salt-induced acidification in western Norway: effects on different life stages within three populations. Can. J. Fish. Aquat. Sci. 53: 1835-1843.
Low pH (4.5-5.1) and high Al (200-350 $\mu g \cdot L^{-1}$) due to sea salt acidification during storms. Fish kills occurred, with effects on age-class structures.

Battram, J.C. 1988. The effects of aluminium and low pH on chloride fluxes in the brown trout, *Salmo trutta* L. J. Fish Biol. 32: 937-947.
Battram (1988) showed that the most severe effects of 175 $\mu g \cdot L^{-1}$ Al on Cl fluxes in juvenile brown trout were at pH 5.5 (pH 4.0, 5.5, 7.0, 2-h experiments; 150 µM Ca).

Baker, J.P., W.J. Warren-Hicks, J. Gallagher, and S.W. Christensen. 1993. Fish population losses from Adirondack lakes: the role of surface water acidity and acidification. Wat. Res. Res. 29: 861-874.
In what was likely a conservative estimate of Adirondack lakes losing one or more fish populations due to acidic precipitation (16-19%), it was found that fish loss was correlated with lower water pH, higher concentrations of ionorganic Al, and higher elevation.

Berntssen, M.H.G., F. Kroglund, B.O. Rosseland, and S.E. Wendelaar Bonga. 1997. Responses of skin mucous cells to aluminum exposure at low pH in Atlantic salmon (*Salar salar*) smolts. Can. J. Fish. Aquat. Sci. 54: 1039-1045.

Birchall, J.D., C. Exley, J.S. Chappell, and M.J. Phillips. 1989. Acute toxicity of aluminium to fish eliminated in silicon-rich acid waters. Nature 338: 146-148.

Silicon can also reduce Al toxicity to fish. At pH 5, Ca ~50 µM, Al =170-190 $\mu g \cdot L^{-1}$ (~7 µM), a 13:1 molar ratio of Si:Al eliminated Al toxicity to 1 g Atlantic salmon fry (96-h exposures). They suggested that the formation of hydroxy-aluminosilicate species, enhanced by more alkaline conditions at the gills, blocks the binding (or precipitation) of Al-OH species at the gills. These workers emphasized that Si has been neglected in Al toxicity studies, and could be used to ameliorate Al toxicity.

Booth, C.E., D.G. McDonald, B.P. Simons, and C.M. Wood. 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 45: 1563-1574.
In adult brook trout (*Salvelinus fontinalis*), 110 to 1000 $\mu g \cdot L^{-1}$ Al (pH 4.4 to 5.2) for up to 11 d reduced Na influx as well as stimulated its efflux, the latter presumably due to displacement of Ca from gill membranes by Al, weakening tight junctions. Calcium (200 *versus* 12 µM) was partially protective against Na losses, and reduced ion efflux was part of an incomplete recovery phase. They suggested either Al precipitation or Al speciation changes at more alkaline gills as the cause of greater Al toxicity at higher pH and higher Al concentrations.

Brezonik, P.L, J.G. Eaton, T.M. Frost, P.J. Garrison, T.K. Kratz, C.E. Mach, J.H. McCormick, J.A. Perry, W.A. Rose, C.J. Sampson, B.C.L. Shelley, W.A. Swenson, and K.E. Webster. 1993. Experimental acidification of Little Rock Lake, Wisconsin: chemical and biological changes over the pH range 6.1 to 4.7. Can. J. Fish. Aquat. Sci. 50: 1101-1121.
This review paper of the Little Rock Lake project indicated that rock bass was most sensitive to lower pH, followed by largemouth bass, black crappie, and perch (least sensitive). In general, juvenile stages of fish were most susceptible to lake acidification: only rock bass (most sensitive fish) were affected as adults. During the study dissolved Al increased from about 5 $\mu g \cdot L^{-1}$ (pre-acidification) to about 40 $\mu g \cdot L^{-1}$ at pH 4.7.

Brown, D.J.A. 1983. Effect of calcium and aluminum concentrations on the survival of brown trout (*Salmo trutta*) at low pH. Bull. Enviro. Contam. Toxicol. 30, 582-587.
The influences and interactions of Ca, Al, and H^+ on 16-d survival of brown trout (*Salmo trutta*) yolk sac fry were studied. She found that Ca (25, 50 µM *versus* 6, 12 µM) ameliorated Al toxicity (250, 500 $\mu g \cdot L^{-1}$). The order of Al toxicity with respect to pH was pH 5.4 >5.1 >4.8 >4.5. She suggested that Ca protected against ionoregulatory effects of Al.

Brown, J.A., and C. Whitehead. 1995. Catecholamine release and interrenal response of brown trout, *Salmo trutta*, exposed to aluminium in acidic water. J. Fish Biol. 46: 524-535.
Brown and Whitehead showed that brown trout exposed to pH 5 and about 200 to 300 $\mu g \cdot L^{-1}$ total Al showed elevated plasma glucose, cortisol, noradrenaline, and adrenaline concentrations during 36 h exposures (Ca=50 µM).

Buckler, D.R., L. Cleveland, E.E. Little, and W.G. Brumbaugh. 1995. Survival, sublethal responses, and tissue residues of Atlantic salmon exposed to acidic pH and aluminum. Aquatic Toxicol. 31: 203-216.
In a 60 d study starting with eyed Atlantic salmon, they showed that feeding and swimming activity were most sensitive to Al (33 $\mu g \cdot L^{-1}$ Al), significant mortality occurred at 71 $\mu g \cdot L^{-1}$ Al, and egg hatching was not affected by 264 $\mu g \cdot L^{-1}$ Al (pH 5.5, Ca =75 µM).

Bulger, A.J., L. Lien, B.J. Cosby, and A. Henriksen. 1993. Brown trout (*Salmo trutta*) status and chemistry from the Norwegian Thousand Lake Survey: statistical analysis. Can. J. Fish. Aquat. Sci. 50: 575-585.
A survey of 584 Norwegian lakes. They found that lakewater pH, monomeric inorganic aluminum concentration, and alkalinity (with Ca and SO_4 as components) best predicted brown trout populations. Lakes with 133 $\mu g \cdot L^{-1}$ Al, pH 4.8, and an alkalinity of -34 $\mu eq \cdot L^{-1}$ had extinct brown trout populations (39% of the 584 lakes), whereas 11 $\mu g \cdot L^{-1}$ Al, pH 6.0, and +27 $\mu eq \cdot L^{-1}$ alkalinity indicated healthy brown trout populations. Organically bound Al was not important in determining brook trout population status, as expected if organically bound Al is not toxic to fish.

Campbell, P.G.C. 1995. Interactions between trace metals and aquatic organisms: a critique of the free-ion model. In: Tessier, A. and D.R. Turner, Metal speciation and bioavailability in aquatic systems. John Wiley & Sons, New York. pp. 45-102.

Carline, R.F., D.R. DeWalle, W.E. Sharpe, B.A. Dempsey, C.J. Gagen, and B. Swistock. 1992. Water chemistry and fish community responses to episodic stream acidification in Pennsylvania, USA. Environ. Pollut. 78: 45-48.
Free-ranging fish in the field, unlike laboratory or caged fish, can sometimes avoid acid and Al pulses. During an autumn rainstorm, caged brook trout adults suffered ~80% mortality over 20 d (lowest pH ~5.0, highest total dissolved Al ~50 $\mu g \cdot L^{-1}$, Ca ~80 µM), whereas free-ranging brook trout fitted with radio transmitters showed only 33% mortality (number of fish implanted not stated). Surviving free-ranging fish moved downstream or into refuges of groundwater entering the stream.

Conlon, M., J.M. Gunn, and J.R. Morris. 1992. Prediction of lake trout (*Salvelinus namaycush*) presence in low-alkalinity lakes near Sudbury, Ontario. Can. J. Fish. Aquat. Sci. 49 (Suppl. 1): 95-101.
Presence of lake trout (*Salvelinus namaycush*) in Sudbury area lakes has been predicted by a combination of pH, alkalinity, and conductivity: concentration of Al in water also correlates with these variables. Lake trout were not usually found in lakes of pH $\leq$5.4 or which contained $\geq$160 $\mu g \cdot L^{-1}$ Al.

DeWalle, D.R., B.R. Swistock, and W.E Sharpe. 1995. Episodic flow-duration analysis: a method of assessing toxic exposure of brook trout (*Salvelinus fontinalis*) to episodic increases in aluminum. Can. J. Fish. Aquat. Sci. 52: 816-827.

DeWalle et al. used a combination of stream flow data, total dissolved Al concentrations, and field and laboratory toxicity data to determine the duration of elevated Al concentrations which would be toxic to brook trout. For example, their data indicated that total dissolved Al concentrations greater than 200 $\mu g \cdot L^{-1}$ would be lethal to brook trout if the exposures lasted 24 to 48 h, and 100 to 200 $\mu g \cdot L^{-1}$ Al would be lethal after 48 to 400 h. Stream acidity was not considered in their analysis; it must be assumed that acidic conditions played some part in the observed toxicity.

Delonay, A.J., E.E. Little, D.F. Woodward, W.G. Brumbaugh, A.M. Farag, and C.F. Rabeni. 1993. Sensitivity of early-life-stage golden trout to low pH and elevated aluminum. Environ. Toxicol. Chem. 12: 1223-1232.

In a study using early life stages of golden trout (*Oncorhynchus aguabonita aguabonita*), DeLonay et al. found that swim-up larvae were most sensitive to Al, with significant, additional mortality due to Al occurring at pH 5.5, 100 $\mu g \cdot L^{-1}$ Al and pH 5.0, 100 and 300 $\mu g \cdot L^{-1}$ Al (Ca ~34 µM, 7 d exposures). Whole-body Na concentrations declined, and locomotory and feeding impairment due to Al was an even more sensitive indication of Al toxicity than was mortality.

Dietrich, D,. and C. Schlatter. 1989a. Aluminium toxicity to rainbow trout at low pH. Aquat. Toxicol. 15: 197-212.

Dietrich and Schlatter demonstrated the dual ionoregulatory (lower pH, lower Al conditions) and respiratory nature (higher pH, higher Al conditions) of Al toxicity in adult rainbow trout, shown by mortality, plasma ion concentrations, and gill pathology (pH 5.2 to 5.6, 0 to 530 $\mu g \cdot L^{-1}$ Al, Ca =370 µM, 96-h). They thought that Al precipitating onto the gills, enhanced by higher pH near the gills due to ammonia release, was the likely toxic mechanism in most of their exposures. In addition, they measured plasma Al concentrations using graphite furnace atomic absorption spectrometry (AAS), and showed significant Al entry into the blood, from about 31-56 $ng \cdot ml^{-1}$ Al (controls) to 50-105 $ng \cdot ml^{-1}$ Al (exposed to 140-530 $\mu g \cdot L^{-1}$ Al for 46-96 h). They emphasized that Al entry into fish blood was not the toxic mechanism of Al: instead, Al toxicity was due to effects of Al directly on the gills. To our knowledge their paper is the only demonstration of Al entry into fish blood.

Dietrich, D., and C. Schlatter. 1989b. Low levels of aluminium causing death of brown trout (*Salmo trutta fario*, L.) in a Swiss alpine lake. Aquat. Sci. 51: 279-295.

