

## Comparison of size-dependent carbon, nitrate, and silicic acid uptake rates in high- and low-iron waters

Valerie M. Franck<sup>1</sup>

Marine Science Institute and Department of Ecology, Evolution and Marine Biology, University of California Santa Barbara, Santa Barbara, California 93106

Geoffrey J. Smith and Kenneth W. Bruland

Ocean Sciences Department, University of California Santa Cruz, Santa Cruz, California 95064

Mark A. Brzezinski

Marine Science Institute and Department of Ecology, Evolution and Marine Biology, University of California Santa Barbara, Santa Barbara, California 93106

### Abstract

We compare the relative contribution of large phytoplankton (>5 and >10  $\mu\text{m}$ ) to uptake rates of carbon (C), nitrate ( $\text{NO}_3^-$ ), and dissolved silicon (Si) and uptake ratios of  $\text{Si}:\text{NO}_3^-$  and  $\text{Si}:\text{C}$  in Monterey Bay, California (a high-iron region) with three low-iron regions in the eastern tropical Pacific: the Costa Rica upwelling dome, Humboldt current, and Peru upwelling. We also demonstrate the effect of iron enrichment on the above parameters in the latter three regions. In Monterey Bay waters, the >5- $\mu\text{m}$  size fraction accounted on average for the majority of particulate organic C and was responsible for 92% of total C uptake, 81% of total  $\text{NO}_3^-$  uptake, and 98% of total Si uptake. In contrast, in low-iron eastern tropical Pacific waters, the >5- $\mu\text{m}$  size fraction accounted for less than half of the particulate organic C and was responsible for a substantially smaller proportion of total C and  $\text{NO}_3^-$  uptake: 27–43% for C and 34–59% for  $\text{NO}_3^-$ . Iron enrichment experiments in the eastern tropical Pacific resulted in much higher  $\text{NO}_3^-$ , C, and Si uptake rates, although increases were restricted to cells in the >5- $\mu\text{m}$  size fraction.  $\text{Si}:\text{NO}_3^-$  and  $\text{Si}:\text{C}$  uptake ratios decreased after iron addition at most locations, and decreases were a direct result of lower  $\text{Si}:\text{NO}_3^-$  and  $\text{Si}:\text{C}$  uptake ratios in the >5- $\mu\text{m}$  size fraction. Our results suggest that iron availability is a major factor regulating primary production, new production, Si uptake, and  $\text{Si}:\text{NO}_3^-$  and  $\text{Si}:\text{C}$  uptake ratios in the larger phytoplankton size classes in high-nitrate, low-chlorophyll (HNLC) regions in the eastern tropical Pacific.

Plankton community structure can determine biomass, new production, and carbon (C) export in marine systems. In highly productive upwelling ecosystems, the abundance of large (>5  $\mu\text{m}$ ) phytoplankton often leads to high values of primary production, new (or nitrate based) production, and carbon export (Michaels and Silver 1988; Probyn 1992; Owens et al. 1993; Chavez 1996; Wilkerson et al. 2000). In marine ecosystems where phytoplankton biomass is dominated by bacteria and picophytoplankton, such as oligotro-

phic open-ocean gyres, biomass is low and production is fueled by recycled nutrients, resulting in limited new production and little if any C flux (Michaels and Silver 1988).

Dissolved iron (Fe) availability is an important factor regulating community structure in many marine ecosystems. The effect of dissolved Fe concentrations on phytoplankton biomass and size structure can be seen by contrasting upwelling centers along the central California coast with widely different Fe availability due to local differences in the width of the continental shelf (Bruland et al. 2001). Long-term monitoring studies in Monterey Bay—a high-Fe, high-productivity upwelling center with a broad continental shelf—show that phytoplankton biomass and new and primary productivity are dominated by large cells, especially diatoms (see Wilkerson et al. 2000). Just a few miles to the south, where the shelf narrows dramatically along the Big Sur coast, is a high-nitrate, low-chlorophyll (HNLC) upwelling center with relatively low dissolved Fe concentrations dominated by smaller phytoplankton, including cyanobacteria, eukaryotic nanoplankton, and dinoflagellates (Hutchins et al. 1998; Bruland et al. 2001).

