Effects of iron and zinc deficiency on elemental composition and silica production by diatoms

Christina L. de La Rocha, D. A. Hutchins, Mark A. Brzezinski, Y. Zhang

Vol. 195: 71–79, 2000 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published March 31

Effects of iron and zinc deficiency on elemental composition and silica production by diatoms

Christina L. De La Rocha[1,*], David A. Hutchins[2], Mark A. Brzezinski[3], Yaohong Zhang[2]

[1]Berkeley Center for Isotope Geochemistry, Department of Geology and Geophysics, MC4767, University of California, Berkeley, California 94720-4767, USA

[2]College of Marine Studies, University of Delaware, Lewes, Delaware 19958, USA

[3]Marine Science Institute and Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106, USA

ABSTRACT: The cellular silicon, nitrogen, and carbon content and the kinetics of silicic acid use were determined for *Thalassiosira weissflogii* grown under nutrient-replete, iron-deficient, and zinc-deficient conditions to assess the effect of metal deficiency on diatom silicon metabolism. Iron- and zinc-deficient *T. weissflogii* cells contained 40 and 66% more silicon, respectively, than their nutrient-replete counterparts. Low Zn and low Fe also increased cellular C and N content. Low Zn increased cellular carbon by 55% and cellular N by 41%. Low Fe increased cellular C and N by 68 and 45%, respectively. Fe stress did not alter cellular Si/N ratios significantly, but Si/C ratios declined by 17%. In contrast, Zn stress increased Si/C and Si/N ratios by 41 and 53%, respectively. Both Zn and Fe stress dramatically altered the kinetics of silica production by *T. weissflogii*. Zn deficiency increased the half saturation constant (K_s) 64% and decreased the maximum specific uptake rate (V_{max}) by 60%. In contrast, Fe stress did not affect the value of K_s, but decreased V_{max} by 66%, similar to the decrease observed under low Zn. The decrease in V_{max} in Zn-deficient cells was almost entirely due to the higher biogenic silica content of the metal-deficient cells. The decline in V_{max} under Fe stress resulted from both the increase in cellular silica content and a 50% decline in the cellular uptake rates for silicic acid. The results indicate that Fe and Zn availability can significantly alter silicification in diatoms and affect the number and efficiency of silicon transport molecules in the cell membrane.

KEY WORDS: Diatoms · Silicon · Iron · Zinc · Silica production

INTRODUCTION

Beginning with the first demonstration of iron limitation in the ocean (Martin & Fitzwater 1988) diatoms have repeatedly shown a substantial response to iron inputs, resulting in post iron-addition phytoplankton communities dominated by diatoms. Subsequent work in both oceanic (Martin et al. 1989, 1991, de Baar et al. 1990, Price et al. 1991, DiTullio et al. 1993, Zettler et al. 1996, Takeda 1998) and coastal high-nutrient, low-chlorophyll (HNLC) regions (Hutchins & Bruland 1998, Hutchins et al. 1998) has confirmed this Fe-induced shift in the phytoplankton community towards diatoms. A dramatic drawdown of nitrate and large increases in carbon fixation typically occur in conjunction with Fe-induced diatom blooms. The drawdown of silicic acid should be concomitantly marked, but often it is not. For example, the drawdown of silicic acid occurs to a similar degree in Fe-enriched treatments and controls, which contain no added metals, during shipboard incubations of Fe-deficient waters. This occurs despite the abundant growth of diatoms in the Fe-enriched treatments as compared to the controls (e.g. Martin & Fitzwater 1988). This suggests that Fe affects silicon metabolism in a more complex manner

*Present address: Harvard University, Department of Earth and Planetary Sciences, 20 Oxford St, Cambridge, Massachusetts 02138, USA. E-mail: clla@fas.harvard.edu

than simply an Fe requirement for silicic acid utilization. The above observations suggest, in fact, that Fe hinders silicification, while paradoxically stimulating the growth of diatoms.

The physiological basis for Fe effects on diatom silicon metabolism is poorly understood. Limited laboratory data suggest that the silicon content of diatoms may either increase (Harrison & Morel 1983) or decrease (Takeda 1998) at elevated free ferric ion concentrations. The laboratory experiments of Rueter & Morel (1981) indicate that another metal, zinc, may also be involved in diatom silicon metabolism. Based on an observed increase in the rate of silicic acid uptake with increasing zinc concentration (from 0 to 10 nM) and the observation that zinc-starved diatoms appeared thinly silicified, this study concluded that zinc is required by diatoms for silicification.

In an attempt to further assess the role of Fe and Zn in silicon metabolism, we compared the silicon, carbon, and nitrogen content of Fe- and Zn-deficient and nutrient-replete *Thalassiosira weissflogii* grown in batch culture. To directly evaluate the effect of metal limitation on silicic acid use, we measured the kinetics of Si use of nutrient-replete, low Fe and low Zn cells. The results, which like the field data are at first glance contradictory, yield insight into the roles of Fe and Zn in diatom silicon metabolism.

METHODS

Axenic cultures were maintained in media containing 200 µM NO_3^-, 20 µM PO_4^{3-}, 200 µM $Si(OH)_4$, and Aquil concentrations of trace metals (e.g. 4.51×10^{-7} Fe, 4.00×10^{-9} Zn) and vitamins (Price et al. 1988/89). All nutrient and vitamin stocks were run through a Chelex 100 column prior to use (Rueter & Morel 1981, Price et al 1988/89). Media were made up in oligotrophic Gulf Stream seawater collected using trace metal clean methods and then filtered through 0.2 µm polycarbonate (PCTE) filters (Poretics), passed through Chelex resin, and microwave sterilized (Keller et al. 1988). Preparation of media and handling of cultures were carried out using standard trace metal clean techniques as outlined in Rueter & Morel (1981). Cells were grown under continuous illumination under 'cool white' fluorescent lights at a photon fluence rate of 100 µEin m^{-2} s^{-1}.

