

## The incorporation of zinc and iron into the frustule of the marine diatom *Thalassiosira pseudonana*

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### Abstract

Zinc and iron uptake experiments were conducted with the marine diatom *Thalassiosira pseudonana*, to investigate whether Zn and Fe are incorporated into the frustule of this diatom. Our results show that the uptake and deposition of Zn into opal has a sigmoidal relationship with the free  $Zn^{2+}$  concentration of the culture medium. The amount of Zn incorporated into the opal represents only 1–3% of the total amount of Zn taken up by the diatom; however, the exact reasons for Zn incorporation into the opal are not known. Even so, the consistent relationship between Zn levels in the opal and the culture medium suggests that fossil diatoms may be used as a recorder for historical changes in oceanic free  $Zn^{2+}$  concentrations. These results also demonstrate that the Zn:Si(OH)<sub>4</sub> relationship observed in the Pacific and Antarctic Oceans is not a result of Zn released from biogenic opal. Fe uptake experiments also revealed that Fe is incorporated into diatom opal; however, the amount incorporated appears to be regulated by the diatom and did not increase with increasing Fe concentration within the diatom culture medium. This therefore eliminates the use of Fe incorporated within diatom opal as a possible proxy for dissolved Fe concentrations.

It is well known that biological uptake and regeneration processes control the concentrations of the major nutrients in seawater. Macronutrients such as  $NO_3^-$ ,  $PO_4^{3-}$ , and Si(OH)<sub>4</sub> are frequently considered to limit phytoplankton growth in the surface waters of the ocean because of their low concentrations. There is now mounting evidence to suggest that low surface-water concentrations of certain micronutrients can also limit phytoplankton growth in some regions of the world ocean. For example, Fe, Zn, and Mn are thought to limit phytoplankton growth in the Southern Ocean because of their extremely low dissolved concentrations (e.g., Martin et al. 1990; Morel et al. 1994). Concentration-depth profiles of dissolved Zn and Fe show that these two metals have nutrient-type behavior characterized by surface depletion and an increase in concentration with depth (Bruland et al. 1978; Martin et al. 1990; Johnson et al. 1997).

Interestingly, concentration-depth profiles of dissolved Zn and Si(OH)<sub>4</sub> in the Pacific and Antarctic Oceans reveal that the increase in Zn concentration with depth is very similar to that of Si(OH)<sub>4</sub> (Bruland et al. 1978; Bruland 1980; Martin et al. 1989, 1990), which implies that a significant fraction of Zn is taken up and regenerated from phytoplankton in a manner similar to Si(OH)<sub>4</sub>. However, few studies have been conducted to determine whether Si(OH)<sub>4</sub> users, such as diatoms, deposit significant amounts of Zn into their opal structure. One such study has shown that a significant amount of Zn in phytoplankton is associated with phytoplankton organic tissue but is not rapidly exchangeable in seawater, unlike P, Cu, Cd, Ni, and Mn, which do rapidly exchange into seawater (Collier and Edmond 1984).

Like Zn, Fe has also been linked to the Si(OH)<sub>4</sub> cycle. Shipboard incubation experiments reveal that the addition of Fe to Fe-limited surface seawater increases phytoplankton productivity, with diatoms appearing to benefit most from the added Fe (Martin et al. 1989; Coale et al. 1996). Recently, Hutchins and Bruland (1998) and Takeda (1998) have presented compelling evidence that Fe is directly linked to the Si(OH)<sub>4</sub> cycle. The results from these two studies demonstrate that diatoms grow more slowly in Fe-limited waters and are generally smaller in size than in non-Fe-limited waters. However, Fe-stressed diatoms appear to have thicker frustules than non-Fe-stressed diatoms—that is, the Fe-stressed diatoms contained more opaline silicon per cell (Hutchins and Bruland 1998; Takeda 1998). Thus, like Zn, Fe also appears to play an important role in diatom growth and, in particular, in the formation of their frustule.

To test whether significant amounts of Zn and Fe are incorporated into diatom opal, we cultured the marine diatom *Thalassiosira pseudonana* in trace metal ion-buffered seawater in which the free ion concentrations of either Zn or Fe were systematically varied. Similar experiments were reported by Sunda and Huntsman (1992, 1995) for the uptake of Zn and Fe by phytoplankton; however, in our study, we are concerned with determining whether Zn and Fe are incorporated into diatom opal and whether these two metals play a role in opal formation.

### Materials and methods

The marine diatom *Thalassiosira pseudonana* was obtained from the Portobello Marine Laboratory (Dunedin, New Zealand) and maintained, by use of sterile techniques, in an f/2 culture media until required (Guillard and Ryther 1962). Zn and Fe uptake experiments were carried out in 500-ml polycarbonate bottles that had been extensively cleaned. New polycarbonate bottles (Nalgene) were rinsed with laboratory-grade methanol, followed by Milli-Q water

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Table 1. Nutrient and metal concentration in the diatom culture medium.

Nutrient or metal added	Concentrations within the medium	
	Zn uptake experiments	Fe uptake experiments
NO <sub>3</sub> <sup>-</sup>	73 μM	89 μM
PO <sub>4</sub> <sup>3-</sup>	3.5 μM	3.6 μM
Si(OH) <sub>4</sub>	40 μM	40 μM
Vitamin B <sub>12</sub>	0.9 nM	0.6 nM
Biotin	4 nM	4 nM
Thiamine	0.3 μM	0.1 μM
EDTA	0.10 mM	0.10 mM
Fe	1.0 μM	Various amounts
Zn	Various amounts	105 nM
Cu	40 nM	40 nM
Mo	100 nM	Not added
Mn	123 nM	50 nM
Co	100 nM	40 nM
Se	10 nM	10 nM
Ni	Not added	100 nM

(Millipore; resistivity >18.2 MΩ cm<sup>-1</sup>). The bottles were soaked for a week in 1 M HCl, after which they were rinsed twice with Milli-Q water and placed in a second HCl solution (1% v/v) for a further week. Before the start of each experiment, the polycarbonate bottles were rinsed three times with Milli-Q water. They were not sterilized for Zn and Fe uptake experiments, because autoclaving is a potential source of Zn and Fe contamination (Rueter and Morel 1981).

