

Silicic acid depletion and silicon limitation in the plume of the Mississippi River: evidence from kinetic studies in spring and summer

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ABSTRACT: The surface distributions of dissolved silicic acid, chlorophyll and diatom abundance were measured in the plume of the Mississippi River and adjacent waters during spring (late April and early May 1993) and summer (July 1992). In spring, the time of maximum river flow, there was an intense diatom bloom with a mean diatom abundance of 1.5×10^7 cells l^{-1} , more than an order of magnitude higher than in summer. Mixing curves of silicic acid concentration ($[Si(OH)_4]$) versus salinity indicate that biological uptake within the river plume removed >99% of the $Si(OH)_4$ supplied by the river in spring and 80 to 95% in summer. In spring $[Si(OH)_4]$ was occasionally depleted to $<0.2 \mu M$ —among the lowest values ever reported from the ocean—with extensive depletion to $<0.5 \mu M$ over the shelf. In summer $[Si(OH)_4]$ was less severely depleted; the lowest measured was $0.93 \mu M$ and all others were $\geq 2.4 \mu M$. ^{30}Si kinetic experiments were performed during both spring and summer to measure the degree to which the rate of Si uptake by the natural diatom assemblages was limited *in situ* by substrate availability. In spring the dependence of the specific uptake rate (V) on extracellular $[Si(OH)_4]$ conformed much more closely to the Michaelis-Menten saturation function than has been observed in past studies. Strong dependence of V on $[Si(OH)_4]$ was observed throughout the most $Si(OH)_4$ -depleted ($<0.5 \mu M$) region, where V was limited to 12 to 45% of the diatom assemblages' maximum uptake rate (V_{max}). Half-saturation concentrations for Si uptake (K_s) averaged $0.85 \mu M$ (range = 0.48 to 1.71 ; $n = 7$) in spring, with the lowest values equal to the lowest previously reported for natural diatom assemblages. There was only 1 station in summer where V was limited by $[Si(OH)_4]$, and at that station K_s was $5.3 \mu M$ —quite high in comparison with previous studies. At stations where V was limited by $[Si(OH)_4]$, in both spring and summer, *Chaetoceros* spp. were numerically dominant; where there was no Si limitation other diatoms, usually *Skeletonema costatum*, dominated. The data thus indicate strong Si limitation in spring, with diatom assemblages well adapted to low $[Si(OH)_4]$, but little or no Si limitation in summer. Historical data suggest that coastal $Si(OH)_4$ depletion and Si limitation may be recent phenomena in the northern Gulf of Mexico, resulting from increasing $[NO_3^-]$ and decreasing $[Si(OH)_4]$ in the Mississippi River during the past 30 to 50 yr.

KEY WORDS: Coastal eutrophication · Diatoms · Gulf of Mexico · Mississippi River · Nutrients · Si limitation

INTRODUCTION

The lowest concentrations of most nutrients in the ocean occur in offshore surface waters of tropical regions and subtropical mid-ocean gyres, where biological uptake often depletes nitrate, ammonium and phosphate to <20 nM (e.g. Garside 1985, Brzezinski 1988, Karl et al. 1993). Silicic acid ($Si(OH)_4$), which is

required in significant quantities only by diatoms, provides a clear exception to that general pattern; concentrations are almost never $<0.6 \mu M$ in the Atlantic, or $<1.0 \mu M$ in the Pacific (Bainbridge 1980, Craig et al. 1981). Unlike the other major nutrients, $Si(OH)_4$ shows the most severe depletion in nearshore areas of high productivity, generally associated with either the offshore edges or the late stages of coastal diatom blooms (e.g. Friederich & Codispoti 1981, Brzezinski & Nelson 1989, Ragueneau 1994). Under those bloom-depleted

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conditions, rates of Si uptake by natural diatom assemblages have been shown to become limited by Si(OH)_4 availability (e.g. Goering et al. 1973, Nelson et al. 1981, Nelson & Brzezinski 1990).

Because only the diatoms have a known growth requirement for Si (e.g. Guillard & Kilham 1978), depletion of Si(OH)_4 in surface waters would not be expected *a priori* to limit primary productivity. Instead, it should limit only diatom growth, resulting in floristic changes (diminished relative abundance of diatoms and correspondingly greater abundance of non-siliceous autotrophs). It has been suggested, for example, that coastal eutrophication resulting from increased N and P runoff from the continents may result in Si limitation of the diatoms and hence in blooms of other algae, including toxic species (Smayda 1989, 1990). There is now clear evidence that nitrate concentrations ($[\text{NO}_3^-]$) in the Rhine, Seine, Mississippi and several other major rivers draining temperate regions in the northern hemisphere have increased by factors of 2 to 3 during this century, while silicic acid concentrations ($[\text{Si(OH)}_4]$) in these same rivers have either remained constant or decreased (e.g. van Bennekom & Salomons 1981, Meybeck 1982, Turner & Rabalais 1991, Justic' et al. 1995). Similar phenomena have been reported in smaller European rivers as well (e.g. Ragueneau 1994). These observations have led to speculation that increased N supply from rivers, and the consequently decreased Si:N supply ratios from those rivers, might result in Si limitation and floristic shifts away from diatoms in the adjacent coastal ocean (e.g. Smayda 1990), a phenomenon that has been documented in the North American Great Lakes (e.g. Schelske et al. 1983).

In the northern Gulf of Mexico, nutrient concentrations and ratios in the plume of the Mississippi River suggest that at present Si limitation is more likely than either N or P limitation, especially in spring (Dortch & Whittedge 1992, Smith & Hitchcock 1994, Dortch et al. 1995). This region may provide a particularly clear case in which development of Si limitation over historical time can be hypothesized. $[\text{NO}_3^-]$ at the river mouth has increased from ~40 μM in 1960 to ~100 μM at present, while $[\text{Si(OH)}_4]$ has decreased from ~170 to ~120 μM since 1950 (Turner & Rabalais 1991). As a consequence, the Si:N supply ratio from the river has decreased from ~4.0 to ~1.2. The Si:N composition ratio of most marine diatoms is ~1.0 (e.g. Brzezinski 1985). Thus in the late 1950s and early 1960s the Mississippi River was supplying Si in approximately a 4-fold excess over N in comparison with diatom requirements, and that excess is now almost gone. With Si no longer being supplied by the river in large excess over N, the potential for Si limitation of diatom growth in the northern Gulf of Mexico has increased during the past 30 to 50 yr (Justic' et al. 1995).

Limitation of diatom metabolism and growth by Si availability has been examined in 2 distinct contexts: studies performed on diatom cultures have assessed the conditions under which Si limits the rates of diatom growth, and the effects of Si limitation on cell size, chemical composition and the timing of events in the cell cycle (e.g. Paasche 1973a, Olsen & Paasche 1986, Brzezinski et al. 1990). In a different approach, Si uptake rates have been measured as a function of extracellular $[\text{Si(OH)}_4]$, both in unialgal diatom cultures (e.g. Paasche 1973b, Nelson et al. 1976) and in natural mixed diatom assemblages (e.g. Goering et al. 1973, Nelson & Brzezinski 1990, Banahan & Goering 1994) to determine the concentration ranges that impose substrate limitation on the uptake rate. In those studies the specific rate of Si uptake (V ; the uptake rate per unit of diatom silica present) has been shown to depend on the extracellular $[\text{Si(OH)}_4]$ in a manner that can be described by the Michaelis-Menten saturation function:

$$V = V_{\max} \frac{[\text{Si(OH)}_4]}{K_s + [\text{Si(OH)}_4]} \quad (1)$$

where V_{\max} represents the maximum uptake rate achievable in the absence of substrate limitation and K_s the $[\text{Si(OH)}_4]$ at which V is limited to $V_{\max}/2$. Similarly, the specific cell-division rate (μ ; the exponential rate of increase in cell abundance in the absence of losses) appears to obey a mathematically similar saturation function, first derived to describe the substrate-limited growth of bacteria (Monod 1942):

$$\mu = \mu_{\max} \frac{[\text{Si(OH)}_4]}{K_d + [\text{Si(OH)}_4]} \quad (2)$$

where μ_{\max} represents the division rate when Si is not limiting and K_d the extracellular $[\text{Si(OH)}_4]$ at which μ is limited to $\mu_{\max}/2$. Culture studies of marine diatoms have shown an important difference between the kinetics of Si uptake and Si-limited growth: K_d , the half-saturating $[\text{Si(OH)}_4]$ for Si-limited growth, is generally 5 to 10 times lower than K_s , the half-saturating $[\text{Si(OH)}_4]$ for Si uptake. Reported K_d values range from 0.04 to 1.0 μM (Guillard et al. 1973, Olsen & Paasche 1986) while K_s values have ranged from ca 0.5 to 5.0 μM (numerous studies, summarized by Ragueneau 1994). This difference implies that many diatoms can sustain maximum or near-maximum division rates at extracellular $[\text{Si(OH)}_4]$ that is moderately, or even severely, limiting to uptake. In culture studies diatoms accomplish this by depositing thinner, or otherwise reduced, frustules and this effect is readily observable morphologically (e.g. Harrison et al. 1976, Brzezinski et al. 1990). This flexibility of cellular Si content means that even direct experimental evidence of uptake-rate limitation in a given habitat indicates only that limitation of diatom growth is a possibility in that system.

If the Si uptake rate is limited by the extracellular $[\text{Si}(\text{OH})_4]$, then the division rate may or may not be Si-limited; if the Si uptake rate is not Si-limited, then the division rate cannot be. Direct information on the Si-limited growth kinetics of individual diatom species at sea would be of great value in evaluating the role of Si limitation in controlling diatom growth, abundance and species composition. However, no reliable method has yet been developed to perform experimental studies of Si-limited diatom growth within natural mixed phytoplankton assemblages.