Cultures of nutrient-replete (designated Complete), Fe-limited (–Fe), and Zn-limited (–Zn) diatoms were prepared by inoculation of cells into 1 of 3 different seawater growth media. Macronutrient, vitamin, and trace metal additions to these media were the same as in the medium described above except that no Fe was added to the –Fe media, and no Zn to the –Zn media.

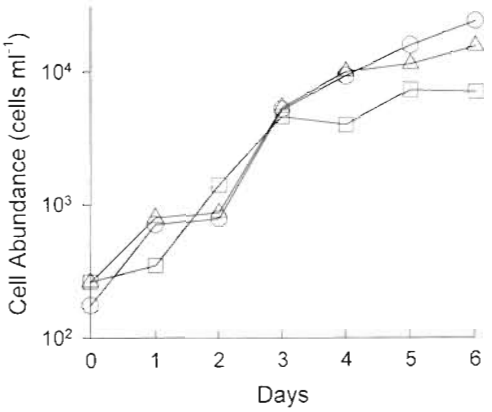

Fig. 1. Log scale plot of the growth of nutrient-replete (○), Fe-stressed (□), and Zn-stressed (△) *Thalassiosira weissflogii* (CCMP 1049) prepared for Si, C, N per cell analysis and silica production experiments. Cells used in the incubations were collected and used on Day 6. Growth curves for cells used in all BSi/cell determinations followed similar patterns

Complete media contained full additions of macronutrients, vitamins, and metals. After several days, cells were transferred using trace metal clean techniques into a fresh batch of the same medium and allowed to grow for several days prior to experimentation to ensure metal limitation in the –Fe and –Zn cultures (e.g. Fig. 1). Four replicate batch cultures were established to measure the change in cellular silicon content of *Thalassiosira weissflogii* induced by Zn and Fe stress. One set was also used to determine the effect of metal deficiency on Si:C and Si:N elemental ratios and the kinetics of Si use. Growth in the cultures was monitored by daily cell counts using a Neubauer type hemacytometer. Specific growth rates of the culture used in the silica production incubation (Culture 4) over the second-to-last day prior to the experimental work on Day 6 (Fig. 1) were 0.53 d^{-1} (Complete), 0.41 d^{-1} (–Fe), and 0.13 d^{-1} (–Zn). Growth rates over the last day before these experiments were 0.41 d^{-1} (Complete), –0.04 d^{-1} (–Fe), and 0.30 d^{-1} (–Zn). Growth in all cultures followed similar patterns (Table 1).

Table 1. Growth rates (d^{-1}) of *Thalassiosira weissflogii* (CCMP 1049) grown under nutrient-replete, Fe-stressed, and Zn-stressed conditions on the second to last day before sampling (μ_2) and on the last day prior to sampling (μ)

Culture	Complete		–Fe		–Zn	
	μ_2	μ	μ_2	μ	μ_2	μ
1	0.60	**0.42**	0.19	**0.39**	0.13	**–0.16**
2	0.44	**0.55**	0.55	**–0.62**	0.18	**0.38**
3	0.51	**0.39**	0.30	**–0.06**	–	–
4	0.53	**0.42**	0.41	**–0.04**	0.13	**0.30**

Each experimental culture was sampled for biogenic silica (BSi) concentration, particulate carbon and nitrogen concentration, and cell abundance. Replicate 25 to 50 ml volumes of culture were filtered onto 0.6 µm PCTE filters for BSi concentration analysis. Filters were rinsed with 0.2 µm filtered seawater, folded into quarters, placed in individual plastic petri dishes, and dried overnight in air at 65°C. BSi on the filters was digested in 0.2 M NaOH at 85°C for 2 h (Paasche 1973), neutralized with 1 N HCl, and diluted. The resulting dissolved silicic acid was quantified via a standard colorimetric analysis (Strickland & Parsons 1972) modified to use a reverse order reagent blank (Brzezinski & Nelson 1986). Two replicate 25 ml samples were filtered through GFF filters for particulate C and N analyses determined according to Sharp (1991), using a Leeman Labs, Inc., CE CHN analyzer (Model 440).

Silica production kinetics were measured on Day 6 using the radioisotope ^{32}Si as a tracer (Brzezinski & Phillips 1997). Cells from the Complete, –Fe and –Zn cultures were gently filtered onto 0.6 µm PCTE filters, and then rinsed with and resuspended in 0.2 µm-filtered and chelexed seawater. Aliquots of the concentrated cells were dispensed into experimental bottles containing 130 ml Complete, –Fe, or –Zn media with 0, 1, 3, 7, 11, 17, 25, or 40 µM silicic acid as sodium metasilicate that had been passed through Chelex resin. The background silicic acid concentration in the oligotrophic seawater was 1.48 µM, and cell additions were adjusted to yield final densities of ~3.2×10^4 cells ml^{-1}. All reported production rate measurements are averages based on 2 duplicate incubations per silicic acid concentration per Complete, –Fe, or –Zn treatment. Three subsamples of concentrated cells per incubation bottle were set aside for BSi analysis. A total of 377 Bq of ^{32}Si were added to each bottle, and the bottles were incubated for 11 h. Incubations were terminated by filtering the cells onto 0.6 µm PCTE filters and rinsing away excess tracer using 0.2 µm filtered and chelexed seawater. Filters were transferred to scintillation vials and prepared for liquid scintillation counting as in Brzezinski & Phillips (1997).

