

## Effect of iron deficiency on diatom cell size and silicic acid uptake kinetics

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### Abstract

We studied the silicic acid uptake kinetics of the pennate diatom *Cylindrotheca fusiformis* grown under a wide range of iron concentrations (from Fe-limiting to Fe-sufficient conditions) to assess the effect of iron availability on diatom cell size, silicon content, and silicic acid uptake kinetic parameters. As the iron stress increased, the growth rate slowed, cell size decreased, and silicification increased. A series of Si kinetic uptake experiments (from Si-limiting to Si-sufficient conditions) performed at different iron concentrations demonstrated the extent of the colimitation domain, where the specific Si uptake rate ( $V_{Si}$ ) varied as a function of both silicic acid and Fe availability. A decrease in maximal specific uptake rate of silicic acid ( $V_{Si-max}$ ) under iron limitation was observed along with a decrease in half-saturation constant for silicic acid uptake ( $K_{Si}$ ). Because  $V_{Si-max}$  and  $K_{Si}$  vary in the same direction, the specific affinity for silicic acid does not change under iron stress. The variation in cell size is an acclimation to low nutrient concentrations. Our study shows that Si uptake kinetics parameters, especially  $V_{Si-max}$ , are strongly related to cell size, which is itself constrained by the degree of iron limitation. Thus, the surface to volume ratio should be related to silicic acid flows in size-based biogeochemical models of planktonic ecosystems. Another way to describe the observed variations of Si uptake over the full range of Si and Fe concentrations tested in the present study would be to implicitly take into account the variations in cell size through a scaling of the maximal specific uptake rate ( $V_{max}$ ) by the iron-limitation term in a multiplicative manner.

Diatoms account for about 40% of the primary production in highly productive coastal and nutrient-replete oceanic waters (Nelson et al. 1995) and play a major role in the export of organic carbon (Buesseler 1998). Hence, they are important in aquatic ecosystems and in the global carbon cycle. Studying the factors controlling the contribution of diatoms to total production is therefore essential for our understanding of the mechanisms controlling the efficiency of the biological pump of carbon in various regions of the oceans. In particular, their role in high-nutrient low-chlorophyll (HNLC) regions is unclear. These areas have elevated nitrate and phosphate concentrations throughout the year in surface waters, but chlorophyll levels remain “lower than expected” (Minas et al. 1986). Several hypotheses have been proposed to explain the paradox of these areas: high grazing pressure (Landry et al. 1997), unfavorable light-mixing regime (Nelson and Smith 1991), and limitation by nutrients other than nitrate or phosphate, in particular by the micronutrient iron

(Martin and Fitzwater 1988) or by silicic acid (Dugdale and Wilkerson 1998). Because amorphous silica (BSi) is an essential component of the diatom cell wall, silicic acid availability is a key factor in the regulation of diatom growth (Paasche 1980) and in food web structure (Egge and Aksnes 1992). Iron is also an essential element for phytoplankton and plays an important role in many physiological processes involved in cell growth. In particular, it is essential for nitrate assimilation and chlorophyll biosynthesis (Sunda 1988/1989).

Field experiments in the Equatorial Pacific and in the Southern Ocean have shown that the phytoplankton biomass and the growth of diatoms are stimulated over the short term (weeks) by iron fertilization (Ironex I and II, Soiree, Eisenex). However, the limitation by iron does not preclude simultaneous limitation by other factors. Indeed, in the same areas, silicic acid limitation of diatom production was also pointed out (Franck et al. 2000; Leynaert et al. 2001). Such field observations clearly indicate that an understanding of the pathways regulating the effect of nutrients on phytoplankton metabolism is needed.

So far, only few of the physiological responses of phytoplankton to iron stress have been studied experimentally in the laboratory. De La Rocha et al. (2000) studied cultures of *Thalassiosira weissflogii* and reported that Fe stress did not affect the half-saturation constant but decreased the maximum Si uptake rate by 66% between Fe-replete and Fe-

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deficient conditions. However, the relationship between iron availability and silicic acid uptake by various diatom species is far from being well understood.

In this study, we examined the silicic uptake kinetics of *Cylindrotheca fusiformis* grown under a wide range of iron concentrations. The experiments were designed to determine the range of iron and silicic acid concentrations where co-limitation can occur and to study the effect of iron stress on diatom cell size, silicon content, and silicic acid uptake kinetic parameters (half-saturation constant and maximal uptake rate).

## Materials and methods

*Culture and adaptation of Cylindrotheca fusiformis to different iron concentrations*—The pennate diatom *C. fusiformis*, purchased from the Center for Culture of Marine Phytoplankton (CCMP 343, Bigelow Laboratory for Ocean Sciences) was grown in artificial seawater medium Aquil containing standard enrichments of nitrates, phosphates, silicates, ethylenediaminetetraacetic acid (EDTA), trace metals, and vitamins (Price et al. 1988/1989). This medium was prepared using ultraclean and sterile techniques. It contained  $100 \mu\text{mol L}^{-1}$  EDTA, which controlled trace metal activity. Iron was added in a range of concentrations varying from iron-limiting to iron-sufficient conditions: from 5 through 15, 30, 50, 80, and  $500 \text{ nmol L}^{-1}$  of total [Fe], corresponding to 0.008, 0.046, 0.076, 0.120, 0.170, and  $0.760 \text{ nmol L}^{-1}$  of inorganic iron ( $\text{Fe}'$ ) concentrations in the final medium (Bucciarelli unpubl. data). Media were sterilized by microwave. Sterile techniques were used for all culture work and the axenicity of each culture was tested prior to inoculation.

Diatoms were grown in 1-liter polycarbonate bottles at  $20^\circ\text{C}$  under a saturating photon flux density of  $123 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  under cool white lights and light:dark cycle equal to 14:10 h. The cells were first adapted to the lowest iron concentration until their growth rate remained constant. They were then transferred into the media at the six different iron concentrations mentioned above. Cells were counted daily under the microscope. Specific growth rate was determined from linear regressions of  $\ln$  (cell density) versus time during the exponential phase of growth. Once this exponential growth phase was reached, cellular volume, biogenic silica, and dissolved nutrient concentrations were measured. At that point, the silicic acid kinetic uptake experiments could be carried out.

*Silicic acid kinetic uptake experiments*—Cells from each medium were concentrated by gentle centrifugation and rinsed with Aquil medium lacking silicic acid. Aliquots of the concentrated cells were then dispensed into eight experimental bottles containing 60 ml of Aquil medium with rising concentrations (from 0 to  $15 \mu\text{mol L}^{-1}$ ) of silicic acid, as sodium metasilicate passed through Chelex resin. Two additional subcultures were set aside for duplicate analysis of biogenic silica (BSi).