Although sterile techniques were used for sterilizing the culture medium and during diatom transfers, the potential for bacterial contamination still existed, since the cultures were not axenic. Daily microscopic inspection of each culture revealed no observable growth of bacteria in the Zn uptake experiments (experiments 1 and 2); however, in the first set of Fe uptake experiments (experiment 101), bacteria appear to be present in bottles with a total Fe concentration <100 nM. In the second set of Fe uptake experiments (experiment 102), the culture bottles were microwave sterilized by use of the method described by Keller et al. (1988), which proved successful in eliminating bacterial growth.

All experiments were carried out at 20 ± 2°C, with the bottles loosely capped (Yee and Morel 1996). Lighting was provided by cool white fluorescent lights (120 μmol quanta s<sup>-1</sup> m<sup>-2</sup>) on a 14:10-h light:dark cycle.

The diatom culture medium was prepared from Subantarctic surface seawater collected from either off the Otago coast (45°47.540 S 171°06.126 E) by use of a trace metal clean peristaltic pumping system (Flegal et al. 1991; Kim et al. 1996) or from over the Chatham Rise (45° S, 178°30 E) by use of an acid-cleaned 30-liter Go-Flo bottle suspended on a Kevlar line to a depth of 25 m. The seawater was stored in an acid-cleaned 8-liter polyethylene bottle in a dark room at 4°C until required (Sunda and Huntsman 1992).

To prepare the diatom culture medium, Subantarctic seawater was enriched with nutrients and trace metals. Listed in Table 1 are the metal and nutrient concentrations added to the culture medium. The culture medium was sterilized

by passing it through an acid-cleaned 0.4-μm Nuclepore filter into the acid-cleaned polycarbonate bottles, after which an appropriate amount of Zn or Fe was added to each bottle in a 1:1 ratio with ethylenediaminetetraacetic acid (EDTA). Bottles containing the media were then left to equilibrate for 12–15 h (Price et al. 1989). The pH of the medium for the two sets of Zn experiments (1 and 2) was 7.2 ± 0.1 and 8.3 ± 0.1, respectively, and for the two sets of Fe experiments (101 and 102), the pH was in the range 8.0 ± 0.1.

Before Zn and Fe uptake experiments were conducted, *T. pseudonana* was transferred into a bottle containing the experimental medium, without any added Zn for the Zn uptake experiments or without any added Fe for the Fe uptake experiments (Sunda and Huntsman 1992, 1995). This allowed *T. pseudonana* to acclimatize to the polycarbonate bottle and to the reduced metal concentrations within the experimental medium. When *T. pseudonana* reached a cell density of 5 × 10<sup>5</sup> cells ml<sup>-1</sup>, the experimental media were inoculated. After inoculation, cells were counted daily by use of a Spencer haemocytometer, after the addition of Lugols solution to the cell suspension. As the cells neared the end of the exponential phase of growth, they were collected on an acid-cleaned 2-μm Nuclepore filter under a low vacuum. Immediately after collection, the cells were rinsed with trace metal “clean” seawater. The specific growth rate of each bottle was calculated from the slope of a logarithmic plot of cell counts versus time (Sunda and Huntsman 1992).

To determine metal concentrations within diatom organic tissue, each Nuclepore filter was digested with 4 ml of 50% quartz-distilled HNO<sub>3</sub> (Q-HNO<sub>3</sub>) at 75°C in a 5-ml acid-cleaned vial. After 1 h, each vial was removed from the 75°C water bath, centrifuged, and two 1.5-ml aliquots of digest were removed and stored for metal and PO<sub>4</sub><sup>3-</sup> determinations. Before metal determinations were performed, the HNO<sub>3</sub> sample matrix was removed by evaporating the sample to dryness and then redissolving the residue with two 0.5-ml aliquots of 1% v/v of Q-HNO<sub>3</sub>. Phosphate was determined in both Fe experiments but only in the first Zn uptake experiment.

The method used to determine metals within *T. pseudonana* opal is similar that used by Ellwood and Hunter (1999) to determine Zn in fossil diatom frustules. The diatom frustules that remained after the HNO<sub>3</sub> oxidation process were rinsed three times with Milli-Q water and then dissolved by heating to 85°C with 4.5 ml of NaOH (1% w/w; Merck, Suprapur; DeMaster 1991). After 5 h, a 1-ml aliquot of the alkaline digest was quickly removed and saved for Si(OH)<sub>4</sub> determination. The remaining digest was acidified with doubly-distilled Q-HNO<sub>3</sub>.

To determine the blank contribution from chemical reagents, Nuclepore filters, and vials, two filters were treated as samples and carried through the above procedures. Blanks for Co, Cu, Mn, and Fe were all below the limit of detection by graphite furnace atomic absorption. A Zn blank was detected for the digestion step, but its concentration was well below sample concentrations (<5%). No Zn blank was detected in the alkaline digest (limit of detection for Zn = 0.035 μg L<sup>-1</sup>).

