Iron and zinc effects on silicic acid and nitrate uptake kinetics in three high-nutrient, low-chlorophyll (HNLC) regions

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ABSTRACT: Effects of Fe and Zn availabilities on silicic acid ($Si(OH)_4$) and nitrate (NO_3^-) uptake kinetics in natural phytoplankton assemblages were investigated in 3 high-nutrient, low-chlorophyll (HNLC) regions: off central California, the Southern Ocean and the eastern tropical Pacific. Fe addition significantly increased the maximum potential uptake rate (V_{max}) of $Si(OH)_4$ (Si V_{max}) by 2 to 3 times relative to controls in 8 of 10 experiments. Fe addition significantly increased the maximum potential uptake rate of nitrate (NO_3^- V_{max}) by 2 to 3 times in all 6 experiments measuring the effect of Fe on NO_3^- V_{max}. Four experiments quantified the effect of Zn addition on Si V_{max} and NO_3^- V_{max}. Zn had a significant effect on Si V_{max} in only 1 experiment, increasing values 2-fold relative to controls. Zn addition had significant effects on NO_3^- V_{max} in 3 experiments, increasing NO_3^- V_{max} almost 2-fold in 1 instance and decreasing NO_3^- V_{max} by 23 to 46% in 2 instances. Combined Fe and Zn additions had effects on Si and NO_3^- V_{max} that were similar to those of Fe addition alone. In 4 experiments measuring Fe and Zn effects on the half-saturation constant (K_s) for $Si(OH)_4$ uptake (Si K_s), Fe addition increased Si K_s over 3-fold relative to controls in one experiment and Zn addition decreased it by 61% in another. The effect of Fe and Zn addition on the half-saturation constant for NO_3^- uptake (NO_3^- K_s) could only be measured at 1 location. Here, Fe addition had no effect, and Zn addition alone and in combination with Fe decreased NO_3^- K_s by 68%. $Si(OH)_4$:NO_3^- drawdown ratios calculated from changes in nutrient concentrations during the experiments were very similar to Si V_{max}:NO_3^- V_{max} ratios measured at the end of the experiments using tracers, and the effects of Fe and Zn addition on drawdown ratios paralleled the effects of Fe and Zn on V_{max} ratios. In general, changes in $Si(OH)_4$:NO_3^- drawdown and V_{max} ratios were driven by changes in NO_3^- drawdown and NO_3^- V_{max}. Our data show that Fe and Zn availabilities have a direct effect on $Si(OH)_4$ and NO_3^- uptake kinetics in natural phytoplankton assemblages in HNLC regions, and that changes in kinetic parameters may be driving changes in nutrient drawdown ratios, providing further proof that Fe limitation may accelerate the 'silica pump' and lead to seasonal $Si(OH)_4$ limitation. These results have significant implications for Si and N biogeochemistry and new production in low-Fe regions. Changes in nutrient uptake kinetics can also have important implications for phytoplankton species succession and ecological dynamics.

KEY WORDS: Nutrient uptake kinetics · Iron limitation · Zinc limitation · California · Eastern tropical Pacific · Southern Ocean

INTRODUCTION

Low Fe availability has been shown to limit phytoplankton growth and modify nutrient uptake ratios in high-nutrient, low-chlorophyll (HNLC) waters. New production, which governs carbon flux over seasonal time scales, is usually regulated by NO_3^- availability, although studies have shown that in HNLC regions,

new production may be regulated by $Si(OH)_4$ availability (Dugdale et al. 1995, Dugdale & Wilkerson 1998). Fe limitation can reduce both NO_3^- and $Si(OH)_4$ uptake (e.g. Price et al. 1991, Coale et al. 1996, Boyd et al. 1999, Franck et al. 2000), suggesting that Fe availability may also be regulating new production and C flux in HNLC regions.

While Fe limitation has received a lot of attention, previous laboratory studies have demonstrated that Zn limitation can also limit phytoplankton growth in culture (e.g. Anderson et al. 1978, Brand et al. 1983, Sunda & Huntsman 1992). The link between Zn availability and $Si(OH)_4$ and NO_3^- uptake, and thus new production, is less well known than that for Fe, but there is laboratory evidence that Zn limitation can decrease $Si(OH)_4$ uptake efficiency in diatoms (Rueter & Morel 1981, De La Rocha et al. 2000), and reduce N uptake and protein synthesis in the autotrophic flagellate *Euglena gracilis* (Wacker 1962). These studies suggest that Zn as well as Fe availability might be regulating phytoplankton growth, nutrient drawdown and possibly new production in HNLC waters. Zn limitation has not yet been demonstrated in the field, but this may be because Zn limitation is more prevalent in coastal HNLC regions, which are not as well-studied as open-ocean HNLC regions.

Liebig's law of the minimum sates that co-limitation, where phytoplankton growth and/or nutrient uptake is simultaneously limited by more than one factor, does not occur in natural phytoplankton populations. The possibility for co-limitation by Fe and another micro-nutrient such as Zn, however, has been discussed in theory (Bruland et al. 1991). Fe and macronutrient co-limitation may also occur. If Fe not only regulates phytoplankton growth rates but also has a direct effect on macronutrient uptake kinetics — for example, if Fe availability regulates the maximum capacity (V_{max}) or uptake efficiency (K_s) of a nutrient essential for growth — phytoplankton growth rates may be co-limited by both Fe and nutrient availability. Recent evidence suggests that phytoplankton production in certain HNLC regions may in fact be simultaneously limited by both Fe and $Si(OH)_4$ (Boyd et al. 1999, Franck et al. 2000, Hutchins et al. 2001).

Several studies in HNLC waters have documented that phytoplankton assemblages growing at ambient Fe concentrations have higher $Si(OH)_4:NO_3^-$ drawdown ratios than do those growing in Fe enrichments, despite reduced diatom abundance (Hutchins & Bruland 1998, Takeda 1998). Higher $Si(OH)_4:NO_3^-$ drawdown ratios have the potential to alter regional Si biogeochemistry. For example, the occurrence of high NO_3^-, low $Si(OH)_4$ surface waters, and the potential for Si limitation has been observed in most HNLC regions, including the subantarctic Southern Ocean (Boyd et al.

1999, Hutchins et al. 2001), the subarctic NE Pacific (Wong & Matear 1999), the eastern tropical Pacific (Dugdale et al. 1995) and upwelling centers off central California (Bruland et al. 2001). One explanation for the occurrence of high-NO_3^-, low-$Si(OH)_4$ regions is a process described in Dugdale et al. (1995) termed the 'silicate pump'. The silicate pump works to export $Si(OH)_4$ to deeper waters more efficiently than NO_3^-, due to the faster regeneration rates of N in surface waters relative to Si, and to the enhanced export of Si that can occur when frustules are repackaged into fecal pellets. Higher $Si(OH)_4:NO_3^-$ drawdown ratios, however, could also explain the presence of these low-Fe, low-$Si(OH)_4$, high-NO_3^- HNLC regions (De La Rocha et al. 2000, Firme et al. 2003).

Despite the biogeochemical importance of high $Si(OH)_4:NO_3^-$ drawdown ratios, the processes driving them remain unclear. Several theories have been used to explain usually high $Si(OH)_4:NO_3^-$ drawdown and uptake ratios, including the growth of more heavily silicified diatoms or a reduction in N and C uptake relative to Si uptake. In this paper, we test the hypothesis that a differential effect of Fe on the maximum potential uptake rates of $Si(OH)_4$ and NO_3^- — namely, that low-Fe conditions lead to stronger reductions in NO_3^- uptake capacity relative to Si uptake capacity — could be causing higher $Si(OH)_4:NO_3^-$ drawdown ratios. Field studies were conducted in 3 HNLC regions: upwelling areas off California, the eastern tropical Pacific and the Pacific sector of the Southern Ocean. These regions, although potentially highly productive, in general have high macronutrient concentrations, low dissolved Fe concentrations and relatively low biomass (e.g. Southern Ocean: Martin et al. 1990; eastern tropical Pacific: Coale et al. 1996; California: Bruland et al. 2001). Although the upwelling center off central California is not regarded as a classic HNLC region, areas within this upwelling center consistently have the high concentrations of $Si(OH)_4$, NO_3^- and PO_4^{3-} and relatively low concentrations of phytoplankton biomass that are typical of other traditionally defined HNLC areas (Bruland et al. 2001). In addition, several studies have demonstrated that Fe availability can regulate nutrient drawdown and chlorophyll concentration in these upwelling regions (Hutchins & Bruland 1998, Hutchins et al. 1998, Firme et al. 2003).

In this study we document the first field evidence that both Fe and Zn availabilities can significantly affect $Si(OH)_4$ and NO_3^- uptake kinetics in natural phytoplankton assemblages. The persistently high nutrient concentrations observed in HNLC regions have been attributed to low phytoplankton growth rates, low phytoplankton biomass and the dominance of pico-phytoplankton as a result of micronutrient limitation.

Our data suggest that Fe and Zn availabilities may have a more direct role than previously thought in causing high nutrient concentrations in HNLC regions through the regulation of nutrient uptake kinetics. By regulating NO_3^- uptake kinetics in particular, Fe and Zn availabilities may have a direct influence on the rate of new production and potential C export as well. We suggest that Fe effects on $Si(OH)_4$ and NO_3^- kinetics may also explain the unusually high $Si(OH)_4:NO_3^-$ drawdown and uptake ratios observed in HNLC regions.

MATERIALS AND METHODS

Ten shipboard bottle enrichment experiments were conducted from 1997 to 2000 in waters with variable concentrations of $Si(OH)_4$, NO_3^- and PO_4^{3-}, and relatively low concentrations of dissolved Fe and Zn (Table 1). Experimental locations included 3 areas in an upwelling region off the central California coast (Fig. 1a), 4 areas in the Pacific sector of the Southern Ocean visited during the US JGOFS AESOPS study (Fig. 1b), and 3 upwelling regions in the eastern tropical Pacific (Fig. 1c). In order to measure the effect of Fe and Zn availability on nutrient uptake kinetics, Fe and Zn enrichments were started by incubating whole seawater collected using trace-metal clean techniques in 10 to 20 l carboys on deck, either unaltered (as controls) or with various additions of dissolved Fe and/or Zn. After several days, nutrient uptake kinetics were measured in each enrichment. In general, kinetics parameters were measured in controls and at one concentration of added Fe and Zn. In the Southern Ocean

experiments, however, Si V_{max} was measured at 4 concentrations of added Fe. Concentrations of added Fe and Zn, as well as the kinetic parameters measured, varied among experiments (Table 2). Fe and Zn additions were higher at the sites located off the California coast, as coastal phytoplankton typically have higher metal requirements than open-ocean phytoplankton (e.g. Brand et al. 1983, Sunda et al. 1991, Sunda & Huntsman 1992).

Shipboard enrichment experiments. *California upwelling region:* A total of 3 experiments were conducted in newly upwelled coastal waters at 3 different locations off central California: Big Sur (1997), Pt Arena (1999) and Pt Conception (1999) (Table 1, Fig. 1a). Initial concentrations of total dissolved Fe and Zn (< 0.2 μm fraction) in the seawater used in these experiments varied from 0.03 to 0.10 nM Fe and 0.07 to 0.2 nM Zn (Table 1). Concentrations of dissolved Fe and Zn were quantified at sea under trace-metal clean conditions using cathodic stripping voltammetry (Rue & Bruland 1995, 1997) or in the laboratory using solvent extraction and graphite furnace atomic absorption spectrometry (Bruland et al. 1979).

