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The effects of pH and aluminum on the growth of the acidophilic diatom *Asterionella ralfsii* var. *americana*

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Abstract

The effects of pH and Al on the acidophilic diatom *Asterionella ralfsii* var. *americana* Körn. were examined in axenic batch cultures. Experiments were performed under conditions of both high (pH 5) and low (pH 6) Al solubility over a range of concentrations from 0 to 30 μmol liter^{-1} total Al. Growth rates were analyzed with respect to analytically determined Al concentrations and to predicted changes in dissolved metal ion speciation in response to Al additions.

Growth rates of *A. ralfsii* were significantly reduced above 15 μmol liter^{-1} total Al at both pH 5 and 6. Al additions increased estimated free ion activities of Al^{3+}, Fe^{3+}, and Cu^{2+} through indirect chelator interactions at pH 5 and 6; therefore, all three were significantly correlated with growth rate reductions. Independent manipulations of total Fe and Cu, however, suggested that Al was not indirectly increasing either Fe^{3+} or Cu^{2+} free ion activities to toxic levels. Relationships of growth rates to both inorganic monomeric Al (Al_i) and to estimates of pAl were strongly pH-dependent with toxicity being greater per unit of dissolved Al concentration at pH 6 than at pH 5. These results are consistent qualitatively with predictions that H^+ ions can ameliorate dissolved metal toxicity by competitively excluding Al^{3+} ions from binding to cell-surface ligands. The impact of Al on natural phytoplankton populations therefore is likely to depend on a combination of pH-dependent Al solubility, the protonation of cell-surface ligands, and chelator-mediated metal speciation.

The effects of Al on aquatic biota have received much recent attention because of the potential link between acidic precipitation and Al toxicity. Acidic precipitation significantly increases mineral weathering rates in some drainage basins thereby increasing loadings of dissolved Al to surface waters (Driscoll et al. 1984). Increased Al loading has been related to the reduced abundance of fish (Dillon et al. 1984) and amphibians (Clark and LaZerte 1985) in acidified systems.

One important aspect of the impact of Al on aquatic biota is its effect on acidophilic phytoplankton populations. Several recent studies have investigated the effect of Al at acidic pH (Hörnström et al. 1984; Folsom et al. 1986; Claesson and Törnqvist 1988), but not all have considered the effects of chemical speciation. Chemical speciation is important because metal bioavailability is strongly coupled to the activity of particular chemical species rather than total metal concentrations (Morel 1983). Studies that consider Al speciation have given contradictory conclusions. For example, free Al^{3+} ion activity has been assumed to be the best predictor of biological response in some cases (Folsom et al. 1986; Riseng 1989), but dissolved Al hydroxide concentrations (Helliwell et al. 1983) and even indirect increases in Cu^{2+} activity (Rueter et al. 1987) have also been suggested as being responsible for Al toxicity.

Because chemical speciation of certain metals changes with pH, it has long been accepted that ambient pH levels could influence trace metal toxicity or growth limitation (Campbell and Stokes 1985). Gensemer and Kilham (1984) found that growth-rate response to pH varied with algal species and probably involved pH-induced changes

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in trace metal speciation. Al speciation is strongly affected by H^+ activity; therefore, biological responses to total Al manipulations are likely to be pH-dependent. Because dissolved, monomeric Al is usually considered to be the most biologically reactive fraction (Helliwell et al. 1983; Clark and LaZerte 1985), Al may be most toxic at pH < 6 where monomeric forms predominate (Driscoll et al. 1984).

Increased concentrations of H^+ ions can also compete with and potentially displace metal cations from cell-surface binding sites. This mechanism has been proposed as a means by which metal toxicity is ameliorated at low pH (Peterson and Healey 1985; Campbell and Stokes 1985). Because this process can counteract the effects of increased metal bioavailability at low pH, it potentially obscures pH-dependent changes in the toxicity of any given amount of total metal. Although these opposing effects have been observed for several metals (Campbell and Stokes 1985), similar effects have not yet been described for Al toxicity to phytoplankton.

Diatoms are particularly significant in bioassays of the chemical consequences of acidification because of their widespread use as paleoecological indicators of lake acidity (Charles 1985; Davis 1987). Assessments of the rate and extent of surface-water acidification have relied heavily on composite indices of diatom community structure (Schindler 1988), yet virtually no causal mechanisms linking diatom community composition to epilimnetic pH have been identified. Most diatoms from lakes of pH < 5.8 are periphytic (Charles 1985), which presents technical problems in performing quantitative culture experiments because of their tendency to adhere to substrates. *Asterionella ralfsii* var. *americana* is an interesting exception because it occurs in the plankton at pH values well below 5.8 (Davis 1987). This feature has made *A. ralfsii* a valuable indicator of early acidification (Findlay and Kasian 1986), and its planktonic life history makes it amenable to quantitative laboratory investigation.