Following Brzezinski & Phillips (1997), BSi produced during the incubation with ^{32}Si (BSi_{new}) was calculated as:

$$BSi_{new} = (Bq\ ^{32}Si_{PSi}/Bq\ ^{32}Si_{tot})[Si(OH)_4] \quad (1)$$

where Bq $^{32}Si_{PSi}$ is the amount of ^{32}Si present in biogenic silica at the end of the incubation, Bq $^{32}Si_{tot}$ is the amount of ^{32}Si added to the sample, and $[Si(OH)_4]$ is the concentration of silicic acid in the sample. Rates of silica production per cell (ρ) were calculated as:

$$\rho = \frac{[BSi_{new}]}{\text{cells } l^{-1} \times t} \quad (2)$$

where t is the incubation time in hours. Specific rates of silica production (V_b) were calculated from ρ and the cellular BSi content (BSi_{cell}) as:

$$V_b = \rho \times BSi_{cell} \quad (3)$$

Kinetic parameters and their associated uncertainty were calculated by fitting the kinetic curves to the Michaelis-Menten equation for enzyme kinetics by nonlinear regression using a procedure that accounts for errors induced by changes in substrate concentrations during incubation, outlined in Del Amo (1996). Values of V_b were plotted against the average values for the silicic acid concentrations,

$$[DSi]_{avg} = \frac{[DSi]_i + [DSi]_f}{2} \quad (4)$$

where $[DSi]_i$ and $[DSi]_f$ are the initial and final dissolved silicon concentrations during the experiment and the mean values for the kinetic parameters, K_s and V_{max}, are calculated by nonlinear regression using the Marquandt-Levenberg algorithm (Press 1992).

Errors associated with the change in substrate concentration during the experiment were determined by first integrating the Michaelis-Menten equation

$$V = V_{max}\frac{[DSi]}{[DSi] + K_s} \quad (5)$$

to give an estimate of the uncertainty on V:

$$\frac{\Delta V}{V_{avg}} = \frac{K_{avg}}{[DSi]_{avg} + K_{avg}} \times \frac{\Delta[DSi]}{[DSi]_{avg}} \quad (6)$$

where ΔV and $\Delta[DSi]$ are the uncertainties in V_{avg} (= V_b as defined in Eq. 3) and [DSi], respectively. K_{avg} is the value of K_s obtained from fitting the V_b (Eq. 3) and $[DSi]_{avg}$ data to the Michaelis-Menten function as described above. $\Delta[DSi]$ is the difference between $[DSi]_{avg}$ and either the initial or final concentration of silicic acid in the incubation:

$$\frac{\Delta[DSi]}{[DSi]_{avg}} = \frac{[DSi]_i - [DSi]_{avg}}{[DSi]_{avg}} \quad (7)$$

Minimum and maximum estimates for V_{avg} (V^- and V^+, respectively) may then be made:

$$V^+ = V_{avg} + \Delta V \quad (8)$$

and

$$V^- = V_{avg} - \Delta V \quad (9)$$

and from them, minimum and maximum estimates for V_{max} and K_s that take changing substrate concentrations into consideration are derived from nonlinear regression of V^+ and V^- against $[DSi]_{avg}$.

RESULTS

Cellular elemental composition

Both iron- and zinc-stressed *Thalassiosira weissflogii* (CCMP 1049) cells consistently contained significantly more silicon per cell than their nutrient-replete counterparts (Table 2; *t*-test, p = 0.04). On average, iron- and zinc-deficient cells contained 1.4 and 1.7 times more silicon, respectively, than those grown under nutrient-replete conditions.

Cellular carbon and nitrogen also increased under metal stress in *Thalassiosira weissflogii* (*t*-test, p = 0.03, Table 3). Low Fe increased cellular carbon and nitrogen by 68 and 45%, respectively. Low Zn produced increases of 55 and 41%, respectively, for cellular carbon and nitrogen. Si/N ratios of Fe-deficient *T. weissflogii* were not statistically different from those of nutrient-replete cells (*t*-test, p = 0.28); however, Si/C ratios declined significantly (*t*-test, p = 0.03), being 17% lower in Fe-stressed cells. Si/C and Si/N ratios of Zn-deficient *T. weissflogii* were higher than those of nutrient-replete cells (*t*-test, p = 0.03), increasing by 41 and 53%, respectively, in response to low Zn availability (Table 3).

Table 2. Cellular silicon content (pmol Si cell^{-1}) of *Thalassiosira weissflogii* (CCMP 1049) grown in batch culture under nutrient-replete, Fe-stressed, or Zn-stressed condtions. Errors are standard deviations on 2 to 3 replicate samples

Culture	Treatment		
	Complete	–Fe	–Zn
1	0.47 ± 0.02	0.90 ± 0.00	0.86 ± 0.01
2	0.58 ± 0.02	0.71 ± 0.01	0.81 ± 0.21
3	0.58 ± 0.04	0.66 ± 0.09	–
4	0.41 ± 0.00	0.57 ± 0.06	0.88 ± 0.02
Average	0.51 ± 0.20	0.71 ± 0.13	0.85 ± 0.04

Silica production kinetics

The kinetic constants from each kinetic experiment are presented in Table 4 with an analysis of the errors associated with the changes in substrate concentration during each experiment presented in Table 5. The error introduced by changes in the substrate concentration is at most ±1.5% for K_s and negligible for V_{max} (Table 5). By comparison the standards errors for the K_s associated with the curve fitting alone are about 1 order of magnitude greater (ca ±10 to 20% of K_s; Tables 4 & 5). All statistical analyses were thus performed using only the standard errors derived from the curve fitting procedure.