Adult brown trout exposed *in situ* to a Swiss alpine lake of pH 5.4, 120 $\mu g \cdot L^{-1}$ Al, and 12 µM Ca suffered gill damage, electrolyte losses, mucus clogging of the gills, had high hematrocrit values and high mortalities, all presumably caused by Al in the lake. The authors commented on the discrepancy between the then-generally accepted view that waters of Al content <200 $\mu g \cdot L^{-1}$ should not be toxic to brown trout at pH ~5.4; however, the soft water, moderately-high Al concentration, and moderately acidic pH would be ideal conditions for Al precipitation in the more alkaline gill micro-environment.

Eaton, J.G., W.A. Swenson, J.H. McCormick, T.D. Simonson, and K.M. Jensen. 1992. A field and laboratory investigation of acid effects on largemouth bass, rock bass, black crappie, and yellow perch. Trans. Am. Fish. Soc. 121: 644-658.
Laboratory toxicity studies and field studies of H^+ and Al usually agree, but do not necessarily duplicate one another. During the Little Rock Lake acidification project, in which half the lake was acidified to pH ~4.8 and the other half was left at pH ~6.0, toxicity studies were also run in the laboratory (pH 4.5, 5.0; Ca ~38 µM; Al 10-90 $\mu g \cdot L^{-1}$). Test fish were rock bass (*Ambloplites rupestris*), largemouth bass (*Micropterus salmoides*), black crappie (*Pomoxis nigromaculatus*; most sensitive), and yellow perch (*Perca flavescens*; least sensitive). Although field data and *in situ* and laboratory toxicity tests generally agreed - especially for adult fish - the authors suggested that laboratory tests should be validated in the field whenever possible. Further, they believe that whole-ecosystem experiments provide unique information which more than compensates for their cost and lack of replication.

Exley, C. 1996. Aluminium in the brain and heart of the rainbow trout. J. Fish Biol. 48: 706-713.
Fish at a fish farm have been exposed to high concentrations of Al (added as alum, 1 tonne per week) for years. They had elevated Al in brain, liver, gonad, gills, heart (~10x higher). Water probably contained 5 to 10x more Al for these fish (~250 $\mu g \cdot L^{-1}$ *versus* <50 $\mu g \cdot L^{-1}$). However, no effects of these high Al concentrations on the fish. Could have great relevance to alum in wastewater. This is a crossover paper, in which Exley proposes that fish could be a good model for study of Alzheimer's disease in humans (e.g. his articles in Coord. Chem. Rev. 149).

Exley, C., J.S. Chappell, and J.D. Birchall. 1991. A mechanism for acute aluminium toxicity in fish. J. Theor. Biol. 151: 417-428.
A good summary of Al toxicity to fish under different water chemistry conditions. The model presented is similar to the gill pH model of more basic conditions next to the gill, and associated Al interactions at the gill. Their model adds the point that Al bound to ligands such as DOC in water may still be toxic if bound Al, in equilibrium with free Al, is passed to negatively charged sites on gills which bind free Al more strongly. This part agrees with the gill modelling using log*K* values of Playle's lab.

Exley, C., A.J. Wicks, A.J. Hubert, and J.D. Birchall. 1996. Kinetic constraints in acute aluminium toxicity in the rainbow trout (*Oncorhynchus mykiss*). J. Theor. Biol. 179: 25-31.
Work by theses workers suggests that silicon concentrations must be greater than 500 µM to significantly reduce Al-OH formation (total Al =4 µM), as shown by filtration through 0.04 µm membranes. More notes in the actual text. Good reference; indirectly, is more gill pH work. They talk about polymerization/precipitation at gill, but a bit differently; speak about "tanning" of mucus layer (pg. 30).

Farag, A.M., D.F. Woodward, E.E. Little, B. Steadman, and F.A. Vertucci. 1993. The effects of low pH and elevated aluminum on Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*). Environ. Toxicol. Chem. 12: 719-731.
Western United States; high elevation. Low pH affected eggs most, but if sublethal effects were considered, then alevins and swim-up larvae were sensitive to low pH and elevated Al. 50 $\mu g \cdot L^{-1}$ and pH 6.0 was enough to cause a problem. Tissue Na and Ca concentrations were also affected.

Freda, J., D.A. Sanchez, and H.L. Bergman. 1991. Shortening of branchial tight junctions in acid-exposed rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 2028-2033.
The effect of Al at lower pH is viewed by these workers as the same mechanism as H^+ toxicity alone, displacement of Ca from tight junctions of the gill membrane.

Freeman, R.A., and W.H. Everhart. 1971. Toxicity of aluminum hydroxide complexes in neutral and basic media to rainbow trout. Trans. Amer. Fish. Soc. 4: 644-658.

Gagen, C.J., W.E. Sharpe, and R.F. Carline. 1994. Downstream movement and mortality of brook trout (*Salvelinus fontinalis*) exposed to acidic episodes in streams. Can. J. Fish. Aquat. Sci. 51: 1620-1628.
In the same streams as those in Dewalle et al. (1995), radio-tagged brook trout showed considerable, probably passive movement downstream during severe acidic episodes (pH <5.0, >200 $\mu g \cdot L^{-1}$ Al), and one third of the fish died during the severe episodes. Gagen et al. suggested that the ability of fish to exploit refuge areas of higher pH and lower Al concentrations during acidic episodes is limited. Note: how does this square with the results of Dewalle et al?

Gloss, S.P., C.L. Schofield, R.L. Spateholts, and B.A. Plonski. 1989. Survival, growth, reproduction, and diet of brook trout (*Salvelinus fontinalis*) stocked into lakes after liming to mitigate acidity. Can. J. Fish. Aquat. Sci. 46: 277-286.

Goossenaerts, C., R. Van Grieken, W. Jacob, H. Witters, and O. Vanderborght. 1988. A microanalytical study of the gills of aluminium-exposed rainbow trout (*Salmo gairdneri*). Intern. J. Environ. Anal. Chem. 34: 227-237.
Adult rainbow trout exposed for 3 d to 200 $\mu g \cdot L^{-1}$ Al at pH 5 (Ca ~28 µM) had fused lamellae, disrupted chloride cells, and separation of epithelial cells from basement tissue (TEM). Aluminum was localized mostly on the surface of lamellae, with some Al in epithelial cells.

Goss, G.G., and C.M. Wood. 1988. The effects of acid and acid/aluminum exposure on circulating plasma cortisol levels and other blood parameters in the rainbow trout, *Salmo gairdneri*. J. Fish Biol. 32: 63-76.
Goss and Wood found that plasma cortisol, lactate, and glucose concentrations increased by 24 to 30 h in cannulated rainbow trout exposed to pH 4.8 plus 110 $\mu g \cdot L^{-1}$ Al (Ca =25 µM) in comparison to fish exposed to pH 4.8 alone, presumably due to the combined respiratory and ionoregulatory effects of Al.

Grippo, R.S., and W.A. Dunson. 1996a. The body ion loss biomarker. 1. Interactions between trace metals and low pH in reconstituted coal mine-polluted water. Environ. Toxicol. Chem. 15: 1955-1963.
Net body ion loss of brook charr as a method of evaluating potential toxicity of coal mine pollution. Al, Fe, Zn, Mn, Ni, and Cd or Pb mixtures. Hard water, high Al concentrations. They mention the need for metal-gill affinities (pg. 1960).

Grippo, R.S., and W.A. Dunson, W.A. 1996b. The body ion loss biomarker. 2. Field validation in coal mine-polluted streams. Environ. Toxicol. Chem. 15: 1964-1972.
Field validation of the 1996a paper (above). Aluminum ended up in both models that predicted Na and Ca losses in the fish.

Gundersen, D.T., and L.R. Curtis. 1995. Acclimation to hard or soft water at weakly alkaline pH influences gill permeability and gill surface calcium binding in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 52: 2583-2593.
A very nice paper regarding acclimation of fish gills to low and high Ca concentrations. Fish acclimated to low Ca had less permeable gills, which agrees with conventional theory. They calculated the number of binding sites for Ca at 0.32 μmol Ca^{2+} per g (wet or dry wt?) for low Ca conditions, and about 0.20 (etc) for high Ca conditions. Aluminum had little effect on gill permeability and gill Ca binding. Check the Al effect (or lack of it) again.

Gundersen, D.T., S. Bustaman, W.K. Seim, and L.R. Curtis. 1994. pH, hardness, and humic acid influence aluminum toxicity to rainbow trout (*Oncorhynchus mykiss*) in weakly alkaline waters. Can. J. Fish. Aquat. Sci. 51: 1345-1355.
Modifying effects of DOC and water hardness against Al toxicity to juvenile rainbow trout were studied. Calcium concentrations of about 830 and 1,190 μM Ca increased survival of trout exposed to approximately 4 and 9 $mg \cdot L^{-1}$ Al for 96 h, compared to Ca concentrations of <380 μM (pH 8.0-8.6). Survival under similar conditions but at pH ~7.6 was always 100%, indicating that high concentrations of Al under slightly basic conditions are more toxic to trout than at near-neutral conditions, indicating that $Al(OH)_4^-$ was more toxic than insoluble Al species. Sixteen day experiments showed similar results. Humic acid (Aldrich Chemical Co.) at $\geq$ 3 mg $C \cdot L^{-1}$ showed protective effects against Al toxicity of up to ~5 $mg \cdot L^{-1}$ Al, but not to ~9 $mg \cdot L^{-1}$ Al. Five mg $C \cdot L^{-1}$ humic acid protected against toxic effects of 0.5 to 2 $mg \cdot L^{-1}$ Al over 16 d. The authors warned that extrapolation of their humic acid results to natural conditions may be difficult, because of the high metal binding of commercial, terrestrial humic acids compared to aquatic humic acids.

Gunn, J.M., J.G. Hamilton, G.M. Booth, C.D. Wren, G.L. Beggs, H.J. Rietveld, and J.R. Munro. 1990. Survival, growth, and reproduction of lake trout (*Salvelinus namaycush*) and yellow perch (*Perca flavescens*) after neutralization of an acidic lake near Sudbury, Ontario. Can. J. Fish. Aquat. Sci. 47: 446-453.
Lime additions reduced acid and Al toxicity to lake trout (*Salvelinus namaycush*). Could be useful, but acid, soft water lakes.

Haaparanta, A., E.T.Valtonen, and R.W. Hoffmann. 1997. Gill anomalies of perch and roach from four lakes differing in water quality. J. Fish Biol. 50: 575-591.
Fish were collected over five seasons in Finland. Gill alterations of interest were mainly chlorid cell anomolies, which appeared to be related to the soft water, acidic pulses, and low water temperatures in the lakes. Similar to Leino and McCormick (1993) on bass.

Hamilton, S.J., and T.A. Haines. 1995. Influence of fluoride on aluminum toxicity to Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 52: 2432-2444.
pH 5.5, 200 $\mu g \cdot L^{-1}$ Al. Addition of <100 $\mu g \cdot L^{-1}$ F protected; >100 $\mu g \cdot L^{-1}$ F did not reduce Al-induced effects. If convert these numbers to molar amounts, there is excess F at the higher concentrations - is it the F that is toxic? The authors think that at higher F concentrations, more Al is kept in solution, so Al has more opportunity to have a toxic effect. Some electron micrographs.

Handy, R.D. 1994. Intermittent exposure to aquatic pollutants: assessment, toxicity and sublethal responses in fish and invertebrates. Comp. Biochem. Physiol. 107C: 171-184.
Look at this review again, to see if there are any ideas related to the Florida.