This study examines growth responses of the acidophilic diatom *A. ralfsii* to Al under conditions of relatively high (pH 5) and low (pH 6) Al solubility. It is done by comparing batch-culture growth-rate responses to Al using empirically determined total and dissolved Al concentrations, modeled monomeric Al concentrations, and modeled free metal ion activity. Free ion activity predictions are also used to examine whether Al^{3+} is directly or indirectly toxic under these experimental conditions and to what extent changes in pH affect growth responses to Al manipulations.

Materials and methods

Culture conditions—A clone of *A. ralfsii* var. *americana* Körn. was isolated from an epilimnetic water sample from Andrus lake, Chipewa County, Michigan (T.50N, R.6W, Sec. 27). Axenic cultures (also submitted to University of Toronto Culture Collection, UTCC No. 170) were maintained in 25- × 150-mm Pyrex tubes with 30 ml of a modified Fraquil medium (Morel et al. 1975) buffered at about pH 5.4 with 200 mg liter^{-1} MES [2-(N-morpholino)ethanesulfonic acid, $pK_a = 6.15$]. MES was chosen for its ability to buffer pH in algal cultures while apparently not complexing significant amounts of trace metal ions (Wehr et al. 1986). Modifications consisted of lowering synthetic metal chelator Na_2EDTA (ethylenediaminetetraacetic acid, disodium salt) concentration to 0.5 μmol liter^{-1} without changing total metal concentrations, adding 400 μmol liter^{-1} B (sodium salt), and reducing P concentrations to 4 μmol liter^{-1} PO_4^{3-}. EDTA concentrations were reduced because higher levels seem to induce trace metal limitation for *A. ralfsii* in Fraquil (Riseng 1989). All media stocks were prepared from reagent-grade chemicals and double-deionized, distilled water (DDW). Experimental media were sterilized by filtration through Gelman GA-8S 0.2-μm membrane filters. All culture flasks and tubes were sterilized by autoclaving while partially filled with DDW, which was then rinsed and discarded before use. All experiments were incubated in a constant-environment chamber at 20°C, and lighted with "cool-white" fluorescent bulbs that provided ~100 μEinst m^{-2} s^{-1} on a 14:10 L/D cycle.

Experiments were performed over a range

of total Al additions from 0 to 30 μmol liter^{-1}. This range was chosen to simulate total Al levels commonly encountered in acidic, north temperate lakes (LaZerte 1984; Driscoll et al. 1984). Al stock solutions were prepared from a hydrated chloride salt and filter sterilized separately from the growth medium to prevent removal of insoluble Al complexes during medium sterilization. Al stock solutions were added to the medium at least 24 h before inoculation to allow monomeric and polymeric forms of Al to come into equilibrium (Campbell et al. 1986). Al treatments were performed at pH 5 and 6 in order to compare conditions of high Al solubility at pH 5 to near-minimum solubility at pH 6 (Driscoll et al. 1984). The pH was set before sterilization by titrating with either 2 N NaOH or HCl. Variations in pH after sterilization and Al additions did not exceed 0.2 pH units. All experiments were performed with three replicates for each treatment.

Growth rate determination—Growth rates were measured in batch culture by in vivo fluorescence with a technique similar to that of Brand et al. (1981). About 30 ml of sterile medium was inoculated with <1 ml of an exponentially growing culture of *A. ralfsii*, thereby yielding an initial culture density of ~500 cells ml^{-1}. Fluorometer readings (Turner Designs model 10) were taken at the same time daily (±1 h) for 7–9 d or until growth ceased. Growth rates were determined as the slope of the linear portion (for at least 4 d growth) of natural log-transformed fluorescence units per day.

In order for changes in fluorescence to represent cellular division rates, in vivo fluorescence per cell must remain constant throughout logarithmic growth (Brand et al. 1981). This relationship was tested by taking subsamples from a representative set of treatments to obtain cell counts for comparison to fluorescence units. These subsamples were preserved in Lugol's solution, then counted for cell numbers in a Sedgwick-Rafter chamber. Cell counts (cells ml^{-1}) were then compared to fluorescence units obtained for the same tube on the same day. During exponential growth, the ratio of fluorescence units : cells ml^{-1} did not change significantly (ANOVA, $P < 0.05$). Therefore, at least between days 2 and 6, cell numbers and in vivo fluorescence were considered to increase at the same rate.