Metal stress caused a dramatic decrease in maximum specific silica production rates (V_{max}; *t*-test, p = 0.05 both low Fe and low Zn). V_{max} declined by 66% in low Fe cells and by 60% in Zn-stressed cells compared to the nutrient-replete controls (Fig. 2B, Table 4). In the case of Zn stress, the maximum cellular silica production rate (ρ_{max}) was statistically indistinguishable from that of the nutrient-replete control (*t*-test, p = 0.05). In contrast, the ρ_{max} of Fe-deficient cells was about half that observed under nutrient-replete conditions (Table 4; *t*-test, p = 0.05). The half saturation constant for silica production was not affected by Fe stress (*t*-test, p = 0.05), but increased by 64% under Zn deprivation (Table 4; *t*-test, p = 0.01).

Table 3. Cellular carbon and nitrogen in relation to cell silicon content in nutrient-replete, Fe-stressed, and Zn-stressed *Thalassiosira weissflogii* (Culture 4). Errors are standard deviations between replicate analyses

Treatment	pmol Si	pmol C	pmol N	C/N	Si/C	Si/N
Complete	0.41 ± 0.00	4.4 ± 0.1	0.56 ± 0.00	7.8 ± 0.18	0.092 ± 0.002	0.72 ± 0.00
–Fe	0.57 ± 0.06	7.4 ± 0.2	0.81 ± 0.02	9.1 ± 0.33	0.076 ± 0.008	0.69 ± 0.07
–Zn	0.88 ± 0.02	6.8 ± 0.1	0.79 ± 0.02	8.6 ± 0.25	0.13 ± 0.004	1.1 ± 0.04

Table 4. Kinetic parameters for silica production by nutrient-replete, Fe-stressed, and Zn-stressed *Thalassiosira weissflogii* (Culture 4). Values and standard errors of replicate incubations were calculated using the Marquandt-Levenberg algorithm for non-linear regression (Press 1992)

Treatment	K_s μM	ρ_{max} (fmol Si cell^{-1} h^{-1})	V_{max} (h^{-1})	V_{max}/K_s
Complete	2.35 ± 0.44	6.11 ± 0.25	0.015 ± 0.000	0.006 ± 0.001
–Fe	2.69 ± 0.37	2.88 ± 0.01	0.005 ± 0.000	0.002 ± 0.000
–Zn	3.95 ± 0.54	5.51 ± 0.21	0.006 ± 0.000	0.001 ± 0.000

Table 5. Kinetic parameters for silica production adjusted for changing concentrations of silicic acid. Errors are standard errors calculated using the Marquandt-Levenberg algorithm for non-linear regressions (Press 1992)

Treatment	K_s (μM)		V_{max} (h^{-1})	
	V^+	V^-	V^+	V^-
Complete	2.32 ± 0.43	2.39 ± 0.45	0.015 ± 0.000	0.015 ± 0.000
-Fe	2.68 ± 0.37	2.72 ± 0.38	0.005 ± 0.000	0.005 ± 0.000
-Zn	3.93 ± 0.54	3.97 ± 0.55	0.006 ± 0.000	0.006 ± 0.000

DISCUSSION

Effects of Fe and Zn on cellular silicon content

Other laboratory and field studies support the increased silicification of at least some diatoms under Fe limitation. Martin & Fitzwater (1988) observed significant diatom growth in their field experiments with Fe-amended seawater, and yet no great difference in the drawdown of silicic acid between the Fe-enriched and control treatments, consistent with the increased silicification of *Thalassiosira weissflogii* under Fe stress observed here (Table 2). More recently, Takeda (1998) reported an increase in cellular Si in *Nitzschia* spp. with Fe stress, but the effect was not universal for all 3 species examined. Additionally, Hutchins et al. (1998) observed 2 to 3 times greater silicon use per diatom cell in controls than in +Fe treatments in a in a low Fe coastal region dominated by *Chaetoceros* sp.

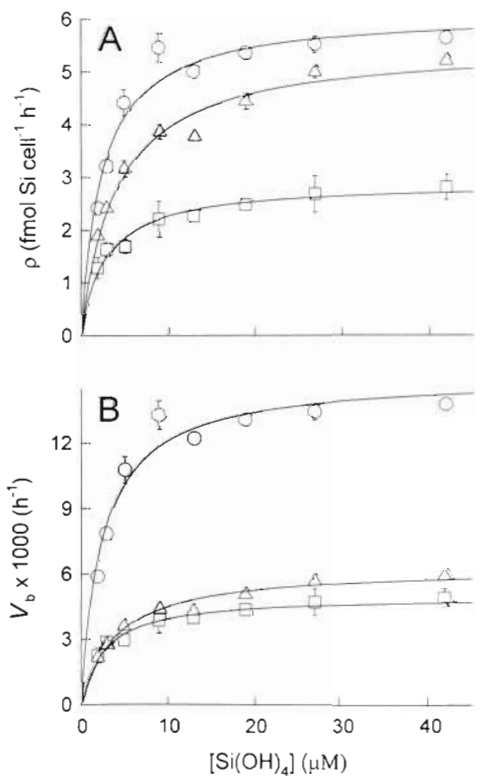

Fig. 2. Silica production by *Thalassiosira weissflogii* is plotted against dissolved silicic acid concentration (μM). (A) fmol Si cell^{-1} h^{-1} and (B) Si produced per biogenic Si h^{-1}. ○: nutrient-replete cells, □: iron-limited cells, and △: zinc-limited cells. Values represent the average of 2 incubations and error bars represent the standard deviations. Absent error bars fall within the boundaries of the symbol. Curves were calculated from the Marquandt-Levenberg algorithm for non-linear regressions (Press 1992)

Zinc-stressed cells also displayed increased cellular silicon content relative to nutrient-replete cells (Table 2). In *Thalassiosira weissflogii* the increase in cellular silicon is more pronounced with Zn stress than with Fe stress, with Zn-stressed cells containing 1.2 times as much silicon as Fe-stressed cells (*t*-test; $p = 0.06$). The increased cellular silicon levels that follow both Fe and Zn stress are unusual and noteworthy. Limitation by macronutrient availability, for example, results in decreased cellular silicon contents, as reported for both silicon-limited and nitrogen-limited cells (e.g. Harrison et al. 1976, 1977).