Metal determinations were carried out, after appropriate dilution, by graphite furnace atomic absorption on a Perkin-

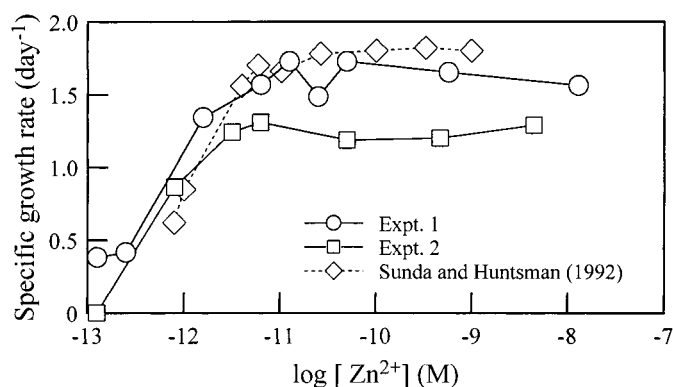


Fig. 1. Specific growth rate of *T. pseudonana* versus free  $Zn^{2+}$  concentration of culture medium. Also shown are results reported by Sunda and Huntsman (1992) for the same diatom species grown under similar conditions.

Elmer 4100ZL spectrometer with Zeeman background correction. Working standards were matrix matched to samples and were made by dilution of commercially available standards (Ajax Chemicals). Standards and samples were all measured in duplicate with excellent reproducibility ( $\pm 5\%$ ).

For the  $PO_4^{3-}$  determination, 0.5 ml of the  $HNO_3$  ( $PO_4^{3-}$ ) aliquot was neutralized with NaOH and then digested with 2 ml of  $K_2S_2O_8$  (5% w/v) for 1 h, to oxidize any organo-phosphate compounds present (Menzel and Corwin 1965). Samples were diluted to 30 ml with distilled water, and the concentration of  $PO_4^{3-}$  was determined by use of the method described by Parsons et al. (1984).

$Si(OH)_4$  was determined by the method described by Korolett (1976), after the sample was diluted with distilled water.

Free metal ion concentrations within the culture medium were calculated from total metal concentrations and their extent of complexation with EDTA by use of the speciation program MINEQL (Westall et al. 1979), with metal-EDTA equilibrium stability constants taken from Martell and Smith (1974). Before calculations were performed, metal-EDTA stability constants were corrected to an ionic strength of 0.6 M by use of the Davies equation. The final free metal ion concentrations computed for Zn uptake experiments were  $\log[Cu^{2+}] = -13.5$ ,  $\log[Co^{2+}] = -10.6$ , and  $\log[Fe^{3+}] = -19.1$ . In the Fe uptake experiments, the corresponding values were  $\log[Cu^{2+}] = -13.5$ ,  $\log[Mn^{2+}] = -8.5$ , and  $\log[Zn^{2+}] = -10.8$ . The dissolved inorganic Fe (III) concentration ( $Fe'$ ) in the Fe uptake experiments was calculated by multiplying the total Fe concentration within the culture medium by the conditional formation, dissociation, and photodissociation ratio of  $[Fe']:[Fe_{total}] = 0.00166$  for the interaction of Fe with EDTA in seawater irradiated on a 14:10-h light:dark cycle (Sunda and Huntsman 1995). Note that we ignored the precipitation of Fe hydroxides at  $Fe'$  concentrations  $>700$  pM (Sunda and Huntsman 1995). In the Zn uptake experiments, the background concentration of Zn was  $<0.05$  nM, whereas in the Fe uptake experiments, the background concentration of Fe was 0.4 nM.

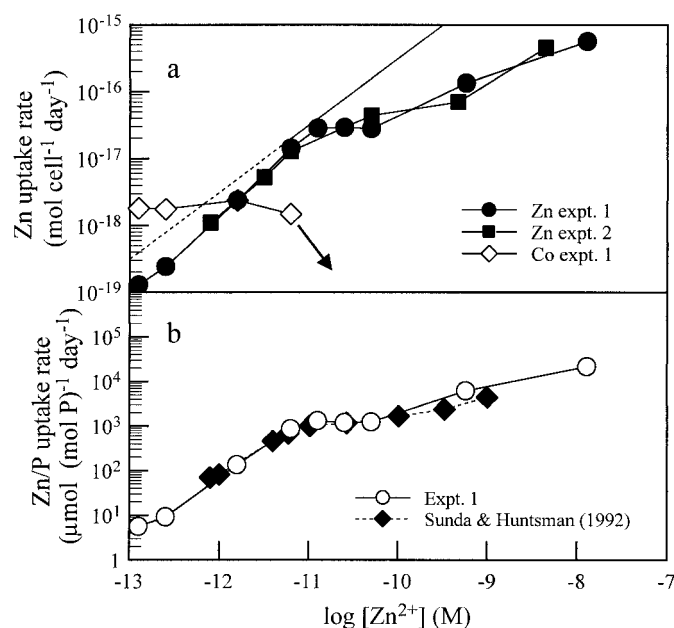


Fig. 2. (a) The cellular uptake rate of Zn and Co into *T. pseudonana* as a function of the free  $Zn^{2+}$  concentration of the growth medium. The straight line is the maximum uptake rate of Zn for *T. pseudonana* under diffusion-limited conditions. This line was calculated from the equation  $\rho = 4\pi rD[Zn^{2+}]$ , where  $\rho$  = maximum diffusion rate,  $D$  is the diffusion rate constant for  $Zn^{2+}$  at  $20^\circ C$  ( $6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ ), and  $r$  is the radius of a spherical diatom. For *T. pseudonana*,  $r = 2.1 \mu\text{m}$ . Data for the calculation are taken from Sunda and Huntsman (1992). (b) Specific uptake rate of Zn:P as a function of the free  $Zn^{2+}$  concentration in the growth medium for experiment 1 cultures. Values calculated from the results of Sunda and Huntman (1992) for the same diatom species are also presented.