Silicic acid concentrations were measured in each flask prior to  $^{32}\text{Si}$  addition. The  $^{32}\text{Si}$  solution was purchased from Los Alamos National Laboratory, with a specific activity of  $27,700 \text{ Bq } \mu\text{g}^{-1}$ . To remove contaminant trace metals, the

$^{32}\text{Si}$  solution was also passed through Chelex 100 ion exchange resin prior to use as described in Price et al. (1988/1989). The silicic acid concentration of the  $^{32}\text{Si}$  solution measured manually was  $24.0 (\pm 0.1) \mu\text{mol L}^{-1}$ . The  $^{32}\text{Si}$  volume added to each of the 60-ml subcultures ranged between 36 and  $400 \mu\text{l}$ , corresponding to an increase in silicic acid concentration of  $0.16 \mu\text{mol L}^{-1}$  at the most. The amount of  $^{32}\text{Si}$  used was the minimum needed to yield, at the end of the incubation, 5 times the background activity on the filter. It corresponded to an initial activity in the bottle ranging from 60 to  $600 \text{ Bq}$ .

After incubation for 4 h, the contents of the bottles were gently filtered through a  $0.6\text{-}\mu\text{m}$  polycarbonate filter. Each filter was then rinsed with filtered seawater to remove excess tracer and placed in a 20-ml plastic scintillation vial. The amount of  $^{32}\text{Si}$  retained on the filter was determined by liquid scintillation counting, as described by Leynaert et al. (1996). The silicic acid uptake rate ( $V_{\text{Si}}$ ) was determined for each subculture. The maximal silicic acid uptake rate ( $V_{\text{Si-max}}$ ) and the half-saturation constant ( $K_{\text{Si}}$ ) were determined by fitting the Michaelis–Menten equation to the data.

*Experiment performed without ultraclean conditions*—Silicic acid kinetic experiments performed in ultraclean conditions, as described above, require difficult, time-consuming procedures and are not always possible, especially on board ships. We therefore investigated the potential effect of metal contamination on  $^{32}\text{Si}$  incubations of an iron-controlled culture. We did this by performing another set of silicic acid kinetic experiments, for cells adapted to  $0.046 \text{ nmol Fe}' \text{ L}^{-1}$ , which was in the middle range of  $\text{Fe}'$  concentrations. The experiment was designed in the same way as described above: ultraclean techniques were used until the cells were adapted to  $0.046 \text{ nmol Fe}' \text{ L}^{-1}$ . Subsequently, no particular precautions were taken to avoid metal contamination of the silicic acid tracer solutions and  $^{32}\text{Si}$  incubations.

*Cellular volume*—Ten milliliters of sample were fixed by  $600 \mu\text{l}$  of 25% glutaraldehyde. Fifty randomly selected cells of each culture were digitized on an inverted microscope using an analogic Leica camera and analyzed with software image analysis (Visilog 5) to determine the maximal width ( $a$ ,  $\mu\text{m}$ ) and length ( $A$ ,  $\mu\text{m}$ ). Menden-Deuer and Lessard (2000) did not find it necessary to consider composite shapes (e.g., one cylinder plus two cones), the error being as important when considering the intrinsic error of microscopic volume measurements and the sometimes inaccurate designation of composite shapes. Thus, the average cell volume was determined using the geometric formula for an ellipsoid.

$$\text{Volume} = (\pi a^2 A)/6$$

$$\text{Surface} = 0.5\pi a^2 + [\pi a A \arcsin(e)]/(2e)$$

$$\text{Width } e = (1 - a^2/A^2)^{1/2}$$

*Biogenic silica concentrations*—Sixty milliliters of exponential phase cultures were filtered onto a polycarbonate membrane (Nuclepore  $0.6 \mu\text{m}$ ) and rinsed with Aquil medium without nutrient. The filters were oven dried at  $60^\circ\text{C}$  for 24 h digested for 7 d in  $2.9 \text{ mol L}^{-1}$  HF (hydrofluoric

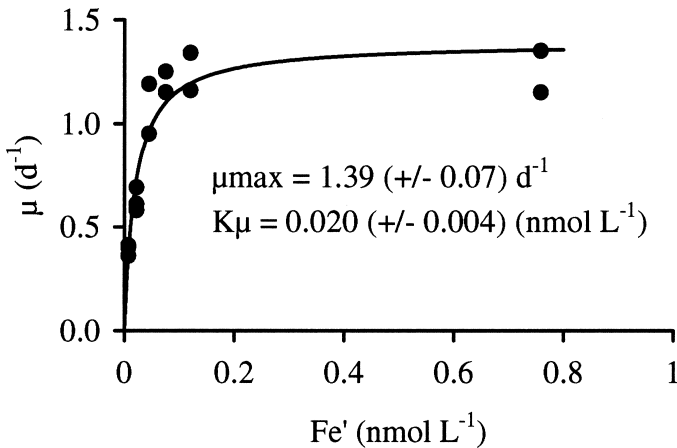


Fig. 1. Specific growth rate ( $\mu$ ) of *C. fusiformis* grown at different dissolved inorganic iron concentrations ( $\text{Fe}'$ ).

acid), and the resulting silicic acid ( $\text{Si}(\text{OH})_4$ ) was measured by spectrophotometry (Strickland and Parsons 1972).

## Results

*Experiment performed in ultraclean conditions*—Growth rate: One of the consequences of a limitation is by definition a decrease of the growth rate. The specific growth rate of *C. fusiformis* was highly dependent on the iron concentration, decreasing with iron stress (Fig. 1) from  $1.35 \text{ d}^{-1}$  at  $0.76 \text{ nmol Fe}' \text{ L}^{-1}$  to  $0.36 \text{ d}^{-1}$  at  $0.008 \text{ nmol Fe}' \text{ L}^{-1}$ . The maximum specific growth rate ( $\mu_{\text{max}}$ ) and the half-saturation constant for growth with respect to iron ( $K_{\mu\text{Fe}'}$ ), determined using a Monod saturation function, were  $1.39 (\pm 0.07) \text{ d}^{-1}$  and  $0.020 (\pm 0.004) \text{ nmol Fe}' \text{ L}^{-1}$ , respectively. Maximum specific growth rates reported in the literature for different diatom species grown in culture vary from 0.2 to  $3.3 \text{ d}^{-1}$  (Geider et al. 1986).  $K_{\mu\text{Fe}'}$  varied by more than 5 orders of magnitude (between  $0.94 \times 10^{-6}$  and  $0.43 \text{ nmol Fe}' \text{ L}^{-1}$ ) (Timmermans et al. 2001). Despite such a large range of variation, our results are very similar to values reported for the same species by Bucciarelli (unpubl. data) ( $\mu_{\text{max}} = 1.89 \pm 0.07 \text{ d}^{-1}$ ;  $K_{\mu\text{Fe}'} = 0.020 \pm 0.004 \text{ nmol Fe}' \text{ L}^{-1}$ ).