Handy, R.D. 1993. The accumulation of dietary aluminum by rainbow trout, *Oncorhynchus mykiss*, at high exposure concentrations. J. Fish. Biol. 42: 603-606.
Dietary Al has no significant adverse or beneficial effects; see also Poston (1991).

Handy, R.D., and F.B. Eddy. 1989. Surface absorption of aluminium by gill tissue and body mucus of rainbow trout, *Salmo gairdneri*, at the onset of episodic exposure. J. Fish Biol. 34: 865-874.
They did not see Al entry into blood of adult rainbow trout (950 $\mu g \cdot L^{-1}$ Al, pH ~5.4, Ca ~10 µM), but the 1 h exposure time may have been too short. The 1 h exposure was, however, long enough to stimulate mucus production at the gills. Gills of juvenile rainbow trout exposed to 950 $\mu g \cdot L^{-1}$ Al at pH 5.4 (Ca =10 µM) accumulated significant amounts of Al in 30 min, and skin mucus accumulated significant Al in 5 min.

Harris, W.R., G. Gerthon, J.P. Day, C. Exley, T.P. Flaten, W.F. Forbes, T. Kiss, C. Orvig, and P.F. Zatta. 1996. Speciation of aluminum in biological systems. J. Toxicol. Environ. Health 48: 543-568.

Hickie, B.E., N.J. Hutchinson, D.G. Dixon, and P.V. Hodson. 1993. Toxicity of trace metal mixtures to alevin rainbow trout (*Oncorhynchus mykiss*) and larval fathead minnow (*Pimephales promelas*) in soft, acidic water. Can. J. Fish. Aquat. Sci. 50: 1348-1355.
Laboratory work with trace metal mixtures (Al, Mn, Fe, Ni, Zn, Cu, and Pb) in soft water showed that Al, Zn, and especially Cu were responsible for all toxicity of the full metal mixture, and that Al was alone responsible for observed toxicity at pH 4.9 (Ca =60 µM, alevin rainbow trout and larval fathead minnow. Thus, combinations of metal toxicity are not necessarily intractable.

Hollis, L., K. Burnison, and R.C. Playle. 1996. Does the age of metal-dissolved organic carbon complexes influence binding of metals to fish gills? Aquat. Toxicol. 35: 253-264.

Hollis, L., L. Muench, and R.C. Playle. 1997. Influence of dissolved organic carbon on copper binding, and calcium on cadmium binding, by gills of rainbow trout (*Oncorhynchus mykiss*). J. Fish Biol. In press.

Howells, G.D., D.J.A. Brown, and K. Sadler. 1983. Effects of acidity, calcium, and aluminium on fish survival and productivity - a review. J. Sci. Food Agric. 34: 559-570.
The importance of Ca in reducing the toxicity of acidity and Al was stressed. The reviewers suggested that lakes of pH 4.5 and 20 µM Ca would be fishless, and that 250 $\mu g \cdot L^{-1}$ Al would be toxic.

Hutchinson, N.J., and J.B. Sprague. 1986. Toxicity of trace metal mixtures to American flagfish (*Jordanella floridae*) in soft, acidic water and implications for cultural acidification. Can. J. Fish. Aquat. Sci. 43: 647-655.
Al is not the only potentially toxic metal to be mobilized in acidic water, and that there may be synergistic effects between metals. Alone, 95 $\mu g \cdot L^{-1}$ Al was the threshold of acute toxicity to flagfish (*Jordanella floridae*) held in soft water (~60 µM Ca, pH 5.8), whereas in the presence of 5 $\mu g \cdot L^{-1}$ Zn and 2 $\mu g \cdot L^{-1}$ Cu, only 29 $\mu g \cdot L^{-1}$ Al was needed to reach toxicity threshold. Other metals at low concentrations (Mn, Fe, Ni, and Pb) did not have additional effects to those of Al, Zn, and Cu on reproduction over 1.3 generations of flagfish.

Hutchinson, N.J., and J.B. Sprague. 1987. Reduced lethality of Al, Zn and Cu mixtures to american flagfish by complexation with humic substances in acidified soft waters. Environ. Toxicol. Chem. 6: 755-765.
As with single metal toxicity, toxicity of metal mixtures was reduced by humic substances. See Hutchinson and Sprague (1986) for exposure conditions and metals.

Jagoe, C.H., V.E. Matey, T.A. Haines, and V.T. Komov. 1993. Effect of beryllium on fish in acid water is analogous to aluminum toxicity. Aquat. Toxicol. 24: 241-256.

Janes, N., and R.C. Playle. 1995. Modeling silver binding to gills of rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 14: 1847-1858.
Use when discuss Wilkinson et al. (1990). The strength of the Al-ligand complex relative to the Al-gill complex is important in considering free Al available to interact at the gills, whether the ligand be F or citrate. As a recent paper on this approach to modelling metal-gill interactions using conditional stability constants, see Janes and Playle (1995). Should we also mention Playle et al. 1993a,b? Maybe the recent SETAC book, too.

Janes, N., and R.C. Playle. In preparation. Opercular windows for viewing dynamic changes at gills of fish.
Possibly include, because it shows photographically the effects of Al at the gills (mucus/precipitation). Mainly depends if we get it submitted soon.

Jensen, F.B., and R.E. Weber. 1987. Internal hypoxia-hypercapnia in tench exposed to aluminium in acid water: effects on blood gas transport, acid-base status and electrolyte composition in arterial blood. J. exp. Biol. 127: 427-442.
Fish used: tench (*Tinca tinca*). High Al concentrations. Large respiratory effects.

Jones, D.L. and L.V. Kochian. 1997. Aluminum interaction with plasma membrane lipids and enzyme metal binding sites and its potential role in Al cytotoxicity. FEBS Lett. 400: 51-57.
This is an important paper regarding the possible mechanism of Al interaction with membranes, both animal and plant. It indicates that Al may interact with membrane lipids, and thus cause toxicity, more than interacting with enzymes. Citrate and malate reduced Al binding to membranes.

Karlsson-Norrgren, L., W. Dickson, O. Ljungberg, and P. Runn. 1986a. Acid water and aluminium exposure: gill lesions and aluminium accumulation in farmed brown trout, *Salmo trutta* L. J. Fish Dis. 9: 1-9.
Gills of one-year old brown trout exposed to 50-500 $\mu g \cdot L^{-1}$ Al, pH ~5 in soft water (Ca <250 µM) for a minimum of 3 wk in either farmed or experimental conditions were studied by Karlsson-Norrgren et al. (1989a, b; scanning EM and TEM). Gill damage included swelling of gill lamellae due to increased number of chloride cells, resulting in increased diffusion distance from water to blood. More Al accumulated on the gills at pH 5.5, with or without added humus, than at pH 7.

Karlsson-Norrgren, L., I. Björklund, O. Ljungberg, and P. Runn. 1986b. Acid water and aluminium exposure: experimentally induced gill lesions in brown trout, *Salmo trutta* L. J. Fish Dis. 9: 11-25.
Aluminum was localized on and in epithelial cells; for conditions of exposure, see Karlsson-Norrgren et al. (1989a).

Kelso, J.R.M., M.A. Shaw, C.K. Minns, and K.H. Mills. 1990. An evaluation of the effects of atmospheric acidic deposition on fish and the fishery resource of Canada. Can. J. Fish. Aquat. Sci. 47: 644-655.
Probably not useful for this review; check again.

Lacroix, G.L. 1996. Long-term enhancement of habitat for salmonids in acidified running waters. Can. J. Fish. Aquat. Sci. 53 (Suppl. 1): 283-294.
Crushed limestone treatment on the bed of brook trout spawning streams in Nova Scotia. It worked well, and the limestone was replenished after 4 yr to replace some lost to washout. Worked for 8 years. Good mitigation.

Lacroix, G.L., D.J. Hood, C.S. Belfry, and T.G. Rand. 1990. Plasma electrolytes, gill aluminum content, and gill morphology of juvenile Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) indigenous to acidic streams of Nova Scotia. Can. J. Zool. 68: 1270-1280.
A field survey involving juvenile Atlantic salmon and brook trout gill histology. >5 mg $C \cdot L^{-1}$ DOC appeared to mitigate the effects of up to ~310 $\mu g \cdot L^{-1}$ total Al, even though gill Al concentrations increased with increasing exchangeable Al in water (<40 $\mu g \cdot L^{-1}$ in all streams surveyed, Ca ≤32 µM).

Lacroix, G.L., and J. Korman. 1996. Timing of episodic acidification in Atlantic salmon rivers influences evaluation of mitigative measures and recovery forecasts. Can. J. Fish. Aquat. Sci. 53: 589-599.
This is more of a fisheries modelling paper. Not of too much use to us here.

Lacroix, G.L., R.H. Peterson, C.S. Belfry, and D.J. Martin-Robichaud. 1993. Aluminum dynamics on gills of Atlantic salmon fry in the presence of citrate and effects on integrity of gill structures. Aquatic Toxicol. 27: 373-402.
The results of Lacroix et al. agree with those of Wilkinson and Campbell (1993), in that Al depuration was rapid and related to the physical process of sloughing from the gills. The addition of citrate to exposure water decreased the amount of Al binding to the gills, but did not accelerate its removal (0.3 g Atlantic salmon, pH ~5.5, Ca~90 μM, 15 d, Al ~270 $\mu g \cdot L^{-1}$). X-ray microanalysis located Al on the the surface of gills, presumably associated with mucus. Although Al content of the gills fell to background in 2 to 4 d, gill damage persisted, as shown by light and scanning electron microscopy, indicating that sloughing of Al is faster than gill tissue repair. Rapid Al depuration from gill epithelia, but slower repair mechanisms, would contribute to observations of intermittent exposure to high concentrations of Al being more toxic than longer exposures to lower concentrations of Al (e.g. review by Handy, 1994), because damage due to Al precipitation or polymerization (at high Al concentrations) in the gill microenvironment may take time to be repaired even after the Al exposure ends.

Lacroix, G.L. 1992. Mitigation of low stream pH and its effects on salmonids. Environ. Pollut. 78: 157-164.
Lime additions reduced acid and Al toxicity to Atlantic salmon.

Lacroix, G.L,. and D.R. Townsend. 1987. Responses of juvenile Atlantic salmon (*Salmo salar*) to episodic increases in acidity of Nova Scotia rivers. Can. J. Fish. Aquat. Sci. 44: 1475-1484.
The difficulty in attributing toxicity observed in the field to either H^+ or Al is nicely illustrated by this study, in which Atlantic salmon parr were held in floating pens for 54 d in four acidic streams during natural acidification in November and December. All fish died in the two streams where pH decreased below pH 4.7; none died in 2 streams where pH did not decrease below pH 4.9. Total Al (200-350 $\mu g \cdot L^{-1}$) and inorganic Al (10-50 $\mu g \cdot L^{-1}$) were similar in all four brownwater streams (DOC 12-18 $mg \cdot L^{-1}$); acute toxicity was attributed to Na and Cl losses due primarily to H^+, not to accumulation of Al on the gills.