Medium chemistry—Al measurements were made via a colorimetric reaction with pyrocatechol violet (PCV, Sigma Chemical Co.) as described by Sullivan et al. (1986) and LaZerte et al. (1988). Three operationally defined Al fractions were measured: total Al (Al_t) was defined as that fraction reactive with PCV after unfiltered media was acidified to pH 1 for at least 24 h; PCV-reactive Al (Al_r) was the fraction reactive with PCV at ambient pH and without filtration; and, total dissolved Al (Al_{td}) was the fraction that reacted with PCV after the filtrate from a 0.45-μm pore-size Gelman GA-6 membrane filter was acidified to pH 1 for at least 24 h. PCV-reactive Al measures inorganic monomeric Al (IMAl) concentrations over a wide range of pH in natural waters after correction for organic Al (Sullivan et al. 1986; LaZerte et al. 1988). Because EDTA-bound Al is not detectable by reaction with PCV at ambient pH (Gensemer 1989), the pretreatments of filtration and acidification are necessary to estimate without interference by insoluble Al complexes the amount of Al bound to EDTA (*see Table 3*).

Chemical speciation was estimated with the equilibrium-based program MINEQL (Westall et al. 1976) release 2.0. Dissolved Al equilibrium constants were modified to conform to those of LaZerte (1984), and Al_{td} concentrations were used for total Al component inputs (Gensemer 1989). Calculations, therefore, were constrained not to allow the formation of insoluble Al complexes. Empirical Al_{td} concentrations were chosen rather than estimating monomeric Al concentrations from the theoretical solubility of $Al(OH)_3$ because of uncertainty in accurately predicting the formation of this complex. Choice of $Al(OH)_3$ equilibrium constants strongly affects estimates of Al^{3+} activity (Driscoll et al. 1984; Gensemer 1989) and can introduce the greatest uncertainty to dissolved Al speciation calculations (Schecher and Driscoll 1987). Nominal concentrations were used for all other chemical component inputs.

Results

Total Al concentrations $\gtrsim 15$ μmol liter^{-1} significantly reduced growth rates of *A. ralfsii* (Fig. 1A). This pattern was independent of pH except near 15 μmol liter^{-1} where growth was more strongly reduced at pH 5 than at 6. Two-way ANOVA indicated both that there was a significant effect of pH and Al treatments and that a significant interaction existed ($F_{9,41} = 41.5$, $r^2 = 0.90$, $P < 0.0001$). Control growth rates (defined here as treatments with no added Al at each pH) did not vary significantly with pH and represented an average overall maximal growth rate of 0.634 d^{-1} ($N = 18$, SE = 0.023 d^{-1}). Growth rates were also negatively related with increasing Al_r concentrations (Fig. 1B). An effect of pH on growth response to Al was also observed with Al_r being more toxic per mole at pH 6 than at pH 5 at virtually all levels of Al addition. Growth rates at pH 6 approached zero at 1.40 μmol liter^{-1} Al_r, whereas growth at pH 5 did not approach zero until 23.07 μmol liter^{-1} Al_r. Fluorescence units per unit of cell density remained constant over all Al treatment groups tested. Neither a pH range of 5–6 nor a range of Al_r from 0 to 9.5 μmol liter^{-1} had significant effects on intensity of fluorescence per unit of cell density (ANOVA, $P > 0.05$). Growth rate determinations using daily in vivo fluorescence, therefore, were not biased by pH or Al treatments.

Similar to Al_r, growth rates plotted as a function of pAl showed Al to be more toxic at pH 6 (Fig. 1C). The slopes and intercepts of regression lines representing growth rate at each pH were significantly different, and the Al^{3+} activity at which growth was reduced to 50% of control rates differed by a factor of $10^{3.26}$; i.e. decreasing pH from 6 to 5 increased the amount of Al^{3+} required to reduce growth of *A. ralfsii* 50% by over three orders of magnitude.

MINEQL simulations predicted that adding Al would increase the activity of other trace metal ions present in Fraquil. At the lowest Al addition, the predicted activity of all trace metals (excluding Mn) increased by at least two orders of magnitude relative to activities without Al addition (Table 1). At pH 5, Mn, Zn, and Co activities did not increase further with Al addition, whereas Fe and Cu activities continued to increase at all Al levels. At pH 6, all the metal ion activities increased across all levels of Al addition, although less so for Mn, Zn, and Co than for Fe and Cu.

