THE EFFECT OF PH, ALUMINUM, AND CHELATOR MANIPULATIONS ON THE GROWTH OF ACIDIC AND CIRCUMNEUTRAL SPECIES OF *ASTERIONELLA*

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Abstract. The growth rates of two diatoms, acidophilic *Asterionella ralfsii* and circumneutral *A. formosa*, were differentially affected by varying pH, Al, and EDTA in chemically defined media. Free Al ion concentration increased as pH and EDTA concentration decreased. Free trace metal ion concentration decreased as EDTA levels increased but increased by orders of magnitude upon addition of Al. pH had an overriding species specific effect on growth rate; at low pH *A. ralfsii* had higher growth rates than *A. formosa* and *vice versa* at high pH. For both species higher EDTA levels depressed growth rates. Moderate additions of Al generally resulted in growth stimulation. The growth rate stimulations, especially at 200 and 400 $\mu g\ L^{-1}$ Al additions, correlate to increases in free trace metal ion concentrations. The EDTA-Al interaction effects on growth rate were both pH and concentration dependent: at pH 7 both species were stimulated by addition of Al at all EDTA levels (except *A. ralfsii* at 5.0 mM EDTA and *A. formosa* at 0.5 mNM EDTA); at pH 6 Al addition either stimulated or had no effect on the growth rates of both species (except at low EDTA and high Al levels); at pH 5 *A. formosa* did not grow and additions of 200 $\mu g\ L^{-1}$ Al stimulated growth of *A. ralfsii*. It is likely that the effect of pH, Al, and EDTA on speciation of essential or toxic trace metals affects growth rates of these diatoms in a species specific manner.

1. Introduction

Acidification and atmospheric pollution of freshwater lakes has increased the stress on freshwater biota greatly. pH is a variable influencing biochemical reactions and possibly affecting species distributions of algae (Schindler *et al.*, 1985). pH also affects trace metal speciation and potentially trace metal toxicity or limitation (Gensemer and Kilham, 1984; Campbell and Stokes, 1985). With increased inputs of mineral acids in precipitation and the subsequent dissolution of elements from the sediments and watershed, the levels of toxic Al ions in acidified lakes has markedly increased (Driscoll and Newton, 1985). Aluminum appears to have direct and indirect toxic effects on phytoplankton. Aluminum may act directly as a toxin in concentrations as low as 135 $\mu g\ L^{-1}$ (Nalewajko and Paul, 1985) by interfering with essential intracellular functions such as ion transport and metabolic enzyme activity. Rueter *et al.* (1987) suggest that Al toxicity results from indirect increases in Cu^{+2} activity.

Biological response generally is due to free metal ion activity (Sunda and Guillard, 1976; Peterson and Healey, 1985). Bioavailability of metals is in turn a function of chelation and the pH of the water column (Peterson *et al.*, 1984). Metals can be classified in one of two groups: 1) metals whose effects on organisms are reduced

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at lower pH owing to competition with H^+ for active sites (type I); 2) metals whose effects on organisms are increased at lower pH owing to increased concentration of the free metal ion species in solution and at biologicaly active sites (Type II). Cu, Cd, and Zn exhibit Type I behavior and Pb, Al, and Hg appear to conform to Type II behavior (Campbell and Stokes, 1985). Studies of Al speciation have indicated biological response could be due to various Al species or to indirect effects (Folsom *et al.*, 1986; Helliwel *et al.*, 1983; Rueter *et al.* 1987). For organisms that tolerate/grow in the pH 4 to 7 range the Type II metals could have significant effects.

Experiments growing five freshwater algae at pH 5 to 7.5 showed reduced growth rates at pH's below 6. Reducing trace metals by 1 to 2 orders of magnitude restored growth rates to control levels in some cases (Gensemer and Kilham, 1984). Preliminary experiments testing Al toxicity on *Asterionella formosa* and *A. ralfsii* indicated a growth stimulation at 200 $\mu g\ L^{-1}$ Al (unpubl., Gensemer and Riseng). These results led to speculation about the possible interactive effects of pH, Al, and trace metals in experimental FRAQUIL medium (Morel *et al.*, 1975). Because the toxicity or limitation of trace metals is determined by their activity, not their total concentration, it is esssential to examine the behavior of EDTA, the complexing ligand in the experimental medium that controls trade metal ion activity. EDTA has different affinities for different metal ions and therefore competition between metal ions in solution for EDTA binding sites will affect their activity. Toxic metals in less than toxic concentrations may indirectly affect growth rates by competing for EDTA binding sites and displacing metal ions that may be deleterious or beneficial to growth depending on concentrations (Rueter *et al.*, 1987).