Seawater for the enrichment experiments was collected from 5 to 15 m depth using a clean pumping system (Hutchins et al. 1998). Unfiltered water was first mixed in a 50 l acid-cleaned carboy, and a subsample of surface water was retained for measurements of *in situ* nutrient and biomass concentrations and $Si(OH)_4$ and NO_3^- uptake rates. Enrichments were conducted in 10 l acid-cleaned polycarbonate carboys. The Big Sur experiment involved 3 enrichment carboys: 1 control (no additions), 1 Fe addition (+10 nM Fe) and 1 Zn

Table 1. Sampling dates, locations and ambient micro- and macronutrient (Fe, Zn, NO_3^-, Si, PO_4) and biomass (chl *a*, particulate organic carbon, POC, and nitrogen, PON, and biogenic silica, $bSiO_2$) concentrations for 10 trace-metal enrichment experiments conducted in waters off the coast of central California, in the Pacific sector of the Southern Ocean and in the eastern tropical Pacific. Southern Ocean ambient Fe, Zn, NO_3^-, PO_4, chl *a*, POC and PON concentrations are from Coale et al. (2003). na: data not available

Region Experiment	Date	Location	Fe (nM)	Zn (nM)	NO_3^- (μM)	Si (μM)	PO_4 (μM)	Chl *a* (mg m^{-3})	POC (μmol l^{-1})	PON (μmol l^{-1})	$bSiO_2$ (μmol l^{-1})
California											
Big Sur	21 Jun 97	37.0°N, 122.0°W	0.10	0.07	12.3	15.1	na	0.54	7.8	0.8	0.2
Pt Arena	25 Jun 99	38.3°N, 124.3°W	0.03	0.2	8.3	3.4	0.4	1.62	19.4	3.1	3.8
Pt Conception	28 Jun 99	35.0°N, 121.5°W	0.06	0.1	8.7	8.8	0.7	1.23	20.1	3.2	2.8
Southern Ocean											
Expt 1	28 Oct 97	62.3°S, 170.0°W	0.10	2.84	31.1	47.2	2.1	0.33	4.0	0.7	0.4
Expt 2	05 Nov 97	59.2°S, 170.0°W	0.06	0.92	26.8	15.4	1.8	0.29	2.8	0.6	0.2
Expt 3	17 Jan 98	67.8°S, 170.1°W	0.02	1.01	25.1	60.8	1.6	0.96	17.2	2.5	3.3
Expt 4	20 Jan 98	62.0°S, 170.1°W	0.11	0.18	22.5	5.1	1.4	0.36	10.3	1.6	5.1
Eastern tropical Pacific											
Costa Rica	30 Aug 00	08.7°N, 090.6°W	0.13	na	4.4	7.2	0.9	0.49	25.5	3.7	0.1
Humboldt	08 Sep 00	03.8°S, 085.3°W	0.10	0.06	11.0	6.3	0.6	0.54	12.9	1.3	0.7
Peru	19 Sep 00	14.3°S, 078.4°W	0.30	0.10	3.2	4.3	0.7	0.90	16.4	1.6	0.4

addition (+2 nM Zn). The Pt Arena and Pt Conception experiments each involved 5 enrichment carboys: 2 controls (no additions), 1 Fe addition (+10 nM), 1 Zn addition (+2 nM) and 1 combined Fe and Zn addition (+10 nM Fe, +2 nM Zn). Metal additions were made from 26 µM inorganic Fe^{3+} and Zn^{2+} stocks prepared in 0.01 N Ultrex HCl. All enrichment carboys were handled under a Class 100 laminar flow hood using trace-metal clean techniques. After filling and handling, enrichment carboys were sealed with parafilm and incubated at surface temperatures and 40% ambient light in flow-though incubators lined with blue plexiglass (Hutchins et al. 1993). Enrichments were incubated for at least 3 d prior to measuring uptake kinetics, as trace-metal bottle enrichments typically show a 1 to 3 d lag period before a response to metal addition is observed. Details of all 3 experiments are listed in Table 2.

Eastern tropical Pacific: Three enrichment experiments were conducted in September 2000 in 3 different regions of the eastern tropical Pacific: the Costa Rica Upwelling Dome, the Humboldt Current region and the Peruvian upwelling region (Table 1, Fig. 1b). Initial dissolved Fe concentrations were 0.10 to 0.30 nM Fe and 0.06 to 0.10 nM Zn (Table 1). No data yet exists for initial dissolved Zn concentrations in the Costa Rica Upwelling Dome experiment.

Experimental methods were almost identical to those in waters off California, except that here all enrichments were replicated in triplicate and concentrations of added Fe and Zn were lower. Briefly, six 10 l acid-cleaned polycarbonate carboys were filled with whole seawater under trace-metal clean conditions. Three enrichment carboys were treated as controls (no additions) and 3 were treated as Fe enrichments (+2 nM Fe). At the Costa Rica Upwelling Dome location, an
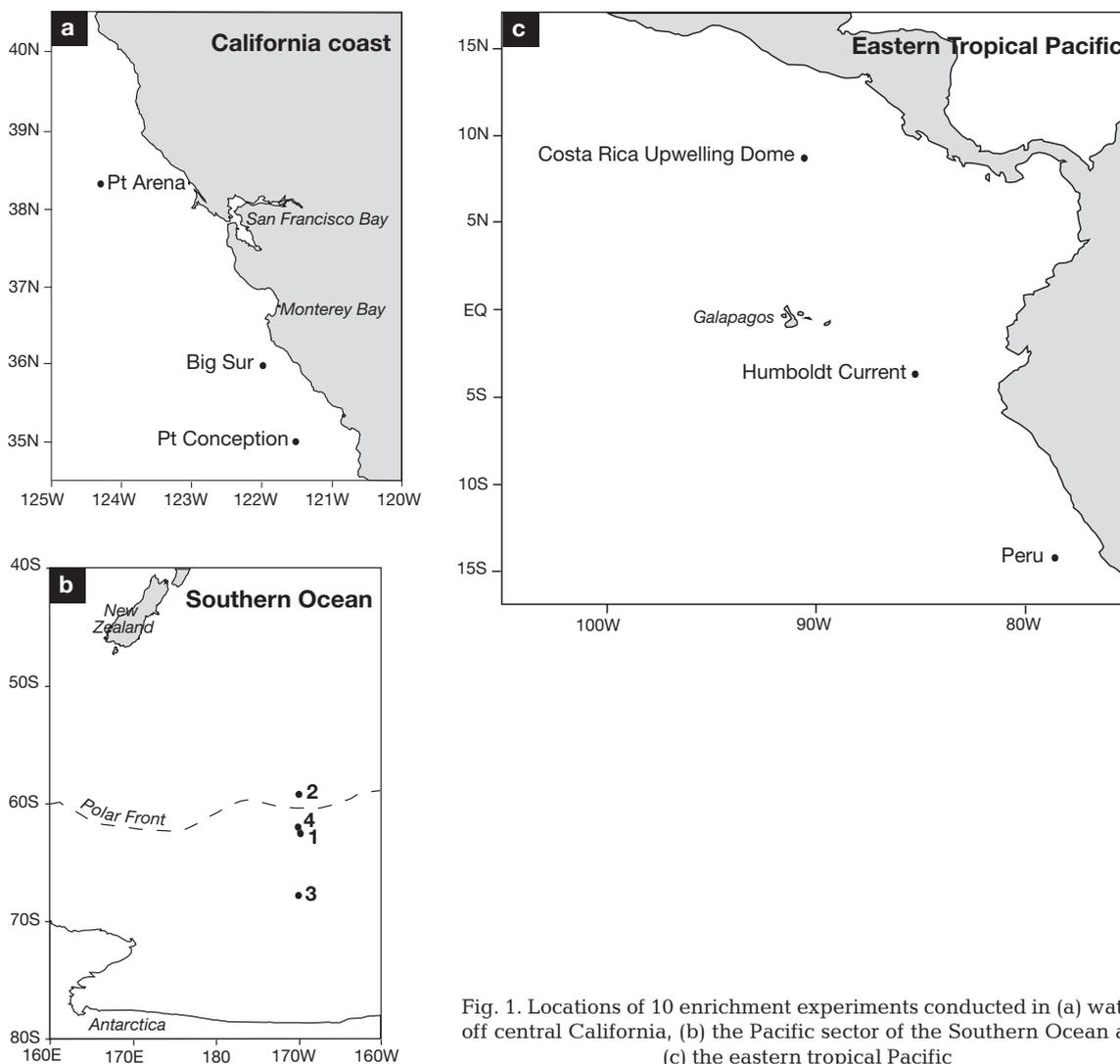

Fig. 1. Locations of 10 enrichment experiments conducted in (a) waters off central California, (b) the Pacific sector of the Southern Ocean and (c) the eastern tropical Pacific

additional 6 enrichment carboys were filled as described above. Three of these were treated as Zn additions (+1 nM Zn) and the remaining 3 were treated as combined Fe and Zn additions (+2 nM Fe, +1 nM Zn). At all locations, carboys were sealed with parafilm and incubated for 3 to 6 d in flow-through incubators on deck with neutral density screening at ~50% ambient light. For additional details, see Tables 1 & 2.

Southern Ocean: Four enrichment experiments were conducted in austral spring and summer 1997–98 in the Pacific sector of the Southern Ocean near the Polar Frontal Zone as part of the US Southern Ocean AESOPS study (Smith et al. 2000): 1 north of the Polar Front (Expt 2), 2 within the Frontal Zone (Expts 1 and 4) and 1 south of the Front (Expt 3) (Table 1, Fig. 1c). Initial dissolved Fe concentrations ranged from 0.02 to 0.06 nM north and south of the front and from 0.10 to 0.11 nM at the front. Initial dissolved Zn concentrations ranged from 0.18 to 2.84 nM. Enrichment experiments were conducted using methods similar to those described in Coale 1991. Whole seawater was collected using trace-metal clean GoFlo bottles suspended on Kevlar line and distributed into 8×20 l acid-washed polycarbonate carboys under Class 100 conditions. Two enrichment carboys were treated as controls (no additions), 4 carboys were treated as Fe additions (+0.2 nM Fe, +0.5 nM Fe, +1.0 nM Fe, +2.5 nM Fe), 1 carboy was treated as a Zn addition (+5 nM Zn) and 1 carboy was treated as a combined Fe and Zn addition (+2.5 nM Fe, +5 nM Zn). Enrichment carboy lids were sealed with plastic bags and incubated at full ambient light in on-deck flow-through incubators for 8 to 16 d. Subsamples for $Si(OH)_4$, NO_3^- and biogenic silica (SiO_2) concentrations, $Si(OH)_4$ and NO_3^- uptake rates and Si V_{max} were withdrawn at 1 to 5 d intervals using clean techniques after an initial lag period of 5 to 8 d. See Table 2 and Franck et al. (2000) for details. Light levels inside the carboys during incubation were higher than those *in situ* (Franck et al. 2000), and white-light photoinhibition could have occurred in the enrichments on clear days. Overcast conditions predominated during each experiment, however.

$Si(OH)_4$ and NO_3^- uptake. Nutrient uptake rates and uptake kinetics were measured using the radiotracer ^{32}Si and the stable isotope ^{15}N. $Si(OH)_4$:NO_3^- drawdown ratios were calculated from changes in $Si(OH)_4$ and NO_3^- concentrations in the enrichments during the course of the experiments. Si uptake kinetics were measured by distributing water from enrichment carboys into 125 to 250 ml acid-cleaned polycarbonate bottles. Bottles received 1 addition of cold $Si(OH)_4$, ranging from 0 to 50 µM, and were then spiked with 1000 Bq of trace-metal clean ^{32}Si (adding <18 nM Si[OH]$_4$). Cold $Si(OH)_4$ additions were made from a trace-metal clean stock solution of Na_2SiO_3. NO_3^- uptake kinetics were measured by distributing water from the same enrichment carboys into 250 to 500 ml acid-cleaned polycarbonate bottles. Each bottle received 1 addition of $^{15}NO_3^-$ ranging from 0.25 to 30 µM. Additions were made from a trace-metal clean stock solution of $Na^{15}NO_3$.