## Results

*Effects of  $[Zn^{2+}]$  on growth rate, Zn uptake rate, and Zn/Si in opal.*—The specific growth rates of *T. pseudonana* grown at various free  $Zn^{2+}$  concentrations are presented in Fig. 1, along with results reported by Sunda and Huntsman (1992) for the same diatom grown under similar conditions to ours. In all cases, the results showed maximal growth rates independent of free  $Zn^{2+}$  concentration in the medium when the latter was  $>10^{-11}$  M and decreasing growth rates at lower free  $Zn^{2+}$  concentrations. At the lowest free  $Zn^{2+}$  concentration ( $10^{-12.9}$  M), the growth rate was at most 25% of the maximal rates.

Figure 2a shows the measured cellular uptake rate of total Zn (i.e., total Zn cell $^{-1}$  day $^{-1}$ ) as a function of free  $Zn^{2+}$  concentration, along with an estimate of the maximum uptake rate that is fixed by the rate of  $Zn^{2+}$  diffusion from solution to the cell surface. Our Zn uptake data agree well with Sunda and Huntsman's (1992) that, at low free  $Zn^{2+}$  concentrations, the rate of Zn uptake is limited by diffusion, whereas at higher Zn levels, *T. pseudonana* was able to regulate the uptake of Zn, and thus the Zn uptake rate becomes relatively constant at  $2\text{--}5 \times 10^{-17}$  mol Zn cell $^{-1}$  day $^{-1}$ . The opposite occurred for the uptake of Co by the diatom. Co uptake decreased at free  $Zn^{2+}$  concentrations  $>10^{-11.8}$  M,

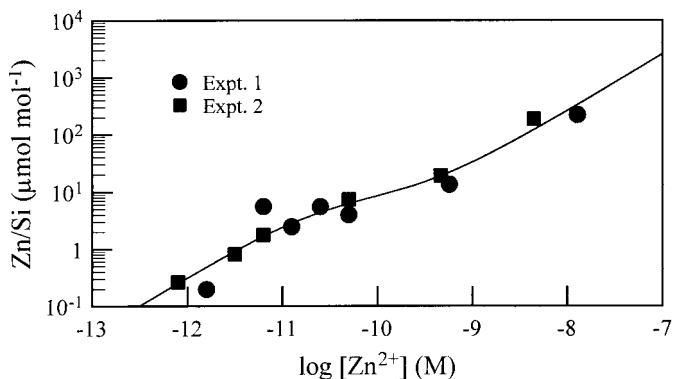


Fig. 3. Zn:Si in opal of *T. pseudonana* versus free  $Zn^{2+}$  concentration of the culture medium. The solid line is the line of fit calculated with use of Eq. 1.

until it was no longer detectable at concentrations  $>10^{-11}$  M. When free  $Zn^{2+}$  concentrations were increased above  $10^{-9}$  M, the uptake of Zn increased as secondary Zn uptake system became dominant (Sunda and Huntsman 1992). Indeed, comparison of uptake rates of Zn to P indicated excellent agreement with results of Sunda and Huntsman (1992).

Figures 2a, 2b both refer to the total amount of Zn taken up by the diatom cells. Figure 3 shows the results for the specific fraction of Zn that was found in the opal frustules. These Zn concentrations were normalized to Si, to eliminate biasing that might arise from differences in frustule size. For example, when *T. pseudonana* is grown under Zn-limiting conditions, the diatom is smaller in size, compared with diatoms grown under non-Zn-limited conditions. The results in Fig. 3 show that Zn:Si increased with increasing free  $Zn^{2+}$  concentration of the culture medium in a fashion similar to the specific Zn:P uptake rate (Fig. 2b). The amount of Zn incorporated within the opal represented only 1–3% of the total Zn taken up by the diatom in these experiments. Note that for cells grown at free  $Zn^{2+}$  concentration  $<10^{-12}$  M we were not able to detect Zn in the alkaline digest (i.e., Zn concentrations were  $<0.035 \mu\text{g L}^{-1}$ ). To describe the Zn/Si data, we fitted the following equation:

$$\text{Zn:Si } (\mu\text{mol mol}^{-1}) = \frac{A[Zn^{2+}]}{([Zn^{2+}] + B) + C[Zn^{2+}]}, \quad (1)$$

where  $A = 7.6 \mu\text{mol (mol Si)}^{-1}$ ,  $B = 2.5 \times 10^{-11}$  M and  $C = 2.5 \times 10^{10} \mu\text{mol Zn (mol Si)}^{-1} \text{ M}^{-1}$ . This equation is similar to the modified Michaelis-Menten uptake kinetics equation that Sunda and Huntsman (1992) used to describe the uptake of Zn by phytoplankton versus free  $Zn^{2+}$  concentration. A 10% error in free  $Zn^{2+}$  concentration at  $10^{-12}$  M gives a Zn:Si error of  $0.03 \mu\text{mol mol}^{-1}$ , whereas at  $10^{-10}$  M, it gives an error of  $0.5 \mu\text{mol mol}^{-1}$ , and at  $10^{-8}$  M it gives an error of  $25 \mu\text{mol mol}^{-1}$ .

The relationship between the specific uptake rates for total Zn:P and the Zn:Si opal data is further illustrated by Fig. 4, which directly compares the Zn:Si of the opal with the Zn:P of the diatom organic tissue for experiment 1. The data set presented is small; however, there appears to be a

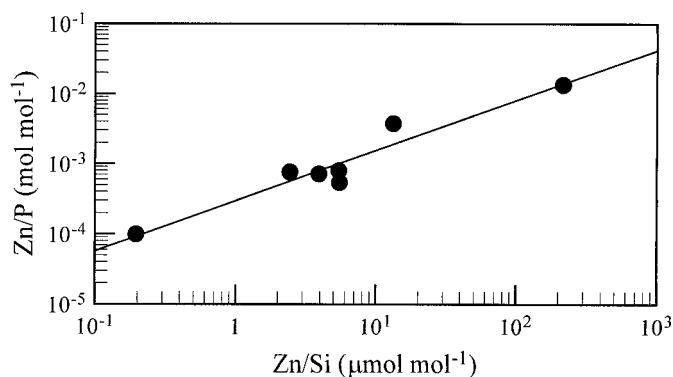


Fig. 4. Zn:P of *T. pseudonana* organic tissue versus Zn:Si of the diatom opal for the cultures used in experiment 1.

consistent relationship between the parameters. Although the amount of Zn incorporated into the opal structure is low, like Zn:P of the organic tissue, it appears to be directly linked to the free  $Zn^{2+}$  concentration of the culture medium. This relationship between Zn:P and Zn:Si also suggests that the Zn being incorporated into the opal structure is from the diatom's internal cellular pool and not from an external source.