Laitinen, M., and T. Valtonen. 1995. Cardiovascular, ventilatory and haematological responses of brown trout (*Salmo trutta* L.), to the combined effects of acidity and aluminium in humic water at winter temperatures. Aquat. Toxicol. 31: 99-112.
Laitinen and Valtonen studied the effects of 280 and 450 $\mu g \cdot L^{-1}$ Al on adult brown trout in cold (1-3°C) humic river water (pH 4.7, Ca~90 μM, 7 d exposures). Heart and ventilation rates were monitored by a non-contact bioelectical monitoring system. Heart and ventilation rates increased in all treatments, with greatest increases in the pH 4.7 plus 450 $\mu g \cdot L^{-1}$ Al treatments, with greater

effects on ventilation rate and ventilation in the Al-exposed fish. Blood samples at the end of the experiment showed a few hematological disturbances in the acid and acid plus Al exposures, the most notable and consistent effect being increases in blood glucose concentration. In general, these fish showed respiratory effects of Al consistent with the relatively high Al concentrations and the pH of the exposures, and only minimal ionoregulatory effects. In addition, the effects of Al were relatively minor compared to experiments run in synthetic soft water, because of the humic nature of the river water used.

Leino, R.L., and J.H. McCormick. 1993. Responses of juvenile largemouth bass to different pH and aluminum levels at overwintering temperatures: effects on gill morphology, electrolyte balance, scale calcium, liver glycogen, and depot fat. Can. J. Zool. 71: 531-543.

When overwintering in laboratory conditions of ~4°C, largemouth bass (*Micropterus salmoides*) showed thickened respiratory epithelia, presumably a mechanism to decrease ion losses at the gills during torpor (LM, TEM). Thickening of the gill epithelium was exacerbated under acidic conditions, as were decreases in blood osmolality (pH 4.5 or 5.0, Ca =38 or 335 μM), and both were affected more in the presence of ~30 $\mu g \cdot L^{-1}$ monomeric Al (50 $\mu g \cdot L^{-1}$ total Al). In addition, Al interfered with Ca resorption from fish scales during overwintering conditions, perhaps by stabilizing Ca crystals in the scales, further reducing the ability of bass to replace Ca lost at the gills.

Leino, R.L., J.H. McCormick, and K.M. Jensen. 1990. Multiple effects of acid and aluminum on brood stock and progeny of fathead minnows, with emphasis on histopathology. Can. J. Zool. 68: 234-244.

Gills of fathead minnows reared at pH 6.0, 5.5, and 5.2, in the presence (30-60 $\mu g \cdot L^{-1}$ Al) or absence (15 $\mu g \cdot L^{-1}$ Al) of added Al had major histopathological changes at the gills compared to pH 7.5 (with or without Al; Ca =200 μM). Aluminum ameliorated slightly the gill damage seen at pH 5.2 in the absence of added Al. Fathead minnows are highly sensitive to H^+ itself, associated with the high sensitivity of the minnow to low Ca concentrations.

Lemly, A.D. 1996. Wastewater discharges may be most hazardous to fish during winter. Environ. Pollut. 93: 169-174.

"Winter Stress Syndrome". Seasonal metabolic changes. Wastewater discharges may be a greater toxic threat in winter. Worth mentioning seasonal effects in the review.

Lin, H., and D.J. Randall. 1990. The effect of varying water pH on the acidification of expired water in rainbow trout. J. exp. Biol. 149: 149-160.

Acidic water (~pH<6) is made more basic as it passes over the gills of rainbow trout. For Al precipitation theory.

Malte, H. 1986. Effects of aluminium in hard, acid water on metabolic rate, blood gas tensions and ionic status in the rainbow trout. J. Fish Biol. 29: 187-198.

Very high Al concentrations (2 $mg \cdot L^{-1}$) were used in very hard water (~3,500 μM Ca; 4-6 d exposures, pH 5). Under these extreme conditions, the cannulated

fish showed large respiratory disturbances due to Al precipitation and mucus clogging of the gills.

Malte, H., and R.E. Weber. 1988. Respiratory stress in rainbow trout dying from aluminium exposure in soft acid water, with or without added sodium chloride. Fish Physiol. Biochem. 5: 249-256.
Respiratory effects of Al were clearly demonstrated by Malte and Weber. They exposed cannulated adult rainbow trout to high Al concentrations (860 $\mu g \cdot L^{-1}$) at pH 5.0 in soft water (Ca =50 µM). They eliminated the ionoregulatory effects of Al by adding 150 mM NaCl to the water (~same concentration as fish plasma) but hyperventilation, low arterial PO_2 and high arterial PCO_2, acidosis, and high blood lactate concentrations still occurred within 24 h. These results confirmed for soft water earlier work by Malte (1986) and Jensen and Weber (1987).

McCahon, C.P., D. Pascoe, and C. Mc Kavanagh. 1987. Histochemical observations on the salmonids *Salmo salar* L. and *Salmo trutta* L. and the ephemeropterans *Baetis rhodani* (Pict.) and *Ecdyonurus venosus* (Fabr.) following a simulated episode of acidity in an upland stream. Hydrobiol. 153: 3-12.
Atlantic salmon and brown trout (0^+ year) both showed Al deposition and mucus production on gills when exposed, in a stream, to an experimental regime of pH 5, ~350 $\mu g \cdot L^{-1}$ Al for 24 h.

McCahon, C.P., A.F. Brown, M.J. Poulton, and D. Pascoe. 1989. Effects of acid, aluminium and lime additions on fish and invertebrates in a chronically acidic Welsh stream. Wat. Air Soil Pollut. 45: 345-359.
More useful for the invertebrate section than here.

McCormick, J.H., and K.M. Jensen. 1992. Osmoregulatory failure and death of first-year largemouth bass (*Micropterus salmoides*) exposed to low pH and elevated aluminum, at low temperature in soft water. Can. J. Fish. Aquat. Sci. 49: 1189-1197.

McDonald, D.G. 1983. The effects of H^+ upon the gills of freshwater fish. Can. J. Zool. 61: 691-703.
In acidic water H^+ ions reduce the active uptake of Na and Cl, and increase their passive effluxes, possibly by displacing Ca from tight junctions. See also Freda et al. (1991).

McDonald, D.G., and C.L. Milligan. 1988. Sodium transport in the brook trout, *Salvelinus fontinalis*: effects of prolonged low pH exposure in the presence and absence of aluminum. Can. J. Fish. Aquat. Sci. 45: 1606-1613.
Fish may be at least partially able to acclimate to Al through increased synthesis of gill Na,K-ATPase.

McDonald, D.G., C.M. Wood, R.G. Rhem, M.E. Mueller, D.R. Mount, and H.L. Bergman. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). I. Physiology. Can. J. Fish. Aquat. Sci. 48: 2006-2015.
McDonald et al. were able to follow the acclimation process of brook trout to Al. There was an initial damage (shock) phase, in which juvenile brook trout

exposed for up to 42 d to 75-150 $\mu g \cdot L^{-1}$ Al (pH 5.2, Ca =15 µM) accumulated Al on their gills, showed a reduction in a gill mucus indicator (sialic acid), and showed ionoregulatory and respiratory effects of Al. After 10 d there was a recovery phase, shown by less Al on the gills, normal gill sialic acid content, and decreased ionoregulatory and respiratory effects. Gill damage and repair followed this general time course (see Mueller et al. 1991). After 4 wk acclimation, fish resistance to a 2 wk exposure to 100 or 300 $\mu g \cdot L^{-1}$ Al was enhanced (pH 4.8 or 5.2) as the recovery and acclimation process continued, with less mortality, less ionoregulatory disturbance, and only about half the Al accumulation on gills as Al-naïve fish.

Milligan, C.L., and C.M. Wood. 1982. Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. J. exp. Biol. 99: 397-415.
The hemoconcentration results of Milligan and Wood for fish at low pH have been confirmed for Al (e.g. similar effects) and probably need no longer be addressed specifically.

Mount, D.R., M.J. Swanson, J.E. Breck, A.M. Farag, and H.L. Bergman. 1990. Responses of brook trout (*Salvelinus fontinalis*) fry to fluctuating acid, aluminum, and low calcium exposure. Can. J. Fish. Aquat. Sci. 47: 1623-1630.
Brook trout fry pre-exposed for up to 18 d to 50 $\mu g \cdot L^{-1}$ Al at pH 5.2 (Ca =64 µM) showed better survival during a 6 d, 330 $\mu g \cdot L^{-1}$ Al, pH 4.6, Ca =1.6 µM pulse as the pre-exposure period lengthened. Unfortunately, as the authors realized, their results were complicated by growth of the fry during the pre-exposure period, which may have added to the apparent acclimation effect.

Mueller, M.E., D.A. Sanchez, H.L. Bergman, D.G. McDonald, R.G. Rhem, and C.M. Wood. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). II. Gill histology. Can. J. Fish. Aquat. Sci. 48: 2016-2027.
Gill damage and repair followed the general time course seen in the companion article of McDonald et al. (1991).

Muniz, I.P., and H. Leivestad. 1980. Acidification - effects on freshwater fish. In: Ecological impact of acid precipitation, pp. 84-92. (Drablös, D. and Tollan, A., Eds,) Proc. Int. conf. ecol. impact acid precip., Norway, SNSF project.
Muniz and Leivestad described two mechanisms of Al toxicity to fish. They were 1) ionoregulatory, involving losses of plasma chloride, and 2) respiratory effects, shown by lowered plasma oxygen tension, due to clogging of gills by mucus at high Al concentrations (150-900 $\mu g \cdot L^{-1}$ Al). A good, early survey paper on Al and fish.

Neville, C.M. 1985. Physiological response of juvenile rainbow trout, *Salmo gairdneri*, to acid and aluminum - prediction of field responses from laboratory data. Can. J. Fish. Aquat. Sci. 42: 2004-2019.
Christine Neville was one of the early investigators to systematically show the dual respiratory and ionoregulatory effects of Al at fish gills. In a complex set of

experiments, cannulated juvenile rainbow trout primarily showed hypoxia at pH 6.1 when exposed 6 to 11 d to 75 $\mu g \cdot L^{-1}$ Al in soft water (50 to 140 µM Ca), suffered electrolyte loss at pH 4.5, and showed a combination of the two responses to Al at pH 5.5 and 5.0. Aluminum provided some protection against the effects of H^+ at pH 4.0. All fish had acidified blood, either from CO_2 accumulation (respiratory acidosis), lactic acid accumulation due to anaerobic respiration (metabolic acidosis), or a mixture of the two. In an effort to explain some of her results, Neville suggested that gill surface pH may be more basic than ambient (acidic) pH due to acid uptake or base release at the gills.

Neville, C.M., and P.G.C. Campbell. 1988. Possible mechanisms of aluminum toxicity in a dilute, acidic environment to fingerlings and older life stages of salmonids. Wat. Air Soil Pollut. 42. 311-327.

A later, re-interpretation of the results of Neville (1985). Re-emphasized the dual ionoregulatory and respiratory nature of Al toxicity. Neville and Campbell stated that Al speciation theories of Al toxicity may not be useful because rapid equilibria between chemical species makes any species of Al a source of free Al to interact with binding sites on gill membranes. Precipitation phenomena, a "physical" process at the gill surface, was considered a likely explanation of the respiratory effects of Al at pH 6.1, especially if gill surfaces were more basic than the acidic bulk water, and the protective effect of Al^{3+} against H^+ (pH 4.0) was seen as competition for binding sites on the gills.

Norrgren, L., A. Wicklund Glynn, and O. Malmborg. 1991. Accumulation and effects of aluminium in the minnow (*Phoxinus phoxinus* L.) at different pH levels. J. Fish Biol. 39: 833-847.