*Cell size*: Pictures of the cells grown at different iron concentrations show the dramatic decrease in cells size with decreasing iron concentrations (Fig. 2). Width and length measurements performed using image analysis indicated that there was a threefold decrease of the cell volume, leading to an important increase of the surface to volume ratio with iron stress (Fig. 3). Reduction of cell size in response to iron limitation seems to be a widespread trend and has been reported by many authors (Timmermans et al. 2001). However, an increase in cell size under iron stress has been reported once, by Takeda (1998) for *Nitzschia* sp. The reason for the different response between two species is unknown. It seems that iron supplies may affect the cell size of algal species differently.

*Cellular silicon content*: The cellular silicon content of the cells grown under a wide range of iron concentrations did

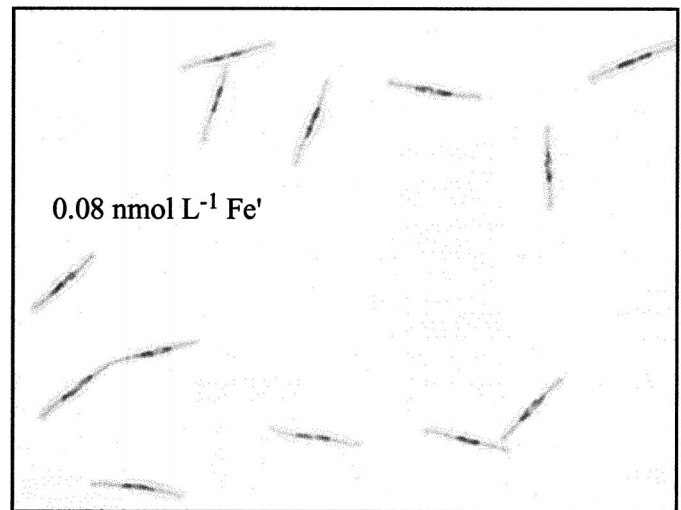
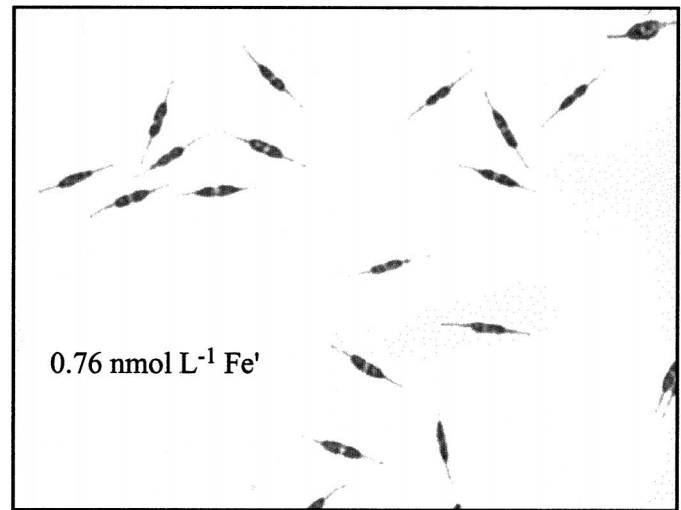


Fig. 2. Picture taken on an inverted microscope, showing the decrease in *C. fusiformis*'s cell size when grown in replete ( $0.76 \text{ nmol L}^{-1} \text{ Fe}'$ ) and depleted ( $0.008 \text{ nmol L}^{-1} \text{ Fe}'$ ) iron conditions.

not vary markedly (Fig. 4). The overall mean for the experiment was  $0.11 \pm 0.02 \text{ pmol BSi cell}^{-1}$ . However, a fourfold increase in BSi per unit of cell volume (from  $0.3 \times 10^{-3}$  to  $1.1 \times 10^{-3} \text{ pmol BSi } \mu\text{m}^{-3}$ ) was observed from replete to depleted iron conditions (Fig. 4). This increase in the degree of silicification under iron stress is similar to that observed by Bucciarelli (pers. comm.) for the same species. Takeda (1998) also observed a 1.7-fold increase in BSi content per unit of cell volume for *Chaetoceros dictyota* grown in a Fe-deficient medium. For *Nitzschia* sp., the increase in silicification under Fe stress was also marked (1.7-fold) when reported per unit of cell volume, in spite of an increase in cell volume in this particular case. De La Rocha et al. (2000) did not measure cell volume but found a 1.4-fold increase in BSi per cell for *T. weissflogii* in iron-stressed conditions. In situ, the same trend was observed for diatom communities. Hutchins and Bruland (1998) reported a 2.9-fold increase in BSi per cell in controls (Fe depleted) as compared to Fe-enriched incubations.

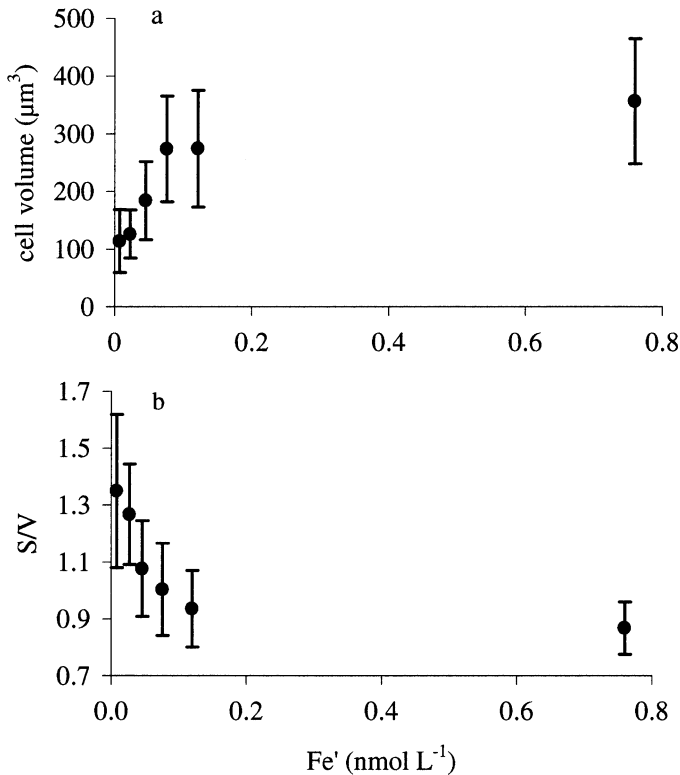


Fig. 3. Variation of (a) cell volume and (b) surface to volume ratio of *C. fusiformis*, as a function of iron stress ( $\text{Fe}'$ ).

Thus, our culture study supports the general observation of an increased silicification of diatoms under Fe stress. A review of the literature on that subject shows that if this increase in silicification under iron stress is not always noted strongly at the cell level (our study, Takeda 1998), it is always observed when reported per unit of cell volume, whether in culture or in situ (Hutchins and Bruland 1998; De La Rocha et al. 2000).