In this paper we report the almost complete (>99%) non-conservative removal of $\text{Si}(\text{OH})_4$ in the coastal plume of the Mississippi River under conditions of high river flow in spring, significant but less complete (80 to 95%) removal during a period of low river flow in summer, and the abundance and species composition of diatoms under both flow regimes. We present kinetic data on the degree to which rates of Si uptake by natural diatom assemblages are limited *in situ* by substrate availability under high and low river-flow conditions, and compare the kinetic parameters for Si uptake in the Mississippi River plume with those observed in other systems. Differences in phytoplankton composition, especially within the diatom assemblage, are associated with spatial and seasonal differences in Si availability and Si uptake kinetics.

METHODS

We conducted experiments aboard the RV 'Pelican' during a period of low river discharge in summer (July 24 to 29) 1992 and a period of near-maximum discharge in spring (April 24 to May 2) 1993. Station locations from which each of several data sets were obtained are shown in Figs. 1–3. This general study area was selected on the basis of historical hydrographic and biological data indicating the spatial extent of the Mississippi River plume (Dinnel & Wiseman 1986, Rabalais et al. 1991). Most stations were sampled during the first 3 to 5 d of each cruise, occasionally with repeated sampling during the same day. The ship then went on to sample to the west of the area shown in Figs. 1–3, after which more stations were taken in the study area. If a station was occupied more than once during a 24 h period, the values were averaged to produce Figs. 1–3, but if the period between occupations of a station exceeded a day, the values from the earlier, more nearly synoptic part of the cruise were used. Additional stations were occupied within the river itself in April 1993. At all stations seaward of the river mouth casts were made to the bottom with a Seabird™ SBE9/11 CTD mounted with a SeaTech™ Fluorometer and a rosette sampler with 12, 10 l Niskin bottles, and 1 to 5 sub-

surface depths sampled. Due to high turbidity and the risk of hitting submerged objects in the river, CTD/fluorometer/Niskin bottle casts were not made there and only surface bucket samples were obtained. Near the river the low-salinity plume is often a thin layer overlying more saline shelf water, and is difficult to sample with Niskin bottles on a CTD/rosette. Our observations confirmed this in both April and July, so bucket samples were collected in preference to bottle samples at all stations to obtain the most reliably undiluted surface water. At each station 10 to 15 l of surface water was collected in a non-toxic polypropylene bucket, poured into a spigoted 20 l polypropylene carboy and all samples were drawn from the carboy, which was agitated frequently. This sample-handling method is necessary to obtain representative samples of suspended particulate matter, especially the larger particles (e.g. Calvert & McCartney 1979, Nelson et al. 1985).

At selected stations, including all at which Si uptake kinetic experiments were performed, $[\text{Si}(\text{OH})_4]$ was measured by a high-sensitivity manual method (Brzezinski & Nelson 1995). Samples were drawn from the agitated carboy and gently syringe filtered through 0.6 μm pore diameter polycarbonate membrane filters (Nuclepore™) using plastic syringes. Then duplicate 10.0 ml samples of the filtered surface water were analyzed by the method of Strickland & Parsons (1972), using the reverse-order reagent addition method described by Brzezinski & Nelson (1989) to determine blank absorbances. Absorbance was measured at 810 nm in 1 or 5 cm cells, depending on visual inspection of the amount of blue silicomolybdic acid complex that had formed during the 2 h development time. Standard-addition curves were run daily on board, using filtered Sargasso Sea surface water (FSSW) whose $[\text{Si}(\text{OH})_4]$ was $0.78 \pm 0.02 \mu\text{M}$ as determined from the standard-addition curves and reverse-order reagent blanks. For low-salinity samples (which were also very high in $[\text{Si}(\text{OH})_4]$) this analysis was performed on samples diluted 1:10 with FSSW and concentrations were calculated on the basis of 1 part ambient surface water and 9 parts FSSW at $0.78 \mu\text{M}$. Using this procedure, all samples analyzed had salinities >31.5 psu—high enough that no correction for salt effects in the $[\text{Si}(\text{OH})_4]$ analysis is required. Minimum detection limits of this analysis for undiluted samples are 0.25 and $0.05 \mu\text{M}$ in 1 and 5 cm cells, respectively, and analytical precision is $\pm 0.25 \mu\text{M}$ (1 cm cells), $\pm 0.05 \mu\text{M}$ (5 cm cells) or $\pm 1\%$, whichever is greater. All $[\text{Si}(\text{OH})_4]$ data used in calculating Si uptake rates from ^{30}Si tracer kinetic experiments (described below) were obtained by this high-sensitivity manual analysis.

Additional $[\text{Si}(\text{OH})_4]$ data were obtained for water collected on CTD casts at all stations outside the river, and from surface samples within the river. $[\text{Si}(\text{OH})_4]$ was

measured by automated colorimetric analysis of unfiltered, frozen samples on a Technicon AutoAnalyzer II™ using Technicon's recommended method (Anonymous 1977), which is based on the hand methods described by Parsons et al. (1984). To accommodate the very high (>100 μM) $[\text{Si}(\text{OH})_4]$ found within the river and in the near-bottom waters over the shelf, a wide-range but low-sensitivity configuration of the AutoAnalyzer was used. For $[\text{Si}(\text{OH})_4]$ analysis the detection limit was approximately 1.0 μM and the precision was $\pm 1.0 \mu\text{M}$ or $\pm 1\%$, whichever is greater. This method was used only for surface $[\text{Si}(\text{OH})_4]$ mapping (Fig. 3).

Chlorophyll *a*, corrected for phaeopigments, was measured fluorometrically on shipboard after filtration onto Whatman GF/FT™ or Micro Filtration Systems GF75™ glass fiber filters (nominal pore size 0.7 μm) and extraction with 40:60 DMSO:90% acetone, as modified from Parsons et al. (1984).

At the stations indicated in Figs. 1–3 tracer kinetic experiments were performed on surface water samples, using the stable isotope ^{30}Si to measure the uptake rate of Si by the natural *in situ* diatom assemblage as a function of $[\text{Si}(\text{OH})_4]$ (e.g. Nelson & Brzezinski 1990, Nelson & Tréguer 1992). Stations were selected for kinetic experiments based on preliminary results of the manual $[\text{Si}(\text{OH})_4]$ analyses described above. While the samples for $[\text{Si}(\text{OH})_4]$ analysis were being filtered and reagents added, a series of nine 0.5 l samples was drawn from the agitated carboy into clear polycarbonate incubation bottles. Approximately 5 min after all reagents had been added to the samples for $[\text{Si}(\text{OH})_4]$ analysis, those samples were inspected visually in comparison with standards to estimate the concentration of blue reduced silicomolybdic acid complex that had formed. If this rapid visual estimate indicated that the surface $[\text{Si}(\text{OH})_4]$ was $\leq 5 \mu\text{M}$, a tracer kinetic experiment was initiated. This procedure permitted us to focus our kinetic studies on those surface waters where there was a reasonable chance that Si uptake rates might be limited by substrate availability, while still beginning each experiment within 30 min of the time the original surface water sample was collected. The $[\text{Si}(\text{OH})_4]$ value used in all rate calculations was determined spectrophotometrically after the full 2 h color development time, as described above.

For each kinetic experiment we added ^{30}Si -labeled $\text{Si}(\text{OH})_4$ (95.20 atom % ^{30}Si) at a series of 8 concentrations ranging from 0.25 to 10.0 μM above ambient. Samples were then incubated 3 to 4 h under natural sunlight in a shipboard incubator maintained at sea-surface temperature by a circulating water bath. After incubation, samples were vacuum-filtered through 0.6 μm pore diameter Nuclepore filters. Filters were folded in quarters, dried for 12 to 24 h at 60°C and analyzed isotopically by conversion of all particulate Si to

BaSiF_6 , followed by solid-sample mass spectrometry on a Nuclide Corp. 3-60 (3" radius, 60° deflection) magnetic sector mass spectrometer as described by Nelson et al. (1991). An additional 0.5 l surface sample was collected at each station for analysis of biogenic and lithogenic particulate SiO_2 by the sequential-digestion method described by Brzezinski & Nelson (1989). The specific uptake rate of Si (V) measured from ^{30}Si enrichment of particulate SiO_2 during the incubation was corrected for the contribution of biologically inactive lithogenic material as described by Brzezinski & Nelson (1989). This correction was considerable at most stations, as lithogenic material (suspended river mud) comprised 29 to 73% of the total particulate SiO_2 at the stations selected for kinetic experiments.

In those experiments showing concentration-dependent Si uptake the kinetic parameters V_{max} and K_s were calculated by direct non-linear regression of the Michaelis-Menten hyperbola (Eq. 1) to the data by the iterative procedure described by Wilkinson (1961). This curve-fitting algorithm has 2 advantages over others using linear regression methods based on any of several linear transformations of Eq. (1): It weights all data points equally by fitting the Michaelis-Menten function directly to the data, and it permits 95% confidence intervals to be calculated for both V_{max} and K_s .

