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ROLE OF ALUMINUM AND GROWTH RATE ON CHANGES IN CELL SIZE AND SILICA CONTENT OF SILICA-LIMITED POPULATIONS OF *ASTERIONELLA RALFSII* VAR. *AMERICANA* (BACILLARIOPHYCEAE)[1]

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ABSTRACT

Changes in cell size and silica content were examined in response to aluminum additions in cultures of the acidophilic diatom Asterionella ralfsii *var.* americana *Körn at pH 6.0. The effects of Al were examined over a range of steady-state growth rates using silica-limited semicontinuous cultures (Si:P = 8.0). Additions of* ≥ 2.8 *μmol·L*$^{-1}$ *total Al decreased mean cell length, total surface area, and biovolume up to 40–50%. The effects of Al were dependent on growth rate with the magnitude of size reduction increasing at higher growth rates. The proportion of small (approximately 15–20 μm) cell length classes increased relative to large (approximately 50 μm length) cell length classes when total Al exceeded 2.8 μmol·L*$^{-1}$*, particularly at higher growth rates. The relationship between cell quota and steady-state growth rate fit a Droop relationship at 0 and 2.8 μmol·L*$^{-1}$ *total Al, but this fit was highly variable in the presence of Al. Cell quotas in the 6.22 μmol·L*$^{-1}$ *total Al treatment were highest at low growth rates; therefore, a Droop relationship was an inappropriate descriptor of growth rate. Cells also became 30–40% more heavily silicified per unit surface area in the presence of Al and at growth rates* ≥ 0.22 *day*$^{-1}$*. Although the mechanisms responsible for size reductions in response to Al additions are unclear, the relationship between metal concentration and frustule morphology may be useful as an indicator of Al loading to acidified lakes.*

Key index words: aluminum; Asterionella ralfsii *var.* americana; *cell quota; metals; morphology; semicontinuous culture; silica; silicification; Si-limitation*

Acidic precipitation enhances the mobilization of trace metals to surface waters (Dillon et al. 1984, Driscoll and Newton 1985, LaZerte 1986); therefore, it is important to understand the potential function of metals in the responses of aquatic biota to acidification. Metal bioavailability tends to increase in acidic solutions, thus making some metals potentially more toxic to a variety of taxa (Campbell and Stokes 1985). One of the more important metals in this regard is aluminum. Aluminum toxicity in acidic habitats has often been implicated as having a significant negative effect on populations of fish (Driscoll et al. 1980, Dillon et al. 1984), amphibians (Clark and LaZerte 1985, 1987, Andrén et al. 1988), and phytoplankton (Hörnström et al. 1984, Folsom et al. 1986, Claesson and Törnqvist 1988).

The association of diatom species with particular pH conditions has stimulated the development of several composite indices of community structure which are highly correlated with lake pH (Charles 1985, Battarbee et al. 1986, Davis 1987). These empirical relationships have proven to be useful indicators of both the rate and extent of surface water acidification from anthropogenic pollutant sources (Dickman et al. 1984, Charles and Whitehead 1986, Schindler 1988). Composite community structure indices also have been used for predicting lake alkalinity (Charles et al. 1987), and the potential exists for the development of similar relationships with trace metals (Charles and Whitehead 1986). Paleoecological indices involving acidophilic diatoms are, however, based purely on correlations of environmental factors to the distribution of field populations. To enhance confidence in the predictions of these indices, experiments are needed to describe the relevant physiological and ecological mechanisms.

Morphological features of the diatom frustule also exhibit variation in response to external environmental factors. Changes in cell morphology and silica cell content have been observed for diatoms in response to changes in environmental factors including inorganic nutrient dynamics, salinity, temperature, and light (Tilman et al. 1976, Paasche 1980, Turpin and Harrison 1980, Theriot and Stoermer 1984, Taylor 1985, Conley et al. 1989). Far less is known, however, about the morphological responses of diatoms to trace elements. Lead and copper affected organelle morphometry in *Diatoma tenue* (Sicko-Goad and Stoermer 1979), and ammonium cuprate pollution induced valve distortion in *Synedra acus* (Tonolli 1961). That aluminum may affect the cell structure of diatoms has been observed only for *Synedra* cf. *nana* (Hörnström et al. 1984). The effects of Al on the growth and physiology of diatoms are also poorly understood; only a few recent studies on *Asterionella* species have been performed (Gensemer 1989, Pillsbury and Kingston 1989, Riseng 1989).

The acidophilic diatom *A. ralfsii* var. *americana* is of particular interest because it occurs in nature at pH values below those usually tolerated by planktonic diatoms (ca. pH 6; Charles 1985, Davis 1987). This feature has made *A. ralfsii* a valuable indicator of early acidification (Schindler et al. 1985, Findlay and Kasian 1986), and it can be quantitatively important in paleolimnological pH reconstructions

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(DeNicola 1986, Duthie 1989). *A. ralfsii* has been described as exhibiting two morphological forms: long (>45 μm length) and short (<45 μm length). The distribution of these morphotypes is probably related to environmental factors (D. F. Charles, pers. comm.), yet little is known about the extent and causes of size variation.

This study examines changes in the cellular morphology and cellular silica content of *A. ralfsii* in response to Al additions under controlled laboratory conditions. Because silicification can be affected both by generation time and the extent to which cells are silica-limited (Paasche 1980, Taylor 1985), these responses also were examined at fixed growth and nutrient supply rates using semicontinuous cultures.