Fe and Zn stress and cellular carbon and nitrogen

Similar to the case with silicon, cellular carbon and nitrogen contents of metal-stressed *Thalassiosira weissflogii* (CCMP 1049) cells were greater than in their nutrient-replete counterparts (Table 3). These are the first results demonstrating that Zn stress affects diatom elemental composition by increasing cell C and N and increasing Si/C and Si/N ratios. Observation of increased cellular carbon and/or nitrogen in nutrient-stressed diatoms is not unprecedented. Increased cellular carbon and nitrogen has been observed in both Si-limited and Si-starved cells of *Skeletonema costatumco*, *Chaetoceros debilis*, and *T. gravida* (Harrison et al. 1977). High levels of cellular carbon and nitrogen have also been observed in Fe-stressed cells of *S. costatum* (Sakshaug & Holm-Hansen 1977) and the oceanic diatom *Actinocyclus* sp. (Muggli & Harrison 1997). However, these results stand in marked contrast to the decrease in C and N per cell in Fe-limited *T. pseudonana* and *T. weissflogii* observed by Sunda & Huntsman (1995) and Maldonado & Price (1996).

One potential explanation for the increase in cellular carbon and nitrogen is that metal stress may be retarding the cells' ability to divide. This would lead to a greater portion of the metal-stressed cells being in later phases of the cell cycle compared to metal-replete cells. Cells at later stages of the cell cycle would contain more cellular materials than cells that have just divided. Because silicic acid is taken up at specific times rather than uniformly over the life of a diatom cell (Lewin et al. 1966, Eppley et al. 1967, Coombs & Volcani 1968, Azam et al. 1974, Darley et al. 1976, Sullivan 1977, Brzezinski et al. 1990), the same explanation may or may not hold for the observed increase in cellular silicon with metal stress.

Our observation that Fe stress did not affect Si:N ratios disagrees with the observed increase in Si/N utilization in the no-Fe controls in Fe-addition experiments (Hutchins & Bruland 1998, Hutchins et al. 1998, Takeda 1998). However, our observations of elevated Si/C ratios under Fe stress agree with Hutchins et al. (1998), who observed an increase in Si/POC in the no-Fe controls, and Takeda (1998), who inferred increased Si/C ratios in low Fe diatom cultures.

One possible explanation for the difference in the response of diatom Si/C and Si/N ratios in our experiments compared to those of Takeda (1998) and Hutchins et al. (1998) lies in the methods used to acclimate the cells to low metal conditions. Diatoms in the field experiments of Hutchins & Bruland (1998), Hutchins et al. (1998) and Takeda (1998) were either acclimatized to low Fe conditions (<0.13 nM Fe) prior to the experiments or were taken from chronically low Fe waters. Diatoms used in this study were grown into metal stress from metal-replete conditions. Their characteristics thus represent the initial response to low metal availability rather than an adaptation to chronically low Fe concentrations. The short-term physiological response of diatoms to Fe stress appears to be different than their long-term physiological adjustment to low Fe conditions. Alternatively, increased C and N per cell in the metal-limited cultures could be an artifact due to larger amounts of non-living organic debris than in the complete cultures, but no indication of a significant amount of debris was observed under the microscope. Monitoring Si, C, and N uptake with ^{32}Si, ^{13}C or ^{14}C, and ^{15}N is the next step in understanding the Si:C:N ratios of metal-stressed diatoms.

The effect of Fe and Zn deficiency on silica production kinetics

The fact that Fe and Zn stress inhibits the uptake of silicic acid adds further intrigue to the increased silicon content of these cells. Maximum specific silica production rates (V_{max}) are 2.5- to 3.0-fold lower in the metal-stressed cells than in the nutrient-replete cells (Table 4). However, due to the increased cellular silicon content of the metal-stressed cells, reductions in V_b (Fig. 2B) and V_{max} (Table 4) may not be due to a decrease in silica production rates per cell.

Normalization of silica production on a per cell basis reveals that increased cellular silicon under Fe stress only partly accounts for the observed 3-fold drop in V_{max} in the Fe treatment. Additionally, maximum cellular production rates (ρ_{max}) are half what they are in nutrient-replete cells (Table 4), suggesting a reduction in the number of active Si transporters in the cell membrane.

Fe availability did not alter the half saturation constant for silica production (K_s) (Table 4). This suggests that while the number of active Si transporters has decreased, their affinity for silicic acid is not Fe dependent. It may be that Fe is directly required for silicon transport, with Fe serving as a co-factor for silicic acid transport molecules. Alternatively the synthesis of the Si transport system may depend on energy or materials produced by cellular processes such as photosynthesis and respiration that are impacted by Fe limitation.