**Kinetics of silicic acid uptake rate (ultraclean methods):** Figure 5 shows the specific silicic acid uptake rate  $V_{\text{Si}}$  ( $\text{h}^{-1}$ ) plotted as a function of silicic acid concentrations for the six different  $\text{Fe}'$  concentrations. Two out of the six experiments were performed in duplicate (at 0.760 and 0.046  $\text{nmol Fe}' \text{L}^{-1}$ ) and exhibit good reproducibility (Fig. 5, Table 1): the standard errors for the determination of  $V_{\text{Si-max}}$  and  $K_{\text{Si}}$  for each duplicate ( $\text{SE}_{\text{dupl}} = 0.001 \text{ h}^{-1}$  and  $0.10 \mu\text{mol Si L}^{-1}$ , respectively) being lower than the average standard error introduced by the curve fitting procedure alone ( $\text{SE} = 0.002 \text{ h}^{-1}$  and  $0.12 \mu\text{mol Si L}^{-1}$ , respectively). The high consistency between duplicates gives confidence in the measurement of  $V_{\text{Si}}$  and  $K_{\text{Si}}$  presented below.

$V_{\text{Si}}$  and  $V_{\text{Si-max}}$ : The increase in  $V_{\text{Si}}$  when silicic acid concentration increased was barely detectable for the lowest  $\text{Fe}'$  concentrations (0.008  $\text{nmol Fe}' \text{L}^{-1}$ ). The maximum silicic acid uptake rate was well defined, but silicic acid concentrations were not low enough to observe an effect of the silicic acid limitation upon silicic acid uptake rates. Above 0.008  $\text{nmol Fe}' \text{L}^{-1}$ , the Michaelis–Menten saturation func-

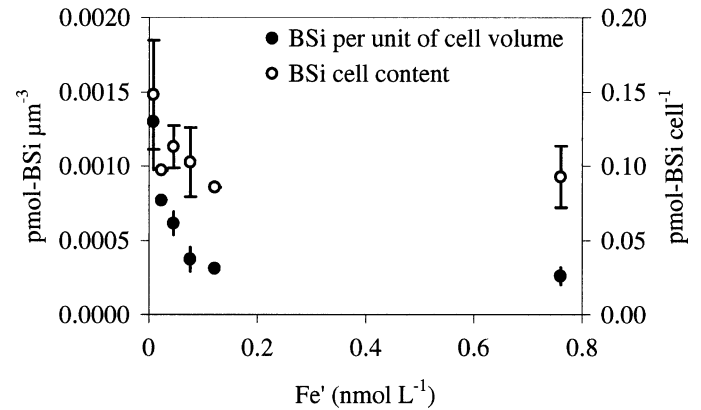


Fig. 4. Variation of the biogenic silica content ( $\text{pmol BSi cell}^{-1}$ ) and the biogenic silica per unit of cell volume ( $\text{pmol BSi } \mu\text{m}^{-3}$ ), versus iron concentration ( $\text{Fe}'$ ).

tion provided an excellent description of the dependence of  $V_{\text{Si}}$  on  $\text{Si(OH)}_4$  concentrations, with low scatter in the data. There was large variation of the maximum specific silicic acid uptake rate ( $V_{\text{Si-max}}$ ) as a function of ambient  $\text{Fe}'$  concentrations. The  $V_{\text{Si-max}}$  considered here is the maximum specific uptake rate for silicic acid reached for each  $\text{Fe}'$  concentration. It is calculated as the maximal uptake of silicic acid ( $\text{PBSi}_{\text{max}}$ , in  $\mu\text{mol L}^{-1} \text{d}^{-1}$ ) normalized to the biogenic silica concentration (BSi, in  $\mu\text{mol L}^{-1}$ ):

$$V_{\text{Si-max}} = \frac{\text{PBSi}_{\text{max}}}{\text{BSi}}$$

$V_{\text{Si-max}}$  varied from 0.011 ( $\pm 0.002$ )  $\text{h}^{-1}$  in the more iron-stressed culture up to 0.072 ( $\pm 0.002$ )  $\text{h}^{-1}$  (sevenfold higher) in nonlimiting conditions. Above 0.120  $\text{nmol Fe}' \text{L}^{-1}$ , there is no additional influence of iron on the specific uptake of silicic acid: the  $V_{\text{Si-max}}$  and  $K_{\text{Si}}$  determined by curve fitting at 0.120  $\text{nmol Fe}' \text{L}^{-1}$  and at 0.760  $\text{nmol Fe}' \text{L}^{-1}$  were statistically indistinguishable. Thus, at these nonlimiting  $\text{Fe}'$  concentrations, the silicic acid uptake rate was only a function

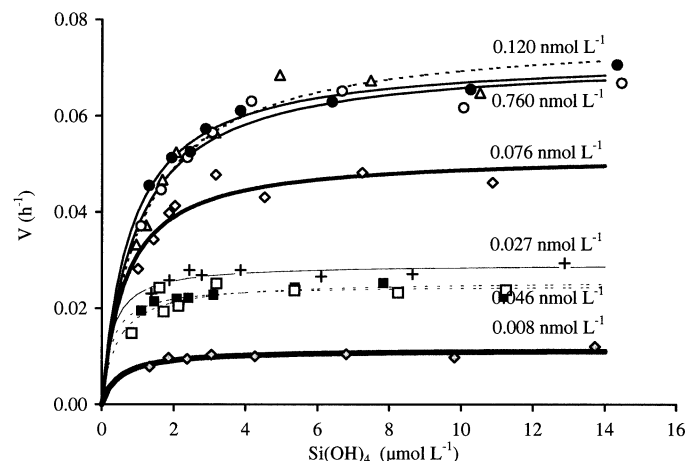


Fig. 5. Specific uptake rates  $V$  ( $\text{h}^{-1}$ ) plotted as a function of silicic acid concentrations ( $\mu\text{mol Si L}^{-1}$ ) for the six different iron conditions. Note duplicate experiments performed at 0.046 and 0.760  $\text{nmol L}^{-1} \text{Fe}'$ .

Table 1. Summary of the maximal uptake rates ( $V_{\max}$ ) and the half-saturation constant ( $K_{\text{Si}}$ ) determined from silicic acid kinetic experiments performed in ultraclean conditions at different iron concentrations ( $\text{Fe}'$ ). The results from standard method are in bold. SE is the standard error determined from the curve fitting procedure. SE dupl is the standard error between duplicates.