MATERIALS AND METHODS

Culture conditions and experimental design. A clone of *Asterionella ralfsii* var. *americana* Körn. was isolated from an epilimnetic water sample from Andrus Lake, Chipewa County, Michigan, U.S.A. (T.50N., R.6W., SEC 27). An axenic clone (submitted to the University of Toronto Culture Collection, UTCC #170) was maintained in a modified FRAQUIL medium (Morel et al. 1975) buffered at about pH 5.4 with 200 mg·L^{-1} MES (2-(N-morpholino) ethanesulfonic acid, $pK_a = 6.15$). Modification consisted of lowering Na_2EDTA (ethylenediaminetetraacetic acid, disodium salt) to 0.5 μmol·L^{-1} without changing total metal concentrations, and adding 400 μmol·L^{-1} boron (Na salt). The EDTA concentration was reduced to prevent trace metal limitation at the 10× higher concentration of unmodified FRAQUIL (Riseng 1989). Media stock solutions were prepared from reagent grade chemicals and double-deionized distilled water (DDW). Experiments were incubated in a constant environment chamber at 20° C and lighted with cool white fluorescent bulbs which provided approximately 115 μE·m^{-2}·s^{-1} on a 14:10 h LD cycle.

Media pH was set to 6.0 with additions of 2N HCl or NaOH to examine the responses of *A. ralfsii* under conditions of maximum dissolved Al toxicity (Helliwell et al. 1983, Claesson and Törnqvist 1988, Gensemer 1989). Experimental media then were filter sterilized through 0.2 μm pleated polysulfone membrane filters (Gelman Sciences). Aluminum was added separately from a filter-sterilized stock solution of $AlCl_3 \cdot 6H_2O$ to produce final concentrations of 2.8 and 5.6 μmol·L^{-1} nominal Al. All media were allowed to equilibrate at ambient temperature for at least 24 h prior to use. Final pH after sterilization and equilibration was 6.0.

Experiments were performed using a semicontinuous dilution rate design set at turnover rates of 0.1, 0.2, 0.4, and 0.5 day^{-1}. These turnover rates corresponded to steady-state growth rates of 0.11, 0.22, 0.51, and 0.69 day^{-1} using a conversion formula from Kilham (1978):

$$D = \ln[1/(1 - f)] \quad (1)$$

where D = steady-state growth rate, and f = semicontinuous dilution rate. These rates were chosen to represent a range of growth rates up to a maximum for *A. ralfsii* in batch culture of 0.6–0.7 day^{-1} (Gensemer 1989, Riseng 1989). Cells were preconditioned in 500 mL batches of media without Al for at least 10 days before inoculation. Approximately 1 mL of this stock culture was transferred into each 500 mL polycarbonate experimental flask which produced a starting density of about 350 cells·mL^{-1}.

Semicontinuous dilutions were performed by discarding an appropriate volume of the culture suspension and replacing it with the same amount of fresh, sterile medium at the same time each day. In vivo fluorescence of the effluent from each flask was checked daily to follow the development of steady state. Steady state was achieved after three weeks (as determined by stable fluorescence values over at least 3–4 days), after which portions of the effluent were sampled either for cell counts or for chemical analysis. Aliquots for cell counts were preserved in Lugol's solution and counted for cell numbers in a Sedgwick-Rafter chamber. Transects were counted until at least three transects and 100 colonies were encountered. If a chamber contained significantly more than 100 colonies, counting continued until the coefficient of variation between replicate transects was below 20%. Aliquots for cellular Si analysis were filtered onto 0.4 μm poresize polycarbonate membranes (Nuclepore Co.) and frozen for later analysis. Because samples for cell counts (day 22) and cellular Si analysis (day 24) were taken two days apart, cell densities for day 24 were estimated from fluorescence per unit cell density values on day 22. Owing to variation among treatments, the mean ratio of fluorescence units:cells·mL^{-1} from each treatment (Table 1) was used to convert fluorescence units to cells·mL^{-1} for each corresponding treatment flask on day 24.

TABLE 1. *Ratios of day 22 fluorescence units : cells·mL^{-1} expressed as the mean (and standard error) for each treatment (n = 2).*

Growth rate (day^{-1})	μmol·L^{-1} total Al		
	0.0	2.8	6.22
0.11	0.0242 (0.0008)	0.0176 (0.0019)	0.0267 (0.0023)
0.22	0.0178 (0.0002)	0.0169 (0.0007)	0.0160 (0.0016)
0.51	0.0131 (0.0013)	0.0066 (0.0008)	0.0094 (0.0005)
0.69	0.0132 (0.0022)	0.0062 (0.0004)	0.0084 (0.0003)

Influent nutrient concentrations were 12 μmol·L^{-1} $Si(OH)_4$ and 1.5 μmol·L^{-1} PO_4 which provided a molar Si:P ratio of 8.0. This ratio was set far below optimum Si:P ratios of 53–96 observed for the circumneutral congener *A. formosa* (Tilman and Kilham 1976, Holm and Armstrong 1981, Tilman 1981) to produce Si-limited populations. However, the extent to which these optimum ratios apply to acidophilic *Asterionella* species is unknown. Therefore, nutrient addition bioassays were performed to ensure that *A. ralfsii* was Si-limited after reaching steady-state. Effluents from both the 0 Al and the highest Al treatments at 0.11 day^{-1} growth rate were divided into 10 mL aliquots and distributed into sterile 25 × 150 mm Pyrex tubes. To each of these tubes was added 20 mL of either 0 Al medium (control), 0 Al medium with 2× normal Si concentration (+Si), or 0 Al medium with 2× normal P concentration (+P) with each treatment containing two replicates. These tubes were then incubated for 8 days; in vivo fluorescence readings were taken on each tube at the same time daily. When the batch cultures reached stationary phase, the bioassay was terminated and day 8 fluorescence readings were used to determine final yield.