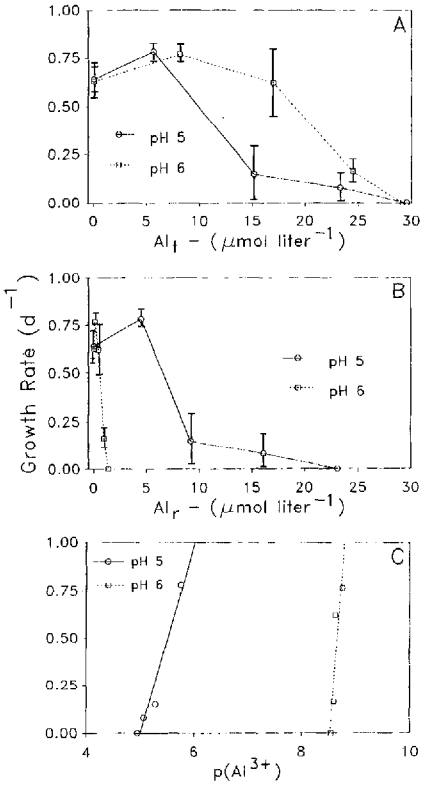

Fig. 1. Growth rate (d^{-1}) of *Asterionella ralfsii* as a function of Al_t, Al_r concentrations, and MINEQL-predicted pAl [$-\log_{10}(Al^{3+})$ activity in mol liter^{-1}] at pH 5 and 6. Least-squares regression lines are fitted through pAl data (panel C only) at each pH. Error bars represent ±2 SE of the mean growth rate. Growth rate errors for pAl data are the same as shown in panels A and B.

Because Al had a consistent effect on predicted Fe and Cu activity at both pH 5 and 6, reductions in growth rate could potentially be a function of changes in estimated pFe and pCu. When the same growth rates were plotted against Al-induced increases in Fe^{3+} (Fig. 2, dashed lines) and Cu^{2+} (Fig. 3, dashed lines) activity, responses similar to that of Al^{3+} were observed. For pFe, slopes and intercepts were significantly different between pH 5 and 6, and the points at which

Table 1. MINEQL-predicted activity of free aquo metal ions in response to additions of Al at pH 5 and 6. All results are presented as $-\log_{10}$ of free metal ion concentration in mol liter^{-1} (pM).

Nominal Al (μmol liter^{-1})	pAl	pFe	pMn	pCu	pZn	pCo
pH = 5						
0.00		17.59	7.75	13.22	10.32	10.33
7.41	5.75	13.52	7.65	9.44	8.41	8.62
14.81	5.28	13.11	7.65	9.28	8.41	8.62
22.22	5.07	12.94	7.65	9.24	8.41	8.62
29.63	4.95	12.85	7.65	9.22	8.41	8.62
pH = 6						
0.00		17.71	7.76	13.27	10.37	10.38
7.41	8.75	15.81	7.65	11.45	8.78	8.88
14.81	8.63	15.76	7.65	11.34	8.72	8.83
22.22	8.60	15.73	7.65	11.31	8.70	8.82
29.63	8.55	15.68	7.65	11.26	8.68	8.81

growth was reduced to 50% of control differed by a factor of $10^{2.60}$. For pCu, a similar relationship existed except that slopes and intercepts were not significantly different ($P > 0.05$). The pCu at which a 50% reduction

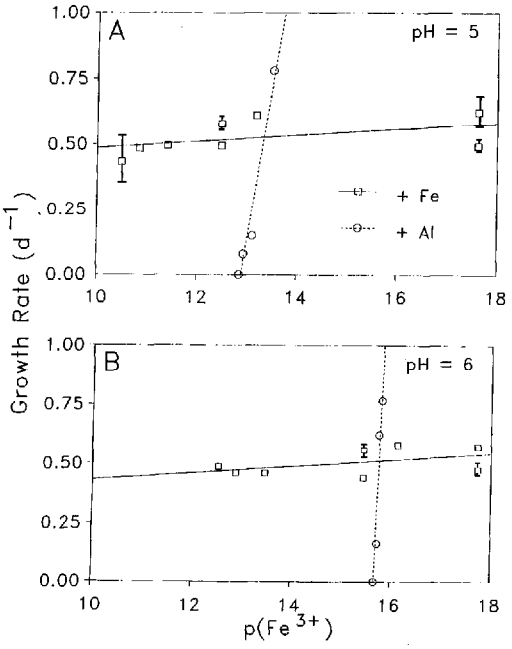

Fig. 2. Mean growth rate of *Asterionella ralfsii* as a function of pFe [$-\log_{10}(Fe^{3+})$ activity in mol liter^{-1}] based on manipulations of Al (dashed lines) and Fe (solid lines) at pH 5 and 6. Error bars represent ±2 SE of the mean growth rate. Growth rate errors for Al manipulations are the same as shown in Fig. 1A and B.