Changes in silica production kinetics under Zn deficiency differ from those observed under Fe-deficiency. Whereas the decline in maximum production rates under Fe deficiency was due to a combination of changes in cellular silica content and a physiological response to Fe stress, the lower V_{max} value of Zn deficient *Thalassiosira weissflogii* (Table 4) was entirely due to the increased silicon content of the cell under Zn deficiency. ρ_{max} for Zn deficient and nutrient-replete cells of *T. weissflogii* are nearly identical (5.45 ± 0.19 vs 6.01 ± 0.20 fmol Si cell^{-1} h^{-1}), indicating a similar maximum capacity for silicon use under low Zn conditions. At the same time, Zn-limited cells have a decreased affinity (higher K_s) for silicic acid (Table 4).

Rueter & Morel (1981) provided the first evidence that zinc may be linked to silicon metabolism in diatoms. They observed an increase in the uptake rate of a 10 µM pulse of silicic acid as a function of Zn availability in *Thalassiosira pseudonana*. That result is consistent with the observed increase in the K_s for silicic acid uptake with Zn stress. Changes in production rates in the 0 to 10 µM $Si(OH)_4$ range could be the result of an increase in K_s with Zn stress as the K_s for silicic acid uptake in nutrient-replete *T. pseudonana* is 2.3 µM (Nelson et al. 1976). Our results with *T. weissflogii* support the hypothesis that zinc is required for silica production by showing that the affinity of the transport system is zinc dependent (Table 4). In our experiments, Zn-limited cells were nonetheless able to

attain maximum cellular silica production rates nearly equivalent to those of nutrient-replete *T. weissflogii* (ρ_{max}; Table 4), indicating that Zn stress does not diminish the number of active transporters on the cell surface.

Analysis of the kinetic constants shows that Zn and Fe deprivation have similar effects on a cell's ability to compete for silicic acid. Approximating the initial slope of the kinetic curves using the ratios of V_{max}/K_s (Healey 1980) shows that both Zn and Fe stress diminish the initial slope of the kinetic curve compared to metal-replete cells and that the reduction in slope is similar for each metal ion (Table 4). Zn- and Fe-stressed cells would be thus equally impaired when competing for low concentrations of silicic acid. The nearly equal reduction in V_{max} observed under both low Zn and low Fe indicates that Zn- or Fe-stressed cells are also equally inferior to metal-replete cells when competing for high concentrations of silicic acid. Thus, while metal limitation has the potential to significantly alter diatom species succession *in situ*, our results suggest that the effects of Zn and Fe stress will be similar.

Potential advantages of increased cellular silicon

As we have observed for *Thalassiosira weissflogii*, increased cellular silicon may co-occur with the depressed capacity for silica production. We are led by this seemingly paradoxical response to speculate as to the advantage that increased cellular silicon content may confer on metal-stressed cells. One such advantage may lie in the proposed faster sinking rates for these cells.

Diatoms are known to sink in response to nutrient stress (Smayda 1970, Bienfang et al. 1982, Bienfang & Harrison 1984, Harrison et al. 1986) and iron-limited diatoms are known to have higher sinking rates than their nutrient-replete counterparts. For example, Fe-limited cells of the oceanic diatom *Actinocyclus* sp. sink 5 times faster than nutrient-replete cells of the same species (Muggli et al. 1996). Muggli et al. (1996) suggested that large diatoms like *Actinocyclus* sp. actively control their buoyancy through processes that require energy. Hence, as a lack of Fe diminishes a cell's ability to generate ATP (Sakshaug & Holm-Hansen 1977), the sinking rates of the cells increase. The increased silicification observed in some diatoms in response to Fe stress will exacerbate the sinking rate increase, because the density of silica is greater than that of both seawater and organic carbon. It should be noted, however, that increased silicification on its own is not necessarily followed by faster sinking. The physiological state of a diatom cell can have a greater effect on its buoyancy than does its degree of silicification. Si limitation, with its accompanying drop in cellular silicon content, for example, increases sinking rates in diatoms more than either N or P limitation (Bienfang et al. 1982).

Rapid sinking of diatoms may be a mechanism for the preservation of a 'seed population' of cells in cool, dark, subsurface waters once surface conditions have become inhospitable to diatom growth (Smetacek 1985). Another potential advantage of increased sinking rates of nutrient-stressed cells is that it gives them access to the abundant nutrients of the subsurface nutricline (Villareal et al. 1993, Villareal & Carpenter 1994). The 'nutriclines' for iron and zinc occur at similar depths as those for macronutrients (Bruland 1980, Martin et al. 1989). If metal deficiency (or at least Fe deficiency) causes cells to sink, and metal availability allows them to regain control over their buoyancy, this may allow cells to exploit these subsurface supplies of metal.

Implications concerning the silica pump

Dugdale et al. (1995) pointed out that BSi tends to be remineralized at deeper depths in the water column than the nitrogen, carbon, and phosphorous in organic matter. As a result, silica is preferentially 'pumped' from the euphotic zone relative to POC and PON. Dugdale et al. (1995) provided evidence for this process in HNLC regions of the ocean, and proposed that the pumping of silica relative to nitrogen out of surface waters should drive HNLC regions of the ocean towards silicon limitation. Increased silicification of Fe-stressed diatoms should accelerate the onset of Si limitation in low iron HNLC regimes when accompanied by increased the Si/N and Si/P utilization ratios of diatoms (Hutchins & Bruland 1998, Takeda 1998), thereby enhancing the efficiency of the silica pump.

Our results indicate that increases in Si/C and Si/N utilization ratios under low Fe may not be a universal phenomenon among diatoms. This concurs with evidence from recent field experiments. When diatom communities from Fe-replete coastal areas are artificially Fe-limited by adding strong Fe chelators, Si/N and Si/C ratios do not increase as they do in communities from naturally Fe-limited areas (Hutchins et al. 1999). Thus different types of Fe limitation (e.g. chronic vs acute) and the resulting variability in cell physiological state may affect diatom elemental ratios in different ways. Further examination of clones from HNLC regions would be of interest to ascertain whether diatoms from these areas increase their Si/N and Si/C utilization ratios under low Fe more readily than do species from other regions.