Medium chemistry. Aluminum concentrations were determined colorimetrically with pyrocatechol violet (PCV, Sigma Chemical Co.) as described by Dougan and Wilson (1974) and refined by Seip et al. (1984) and LaZerte et al. (1988). Two Al fractions were measured depending on how the samples were treated prior to analysis: 1) total Al (Al_t) was that fraction which was reactive with PCV after unfiltered media was acidified to pH 1 for at least 24 h, and 2) PCV-reactive Al (Al_r) was that fraction which was reactive with PCV at ambient pH without filtration. PCV-reactive Al measures inorganic monomeric Al (IMAl) concentrations in natural waters after correction for organic Al (Sullivan et al. 1986, LaZerte et al. 1988), and without such correction in media containing EDTA-bound Al as the only organic form (Gensemer 1989). Al_r was used because IMAl rather than polymeric or organically-bound Al has been shown to be the most toxic fraction for vertebrates (Driscoll et al. 1980, Dillon et al. 1984, Clark and LaZerte 1985). Nominal Al additions of 0, 2.8, and 5.6 μmol·

TABLE 2. *Mean (and standard error, n = 2) in vivo fluorescence unit yields for the nutrient addition bioassay experiments. See text for details of experimental design. High Al denotes 6.22 μmol·L⁻¹ Al_t.*

Treatment	Fluorescence units
0 Al	
Control	380 (20.0)
+ Si	760 (40.0)
+ P	360 (40.0)
High Al	
Control	390 (30.0)
+ Si	810 (30.0)
+ P	470 (10.0)

L^{-1} Al produced mean Al_t concentrations of 0.00 (all measurements below limit of detection), 2.80 (SE = 0.03), and 6.22 (SE = 0.03) μmol·L^{-1}, and Al, concentrations of 0.00 (all measurements below limit of detection), 0.32 (SE = 0.02), and 0.43 (SE = 0.13) μmol·L^{-1}.

Frozen filters from day 24 effluents were transferred to polycarbonate tubes and pre-treated for particulate silica analysis using the NaOH dissolution technique of Paasche (1973). After digestions were complete and remaining particulates were removed by centrifugation, dissolved Si was determined for the supernatant using the molybdate assay of Strickland and Parsons (1972). Particulate Si concentrations from each treatment flask were then divided by estimated day 24 cell densities to calculate total Si cell quota.

Cell morphology. Cells from day 22 effluent were cleaned using concentrated chromic-sulfuric acid. Cleaned cells were dried onto coverslips and mounted in Hyrax for examination at 1000× using Nomarski illumination. Length and width measurements were taken on cells in girdle and valve view using an eyepiece micrometer. Length measurements were taken on the longest dimension of the cell along the longitudinal axis, and width measurements were taken perpendicular to the longitudinal axis at the midpoint of the cell. All cells that were encountered within the eyepiece field were measured until at least 25 cells were encountered, and until the standard error of cell length was less than or equal to 5% of the mean length. Measurements for girdle view and valve view cells were tabulated separately; therefore the number required to satisfy the 5% standard error rule was not necessarily the same for valve or girdle view cells on any given slide.

Area and biovolume calculations were made using a simplifying assumption that frustule shape could be approximated by a rectangle of area = L × W and volume = L × W × D. Mean lengths for girdle view and valve view cells were pooled for valve length, total surface area and biovolume calculations; therefore, the formulas used were:

$$\text{Valve length} = (L_g + L_v)/2 \quad (2)$$

$$\text{Total surface area} = 2(L_g \cdot W_g) + 2(L_v \cdot W_v) \quad (3)$$

$$\text{Biovolume} = [(L_g + L_v)/2] \cdot W_v \cdot W_g \quad (4)$$

where L_g and L_v = mean girdle and valve view length, respectively, and W_g and W_v = mean girdle and valve view widths, respectively.

The significance of Al and growth rate treatments on cell size and cell Si content was analyzed using 2-way ANOVA (PROC GLM, SAS Institute, Inc. 1985) on a design of 4 growth rates ×3 Al concentrations with two replicates per treatment unless otherwise specified. Mean values for valve dimensions, total surface area, and biovolume from each experimental flask were used in all analyses. If ANOVA's were significant, differences between individual treatment means were determined using Tukey's Studentized Range Test (TSR). A significance level of $P < 0.05$ was chosen for all analyses.