Diatom community indices have been used as paleoecological indicators of lake acidification, although the causal mechanisms are largely unknown (Charles, 1985; Schindler, 1988). *A. ralfsii* is unusual in that it is a planktonic, acidophilic diatom making it useful both as an index of surface water acidification and for laboratory study. Circumneutral and acidiphilic species of *Asterionella* were chosen in order to compare the effects of Al, pH and chelator manipulations on growth rates of these two diatoms and analyze potential species specific effects.

This study examines an apparent growth stimulation of *A. ralfsii* and *A. formosa* upon addition of low concentrations of Al. Growth rates of the two diatoms, grown under varying batch culture conditions, were analyzed and compared with empirical and modeled levels of the culture components to determine if Al, chelator and pH interact to produce a growth stimulation.

2. Methods

The diatoms used were: *Asterionella ralfsii* var. *americana* W. Sm. from acidified Lake Andrus, Chippewa County, Michigan, U.S.A. (T. 50N., R. 6W, SEC 27; clone isolated by R.W. Gensemer and submitted to Toronto collection), and *Asterionella formosa* Hass, a circumneutral species from Lake Michigan (clone LMAF2 isolated

by S. S. Kilham). Axenic stocks were maintained in sterile FRAQUIL media, buffered with 200 $\mu g\ L^{-1}$ 2-(N-morpholino) ethanesulfonic acid (MES, pKa 6.82). Prior to inoculation aliquots of the axenic stock cultures of *A. formosa* and *A. ralfsii* were transferred to 250 mL Pyrex flasks in 200 mL sterile FRAQUIL medium. *A. ralfsii* stock cultures were grown with MES at a pH of approximnately 5.3, and *A. formosa* was grown in stock culture media without MES at a pH of about 7.2. Algae were incubated in an environmental chamber at 20 °C, on a 14 hr light: 10 hr dark schedule with cool white fluorescent bulbs (100 $\mu E\ m^{-2}\ s^{-1}$).

Experiments were run to measure the growth rates of *A. formosa* and *A. ralfsii* in response to varying pH, trace metals, and Al concentrations. Because EDTA is the FRAQUIL component that controls availability of trace metals, changes in EDTA concentrations were used to highlight possible trace metal activity effects. Trace metal concentrations were varied using order of magnitude EDTA dilutions (0.1, 1, and 10× dilutions resulting in final concentrations of 0.5, 5.0, and 50 mM) in the trace metal stock solution. The pH of the treatment groups was adjusted to within 0.04 pH units of 5, 6, and 7 with HCl and NaOH. The medium was sterilized by filtration through 0.2 mm membrane filters to avoid contamination of trace metals through autoclaving. Filter sterilized Al was added separately to the filtered media after initial sterilization to final concentrations of 0, 200, 400 and 800 $\mu g\ L^{-1}$ Al. Approximately 2 mL aliquots of stock culture were inoculated into sterile 25 × 150 mm Pyrex treatment tubes containing 35 mL of experimental media. Growth rates were measured by *in vivo* fluorescence using a Turner Designs Model 10 fluorometer after a technique similar to Brand *et al.* (1981). Daily fluorometric measurements were taken until the exponential growth phase stopped, usually 4 to 8 days. Fluorometric readings were natural log transformed and plotted against days to calculate growth rates by least squares regression. The straightest part of the curve (at least 4 d.) was used, representing log phase growth. Mean growth rate and standard deviation per treatment group were established from three replicates. Aliquots were taken from randomly selected tubes on days 2, 4, and 6 to compare fluorescence/cell at different days in the growth cycle.

Aluminum concentrations were measured using a pyrocatechol violoet (PCV) colorimetric technique (Dougan and Wilson, 1974; Sullivan and Seip, 1986; LaZerte *et al.*, 1988; Gensemer, 1990). Total Al concentrations were determined from unfiltered, acidified samples (pH= 1) by the PCV method. Total acid soluble Al includes colloidal and nonlabile organic complexes (Driscoll *et al.*, 1984). Reactive Al concentrations were determined from PCV analysis on unfiltered media at treatment pH (Sullivan and Seip, 1986). PCV reactive Al measures only the inorganic Al fraction in FRAQUIL media presumably due to the strong affinity of EDTA for Al (Gensemer, 1989).

The behavior and activity of trace metal speciation was modeled using the equilibrium-based MINEQL program (Westall *et al.*, 1976) release 2.0. Total PCV measured Al was used as the total Al component input (Gensemer, 1989) and nominal concentrations were used for all other chemical component inputs. Input for the

dissolved Al equilibrium constants were taken from LaZerte (1984). This qualitative analysis was undertaken to ascertain trends in trace metal concentrations with changes in treatment variables.