Norrgren et al. showed gross deformities of minnow gills (*Phoxinus phoxinus* L.) after 12 to 48 d exposures to 160-170 $\mu g \cdot L^{-1}$ Al at pH 5 and 6 (Ca =90 µM). Aluminum on the gills was localized mainly in mucus between gill lamellae, and there were changes in the morphology of cilia in the olfactory mucosa (LM, SEM). After 48 d there was some additional Al in liver and kidney if fish were exposed to pH 6 plus Al, but mortality at pH 5 and 6 in the presence of Al was likely due to gill effects.

Orr, P.L., R.W. Bradley, J.B. Sprague, and N.J. Hutchinson. 1986. Acclimation-induced change in toxicity of aluminum to rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 43: 243-246.

Orr et al. found that juvenile rainbow trout doubled their tolerance to Al when exposed for 2-3 weeks to ~90 and 150 $\mu g \cdot L^{-1}$ Al (pH 5.1-5.3, Ca =72 µM), but did not speculate as to what the acclimation process was.

Østbye, K., S.A. Øxnevad, and L.A. Vøllestad. 1997. Developmental stability in perch (*Perca fluviatilis*) in acidic aluminium-rich lakes. Can. J. Zool. 75: 919-928.

Oughton, D.H., B. Salbu, H.E. Bjørnstad, and J.P. Day. 1992. Use of aluminium-26 tracer to study the deposition of aluminium species on fish gills following mixing of limed and acidic waters. Analyst 117: 619-621.

The expensive ^{26}Al isotope was used to trace Al accumulation on fish gills (see also Playle (1987) and Bjørnstad et al. (1992). The usefulness of these laboratory studies suffered from lack of replication, due mainly to the extremely high cost of ^{26}Al. Except perhaps for very particular experiments, graphite furnace AAS is as good a method for Al localization and is certainly cheaper.

Palmer, R.E., R.J. Klauda, M.A. Jepson, and E.S. Perry. 1989. Acute sensitivity of early life stages of fathead minnow (*Pimephales promelas*) to acid and aluminum. Wat. Res. 23: 1039-1047.

If all Al species are in equilibrium (e.g. Neville and Campbell 1988), then attempts made to determine which individual Al species is responsible for Al toxicity, such as this are of questionable value (and those made by Sadler and Lynam (1987) and Playle and Wood (1991)).

Parkhurst, B.R, H.L. Bergman, J. Fernandez, D.D. Gulley, J.R. Hockett, and D.A. Sanchez. 1990. Inorganic monomeric aluminum and pH as predictors of acidic water toxicity to brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 47: 1631-1640.

Twenty-one day survival of brook trout fry was consistently enhanced by $\geq$3.1 mg C·L^{-1} DOC (Aldrich humic acid) during exposures to $\leq$340 µg·L^{-1} total Al at pH 5.1 and 5.6 (Ca =55 µM). The protective effect of DOC was less at pH 4.8 and was virtually eliminated at pH 4.4 and high Al concentrations (~1,200 µg·L^{-1}). Dissolved organic carbon was able to bind Al best at higher pH (as shown by low inorganic, monomeric Al concentrations); presumably DOC did not bind Al well at lower pH because of H^+ competition for binding sites on DOC (=protonation of binding sites), because of Al^{3+} predominance at lower pH, and higher Al solubility at lower pH. Monomeric (available) Al best predicted Al toxicity to fish.

Peterson, R.H., R.A. Bourbonnière, G.L. Lacroix, D.J. Martin-Robichaud, P. Takats, and G. Brun. 1989. Responses of Atlantic salmon (*Salmo salar*) alevins to dissolved organic carbon and dissolved aluminum at low pH. Wat. Air Soil Pollut. 46: 399-413.

Dissolved organic carbon was protective against Al toxicity of Atlantic salmon alevins as long as inorganic Al not complexed by natural DOC was less than 50 µg·L^{-1} (Ca ~20 µM, pH 4.8-5.8, 30-d exposures). Aluminum accumulation by the fish (and hence toxicity) was related to the amount of free Al.

Playle, R.C. 1987. Methods and feasibility of using aluminum-26 as a biological tracer in low pH waters. Can. J. Fish. Aq. Sci. 44 (Suppl. 1): 260-263.

^{26}Al work. Expensive. Low replication. See Oughton et al. (1992).

Playle, R.C., and C.M. Wood. 1989a. Water chemistry changes in the gill micro-environment of rainbow trout: experimental observations and theory. J. Comp. Physiol. B 159: 527-537.

Playle and Wood (1989a) and Playle et al. (1992) showed that soft, acidic water (~pH<6) is made more basic as it passes over the gills of rainbow trout and fathead minnows (*Pimephales promelas*), primarily due to NH_3 release at the

gills. This partial neutralization of acidic water is particularly important with respect to acidic soft water containing Al. In theory, the various species of Al in acidic water passing over fish gills would shift from mostly Al^{3+} in favour of Al-hydroxides, and Al would be less soluble in the more basic water in the gill micro-environment.

Playle, R.C., and C.M. Wood. 1989b. Water pH and aluminum chemistry in the gill micro-environment of rainbow trout during acid and aluminum exposures. J. Comp. Physiol. B 159: 539-550.
Soft water at pH 5.2 can hold ~150 $\mu g \cdot L^{-1}$ Al, and its composition is about 23% Al^{3+}, 40% $AlOH^{2+}$, 25% $Al(OH)_2^+$, 10% $Al(OH)_3^\circ$, and only about 2% $Al(OH)_4^-$. As this water passes over the gills, its pH increases to pH ~5.5, which can only hold about 30 $\mu g \cdot L^{-1}$ Al, and the Al composition is now about 8% Al^{3+}, 30% $AlOH^{2+}$, 40% $Al(OH)_2^+$, 15% $Al(OH)_3^\circ$, and 8% $Al(OH)_4^-$.

Playle, R.C., and C.M. Wood. 1990. Is precipitation of aluminum fast enough to explain aluminum deposition on fish gills? Can. J. Fish. Aquat. Sci. 47: 1558-1561.
Playle and Wood favoured the interpretation that soft water at pH 5.0 to 5.5 containing 100 to 200 $\mu g \cdot L^{-1}$ Al would become oversaturated with Al in the more basic gill micro-environment, with the precipitated Al being responsible for the respiratory effects of Al. These workers used a filtration apparatus to show that Al can polymerize and precipitate from solution in about 1-4 s after base (NH_3) addition, likely fast enough for Al accumulation on fish gills to occur through precipitation. There was ~10% of 160 $\mu g \cdot L^{-1}$ total Al which did not pass through 0.2 µm filters at pH 5.3; 1-4 s after NH_3 was added to raise the pH to 5.7, ~35% of the Al was retained by the filters. See also the new work by Exley et al.

Playle, R.C., and C.M. Wood. 1991. Mechanisms of aluminium extraction and accumulation at the gills of rainbow trout, *Oncorhynchus mykiss* (Walbaum), in acidic soft water. J. Fish Biol. 38: 791-805.
If all Al species are in equilibrium (Neville and Campbell 1988), then attempts made to determine which individual Al species is responsible for Al toxicity, such as those made by Sadler and Lynam (1987), Palmer et al. (1989), and Playle and Wood (1991), are of questionable value. Perhaps this is why the Al precipitation theory at fish gills is so attractive. Which specific Al polymer produced at the gills is not necessarily important to know, nor is it necessary to know which positively-charged Al species binds to the negatively charged gill surfaces when precipitation does not occur: it is enough to know that binding and precipitation can occur at fish gills.

Playle, R.C., G.G. Goss, and C.M. Wood. 1989. Physiological disturbances in rainbow trout (*Salmo gairdneri*) during acid and aluminum exposures in soft water of two calcium concentrations. Can. J. Zool. 67: 314-324.
Continuing where Neville (1985) stopped, Playle et al. again showed the dual ionoregulatory and respiratory effects of Al on cannulated adult rainbow trout (Al =105 $\mu g \cdot L^{-1}$; pH 4.4 to 5.2; Ca =22 or 205 µM; 66 h exposures). Ionoregulatory toxicity of Al, possibly due to Al^{3+}, was seen as additional to the ionoregulatory

effects of acidity alone, but the respiratory effects (low arterial PO_2, high arterial PCO_2) were seen as being unique to Al, possibly due to Al precipitation on the gills. Blood acidification was a combination of respiratory and metabolic acidosis, and Ca reduced the ionoregulatory disturbances of Al at lower pH, but did not reduce the respiratory effects of Al at higher pH.

Playle, R.C., R.W. Gensemer, and D.G. Dixon. 1992. Copper accumulation on gills of fathead minnows: influence of water hardness, complexation and pH of the gill micro-environment. Environ. Toxicol. Chem. 11: 381-391.
Mainly shows that pH changes in the gill micro-environment also happen in fish besides rainbow trout. Interesting because of the small scale of the experiments.

Poléo, A.B.S. 1995. Aluminium polymerization - a mechanism of acute toxicity of aqueous aluminium to fish. Aquat. Toxicol. 31: 347-356.
A nice compilation of relevant fish experiments. Poléo proposes that it is the formation of Al polymers is responsible for acute, hypoxic death of fish (see Witters et al. 1996, too). So, more-or-less agrees with the idea of ionoregulatory effects at low pH, and a polymerization effect (positive Al binding to negative gills, then polymerizing) at more neutral, liming conditions. However, did not consider pH changes at the gills, which I consider a great oversight.

Poléo, A.B.S., E. Lydersen, and I.P. Muniz. 1991. The influence of temperature on aqueous aluminium chemistry and survival of Atlantic salmon (*Salmo salar* L.) fingerlings. Aquat. Toxicol. 21: 267-278.
The effect of temperature on Al toxicity to Atlantic salmon fingerlings was studied. They found that toxicity of a 270 or 400 $\mu g \cdot L^{-1}$ Al, pH 5.0, 25 µM Ca solution increased as temperature increased (1 to 19°C; 12 d exposures). They interpreted their data as being due to Al polymerization, which is faster at higher temperature, but these workers did not dismiss the possibility of greater Al toxicity at higher temperature being due to higher metabolic rate at higher temperature (e.g. Roch and Maly 1979 for Cd; Q_{10}) or to heat stress of the fish (they were all acclimated to 6°C).

Poléo, A.B.S., S.A. Øxnevad, K. Østbye, R.A. Anderson, D.H. Oughton, and L.A. Vøllestad. 1995. Survival of crucian carp, *Carassisus carassius*, exposed to a high low-molecular weight inorganic aluminium challenge. Aquat. Sci. 57: 350-359.
All fish survived 25 d in pH 5.2 and 240 $\mu g \cdot L^{-1}$ Al. Plasma Cl decreased, and Al accumulated on the gills. They did not measure blood PO_2, but said that, because blood hematocrit did not change, that this supported the Al polymerization theory of Al toxicity because these anoxia tolerant fish survived. Indirectly it does, but not as good as measuring blood PO_2.

Poston, H.A. 1991. Effects of dietary aluminum on growth and composition of young Atlantic salmon. Progress. Fish-Cultur. 53: 7-10.
Dietary Al has no significant adverse or beneficial effects.

Rahaman-Noronha, E., M.J. O'Donnell, C.M. Pilley, and P.A. Wright, P.A. 1996. Excretion and distribution of ammonia and the influence of boundary layer acidification in embryonic rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 199: 2713-2723.
Similar idea of unstirred boundary layer next to embryos (more acidic in more basic conditions due to CO_2; more basic in acidic conditions due to NH_3. Could be useful for the gill micro-environment section.