Samples for phytoplankton identification and counting were collected from the surface bucket sample at approximately half the stations sampled for chlorophyll and nutrients (Figs. 1–3). Those stations include all that were sampled for ^{30}Si uptake experiments, the station at the northern and southern end of each transect, and every alternate station along each transect line. Samples were preserved in 0.5% glutaraldehyde and refrigerated for 1 to 24 h. They were then size fractionated by filtration onto 0.2, 3, and 8 μm polycarbonate filters (Poretics™), with 0.03% proflavine hemisulfate used to stain the latter 2 fractions, and the filters were mounted in immersion oil (Murphy & Haugen 1985, Shapiro et al. 1989). The 0.2 to 3 μm fractions were counted immediately on shipboard; the 3 to 8 μm fractions were counted immediately if possible and, if not, refrigerated and counted within days of returning. The >8 μm fractions were frozen and counted later. All samples were counted using an Olympus BH2-RFCA epifluorescence microscope with blue and green excitation light and, as necessary, transmitted light. The autotrophic cells were identified to the nearest possible taxon. As described in detail by Buck et al. (1992), epifluorescence microscopy is not usually used for enumerating the entire size range of phytoplankton, but large cells can usually be identified to the genus, and often the species level. Small forms such as cyanobacteria and small autotrophic flagel-

lates can be counted by this method, but not identified to species. Diatom taxa are reported according to Hasle & Syvertsen (1996).

RESULTS

Phytoplankton and $[\text{Si}(\text{OH})_4]$ distributions in the river plume

Figs. 1 & 2 show surface distributions of salinity, chlorophyll *a* and the numerical abundance of diatoms in spring (April/May 1993) and summer (July 1992),

respectively. During both seasons there was a phytoplankton bloom evident in the low-salinity river plume, with chl *a* reaching maximum concentrations of $76 \mu\text{g l}^{-1}$ in spring and $31 \mu\text{g l}^{-1}$ in summer. The spatial distribution of diatom abundance closely matched that of chlorophyll in both spring and summer with abundances approximately an order of magnitude higher in spring (Table 1, Figs. 1c & 2c). Despite the large variation for each cruise due to the strong lateral gradients in diatom abundance (Figs. 1c & 2c), the differences between seasons was significant statistically (Table 1). In both spring and summer there was a very strong gradient of decreasing $[\text{Si}(\text{OH})_4]$ with distance from the river mouth in the mid-salinity (20 to 25 psu) range of the plume (Fig. 3). The lowest $[\text{Si}(\text{OH})_4]$ values,

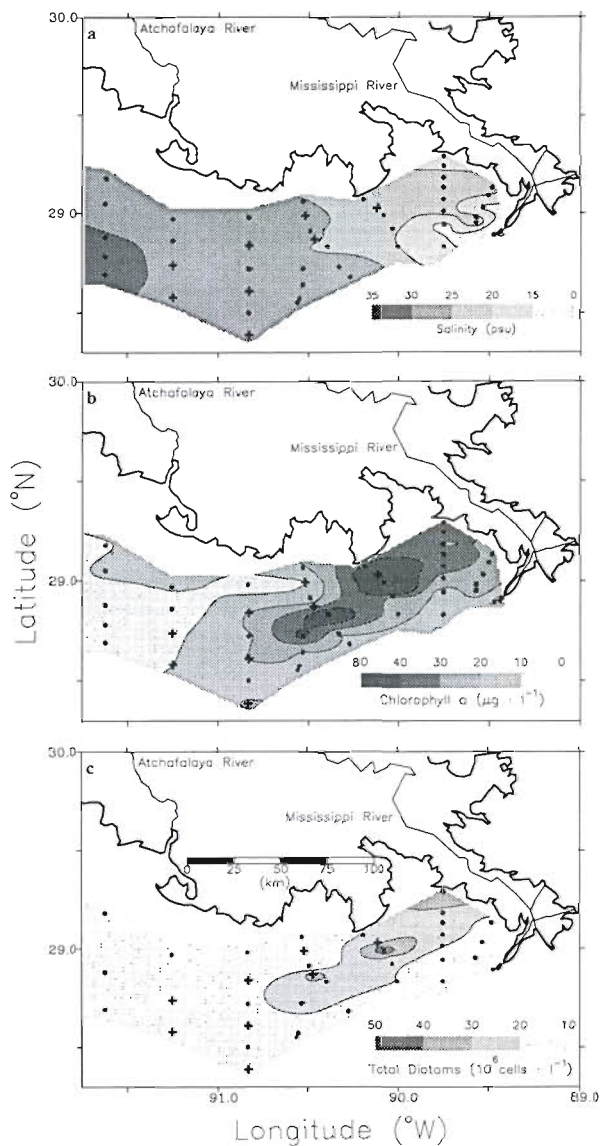


Fig. 1. Surface distributions of (a) salinity; (b) chlorophyll *a*; and (c) diatom abundance in the northern Gulf of Mexico; April 26 to May 1, 1993. ^{30}Si uptake kinetics experiments were conducted at stations designated by (+)

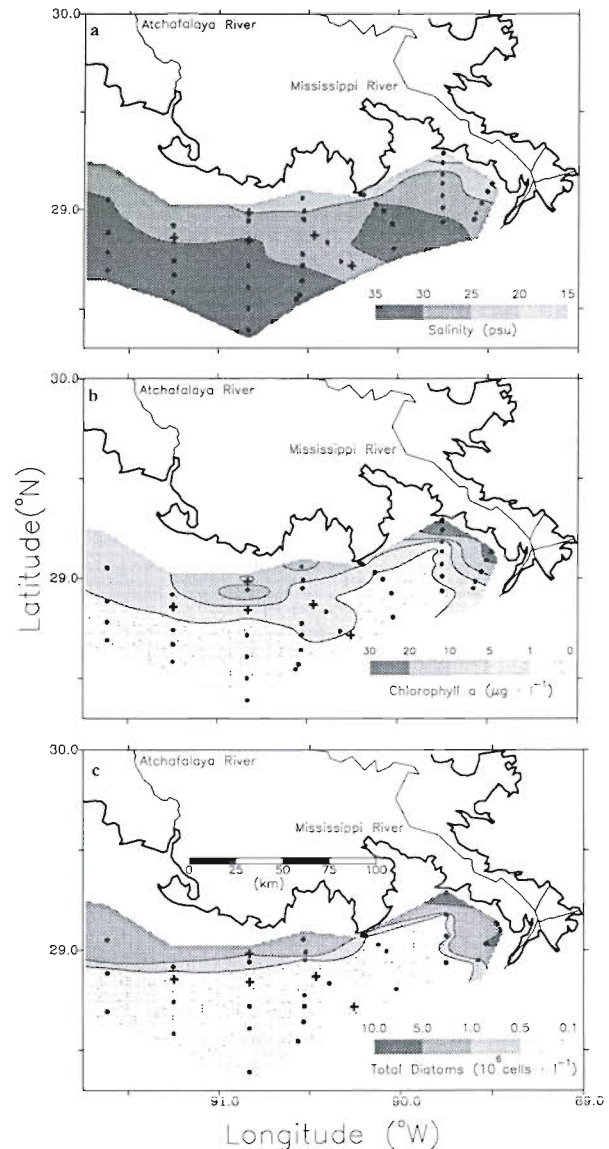


Fig. 2. As in Fig. 1, but for July 24 to 28, 1992

Table 1. Comparison between surface phytoplankton composition in spring and summer. Spring and summer are significantly different ($p \leq 0.05$) for all variables according to a non-parametric Mann-Whitney test (Sokal & Rohlf 1969). Both median and interquartile range (IQR) and mean and standard deviation (SD) are given because some data are not normally distributed, as indicated by * after n

		Spring (April 24–May 2, 1993)	Summer (July 24–29, 1992)
Total autotrophs (cells l ⁻¹)	Median	2.04×10^7	3.33×10^8
	IQR	2.04×10^7	3.79×10^8
	Mean	2.59×10^7	5.00×10^8
	SD	1.67×10^7	4.12×10^8
	n	40	36
Total diatoms (cells l ⁻¹)	Median	1.10×10^7	2.49×10^5
	IQR	1.88×10^7	6.25×10^5
	Mean	1.52×10^7	1.01×10^6
	SD	1.19×10^7	2.03×10^6
	n	40	36*
Total cyanobacteria (cells l ⁻¹)	Median	5.31×10^6	3.28×10^8
	IQR	6.13×10^6	3.79×10^8
	Mean	8.26×10^6	4.97×10^8
	SD	1.45×10^7	4.11×10^8
	n	40	36*
Diatoms/total autotrophs (%)	Median	67.5	0.075
	IQR	35.2	0.011
	Mean	59.4	0.20
	SD	25.0	0.36
	n	40	36*
Cyanobacteria/total autotrophs (%)	Median	22.2	99.57
	IQR	30.9	0.78
	Mean	29.4	99.24
	SD	23.8	0.80
	n	40*	36

measured with the high-sensitivity analysis in surface waters over the shelf, were much lower in spring than in summer. Surface waters were depleted to $<0.5 \mu\text{M}$ over an extensive portion of the shelf in the spring

of 1993, with one entire transect line depleted to $<0.2 \mu\text{M}$ (Fig. 3a). In contrast, the lowest $[\text{Si}(\text{OH})_4]$ measured in the river plume in summer 1992 was $0.93 \mu\text{M}$, with all other values $>2.4 \mu\text{M}$. The area of $<2 \mu\text{M}$ $[\text{Si}(\text{OH})_4]$ over the outer shelf in summer was high-salinity, low-chlorophyll Gulf of Mexico surface water intruding onto the shelf (Figs. 1–3). Because our study was focused on the low-salinity river plume, we did not perform high-sensitivity $[\text{Si}(\text{OH})_4]$ analyses in the Gulf of Mexico surface water over the outer shelf. Previous measurements indicate that its surface $[\text{Si}(\text{OH})_4]$ typically ranges from 0.8 to $1.6 \mu\text{M}$ (Murrell & Dagg 1987, 1988, Toon & Dagg 1989, 1990a, b).