The main effects of pH, EDTA, and Al on growth rates were evaluated using ANOVA (F test), and the significance of different levels within treatment variables were analyzed using ANOVA contrasts (F test) using SYSTAT (1987). Correlation matrices were established from regression equations to determine which variables were correlated with growth rates.

3. Results

Measured reactive Al covaries significantly with pH and EDTA concentration ($p<0.1$) but the patterns that develop are distinctly different from those with free Al ion (Figure 1). At 50 mM EDTA, pH 5 and 6, the concentration of reactive Al remains constant despite increases in added Al. Slight increases in reactive Al were noted upon additions of 400 and 800 $\mu g\ L^{-1}$ Al at pH 7 with 50 mM EDTA. At 0.5 and 5.0 mM EDTA, pH 5, reactive Al shows increases of greater than one order of magnitude over pH 6 as nominal Al increases. At pH 7 reactive Al varies by slightly less than one order of magnitude from pH 6 at all EDTA levels.

Free Al ion concentration (-log $[Al^{+3}]=pAl^{+3}$) was significantly pH dependent (Table I, Figure 1). As pH decreases from 7 to 5, Al ion concentration increases

TABLE I

A. formosa and *A. ralfsii* growth rates for all treatment groups (pH, EDTA, Al). NG = No growth. Standard errors in parentheses. $n = 3$.

	EDTA (mM)	Nominal Al ($\mu g\ L^{-1}$)			
		0	200	400	800
Asterionella formosa					
pH 6	0.5	0.55 (0.05)	0.58 (0.01)	0.59 (0.03)	NG
	5.0	NG	0.50 (0.02)	0.46 (0.01)	NG
	50	NG	0.35 (0.02)	0.37 (0.03)	0.50 (0.03)
pH 7	0.5	0.56 (0.05)	0.63 (0.03)	0.67 (0.01)	0.61 (0.07)
	5.0	0.44 (0.04)	0.54 (0.02)	0.57 (0.01)	0.60 (0.02)
	50	0.18 (0.03)	0.37 (0.05)	0.36 (0.00)	0.30 (0.03)
Asterionella ralfsii					
pH 5	0.5	0.51 (0.01)	0.55 (0.01)	0.40 (0.01)	0.03 (0.04)
	5.0	0.25 (0.04)	0.62 (0.01)	0.28 (0.04)	0.04 (0.01)
	50	NG	0.07 (0.01)	0.04 (0.01)	0.12 (0.01)
pH 6	0.5	0.63 (0.02)	0.61 (0.02)	0.42 (0.02)	0.50 (0.03)
	50	NG	28 (0.01)	NG	NG
pH7	0.5	NG	NG	NG	NG
	5.0	NG	0.20 (0.02)	0.22 (0.03)	0.32 (0.01)
	50	NG	0.21 (0.02)	0.20 (0.03)	0.36 (0.01)

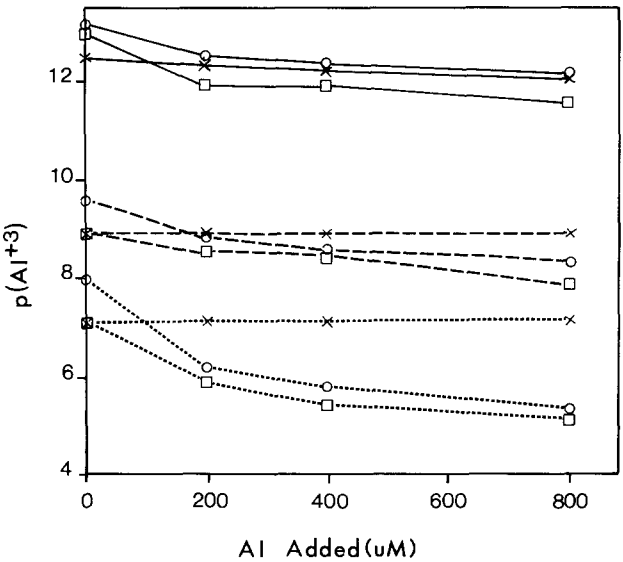


Fig. 1. Plot of pAl^{+3} (calculated -log free Al ion concentration) versus amount Al added in μg L^{-1}. (□) = 0.5 mM EDTA, (○) = 5.0 mM EDTA, (X) = 50 mM EDTA, (——) = pH 7, (— — —) = pH 6, (- - -) = pH 5.

approximately 1 to 4 orders of magnitude illustrating the pH differentiated speciation of Al and EDTA. At pH 6 the $Al(OH)_3$ solid is most likely to form while EDTA's affinity for Ca increases dramatically at pH 7, indicating that Ca could compete with Al or other trace metals for EDTA binding sites at pH 7. Within each pH range EDTA controls the Al ion concentration. As the concentration of EDTA increases less free Al ion is available until at 50 mM EDTA Al ions is essentially 100% EDTA bound at all pH's examined.