Reid, S.D., D.G. McDonald, and R.R. Rhem. 1991. Acclimation to sublethal aluminum: modifications of metal-gill surface interactions of juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 48: 1996-2005.
Changes in binding properties of Al to the gill surface during the acclimation process were studied. A 21 d exposure of juvenile rainbow trout to 30 $\mu g \cdot L^{-1}$ Al (pH 5.2, Ca = 15 µM) reduced the physiological effects and mortality when the fish were later exposed to 120 $\mu g \cdot L^{-1}$ Al. During this time the binding affinity of the gill surface to Al decreased, and that of Ca increased, so that the ability of Al to displace Ca at gills, causing the ionoregulatory effects of Al, would be reduced. There is likely a metabolic cost associated with acclimation.

Richards, J.G., and R.C. Playle. 1998. Cobalt binding to gills of rainbow trout (*Oncorhynchus mykiss*): an equilibrium model. J. Comp. Biochm. Physiol. in press.

Roch, M., and E.J. Maly. 1979. Relationship of cadmium-induced hypocalcemia with mortality in rainbow trout (*Salmo gairdneri*) and the influence of temperature on toxicity. J. Fish. Res. Board Can. 36: 1297-1303.
See Poleo et al. (1991) reference.

Rosseland, B.O., and M. Stuarnes. 1994. Physiological mechanisms for toxic effects and resistance to acidic water: an ecophysiological and ecotoxicological approach. *In* Acidification of Freshwater Ecosystems: Implications for the Future. Steinberg, C.E.W., and Wright, R.F. [eds.]. Environ. Sci. Rep. ES 14, John Wiley & Sons, Ltd., New York.
Rosseland et al. (1990) and Rosseland and Staurnes (1994) are reviews with a more European viewpoint. Rosseland and Staurnes (1994) stressed that declines in fish populations caused by acidic precipitation are due to the sum of the effects of elevated H^+ and Al concentrations and low Ca concentrations, affecting critical life processes during critical life stages of fish. They felt that the main effect of precipitated Al on fish gills is osmoregulatory failure; different water chemistry in the gill micro-environment compared to the bulk (acidic) water can lead to Al precipitation on the gills (Rosseland and Staurnes 1994, citing Exley et al. 1991).

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

Accumulation of Al on gills of caged Atlantic salmon and sea trout (*S. trutta*) smolts, placed in the mixing zone of acid water meeting water limed with $CaCO_3$, was thought to be due to polymerization of Al in the mixing zone. The mixing zone was more toxic than the acid water alone (containing about 250 $\mu g \cdot L^{-1}$ monomeric Al, pH ~4.8, Ca ~32 µM) or the limed water (~90 $\mu g \cdot L^{-1}$ monomeric Al, pH ~7.0, Ca ~92 µM).

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

Sadler, K., and S. Lynam. 1987. Some effects on the growth of brown trout from exposure to aluminium at different pH levels. J. Fish Biol. 31: 209-219.
Another of these speciation papers which could be of limited value if all Al species are in equilibrium (e.g. Neville and Campbell 1988).

Sayer, M.D.J., J.P. Reader, and R. Morris. 1991a. Effects of six trace metals on calcium fluxes in brown trout (*Salmo trutta* L.) in soft water. J. Comp. Physiol. B 161: 537-542.
Aluminum can interfere with Ca balance. Brown trout fry exposed to 40 to ~160 or 490 $\mu g \cdot L^{-1}$ Al at pH 5.8-6.6 (Ca =20 µM, 72 h exposures) showed increased net efflux of Ca, and, at the higher Al concentrations, reduced Ca influx.

Sayer, M.D.J., J.P. Reader, T.R.K. Dalziel, and R. Morris. 1991b. Mineral content and blood parameters of dying brown trout (*Salmo trutta* L.) exposed to acid and aluminium in soft water. Comp. Biochem. Physiol. 99C: 345-348.
Measured plasma Na and hematocrit of dying juvenile brown trout (exposure =320 $\mu g \cdot L^{-1}$ Al, pH 4.5, 20 µM Ca) and once again showed hemoconcentration due to ionoregulatory failure as the primary cause of death, although blood gases were not measured.

Sayer, M.D.J., J.P. Reader, and R. Morris. 1991c. Embryonic and larval development of brown trout, *Salmo trutta* L.: exposure to aluminium, copper, lead or zinc in soft, acid water. J. Fish Biol. 38: 431-455.
They found that Cu (5 $\mu g \cdot L^{-1}$), Pb (10 $\mu g \cdot L^{-1}$), and Al (160 $\mu g \cdot L^{-1}$) caused brown trout fry mortalities and impaired skeletal calcification in synthetic soft water, but Zn (20 $\mu g \cdot L^{-1}$) did not (Ca =20 µM, pH ~5.6).

Simonin, H.A., W.A. Kretser, D.W. Bath, M. Olson, and J. Gallagher. 1993. In situ bioassays of brook trout (*Salvelinus fontinalis*) and blacknose dace (*Rhinichthys atratulus*) in Adirondack streams affected by episodic acidification. Can. J. Fish. Aquat. Sci. 50: 902-912.
A field study involving blacknose dace (*Rhinichthys astratulus*) and brook trout placed in four Adirondack headwater streams. High water flows associated with snowmelt or rain were toxic to the fish, with water pH (pH 4.4-6.8), inorganic Al concentration (20-350 $\mu g \cdot L^{-1}$), episode duration (to ~2 wk), and DOC (3-9 mg $C \cdot L^{-1}$) all being important in determining fish mortality (Ca ~50-150 µM). Blacknose dace were consistently more sensitive than brook trout to the acidic

water episodes, and showed good survival if exposed to inorganic Al concentrations of $\leq$100 $\mu g \cdot L^{-1}$.

Skogheim, O.K., B.O. Rosseland, F. Kroglund, and G. Hagenlund. 1987. Addition of NaOH, limestone slurry and finegrained limestone to acidified lake water and the effects on smolts of Atlantic salmon (*Salmo salar* L.). Wat. Res. 21: 435-443.
If water pH rises too high during liming, the $Al(OH)_4^-$ anion can become toxic. Atlantic salmon smolts in enclosure experiments.

Spry, D.J., and J.G. Wiener. 1991. Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. Environ. Pollut. 71: 243-304.
Spry and Wiener cover the geochemistry of Al, and modifying effects of Ca, pH, dissolved organic carbon, pre-exposure to Al, and life stage on Al toxicity to fish in acidic waters.

Staurnes, M., L.P. Hansen, K. Fugelli, and Ø. Haraldstad. 1996. Short-term exposure to acid water impairs osmoregulation, seawater tolerance, and subsequent marine survival of smolts of Atlantic salmon (*Salmo salar* L.). Can. J. Fish. Aquat. Sci. 53: 1695-1704.
Smolts left in cages showed osmoregulatory failure and high mortality. Gill Na/K/ATPase activities were reduced. Implications for aquaculture. Table 2 and the references need to be examined closely.

Staurnes, M., T. Sigholt, and O.B. Reite. 1984. Reduced carbonic anhydrase and Na-K-ATPase activity in gills of salmonids exposed to aluminium-containing acid water. Experientia 40: 226-227.
They found that 30-60 g rainbow trout (*Salmo gairdneri* =*Oncorhynchus mykiss*) and salmon (*S. salar*) exposed to 200 $\mu g \cdot L^{-1}$ Al at pH 5.0 for 4-7 d had reduced Na,K-ATPase and carbonic anhydrase activities in fish gills. Both these enzymes are important for ion regulation across the gills; the 23-39% reduction in their activities was accompanied by 8-39% declines in plasma Na and Cl concentrations. It was assumed that these effects were due to Al alone, because brown trout exposed to pH 5.0 for 7 d (Ca =10 µM) had normal plasma Cl concentrations (see Muniz and Leivestad 1980).

Staurnes, M., P. Blix, and O.B. Reite. 1993. Effects of acid water and aluminum on parr-smolt transformation and seawater tolerance in Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. 50: 1816-1827.
Here they showed that decreased plasma Na concentrations in 1 yr old Atlantic salmon were linearly related to decreases in gill Na,K-ATPase activity when the fish were exposed to acidic conditions (pH 5) and to 50 $\mu g \cdot L^{-1}$ Al at pH 5 (Ca=25-40 µM; 24 d exposures). In addition, carbonic anhydrase activity decreased in the Al plus acid exposures.

Staurnes, M., L.P. Hansen, K. Fugelli, and O. Haraldstad. 1996. Short-term exposure to acid water impairs osmoregulation, seawater tolerance, and subsequent marine survival of smolts of Atlantic salmon (*Salmo salar* L.). Can. J. Fish. Aquat. Sci. 53: 1695-1704.

Suter, G.W. II. 1996. Toxicological benchmarks for screening contaminants of potential concern for effects on freshwater biota. Environ. Toxicol. Chem. 15: 1232-1241.

Thomsen, A., B. Korsgaard, and J. Joensen. 1988. Effect of aluminium and calcium ions on survival and physiology of rainbow trout *Salmo gairdneri* (Richardson) eggs and larvae exposed to acid stress. Aquat. Toxicol. 12: 291-300.

Tietge, J.E., R.D. Johnson, and H.L. Bergman. 1988. Morphometric changes in gill secondary lamellae of brook trout (*Salvelinus fontinalis*) after long-term exposure to acid and aluminum. Can. J. Fish. Aquat. Sci. 45: 1643-1648.
Aluminum effects on gills of adult brook trout were seen after ~5 mo exposure to pH 4.45 plus 390 $\mu g \cdot L^{-1}$ Al (Ca ~40 µM; light microscopy).

Van Offelen, H.K., C.C. Krueger, C.L. Schofield, and C. Keleher. 1994. Survival, distribution, and ion composition in two strains of brook trout (*Salvelinus fontinalis*) fry after exposure to episodic pH depressions in an Adirondack lake. Can. J. Fish. Aquat. Sci. 51: 792-799.
Two strains of brook trout fry within long enclosures survived spring snowmelt by moving into the deeper, less acidic, less Al, and higher Ca water (pH 6.3, Al ~480 $\mu g \cdot L^{-1}$, Ca ~160 µM at >2 m depth; pH 4.8, Al ~1,200 $\mu g \cdot L^{-1}$, Ca ~60 µM at 0.7 m).

Verbost, P.M., M.H.G. Berntssen, F. Krogland, E. Lydersen, H.E. Witters, B.O. Rosseland, B. Salbu, and S.E.W. Bonga. 1995. The toxic mixing zone of neutral and acidic river water - acute aluminum toxicity in Brown trout (*Salmo trutta* L.). Water Air Soil Pollut. 85: 341-346.
They thought that resulting water after mixing would not be toxic, on the basis of "current Al toxicity models". The chemistry was pH 6.4, 245 $\mu g \cdot L^{-1}$ Al. (I would say - "oversaturated", so of course it would be toxic!) In fact, it was, and with greater toxicity right after mixing (e.g. 12 s) and less toxicity ~5 min after mixing. Proposed mechanism: suffocation due to Al precipitates on the gills (or, polymerization) and loss of plasma Na and Cl. See Witters et al. (1996) and Poleo, too. Very useful because it discusses transient mixing zone effects.