Full $Si(OH)_4$ and NO_3^- kinetic curves, including V_{max} and K_s measurements, were conducted in 4 enrichment experiments: Big Sur, Pt Arena and Pt Conception off California and the Costa Rica Upwelling Dome in the eastern tropical Pacific (Table 2). Curves included 8 concentrations of added $Si(OH)_4$ (0 to 20 µM) and 7 concentrations of added NO_3^- (0.25 to 10 µM) and bottles were incubated for ~7 h, spanning local-time noon.

In the Southern Ocean experiments and in the Humboldt and Peru experiments, K_s values were not measured (Table 2). For the Southern Ocean experiments, $Si(OH)_4$ uptake rates were measured in seawater samples incubated for 24 h at ambient and saturating $Si(OH)_4$ concentrations (+0 µM and +40 to 50 µM,

Table 2. Enrichment volumes, durations and Fe and Zn additions in 10 enrichment experiments conducted off California, in the Southern Ocean and in the eastern tropical Pacific. –: no enrichment done

Region	Experiment	Volume (l)	Duration (d)	Enrichment additions		
				Fe (nM)	Zn (nM)	Fe + Zn (nM Fe/nM Zn)
California	Big Sur	10	3	10	2	–
	Pt Arena	10	3	10	2	10/2
	Pt Conception	10	3.5	10	2	10/2
Southern Ocean	Expt 1	20	5–16	0.2, 0.5, 1.0, 2.5	5	2.5/5
	Expt 2	20	5–8	0.2, 0.5, 1.0, 2.5	5	2.5/5
	Expt 3	20	6–10	0.2, 0.5, 1.0, 2.5	5	2.5/5
	Expt 4	20	8–16	0.2, 0.5, 1.0, 2.5	5	2.5/5
Eastern tropical Pacific	Costa Rica	10	6	2	1	2/1
	Humboldt	10	3	2	–	–
	Peru	10	4	2	–	–

respectively), yielding measurements of ambient $Si(OH)_4$ uptake rates and Si V_{max}. NO_3^- uptake was measured at ambient concentrations only. Measurements were not replicated. Ambient $Si(OH)_4$ and NO_3^- uptake rates for the Southern Ocean experiments have been published elsewhere (Franck et al. 2000). In Humboldt and Peru experiments, $Si(OH)_4$ and NO_3^- uptake rates were measured in triplicate seawater samples incubated for 12 to 24 h at ambient and saturating $Si(OH)_4$ and NO_3^- concentrations (+0 μM and +25 to 30 μM $Si(OH)_4$; +0.25 μM and +15 to 30 μM NO_3^-), yielding measurements of ambient $Si(OH)_4$ and NO_3^- uptake rates and Si V_{max} and NO_3^- V_{max}.

After incubation, samples were vacuum-filtered (<150 mm Hg) onto 0.6 μm polycarbonate filters and allowed to air dry (^{32}Si) or filtered onto pre-combusted Whatman GF/F glass fiber filters (~0.7 mm) and stored frozen in pre-combusted aluminum foil packets (^{15}N). Samples with ^{32}Si were stored until ^{32}Si and ^{32}P reached secular equilibrium (>3 mo) and ^{32}Si activity was assayed according to Brzezinski & Phillips (1997). $Si(OH)_4$ uptake rates were calculated as described therein. Samples with ^{15}N were dried for 3 d at 65°C and atom% ^{15}N was analyzed on a Europa Scientific Tracer Mass or 20-20 mass spectrometer. NO_3^- uptake rates were calculated using equations in Dugdale & Goering (1967). These values compared well with NO_3^- uptake rates calculated using equations adapted from Brzezinski & Phillips (1997).

Kinetics parameters were calculated using the Michaelis-Menten equation:

$$V = \frac{V_{max} \times S}{K_s + S}$$

where V is the uptake rate (d^{-1}), V_{max} is the maximum potential uptake rate (d^{-1}), S is the substrate concentration (μM) and K_s is the half-saturation constant (μM), or the substrate concentration at which V is one-half V_{max}. V_{max} and K_s parameters and their standard errors were estimated by fitting the data points in each kinetic curve to a rectangular hyperbolic non-linear regression using SigmaPlot software (SPSS). Kinetics parameters from metal enrichments were compared with kinetics parameters from corresponding control enrichments using paired Student's t-tests. In all cases but one, data for individual kinetic curves passed normality tests incorporated into the Sigma-Plot non-linear regression analysis. When treatments were replicated, kinetic data from all curves were combined for 1 averaged curve fit. Unpublished data from kinetic experiments conducted in oligotrophic waters off Baja, Mexico show that replicate $Si(OH)_4$ and NO_3^- kinetics using this method yield results with <10% variability.

Phytoplankton community composition and cell counts. In order to determine whether changes in

$Si(OH)_4$ and NO_3^- uptake kinetics could be due to changes in phytoplankton species composition, samples for phytoplankton cell counts were taken in experiments off California (Pt Arena and Pt Conception) and in the eastern tropical Pacific (Costa Rica Upwelling Dome, Humboldt and Peru). Phytoplankton samples were preserved and analyzed using methods to ensure accurate diatom counts, as diatoms are the major contributors to $Si(OH)_4$ uptake and tend to dominate NO_3^- uptake in high-nitrate environments. Whole-water samples were preserved with acid formalin and stored in the dark at 10°C until analyzed (Throndsen 1978). In the laboratory, 10 to 50 ml subsamples were settled in Hydrobios Utermöhl chambers and cells were identified and counted in randomized fields of view using an inverted light microscope (Leitz/Wetzlar Diavert) at 320× magnification, until a total of 400 cells or 20 fields were counted. When further magnification was necessary, valves were acid-cleaned and permanent mounts were made in mounting media with a high index of refraction (MeltMount 1.704, Cargille). Valves were then identified and enumerated at 1000×. Identification was made using Cupp (1943) and Tomas (1997). Standard errors were calculated from replicate fields (5 to 20). *Synechococcus* spp. as well as bacteria are also known to utilize NO_3^- (Glover et al. 1988, Kirchman et al. 1992), although both groups exhibit a strong preference for NH_4^+ and probably rely almost entirely on NH_4^+ as a nitrogen source in low-Fe waters (Kudo & Harrison 1997). For this reason, no effort was made to enumerate picophytoplankton and bacteria in the enrichments.

RESULTS

Values for $Si(OH)_4$ and NO_3^- V_{max} varied widely among the 10 experiments in our study, but fall well within the range of previously reported values from other field and laboratory experiments. Values for Si V_{max} varied almost 30-fold, ranging from 0.049 to 0.699 d^{-1} in the controls and 0.075 to 1.397 d^{-1} in treatments with added Fe and/or Zn (Table 3, Figs. 2 & 3). The lowest Si V_{max} values were observed in the Southern Ocean experiments (overall range 0.049 to 0.471 d^{-1}) (Fig. 2). Other studies on $Si(OH)_4$ uptake kinetics in the Southern Ocean report similarly low V_{max} values (e.g. Nelson et al. 2001). The highest Si V_{max} values (0.699 to 1.397 d^{-1}) were observed in the Costa Rica Upwelling Dome experiment conducted in the eastern tropical Pacific. Values for NO_3^- V_{max} in this study varied almost 40-fold, ranging from 0.109 to 0.299 d^{-1} in the controls to 0.025 to 0.759 d^{-1} in treatments with added Fe and/or Zn (Table 3, Fig. 4). Some of the lowest NO_3^- V_{max} values were observed in the Zn

enrichments. The range in NO_3^- V_{max} values was similar among the 6 experiments conducted in the California and eastern tropical Pacific upwelling zones. There are no NO_3^- V_{max} values from the Southern Ocean experiments for comparison.

K_s values for $Si(OH)_4$ uptake in this study ranged from 0.75 to 7.11 µM (Table 4, Fig. 3), and agree well with the mostly commonly reported Si K_s values in the literature (~0.5 to 5 µM; Martin-Jézéquel et al. 2001, Nelson et al. 2001). Estimates of NO_3^- K_s values ranged from 0.46 to 1.93 µM (Table 4, Fig. 4), again agreeing well with the most commonly reported values for NO_3^- K_s (~0.02 to 4 µM; e.g. Eppley et al. 1969, Kanda et al. 1985, Harrison et al. 1996).

Initial concentrations of dissolved Fe and Zn varied 10- and 40-fold, respectively, in the 10 enrichment experiments in this study, ranging from 0.03 to 0.30 nM Fe and 0.07 to 2.84 nM Zn (Table 1). The central California experiments had some of lowest initial concentrations of dissolved Fe and Zn measured in this study (0.03 to 0.10 nM Fe and 0.07 to 0.2 nM Zn). Many of the highest initial concentrations of dissolved Fe were measured in the eastern tropical Pacific experiments (0.10 to 0.30 nM Fe) and the highest initial concentrations of dissolved Zn were measured in the Southern Ocean experiments.

California upwelling region

$Si(OH)_4$ and NO_3^- uptake kinetics

Figs. 3 & 4 show $Si(OH)_4$ and NO_3^- kinetic curves measured in 3 experiments conducted in waters off California, and Tables 3 & 4 summarize the kinetics parameters calculated from the curve fits shown in the figures. Enrichments included 2 controls, 1 Zn addition, 1 Fe addition and 1 Fe + Zn addition (Table 2). Fe addition significantly increased Si V_{max} in the Big Sur and Pt Arena experiments by 2.4 and 2.8 times, respectively, and significantly decreased Si V_{max} in the Pt Conception experiment, to 59% of the control value (Fig. 3, Table 3). Fe addition significantly increased NO_3^- V_{max} in all 3 experiments off California, by 2.1 to 2.7 times (Fig. 4, Table 3).