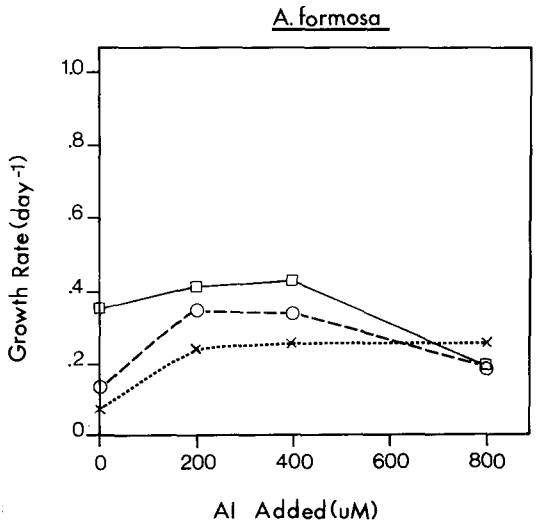
With each order of magnitude increase in EDTA concentration and no added Al, free ion activities of trace metals declined 1 to 3 ordes of magnitude (Table I). Aluminum additions increased the free ion concentration of Fe, Cu, Zn, and Co by 2 to 4 orders of magnitude. Increases in trace metal concentrations peaked after additions of 200 μg L^{-1} Al and leveled off or more slowly increased upon further addition of Al. Trace metal activities increased in the following cases upon addition of Al: Mn at 5.0 mM EDTA and pH of 5; Fe, Cu, Zn, and Co at 0.5 and 5.0 mM EDTA at pH 5; and Fe, Cu, Zn, and Co at 0.5 mM EDTA at pH 6 and 7. At 50 mM EDTA all trace metals are 100% EDTA bound.

Mean growth rates for *A. formosa* and *A. ralfsii* were relatively constant within each treatment. Standard error of the treatment growth rate means varied from 0.002 to 0.051 d^{-1}. The *A. formosa* clone died shortly after termination of the experiments, and *A. formosa* fluorescence $cell^{-1}$ data revealed a significant difference between day 2 and day 6 fluorescence with treatment, therefore less confidence is assured with *A. formosa* data. The problem could be due to a small data set for cell counts or to changes in diatom reproductive rates with changes in cell

size as the clone senesced (Brand *et al.*, 1981). Selected experiments were repeated using a different *A. formosa* clone (S. S. Kilham, Douglas Lake, Michigan) for pH 6,50 mM EDTA treatments. Analysis of covariance using measurements of fluorescence cell^{-1} showed no significant changes with time or treatment for *A. ralfsii*.

pH had significant overall effects on the growth rates of both species ($p < 0.0005$). The pH effect was species specific as exhibited by the zero growth of *A. formosa* at pH 5 and significantly reduced growth of *A. ralfsii* at pH 7. The optimum growth

(a)



(b)

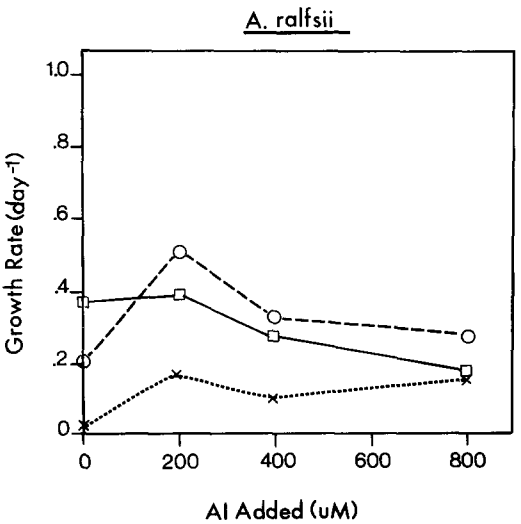


Fig. 2. (a) *A. formosa* growth rates vs. Al (μg L^{-1}) added summed over pH 5, 6, & 7 to illustrate the effect of EDTA on growth rates. (□) = 0.5 mM EDTA, (○) = 5.0 mM EDTA, (X) = 50 mM EDTA; (b) *A. ralfsii* growth rates vs Al (μg L^{-1}) added summed over pH 5, 6 & 7 to illustrate the effect of EDTA on growth rates. (□) = 0.5 mM EDTA, (○ = 5.0 mM EDTA, (X) = 50 mM EDTA.

rates of control cultures (0 μg L^{-1} Al, 5.0 mM EDTA) were m=0.35 d^{-1} at pH 6 for *A. ralfsii* and m=0.44 d^{-1} at pH 7 for *A. formosa*.