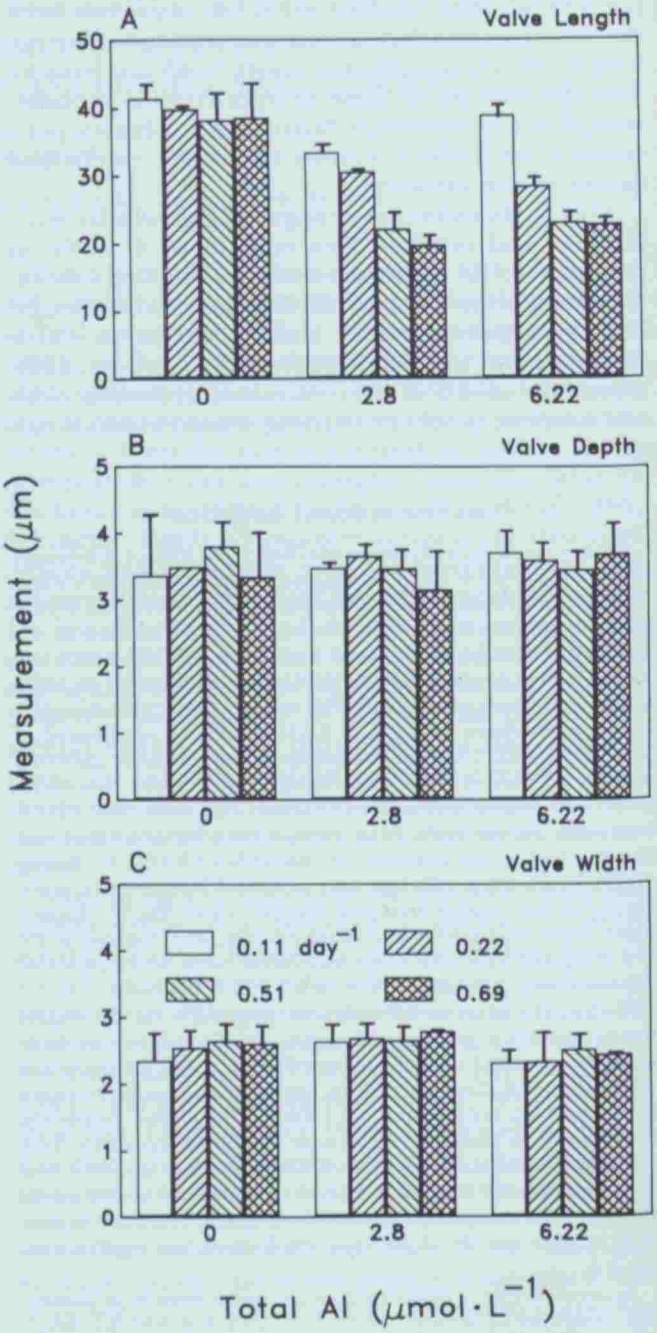


FIG. 1A–C. Mean frustule measurements (μm) as a function of Al, (μmol·L^{-1}) at all four steady-state growth rates (day^{-1}). Measurements are expressed as treatment means (vertical bars = two standard errors) of valve A) length B) depth, and C) width.

RESULTS

Nutrient limitation bioassay. Fluorescence-based culture yields from the nutrient addition bioassays confirmed that Si was limiting growth (Table 2). For both Al treatments, Si stimulated final culture yield, whereas P had no stimulatory effect (1-way ANOVA; 0 Al: $F_{2,3} = 42.33$, $P < 0.006$; 6.22 μmol·L^{-1} Al_t: $F_{2,3} = 78.53$, $P < 0.003$). Doubling Si concen-

trations doubled culture yields, whereas P additions did not result in significant increases from control yields (TSR, df = 3).

Cell dimensions. Valve lengths decreased up to about 50% as Al_t concentrations and steady-state growth rates increased (Fig. 1A). Both Al and growth rate treatments significantly affected mean valve length ($F_{11,12} = 43.42$, $P < 0.0001$), and a significant interaction existed between the effects of Al and growth rate ($F_{6,12} = 8.57$, $P < 0.0009$). Al_t concentrations ≥ 2.8 $\mu mol \cdot L^{-1}$ decreased mean length at 0.22 relative to 0.11 day^{-1}, and this effect was more pronounced at 0.51 and 0.69 day^{-1} (TSR, df = 12). In contrast, both valve depth (Fig. 1B) and valve width (Fig. 1C) exhibited no significant relationship to either Al or growth rate ($F_{11,12} = 0.77$, $P < 0.66$; $F_{11,12} = 2.64$, $P < 0.055$, respectively).

Mean total surface area and biovolume decreased in the presence of Al at all growth rates (Fig. 2). Both Al and growth rate treatments combined to reduce total surface area nearly 50% (Fig. 2A; $F_{11,12} = 39.76$, $P < 0.0001$). Al-induced size reductions were minor at 0.22 day^{-1} and maximal at 0.51 and 0.69 day^{-1} (TSR, df = 12). Biovolume decreased as much as 40% in the presence of Al (Fig. 2B; $F_{11,12} = 12.41$, $P < 0.001$), and this reduction was more pronounced at higher steady state growth rates (TSR, df = 12).

Size frequency analysis. Frequency distributions of valve lengths were compiled to examine changes in the distribution of frustule sizes in response to Al and growth rate. Individual valve lengths from each treatment were pooled into 5 μm size classes from 5–60 μm and expressed as the percentage of total observations in each size class (Fig. 3). Al_t additions ≥ 2.8 $\mu mol \cdot L^{-1}$ changed the distribution of valve lengths at all growth rates, and as with mean length, this effect was more pronounced at the highest growth rates. At 0.11 day^{-1}, size frequency distributions for all Al treatments tended to be negatively skewed with most cells being 50–55 μm in length (Fig. 3A), but Al additions increased the proportion of valves in the smaller size classes ($\chi^2 = 42.8$, df = 18). Aluminum additions had a stronger effect at 0.22 day^{-1} where the distributions of cells in Al-treated populations became positively skewed with most cells occurring within the 20 μm size class (Fig. 3B; $\chi^2 = 95.1$, df = 18). This effect of Al addition on size frequency distribution was strongest at 0.51 and 0.69 day^{-1} growth rates (Fig. 3C, D; $\chi^2 = 174.6$, df = 18, and $\chi^2 = 166.5$, df = 18, respectively). At both growth rates, the size distribution of populations grown without added Al remained negatively skewed, whereas those at 2.80 and 6.22 $\mu mol \cdot L^{-1}$ Al_t were strongly skewed toward a majority of cells of 15–20 μm in length.