Fig. 4 shows surface $[\text{Si}(\text{OH})_4]$ as a function of salinity in the upper 5 m in spring 1993. Comparison with a conservative mixing line (also shown in Fig. 4) indicates pronounced non-conservative removal of $\text{Si}(\text{OH})_4$ within the river plume. Surface waters in which the lowest $[\text{Si}(\text{OH})_4]$ values (0.13 to $0.4 \mu\text{M}$) were measured would have $[\text{Si}(\text{OH})_4]$ levels of 20 to $50 \mu\text{M}$ on the basis of conservative mixing of Mississippi River water and Gulf of Mexico surface water. Thus Fig. 4 indicates that biological uptake had removed $>99\%$ of the $\text{Si}(\text{OH})_4$ supplied by the river within ca 100 km of the river mouth. The river end-member was not sampled in July 1992, but our spring value of $119 \mu\text{M}$ is very close to the historical average $[\text{Si}(\text{OH})_4]$ in the lower portions of the river since 1985

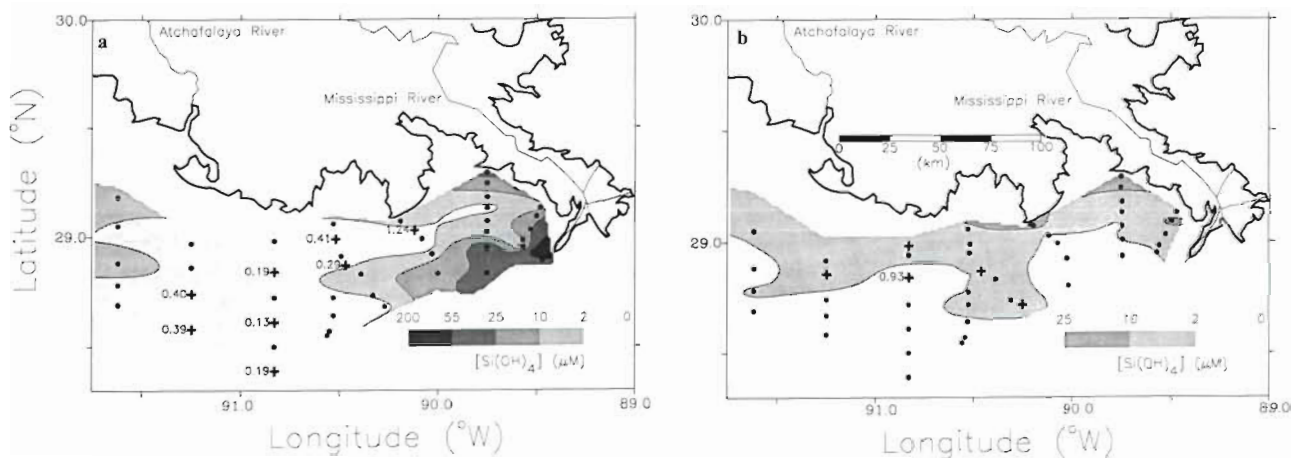


Fig. 3. Surface distribution of $[\text{Si}(\text{OH})_4]$: (a) April 26 to May 1, 1993 and (b) July 24 to 28, 1992. The contours shown are based on a low-sensitivity automated analysis with a detection limit of $1 \mu\text{M}$ (see 'Methods'). The $[\text{Si}(\text{OH})_4]$ measured by a high-sensitivity manual analysis with a detection limit of $0.05 \mu\text{M}$ is shown for each station (+) at which that analysis was used and the surface $[\text{Si}(\text{OH})_4]$ was $<2 \mu\text{M}$.

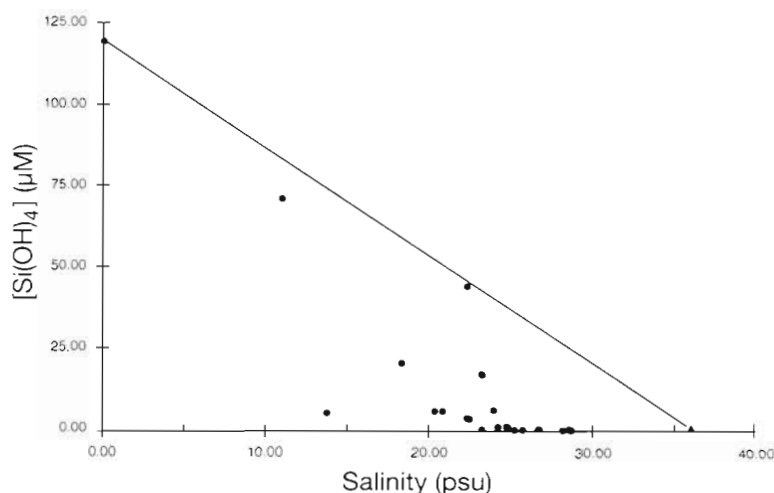


Fig. 4. $[\text{Si}(\text{OH})_4]$ - salinity mixing diagram for surface waters in the northern Gulf of Mexico, April 24 - May 2, 1993. All $[\text{Si}(\text{OH})_4]$ data were obtained by the high-sensitivity manual analysis and only the upper 5 m are considered ($n = 30$). The line denotes conservative mixing between the Mississippi River (0 psu; 119 μM $\text{Si}(\text{OH})_4$) and offshore Gulf of Mexico surface water (36.0 ± 0.34 psu; 1.21 ± 0.41 μM $\text{Si}(\text{OH})_4$). The river end member was measured April 26, 1993. The offshore end member represents the mean and standard deviation for those stations with salinity > 35 psu from 5 cruises (Murrell & Dagg 1987, 1988, Toon & Dagg 1989, 1990a, b). Both the analytical uncertainty and the end-member variability are less than the size of the data points shown

(e.g. Turner & Rabalais 1991). While we have not constructed a mixing diagram for the summer condition the $[\text{Si}(\text{OH})_4]$ values of 0.9 to 4.0 μM observed in the mid-salinity region of the shelf appear to represent ca 80 to 95% non-conservative removal of the river-borne $\text{Si}(\text{OH})_4$ by diatoms.

Si uptake kinetics

Fig. 5 shows V as a function of $[\text{Si}(\text{OH})_4]$ at Stn D4 on May 1, 1993. The ambient $[\text{Si}(\text{OH})_4]$ at that station was 0.13 μM , the lowest observed at any station during either cruise. The kinetic data show that V was quite severely limited by the ambient $[\text{Si}(\text{OH})_4]$, as indicated by the almost linear increase in V with $[\text{Si}(\text{OH})_4]$ at the lowest ^{30}Si additions. Fig. 5 also shows the hyperbolic function fitted to the data by nonlinear regression using Eq. (1), and the V_{max} and K_s values obtained by that curve fit. The Michaelis-Menten saturation function (Eq. 1) provides an excellent description of the dependence of V on $[\text{Si}(\text{OH})_4]$ observed in that experiment. In fact, Fig. 5 shows much less experimental scatter than has been seen in any previous kinetic study of Si uptake by natural diatom assemblages (e.g. Nelson & Brzezinski 1990, Nelson & Tréguer 1992). This greatly diminished experimental scatter was characteristic of the entire spring data set. We performed 9

^{30}Si kinetic experiments at 8 locations in April and early May 1993; of those, 7 showed V to be dependent on $[\text{Si}(\text{OH})_4]$, and in each of those experiments Eq. (1) provides an excellent fit to the observed concentration dependence, with considerably less scatter than has been found in previous studies (Fig. 6). At the 2 other stations where we performed ^{30}Si kinetic experiments in spring, ambient $[\text{Si}(\text{OH})_4]$ was 1.24 and 5.35 μM and there was no detectable increase in V with increasing $[\text{Si}(\text{OH})_4]$ (Table 2).

At the one July station for which ^{30}Si kinetic data provide evidence of uptake-rate limitation (Fig. 7a, Table 2) the ambient $[\text{Si}(\text{OH})_4]$ was 4.7 μM , more than 10 times higher than any ambient concentration that was shown to limit Si uptake rates in spring 1993. However, there were several stations in July at which the surface $[\text{Si}(\text{OH})_4]$ was between 0.9 and 3.5 μM and no substrate limitation of Si uptake rates could be detected (e.g. Fig. 7b). Table 2 summarizes the kinetic data from both cruises. In spring K_s ranged from 0.48 to 1.7 μM and averaged

0.85 μM , which is low in comparison with most values obtained at other locations in the ocean (e.g. Goering et al. 1973, Azam & Chisholm 1976, Nelson & Tréguer 1992). In contrast, the single K_s value obtained in summer was 5.3 μM , one of the highest yet reported from the ocean (ibid.). For each experiment that indicated limitation we have evaluated Eq. (1) at the ambient $[\text{Si}(\text{OH})_4]$ to determine V/V_{max} (Table 2). This calculation provides a measure of the degree to which V

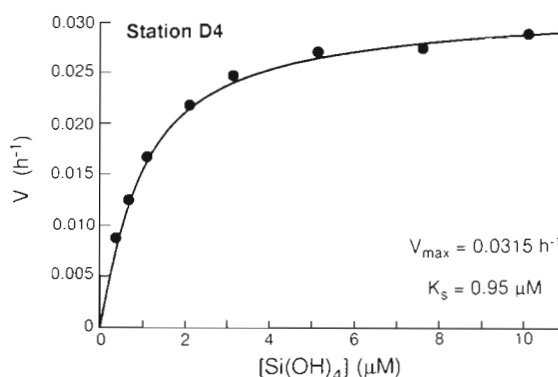


Fig. 5. Specific uptake rate of $\text{Si}(\text{OH})_4$ (V) vs extracellular $[\text{Si}(\text{OH})_4]$ in surface water at Stn D4, May 1, 1993. The ambient $[\text{Si}(\text{OH})_4]$ was 0.13 μM , the lowest measured during this study. The fitted curve represents Eq. (1), fitted to the data by non-linear regression, and the kinetic parameters V_{max} and K_s were generated from that curve fit. See text for details