Treatment with 50 mM EDTA resulted in overall depression of growth and mean growth rates significantly different from 0.5 and 5.0 mM EDA (F, $p<0.0005$) for both species (Table II). The growth responses of both species were not significantly different at 0.5 mM EDTA (m=0.32 d^{-1}) and 5.0 mM EDTA (m=0.28 d^{-1}). At pH 5 and 6 growth rates in the 50 mM EDTA treatment were significantly less than both 0.5 mM EDTA (F, $p<0.004$) and 5.0 mM EDTA (F, $p<0.2$). At pH

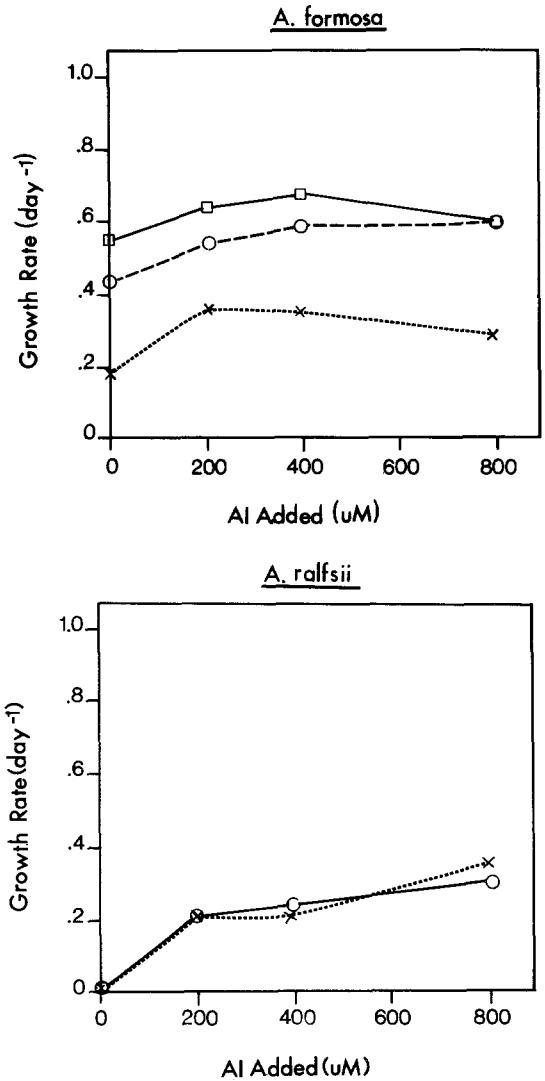


Fig. 3. (a) Growth rate of *A. formosa* vs Al added at pH 7. (□) = 0.5 mM EDTA, (○) = 5.0 mM EDTA, (X) = 50 mM EDTA; (b) Growth rate of *A. ralfsii* vs Al added at pH 7, (□ = 0.5 mM EDTA, (○) = 5.0 mM EDTA, (X) = 50 mM EDTA.

7 growth rates in the 50 mM EDTA treatment were significantly less than the 5.0 mM EDTA treatment (F, $p<0.06$).

EDTA treatment resulted in differences in growth patterns depending upon Al treatment irrespective of pH (Figures 2a and b). At 0 μg L^{-1} Al *A. ralfsii* growth rates were significantly different at all EDTA levels (F, $p<0.05$) and *A. formosa* 0.5 mM EDTA treatment growth rates were significantly greater than 5.0 and 50 mM EDTA (F, $p<0.002$). Growth rates increase significantly at 5.0 and 50 mM EDTA treatments upon Al additions of 200 μg L^{-1} for *A. ralfsii* (F, $p<0.001$)