Silicification. The relationship between Si cell quota and steady-state growth rate at each Al level (Fig. 4) was examined relative to Droop's equation (Droop 1974, Morel 1987):

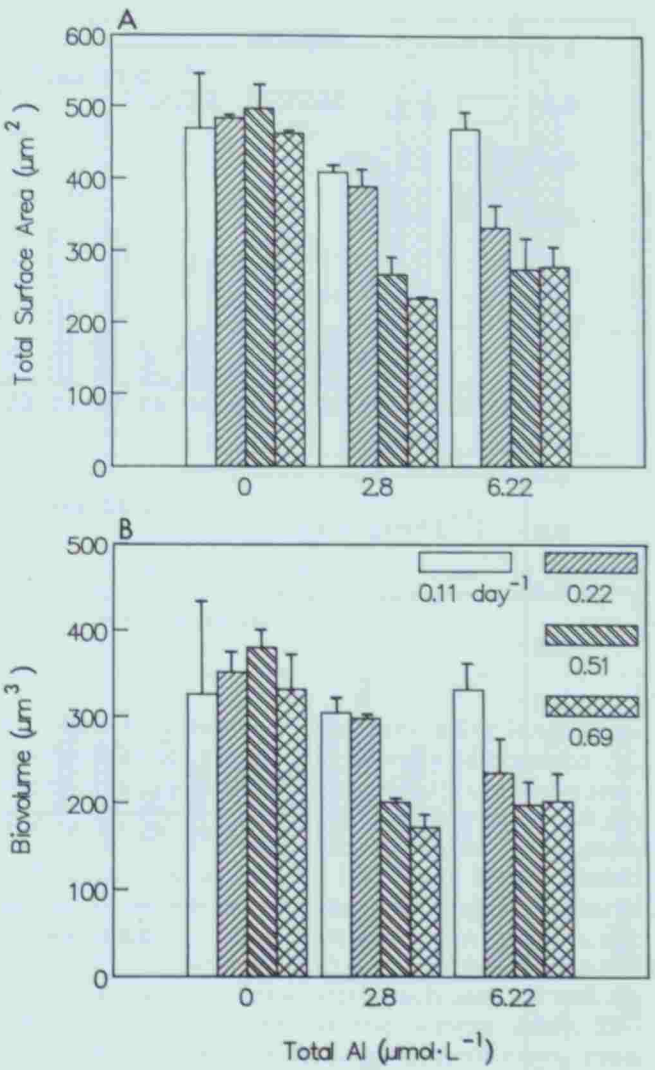
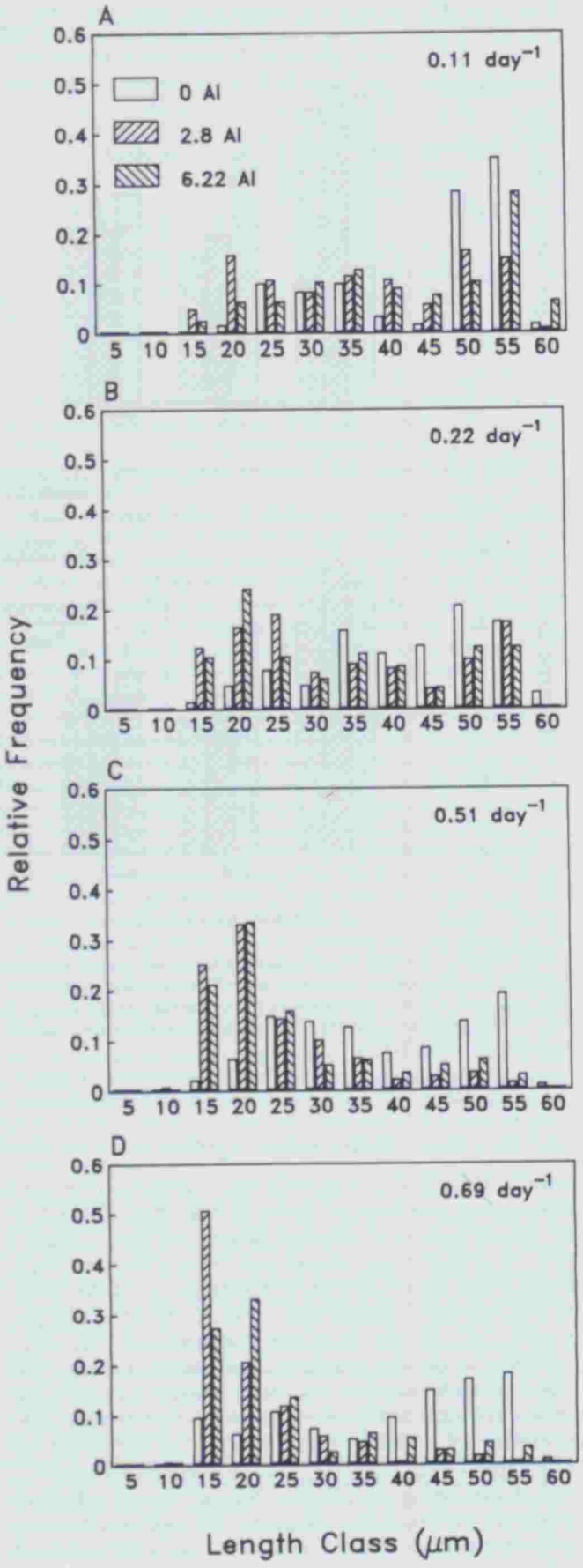


FIG. 2A, B. Changes in cell surface area and biovolume as a function of Al_t ($\mu mol \cdot L^{-1}$) treatments at all four steady-state growth rates (day^{-1}). Data are expressed as treatment means (vertical bars = two standard errors) of A) total cell surface area (μm^2), and B) biovolume (μm^3).