*Effect of Zn on Si(OH)<sub>4</sub> uptake rates*—The rates of  $\text{Si(OH)}_4$  uptake for *T. pseudonana* grown at various free  $Zn^{2+}$  concentrations are presented in Fig. 5. At free  $Zn^{2+}$  levels  $>10^{-11}$  M  $\text{Si(OH)}_4$  uptake was relatively constant at  $\sim 38 \text{ mol cell}^{-1} \text{ day}^{-1} (\times 10^{16})$ ; however, when free  $Zn^{2+}$  concentrations were decreased to  $10^{-12.9}$  M, the rate of  $\text{Si(OH)}_4$  uptake decreased to  $\sim 4 \text{ mol cell}^{-1} \text{ day}^{-1} (\times 10^{16})$ . A similar decrease was seen when the ratio of Si:P was plotted versus free  $Zn^{2+}$  concentration (Fig. 5). At free  $Zn^{2+}$  concentrations  $<10^{-11}$  M, the Si:P decreased from a maximum of  $2.8 \mu\text{mol mol}^{-1}$  to  $1.4 \text{ mol mol}^{-1}$ , indicating that the uptake of  $\text{Si(OH)}_4$  is reduced at a faster rate than that of phosphate.

*Effect of [Fe'] on growth rate, Fe uptake rate, and Fe/Si*—Growth-rate results for *T. pseudonana* grown at various Fe' concentrations are shown in Fig. 6, along with results reported by Sunda and Huntsman (1995) for the same diatom

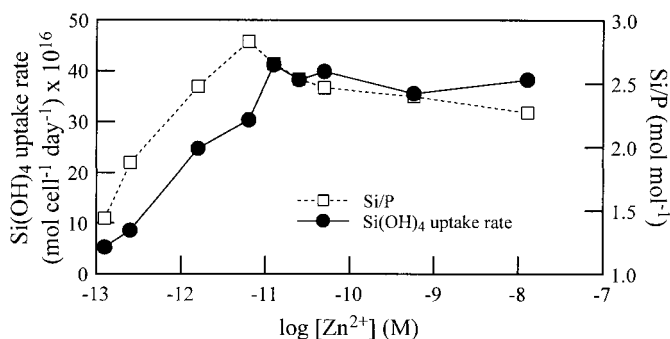


Fig. 5. Si:P versus free  $Zn^{2+}$  concentration and specific uptake rate of  $\text{Si(OH)}_4$  versus free  $Zn^{2+}$  concentration for experiment 1.

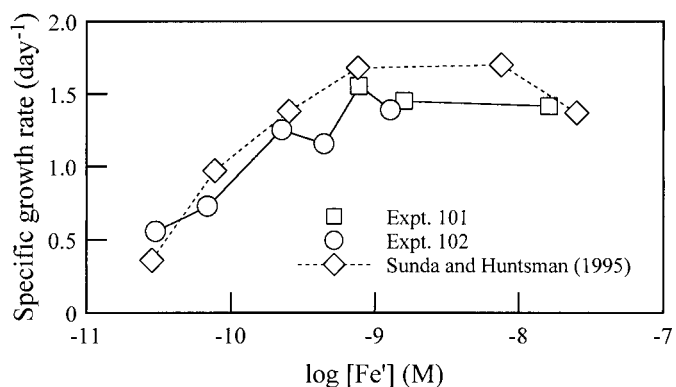


Fig. 6. Specific growth rate of *T. pseudonana* versus  $\text{Fe}'$  concentration of culture medium. Also shown are results reported by Sunda and Huntsman (1995) for the same diatom species grown under similar conditions.

species. The two data sets are in excellent agreement at low  $\text{Fe}'$  concentrations.

The cellular uptake rate of  $\text{Fe}'$  into *T. pseudonana* increased in a linear manner with increasing  $\text{Fe}'$  concentration of the culture medium (Fig. 7a). At no stage did the uptake of  $\text{Fe}'$  by the diatom appear to be diffusion limited. In fact, the cellular uptake rate of  $\text{Fe}'$  by the diatom is only  $\sim 4$ – $6\%$  of the maximum rate that would be expected for a diatom of this size. Similarly, Sunda and Huntsman (1995) found that cellular uptake rates that were 3– $6\%$  of the maximum rate in their experiments. The uptake of  $\text{Fe}'$  into the diatom is thought to be limited by the kinetics of  $\text{Fe}'$  exchange between  $\text{Fe}'$  in solution and the membrane transport ligands which transport  $\text{Fe}'$  into the cell (Hudson and Morel 1990; Sunda and Huntsman 1995).