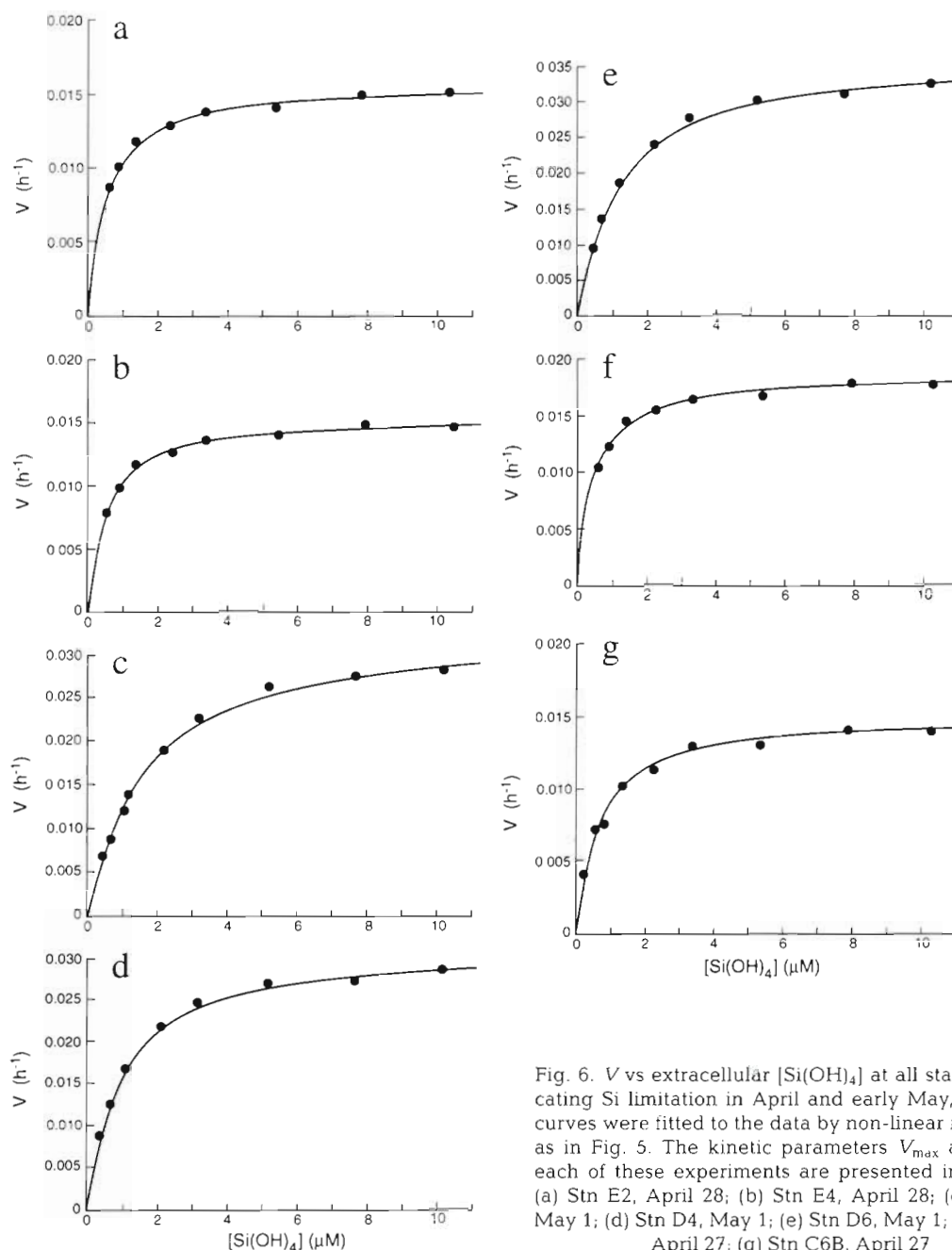


Fig. 6. V vs extracellular $[\text{Si}(\text{OH})_4]$ at all stations indicating Si limitation in April and early May, 1993. All curves were fitted to the data by non-linear regression as in Fig. 5. The kinetic parameters V_{max} and K_s for each of these experiments are presented in Table 2. (a) Stn E2, April 28; (b) Stn E4, April 28; (c) Stn D2, May 1; (d) Stn D4, May 1; (e) Stn D6, May 1; (f) Stn C3, April 27; (g) Stn C6B, April 27

was limited *in situ* by the ambient $[\text{Si}(\text{OH})_4]$. At the lowest concentrations observed in spring (0.13 to 0.2 μM) V was apparently limited to ca 10 to 15% of V_{max} , indicating reasonably severe substrate limitation of Si uptake by diatoms under those conditions.

Phytoplankton abundance and composition

During both cruises, the phytoplankton on the Louisiana shelf was composed almost entirely of

diatoms and small (1 to 2 μm) coccoid cyanobacteria (Table 1). Diatom abundance (both relative and absolute) was significantly higher in spring (Figs. 1c & 2c, Table 1), when chlorophyll *a* was also significantly higher (Figs. 1b & 2b). In summer the cyanobacteria were overwhelmingly dominant numerically (>99%, Table 1). Cyanobacteria should also dominate the autotrophic biomass, in spite of their very small size, when they are 2 orders of magnitude more abundant numerically than the sum of all other autotrophs (Dortch 1994). Differences in species composition

within the diatom assemblage are also apparent (Fig. 8). In spring there were fewer dominant diatom taxa than in the summer, *Skeletonema costatum* and *Chaetoceros* spp. were present at all stations, but their relative abundances changed systematically within the river plume in a way that suggests a response to Si limitation (see 'Discussion').

Using epifluorescence microscopy it is possible to distinguish between dead and living diatoms by the presence of chloroplasts and other cellular contents. The occurrence of significant numbers of empty diatom frustules could bias the specific silicate uptake rates, which are normalized to total biogenic silica (Brzezinski & Nelson 1989). Dead and living diatoms were counted in 5 samples selected from each cruise to represent populations experiencing different degrees of Si limitation or sufficiency. The percentage of living diatoms was always >95% and averaged 97.7%.

DISCUSSION

Biological removal of $\text{Si}(\text{OH})_4$ in the Mississippi River plume

Uptake by diatoms over the Louisiana shelf resulted in the non-conservative removal of at least 80% of the $\text{Si}(\text{OH})_4$ delivered to the ocean by the Mississippi River in July 1992, and 99% of that delivered in April 1993 (Figs. 3 & 4). The diatom bloom responsible for that removal in both seasons was closely associated spatially with the low-salinity river plume

Table 2. Summary of ^{30}Si uptake kinetic results from surface waters in the plume of the Mississippi River in spring 1993 and summer 1992. Italicized entries indicate stations at which no Si limitation was observed (i.e. where addition of Si resulted in no analytically detectable increase of V). nd: not determined

Station (& date)	$[\text{Si}(\text{OH})_4]$ (μM)	V_{max} (h^{-1})	K_s (μM)	V at ambient $[\text{Si}(\text{OH})_4]$ V_{max}
Spring 1993				
B4 (24 Apr)	1.24	0.0114 ^a (± 0.0021)	nd ^a	1.0
C3 (27 Apr)	0.41	0.0188 ^b (± 0.0005)	0.48 ^b (± 0.06)	0.36
C6B (27 Apr)	0.29	0.0155 (± 0.0010)	0.69 (± 0.12)	0.30
E2A (28 Apr)	0.40	0.0156 (± 0.0004)	0.49 (± 0.06)	0.45
E4 (28 Apr)	0.39	0.0155 (± 0.0004)	0.50 (± 0.07)	0.44
D2 (1 May)	0.19	0.0337 (± 0.0019)	1.71 (± 0.29)	0.10
D4 (1 May)	0.13	0.0315 (± 0.0009)	0.95 (± 0.10)	0.12
D6 (1 May)	0.19	0.0366 (± 0.0011)	1.14 (± 0.12)	0.14
C6B (2 May)	5.35	0.0125 (± 0.0032)	nd	1.0
Summer 1992				
C6B (25 July)	3.48	0.0298 (± 0.0042)	nd	1.0
C9 (25 July)	2.42	0.0304 (± 0.0130)	nd	1.0
D1 (26 July)	4.72	0.0327 (± 0.0090)	5.29 (± 3.79)	0.47
D2 (26 July)	0.93	0.0308 (± 0.0099)	nd	1.0
E2 (26 July)	1.24	0.0311 (± 0.0096)	nd	1.0

^aWhere no Si limitation was observed V_{max} represents the mean of the 8 measured V values, and no K_s can be calculated. The (\pm) term represents ± 2 SD in the measured V

^bWhere Si limitation was evident V_{max} and K_s were determined by iterative nonlinear regression of Eq. (1) to the data. The (\pm) term represents the 95% confidence limit in V_{max} or K_s determined by that regression