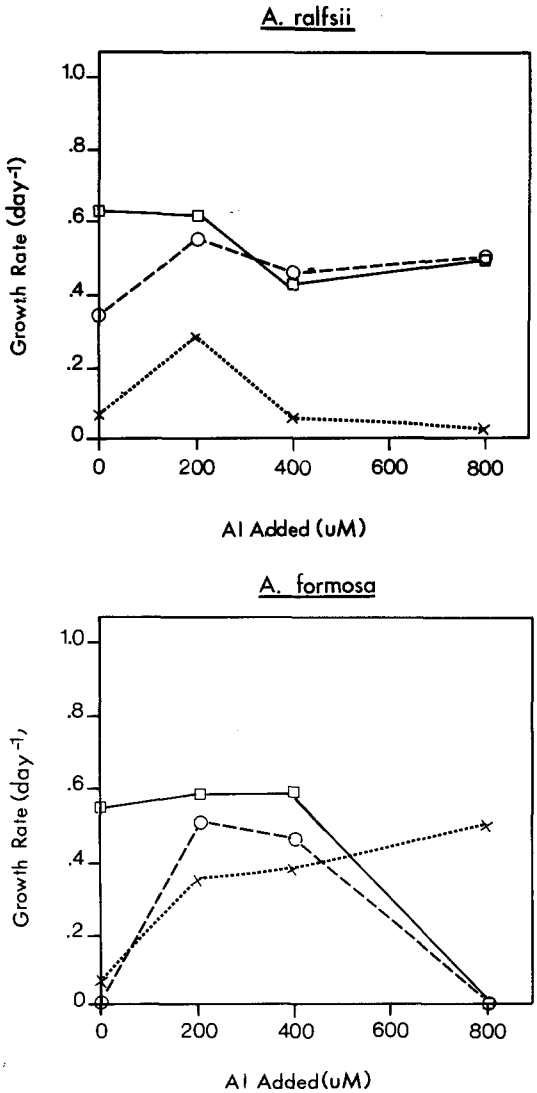


Fig. 4. (a) Growth rate of *A. formosa* vs Al added at pH 6. (□) = 0.5 mM EDTA, (○) = 5.0 mM EDTA, (X) = 50 mM EDTA; (b) Growth rate of *A. ralfsii* vs Al added at pH 6. (□) = 0.5 mM EDTA, (○) = 5.0 mM EDTA, (X) = 50 mM EDTA.

and *A. formosa* (F, $p<0.009$). For both species 50 mM EDTA were significantly less than 0.5 and 5.0 mM EDTA treatment growth rates at 200 μg L^{-1} Al (F, $p<0.007$). *A. ralfsii* 50 mM EDTA treatment growth rates were also lower than the 0.5 and 5.0 mM EDTA treatments at 400 μg L^{-1} Al (F, $p<0.002$). *A. formosa* growth rates were significantly different at all EDTA levels at 400 μg L^{-1} Al (F, $p<0.2$). Growth rates decreased significantly upon addition of 800 μg L^{-1} Al for both species at the 0.5 mM EDTA treatment and for *A. formosa* at the 5.0 mM EDTA treatment (F, $p<0.02$). Addition of 800 μg L^{-1} Al resulted in no significant differences between growth rates owing to EDTA treatment.

Within each treatment group the addition of Al resulted in several growth response patterns depending on species, pH, and EDTA concentration. At pH 7, 50 and 5.0 mM EDTA, *A. formosa* showed a significant increase in growth upon addition of 200 μg $L^{-,1}$ Al (F, $p<0.05$) (Figure 3a). *A. ralfsii* did not grow at pH 7, 5.0 mM EDA. At 0.5 and 50 mM EDTA *A. ralfsii* growth rates increased at 200 μg L^{-1} Al (F, $p<0.006$) and increased significantly upon addition of 800 μg L^{-1} Al at 50 mM EDTA (F, $p<0.015$) (Figure 3b). At pH 6, 5.0 mM EDTA, *A. formosa* exhibited a growth peak with nominal Al concentration of 200 μg L^{-1} and declined to zero growth at 800 μg L^{-1} added Al (F, $p<0.005$). *A. formosa* 50 mM EDTA treatment growth rates increased significantly at nominal Al levels of 200 and 800 μg L^{-1} (F, $p<0.015$) (Figure 4a). At pH 6, 5.0 and 50 mM EDTA, *A. ralfsii* growth rates increased upon addition of 200 μg L^{-1} Al (F, $p<0.0005$) and all EDTA treatments showed a significant growth rate decrease at 400 μg L^{-1} Al (F, $p<0.045$) (figure 4b). At pH 5, and especially 5.0 mM EDTA, *A. ralfsii* growth rates increased upon addition of 200 μg L^{-1} Al (F, $p<0.001$) and declined with further Al additions (F, $p<0.001$). The 50 mM EDTA treatment showed overall depressed *A. ralfsii*