Figure 7b shows the specific uptake rate of  $\text{Fe}'$  relative to P as a function of  $\text{Fe}'$  concentration, calculated in the same manner as the corresponding Zn:P rates. Also shown are specific Fe:P uptake rates, calculated from the corresponding Fe:C rates reported by Sunda and Huntsman (1995) for *T. pseudonana* grown under similar conditions. At low  $\text{Fe}'$  concentrations—that is,  $\text{Fe}' < 10^{-10}$  M—there is a reasonable agreement between our  $\text{Fe}'$  uptake results and those of Sunda and Huntsman (1995). At  $\text{Fe}' > 10^{-10}$  M the two data sets diverge somewhat, possibly because Sunda and Huntsman (1995) measured intracellular  $\text{Fe}'$ , whereas we measured total  $\text{Fe}'$ . Our method would include  $\text{Fe}'$  hydroxides adsorbed onto cell walls; however, we were unconcerned by the adsorption of  $\text{Fe}'$  onto the cell surface, because the aim of these experiments was to investigate the incorporation of  $\text{Fe}'$  into biogenic opal. The strong acid ( $\text{HNO}_3$ ) digestion step eliminates surface  $\text{Fe}'$  and  $\text{Fe}'$  within the organic tissue of the diatom (Ellwood and Hunter 1999).

Our study addresses the amount of  $\text{Fe}'$  incorporated into the opal structure and whether this  $\text{Fe}'$  is directly proportional to the  $\text{Fe}'$  concentration of the culture medium. Figure 8 shows the concentration of  $\text{Fe}'$  in the opal, expressed as Fe:Si, as a function of the  $\text{Fe}'$  concentration of the medium. The amount of  $\text{Fe}'$  incorporated into *T. pseudonana* opal represented only  $\sim 3\%$  of the total amount  $\text{Fe}'$  taken up by the diatom. Interestingly, the Fe:Si data decreased from  $\sim 50$

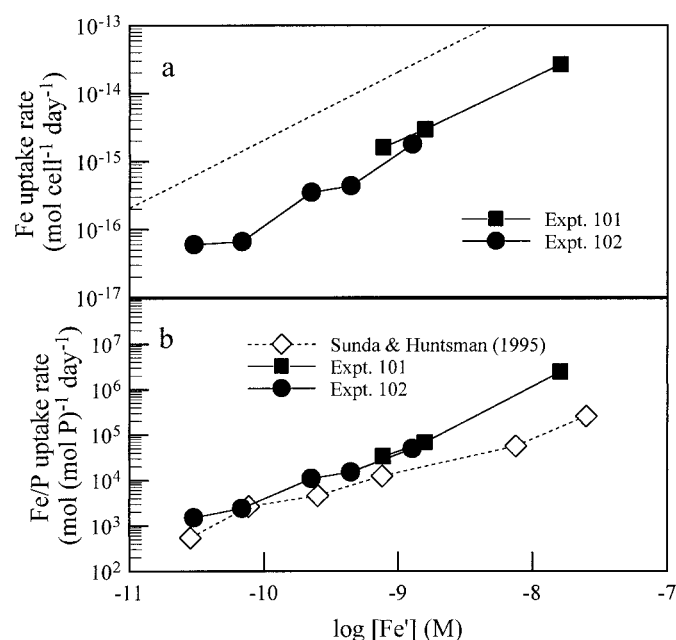


Fig. 7. (a) The cellular uptake rate of  $\text{Fe}'$  as a function of the uncomplexed  $\text{Fe}'$  concentration in the growth medium for experiments 101 and 102. The straight line is the maximum uptake rate of  $\text{Fe}'$  for *T. pseudonana* based on diffusion limitation and was calculated from the equation  $\rho = 4\pi rD[\text{Fe}']$ , where  $D = 9 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  for  $\text{Fe}'$  at  $20^\circ\text{C}$  and  $r = 2.1 \mu\text{m}$  (Sunda and Huntsman 1995). (b) Specific uptake rate of  $\text{Fe}'$  relative to P versus  $\text{Fe}'$  concentration in the culture medium. Values calculated from the results of Sunda and Huntsman (1995) for the same diatom species are presented here.

$\mu\text{mol mol}^{-1}$  at the low  $\text{Fe}'$  concentration ( $10^{-10.5}$  M) to  $\sim 16 \mu\text{mol mol}^{-1}$  at a higher inorganic  $\text{Fe}'$  concentrations ( $10^{-9.7}$  M). At  $\text{Fe}'$  concentrations  $> 10^{-9.7}$  M, the Fe:Si increased again. These results are in contrast to those for Zn (Fig. 3), in which Zn:Si increased consistently with increasing free  $\text{Zn}^{2+}$  concentration, with no minimum in Zn/Si observed.

## Discussion

The results from our Zn and  $\text{Fe}'$  uptake studies agreed well with the radioisotope studies Sunda and Huntsman (1992, 1995) conducted with these two metals (Figs. 2, 7). However, our experiments were extended to show that both Zn and  $\text{Fe}'$  are incorporated into biogenic opal. Indeed, the results here show that both metals are incorporated into diatom frustules but that the amount incorporated is much less than would be predicted from ocean metal–nutrient regeneration models. The results also imply that the mechanisms for the incorporation of Zn and  $\text{Fe}'$  into *T. pseudonana* opal are different.

For Zn, the amount incorporated into the diatom frustule was found to be directly related to the amount of Zn taken up by the diatom and, in turn, to the free  $\text{Zn}^{2+}$  concentration of the medium. For example, if low Zn concentrations were found in diatom organic material, then low Zn concentrations were found in the opal structure (Figs. 2b, 3). The variability seen in the measured Zn:Si ratios is not due to

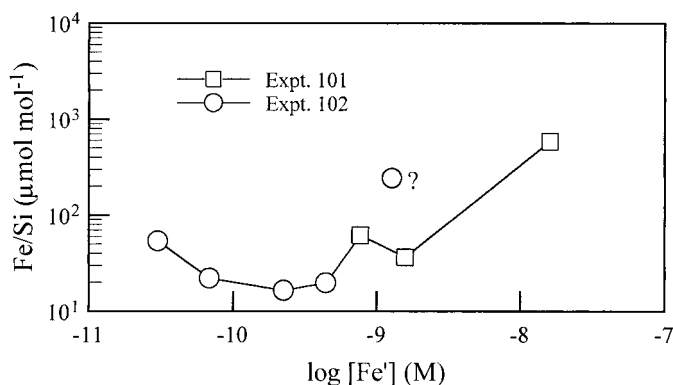


Fig. 8. Fe:Si ratio for opal of *T. pseudonana* versus Fe' concentration of the culture medium. The point marked “?” is suspect, since this sample contained elevated amounts of both Fe and Zn, possibly indicating that the frustule remains were not rinsed properly with Milli-Q water after the HNO<sub>3</sub> oxidation process or that the sample was contaminated with Fe and Zn in some way. All other samples had a low but constant amount of Zn.

analytical artifacts during the measurement of Zn and Si (analytical reproducibility was  $\pm 5\%$ ) but is more probably due to environmental factors.