Zn addition alone had mixed effects on Si and NO_3^- V_{max}. Zn addition significantly increased Si V_{max} by 3.2 times at the Big Sur site (Fig. 3, Table 3). This was the only experiment off California in which Zn addition alone had a significant effect on Si V_{max}. Zn addition had opposing effects on NO_3^- V_{max} in California waters. In the Big Sur experiment, Zn addition significantly increased NO_3^- V_{max} by 1.8 times, while in the Pt Conception experiment Zn addition significantly de-

Table 3. Comparison of maximum potential uptake rates (V_{max}, d^{-1}) for Si and NO_3^- in Fe and Zn enrichments in experiments conducted off California, in the Southern Ocean and in the eastern tropical Pacific. Errors are standard errors. Enrichments in which V_{max} values differed significantly from control values are printed in bold (2-tailed t-test, $\alpha < 0.05$). In the Southern Ocean experiments, statistical comparisons of Si V_{max} in the Zn and Fe + Zn enrichments could not be done because measurements were not replicated (n = 1). n: number of data points used to estimate V_{max}; –: enrichment and/or measurement was not done

| Cruise Experiment	Si V_{max} Control	Zn	Fe	Fe + Zn	NO_3^- V_{max} Control	Zn	Fe	Fe + Zn
California								
Big Sur	0.408±0.024 (n = 7)	**1.296±0.072 (n = 8)**	**0.960±0.072 (n = 8)**	–	0.288±0.024 (n = 7)	**0.528±0.024 (n = 7)**	**0.696±0.048 (n = 7)**	–
Pt Arena	0.233±0.006 (n = 16)	0.242±0.007 (n = 8)	**0.663±0.041 (n = 8)**	**0.757±0.029 (n = 8)**	0.204±0.010 (n = 11)	0.210±0.008 (n = 5)	**0.560±0.042 (n = 6)**	**0.582±0.021 (n = 6)**
Pt Conception	0.379±0.028 (n = 16)	0.353±0.052 (n = 8)	**0.225±0.012 (n = 7)**	**0.242±0.020 (n = 8)**	0.180±0.012 (n = 6)	**0.098±0.002 (n = 6)**	**0.372±0.018 (n = 7)**	**0.675±0.071 (n = 7)**
Southern Ocean								
Expt 1	0.137±0.010 (n = 2)	0.157	**0.246±0.007 (n = 3)**	0.287	–	–	–	–
Expt 2	0.340±0.008 (n = 2)	0.359	**0.410±0.004 (n = 4)**	0.459	–	–	–	–
Expt 3	0.150±0.018 (n = 2)	0.198	**0.357±0.004 (n = 2)**	0.353	–	–	–	–
Expt 4	0.137±0.001 (n = 2)	0.148	**0.180±0.005 (n = 4)**	0.198	–	–	–	–
Eastern tropical Pacific								
Costa Rica	0.699±0.057 (n = 19)	0.758±0.038 (n = 17)	**1.158±0.103 (n = 14)**	**1.397±0.202 (n = 19)**	0.109±0.010 (n = 12)	**0.025±0.002 (n = 11)**	**0.386±0.082 (n = 17)**	**0.600±0.041 (n = 14)**
Humboldt	0.529±0.073 (n = 9)	–	**0.862±0.098 (n = 9)**	–	0.103±0.014 (n = 9)	–	**0.261±0.009 (n = 9)**	–
Peru	0.537±0.021 (n = 9)	–	**0.373±0.027 (n = 9)**	–	0.299±0.015 (n = 9)	–	**0.759±0.117 (n = 9)**	–
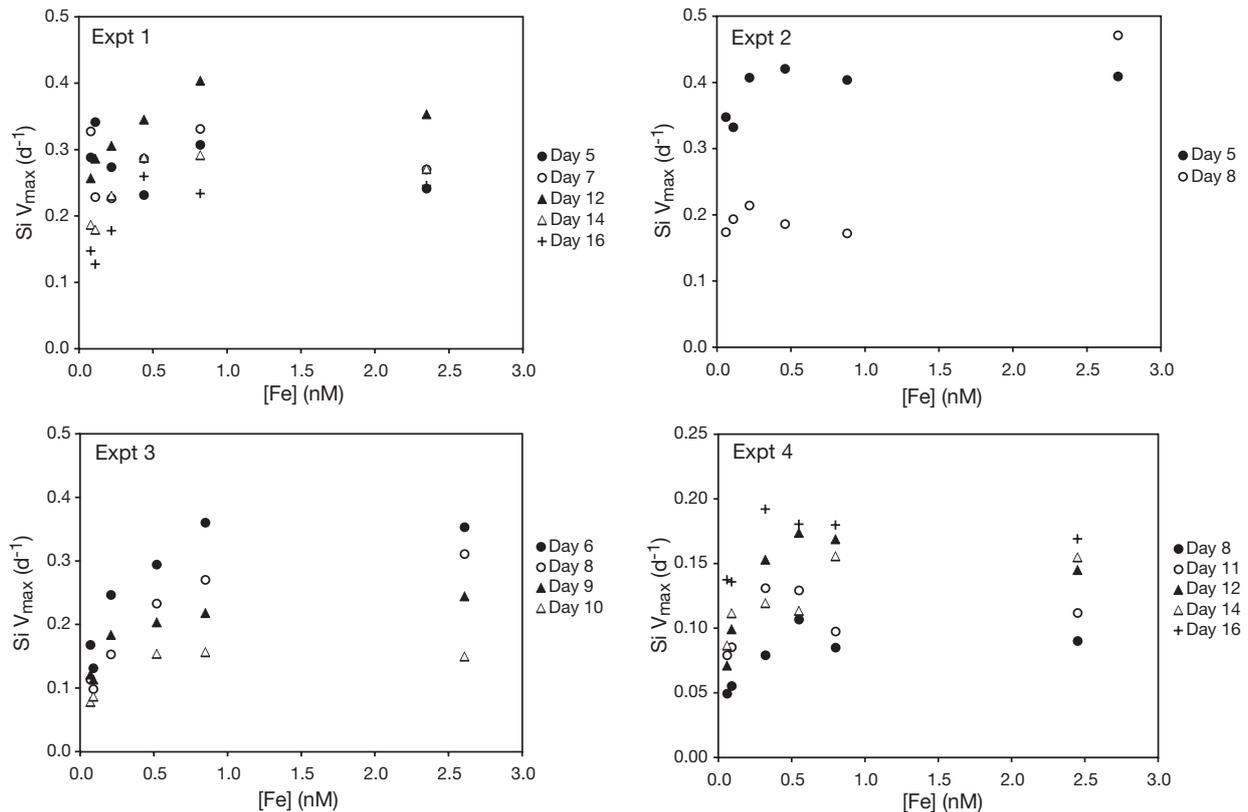
Fig. 2. Maximum potential uptake rate of Si (Si V_{max}) plotted as a function of dissolved Fe concentration 5 to 16 d after Fe addition in 4 enrichment experiments conducted in the Southern Ocean. Symbols indicate days since Fe addition. Note the different scale for Si V_{max} in Expt 4 (lower right panel)

creased NO_3^- V_{max} to 54% of the control value (Fig. 4, Table 3). Combined enrichments with Fe and Zn (Fe + Zn) were conducted at the Pt Arena and Pt Conception locations. The effect of Fe + Zn additions was similar to the effect of Fe addition alone at both locations. Fe + Zn significantly increased Si V_{max} at the Pt Arena site by 3.2 times, and significantly decreased Si V_{max} at the Pt Conception site to 64% of the control value (Fig. 3, Table 3). NO_3^- V_{max} increased by 2.8 and 3.7 times after Fe + Zn addition at the Pt Arena and Pt Conception locations, respectively (Fig. 4, Table 3).

Fe and/or Zn additions had significant a effect on the half-saturation constant for $Si(OH)_4$ uptake (Si K_s) in 2 experiments conducted in waters off California, but the effect varied by location. In the Pt Arena experiment, enrichments with added Fe (Fe, Fe + Zn) had 3.6 and 3.8 times higher Si K_s values, respectively, relative to controls (Fig. 3, Table 4). In the Big Sur experiment, the Si K_s value in the Zn addition was 39% of the control value (Fig. 3, Table 4). The effect of Fe, Zn and Fe + Zn additions on NO_3^- K_s values could not be examined in the experiments off California because ambient NO_3^- values were still saturating (>3.5 µM) in the con-

trol and Zn treatments at the time the kinetic experiments were conducted. It was possible to determine NO_3^- K_s in only 2 experiments, and only in enrichments with added Fe (Fe, Fe + Zn), as NO_3^- concentrations in these enrichments were low enough at the time of sampling to limit uptake (~0.1 µM). These values are reported in Table 4.

$Si(OH)_4:NO_3^-$ drawdown and $Si(OH)_4:NO_3^-$ V_{max} ratios

We sought to compare the effect of Fe and Zn addition on $Si(OH)_4:NO_3^-$ drawdown ratios, calculated from changes in nutrient concentrations over time, with the effect of Fe and Zn on $Si(OH)_4:NO_3^-$ V_{max} ratios (i.e. the ratio of Si V_{max} to NO_3^- V_{max}) in order to determine whether changes in nutrient drawdown ratios could result from changes in nutrient uptake kinetics. Fe addition decreased $Si(OH)_4:NO_3^-$ drawdown ratios in both the Big Sur and Pt Conception experiments off California (Table 5). Fe addition also decreased the $Si(OH)_4:NO_3^-$ V_{max} ratio in the Pt Conception experiment. In the Pt Arena experiment, $Si(OH)_4$ and NO_3^-

concentrations were near the detection limit in the Fe and Fe + Zn enrichments by the time kinetic experiments were done, and thus $Si(OH)_4:NO_3^-$ drawdown ratios in these enrichments represent the initial ratio of $Si(OH)_4$ concentration to NO_3^- concentration in the water collected for this experiment (0.4, Table 1). The $Si(OH)_4:NO_3^-$ uptake ratio in the Pt Arena experiment, measured immediately after water collection using tracers, was somewhat high (1.5) but the final drawdown ratios calculated in the controls were very close to the value reported for trace-metal replete diatoms in culture (~1, Brzezinski 1985). Similarly, $Si(OH)_4:NO_3^-$ V_{max} ratios in this experiment were ~1 in the controls and did not change significantly with either Fe or Zn addition. Contrary to the effect of Fe in the Big Sur and Pt Conception experiments, Zn addition increased $Si(OH)_4:NO_3^-$ drawdown and $Si(OH)_4:NO_3^-$ V_{max} ratios. In the Big Sur enrichment, $Si(OH)_4:NO_3^-$ drawdown and V_{max} ratios increased due to Zn-stimulated increases in $Si(OH)_4$ drawdown and Si V_{max} (Fig. 3, Table 3). In the Pt Conception experiment, the ratios increased due to Zn-stimulated declines in NO_3^- drawdown and NO_3^- V_{max} (Fig. 4, Table 3).

Phytoplankton species composition

Diatoms were the dominant taxonomic group in all the enrichments in the Pt Arena and Pt Conception experiments (Table 6). The most abundant diatoms included *Chaetoceros* spp., as well as *Pseudo-nitzschia* spp. and small (<20 μm) pennate diatoms. In both experiments, total cell numbers were higher in the Fe, Zn and Fe + Zn enrichments than in the controls, and were greatest in the combined Fe + Zn additions. In the Pt Arena experiment, Fe and Zn addition led to similar increases in relative proportion of *Chaetoceros* spp., from 67% in the controls to 80 to 83% in the metal additions, and to slight decreases in the relative abundance of *Pseudo-nitzschia* spp., from 13 to 4–12%. This produced a higher proportion of centric diatoms in the Fe and Zn enrichments relative the controls: 79 to 83% versus 65%. In the Pt Conception experiment, *Chaetoceros* spp. and *Pseudo-nitzschia* spp. were again the dominant phytoplankton groups. *Rhizosolenia* spp. were also present in this experiment, accounting for 0.3 to 4% of total cells. The large size and surface area of *Rhizosolenia*
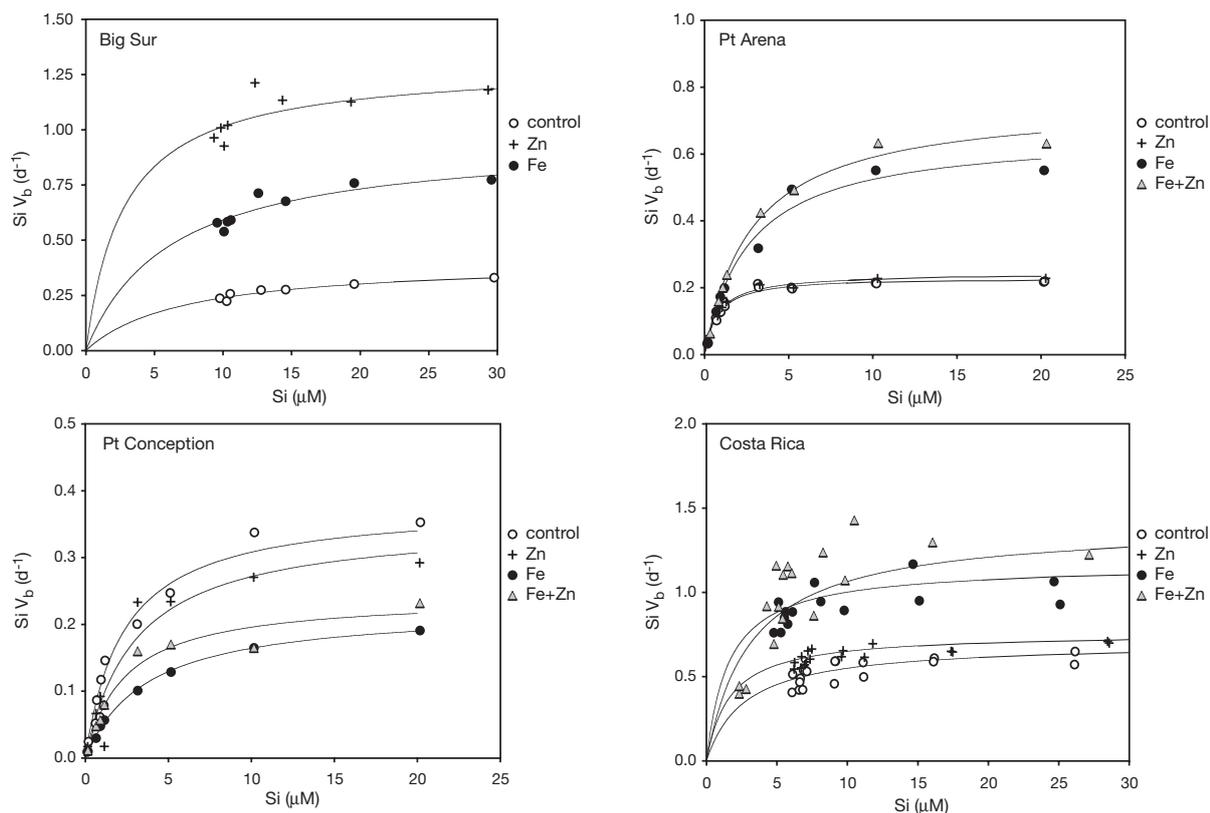