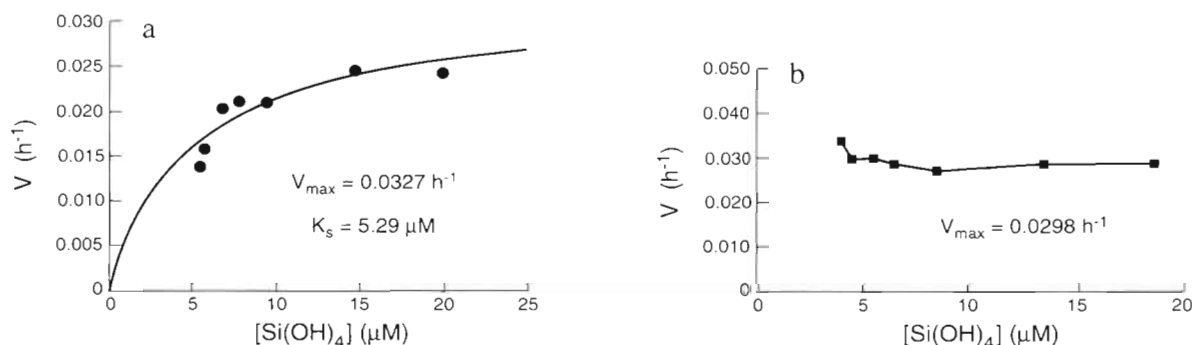


Fig. 7. V vs extracellular $[\text{Si}(\text{OH})_4]$ at 2 stations in July, 1992. (a) Stn D1, July 26 (the only July station showing Si limitation). Curve fit and kinetic parameters V_{max} and K_s obtained by nonlinear regression as in Fig. 5; (b) Stn C6B, July 25 (1 of 4 indicating no Si limitation). No hyperbolic curve can be fit, and no K_s calculated, because V is independent of $[\text{Si}(\text{OH})_4]$. The reported V_{max} represents the mean of the individual V values, each of which is an estimate of V_{max} when no substrate limitation is present

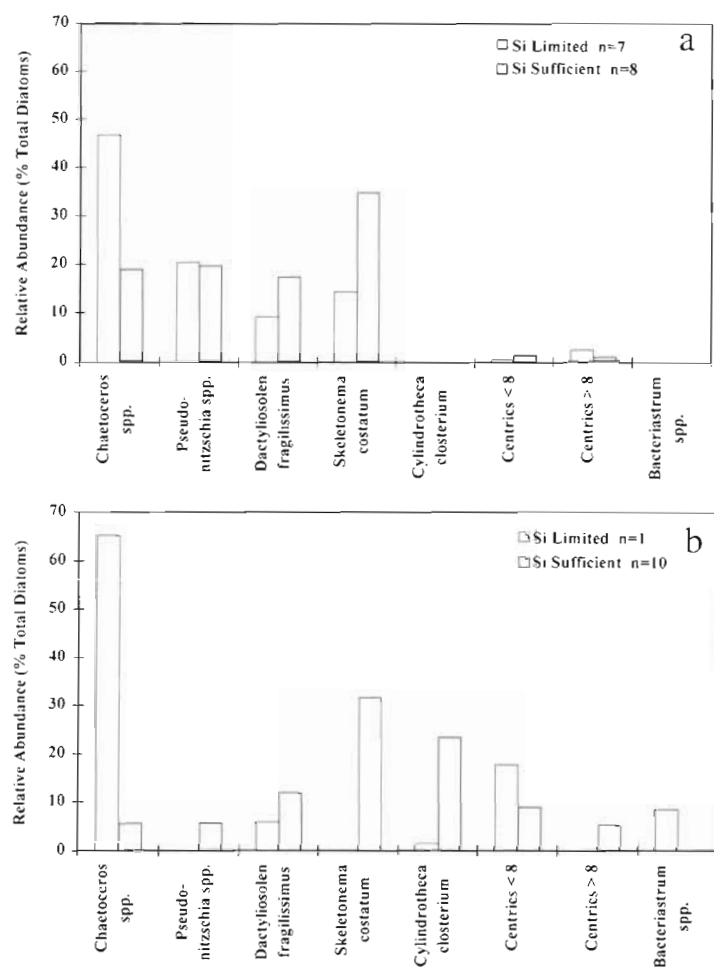


Fig. 8. Relative abundances of taxa accounting for at least 90% of the diatoms, averaged for stations showing no substrate limitation of Si uptake rates (Si-sufficient) and for stations at which Si uptake was limited by $[\text{Si}(\text{OH})_4]$ (Si-limited). (a) April 26 to May 1, 1993; (b) July 24 to 28, 1992. Centrics > 8 μm include *Thalassiosira* spp., *Cyclotella* spp., *Coscinodiscus* spp. and other centric diatoms of similar appearance which are not readily identified by the methods used. *Dactyliosolen fragilissimus* formerly *Rhizosolenia fragilissima* (Hasle & Syvertsen 1996)

(Figs. 2 & 3), and appears to have resulted from the enhanced river inputs of $\text{Si}(\text{OH})_4$ and other nutrients. In April 1993 $[\text{Si}(\text{OH})_4]$ was depleted to $<0.5 \mu\text{M}$ over a large portion of the Louisiana shelf, with surface water in the core of this zone depleted to $<0.2 \mu\text{M}$ (Fig. 3a). Silicic acid concentrations that low are rare anywhere in the ocean. The global ocean data set of the Geochemical Ocean Section Study (GEOSECS) shows only 4 individual stations (in a total of >400) where surface $[\text{Si}(\text{OH})_4]$ was depleted to $0.2 \mu\text{M}$ (Bainbridge 1980, Craig et al. 1981, Spencer et al. 1982). Only a few other studies have encountered similar $\text{Si}(\text{OH})_4$ depletion in open-ocean waters (e.g. Marra et al. 1990, Nelson & Brzezinski 1990). In the northern Gulf of Mexico this severe depletion appears to be a seasonal phenome-

non associated with high river flow, as we observed no depletion of $[\text{Si}(\text{OH})_4]$ to $<0.9 \mu\text{M}$ in any part of the river plume in summer (Fig. 3b).

When observed, $\text{Si}(\text{OH})_4$ depletion to $<0.2 \mu\text{M}$ has almost always been associated with high primary productivity and diatom abundance. Sites from which that degree of depletion has been reported include: the coasts of Peru and northwest Africa during seasonal upwelling periods (Friederich & Codispoti 1979, 1981), the centers of 2 Gulf Stream warm-core rings in which the eddy circulation was generating enhanced upward nutrient transport and productivity (Brzezinski & Nelson 1989, Nelson et al. 1989), an ice-edge diatom bloom in the Barents Sea north of Norway in mid summer (Rey & Skjoldal 1987), the northern Sargasso Sea during a brief wind-driven mixing event in spring (Marra et al. 1990), the Bay of Brest during the summer maximum in primary productivity (Ragueneau 1994) and the eastern Bering Sea during a spring phytoplankton bloom (Banahan & Goering 1994). The notable absence of temporally persistent $\text{Si}(\text{OH})_4$ depletion even to $0.5 \mu\text{M}$ in any of the oligotrophic central-ocean gyres (e.g. Bainbridge 1980, Craig et al. 1981, Brzezinski & Nelson 1995) indicates that concentrations $<0.5 \mu\text{M}$ are reached only when diatoms are relatively abundant, growing actively and taking up $\text{Si}(\text{OH})_4$ at rates exceeding its supply rate. Thus the hypothesis that $\text{Si}(\text{OH})_4$ depletion and Si limitation of diatom growth may characterize areas of coastal eutrophication (e.g. Smayda 1990) is highly consistent with global $[\text{Si}(\text{OH})_4]$ distributions.

Within the Mississippi River plume, $\text{Si}(\text{OH})_4$ was depleted to much lower concentrations in spring 1993 than in summer 1992 (Figs. 1–3). Chlorophyll *a* concentrations and diatom abundances were also considerably higher in spring (Figs. 1 & 2), a period which coincides with the seasonal maximum in river discharge (Boyles & Humphries 1994). Previous studies of nutrient concentrations in the northern Gulf of Mexico have not measured $[\text{Si}(\text{OH})_4]$ with the analytical sensitivity required to identify depletion to $<0.5 \mu\text{M}$, and our 2 sets of observations do not adequately constrain the seasonal cycle of surface $[\text{Si}(\text{OH})_4]$ in the area. However, the data are consistent with the hypothesis that the degree of $\text{Si}(\text{OH})_4$ depletion over the Louisiana shelf oscillates seasonally with the magnitude of the river nutrient source, with the lowest $[\text{Si}(\text{OH})_4]$ occurring during periods of high river discharge and high nutrient input in spring.