Verbost, P.M., F.P.J.G. Lafeber, F.A.T. Spanings, E.M. Aarden, E.M., and S.E. Wendelaar Bonga. 1992. Inhibition of Ca^{2+} uptake in freshwater carp, *Cyprinus carpio*, during short-term exposure to aluminum. J. Exp. Zool. 262: 247-254.
Monitoring of Ca and Na influx and efflux using ^{45}Ca and ^{24}Na indicated a dose dependent inhibition of Ca influx by Al, and stimulation of both Ca and Na efflux at higher Al concentrations (30-440 $\mu g \cdot L^{-1}$ Al, 780 µM Ca, pH 5.2, 1-4 h exposures, carp *Cyprinus carpio*). Stimulation of Ca and Na efflux by Al may have been due to displacement of Ca from tight junctions on the gill epithelium, but inhibition of Ca influx was harder to explain. These authors speculated that Al may inhibit Ca influx by interfering with the second messengers involved in keeping open Ca channels on the apical membrane.

Vuorinen, P.J., M. Vuorinen, S. Peuranen, M. Rask, A. Lappalainen, and J. Raitaniemi. 1992. Reproductive status, blood chemistry, gill histology and growth of perch (*Perca fluviatilis*) in three acidic lakes. Environ. Pollut. 78: 19-27.
Adult perch (*Perca fluviatilis* L.) from four Finnish lakes had more Al deposits (as indicated by hematoxylin staining) in their gills as water Al content increased (to 250 $\mu g \cdot L^{-1}$ Al) and water pH decreased (down to pH 4.3; Ca $\leq$80 µM).

Waring, C.P., and J.A. Brown. 1995. Ionoregulatory and respiratory responses of brown trout, *Salmo trutta*, exposed to lethal and sublethal aluminium in acidic soft waters. Fish Physiol. Biochem. 14: 81-91.
Cannulated brown trout exposed to ~12, 24, and 60 $\mu g \cdot L^{-1}$ Al in synthetic soft water showed moderate to severe respiratory effects of Al (e.g. decreased arterial blood oxygen content) but few ionoregulatory effects (Ca=20 µM, pH 5.0). Brown trout were extremely sensitive to Al in this study, with mortalities occurring at 24 $\mu g \cdot L^{-1}$ Al (67% in 5 d) and at 60 $\mu g \cdot L^{-1}$ Al (100% in 2 d).

Waring, C.P., J.A. Brown, J.E. Collins, and P. Prunet. 1996. Plasma prolactin, cortisol, and thyroid responses of the brown trout (*Salmo trutta*) exposed to lethal and sublethal aluminium in acidic soft waters. Gen. Comp. Endocr. 102: 377-85.
Soft water, 0-50 $\mu g \cdot L^{-1}$ Al, 0-100% mortality in 48 h, cannulated fish. Prolactin down, cortisol up, T_4 up, T_3 up in some. A nice companion paper to Waring and Brown (1995).

Weatherley, N.S., G.P. Rutt, S.P. Thomas, and S.J. Ormerod. 1991. Liming acid streams: aluminium toxicity to fish in mixing zones. Water Air Soil Pollut. 55: 345-353.
Caged brown trout in an acidic stream which was treated with limestone. While total Al stayed approximately constant, the proportion of Al that would not pass through a 0.22 µm filter increased, and toxicity to the fish was high (until further downstream). The authors suggest that $Al(OH)_4^-$, precipitation of Al, or Al polymerization, all could have been the toxic mechanism.

Wilkinson, K.J., P.G.C. Campbell, and P. Couture. 1990. Effect of fluoride complexation on aluminum toxicity towards juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 47: 1446-1452.
They found that 9 µM Al in the presence of 1 to 10 µM F was less toxic to juvenile Atlantic salmon than was Al alone, but was more toxic than predicted by calculated Al^{3+} concentrations (pH 4.5, 4.9; Ca =45 µM; 7 d exposures). They interpreted their data as Al accumulation and toxicity due to an Al-F complex itself, but another interpretation is that fish gills can out-compete the Al-F complex for Al. Estimates of conditional stability constants of Al^{3+} made by Wilkinson et al. (1990) are log $K_{Al\text{-}gill}$=6.5, and log $K_{Al\text{-}F}$=7.0, so the Al^{3+}-F complex is only 3X stronger than the Al^{3+}-gill complex.

Wilkinson, K.J., P.M. Bertsch, C.H. Jagoe, and P.G.C. Campbell. 1993. Surface complexation of aluminum on isolated fish gill cells. Environ. Sci. Technol. 27: 1132-1138.

They used isolated cells from gills of largemouth bass, *Micropterus salmoides*, and determined the electrophoretic mobility of suspended gill cells in the presence and absence of Al. As up to 50 µM Al (=1,350 $\mu g \cdot L^{-1}$) was added to isolated gill cells (solution pH = 4.5), the negatively charged cells, as determined by their electrophoretic mobility, became progressively more positive as Al^{3+} bound to negative sites. For 10 µM Al, the addition of up to 10 µM F kept some Al off the gills, as judged by electrophoretic mobility of negatively charged isolated cells (suspension pH =4.5), but not by as much as predicted by the decrease in free Al^{3+} through complexation by F. These workers interpreted their results as Al-F complexes binding to the gills, as well as free Al^{3+}, but an alternative explanation, is that the gill cells outcompete F for Al^{3+}. At very high Al-F concentrations (10 mM) these workers were able to show some Al-F_n deposition on fish gill cells. Soil fulvic acid added to the isolated cell suspensions (0-20 mg $C \cdot L^{-1}$) was able to reduce the amount of Al binding to the isolated gill cells, as indicated by the greater negative charge of the cells as the fulvic acid concentration was increased. Again, they interpreted their data as Al-DOC complexes binding to the gills, but the DOC was also likely able to keep Al off the gills through complexation.

Wilkinson, K.J., and P.G.C. Campbell. 1993. Aluminum bioconcentration at the gill surface of juvenile Atlantic salmon in acidic media. Environ. Toxicol. Chem. 12: 2083-2095.

Initial, slow accumulation of Al onto gills of Atlantic salmon, and subsequent, rapid loss to water once Al exposure is halted, was mimicked by incorporating mucus production and sloughing into a computer model. In their model, Al binds initially to mucus from where it is passed to gill tissue, and total gill Al increases linearly then reaches a plateau when mucus production and mucus sloughing are equal. Rapid depuration of gill Al occurs as mucus production decreases when the Al stress is removed, but mucus (and therefore mucus-bound Al) sloughing continues.

Williams, R.J.P. 1996. Aluminium and biological systems: an introduction. Coordination Chem. Rev. 149: 1-9.

This is the introductory paper to a special issue on Al and (mainly) human effects. There's a brief introduction to plant and animal Al toxicity. This may be good to reference to get interested readers to a good collection of up to date papers on the human side of the question.

Wilson, R.W., H.L. Bergman, and C.M. Wood. 1994a. Metabolic costs and physiological consequences of acclimation to aluminum in juvenile rainbow trout (*Oncorhynchus mykiss*). 1: Acclimation specificity, resting physiology, feeding, and growth. Can. J. Fish. Aquat. Sci. 51: 527-535.

A continuation of their earlier work. They again showed the costs to fish of acclimation to Al (e.g. reduced feeding and growth), but were also able to show that acclimation to Al was specific to Al, and did not protect fish against acute Cu toxicity (Wilson et al. 1994b). In these studies, juvenile rainbow trout were

exposed for 34 d to soft water at pH 5.2 containing 38 $\mu g \cdot L^{-1}$ Al (for Al acclimation), pH 5.2 water with background Al (3 $\mu g \cdot L^{-1}$), or to pH 6.5 water, 4 $\mu g \cdot L^{-1}$ Al (control; all ~15 µM Ca). Acclimation to Al developed in 5 d, as shown by fish mortalities during exposures to 162 $\mu g \cdot L^{-1}$ Al at pH 5.2, but there was no concurrent, protective effect against a 32 $\mu g \cdot L^{-1}$ Cu challenge (also at pH 5.2; Wilson et al. 1994a). Aluminum acclimated fish had about 22% smaller gill surface area (shorter lamellae and thicker filaments) and increased mucous cell density (Wilson et al. 1994b), which perhaps helped protect against respiratory effects and some of the ionoregulatory effects of the 162 $\mu g \cdot L^{-1}$ Al challenge, but not the ionoregulatory effects presumably caused by the 32 $\mu g \cdot L^{-1}$ Cu challenge. Reduced gill surface area resulted in decreased U_{crit}, decreased maximum rate of oxygen uptake, and decreased aerobic scope in the Al-acclimated fish (Wilson et al. 1994b), again indicating that acclimation to Al has metabolic and physiological costs to a fish.

Wilson, R.W., H.L. Bergman, and C.M. Wood. 1994b. Metabolic costs and physiological consequences of acclimation to aluminum in juvenile rainbow trout (*Oncorhynchus mykiss*). 2: Gill morphology, swimming performance, and aerobic scope. Can. J. Fish. Aquat. Sci. 51: 536-544.
Consider with their 1994a paper, above.

Wilson, R.W., and C.M. Wood. 1992. Swimming performance, whole body ions, and gill Al accumulation during acclimation to sublethal aluminium in juvenile rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 10: 149-159.
They studied the costs of acclimation to Al over 22 d (<3 or 31 $\mu g \cdot L^{-1}$ Al, pH 5.2, Ca =13 µM) by measuring critical swimming speed in juvenile rainbow trout. Acclimation to Al (as shown by reduced mortality when exposed to 210 $\mu g \cdot L^{-1}$ Al, pH 5.2) developed by day 17, and was reflected in recovery of whole body Na^+ and Cl^- (lost in the first 7 d, at ~double the rate of fish exposed to pH 5.2, no Al) to normal by 22 d. Critical swimming speed (U_{crit}), however, did not recover in the Al-exposed fish, and was always significantly lower than U_{crit} for pH 5.2, no Al-exposed fish, which in turn was always significantly less than for control fish (pH 6.5, no Al), although some recovery did occur in both groups after day 7. Gill Al content was elevated and approximately constant after 2 d in the Al-exposed fish, with a temporary decrease at 7 d, and growth was reduced in this group. Their study indicates that fish can acclimate to sublethal concentrations of Al (ion recovery, greater tolerance to Al), but at some cost to overall fitness (U_{crit}, decreased growth). Gill damage and repair, plus mucus production and thicker gill lamellae, could reduce ion losses in Al-exposed fish, but would also reduce gas transfer at the gills and would consume energy that could otherwise be expended on growth. Alternatively, reduced appetite in the Al-exposed fish could have resulted in decreased growth, and decreased food energy ultimately could also explain the persistently-reduced U_{crit} at 22 d, but probably not earlier.

Wilson, R.W., C.M. Wood, and D.F. Houlihan. 1996. Growth and protein turnover during acclimation to acid and aluminum in juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 53: 802-811.
32 d exposures to pH 5.2 and 30 $\mu g \cdot L^{-1}$ Al in soft water. Loss or appetite, reduced growth, then recovery, and actual better growth. The paradoxical better growth in the acid plus Al group may have been a result of reduced activity in these fish.

Witters, H.E. 1986. Acute acid exposure of rainbow trout, *Salmo gairdneri* Richardson: effects of aluminium and calcium on ion balance and haematology. Aquat. Toxicol. 8: 197-210.
Witters showed that adult rainbow trout exposed 3.5 h to 350 $\mu g \cdot L^{-1}$ Al in very acidic conditions (pH 4.1, Ca =20 or 95 µM) lost ions through the gills at about twice the rate as at pH 4.1 in the absence of Al. The higher concentration of Ca did not reduce ion losses, possibly because of the severity of the acid and Al exposures.