Fig. 3. Biomass-specific Si uptake rates (Si V_b) plotted as a function of dissolved Si concentration (i.e. Si uptake kinetics) in 4 enrichment experiments in waters off California (Big Sur, Pt Arena, Pt Conception) and the eastern tropical Pacific (Costa Rica). Legend labels indicate trace-metal enrichment: control (open circle), Zn addition (plus), Fe addition (closed circle), combined Fe + Zn addition (shaded triangle). Note the different scales in each panel
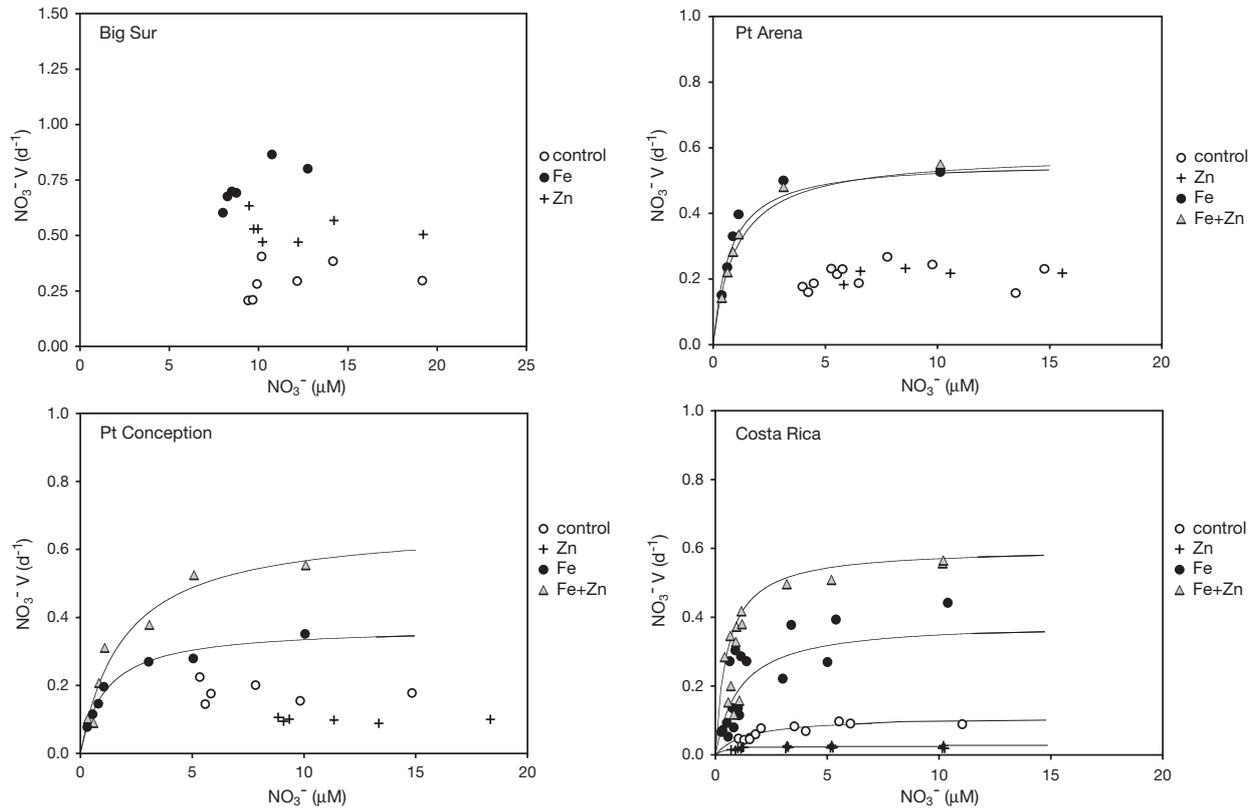
Fig. 4. See Fig. 3 legend for details, except this is for NO_3^- (i.e. NO_3^- uptake kinetics). Note the different scale for biomass-specific NO_3^- uptake (NO_3^- V) in the Big Sur experiment (upper left panel)

spp. diatoms means they can contribute significantly to silicon uptake even at abundances as low as 10 ml^{-1} (Franck et al. unpubl.). In the Pt Conception experiment, Fe addition alone and in combination with Zn increased the relative abundance of *Chaetoceros* spp. cells and decreased the relative abundance of *Pseudo-nitzschia* spp. cells, again resulting in an

higher percentage of centric diatoms: 58 to 63% of total cells in the control and Zn additions and 69 to 72% in the Fe and Fe + Zn additions. The relative abundance of *Rhizosolenia* spp. cells also decreased after Fe addition, from 1 to 4% of total cells in the control and Zn additions to 0.3 to 1% in the Fe and Fe + Zn additions.

Table 4. Comparison of half-satuation constants (K_s, µM) for Si and NO_3^- uptake in Fe and Zn enrichments in experiments conducted off California and in the eastern tropical Pacific. Errors are standard errors. Enrichments in which K_s values differed significantly from control values are printed in bold (*t*-test, $\alpha < 0.05$). n: number of data points used to estimate K_s; –: enrichment not done; * * K_s could not be estimated (NO_3^- concentrations were saturating)

| Cruise | Si:NO$_3$ | | | | NO$_3^-$ K_s | | | |
Experiment	Control	Zn	Fe	Fe + Zn	Control	Zn	Fe	Fe + Zn
California								
Big Sur	7.11±1.04 (n = 7)	**2.78±0.87 (n = 8)**	6.24±1.04 (n = 8)	–	* *	* *	* *	–
Pt Arena	0.75±0.07 (n = 16)	0.78±0.09 (n = 8)	**2.66±0.49 (n = 8)**	**2.83±0.31 (n = 8)**	* *	* *	0.74±0.17 (n = 6)	1.02±0.11 (n = 6)
Pt Conception	2.31±0.54 (n = 16)	3.01±1.29 (n = 8)	3.71±0.76 (n = 7)	2.37±0.60 (n = 8)	* *	* *	1.14±0.17 (n = 7)	1.93±0.55 (n = 7)
Eastern tropical Pacific								
Costa Rica	2.73±0.92 (n = 19)	1.67±0.50 (n = 17)	1.57±0.74 (n = 14)	3.13±1.51 (n = 19)	1.42±0.39 (n = 12)	**0.46±0.19 (n = 11)**	1.09±0.59 (n = 17)	**0.53±0.14 (n = 14)**

Table 5. Si:NO_3^- uptake ratios in initial waters and comparison of Si:NO_3^- drawdown and V_{max} ratios in Fe and Zn enrichments in experiments off California and in the eastern tropical Pacific. Errors are standard errors. Enrichments in which V_{max} ratios differed significantly from control ratios are printed in bold (t-test, $\alpha < 0.001$). Si:NO_3^- drawdown ratios in parentheses represent initial dissolved Si:NO_3^- ratios (see text). –: measurement/enrichment not done

Cruise Experiment	Si:NO_3^- uptake	Si:NO_3^- drawdown				Si:NO_3^- V_{max}			
		Control	Zn	Fe	Fe + Zn	Control	Zn	Fe	Fe + Zn
California									
Big Sur	–	1.7	1.9	1.2	–	1.4±0.0	**2.5±0.1**	1.4±0.1	–
Pt. Arena	1.5	0.9	1.1	(0.4)	(0.4)	1.1±0.0	1.2±0.0	1.2±0.1	1.3±0.0
Pt Conception	1.5	2.2	5.5	1.0	1.0	2.1±0.0	**3.6±0.1**	**0.6±0.0**	**0.4±0.1**
Eastern tropical Pacific									
Costa Rica	6.0	5.8	26.9	3.3	3.9	6.4±0.1	**30.3±0.0**	**3.0±0.1**	**2.3±0.2**
Humboldt	5.8	6.1	–	3.4	–	5.1±0.1	–	**3.3±0.1**	–
Peru	2.5	1.8	–	0.5	–	1.8±0.0	–	**0.5±0.1**	–

Eastern tropical Pacific

Si(OH)$_4$ and NO_3^- uptake kinetics (V_{max}, K_s)

Figs. 3 & 4 show Si(OH)$_4$ and NO_3^- kinetic curves measured in 4 enrichments (controls, +1 nM Zn, +2 nM Fe, +2 nM Fe + 1 nM Zn) at the Costa Rica Upwelling Dome location; Tables 3 & 4 summarize kinetics parameters measured at all locations. (Due to time constraints, smaller kinetic experiments measuring only Si V_{max} and NO_3^- V_{max} values were conducted at the Humboldt and Peru locations.) Fe addition significantly increased both Si V_{max} and NO_3^- V_{max} in the Costa Rica experiment (Fig. 3, Table 3). Relative to controls, Si V_{max} values increased 1.7 times and NO_3^- V_{max} values increased 3.5 times. Fe addition had a sig-

nificant effect on both Si V_{max} and NO_3^- V_{max} values in the Humboldt experiment as well, increasing Si V_{max} by 1.6 times and NO_3^- V_{max} by 2.5 times relative to controls (Table 3). In the Peru experiment, Fe addition resulted in a 31% decrease in Si V_{max} values relative to controls, and a small but significant (1.2×) increase in NO_3^- V_{max} (Table 3).