Analysis of scatter in ^{30}Si kinetic data

Linear regression of $[\text{Si}(\text{OH})_4]/V$ versus $[\text{Si}(\text{OH})_4]$, based on the Woolf linear transformation of the Michaelis-Menten equation (e.g. Nelson et al. 1976) yields a linear correlation coefficient (r^2) of >0.99 for each experimental result shown in Fig. 6. That very high degree of statistical correlation is a very unusual result for any biological field experiment, and reflects both the lack of scatter in the data and the excellent fit of Eq. (1) to the results obtained. The lack of scatter evident in Figs. 5 & 6 can be attributed to the fact that conditions in the northern Gulf of Mexico in spring 1993 were nearly optimal for tracer kinetic studies of Si uptake. Phytoplankton biomass was high and dominated by diatoms, surface $[\text{Si}(\text{OH})_4]$ was very low (resulting in strong labelling of the dissolved $\text{Si}(\text{OH})_4$ with ^{30}Si , even at the lowest isotope additions) and the diatoms were capable of growing quite rapidly, as indicated by relatively high V_{max} values. Each of those conditions increases the precision and sensitivity with which V can be measured, either by increasing the amount of siliceous material available for mass spectrometry or by increasing the ^{30}Si enrichment of the particulate SiO_2 per unit of Si uptake. The cumulative effect is considerable. We calculate, for example, that under the conditions we encountered in spring 1993

the change in the ^{30}Si enrichment of the particulate matter for any given change in V would be 10 to 50 times that obtained in previous studies in either Gulf Stream warm-core rings (where diatoms were considerably less abundant, resulting in lower biogenic silica concentrations and the necessity of adding ^{29}Si carrier to samples prior to mass spectrometry to assure a strong signal; Nelson & Brzezinski 1990) or the Ross Sea, Antarctica (where the lowest ambient $[\text{Si}(\text{OH})_4]$ values were 6 to 8 μM , resulting in greatly diminished label strength at the lowest ^{30}Si additions; Nelson & Tréguer 1992). In summer 1992 diatoms were typically 10 times less abundant than in spring 1993, and the lowest $[\text{Si}(\text{OH})_4]$ values were considerably higher (Figs. 1c, 2c & 3). The one ^{30}Si kinetic experiment that showed concentration dependence of uptake under those conditions had scatter greater than that observed in any of the spring experiments, and comparable to that observed in previous studies (see Fig. 7a). It thus appears that when conditions in a study area provide a very high signal-to-noise ratio in ^{30}Si tracer kinetic experiments the data obtained conform almost perfectly to the Michaelis-Menten saturation model.

Kinetic evidence of Si limitation in the Mississippi River plume

Seven of the nine ^{30}Si kinetic experiments we performed in spring indicate that the rate of Si uptake by natural diatom assemblages in the plume of the Mississippi River was limited by the extracellular $[\text{Si}(\text{OH})_4]$ (Figs. 5 & 6). The kinetic data show that in spring the ambient $[\text{Si}(\text{OH})_4]$ generally limited V to $<50\%$ of V_{max} within the river plume, and to $<15\%$ of V_{max} within the most $\text{Si}(\text{OH})_4$ -depleted ($<0.2 \mu\text{M}$) zone (Table 2). In addition, the available kinetic data on Si limitation of diatom division rates (Fig. 9) indicate that almost every diatom species that has been examined to date would be measurably growth-rate limited at a $[\text{Si}(\text{OH})_4]$ of $0.5 \mu\text{M}$ and quite severely growth-limited at $0.2 \mu\text{M}$. $\text{Si}(\text{OH})_4$ was depleted to $<0.2 \mu\text{M}$ along one entire transect line in the Mississippi River plume during spring 1993, and depletion to $<0.5 \mu\text{M}$ was quite extensive spatially (see Fig. 3a). These spatial data, taken together with ^{30}Si kinetic results showing that Si uptake rates were severely limited by substrate availability in the plume region, provide strong indirect evidence that diatom growth rates within the river plume were limited by Si during spring 1993.

The kinetic data indicate that uptake-rate limitation was much less common in summer than in spring. Only 1 July station showed V to be measurably dependent on $[\text{Si}(\text{OH})_4]$ (Fig. 6, Table 2), and at that station the ambient $[\text{Si}(\text{OH})_4]$ was $4.7 \mu\text{M}$, which is well above the

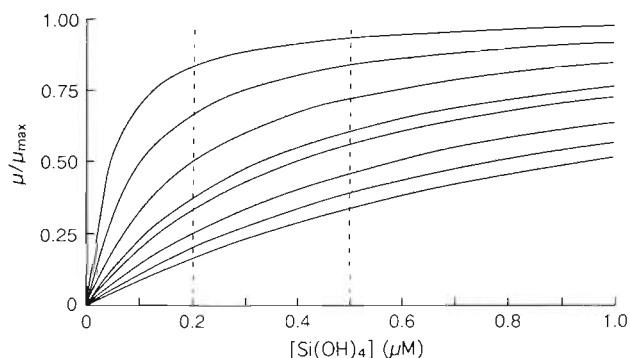


Fig. 9. Dependence of the specific growth rate (μ) on extracellular $[\text{Si}(\text{OH})_4]$ determined in representative culture studies of Si-limited growth kinetics in diatoms. Note factor-of-10 difference in $[\text{Si}(\text{OH})_4]$ axis from Figs. 5 & 6. In all cases Eq. (2) was fitted to the data and the kinetic constants μ_{max} and K_d calculated by the original authors. To permit direct inter-comparison among species with different maximum growth rates μ is normalized to μ_{max} , the original data points are omitted and only the fitted functions are shown. The dashed lines indicate the degree of Si limitation calculated at 0.2 and $0.5 \mu\text{M}$. From the uppermost to lowermost curve (i.e. from the lowest to highest K_d) the species and original data sources are as follows: *Thalassiosira pseudonana* (Olsen & Paasche 1986) *T. nordenskiöldii* (Paasche 1975), *T. oceanica* (Guillard et al. 1973), *Leptocylindrus danicus* (Thomas & Dodson 1975), *Chaetoceros gracilis* (2 curves; Thomas & Dodson 1975), *T. pseudonana* (Paasche 1973a), *T. pseudonana* (Guillard et al. 1973)

range that has been shown to limit the division rates of diatom species from any temperate or tropical habitat (Fig. 9). Thus our data indicate that Si limitation of diatom growth was rare or absent in July 1992.

The lowest K_s values measured in spring 1993 were ca 0.5 μM (Table 2). Those values are equal to the lowest that have been reported previously from natural diatom assemblages anywhere in the ocean (data from various sources, summarized by Ragueneau 1994). The only previous study showing K_s values as low as ca 0.5 μM for natural diatom assemblages was conducted in 2 Gulf Stream warm-core rings in which surface $[\text{Si}(\text{OH})_4]$ was also $<0.2 \mu\text{M}$, and had been depleted to $<0.5 \mu\text{M}$ for at least several days (Nelson & Brzezinski 1990). The low K_s values obtained for diatoms in the Mississippi River plume in spring may thus indicate that the resident diatom species were well adapted to low $[\text{Si}(\text{OH})_4]$.

Data on Si uptake kinetics provide only indirect information on the possibility that diatom growth rates are limited by Si, because of flexibility in the cellular Si content of many diatoms. As discussed earlier, uptake-rate limitation is a necessary, but not sufficient condition for growth-rate limitation. The data from the Mississippi River plume show clearly that Si uptake rates were strongly substrate-limited within the $\text{Si}(\text{OH})_4$ -depleted region of the plume in spring, indicating that diatom growth may have been limited by Si. By the same reasoning, the near absence of uptake-rate limitation over the Louisiana shelf in summer makes it almost certain that diatom growth within the river plume was not limited by Si at that time. Culture data on the kinetics of Si-limited growth of marine diatoms indicate that K_d values range from ca 0.04 to 1.0 μM (Guillard et al. 1973, Olsen & Paasche 1986) with values $>0.2 \mu\text{M}$ reported only rarely (e.g. Paasche 1975). Thus, most diatoms whose Si-limited growth kinetics have been examined to date would have been detectably growth-rate limited throughout the region depleted to $<0.5 \mu\text{M}$ $\text{Si}(\text{OH})_4$ in spring, and quite severely limited at the lowest concentrations (0.13 to 0.19 μM ; see Fig. 9). In contrast, most diatoms whose Si-limited growth kinetics have been examined would experience little or no growth limitation at the $>2.4 \mu\text{M}$ concentrations that were present almost everywhere over the shelf in summer.

In both spring and summer there were stations at which the surface $[\text{Si}(\text{OH})_4]$ was between 0.9 and 3.5 μM but ^{30}Si kinetic experiments provided no evidence that Si uptake rates were limited by substrate availability (Table 2, Fig. 7b). Those results indicate either that the resident diatom assemblages at those sites had very low K_s values for Si uptake or that their rates of growth (and hence silicification) were limited by some other factor. Summer stations showing no Si

limitation at ambient $[\text{Si}(\text{OH})_4]$ between 0.9 and 3.5 μM had ratios of $[\text{Si}(\text{OH})_4]$ to total inorganic N ($[\text{NO}_3^-] + [\text{NO}_2^-] + [\text{NH}_4^+]$) consistently greater than the typical diatom composition ratio 1.0, and in fact greater than the present day river ratio of 1.2 (Dortch et al. 1995). The data thus suggest that diatom assemblages are often limited more strongly by N than by Si in summer. In spring N limitation is far less likely than in summer, but P limitation is somewhat more likely (Dortch & Whitley 1992). Our one spring station that showed no kinetic response to added ^{30}Si at reasonably low $[\text{Si}(\text{OH})_4]$ (Stn B4; see Table 2) had a surface $[\text{HPO}_4^{2-}]$ of 0.1 μM , making P limitation a possible cause of that result.

Changes in phytoplankton composition in relation to Si availability and uptake kinetics

There is a seasonal shift in the phytoplankton composition over the Louisiana shelf, with diatoms dominant in spring and cyanobacteria in summer. This is a dilemma, since spring is the period when our data show Si uptake, and possibly diatom growth, to be limited by low $[\text{Si}(\text{OH})_4]$ over much of the shelf. Smayda (1989, 1990) hypothesized that in systems with high N and P inputs and low Si inputs, blooms of non-siliceous autotrophs would occur, resulting from Si limitation of diatom growth. However, nutrient inputs to the Mississippi River plume system are nearly balanced with diatom growth requirements (Justic' et al. 1995). That is, while the nutrient inputs from the river have changed temporally, that change has been from a large stoichiometric excess of Si over N (relative to diatom requirements) to approximately the 1:1 mole ratio that is characteristic of nutrient-sufficient diatoms (e.g. Brzezinski 1985). Thus Si limitation may not be severe enough or temporally persistent enough in spring to enable non-siliceous forms to become dominant. In summer, when cyanobacteria become overwhelmingly dominant numerically, there is no evidence that this dominance occurs because diatom growth is limited by Si. Quite the opposite in fact—both the $[\text{Si}(\text{OH})_4]$ data and the ^{30}Si kinetic results clearly indicate Si sufficiency throughout the river plume in summer.