$$D/D_m = 1 - k_Q/Q \qquad (5)$$

where D and D_m = steady-state growth rate and maximum steady-state growth rate (day^{-1}), and Q and k_Q = cell quota and minimum cell quota (pmol·cell^{-1}). The Droop model was fit to empirical data from each treatment flask using an iterative nonlinear regression technique (PROC NLIN, SAS Institute, Inc. 1985). At 0 and 2.8 $\mu mol \cdot L^{-1}$ Al_t (Fig. 4A, B), the Droop equation could be fit to empirical results. Both maximum growth rate (D_m) and minimum cell quota (k_Q) were reduced over 50% in the presence of Al, although variance in the constant estimates at 2.8 $\mu mol \cdot L^{-1}$ Al_t prevented this difference from being significant. No significant relationship existed between cell quota (Q) and steady-state growth rate (D) at 6.22 $\mu mol \cdot L^{-1}$ Al_t although cell



quotas were generally higher at low growth rates compared to high growth rates (Fig. 4C).

The amount of silica per unit surface area was enhanced in the presence of Al (Fig. 5). Both Al and growth rate treatments significantly affected mean silica per unit surface area ($F_{11,12} = 10.56$, $P < 0.0001$). Mean $[Si]\cdot\mu m^{-2}$ increased by about 30% in the presence of 2.80 and 6.22 $\mu mol\cdot L^{-1}$ Al_t, and mean $[Si]\cdot\mu m^{-2}$ increased by about 30% at growth rates of 0.22 and 0.51 day^{-1} and by nearly 40% at 0.69 day^{-1} relative to mean levels at 0.11 day^{-1} (TSR, df = 12).

DISCUSSION

Aluminum additions significantly reduced the cell size of *A. ralfsii* populations grown in silica-limited cultures. Size reductions occurred when Al_t concentrations exceeded 2.8 $\mu mol\cdot L^{-1}$ and were represented by decreases in mean cell length while mean cell width and depth remained constant. Decreases in cell length subsequently reduced total cell surface area and biovolume in the presence of Al. The magnitude of size reductions was strongly dependent on steady-state growth rates. Al had only a minor effect on size at the lowest growth rate (0.11 day^{-1}), but the magnitude of cell size reductions increased at 0.22 day^{-1}, and reached their maximum at 0.51 and 0.69 day^{-1}. Growth rate-dependent size reductions, however, were not observed in 0 Al treatments. Therefore, at least when *A. ralfsii* is growth limited by Si at pH 6, Al additions can decrease mean cell size, surface area, and biovolume as much as 50%.

As little as 2.8 $\mu mol\cdot L^{-1}$ Al_t (corresponding to 0.32 $\mu mol\cdot L^{-1}$ Al_r) exerted the maximum effect on cell size, yet all populations in the Al treatments were able to grow at the maximum turnover rate of this experiment (0.69 day^{-1}). In batch culture, 24.5 $\mu mol\cdot L^{-1}$ Al_t (corresponding to 0.98 $\mu mol\cdot L^{-1}$ Al_r) was required to significantly reduce growth rates for *A. ralfsii* at pH 6 (Gensemer 1989). Therefore, *A. ralfsii* was susceptible to morphological changes at Al concentrations in the present study that were below those required to reduce maximum growth rates in vitro. Furthermore, monomeric Al appears to be most toxic to algae at pH 6 (Helliwell et al. 1983, Claesson and Törnqvist 1988, Gensemer 1989, Riseng 1989), even though Al is largely insoluble under these conditions (Burrows 1977, Driscoll et al. 1984).

The concentrations of IMAl in the present study ($Al_r = 0.32, 0.43$ $\mu mol\cdot L^{-1}$) were somewhat lower than those observed in northern temperate lakes of comparable pH. Concentrations of inorganic mo-

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FIG. 3A–D. Size frequency distributions of valve length size classes (μm) for all three Al_t treatments (0, 2.8, and 6.22 $\mu mol\cdot L^{-1}$) at growth rates of A) 0.11, B) 0.22, C) 0.51, and D) 0.69 day^{-1}. Data are expressed as the relative abundance of observations in each 5 μm size class.