The effect of Zn addition on Si and NO_3^- V_{max} was investigated only at the Costa Rica Upwelling Dome location. Zn addition alone had no effect on Si V_{max} in this experiment, and significantly decreased NO_3^- V_{max} by 80% (Figs. 3 & 4, Table 3). Combined additions of Fe + Zn significantly increased both Si V_{max} (2.0×) and NO_3^- V_{max} (5.5×) relative to controls. The effect of combined Fe + Zn addition on Si V_{max} was similar to the effect of Fe addition alone at this site, but the effect

Table 6. Microplankton cell counts in enrichment experiments conducted off California. Errors are standard errors from replicate field counts. Rare: <20 cells per 400 observed; –: no cells of that type observed

Phytoplankton taxa	Pt Arena				Pt Conception			
	Control (cells ml^{-1})	Zn (cells ml^{-1})	Fe (cells ml^{-1})	Fe+Zn (cells ml^{-1})	Control (cells ml^{-1})	Zn (cells ml^{-1})	Fe (cells ml^{-1})	Fe+Zn (cells ml^{-1})
Diatoms								
Centrics	**1286±192**	**6514±503**	**3866±385**	**12142±4726**	**2805±1320**	**3897±725**	**11025±948**	**14625±2066**
Chaetoceros spp.	1261±194	6458±513	3721±391	11732±4711	2503±1249	3534±772	10615±818	13855±2085
Rhizosolenia spp.	Rare	Rare	Rare	Rare	59±4	59±11	107±7	57±5
Coscinodiscineae	Rare	Rare	Rare	Rare	Rare	Rare	Rare	Rare
Corethron spp.	Rare	Rare	Rare	Rare	Rare	Rare	Rare	Rare
Pennates	**621±113**	**1564±111**	**950±163**	**2421±590**	**2000±363**	**2221±360**	**4991±552**	**5413±820**
<20 μm pennates	49±21	56±11	156±83	596±275	134±106	154±56	894±223	670±217
Pseudo-nitzschia spp.	240±50	950±176	190±82	633±203	1318±197	1592±337	931±358	2657±624
Fragilariopsis spp.	Rare	Rare	Rare	Rare	–	–	Rare	–
Thalassiothrix spp.	Rare	125±21	Rare	Rare	Rare	–	–	Rare
T*halassionema* spp.	Rare	Rare	–	–	Rare	–	Rare	Rare
Other cells	**8±5**	**34±24**	**123±94**	**112±76**	**45±45**	**70±36**	**74±47**	**149±79**
Dinoflagellates	Rare	Rare	Rare	–	Rare	Rare	Rare	Rare
Silicoflagellates	Rare	–	–	–	Rare	Rare	Rare	–
Other flagellates	Rare	Rare	Rare	Rare	Rare	Rare	Rare	Rare
Total cells	**1912±251**	**8112±578**	**4872±442**	**14675±5294**	**5632±315**	**6187±1046**	**16004±890**	**20187±2388**

on NO_3^- V_{max} was much stronger than that for Fe addition.

Si and NO_3^- K_s values were measured only at the Costa Rica Upwelling Dome location. None of the trace-metal additions had significant effects on Si K_s values in this experiment (Fig. 3, Table 4). Additions with Zn (Zn and Fe + Zn) significantly decreased NO_3^- K_s, by 68% for Zn alone and by 63% for combined Fe + Zn (Fig. 4, Table 4).

Si(OH)$_4$:NO$_3^-$ drawdown ratios and V_{max} ratios

In situ Si(OH)$_4$:NO$_3^-$ uptake ratios—measured with tracers at the beginning of the experiments—agreed well with Si(OH)$_4$:NO$_3^-$ drawdown ratios in the controls, which were calculated at the end of the experiments from changes in Si(OH)$_4$ and NO_3^- after 3 to 6 d incubation (2.5 to 6.0 vs 1.8 to 6.1, Table 5). Both ratios were much higher than the mean value of ~1 reported for trace-metal replete diatom cultures (Brzezinski 1985), although the values fall within the range of Si(OH)$_4$:NO$_3^-$ uptake ratios measured in low-Fe waters in the Southern Ocean (1.5 to 8, Franck et al. 2000).

Fe addition (Fe and Fe + Zn) resulted in lower Si(OH)$_4$:NO$_3^-$ drawdown ratios relative to *in situ* Si(OH)$_4$:NO$_3^-$ uptake ratios and Si(OH)$_4$:NO$_3^-$ drawdown ratios in both the controls and Zn additions (Table 5). This was true for all 3 experiments in the eastern tropical Pacific. Si(OH)$_4$:NO$_3^-$ V_{max} ratios were also significantly lower in Fe and Fe + Zn additions relative to controls at all 3 locations (Table 5). Si(OH)$_4$:

NO_3^- V_{max} ratios, measured with tracers, were very similar to Si(OH)$_4$:NO$_3^-$ drawdown ratios calculated from changes in Si(OH)$_4$ and NO_3^- concentrations over the course of the experiment. With the exception of the Fe + Zn treatment at the Costa Rica site, these 2 independent measurements varied by <20%. At the Costa Rica and Humboldt sites, drawdown and V_{max} ratios remained high (2.3 to 3.9) even after 3 to 6 d incubation with +2 nM Fe. The effect of Zn addition on Si(OH)$_4$:NO$_3^-$ drawdown and V_{max} ratios was measured only at the Costa Rica site. At this location, Zn additions led to 4.6- to 4.7-fold increases relative to controls in both drawdown and V_{max} ratios, resulting in extremely high ratios (26.9 to 30.3).

Phytoplankton species composition

Total cell counts in the controls were almost an order of magnitude lower than those in the central California experiments (Table 7). In the Costa Rica Upwelling Dome experiment, Fe and Fe + Zn additions substantially increased total cell numbers, to values similar to those seen in the California experiments (10^3 to 10^4 ml^{-1}). Although we did not enumerate picophytoplankton, other studies reveal that this experiment was conducted during a *Prochlorococcus* spp. bloom (~10^4 ml^{-1}, R. Kudela et al. unpubl.). Our phytoplankton counts were dominated by pennate diatoms as well as various flagellate groups, including silicoflagellates (genus *Meringosphaera*). Following Fe addition, the pennate diatoms accounted for most of the observed

Table 7. Microplankton cell counts in enrichment experiments conducted in the eastern tropical Pacific. Errors are standard errors from replicate field counts. Rare: <20 cells per 400 observed; –: no cells of that type observed

Phytoplankton taxa	Costa Rica				Humboldt		Peru	
	Control (cells ml^{-1})	Zn (cells ml^{-1})	Fe (cells ml^{-1})	Fe+Zn (cells ml^{-1})	Control (cells ml^{-1})	Fe (cells ml^{-1})	Control (cells ml^{-1})	Fe (cells ml^{-1})
Diatoms								
Centrics	**9±5**	**24±12**	**31±15**	**319±168**	**62±13**	**63±17**	**59±12**	**44±9**
Chaetoceros spp.	–	Rare	Rare	Rare	51±13	61±18	40±12	33±7
Rhizosolenia spp.	–	–	–	Rare	Rare	Rare	Rare	Rare
Coscinodiscinceae	Rare	Rare	Rare	Rare	Rare	Rare	Rare	Rare
Corethron spp.	Rare	Rare	Rare	Rare	Rare	Rare	Rare	Rare
Pennates	**314±31**	**293±35**	**885±83**	**12163±695**	**82±9**	**137±13**	**166±14**	**92±15**
<20 µm pennates	180±17	192±20	501±56	8716±637	16±4	25±7	Rare	Rare
Pseudo-nitzschia spp.	39±7	55±12	134±35	1213±544	41±8	63±7	26±10	27±11
Fragilariopsis spp.	37±5	40±9	–	–	Rare	Rare	Rare	Rare
Thalassiothrix spp.	–	–	89±11	223±98	–	Rare	Rare	Rare
Thalassionema spp.	–	–	–	–	–	–	Rare	Rare
Other cells	**127±27**	**80±26**	**201±58**	**734±210**	**23±4**	**83±9**	**154±43**	**189±55**
Dinoflagellates	Rare	Rare	Rare	Rare	Rare	Rare	Rare	Rare
Silicoflagellates	38±10	Rare	Rare	Rare	Rare	Rare	Rare	Rare
Other flagellates	68±8	47±12	170±38	670±146	16±4	65±9	133±30	171±33
Total cells	**450±32**	**386±43**	**1117±116**	**13217±863**	**167±14**	**282±24**	**379±16**	**325±26**

increase in cells, and the relative abundance of pennates increased from 70% of total cells in the controls to 80 to 92% in the Fe and Fe + Zn additions. In contrast to the Costa Rica Upwelling Dome experiment, total cell counts remained low even after Fe addition in Humboldt and Peru experiments (10^2 ml^{-1}). Our counts at these locations were dominated by flagellates as well as diatoms, and the major microplankton groups included *Chaetoceros* spp., *Pseudo-nitzschia* spp., small pennates and, in the Peru experiment, colonial flagellates of the genus *Dinobryon*. In the Humboldt experiment, Fe addition resulted in decline in the relative abundance of centric diatoms, due to a decline in the percentage of *Chaetoceros* spp. as well as an increase in the percentage of flagellates. In the Peru experiment, Fe addition resulted in a decline in the relative abundance of pennate diatoms, due to a decrease in the percent of some of the rarer pennates, including *Nitzschia* spp. and *Navicula* spp., as well as to an increase in the abundance of flagellates.

Southern Ocean

Si(OH)$_4$ uptake kinetics (V_{max})

Two of the 4 long-term (8 to 16 d) enrichment experiments conducted in the Southern Ocean showed marked changes in Si(OH)$_4$ and NO$_3^-$ uptake rates and phytoplankton biomass — including biogenic silica, particulate organic nitrogen and carbon and chl *a* — following Fe addition (Expts 1 and 3) (Franck et al. 2000, Coale et al. 2003). In Expt 4, NO$_3^-$ uptake rates responded strongly to Fe addition but Si(OH)$_4$ uptake rates and biogenic silica concentrations did not (Franck et al. 2000). As shown in Fig. 2, Fe addition increased Si V_{max} values in all 3 of these experiments (Expts 1, 3 and 4). In most cases, the relationship between Si V_{max} and dissolved Fe concentration tended to be hyperbolic, similar to the relationship observed between uptake rate and substrate concentration in kinetic experiments (e.g. Figs. 3 & 4).

Si V_{max} values showed a clear response to increasing Fe concentrations in the experiment conducted south of the Polar Frontal Zone in austral summer (Expt 3) (Fig. 2). This was true for all 4 d sampled. V_{max} values were greatest in the higher (1 to 2 nM) Fe additions, and varied from 1.4 to 2.9 times those in the controls, depending on sampling date and Fe addition. Fe had the greatest stimulatory effect on Si V_{max} on Day 6, when V_{max} values were highest, and the smallest effect at the end of the experiment on Day 10, when Si V_{max} values were lowest.

The effect of Fe concentration on Si V_{max} in waters within the Polar Frontal Zone (~62°S) was measured both in austral spring (Expt 1) and summer (Expt 4). Higher dissolved Fe concentrations resulted in higher Si V_{max} values in both experiments, although not on all days. In the spring (Expt 1), Fe addition did not have an apparent effect on V_{max} until Day 12 (Fig. 2). A similarly long lag time occurred before increases were detected in Si(OH)$_4$ and NO$_3^-$ uptake rates and biogenic SiO$_2$ concentrations in this enrichment as well (Franck et al. 2000). V_{max} values in the spring Fe additions were anywhere from 0.7 to 1.9 times those in the controls depending on date and Fe addition, and were greatest at Fe concentrations of ~1 nM. In the summer (Expt 4), Si V_{max} values in the Fe additions were 1.1 to 2.0 times those in the controls and were greatest in the enrichments with ~0.5 nM total dissolved Fe. V_{max} values increased with time in this experiment, and were highest on Day 16, the last day sampled (Fig. 2).

Fe availability had the smallest affect on Si V_{max} in the experiment conducted to the north of the Polar Frontal Zone (Expt 2). On Day 5, the highest V_{max} values were measured in waters with ≥0.1 nM ambient Fe, but Fe concentration had no effect on Si V_{max} on Day 8, the last day sampled (Fig. 2).