A study of the dissolution of diatom frustules separated from marine sediments found that Zn is released at the same rate a Si during dissolution, suggesting that Zn is evenly distributed throughout the opal structure (Ellwood and Hunter 1999). We were not able to carry out such a dissolution study with *T. pseudonana* because of the large sample masses required (20–30 mg of pure opal).

The results presented here also show that the amount of Zn incorporated into diatom opal is much less that would be expected from the Zn:Si(OH)<sub>4</sub> relationship seen down the water column for the Pacific and Antarctic Oceans (Bruland 1980; Martin et al. 1990). Free Zn<sup>2+</sup> concentrations in sur-

face Pacific waters range between  $\sim 1$  and 15 pM (Bruland 1989; Donat and Bruland 1990). With use of the relationship of Zn:Si to free [Zn<sup>2+</sup>] established for *T. pseudonana* (Fig. 3), the Zn:Si ratios for Pacific waters should be correspondingly between 0.3 and 3.2 ( $\mu\text{mol mol}^{-1}$ ). However, the actual total Zn:Si(OH)<sub>4</sub> ratio for the Pacific and Antarctic Oceans averages 58 ( $\mu\text{mol mol}^{-1}$ ), well above this estimate.

One possible drawback in the use of the Zn:Si to free Zn<sup>2+</sup> relationship to predict free Zn<sup>2+</sup> concentrations is that the diatom species we used here is a coastal species. Work by Sunda and Huntsman (1992) showed that coastal phytoplankton tend to have a higher Zn requirement for growth than their open-ocean counterparts. However, overall Zn:C ratios only varied threefold between species, indicating that the total amount of Zn taken up by each species is similar. Indeed, at the lowest free Zn<sup>2+</sup> concentrations, Zn:C ratios only varied twofold. This would suggest the Zn:Si to free [Zn<sup>2+</sup>] relationship of open-ocean diatoms is probably close to that of *T. pseudonana*. The fact that only a small amount of Zn occurs in opal implies that there would be little selective pressure for open-ocean species of reduce the amount of Zn in opal.

The relationship established between Zn:Si and free Zn<sup>2+</sup> concentrations suggests that diatom opal may be a useful paleochemical indicator of oceanic free Zn<sup>2+</sup> concentrations. We explored this idea by estimating free Zn<sup>2+</sup> concentrations for the Southern Ocean and the Pacific Ocean by converting Zn:Si data measured for diatom frustules found in both surface (core top) sediments (Ellwood and Hunter 1999) and for siliceous plankton collected in plankton tows (Martin and Knauer 1973; Collier and Edmond 1984) into free Zn<sup>2+</sup> concentrations using the relationship established in Fig. 3 (Table 2). The sediment samples from the South Atlantic sector of the Southern Ocean have a Zn:Si of  $\sim 3.4$  ( $\mu\text{mol mol}^{-1}$ ), which would correspond to a free Zn<sup>2+</sup> concentration of  $\sim 16$  pM for the surface waters in which these diatoms grew. Si-

Table 2. Zn:Si ratios for opal from core-top sediments (Holocene) and from phytoplankton samples collected in plankton tows from the South Atlantic Ocean and the Pacific Ocean, and the corresponding free Zn<sup>2+</sup> concentrations calculated from each Zn:Si ratio using the Zn:Si versus free Zn<sup>2+</sup> relationship measured for *T. pseudonana* (Fig. 3).

Variable	Position	Zn:Si ( $\mu\text{mol mol}^{-1}$ )	[Zn <sup>2+</sup> ] (pM)
Core data (Ellwood and Hunter 1999)			
AII107-22GGC	54°48S, 03°20W	3.3*	16†
RC13-252	45°05S, 09°09E	3.4*	16†
RC13-259	53°53S, 04°56W	3.4*	16†
RC13-271	51°59S, 04°31E	3.3*	16†
Plankton studies			
Collier and Edmond (1984)	66°44S, 30°00E	2.8	12†
Martin and Knauer (1973)	Northwest Pacific Ocean	1–11‡	3–174†
Measured water column Zn <sup>2+</sup> concentrations			
Donat and Bruland (1990)	North Pacific Ocean (60–150 m)		4–15§
Ellwood and van den Berg (2000)	North Atlantic Ocean (open ocean, 3 m)		6–20

\* Zn:Si data from Ellwood and Hunter (1999).

† Calculated using Zn:Si versus Zn<sup>2+</sup> concentration relationship established for *T. pseudonana*.

‡ Data from table 2 (group I) of Martin and Knauer (1973); reported Zn concentrations in  $\mu\text{g g}^{-1}$  converted to Zn:Si ratio using biogenic opal = SiO<sub>2</sub>·0.3H<sub>2</sub>O (Shemesh et al. 1988).