$\text{Fe}'$ (nmol L <sup>-1</sup> )	$V_{\max}$ (h <sup>-1</sup> )	SE( $V_{\max}$ ) (h <sup>-1</sup> )	avg( $V_{\max}$ ) (h <sup>-1</sup> )	SE dupl (h <sup>-1</sup> )	$K_{\text{Si}}$ ( $\mu\text{mol Si L}^{-1}$ )	SE( $K_{\text{Si}}$ ) ( $\mu\text{mol Si L}^{-1}$ )	avg( $K_{\text{Si}}$ ) ( $\mu\text{mol Si L}^{-1}$ )	SE dupl ( $\mu\text{mol Si L}^{-1}$ )	$V_{\max}/K_{\text{Si}}$
0.008	0.011	0.002			nd				nd
0.023	0.029	0.001			0.27	0.08			0.11
0.046	0.025	0.001	0.026	0.001	0.26	0.06	0.35	0.120	0.10
0.046	0.026	0.001			0.43	0.13			0.06
<b>0.046</b>	<b>0.025</b>	<b>0.002</b>			<b>0.49</b>	<b>0.21</b>			<b>0.05</b>
0.076	0.052	0.002			0.66	0.14			0.08
0.120	0.077	0.003			1.14	0.17			0.07
0.760	0.072	0.002	0.072	0.001	0.94	0.12	0.87	0.092	0.08
0.760	0.072	0.001			0.81	0.07			0.09
Average		0.002				0.12			0.08 (SE 0.02)

of the silicic acid availability. However, below 0.120 nmol  $\text{Fe}' \text{ L}^{-1}$ , a colimitation domain occurred where the specific Si uptake rate ( $V_{\text{Si}}$ ) varied as a function of both silicic acid and  $\text{Fe}'$  availability.

$K_{\text{Si}}$ : Figure 5 also shows that the greater the iron stress, the sooner the maximum uptake rate was reached (i.e., at lower  $\text{Si}(\text{OH})_4$  concentrations). Iron availability thus clearly

altered the half-saturation constant ( $K_{\text{Si}}$ ) for silicic acid uptake; with  $\text{Fe}'$  deprivation  $K_{\text{Si}}$  decreased from 0.87 ( $\pm 0.10$ ) to 0.27 ( $\pm 0.08$ )  $\mu\text{mol Si L}^{-1}$  (Fig. 6). At 0.008 nmol  $\text{Fe}' \text{ L}^{-1}$  the nonresponse of  $V_{\text{Si}}$  to increasing silicic acid concentrations did not allow us to accurately determine a  $K_{\text{Si}}$  value. Above 0.120 nmol  $\text{Fe}' \text{ L}^{-1}$ , iron was no longer limiting and  $K_{\text{Si}}$  reached a maximum that was characteristic of the silicic acid uptake rate of *C. fusiformis* in nonlimiting conditions.

*Comparison between standard and ultraclean methods during the  $^{32}\text{Si}$  incubations*—Once the cells were fully adapted to the iron concentration in their medium there was a high degree of similarity between the results obtained from experiments performed in ultraclean conditions and experiments carried out without regard to metal contamination of silicic acid solutions (Fig. 7).  $V_{\text{Si-max}}$  and  $K_{\text{Si}}$  values between the two experiments were statistically indistinguishable, the differences being included in the standard error calculated from the kinetic uptake curve fitting procedure (Table 1). This allowed us to conclude that (1) the experiment was reproducible and (2) that trace metal contamination of silicic acid solutions had no effect on silicic acid uptake kinetics

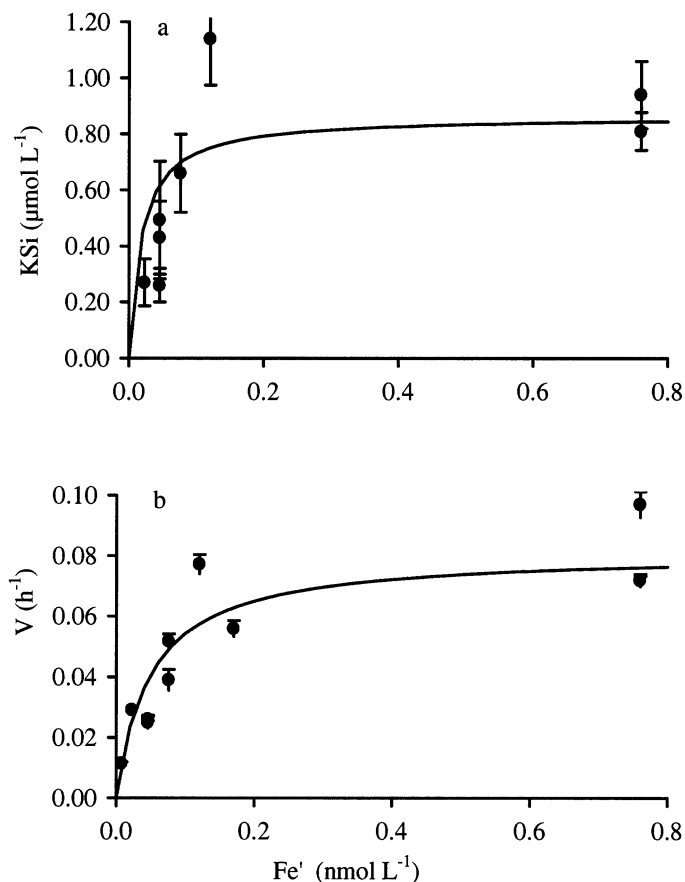


Fig. 6. (a) Half-saturation constant ( $K_{\text{Si}}$ ), and (b) maximum specific uptake rate ( $V_{\max}$ ) variations as a function of iron concentrations ( $\text{Fe}'$ ).

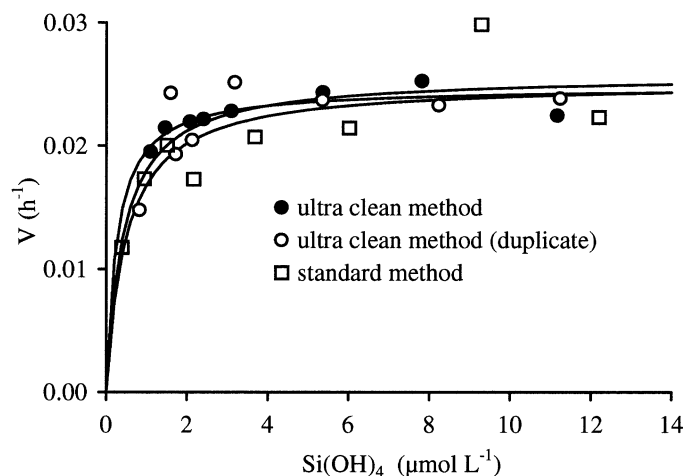


Fig. 7. Comparison of the results obtained from silicic acid kinetic experiments performed with the standard or with ultraclean methods.

on short time scale (<4 h) experiments. This conclusion is of interest for future experiments of this type conducted in potentially iron-limited or colimited areas (HNLC zones or oligotrophic oceanic gyres): ultraclean precautions may not always be necessary. However, water collection must still be done carefully to avoid contamination.

## Discussion

The behavior of a pennate diatom, like *C. fusiformis*, at different degrees of iron limitation is of the greatest interest because in most in vitro (Martin et al. 1991; Takeda and Obata 1995) and in situ experiments (Boyd et al. 2000; Hutchins et al. 2001), the biomass of pennate diatoms is greater than that of centric diatoms after iron addition.