DISCUSSION

Fe-dependent Si(OH)$_4$ uptake kinetics

Iron addition significantly affected Si V_{max} values in experiments conducted in all 3 HNLC areas (Table 3, Figs. 2 & 3). With few exceptions, Fe addition increased Si V_{max} by ~2 to 3 times relative to controls. These experiments, conducted in widely different regions with very different phytoplankton assemblages, suggest that low Fe availability depresses Si V_{max} values in HNLC waters. The data agree well with the one previous study investigating the effect of Fe on Si V_{max}, which reported 2 to 3 times higher values in Fe-replete versus Fe-stressed laboratory cultures of *Thalassiosira weissflogii* (De La Rocha et al. 2000).

Results from the Southern Ocean (Expts 1, 3 and 4) suggest that the effect of Fe addition on Si V_{max} can vary with the level of Fe addition. In these experiments, Si V_{max} values were greatest in enrichments with final Fe concentrations > 0.5 nM, and Si V_{max} values seemed to peak at Fe concentrations of ~0.8 nM (Fig. 2). Phytoplankton growth rates in HNLC regions also showed a similar response to increasing Fe concentrations, with maximum growth rates observed at ~1 nM Fe (Coale et al. 1996, Hutchins et al. 2002). In marine diatoms, the energy for Si biomineralization has been linked to aerobic respiration (Sullivan 1986, Martin-Jézéquel et al. 2001). The respiratory chain has a high Fe requirement, and it may be that reduced

electron transport efficiency in Fe-limited diatoms, occurring over a relatively wide range of dissolved Fe concentrations, is one cause for the observed decrease in Si V_{max} at low Fe availability.

The time-course experiments in the Southern Ocean show that the effect of Fe addition on Si V_{max}, and Si V_{max} values themselves, can vary with time. This could be due to gradual changes in species composition, Si transporter synthesis and/or cellular metabolism. It is common to see long lag times in Fe enrichments conducted in the Southern Ocean. These enrichments were maintained for longer time periods, however, and changes in Si V_{max} over time may also be due to bottle effects, such as sudden declines in phytoplankton abundance or activity. In addition, the Southern Ocean enrichments were not screened and changes in cell physiology due to white-light photoinhibition could have been an issue (Franck et al. 2000). Despite such caveats, the response to Fe seems to be the same in all 3 regions: in general, Fe addition significantly increased the capacity for $Si(OH)_4$ uptake in marine phytoplankton.

Combined Fe and Zn additions, when conducted, resulted in changes in Si V_{max} similar to those produced by Fe addition (Table 3, Fig. 3). In 2 out of 3 experiments with Fe + Zn additions, Si V_{max} was higher in the Fe + Zn treatments than in the Fe treatments, suggesting that Fe and Zn may have had a synergistic effect on $Si(OH)_4$ uptake. Bruland et al. (1991) first discussed the potential for synergistic effects of trace metals on phytoplankton biology. One reason for such an effect may be that Fe-stimulated increases in phytoplankton growth and photosynthetic activity lead to increases in carbonic anhydrase activity and an increased requirement for Zn. It is unlikely that the apparent synergistic effect of Fe + Zn could be due to changes in microplankton species composition. In all 3 experiments with Fe + Zn additions, total cell numbers were greatest in the Fe + Zn enrichments but, for the most part, there was little if any difference in the relative proportion of the major phytoplankton groups among the Zn, Fe and Fe + Zn enrichments (Tables 6 & 7). These groups included *Chaetoceros* spp., <20 μm pennate diatoms, *Pseudo-nitzschia* spp., *Rhizosolenia* spp. and non diatoms. An exception is the higher contribution of small pennate diatoms to total cells in the Fe + Zn enrichment in the Costa Rica Upwelling Dome experiment (66 vs 40 to 50%).

There were 2 experiments in which Fe and combined Fe + Zn additions significantly decreased, rather than increased, Si V_{max} (Table 3). These included the Pt Conception experiment off California and the Peru experiment in the eastern tropical Pacific. It seems unlikely that increased Fe availability would cause a

decline in the synthesis of $Si(OH)_4$ transporters in the cell walls of diatoms from these locations. We hypothesize that Fe-induced changes in diatom species composition caused the observed declines in Si V_{max}. With respect to uptake kinetics, changes in the relative abundance of diatom groups, rather than changes in absolute cell numbers, would have more of an effect. In both experiments, there was a decline in the proportion of larger pennate diatoms in the Fe and Fe + Zn enrichments relative to controls (Tables 6 & 7). In the Pt Conception experiment, the decline in the proportion of pennate diatoms was due to a 13 to 20% drop in the relative abundance of *Pseudo-nitzschia* spp. cells, while in the Peru experiment, the decline in the proportion of pennates was due to a 16% drop in the relative abundance of *Nitzschia* and *Navicula* spp. If certain diatom groups have higher Si V_{max} values in general, then the bulk Si V_{max} value may decline if species compositional changes outweigh environmental effects, including increases in Fe availability. In these 2 experiments, diatoms of the larger *Nitzschioid* and *Naviculoid* types would have had to have ~2-fold higher Si V_{max} than the rest of the diatom assemblage in order for the observed declines in the relative abundance of these groups to account for the decline in Si V_{max}. Such a difference seems reasonable. Pennate diatoms tend to have higher surface area:volume ratios than do centric diatoms and thus may have a higher density of silicon transporters by default. In a review of silicon metabolism in diatoms, published values for Si V_{max} were routinely higher in pennate species (Martin-Jézéquel et al. 2001).

The effect of Fe addition on the half-saturation constant for $Si(OH)_4$ uptake (Si K_s) in natural phytoplankton assemblages was much harder to elucidate in this study given the mixed results of the 4 experiments in which Si K_s was measured. In 3 experiments, Fe addition had no significant effect on Si K_s (Table 4). In one experiment in upwelling waters off California, however, Fe addition alone and in combination with Zn significantly increased Si K_s more than 3-fold. The increase in Si K_s suggests that diatom assemblages would become silicon-limited at higher $Si(OH)_4$ concentrations after Fe addition. Only one published study has investigated the effect of Fe on $Si(OH)_4$ uptake kinetics to date, and this study did not show a significant difference in Si K_s between Fe-limited and Fe-replete laboratory cultures of *Thalassiosira weissflogii* (De La Rocha et al. 2000).

Healey (1980) observed that V_{max} as well as K_s determine nutrient uptake rate rates at low nutrient concentrations, and suggested that $V_{max}:K_s$ ratios would be a better indicator of the relative competitive advantage of a phytoplankton group for nutrient acquisition than K_s estimates alone. An increase in the $V_{max}:K_s$ ratio

would indicate an increased ability to utilize nutrients at low concentrations. Based on changes in this ratio, Fe addition to Fe-stressed waters can substantially increase the competitive advantage of diatom assemblages with respect to $Si(OH)_4$ acquisition, although not consistently. In the Big Sur and Costa Rica experiments, Fe addition increased the V_{max}:K_s ratio about 3-fold relative to the controls. In the Pt Arena experiment, V_{max}:K_s ratios remained relatively constant after Fe addition, despite the fact that Fe addition altered both Si V_{max} and Si K_s. Lastly, in the Pt Conception experiment, Fe addition led to a decrease in the V_{max}:K_s ratio, to 37 % of the control value.

Fe-dependent NO_3^- uptake kinetics

Fe addition significantly increased NO_3^- V_{max} values in enrichment studies conducted at all sites where NO_3^- V_{max} values were measured (Fig. 4, Table 3). For the most part, NO_3^- V_{max} values after Fe addition were more than twice those in the controls. The one experiment in which Fe addition resulted in only a small (1.2×) increase in NO_3^- V_{max} had the highest initial dissolved Fe concentration in this study (Peru, 0.3 nM, Table 1), suggesting the phytoplankton assemblage at this site may not have been severely Fe-stressed. Combined Fe and Zn additions also significantly increased NO_3^- V_{max} values in all 3 experiments in which Fe + Zn enrichments were done. In 2 cases, NO_3^- V_{max} values in the Fe + Zn treatments were significantly higher than those in the Fe treatments. As stated previously, this provides evidence for an apparent synergistic effect of combined Fe and Zn additions on marine phytoplankton.

To our knowledge, no other experiment has investigated the effect of Fe on nitrate uptake kinetics, either in the laboratory or in the field. Fe is a co-factor in the enzymes nitrate and nitrite reductase (Raven 1976, Timmermans et al. 1994, Milligan & Harrison 2000), which are essential for NO_3^- uptake in phytoplankton. Studies have shown that both nitrate and nitrite reductase activities are reduced in Fe-limited phytoplankton in culture (Timmermans et al. 1994, Milligan & Harrison 2000), and that Fe addition stimulates instantaneous NO_3^- uptake rates in bottle enrichment experiments in HNLC regions (Price et al. 1991, Timmermans et al. 1998, Franck et al. 2000). This is the first study to look at the effect of Fe availability on NO_3^- V_{max}, a key indicator of the maximum rate of new production and, by inference, the maximum rate of C export from marine ecosystems. Our results suggest that Fe availability directly regulates NO_3^- V_{max} in HNLC regions.

Whatever the causes for the apparent decline in Si V_{max} after Fe addition in 2 of the enrichment experiments (see above section), NO_3^- V_{max} values significantly increased in all experiments (Table 3). Thus, it appears that NO_3^- uptake kinetics are more likely to show Fe stress than are $Si(OH)_4$ uptake kinetics. This observation supports results from a recent study showing that particulate organic nitrogen production was more sensitive to Fe availability than biogenic silica production in HNLC waters off California (Firme et al. 2003). This is not surprising given that the enzymes required for NO_3^- assimilation are Fe-dependent, and the link between Fe availability and $Si(OH)_4$ assimilation is probably less direct, as mentioned earlier.

Initial NO_3^- concentrations were high in these experiments (Table 1). Due to low NO_3^- drawdown, NO_3^- concentrations remained too high in control and Zn enrichments to measure half-saturation constants for NO_3^- uptake (NO_3^- K_s) in 3 of the experiments in which measurements of NO_3^- K_s were attempted (Table 4). It was possible to measure NO_3^- K_s in all 4 enrichments in the Costa Rica Upwelling Dome experiment. In this experiment, Fe did not have a significant effect on NO_3^- K_s. Fe addition did increase the V_{max}:K_s ratio more than 4-fold, suggesting that Fe addition greatly improved the ability of the phytoplankton in these waters to acquire NO_3^- at low concentrations (Healy 1980).

It should be noted that high NH_4^+ concentrations can inhibit NO_3^- uptake in phytoplankton (Dortch 1990, Harrison et al. 1996). Ammonium concentrations were not monitored in the individual enrichments, and it is possible that differences in NH_4^+ accumulation during the course of the experiments could have contributed to some of the variation observed in NO_3^- V_{max} values. Published values of K_i, the half-saturation constant for NH_4^+ inhibition of NO_3^- uptake, range from 0.40 to 0.6 µM NH_4^+ for high-nitrate environments (Price et al. 1994, Harrison et al. 1996, Elskens et al. 1999). Initial NH_4^+ concentrations in our experiments ranged from 0.1 to 0.7 µM, and so a moderate amount of NH_4^+ inhibition may have occurred in the enrichments. The fact that Fe addition increased NO_3^- V_{max} in all 6 experiments listed in Table 3, however, suggests that Fe is regulating NO_3^- kinetics in low-Fe waters irrespective of potential NH_4^+ inhibition.