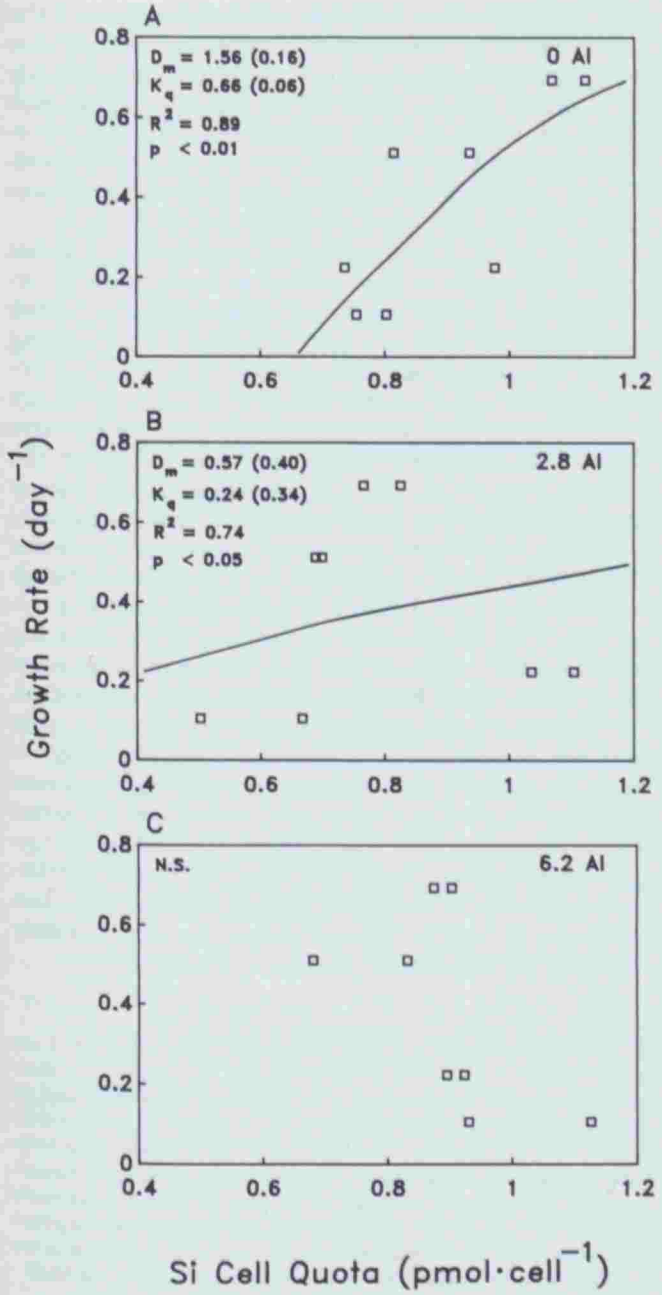

FIG. 4A–C. Relationship between steady-state growth rate (day^{-1}) and Si cell quota (pmol·cell^{-1}) at (A) 0 Al, (B) 2.8 μmol·L^{-1} Al_t, and (C) 6.22 μmol·L^{-1} Al_t. Curves represent a theoretical Droop model (Droop 1974) fit to experimental data by non-linear regression (SAS Institute, Inc. 1985). Data points represent results from each treatment flask.

nomeric Al range from about 1–5 μmol·L^{-1} in Adirondack lakes with pH values of about 6 (Driscoll et al. 1984, Driscoll and Newton 1985). Therefore, Al concentrations are high enough in mildly acidified lakes that morphological effects could be expressed in natural populations.

Changes in the cell size of *A. ralfsii* corresponded to a shift in the proportions of two discrete frustule size classes rather than a gradual shift in mean size.

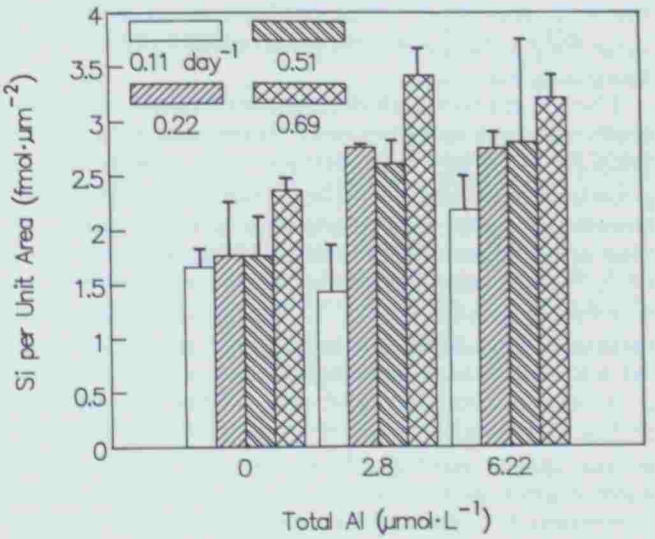

FIG. 5. Mean silica cell content (fmol Si·μm^{-2}) as a function of Al, (μmol·L^{-1}) at all four steady-state growth rates (day^{-1}). Vertical bars = two standard errors.

Particularly at high growth rates, valve length frequency distributions were strongly affected by Al. Size frequency distributions shifted from being negatively skewed with most valves being about 50 μm long at 0 Al to being positively skewed with most cells occurring within the 15–20 μm length classes in the presence of Al. Small cells are commonly found in small proportions as diatom cultures age (S. S. Kilham, pers. comm.) presumably because cell sizes gradually decrease after each asexual cell division (Crawford 1981). In the present study, 20–25 μm cells were present at all growth rates, even in the 0 Al treatments. However, all treatments were started from the same stock culture; therefore, it is unlikely that the enhanced abundance of small cells in the Al treatments was an artifact of culture maintenance.

Types of morphological variation in diatoms have been observed that may correlate to external environmental factors. Frustule morphology in *Synedra acus* became distorted in response to copper pollution in Lago d'Orta (Tonolli 1961), and the number and size of cellular organelles in *Diatoma tenue* var. *elongatum* were significantly affected by Pb and Cu (Sicko-Goad and Stoermer 1979). Other examples include changes in the abundance of three morphotypes of *Phaeodactylum tricornutum* (Borowitzka and Volcani 1977, Volcani 1981) and of two forms of the acidophilic *Tabellaria binalis* (Flower 1986). In both of these cases, no specific environmental factors have yet been confirmed as being responsible for inducing changes between morphotypes. Of particular relevance to this study, *A. ralfsii* var. *americana* exists in both a long (>45 μm) and a short (<45 μm) form in nature (D. F. Charles, pers. comm.). If the two morphotypes reported here correspond to

the long and short forms of this species in nature, these forms may represent environmentally-induced morphotypes.