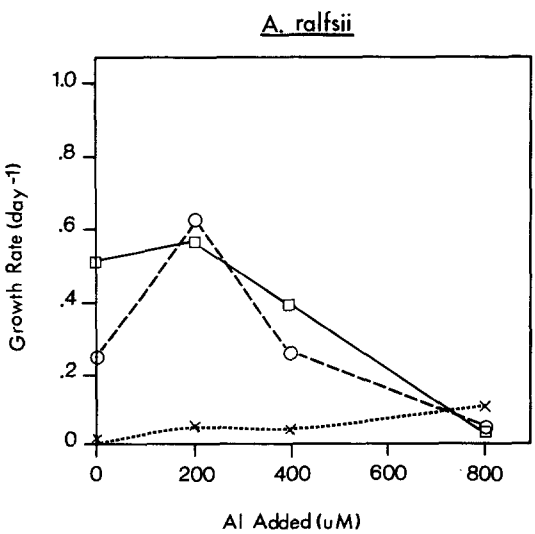


Fig. 5. Growth rate of *A. ralfsii* vs Al added at pH 5. (□) = 0.5 mM EDTA, (○) = 5.0 mM EDTA, (X) = 50 mM EDTA.

growth rates but significant increases in growth with increases in nominal Al (F, $p<0.05$) (Figure 5). *A. formosa* did not grow at all at pH 5.

Estimated trace metal activities increased upon addition of Al in a pH and EDTA dependent fashion (Table I). These increases in free metal ion activities, specifically Fe, Cu, Mn, Zn, and Co, correlate with growth rate patterns in these experiments. Regression of *A. formosa* growth rates with free trace metal ion concentrations indicated a significant correlation between growth rate and pH, Fe, Cu, Mn, and Zn ($r=0.803$, $p<0.0005$). *A. ralfsii* growth rate are correlated to Al and Mn only ($r=0.412$, $p<0.005$). These regression coefficients could be biased because the trace metal activities were estimated from model calculations dependent on pH, EDTA and Al inputs, and thus are not independent variables.

4. Discussion

The growth patterns of the two algae can be attributed to either overriding effects or to interactions of individual parameters depending on conditions of pH, EDTA, and nominal Al. Overall, pH has a species specific effect. More acid tolerant *A. ralfsii* grew well generally at pH 5 while *A. formosa* grew better at pH 6 and 7. pH also controls the speciation of trace metals and, to a lesser extent, chelation with EDTA in the pH range tested, which this study shows may affect phytoplankton growth patterns. In addition, decreasing pH may effectively decrease toxicity at the cell surface via competition between metals and H^+ ions. This may mean that Al acts as a Type I metal and competes with H^+ for the cells' biologically active sites (Peterson *et al.*, 1984, Gensemer, 1990). Aluminum is presently classified loosely as a Type II metal although the complicated nature of Al speciation, solubility, and biological uptake limit generalizations (Campbell and Stokes, 1985).

pH as a master variable controls speciation of both chelators and metals. Within the pH range 5 to 7, EDTA speciation change slightly, mainly by becoming more Ca bound at pH 7. This speciation change in EDTA could have minor effects on the availability of trace metals. There is evidence that natural humid acid speciation changes with pH as well (Jorgensen and Jensen, 1984). A number of studies (Thurman, 1985) have found that the stability constants of humic trace metal complexes follow the Irving-Wallace order of stability at pH 8: $Hg>Pb>Cu>Ni>Co>Zn>Cd>Fe>Mn>Mg$. At pH 3 the stability constants change such that $Fe>Al>Cu$, likely caused by protonation of the binding sites (Thurman, 1985). Baccini (1984) reports that the stability of natural organic complexes with Cu is pH dependant: organocopper complexes reduce bioavailability of Cu to phytoplankton and decreases in pH would release free cupric ion to the ecosystem.

Chelation has both stimulating and inhibitory effects on growth rates depending on concentration and metal additions. In general, higher concentrations of EDTA tended to ameliorate metal toxicity as nominal Al increased. The amelioration of the toxic effect of high concentrations of free Al ion by organic complexation can

be illustrated by significant growth rate increases with increases in nominal Al at 50 mM EDTA (Figures 3b, 4a, 5). Decreasing chelator concentrations at lower nominal Al (and one 800 $\mu g\ L^{-1}$ treatment) proved to be stimulating to growth rates possibly due to release of essential trace metals from EDTA upon addition of Al.

Studies with trace metals and synthetic chelators NTA and EDTA show they can both increase and decrease toxicity of trade metals (Hongve *et al.*, 1980). Some studies of the effects of NTA and metals on algal growth revealed unexplained growth stimulation (Hamilton, 1972). In this study addition of 200 $\mu g\ L^{-1}$ Al resulted in growth stimulation and increased availability of trace metals, suggesting this growth stimulation may result from trace metal interaction and competition for chelator binding sites.