Witters, H.E., S. Van Puymbroeck, I. Van Den Sande, and O.L.J. Vanderborght. 1990a. Haematological disturbances and osmotic shifts in rainbow trout, *Oncorhynchus mykiss* (Walbaum) under acid and aluminium exposure. J. Comp. Physiol. B 160: 563-571.
Witters et al. showed that erythrocytes were released into the blood when adult rainbow trout were exposed to 60 and 200 $\mu g \cdot L^{-1}$ Al for 3 d, after 14 d exposures to low pH alone (pH 5.0, Ca =25 µM), which added to the increased hematocrit due to the osmotic effects of Na and Cl losses from the blood. They proposed, on the basis of spleen appearance, that the spleen released more red blood cells into the blood in response to hypoxia caused by Al exposure, as was found in other studies. However, their spleen weights and spleen hemoglobin contents did not change significantly.

Witters, H.E., S. Van Puymbroeck, J.H.D. Vangenechten, and O.L.J. Vanderborght. 1990b. The effect of humic substances on the toxicity of aluminium to adult rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Biol. 37: 43-53.
They found that 10 mg $C \cdot L^{-1}$ humic material (soil and bog sources) virtually eliminated the toxicity of 180 $\mu g \cdot L^{-1}$ total Al (pH 4.7, Ca =25 µM, 10 d exposure, cannulated rainbow trout), and about 10X less Al accumulated on trout gills in the presence of humic material than in its absence.

Witters, H.E., S. Van Puymbroeck, A.J.H.X. Stouthart, and S.E. Wendelaar Bonga. 1996. Physiocochemical changes of aluminium in mixing zones: mortality and physiological disturbances in brown trout (*Salmo trutta* L.). Environ. Toxicol. Chem. 15: 986-996.
A great paper. Simulated mixing zones of limed rivers with acidic, Al-rich tributaries. Chemical analysis of Al to explain high mortality at pH 6.0. They show that high molecular weight Al (>10 kD), defined as Al polymerization, was a better indicator of Al toxicity than total Al concentration. Aging of the polymerized Al for 8 min reduced Al toxicity. Blood ions were ok, but it looked as

if the fish were dying in the mixing zones through respiratory distress. Histology, too. A very useful paper.

Witters, H.E., S. Van Puymbroeck, and O.L.J. Vanderborght. 1991. Adrenergic response to physiological disturbances in rainbow trout, *Oncorhynchus mykiss*, exposed to aluminum at acid pH. Can. J. Fish. Aquat. Sci. 48: 414-420.
Adult rainbow trout exposed to 60 $\mu g \cdot L^{-1}$ Al at pH 5 in synthetic soft water (Ca =27 µM) for 2.5 d showed, in response to Al, the usual decreases in plasma Na, blood PO_2, and blood pH, increased hematocrit, plus increased plasma concentrations of epinephrine, norepinephrine, and cortisol. Increased plasma catecholamine concentrations might have been to maintain erythrocyte O_2 carrying capacity.

Wood, C.M., and D.G. McDonald. 1987. The physiology of acid/aluminum stress in trout. Ann. Soc. Royale Zool. Belgique 117: 399-410.
This is a fairly good paper giving the ionoregulatory effects of Al in acidic water. Probably use it in the introduction or in a model.

Wood, C.M., R.C. Playle, B.P. Simons, G.G. Goss, and D.G. McDonald, D.G. 1988a. Blood gases, acid-base status, ions, and hematology in adult brook trout (*Salvelinus fontinalis*) under acid/aluminum exposure. Can. J. Fish. Aquat. Sci. 45: 1575-1586.
Respiratory effects of 330 $\mu g \cdot L^{-1}$ Al at pH 4.8 were elucidated using cannulated brook trout. Gill inflammation, mucus production, and thickening of the blood-water barrier was suggested as the mechanism of the respiratory effects of Al, which were manifested as low arterial O_2 tension (PO_2), high arterial CO_2 tension (PCO_2), and blood acidosis.

Wood, C.M., D.G. McDonald, C.E. Booth, B.P. Simons, C.G. Ingersoll, and H.L. Bergman. 1988b. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 1. Blood composition and net sodium fluxes. Can. J. Fish. Aquat. Sci. 45: 1587-1596.
An acclimation study. Adult brook trout exposed for 10 wk to pH 5.2, 12 or 200 µM Ca, and 75 or 150 $\mu g \cdot L^{-1}$ Al were better able to tolerate a 48 h pulse exposure to pH 4.8, 330 $\mu g \cdot L^{-1}$ Al than fish held at pH 5.2 or 6.5 in the absence of Al, owing to a faster recovery phase during the acid and Al pulse. The mechanism of acclimation to Al was unclear, but see McDonald and Milligan (1988) for a possible mechanism.

Wood, C.M., B.P. Simons, D.R. Mount, and H.L. Bergman. 1988c. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*). 2. Blood parameters by cannulation. Can. J. Fish. Aquat. Sci. 45: 1597-1605.
See Wood et al. 1988b.

Woodward, D.F., A.M. Farag, M.E. Mueller, E.E. Little, and F.A. Vertucci. 1989. Sensitivity of endemic Snake River cutthroat trout to acidity and elevated aluminum. Trans. Am. Fish. Soc. 118: 630-643.

Gills of 40-d old cutthroat trout (*Oncorhynchus clarki*) exposed to 50 $\mu g \cdot L^{-1}$ Al at pH 6.0 and 5.5 (7-d exposures as alevins, Ca =35 µM) showed mucous cell proliferation and noticeable mucus production (LM).

Youson, J.H., and C.M. Neville. 1987. Deposition of aluminum in the gill epithelium of rainbow trout (*Salmo gairdneri* Richardson) subjected to sublethal concentrations of the metal. Can. J. Zool. 65: 647-656.

They exposed juvenile rainbow trout for 11 d to 75 $\mu g \cdot L^{-1}$ Al at pH 5.5, 5.0, and 4.5 (Ca =50 to 125 µM), and found Al in and on gill epithelial cells (transmission electron microscopy). No obvious hyperplasia was seen at this relatively low Al concentration.

4.3 Other Vertebrates

Blancher, P.J., and D.K. McNicol. 1991. Tree swallow diet in relation to wetland acidity. Can. J. Zool. 69: 2629-2637.
Acidified waters are often deficient in Ca-rich gastropods, etc., so bird diet can be affected.

Bradford, D.F., C. Swanson, and M.S. Gordon. 1994. Effects of low pH and aluminum on amphibians at high elevation in the Sierra Nevada, California. Can. J. Zool. 72: 1272-1279.
Information in this paper would supplement the information in Freda (1991).

Corain, B., G.G. Bombi, A. Tapparo, M. Perazzolo, and P. Zatta. 1996. Aluminium toxicity and metal speciation: established data and open questions. Coordination Chem. Rev. 149: 11-22.
Check the chemistry, but this is probably too "human" to be useful here.

Freda, J. 1991. The effects of aluminum and other metals on amphibians. Environ. Pollut. 71: 305-328.
The complex toxicity of Al to amphibians has been well reviewed by Freda (1991). Aluminum toxicity to amphibians is modified by water pH, hardness, and dissolved organic carbon concentration, and susceptibility of amphibians varies with life stage.

Freda, J., and D.G. McDonald. 1993. Toxicity of amphibian breeding ponds in the Sudbury region. Can. J. Fish. Aquat. Sci. 50: 1497-1503.
Additional information regarding amphibian distribution and water quality in breeding ponds is found in Freda and McDonald (1993), Bradford et al. (1994), and Whiteman et al. (1995).

Golub, M.A., and J.L. Domingo. 1996. What we know and what we need to know about developmental aluminum toxicity. J. Toxicol. Environ. Health 48: 585-597.
Too human health orientated.

Golub, M.S. 1996. Issues in aluminum toxicity - concluding statement. J. Toxicol. Environ. Health 48: 685-686. (see also pp. R7-R8, this issue).

Gonda, Z., K. Lehotzky, and A. Miklósi. 1996. Neurotoxicity induced by prenatal aluminum exposure in rats. Neurotoxicol. 17: 459-470.
Too neurotoxicological to be useful here. Study was meant to mimic pregnant women taking antacids for morning sickness.

Howard, B.J., F.R. Livens, and C.B. Walters. 1996. A review of radionuclides in tide-washed pastures on the Irish Sea coast in England and Wales and their transfer to food products. Environ. Pollut. 93: 63-74.
Some background information on eH is in this article, which could be useful in the chemistry section. Otherwise not of much interest here.

Miles, A.K., C.E. Grue, G.W. Pendleton, and J.H.Jr. Soares. 1993. Effects of dietary aluminum, calcium, and phosphorus on egg and bone of European starlings. Arch. Environ. Contam. Toxicol. 24: 206-212.
More on the inter-relationship of Al, Ca, and egg formation in birds.

Möller, G. 1996. Biogeochemical interactions affecting hepatic trace element levels in aquatic birds. Environ. Toxicol. Chem. 15: 1025-1033.
Includes a bit on Al, but emphasizes Se more. Perhaps include in the invertebrate section regarding the lack of bioaccumulation of Al in the food chain.

Nybo, S. 1996. Effects of dietary aluminum on chicks *Gallus gallus domesticus* with different dietary intake of calcium and phosphorus. Arch. Environ. Contam. Toxicol. 31: 177-183.

Radunović, A., M.W.B. Bradbury, and H.T. Delves. 1993. Determination of aluminium in different tissues of the rat by atomic absorption spectrometry with electrothermal atomization. Analyst 118: 533-536.
Perhaps of some use in the analysis section of this paper.

Scheuhammer, A.M. 1991. Effects of acidification on the availability of toxic metals and calcium to wild birds and mammals. Environ. Pollut. 71: 329-375.
Again, acidified water can be deficient in Ca rich prey for birds.

St. Louis, V.L., and J.C. Barlow. 1993. The reproductive success of tree swallows nesting near experimentally acidified lakes in northwestern Ontario. Can. J. Zool. 71: 1090-1097.
St. Louis and Barlow demonstrated reduced hatching success of tree swallows (*Tachycineta bicolor*) nesting near acidified lakes at the Experimental Lakes Area, northwestern Ontario.

Tyler, S.J., and S.J. Ormerod. 1992. A review of the likely causal pathways relating the reduced density of breeding dippers *Cinclus cinclus* to the acidification of upland streams. Environ. Pollut. 78: 49-55.
The dippers on acidic streams showed blood chemistry which reflected calcium scarcity. Insufficient dietary Ca can affect egg-shell production in birds.

Walton, J., C. Tuniz, D. Fink, G. Jacobsen, and D. Wilcox. 1995. Uptake of trace amounts of aluminum into the brain from drinking water. NeuroToxicol. 16: 187-190.
Interesting, but I'm not sure we can cover human effects in this review. Getting too broad.

Whiteman, H.H., R.D. Howard, and K.A. Whitten. 1995. Effects of pH on embryo tolerance and adult behavior in the tiger salamander, *Ambystoma tigrinum tigrinum*. Can. J. Zool. 73: 1529-1537.
More on the effects of water quality on amphibians. Would go well with Freda (1991) and Freda and McDonald (1993).

Yokel, R.A. 1994. Aluminum chelation: chemistry, clinical, and experimental studies and the search for alternatives to desferrioxamine. J. Toxicol. Environ. Health 41: 131-174.
This paper may have some use in the chemcial section, but it is human epidemiology with some chemistry.