The extent to which diatoms are Si-limited is generally thought to affect both Si cell quotas and the amount of Si per unit surface area. Silica-limited populations often follow the Droop relationship between cell quota and steady-state growth rate (Tilman and Kilham 1976, Kilham 1978). Growth rates of *A. ralfsii* were consistent with the Droop relationship at 0 and 2.8 $\mu mol \cdot L^{-1}$ Al_t although constant estimates were highly variable in the presence of Al. At 6.22 $\mu mol \cdot L^{-1}$ Al_t, empirical data no longer fit a Droop relationship, and cell quotas were higher at low steady-state growth rates. Taylor (1985) observed that cells limited by factors other than Si will have higher Si cell contents at low growth rates; therefore, *A. ralfsii* may have been limited by another environmental factor in the highest Al treatment. Cells limited by factors other than Si tend to be more heavily silicified per unit surface area (Paasche 1980, Taylor 1985). The increase in $[Si] \cdot \mu m^{-2}$ in response to Al additions is also consistent with the possibility that other factors are limiting growth in the presence of Al. However, nutrient addition bioassays performed on populations from the 6.22 $\mu mol \cdot L^{-1}$ Al_t treatments confirmed that cells were Si-limited; therefore, this is an unlikely explanation for observed changes in silicification.

Many environmental factors other than silica are known to change the silica content of diatom frustules (Paasche 1980, Conley et al. 1989), but the mechanism by which Al or other metals have this effect is unknown. One possibility is that Al disrupts the progress or timing of particular phases of the cell division cycle. Diatoms tend to take up most of the silica they require for frustule formation just prior to cell wall formation and cell division (Paasche 1980, Sullivan and Volcani 1981). If Al were to disrupt some cellular development process prior to Si uptake and frustule formation, cell division could occur before the daughter cells have had sufficient time to develop to normal size. Assuming that the acquisition of Si is not significantly reduced, the daughter cells would be smaller but would contain the same amount of silica. Aluminum additions also may have accentuated cell size reduction normally observed in diatoms after each cell division. Populations growing at 0.69 day^{-1} would have gone through more cellular divisions after three weeks than those at 0.11 day^{-1}; therefore, enhanced cell size reductions in the presence of Al would have the greatest impact at higher growth rates. Törnqvist and Claesson (1987) also proposed that Al disrupted components of the cell cycle for *Monoraphidium dybowskii* and *Stichococcus* sp., because size distribution changes were highly dependent on growth cycle phase. They claimed that this disruption had the potential to produce daughter cells of different sizes depending on where Al exerted its effect in the cell cycle. The semicontinuous culture design and the light : dark cycle used in this experiment may also accentuate the effects of cell cycle disruption. Daily light cycles usually synchronize diatom cell division (Paasche 1980, Sullivan and Volcani 1981); therefore, applying Si in daily pulses to synchronized cultures would emphasize effects Al might have on precisely-timed components of the cell cycle.

Although much remains to be understood about the mechanisms of size reduction in *A. ralfsii* in response to Al additions, this morphological response may prove to be a valuable indicator of Al loading to acidic lakes. Laboratory work is needed to understand the mechanisms by which Al induces change in cell size and silica content. Field populations also should be investigated to determine whether the morphology of *A. ralfsii* is environmentally plastic and whether Al (or perhaps other chemical factors) is responsible for this process. Reductions in cell size could affect natural populations indirectly by changing resource acquisition rates due to changes in cellular surface area to volume ratios, or by enhancing loss rates through the increased sedimentation rates of smaller, more heavily silicified cells. If verified, metal-induced changes in diatom size may provide another technique to assess the paleolimnological conditions of acidified lakes that would compliment analyses involving community structure (Charles 1985, Battarbee et al. 1986, Davis 1987). Using frustule morphology as an indicator of metal perturbation has rarely been attempted (Tonolli 1961), but the potential application of this approach certainly merits further consideration.

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STRUCTURE AND CHEMISTRY OF A NEW CHEMICAL RACE OF *BOTRYOCOCCUS BRAUNII* (CHLOROPHYCEAE) THAT PRODUCES LYCOPADIENE, A TETRATERPENOID HYDROCARBON[1]

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ABSTRACT

New strains of the hydrocarbon rich alga Botryococcus braunii *Kützing were isolated from water samples collected in three tropical freshwater lakes. These strains synthesize lycopadiene, a tetraterpenoid metabolite, as their sole hydrocarbon. The morphological and ultrastructural characteristics of these algae are similar to those reported for previously described strains which produce either alkadienes or botryococcenes. The pyriform shaped cells are embedded in a colonial matrix formed by layers of closely appressed external walls; this dense matrix is impregnated by the hydrocarbon and some other lipids. We believe the new strains synthesizing lycopadiene form a third chemical race in* B. braunii, *besides the alkadiene and botryococcene races, rather than a different species.*

Like the other two types of hydrocarbons, lycopadiene was produced primarily during the exponential and linear growth phases. The major fatty acid in the three races was oleic acid. This fatty acid was predominant in the alkadiene race; palmitic and octacosenoic acid also were present in appreciable amounts in the three races. Cholest-5-en-3β-ol, 24-methylcholest-5-en-3β-ol and 24-ethylcholest-5-en-3β-ol occurred in the three races; three unidentified sterols also were detected in the lycopadiene race. Moreover, the presence of very long chain alkenyl-phenols in the lipids of algae of the alkadiene race was not observed in the botryococcene and lycopadiene races. Of the polysaccharides released in the medium, galactose appeared as a primary component: it predominated in the botryococcene race. The other major constituents were fucose for the alkadiene race and glucose and fucose for the lycopadiene race. Although morphologically similar, some important chemical differences exist among algae classified as B. braunii.

Key index words: alkadienes; botryococcenes; Botryococcus braunii; *fatty acids; hydrocarbons; lycopadiene; phenols; polysaccharides; sterols; ultrastructure*

The green colonial alga *Botryococcus braunii* Kützing is characterized by its unusually high hydrocarbon content; levels ranging from 25–40% of the dry

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