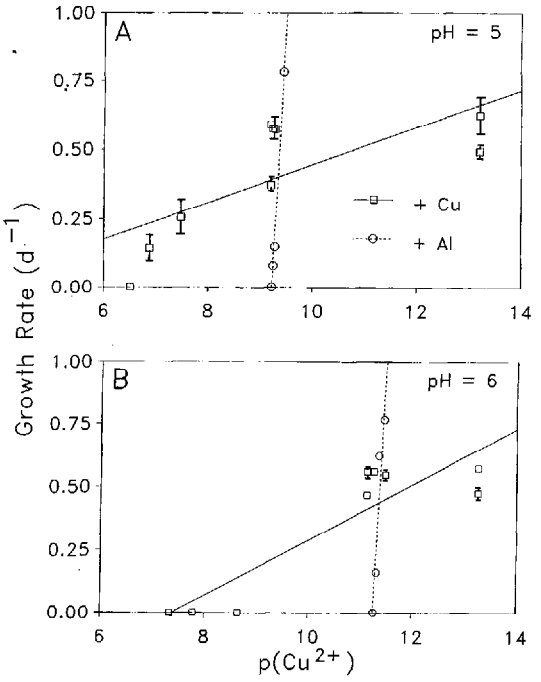

Fig. 3. As Fig. 2, but of pCu [$-\log_{10}(Cu^{2+})$ activity in mol liter^{-1}] based on manipulations of Al (dashed lines) and Cu (solid lines).

in control growth rate occurred differed by a factor of $10^{2.01}$ between pH 5 and 6. For both pFe and pCu, growth toxicity was greater at pH 6 than at 5.

Al additions may, therefore, reduce growth either by directly increasing Al^{3+} activity or by indirectly increasing Fe^{3+} or Cu^{2+} activity to toxic levels. To test among these alternatives, I repeated growth rate experiments under conditions identical to those of the Al treatments except that total Fe and Cu concentrations were manipulated in the absence of Al. Total metal concentrations were increased to achieve free ion activities that simulated those predicted to occur in response to Al addition and exceed them by at least one order of magnitude (Table 2). Total metal levels were increased by preparing more concentrated Fe or Cu stock solutions to achieve the desired estimated metal ion activities. Because Fe additions were also predicted to increase Cu^{2+} ion activity, normal total Cu levels were reduced 10,000× at pH 5 and 100× at pH 6 by serial dilution of the concentrated Cu stock so-

Table 2. Experimental conditions used to test effects of Al-induced changes in Cu and Fe free ion activity. Cu and Fe additions are listed as total nominal concentration (mol liter^{-1}), and calculated free ion activities based on these combinations are listed as $-\log_{10}$ free ion activity in mol liter^{-1} (pM). Control Cu and Fe concentrations are 9.97×10^{-10} and 4.51×10^{-7} mol liter^{-1}.

	Total Cu	Total Fe	pCu	pFe
Cu addition				
pH = 5	9.97×10^{-10}	4.51×10^{-7}	13.21	17.62
	4.96×10^{-8}	4.51×10^{-7}	9.27	15.33
	4.97×10^{-8}	4.51×10^{-7}	9.23	15.29
	4.98×10^{-8}	4.51×10^{-7}	9.19	15.25
	9.96×10^{-8}	4.51×10^{-7}	7.47	13.57
	2.49×10^{-7}	4.51×10^{-7}	6.87	13.05
	4.98×10^{-7}	4.51×10^{-7}	6.51	12.78
pH = 6	9.97×10^{-10}	4.51×10^{-7}	13.26	17.73
	3.10×10^{-8}	4.51×10^{-7}	11.47	17.40
	3.65×10^{-8}	4.51×10^{-7}	11.25	17.24
	3.90×10^{-8}	4.51×10^{-7}	11.12	17.15
	7.80×10^{-8}	4.51×10^{-7}	8.64	14.92
	1.95×10^{-7}	4.51×10^{-7}	7.78	14.35
	3.90×10^{-7}	4.51×10^{-7}	7.33	14.11
Fe addition				
pH = 5	9.97×10^{-10}	4.51×10^{-7}	13.21	17.62
	9.97×10^{-14}	5.10×10^{-7}	13.32	13.18
	9.97×10^{-14}	5.50×10^{-7}	13.18	12.48
	9.97×10^{-14}	1.10×10^{-6}	13.14	11.40
	9.97×10^{-14}	2.75×10^{-6}	13.14	10.83
	9.97×10^{-14}	5.50×10^{-6}	13.14	10.48
pH = 6	9.97×10^{-10}	4.51×10^{-7}	13.26	17.73
	9.97×10^{-12}	5.00×10^{-7}	13.75	16.13
	9.97×10^{-12}	5.05×10^{-7}	13.08	15.45
	9.97×10^{-12}	1.01×10^{-6}	11.78	13.47
	9.97×10^{-12}	2.53×10^{-6}	11.71	12.87
	9.97×10^{-12}	5.05×10^{-6}	11.69	12.52

lution with DDW to maintain estimated pCu as close to control levels as possible.