§ Free Zn<sup>2+</sup> concentrations calculated by use of data for Zn-binding ligand concentrations and stability constants reported for surface waters by Donat and Bruland (1990) using cathodic stripping voltametry.

liceous plankton collected in Southern Ocean waters had a Zn:Si of 2.8 ( $\mu\text{mol mol}^{-1}$ ), whereas for Pacific water the Zn:Si data range between 1 and 11 ( $\mu\text{mol mol}^{-1}$ ). These ratios would correspond to free  $\text{Zn}^{2+}$  concentrations of 12 and 3–174 pM, respectively (Table 2). Actual free  $\text{Zn}^{2+}$  measurements made for the Pacific and Atlantic Oceans range between 4 and 20 pM (Donat and Bruland 1990; Ellwood and van den Berg 2000). This suggests that the diatom-based estimates for free  $\text{Zn}^{2+}$  concentration (Table 2) are probably close to the “true” free  $\text{Zn}^{2+}$  concentrations for Southern Ocean and Pacific waters. This also implies that the Zn content of fossil diatoms may be useful for reconstructing historical changes in seawater free  $\text{Zn}^{2+}$  concentrations. Morel et al. (1994) have suggested that parts of the present-day ocean are Zn limited and that during glacial times Zn limitation was overcome by increased dust inputs to the ocean. Measurements of Zn within diatom frustules spanning the last glacial–interglacial transition will help test this hypothesis.

Why Zn should be incorporated into diatom opal is unknown, but two possible explanations are (1) Zn may be deposited into the opal structure via a passive incorporation process, with the amount deposited purely reflecting the amount present within the diatom’s internal cellular pool; and, alternatively, (2) Zn may play a role in the formation of the opal structure itself. Previous evidence from Rueter and Morel (1981) and Rueter et al. (1981) tends to suggest that Zn is actually serving a specific function in either the transport of Si into the cell or within the cell.

If the incorporation of Zn into diatom opal was a purely passive process, then one might expect other metals to also be incorporated into the opal structure. Apart from Zn and Fe, we not able to detect any other metals (Cu, Co, or Mn) present within biogenic opal. Specifically, one might expect Co to be incorporated into the opal structure when *T. pseudonana* is grown under Zn-limiting conditions, because Co is known substitute for Zn in certain enzymatic processes (Price and Morel 1990; Morel et al. 1994); however, no Co was detected within the opal structure.

The uptake of  $\text{Si(OH)}_4$  by *T. pseudonana* from solution appears to involve a Zn-sensitive enzyme (Rueter and Morel 1981). Rueter and Morel (1981) found that, when the Zn concentration of the culture medium was lowered, the uptake of  $\text{Si(OH)}_4$  also decreased, and when Cu was added to the medium, a similar decrease in the uptake of  $\text{Si(OH)}_4$  was observed. Our results also indicate that the uptake of  $\text{Si(OH)}_4$  also is via a Zn dependent-sensitive active site. We also observed a decrease in the uptake of  $\text{Si(OH)}_4$  at low free  $\text{Zn}^{2+}$  concentrations (Fig. 5). The fact that the Si:P ratio within the cell decreased in a similar fashion to the  $\text{Si(OH)}_4$  uptake rate implies that the decrease in the uptake of  $\text{Si(OH)}_4$  was not just a function of diatom growth but rather of  $\text{Si(OH)}_4$  transport into the cell. If a Zn-based protein/active site is involved in the transport of  $\text{Si(OH)}_4$  into and within the diatom, there is the possibility that the Zn trapped in the  $\text{SiO}_2$  matrix is from such a transport protein being randomly incorporated during opal formation. To date,  $\text{Si(OH)}_4$  transport proteins/ionophores have been isolated, but whether Zn is present within these molecules has not yet been confirmed (Bhattacharyya and Volcani 1980, 1983).

Another possibility is that Zn plays a structural role in the formation of diatom opal. There is strong evidence to suggest that the formation of the opal structure involves an organic templating process of some kind (Hecky et al. 1973; Swift and Wheeler 1992; Shemesh et al. 1993). Again, however, more research is needed confirm whether Zn is involved in such a templating process.

In contrast to Zn:Si, the Fe:Si ratio of the *T. pseudonana* opal only increased at high Fe’ medium concentration. It is particularly interesting that when Fe’ concentrations were decreased from moderately Fe-limiting conditions ( $\text{Fe}' = 10^{-9.5}$  M) to extremely Fe-limited conditions ( $\text{Fe}' = 10^{-10.5}$  M), the Fe:Si ratio within the opal increased (Fig. 8). It is perplexing that the iron content of diatom did not increase with increasing Fe’ concentration, but this does suggest that the amount of Fe being incorporated into the opal structure is being regulated by the diatom. This also eliminates the use of Fe within diatom frustules as a proxy for Fe’.

Fe enrichment experiments involving the addition of Fe to Fe-deficient waters have shown that diatoms benefit most from its addition and that they tend to form more silicified frustules than non-Fe-limited diatoms (Martin et al. 1989; Coale et al. 1996; Hutchins and Bruland 1998; Takeda 1998). It is not known why diatoms should produce thicker frustules when Fe stressed, but perhaps Fe is playing role in maintaining the integrity of shell wall.

## Conclusions

A small fraction of the Zn and Fe taken up by the diatom *T. pseudonana* is incorporated into the opal frustules. However, the metals appear to serve different roles within the opal structure. The concentration of Zn in the opal is directly related to total Zn concentration within the cell and is sigmoidally related to the free  $\text{Zn}^{2+}$  concentration of the culture medium. Zn is incorporated into diatom opal (1) as result of a passive process, (2) to serve a specific function (e.g., as a template ion for frustule formation), or (3) because of involvement in the transport of  $\text{Si(OH)}_4$  within the diatom. The relationship between Zn:Si and free  $\text{Zn}^{2+}$  suggests that the Zn content of diatom shells may be useful in reconstructing changes in oceanic free  $\text{Zn}^{2+}$  concentration.

Unlike Zn, Fe:Si ratios of *T. pseudonana* opal are not directly proportional to Fe’ concentration in the growth medium. This therefore limits the use Fe within diatom opal as a proxy for seawater Fe’ concentration.

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