Alternatively, trace-metal-ligand complexes can reduce growth by removing essential trace metals or indirectly increasing the supply of toxic metals. Rueter *et al.* (1987) manipulated Al and Cu concentrations and attributed toxic effects on the alga *Scenedesmus quadricauda* to indirect Al toxicity which acted to increase Cu concentrations to toxic levels. This study showed manipulation of nominal Al resulted in changes in Fe, Cu, Zn, Co, and Mn free ion concentrations. Changes in concentrations of free metal ions from 2 to 4 orders of magnitude can result in toxicity to phytoplankton (Eichenberger, 1985). Changes of this magnitude occurred for Fe, Cu, Zn and Co at 0.5 and 5.0 mM EDTA and at 5.0 mM EDTA for Mn upon addition of 200 $\mu g\ L^{-1}$ Al (Table I). These 2 to 4 order of magnitude changes in trace metal concentrations could account for changes in growth rates.

Both synthetic and natural chelators can significantly alter bioavailability of trace metals depending on pH and ionic conditions (Huljev and Strohal, 1983; Mantoura *et al.*, 1977; Hongve *et al.*, 1980; Jackson and Hecky, 1980). Driscoll *et al.* (1980) hypothesize that organic complexation *vs* pH may control the natural fluctuations of labile Al. Sunda and Lewis (1978) found that complexation of Cu by natural organic ligands reduced toxicity to an alga *Monochrysis lutheri* by reducing free cupric ion activity. Hongve *et al.* (1980) experimented with metal mixture complexes with humic acid and NTA in a natural phytoplankton community and found that complexation with humic acids led to partial detoxification of individual metals Cd, Zn, Pb, and Cu while complexation with Hg and the metal mixture increased toxicity. Within specific pH ranges organic complexation may have differing effects. At pH 7, where free Al ion concentration is small, high levels of EDTA have a negative effect on growth, possibly due to the complexation and reduced bioavailability of essential trace metals (Table I, Figure 3a).

Application of these experiments to natural waters requires the assumption that EDTA-trace metal behavior accurately models natural chelators such as humic and fulvic acids (Gamble and Schnitzer, 1973; Steinberg and Meunster, 1985). While some feel confident that EDTA could mimic a natural system (Rueter *et al.*, 1987; Sunda and Lewis, 1978) others feel less so because of the variable, site specific stability constants that dictate humic metal interactions (Mantoura *et al.*, 1977).

In both natural waters and laboratory culture, free ion concentrations are regulated by both pH via solubility chemistry and organic complexation (Driscoll *et al.*, 1984). Limited comparisons may perhaps be made along this line.

The ultimate importance of pH, Al and chelator effects on aquatic biota are reflected in species composition, diversity and biomass. In natural environments organisms are surrounded by dissolved organic matter, including many types of chelators, resulting in spacial and temporal heterogeneity or patchiness of metal availability and/or toxicity (Eichenberger, 1985). Species exhibit a wide range of tolerances to trace metal availability: *Fucus* and *Ascophyllum* are highly tolerant to trace metal concentrations, whereas *Skeletonema costatum* is very sensitive (Eichenberger, 1985); metal stressed aquatic ecosystems undergo species shifts from diatoms to filamentous green algae in Swiss river water (Baccini, 1984); species may differ in their ability to use alumino-phosphate complexes which could result in a change in species composition with a change in pH and in metal concentration and speciation (Nalewajko and Paul, 1985).

A. formosa and *A. ralfsii* were stimulated by additions of 200 $\mu g\ L^{-1}$ Al at pH 5, 6 and 7. At pH 7 further Al additions had only slight effects: depressed growth rates for *A. formosa*; stimulated growth rates for *A. ralfsii*. As pH decreased, growth was depressed with further addition of Al for both species (except pH 6, 50 μM EDTA, *A. formosa*). Although *A. formosa* and *A. ralfsii* exhibit a species specific response to pH, they did not exhibit species specific responses to Al addition.

In nature, response to increased Al concentrations is likely to be mediated by pH and chelation. With changes in pH one observes major species shifts in the aquatic environment. The changes in species assemblies upon acidification of natural waters is likely a complex event. Acid precipitation and spring snowmelt often result in a double insult to the aquatic environment by contributing excess Al as well as H^+ ions with runoff. The quality of natural waters affects the type and extent of trace metal binding with organic ligands. It is likely that sources of pollution will contribute a composite slew of metals as well as nutrients and ligands that regulate bioavailability (Eichenberg, 1985). The ligands may be natural organic material resulting from decomposition or may be synthetic chelators released as waste products.

This study indicate that Al effects on phytoplankton involve complex pH-chelator-trace metal interactions. These interactions can influence the growth of phytoplankton both directly and indirectly resulting in a variety of growth rate responses.Species may thrive or become locally extinct as a result of interactions of pH with chemical constituents in the environment.

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