*The effect of iron stress on silicic acid uptake rate parameters and cellular silicon content— $V_{\text{Si-max}}$  and  $V_{\text{Si}}$ :* There is a general agreement about the decrease in the maximal uptake rate for silicic acid ( $V_{\text{Si-max}}$ ) with increasing iron stress. In culture studies, De La Rocha et al. (2000) found a threefold (from 0.015 h<sup>-1</sup> to 0.005 h<sup>-1</sup>) decrease in  $V_{\text{Si-max}}$  of *T. weissflogii* between replete and depleted iron conditions. Bucciarelli (unpubl. data) for *Thalassiosira pseudonana* observed a 3.2-fold decrease between 0.760 and 0.045 nmol Fe' L<sup>-1</sup>. Hutchins et al. (1999), by inducing iron limitation in waters of the California upwelling regime, observed a twofold decrease in  $V_{\text{Si-max}}$  under iron stress conditions. During field experiments, in vitro incubations of a natural diatom population of the Southern Ocean enriched with Fe also showed a 2.7-fold increase (from 0.10 to 0.27 d<sup>-1</sup>) of  $V_{\text{Si-max}}$  under nonlimiting Si conditions (Franck et al. 2000).

The  $V_{\text{Si-max}}$  parameters were determined in Si-replete conditions but under different iron concentrations (Fig. 5). Thus, these are not "true"  $V_{\text{Si-max}}$  values, since  $V_{\text{Si-max}}$  should be determined when no other nutrient is limiting. The  $V_{\text{Si-max}}$  values determined for each Fe' concentration will now be called "apparent  $V_{\text{Si-max}}$ ." It represents the maximum silicic acid uptake rate that diatom can reach in non-Si-limiting conditions in a given environment, i.e., in our study, at a given Fe' concentration. Thus the variations of the apparent  $V_{\text{Si-max}}$  evident in our study for *C. fusiformis* demonstrated the effect of a single nutrient limitation (iron) on the uptake kinetics of another nutrient (silicic acid). In these cases, the uptake rate of silicic acid (like that of any other nutrient uptake rate) is set by the Michaelis–Menten kinetics term for Fe uptake:

$$\text{app}V_{\text{Si-max}} = V_{\text{Si-max}} \frac{[\text{Fe}]}{K_{\text{Fe}} + [\text{Fe}]} \quad (1)$$

We plotted the apparent  $V_{\text{Si-max}}$  measured in this study as a function of Fe' concentrations (Fig. 6). Results from the kinetic experiment performed without ultraclean cautions are also reported on this graph but were not used to fit the curve. A Michaelis–Menten curve was fitted to the data, and the kinetic parameters  $K_{\text{Fe}}$  (0.049 ± 0.011 nmol Fe' L<sup>-1</sup>) and  $V_{\text{Si-max}}$  (0.081 ± 0.008 h<sup>-1</sup>) were determined by the nonlinear regression method of Wilkinson (1961). The  $K_{\text{Fe}}$  determined in this manner is slightly higher than the half-saturation con-

stant for growth with respect to iron, determined from cells counts ( $K_{\mu\text{Fe}} = 0.020 \pm 0.004$  nmol Fe' L<sup>-1</sup>). This is not surprising because it is well known that half-saturation constants for uptake are typically greater than half-saturation constants for growth (Paasche 1973; Martin-Jézéquel et al. 2000).

The cellular silicon content: Along with the reduction in apparent  $V_{\text{Si-max}}$ , we noticed an increase in the cell silicification under iron stress. This can be explained by the fact that the incorporation of Si is mainly linked to the duration of the cell wall synthesis phase and does not require concurrent photosynthesis (Martin-Jézéquel et al. 2000). Thus, under nonlimiting Si conditions, mineralization is generally inversely correlated to growth rate (Flynn and Martin-Jézéquel 2000) with low growth rate leading to higher silicification. Indeed, experiments performed by Claquin et al. (2002) in continuous culture of *T. pseudonana*, either under light, nitrogen, or phosphorus control, also evidenced a highly significant increase in the amount of biogenic silica (BSi) per cell, and per cell surface, with decreasing growth rates. Therefore, it is not surprising that when iron regulates the growth rate, it controls indirectly the silicification. Thus, the increase of silicification under Fe-deficient conditions does not seem to be a straightforward effect, neither is it iron specific, but rather it is linked to the cell growth rate.

$K_{\text{Si}}$ : Until now, only two studies have reported  $K_{\text{Si}}$  values for silicic acid uptake under different iron conditions, and neither was able to show variation of this parameter. De La Rocha et al. (2000) did not find any change of the half-saturation constant of *T. weissflogii* grown in low Fe conditions. It must, however, be noted that their iron-limited cultures exhibited a negative growth rate at the time of the experiment, which might indicate that their cells were not adapted but rather senescent. In the second study, Franck et al. (2000) considered the role of Fe availability in regulating Si uptake for natural diatom population in the Polar Front Zone of the Southern Ocean. They reported that  $K_{\text{Si}}$  values in Fe additions were similar to those in the controls. However, as pointed out by the authors, these  $K_{\text{Si}}$  values were only approximated by solving the Michaelis–Menten equation at only two substrate concentrations and were probably marked with important uncertainties that did not allow them to observe any variation.

Ours is the first study using a series of kinetic uptake experiments over a large range of Fe concentrations that has been able to demonstrate a decrease of  $K_{\text{Si}}$  with iron stress.

The effect is opposite of what was expected. Until now, it was thought that the more the diatoms were limited by iron, the less would be their affinity for silicic acid (higher  $K_{\text{Si}}$ ). This probably came from the idea that under iron limitation, the physiological state of the cell would be deficient, which would prevent them from taking up silicic acid from very low ambient concentrations. For example, the high  $K_{\text{Si}}$  values observed in the Southern Ocean (up to almost 90 μmol L<sup>-1</sup>) were considered to be biased because of a possible iron limitation (Aumont et al. 2003). Our results clearly show the contrary, i.e., that the  $K_{\text{Si}}$  values decrease with increased Fe limitation. It means that to overcome nutrient

stress and stay competitive at a lower growth rate (lower  $V_{\text{Si-max}}$ ), cells must increase their affinity for the substrate (lower  $K_{\text{Si}}$ ). It can be analyzed as follows:  $K_s$  is one factor determining the affinity of the cell for its substrate. To be of more general application, the specific affinity,  $a_s^0$  (Button 1991), which is the slope of the Michaelis–Menten equation at lowest substrate concentration, can be calculated according to Eq. 2:

$$a_s^0 = V_{\text{Si-max}}/K_{\text{Si}}$$

$a_s^0$  for *C. fusiformis* did not vary significantly with inorganic iron concentrations in the medium (the overall mean was  $0.08 \pm 0.02$  for all iron concentrations, Table 1). Thus the  $V_{\text{Si-max}}$  was reduced to match the reduction in cellular growth rate resulting from Fe limitation. Eq. 1 shows that indirectly Fe-induced reductions in  $V_{\text{Si-max}}$  (maximum growth rate) will be matched equally with a reduction in  $K_{\text{Si}}$ . Fe-induced reduced growth rates did not affect the  $\text{Si}(\text{OH})_4$  uptake mechanism directly.