Despite the wide variation in initial dissolved Fe concentrations observed among our study sites, it does not appear that initial Fe concentrations are a good predictor of the effect of Fe addition on kinetics parameters. For example, when increases in Si V_{max} and NO_3^- V_{max} observed after Fe addition were plotted as a function of initial Fe concentration, no clear trend emerged. It may be that total dissolved Fe and Zn measurements, while good indicators of the potential for Fe and Zn stress, are not good indicators of subtle differences in Fe and Zn bioavailability.

Zn-dependent Si(OH)$_4$ uptake kinetics

The effects of Zn enrichment on Si(OH)$_4$ uptake kinetics varied widely among experiments. Zn limitation was apparent only in the Big Sur experiment off California, where Zn addition by itself significantly increased Si V_{max} (Table 3, Fig. 3). In addition to a lower Si V_{max} value, the control treatment also had a significantly higher Si K_s value relative to the Zn treatment in this experiment (Table 4, Fig. 3). De La Rocha et al. (2000) reported similar differences in Si V_{max} and Si K_s between Zn-limited and Zn-replete cultures of the diatom *Thalassiosira weissflogii*, with Si V_{max} values 2.5 times lower in the Zn-limited cultures, and Si K_s values 1.7 times higher (4.0 vs 2.4 µM). The results from the Big Sur region, together with the laboratory data from De La Rocha et al. (2000), support the hypothesis of a zinc-dependent active site for silicon uptake in diatoms, proposed by Rueter & Morel (1981). A Zn-dependent Si transporter may explain why oceanic profiles of dissolved Zn co-vary with those of Si(OH)$_4$ (Bruland 1980, Bruland et al. 1994).

Zn addition alone had no significant effect on Si V_{max} or Si K_s in any of the other enrichment experiments with Zn, implying that Zn was limiting only at the Big Sur site. Neither the initial Zn concentration nor the amount of Zn added seemed to have any bearing on the response to Zn enrichment in these experiments, although the Big Sur experiment had some of the lowest dissolved Zn concentrations in this study. The Big Sur experiment was conducted in coastal waters, and previous studies have shown that coastal phytoplankton have higher requirements for Zn than in open-ocean regions (Brand et al. 1983, Sunda & Huntsman 1992, Tortell & Price 1996).

Zn-dependent NO$_3^-$ uptake kinetics

In addition to increasing Si V_{max}, Zn addition also significantly increased NO$_3^-$ V_{max} in the Big Sur experiment, further supporting the idea that this site was Zn-limited (Table 3, Fig. 4). No previous studies on the effect of dissolved Zn concentrations on NO$_3^-$ uptake kinetics, either in the laboratory or in the field, have been reported. Early work by Wacker (1962) linked Zn deficiency with decreased protein synthesis and nitrogen content in the autotrophic flagellate *Euglena gracilis*, and suggested that Zn was important for protein synthesis. This could explain the enhancement of NO$_3^-$ V_{max} values after Zn addition in the Big Sur experiment.

In the remaining 3 experiments in which Zn enrichments were conducted, Zn either had no significant effect on NO$_3^-$ V_{max} (Pt Arena), or significantly decreased NO$_3^-$ V_{max} values relative to controls (Pt Conception, Costa Rica) (Table 3, Fig. 4). This response to Zn—either no effect or an actual decline—is similar to previous experiments investigating the effect of Zn on NO$_3^-$ assimilation. These experiments either show no change in NO$_3^-$ drawdown after Zn addition (Buma et al. 1991, Coale 1991) or show a decrease in NO$_3^-$ uptake rates (Franck et al. 2000).

It is possible that Zn toxicity may have caused the depressed NO$_3^-$ V_{max} values observed in some of our experiments, although studies show that Zn toxicity is unlikely in marine phytoplankton at concentrations <1 µM (Fisher & Frood 1980, Stauber & Florence 1990, Tadros et al. 1990). Decreases in NO$_3^-$ V_{max} in these experiments are not likely to be a result of shifts in species composition, as the proportions of the dominant phytoplankton groups in the Zn enrichments were somewhere in between the controls and Fe enrichments (Tables 5 & 6). When Zn was added at the same concentration in combination with Fe at the Pt Conception and Costa Rica locations, NO$_3^-$ V_{max} increased relative to the Fe additions. If lower NO$_3^-$ V_{max} values in the Zn additions were due to Zn toxicity, the lack of Zn toxicity in the combined Fe + Zn additions may be because Fe increases the cellular demand for Zn, as hypothesized previously. Alternately, Zn may competitively inhibit Fe uptake, resulting in a higher likelihood for Zn toxicity at low Fe concentrations. It has been shown that Zn can be toxic for this reason at low Mn concentrations (Sunda 1991), although no work has demonstrated a competitive effect at low Fe concentrations.

The conflicting effects of Zn addition on NO$_3^-$ kinetics in the Costa Rica Upwelling Dome enrichment (lower V_{max} but higher substrate affinity) and the lack of a clear trend among the other 3 experiments suggest that the effects of Zn addition may be mediated by complicating factors such as phytoplankton species composition, organic complexation, intracellular Zn reserves or the availability of other trace metals such as Fe.

Fe- and Zn-dependent Si(OH)$_4$:NO$_3^-$ drawdown ratios and Si(OH)$_4$:NO$_3^-$ V_{max} ratios

In general, Fe and Fe + Zn additions significantly decreased Si(OH)$_4$:NO$_3^-$ drawdown ratios. Similar changes were seen in Si(OH)$_4$:NO$_3^-$ V_{max} ratios. The decline in drawdown and V_{max} ratios after Fe addition was due mainly to the fact that Fe stimulated NO$_3^-$ drawdown and NO$_3^-$ V_{max} more so than Si(OH)$_4$ drawdown and Si V_{max}. A similar cause has been postulated for decreases in Si(OH)$_4$:NO$_3^-$ uptake ratios after Fe addition in experiments conducted in the Southern Ocean (Franck et al. 2000) and in waters off California (Firme et al. 2003).

Zn addition increased rather than decreased $Si(OH)_4$:NO_3^- drawdown and V_{max} ratios. In 2 experiments, at the Pt Conception and Costa Rica Upwelling Dome locations, the increase in $Si(OH)_4$:NO_3^- drawdown and V_{max} ratios was due to a decline in NO_3^- drawdown and NO_3^- V_{max} following Zn addition, perhaps due to Zn toxicity (see 'Zn-dependent NO_3^- uptake kinetics' above). At the Big Sur location in waters off California, the increase in $Si(OH)_4$:NO_3^- drawdown and V_{max} ratios was due instead to a Zn-stimulated increase in $Si(OH)_4$ drawdown and Si V_{max} (Table 3).

Previous Fe enrichment experiments have also reported higher $Si(OH)_4$:NO_3^- uptake and drawdown ratios in Fe-stressed versus Fe-replete waters, and shown declines in these ratios after Fe addition. To date, higher $Si(OH)_4$:NO_3^- drawdown and uptake ratios have been reported in upwelling waters off California near Big Sur (Hutchins & Bruland 1998, Hutchins et al. 1998), in the Southern Ocean (Takeda 1998, Franck et al. 2000), in the subarctic North Pacific (Takeda 1998) and in the eastern tropical Pacific (Takeda 1998, Hutchins et al. 2002). Higher $Si(OH)_4$:NO_3^- drawdown ratios and higher cellular Si:N biomass ratios have also been demonstrated in Fe-limited versus Fe-replete diatom cultures (Takeda 1998, De La Rocha et al. 2000).

This study is the first to look at the effect of Zn on $Si(OH)_4$:NO_3^- drawdown ratios, and the effect of Fe and Zn on Si V_{max}:NO_3^- V_{max} ratios. Based on our results, higher $Si(OH)_4$:NO_3^- drawdown ratios in low-Fe and/or high-Zn waters may be due to the effects of Fe and Zn on $Si(OH)_4$ and NO_3^- uptake kinetics, specifically to NO_3^- V_{max}. It appears that lower $Si(OH)_4$:NO_3^- drawdown ratios in HNLC regions are, at least in part, a result of the preferential depression of NO_3^- V_{max} relative to Si V_{max} at low Fe or high Zn concentrations.

The question remains whether higher $Si(OH)_4$:NO_3^- drawdown ratios observed in HNLC regions result in more heavily silicified diatoms. To date, 2 studies report that Fe-limited diatoms grown in culture can have 1- to 2-times higher Si per cell (Takeda 1998, De La Rocha et al. 2000). Higher Si per cell would suggest that Fe-limited diatoms either have higher biomass-specific $Si(OH)_4$ uptake rates, or spend more time in cell cycle stages where $Si(OH)_4$ uptake occurs (e.g. G1, G2 and M, Brzezinski et al. 1990, Martin-Jézéquel et al. 2001). Franck et al. (2000) reported lower biomass-specific $Si(OH)_4$ uptake rates in Fe-limited waters in the Southern Ocean, which are in agreement with the reduced $Si(OH)_4$ uptake capacity of Fe-limited versus Fe-replete phytoplankton assemblages reported in this study. Given lower biomass-specific $Si(OH)_4$ uptake rates and reduced $Si(OH)_4$ uptake capacities, Fe-stressed diatom cells would have to spend significantly more time in cell-cycle stages where Si deposition occurs in order to become more silicified.

It should be noted that higher $Si(OH)_4$:NO_3^- drawdown ratios and higher Si:N and Si:C biomass ratios do not necessarily suggest more Si per cell. Fe limitation can reduce the biovolume (Takeda 1998) as well as the photosynthetic capacity (Milligan & Harrison 2000) of marine phytoplankton cells. Even if the amount of Si per cell remained constant, phytoplankton cells with less biovolume and thus less organic matter would exhibit higher $Si(OH)_4$:NO_3^- drawdown ratios and higher Si:N and Si:C biomass ratios. Similarly, if a reduction in nitrate and/or nitrite reductase activity forced Fe-limited cells to switch from NO_3^- as their primary nitrogen source to NH_4^+, urea or DON, $Si(OH)_4$:NO_3^- drawdown ratios would increase without a corresponding increase in Si per cell.

CONCLUSION

The few laboratory and field studies that have investigated the effects of Fe on $Si(OH)_4$ uptake kinetics to date suggest that Fe availability can directly control the density of $Si(OH)_4$ transporters in diatom cell walls. Based on this study, we suggest that Fe availability can also directly control the density of active NO_3^- transporters in marine phytoplankton. Phytoplankton communities in HNLC regions are probably operating at near-maximum uptake capacities, due to high nutrient concentrations. Our study shows that Fe availability can directly regulate nutrient uptake capacity in low-Fe waters, which may explain why nutrient drawdown is minimal in HNLC regions. By regulating maximum potential NO_3^- uptake rates, Fe availability is also regulating new production and the upper level of export flux from HNLC regions as well. The preferential depression of NO_3^- V_{max} relative to Si V_{max} observed in this study may help explain why $Si(OH)_4$:NO_3^- uptake ratios are higher in low-Fe waters, and why seasonal $Si(OH)_4$ limitation can occur while ambient NO_3^- concentrations remain saturating. Further work is needed to address whether Fe plays a physiological role in transporter synthesis and function, or whether altered $Si(OH)_4$ and NO_3^- uptake kinetics are mostly a result of Fe effects on cell metabolism and protein synthesis. Based on results from this study, it is uncertain whether Fe availability affects the affinity of $Si(OH)_4$ and NO_3^- transporters. Nutrient uptake affinity can control the concentrations at which field populations become nutrient-limited, thereby influencing phytoplankton species composition and plankton succession during seasonal or bloom-induced changes in nutrient concentration. It is important that future studies identify whether Fe availability can alter K_s in individual species and in mixed phytoplankton assemblages.

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