When Fe was manipulated independently from Al, growth rate responses to free ion activity were significantly different from those predicted to occur in response to Al addition (Fig. 2). Total Fe concentrations over 10× in excess of normal Fraquil levels lowered pFe 100–1,000× relative to those predicted to occur in response to Al addition (Table 2). These treatments, however, did not reduce growth below 69% of control rates (Fig. 2). Fe^{3+} activity was linearly related to growth rates at both pH 5 (Fig. 2A, $P < 0.03$) and 6 (Fig. 2B, $P < 0.05$), but <24% of the variance could be explained in either case. The slopes of Fe-induced changes in pFe were significantly lower than those predicted to occur in response to Al additions in both pH treatments.

Unlike Fe, total Cu manipulations significantly reduced growth rates of *A. ralfsii* but only at Cu^{2+} activities significantly higher than those predicted to occur in response to Al addition. Total Cu concentrations nearly 400× in excess of normal Fraquil concentrations increased Cu^{2+} activity to exceed Al-induced levels at least 10× (Table 2). Without Al, higher Cu^{2+} activities were required to reduce growth rates than when Al was added to Fraquil at control Cu concentrations (Fig. 3). At pH 5, both slopes and intercepts between Cu-induced and Al-induced pCu levels were significantly different as predictors of growth rate (Fig. 3A). Levels of pCu that corresponded to 50% control growth rate were $10^{1.15}$ times lower than when total Al was manipulated. At pH 6, pCu levels that reduced growth 50% were $10^{1.05}$ times lower than Al-induced changes in pCu (Fig. 3B). Cu additions also increased estimated Fe^{3+} activities (Table 2), but to levels ~100× below those in the Fe manipulations (which had only a negligible effect on growth, Fig. 2).

Discussion

If concentrations of dissolved inorganic Al alone determine biological response, then toxicity should be greatest at pH 5 because Al is more soluble at this pH. When growth rates were considered as a function of Al_t, Al was more toxic at pH 5, but only at moderate concentrations. The Al_t fraction, however, includes more than simple inorganic Al species, so the effects of Al_t on growth could be obscured by the presence of polymeric $Al(OH)_3$ or EDTA-bound Al. Because Al_r is an empirical descriptor of IMAl (Sullivan et al. 1986; Gensemer 1989), it is the most appropriate fraction with which to examine the toxicity of dissolved Al to *A. ralfsii*. Al_r concentrations demonstrated Al toxicity to be pH-dependent, but, in contrast to Al_t, Al_r was clearly more toxic at pH 6 than at 5.

When Al treatments were analyzed in terms of predicted free metal ion activity, it appeared that increases in either Al^{3+}, Fe^{3+}, or Cu^{2+} activity could have been responsible for the observed growth reductions at both

pH 5 and 6. Total Fe and Cu manipulations, however, suggested that indirect chemical effects of Al addition did not have a significant impact on growth. Although it would have been preferable to verify total metal concentrations empirically, relative Fe and Cu manipulations of control media (Table 2) were sufficient to test among these alternatives. Fe and Cu manipulations were also not completely independent in terms of free ion activity (Table 2), yet concentrations of the metal not being directly manipulated were adjusted to minimize potential indirect effects between Fe and Cu. Therefore, increased Al^{3+} activity was the best overall predictor of reduced growth rates in response to these Al treatments.

In other studies, indirect interactions of Al with other trace metals have been shown to affect algal growth significantly. Rueter et al. (1987) concluded that Al induced indirect Cu toxicity to *Scenedesmus quadricauda* in the same growth medium, but at pH 7.0 and 10× higher EDTA concentration. In contrast, Riseng (1989) concluded that Al stimulated the growth of both *A. ralfsii* and its circumneutral congener *Asterionella formosa* probably because competitive metal ion displacement by Al increased their activities above limiting levels. Riseng (1989) also used Fraquil, and the magnitude of the stimulatory effect was highly dependent on pH and EDTA concentration. These studies point out that chelator concentration and the relative concentration of other trace metals probably have the greatest impact on whether Al^{3+} will have an indirect or direct influence on growth rates in vitro. Furthermore, both Rueter et al. (1987) and Riseng (1989) predicted dissolved Al concentrations based on the theoretical solubility of $Al(OH)_3$ rather than measuring dissolved Al. Experimental interpretations based on predicted Al^{3+} activity will depend strongly on which form of $Al(OH)_3$ was chosen for use in speciation calculations because of the potential uncertainty introduced by differences between equilibrium constants (Schecher and Driscoll 1987). Riseng (1989) used microcrystalline gibbsite in her Al speciation models, but Rueter et al. (1987) did not report which form of $Al(OH)_3$ was used; therefore, it is difficult to compare the specific metal activities at which growth effects occurred.