*How do cells increase their affinity?*—Hildebrand et al. (1998) have isolated and characterized five different types of silicic acid transporter (SIT) genes in *C. fusiformis*. The different forms are present in different amounts during cell wall synthesis. They suggested that these different SITs have a specific role in the overall process of transport and may be important in the control of silicic acid uptake. A decrease in  $K_{\text{Si}}$  could thus be due to the synthesis of higher affinity transporters (Martin-Jézéquel et al. 2000).

We have shown in this study that cells reduced their volume under iron stress. This is probably another way for the cells to increase their ability to take up nutrients. Indeed, it led to an increase in the surface to volume ratio, thereby increasing the capacity of solute influx or efflux of the smaller cell, which should be less diffusion limited than larger cells (Thingstad et al. 1998; Raven and Kübler 2002). This effect is not iron specific and has been reported for various limiting factors, both in the field and in cultures. Cell size of *Stephanodiscus minutulus*, a freshwater diatom, was significantly smaller for Si-, N-, or P-limited cultures than for nonlimited cells (Lynn et al. 2000).

In nature, the same trend is observed at the population level. Small phytoplankton (picoplankton and nanoplankton) tend to dominate in oligotrophic, nutrient-poor systems, whereas large microplankton dominate in nutrient-rich upwelling regions. Such modification of cell size has also been observed during fertilization experiments (Ironex II, Soiree), where the supply of micronutrient led to dramatic changes in the phytoplankton community structure, from one dominated by picophytoplankton or nanophytoplankton to one dominated by large diatoms (de Baar and Boyd 2000).

*Possible implications at the global scale*—There is a similar relationship between cell size and iron concentration in both our culture and field fertilization experiments. In addition, there is general agreement about the increase in  $V_{\text{max}}$  in response to iron enrichment. Previous studies did not find any change of  $K_{\text{Si}}$  in response to iron concentration. However, this result is not surprising since half-saturation constants for nutrient uptake are supposed to increase with algal

size due to surface–volume considerations (Moloney and Field 1991). Although these results relate to a single species, there are some reasons to expect a priori the entire diatom community to evolve in the same way.

Over the past 20 years, the possibility of fertilizing the oceans with iron to mitigate the rise in atmospheric  $\text{CO}_2$  has begun to be taken seriously. Short-term, small-scale experiments in the Equatorial Pacific and in the Southern Ocean started this line of thinking. Those experiments were a valuable tool for understanding the regulation of phytoplankton growth and the role of the oceans in the global carbon cycle, although they did not document any clear impact on the export production or a net transfer of  $\text{CO}_2$  from the atmosphere to the deep sea (Johnson et al. 2002; Buesseler and Boyd 2003). Scaled-up ocean fertilization is now being viewed as an attractive (low cost) way to draw significant quantities of  $\text{CO}_2$  out of the atmosphere, but large uncertainties still remain (Chisholm 2000). These uncertainties relate to the cumulative long-term consequences of fertilization that cannot be foreseen from small-scale experiments. For Chisholm, the known consequences and uncertainties of ocean fertilization already far outweigh hypothetical benefits. Our results raise further considerable doubts about the efficiency of such potential large-scale geoengineering projects. Fertilization would increase the  $V_{\text{max}}$ , leading to higher opal export production. However, in contrast to this effect, the concomitant increase of  $K_s$  would tend to increase preformed  $\text{Si}(\text{OH})_4$  concentrations (Heinze et al. 2003). Thus, if  $V_{\text{max}}$  and  $K_s$  have an opposite effect on the export production, there is reason to wonder whether the  $K_s$  changes could override or at least diminish significantly the effect of the increase in  $V_{\text{max}}$  for sequestering  $\text{CO}_2$ .

*Silicic acid uptake rate and the surface to volume ratio of the cells*—Size can play an important role in structuring marine plankton communities (Moloney and Field 1991), and the size spectrum can thus serve as a convenient basis for exploring the functioning of marine planktonic ecosystem. Moloney and Field (1991) developed a size-based model of carbon and nitrogen flows in plankton ecosystems. More recently, Armstrong (2002) presented a new model based on a size spectrum parameterization of plankton, which should be useful in simulating food web transfers and export fluxes in pelagic ecosystems.

We have plotted the variation of the apparent  $V_{\text{Si-max}}$  and  $K_{\text{Si}}$  as a function of the  $S/V$  of the cell (Fig. 8). The decrease of  $\text{app}V_{\text{Si-max}}$  and  $K_{\text{Si}}$  with iron stress is nicely related to the increase of the surface to volume ratio of the cells. Fitting the data with a power law gives  $V_{\text{Si-max}} = 0.05 \times (S/V)^{-4.014}$  ( $r^2 = 0.84$ ) and  $K_{\text{Si}} = 0.64 \times (S/V)^{-3.66}$  ( $r^2 = 0.87$ ).

These relationships allowed us to fit  $V_{\text{Si}}$ , as a function of the  $S/V$  of the cells:

$$V = V_{\text{max},f(S/V)} \frac{\text{Si}}{K_{s,f(S/V)} + \text{Si}} \quad (3)$$

Using this equation, we calculated the silicic acid uptake rate ( $V$ ) in the full range of Si and Fe' concentrations studied and compared it with the silicic acid uptake rate measured ( $V_{\text{measured}}$ ) during the experiment (Fig. 9). We obtained a good correlation ( $r^2 = 0.95$ ) between  $V_{\text{calculated}}$  and  $V_{\text{measured}}$ . These

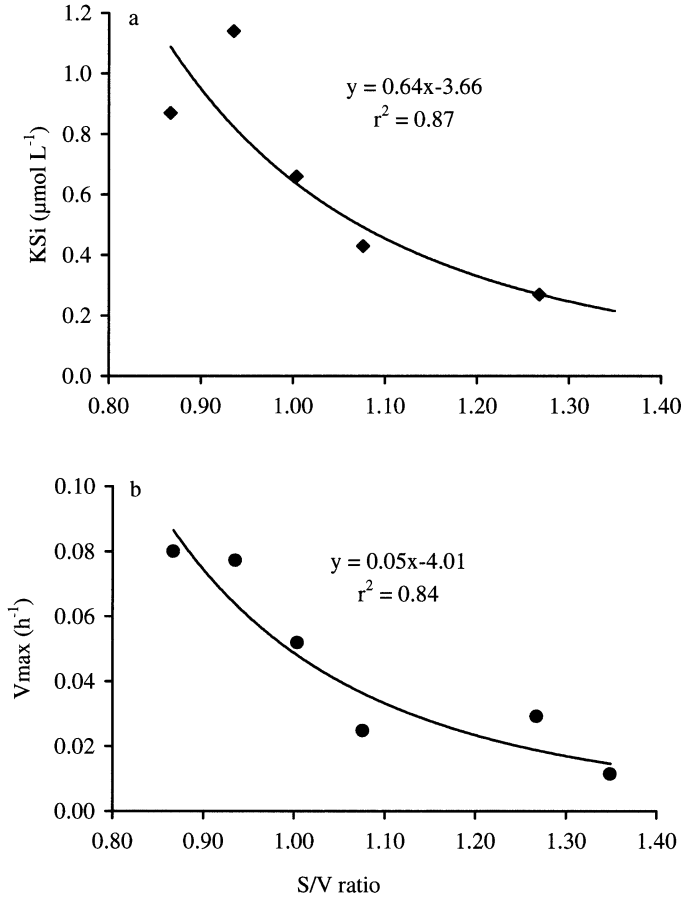


Fig. 8. Plot of (a) the half-saturation constant for silicic acid ( $K_{Si}$ ) and (b) the apparent maximal uptake rate ( $V_{max}$ ), versus the surface to volume ratio ( $S/V$ ) of the cells.