One implication of the observed increase in Si/N utilization in low Fe waters (Hutchins & Bruland 1998, Takeda 1998) and the lack of a Si/N increase reported here is that care should be exercised when estimating nitrogen utilization by diatoms from the uptake of dissolved silicic acid in HNLC regions. The commonly used 1:1 mole ratio of Si/N utilization by diatoms (Brzezinski 1985) that has been applied to low Fe waters of the equatorial Pacific (Dugdale & Wilkerson 1998) may be inappropriate when the diatoms are acclimatized to low Fe conditions. Observation of Si/N utilization ratios 2 to 3 times higher in low iron waters (Hutchins & Bruland 1998) suggest that the use of a 1:1 Si/N mole ratio in such cases will overestimate diatom N utilization by 200 to 300%. However, when supplies of Fe are ample or at the onset of Fe-stress, the 1:1 mole ratio for Si/N will still be useful.

Acknowledgements. We thank M. Schmidt, C. M. Preston, R. Shipe, L. K. Crowder, Y Del Amo, V. Franck, R. Ellis, and W. Golden for support and technical assistance, T Villareal for the offhand comment that inspired this investigation, O. Holm-Hansen and 5 anonymous reviewers for helpful comments. This material is based upon work supported by, or in part by, the US Army Research Office under contract/grant number DAAH04-96-1-0043 and NSF grants OCE 9401990 to M.A.B., by University of Delaware startup funds and OCE 9703642 to D.A.H., and a U.C. Berkeley Chancellor's Fellowship awarded to C.L.D.

LITERATURE CITED

Azam F, Hemmingson BB, Volcani BE (1974) Role of silicon in diatom metabolism. VI. Silicic acid transport and metabolism in the heterotrophic diatom *Nitzschia alba*. Arch Microbiol 97:103–114

Bienfang PK, Harrison PJ (1984) Sinking rate response of natural assemblages and subtropical phytoplankton to nutrient depletion. Mar Biol 83:293–300

Bienfang PK, Harrison PJ, Quarmby LM (1982) Sinking rate response to depletion of nitrate, phosphate and silicate in four marine diatoms. Mar Biol 67:295–302

Bruland KW (1980) Oceanographic distributions of cadmium, zinc, nickel, and copper in the North Pacific. Earth Planet Sci Lett 47:176–198

Brzezinski MA (1985) The Si:C:N ratio of marine diatoms: interspecific variability and the effect of some environmental variables. J Phycol 21:347–357

Brzezinski MA, Nelson DM (1986) A solvent extraction method for the colorimetric determination of nanomolar concentrations of silicic acid in seawater. Mar Chem 19: 139–151

Brzezinski MA, Phillips DR (1997) Evaluation of ^{32}Si as a tracer for measuring silica production rates in marine waters. Limnol Oceanogr 42:856–865

Brzezinski MA, Olson RJ, Chisholm SW (1990) Silicon availability and cell-cycle progression in marine diatoms. Mar Ecol Prog Ser 67:83–96

Coombs J, Volcani BE (1968) Studies on the biochemistry and fine structure of silica shell formation in diatoms. Chemical changes in the wall of *Navicula pelliculosa* during its formation. Planta 82:280–292

Darley WM, Sullivan CW, Volcani BE (1976) Studies on the biochemistry and fine structure of silica shell formation in diatoms. Division cycle and chemical composition of *Navicula pelliculosa* during light-dark synchronized growth. Planta 130:159–167

de Baar HJW, Burma AGJ, Nolting RF, Cadee GC, Jacques G, Tréguer P (1990) On iron limitation of the Southern Ocean: experimental observations in the Weddell and Scotia Seas. Mar Ecol Prog Ser 65:105–122

Del Amo (1996) Dynamique et structure des communautés phytoplanctoniques en écosystème côtier perturbé; cinétiques de l'incorporation de silicium par les diatomées. PhD dissertation, Université de Bretagne Occidentale, Brest

DiTullio GR, Hutchins DA, Bruland KW (1993) Interaction of iron and major nutrients controls phytoplankton growth and species composition in the tropical north Pacific Ocean. Limnol Oceanogr 38:495–506

Dugdale RC, Wilkerson FP (1998) Silicate regulation of new production in the equatorial Pacific upwelling. Nature 391:270–273

Dugdale RC, Wilkerson FP, Minas HJ (1995) The role of a silicate pump in driving new production. Deep-Sea Res 42: 697–719

Eppley RW, Holmes RW, Paasche E (1967) Periodicity in cell division and physiological behavior of *Ditylum brightwelli*, a marine planktonic diatom, during growth in light-dark cycles. Arch Microbiol 56:305–323

Harrison GI, Morel FMM (1983) Antagonism between cadmium and iron in the marine diatom *Thalassiosira weissflogii*. J Phycol 19: 495–507

Harrison PJ, Conway HL, Dugdale RC (1976) Marine diatoms grown in chemostats under silicate or ammonium limitation. I. Cellular chemical composition and steady state growth kinetics of *Skeletonema costatum*. Mar Biol 35: 177–186

Harrison PJ, Conway HL, Holmes RW, Davis CO (1977) Marine diatoms grown in chemostats under silicate or ammonium limitation. III. Cellular chemical composition and morphology of *Chaetoceros debilis*, *Skeletonema costatum*, and *Thalassiosira gravida*. Mar Biol 43:19–31

Harrison PJ, Turpin DH, Bienfang BK, Davis CO (1986) Sinking as a factor affecting phytoplankton species succession: the use of selective loss semi-continuous cultures. J Exp Mar Biol 99:19–30