Applying the results of any culture experiment to natural systems in part depends on how well EDTA-mediated metal interactions simulate those of natural dissolved organic C (DOC). The overall affinity of EDTA for metal ions is probably high compared to simple, well-characterized compounds (e.g. amino acids) and low relative to the more complex biotically released compounds such as Fe siderophores (Morel 1983). It is comparatively difficult to determine how well EDTA simulates the binding affinity of humic and fulvic acids because the complexity of these compounds makes it difficult to define metal binding affinities. Even in the absence of specific thermodynamic predictions, however, competitive binding interactions between metal cations bound to complex forms of DOC have been observed (Perdue and Lytle 1983; Buffle and Altmann 1987) and thus may be relevant to understanding Al toxicity in natural populations.

The pH dependence of Al toxicity to *A. ralfsii* is consistent qualitatively with the prediction that increasing H^+ activity reduces metal toxicity by competitively excluding metal ions from binding with cell-surface ligands (Morel 1983; Campbell and Stokes 1985). The ameliorating effects of H^+ have been demonstrated for Cu, Cd (Peterson and Healey 1985), and other metals (*see* Campbell and Stokes, 1985), but not previously for Al. This effect, however, may be masked by increases in metal toxicity at low pH resulting from increased concentrations of dissolved species. These effects are often difficult to separate, but Al seems to act similarly to Cu in that pH changes both chemical speciation and cellular toxicity (i.e. a type 1 response, Campbell and Stokes 1985). Therefore, even though lowering pH increases the absolute concentrations of the potentially most toxic dissolved Al species (Helliwell et al. 1983; Clark and LaZerte 1985), toxicity per unit of Al^{3+} activity is reduced.

Other investigations generally agree that Al toxicity to phytoplankton is reduced at low pH. Both the growth rates (Hörnström et al. 1984) and P uptake capabilities (Na-

Table 3. Analytical concentrations (±SE of duplicate measurements) of mean Al_t, Al_{td}, Al_r, total filterable Al ($Al_t - Al_{td}$), and the sum of monomeric $Al(OH)_x$ species [= Σ $Al(OH) + Al(OH)_2 + Al(OH)_4 + Al_2(OH)_2 + Al_3(OH)_4$] as predicted by MINEQL using Al_{td} as total Al input with no Al solid formation allowed. Data are tabulated as a function of nominal Al additions at each pH and are expressed in units of μmol liter^{-1}.

Nominal Al	Al_t	Al_{td}	Al_r	Filt. Al	Σ $Al(OH)_x$
pH = 5					
7.41	5.66(0)	4.53(0.02)	4.47(0.02)	1.13	2.45
14.81	15.15(0.03)	13.33(0.04)	9.20(0.01)	1.83	7.33
22.22	23.31(0.03)	21.38(0.03)	16.09(0.02)	1.93	11.78
29.63	29.54(0.18)	28.37(0.10)	23.07(0.07)	1.17	15.64
pH = 6					
7.41	8.13(0.02)	0.26(0)	0.08(0.01)	7.78	0.21
14.81	16.96(0.04)	0.33(0.03)	0.43(0)	16.63	0.28
22.22	24.49(0.07)	0.35(0.01)	0.98(0.01)	24.14	0.30
29.63	29.16(0.01)	0.39(0.03)	1.40(0)	28.77	0.34

lewajko and Paul 1985) of natural phytoplankton communities are less affected by Al at lower pH. Claesson and Törnqvist (1988) tested levels of Al which reduced growth rates 50% for *Monoraphidium dybowskii* and *Stichococcus* sp. in well-buffered, isolated cultures and found that Al was less toxic at lower pH even though speciation was not addressed specifically. Growth responses of *A. ralfsii* and its circumneutral congener *A. formosa* to Al^{3+} activities exhibited reduced toxicity at low pH (Riseng 1989) as did responses of *Chlorella pyrenoidosa* to concentrations of $Al(OH)_2$ (Helliwell et al. 1983). Helliwell et al. (1983) concluded that Al was most toxic at pH 6 but that toxicity was linked to $Al(OH)_2$ rather than either polymeric Al or Al^{3+}.

In the present study, all monomeric $Al(OH)_x$ species were more concentrated in absolute molar terms at pH 5 owing to the solubility of Al at this pH (Table 3); thus in contrast to Helliwell et al. it is doubtful that concentrations of Al(OH) or $Al(OH)_2$ alone determined toxicity to *A. ralfsii*. Alternatively, the enhanced toxicity of dissolved Al at pH 6 may result from contact with suspended or colloidal $Al(OH)_3$, which is more abundant at this pH. Although total insoluble Al, primarily $Al(OH)_3$, was far less concentrated at pH 5 than at 6 (Table 3), growth rate reductions were at least as significant at this pH. Therefore it is also unlikely that insoluble $Al(OH)_3$ was having any direct effect on algal growth at either pH.