results confirm the strong relationship between the structure of the cell and the physiological parameters for silicic acid uptake. They also show that there is a good potential for the  $S/V$  ratio to be used to describe silicic acid flows in size-based biogeochemical models of planktonic ecosystems, as long as different phytoplankton taxa are identified (Arms-trong 2002).

**Multiple nutrient limitations formulation:** The nutrient co-limitation data set obtained in this study is of interest for biogeochemical models that consider multiple nutrient limitation. Indeed, in most of these models, different functions are commonly encountered: the Liebig law (Lancelot et al. 2000; Pondaven et al. 1999) and the multiplicative model (Loukos et al. 1997). The use of one or the other formulation relies on few experimental data. In this context, the data set presented here can be used to test which of these formulations better reports the observed overall response of the diatom Si uptake rate to an iron-silicic acid colimitation.

Equation 4 assumes that the process rate is proportional to the most limiting nutrient, whereas the multiplicative model (Eq. 5) multiplies the respective limitation on uptake by each nutrient. These two functions were tested, along with a third one that takes into account the variation of  $K_{Si}$  with  $Fe'$  concentrations, as determined in this study (Eq. 6).

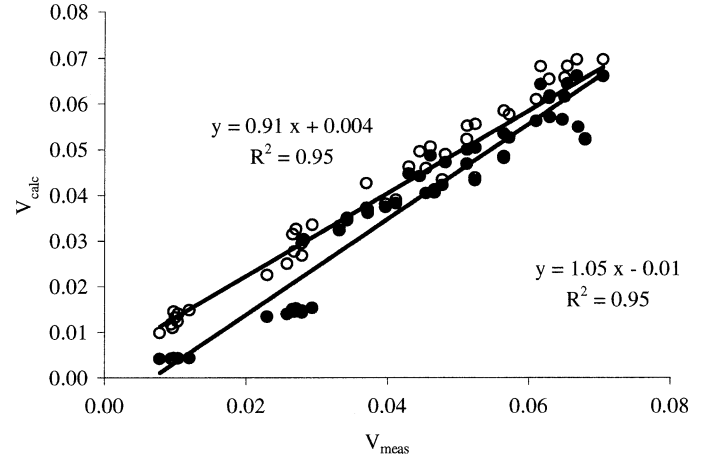


Fig. 9. Relationship between the specific silicic acid uptake rate measured during the experiment ( $V_{meas}$ ) and the  $V$  calculated ( $V_{calc}$ ) as a function of the  $S/V$  ratio of the cells (filled circle) or multiplying the respective limitation on uptake by each nutrient (open circle).

$$V = V_{max} \min\left(\frac{Fe'}{K_{Fe'} + Fe'}, \frac{Si}{K_{Si} + Si}\right) \quad (4)$$

$$V = V_{max} \frac{Fe'}{K_{Fe'} + Fe'} \frac{Si}{K_{Si} + Si} \quad (5)$$

$$V = V_{max} \frac{Fe'}{K_{Fe'} + Fe'} \frac{Si}{K_{Si(Fe')} + Si} \quad (6)$$

Silicic acid uptake rates were calculated using each of these functions over the full range of Si and  $Fe'$  concentrations studied. Then, for each of the data sets obtained, we used the least squares method to estimate  $V_{Si-max}$ ,  $K_{Si}$ , and  $K_{Fe'}$ . Results are presented in Table 2 and compared to the experimentally measured parameters. The three functions gave realistic values for  $V_{Si-max}$ ,  $K_{Si}$ , or  $K_{Fe'}$ . However, they all gave results slightly outside the confidence interval of the  $K_{Fe'}$  value experimentally determined. The minimum function had difficulties in reproducing the  $K_{Si}$  value. The multiplicative model and the function with a variable  $K_{Si}$  both reproduce nicely the experimental  $K_{Si}$  and  $V_{Si-max}$  parameters. Between these two functions, there seems to be very little reason to recommend one over the other. The small differences between results obtained from these two functions

Table 2. The silicic acid kinetic parameters ( $V_{max}$  at  $K_{Si}$ ) estimated from three different formulations of the multiple nutrient limitation, the multiplicative model (multipl, Eq. 5), the Liebig law (Eq 4), and the third one taking into account the variation of  $K_{Si}$  with  $Fe'$  concentrations ( $K_{Si}$  var, Eq. 6), are compared to those determined from experimental data.

	Experimental	Multipl	$K_{Si}$ var	Liebig
$V_{max Si}$	0.081 ( $\pm 0.008$ )	0.077	0.078	0.078
$K_{Si}$	0.86 ( $\pm 0.13$ )	0.80	0.88	1.34
$K_{Fe'}$	0.020 ( $\pm 0.008$ )	0.031	0.036	0.048

show that the range of  $K_{Si}$  variations was not great enough to be significant. In this case, it is probably not worth taking it into account. Whether this remains true when applied to the field is still an open question, since the order of magnitude of  $K_{Si}$  variation in response to iron stress in the field might be much more expanded. Indeed it concerns not only the physiological adaptation of a single species but also the structure and succession of diatom communities. Thus, although we can expect the variation to occur in the same direction, the range of that variation may be much greater. Reported half-saturation constants for silicic acid uptake span at least more than one order of magnitude either for culture studies of single species or in the field (Pondaven et al. 1999; Martin-Jézéquel et al. 2000; Leynaert et al. 2001).

Our study shows that Si uptake kinetics parameters, especially  $V_{max}$ , are strongly related to cell size, which is itself constrained by the degree of iron limitation. In this context, if one wants to model nutrient uptake kinetics, the present study suggests that a simple way to describe the observed variations of Si uptake kinetic parameters within a colimitation context would be either to take into account variations in cell size (Eq. 3) or to implicitly take into account the variations in cell size through a scaling of  $V_{max}$  by the iron-limitation term in a multiplicative manner (Eq. 5). Figure 9 shows that both of these approaches, using either Eq. 3 or Eq. 5, reproduce nicely the observed variations of  $V_{Si}$  over the full range of Si and Fe' concentrations tested in the present study.

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