Healey FP (1980) Slope of the Monod equation as an indicator of advantage in nutrient competition. Microb Ecol 5: 281–286

Hutchins DA, Bruland KW (1998) Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime. Nature 393:561–564

Hutchins DA, DiTullio GR, Zhang Y, Bruland KW (1998) An iron limitation mosaic in the California upwelling regime. Limnol Oceanogr 43:1037–1054

Hutchins DA, Franck VM, Brzezinski MA, Bruland KW (1999) Inducing phytoplankton iron limitation in iron-replete coastal waters with a strong chelating ligand. Limnol Oceanogr 44:1009–1018

Keller MD, Bellows WK, Guillard RRL (1988) Microwave treatment for sterilization of phytoplankton culture media. J Exp Mar Biol Ecol 117:279–283

Lewin JC, Reimann BE, Busby WF, Volcani BE (1966) Silica shell formation in synchronously dividing diatoms. In: Cammerson IL, Pedilla GM (eds) Cell synchrony, studies in biosynthetic regulation. Academic Press, New York, p 169–188

Maldonado MT, Price NM (1996) Influence of N substrate on Fe requirements of marine centric diatoms. Mar Ecol Prog Ser 141:161–172

Martin JH, Fitzwater SE (1988) Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. Nature 331:341–343

Martin JH, Gordon RM, Fitzwater SE, Broenkow WW (1989) VERTEX: phytoplankton/iron studies in the Gulf of Alaska. Deep-Sea Res 36:649–680

Martin JH, Gordon RM, Fitzwater SE (1991) The case for iron. Limnol Oceanogr 36:1793–1802

Muggli DL, Harrison PJ (1997) Effects of iron on two oceanic phytoplankters grown in natural NE subarctic Pacific seawater with no artificial chelators present. J Exp Mar Biol Ecol 212:225–237

Muggli DL, Lecourt M, Harrison PJ (1996) Effects of iron and nitrogen source on the sinking rate, physiology and metal composition of an oceanic diatom from the subarctic Pacific. Mar Ecol Prog Ser 132:215–227

Nelson DM, Goering JJ, Kilham SS, Guillard RRL (1976) Kinetics of silicic acid uptake and rates of silica dissolution in the marine diatom *Thalassiosira pseudonana*. J Phycol 12: 246–252

Paasche E (1973) Silicon and the ecology of marine planktonic diatoms. 1. *Thalassiosira pseudonana* (*Cyclotella nana*) grown in chemostats with silicate as the limiting nutrient. Mar Biol 19:117–126

Press WH (1992) Numerical recipies in C: the art of scientific computing, 2nd edn. Cambridge University Press, New York

Price NM, Harrison GI, Hering JG, Hudson RJ, Nirel PMV, Palenik B, Morel FMM (1988/89) Preparation and chemistry of the artificial algal culture medium Aquil. Biol Oceanogr 6:443–461

Price NM, Anderson LF, Morel FMM (1991) Iron and nitrogen nutrition of equatorial Pacific phytoplankton. Deep-Sea Res 38:1361–1378

Rueter JG Jr, Morel FMM (1981) The interaction between zinc deficiency and copper toxicity as it affects silicic acid uptake mechanisms in *Thalassiosira pseudonana*. Limnol Oceanogr 26:67–73

Sakshaug E, Holm-Hansen O (1977) Chemical composition of *Skeletonema costatum* (Grev.) Cleve and *Pavlova* (*Monochrysis*) *lutheri* (Droop) Green as a function of nitrate-, phosphate-, and iron-limited growth. J Exp Mar Biol Ecol 29:1–34

Sharp JH (1991) Total mass and particulate carbon, nitrogen and phosphorus. In: Hurd DC, Spencer DW (eds) Marine particles: analysis and characterization. Geophysical monograph. American Geophysical Union, Washington DC, p 87–91

Smayda TJ (1970) The suspension and sinking of phytoplankton cells in the sea. Oceanogr Mar Biol Annu Rev 8:353–414

Smetacek VS (1985) Role of sinking in diatom life-history cycles: ecological, evolutionary and geological significance. Mar Biol 84:239–251

Strickland JD, Parsons TR (1972) A practical handbook of seawater analysis. Bull Fish Res Board Can 167

Sullivan CW (1977) Diatom mineralization of silicic acid. II. Regulation of $Si(OH)_4$ transport rates during the cell cycle of *Navicula pelliculosa*. J Phycol 13: 86–91

Sunda WG, Huntsman SA (1995) Iron uptake and growth limitation in oceanic and coastal phytoplankton. Mar Chem 50:189–206

Takeda S (1998) Influence of iron availability on nutrient consumption ratio of diatoms in oceanic waters. Nature 393:774–777

Villareal TA, Carpenter EJ (1994) Chemical composition and photosynthetic characteristics of *Ethmodiscus rex* (Bacillariophyceae): evidence for vertical migration. J Phycol 30:1–8

Villareal TA, Altabet MA, Culver-Rymsza K (1993) Nitrogen transport by vertically migrating diatom mats in the North Pacific Ocean. Nature 363:709–712

Zettler ER, Olson RJ, Binder BJ, Chisholm SW, Fitzwater SE, Gordon RM (1996) Iron-enrichment bottle experiments in the equatorial Pacific: responses of individual phytoplankton cells. Deep-Sea Res II 43:1017–1029

Editorial responsibility: Osmund Holm-Hansen (Contributing Editor), La Jolla, California, USA

Submitted: September 20, 1999; Accepted: October 15, 1999
Proofs received from author(s): March 10, 2000