These results would appear superficially to be contradictory to those obtained by Egge & Aksnes (1992) in large enclosures of Norwegian coastal waters. They found that diatoms were almost always dominant ($>70\%$ of the total numerical abundance) when $[\text{Si}(\text{OH})_4]$ exceeded 2 μM , and could be anywhere from a trace component of the phytoplankton to $>90\%$ dominant when $[\text{Si}(\text{OH})_4]$ was $<2 \mu\text{M}$. Our data from the Mississippi River plume show that cyanobacteria are

the numerically dominant autotrophic form in both spring and summer, but that diatoms dominate the autotrophic biomass in spring. Egge & Aksnes (1992) reported no data on cyanobacteria, and their microscopic methods, based on gravitational sedimentation, would not have yielded data on cyanobacterial abundance. Diatoms would have appeared to be dominant numerically, as well as in biomass, in the Mississippi River plume in spring had we analyzed our samples by their methods. The diatom dominance we observed in spring, during a time of very low surface $[\text{Si}(\text{OH})_4]$, shows that diatoms can persist when $[\text{Si}(\text{OH})_4]$ is considerably $< 2 \mu\text{M}$; a point made by Egge & Aksnes (1992) as well. In fact, very low ($< 0.2 \mu\text{M}$) $[\text{Si}(\text{OH})_4]$ in the ocean is almost always associated with *high* diatom abundance (e.g. Rey & Skjoldal 1987, Brzezinski & Nelson 1989, Marra et al. 1990, this study), and appears to be sustainable only when diatoms are growing actively and taking up $\text{Si}(\text{OH})_4$ at high rates.

The species composition data and the ^{30}Si kinetic results combine to suggest that systematic changes occur *within* the diatom assemblage in response to Si limitation. To investigate that possibility we divided the stations over the shelf at which high-sensitivity $[\text{Si}(\text{OH})_4]$ analyses were performed into 2 groups for each cruise: Si-limited stations, those where ^{30}Si kinetic data showed that V was $< 0.5 V_{\text{max}}$ at the ambient $[\text{Si}(\text{OH})_4]$ (See Table 2), and Si-sufficient stations, those at which $[\text{Si}(\text{OH})_4]$ was $> 5 \mu\text{M}$ or where ^{30}Si kinetic data showed that V was not limited by $[\text{Si}(\text{OH})_4]$. Intermediate cases (i.e. where $0.5 V_{\text{max}} < V < V_{\text{max}}$) were not observed (Table 2). For each station considered, the percentage abundance of each major diatom taxon (no. of individuals $\times 100$ / total no. of diatoms) was calculated, beginning with the most abundant and working downward until $> 90\%$ of the diatoms had been included in the analysis. This procedure included 4 to 6 diatom taxa at each station. Although the same taxa were present at all stations in spring, their relative abundances at Si-limited stations were found to be quite different from those at Si-sufficient stations (Fig. 8). In particular, *Skeletonema costatum* predominated at sites where there was no evidence of Si limitation and *Chaetoceros* spp. dominated where Si limitation was apparent in the kinetic results. At the Si-limited stations in spring about half of the *Chaetoceros* spp. individuals were of the species *C. socialis* and the rest comprised a variable mixture of species. The difference in diatom composition between stations showing no Si limitation and stations at which Si limitation was apparent suggests successional emergence of genetically low- K_s species under conditions of low $[\text{Si}(\text{OH})_4]$. No dominant taxa disappear entirely in spring, so some degree of physiological adaptation to low $[\text{Si}(\text{OH})_4]$ by individual species is also possible.

In summer the total number of diatoms was about an order of magnitude less than in spring, and the diatom assemblage was more diverse. There was only one summer station at which Si limitation was apparent in the ^{30}Si kinetic data. That station had a diatom species composition broadly similar to that found at the Si-limited stations in spring: *Chaetoceros* spp. predominated and *Skeletonema costatum* abundance was low. However, unlike the Si-limited stations in spring, *C. socialis* was not observed in summer. Because there was only one station providing evidence of Si limitation in summer, and that station had $[\text{Si}(\text{OH})_4]$ approximately an order of magnitude higher than was found at Si-limited stations in spring, the similarity in diatom taxonomic composition between Si-limited stations in spring and summer must be interpreted cautiously. However, all data are at least consistent with a general pattern in which *Chaetoceros* spp. predominate when Si is limiting and *S. costatum* when it is not.

In several of the North American Great Lakes increasing Si limitation due to eutrophication has resulted in a shift in the diatom species composition (Stoermer et al. 1985a, b, 1990). However, there is no *a priori* reason to expect a shift from *Skeletonema costatum* to *Chaetoceros* spp. at low $[\text{Si}(\text{OH})_4]$. Neither the Si content (Conley et al. 1989) nor the Si uptake kinetics (Conway & Harrison 1977) of *Chaetoceros* spp. in comparison with *S. costatum* indicate special adaptation to low $[\text{Si}(\text{OH})_4]$. *C. socialis* blooms have also been observed in severely Si-depleted water in the Barents Sea (Rey & Skjoldal 1987) and the Bay of Brest, France (Ragueneau et al. 1994). However the data are insufficient to make generalizations over a wide range of species and environmental conditions, and there are no laboratory data on either the Si content or the Si uptake kinetics of *C. socialis*. There is evidence that several diatom genera other than *Chaetoceros* may be adapted to low $[\text{Si}(\text{OH})_4]$. For example, *Rhizosolenia* spp. have been associated with low $[\text{Si}(\text{OH})_4]$ in the Bay of Brest (Ragueneau et al. 1994) and on the Louisiana shelf during other periods (Dortch et al. 1992); some *Rhizosolenia* species are very lightly silicified (Conley et al. 1989). Understanding the role of Si availability on phytoplankton growth dynamics and species composition in this region will require more information about the responses of individual diatom species to low $[\text{Si}(\text{OH})_4]$.

Historical perspective: is Si limitation in the northern Gulf of Mexico a new phenomenon?

Our observations of severe $\text{Si}(\text{OH})_4$ depletion and the clear evidence of Si limitation in the Mississippi River plume in spring are consistent with the hypothesis that

these phenomena reflect coastal eutrophication caused by increasing $[\text{NO}_3^-]$ in the river. Certainly the river nutrient source has changed since 1950 in a direction that makes coastal Si limitation more likely than it used to be (Turner & Rabalais 1991). The spatial distributions of salinity, chlorophyll, $[\text{Si}(\text{OH})_4]$ and diatom abundance in both spring and summer (Figs. 1–3) provide strong indirect evidence that under present conditions the river is the main nutrient source supporting phytoplankton blooms over the Louisiana shelf during both seasons. In addition, there is now evidence that the biogenic silica content of surface sediments near the Mississippi River delta has increased since 1960, and that increase has been interpreted as a signal of coastal eutrophication (Turner & Rabalais 1994). We cannot say with certainty whether or not the severe $\text{Si}(\text{OH})_4$ depletion and Si limitation we observed in surface waters in spring 1993 are historically recent phenomena associated with the altered nutrient sources from the river. However, our data indicate the nutrient conditions under which Si limitation is most likely: The available K_s data show that V would be $<75\%$ of V_{max} whenever $[\text{Si}(\text{OH})_4]$ is $<1.5 \mu\text{M}$, even for the highest-affinity assemblages encountered in spring (those for which $K_s \approx 0.5 \mu\text{M}$; see Table 2). The absence of detectable Si limitation at some moderately $\text{Si}(\text{OH})_4$ -depleted stations suggests that, regardless of absolute concentrations, Si limitation is quite unlikely unless the ratio of $[\text{Si}(\text{OH})_4]$ to total inorganic N ($[\text{NO}_3^-] + [\text{NO}_2^-] + [\text{NH}_4^+]$) is <1.0 . Thus the waters most likely to impose some degree of Si limitation on the diatoms are those that simultaneously have a dissolved Si:N ratio <1.0 and a $[\text{Si}(\text{OH})_4]$ $<1.5 \mu\text{M}$. While those conditions can, in principle, provide a method for investigating the previous Si-limitation status of the system from historical nutrient data, the scarcity of historical $[\text{Si}(\text{OH})_4]$ data based on methods with enough sensitivity to define the $<1.5 \mu\text{M}$ range adequately may present a severe practical problem to that approach.

If the Si depletion and limitation we report here are in fact conditions that have developed over the past few decades, our results are only partially consistent with the hypothesis that increased N and P inputs have led a diminished relative abundance of diatoms in the phytoplankton by imposing Si limitation on the diatoms. Present data are more consistent with the hypothesis that the greatest floristic changes that have occurred in response to increasing N supply and Si limitation in the northern Gulf of Mexico have been within the diatom community, favoring species with a high affinity for $\text{Si}(\text{OH})_4$ (i.e. low K_s) over those less adapted to $\text{Si}(\text{OH})_4$ -depleted habitats. In other areas, with an excess N input instead of the nearly balanced Si and N inputs that characterize the Mississippi River outflow at present, non-siliceous phytoplankton could still dominate if severe depletion of $\text{Si}(\text{OH})_4$ occurs.

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