This study not only describes the responses of an important acidophilic diatom species to Al but also examines general aspects of pH-dependent Al toxicity to phytoplankton. Although Al has also been shown to reduce growth rates as a result of competitive interactions with EDTA-bound metals (Rueter et al. 1987), no such indirect interaction was detected in the present study. How algae ultimately respond to Al, therefore, is likely to be coupled to a site-specific combination of pH and the metal complexation properties of DOC. At present, it is technically feasible to examine these interactions quantitatively only with well-characterized synthetic compounds, so it is important to examine other metal–DOC interactions before extrapolating to nature. Although artificial culture experiments thus are limited in their potential to predict these effects, they serve to isolate and identify relevant biological processes that merit more careful analysis in natural systems.

References

BRAND, L. E., R. L. L. GUILLARD, AND L. S. MURPHY. 1981. A method for the rapid and precise determination of acclimated phytoplankton growth rates. J. Plankton Res. **3**: 193–201.

BUFFLE, J., AND R. S. ALTMANN. 1987. Interpretation of metal complexation by heterogenous complexants, p. 351–383. *In* W. Stumm [ed.], Aquatic surface chemistry: Chemical processes at the particle–water interface. Wiley.

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.

———, D. THOMASSIN, AND A. TESSIER. 1986. Aluminum speciation in running waters on the Canadian Precambrian Shield: Kinetic aspects. Water Air Soil Pollut. **30**: 1023–1032.

CHARLES, D. F. 1985. Relationships between surface

diatom assemblages and lakewater characteristics in Adirondack lakes. Ecology **66:** 994–1011.

CLAESSON, A., AND L. TÖRNQVIST. 1988. The toxicity of aluminum to two acido-tolerant green algae. Water Res. **22:** 977–983.

CLARK, K. L., AND B. D. LAZERTE. 1985. A laboratory study of the effects of aluminum and pH on amphibian eggs and tadpoles. Can. J. Fish. Aquat. Sci. **42:** 1544–1551.

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.

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.

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, p. 55–75. *In* O. P. Bricker [ed.], Acid precipitation: Geological aspects. Ann Arbor Sci.

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.

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. thesis, Univ. Michigan. 159 p.

———, AND S. S. KILHAM. 1984. Growth rates of five freshwater algae in well-buffered acidic media. Can. J. Fish. Aquat. Sci. **41:** 1240–1243.

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.

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* Inst. Freshwater Res. Rep. 61. Drottingholm, Sweden.

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.

———, C. CHUN, AND D. EVANS. 1988. Measurement of aqueous aluminum species: Comparison of dialysis and ion-exchange techniques. Environ. Sci. Technol. **22:** 1106–1108.

MOREL, F. M. M. 1983. Principles of aquatic chemistry. Wiley.

———, J. C. WESTALL, J. G. RUETER, AND J. P. CHAPLICK. 1975. Description of the algal growth media "Aquil" and "Fraquil." Mass. Inst. Technol. R. M. Parsons Lab. Tech. Note 16.

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.

PERDUE, E. M., AND C. R. LYTLE. 1983. Distribution model for binding of protons and metal ions by humic substances. Environ. Sci. Technol. **17:** 654–660.

PETERSON, H. G., AND F. P. HEALEY. 1985. Comparative pH dependent metal inhibition of nutrient uptake by *Scenedesmus quadricauda* (Chlorophyceae). J. Phycol. **21:** 217–222.

RISENG, C. M. 1989. The effect of pH, aluminum, and chelator manipulations on the growth of acidic and circumneutral species of *Asterionella.* M.S. thesis, Univ. Michigan. 29 p.

RUETER, J. G., K. T. O'REILLY, AND R. PETERSEN. 1987. Indirect aluminum toxicity to the green alga *Scenedesmus quadricauda* through increased cupric ion activity. Environ. Sci. Technol. **21:** 435–438.

SCHECHER, W. D., AND C. T. DRISCOLL. 1987. An evaluation of uncertainty associated with aluminum equilibrium calculations. Water Resour. Res. **23:** 525–534.

SCHINDLER, D. W. 1988. Effects of acid rain on aquatic ecosystems. Science **239:** 149–157.

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.

WEHR, J. D., L. M. BROWN, AND I. E. VANDERELST. 1986. Hydrogen ion buffering of culture media for algae from moderately acidic, oligotrophic waters. J. Phycol. **22:** 88–94.

WESTALL, J. C., J. L. ZACHARY, AND F. M. M. MOREL. 1976. MINEQL, a computer program for the calculation of the chemical equilibrium composition of aqueous systems. Mass. Inst. Technol. Dep. Civ. Eng. Tech. Rep. 18. 91 p.

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