Fe limitation has been well demonstrated in HNLC waters in the eastern tropical Pacific, in both large-scale in situ and mesocosm bottle enrichment experiments (e.g., Price et al. 1994; Coale et al. 1996; Hutchins et al. 2002a). Phytoplankton community structure in eastern tropical Pacific HNLC

<sup>1</sup> To whom correspondence should be addressed. Present address: College of Natural Sciences, Hawai'i Pacific University, Hawai'i Loa Campus, 45-045 Kamehameha Highway, Kaneohe, Hawai'i 96744 (vfranck@hpu.edu).

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waters is dominated by smaller cells typical of oligotrophic, open-ocean gyres despite high concentrations of macronutrients, similar to the Big Sur coast off California (Chavez 1989; Chavez et al. 1990, 1991; Peña et al. 1990; Cavender-Bares et al. 1999). One reason for the dominance of bacteria and picophytoplankton in HNLC regions is that smaller cells are better able to use low dissolved Fe concentrations and thus are able to outcompete larger phytoplankton for Fe (Hudson and Morel 1990). Studies on the effect of Fe addition on community structure in these waters show that the increased phytoplankton biomass and new production stimulated by Fe addition are mainly limited to larger cells, especially diatoms (e.g., Chavez et al. 1991; Fryxell and Kaczmarska 1994; Price et al. 1994; Boyd et al. 1996; Coale et al. 1996; Cavender-Bares et al. 1999).

Information on what governs phytoplankton community size structure in marine ecosystems is crucial to understanding what governs C flux in the ocean, primarily because larger cells tend to use nitrate ( $\text{NO}_3^-$ ) as their main nitrogen source. Diatoms, the major component of large phytoplankton, have an obligate requirement for dissolved silicon [as silicic acid,  $\text{Si}(\text{OH})_4$ ] for the synthesis of silica shells, or frustules (Lewin 1955). Studies comparing  $\text{Si}(\text{OH})_4$  uptake with  $\text{NO}_3^-$  uptake show that diatoms are usually the dominant contributors to new production, even in oligotrophic regions of the ocean (Leynaert et al. 2001). For this reason, researchers generally attribute all  $\text{NO}_3^-$  uptake by large ( $>5 \mu\text{m}$ ) phytoplankton to diatoms, even though comparisons of size-dependent  $\text{Si}(\text{OH})_4$  and  $\text{NO}_3^-$  uptake are lacking. Comparing the effect of Fe availability on size-dependent C,  $\text{NO}_3^-$ , and  $\text{Si}(\text{OH})_4$  uptake will help us better understand how Fe regulates primary production and new production in HNLC regions, which in turn may shed some light on the link between Fe availability and C export in marine systems, both past and present.

In this study we investigate the effect of Fe availability on size-dependent primary production, new production, silica production, and nutrient uptake ratios. Specifically, we compare the relative contribution of large phytoplankton ( $>5$ - and  $>10$ - $\mu\text{m}$  fractions) to C,  $\text{NO}_3^-$ , and  $\text{Si}(\text{OH})_4$  uptake rates in Monterey Bay with those from three HNLC regions in the eastern tropical Pacific, before and after Fe addition. We also estimate the importance of diatoms to new production at these locations by comparing the relative contribution of large phytoplankton to  $\text{Si}(\text{OH})_4$  and  $\text{NO}_3^-$  uptake rates and  $\text{Si}:\text{NO}_3^-$  uptake ratios. To our knowledge, this is the first study to report size-dependent  $\text{Si}(\text{OH})_4$  uptake rates from any region, and one of only a few studies quantifying the effect of Fe addition on size-dependent C and  $\text{NO}_3^-$  uptake from HNLC waters.

## Methods

Uptake rates of C,  $\text{NO}_3^-$ , and  $\text{Si}(\text{OH})_4$  were measured in two size fractions (0.7–5 and  $>5 \mu\text{m}$ ) in Monterey Bay surface waters at in situ Fe concentrations, and in three size fractions (0.7–5,  $>5$ , and  $>10 \mu\text{m}$ ) in eastern tropical Pacific surface waters at in situ and amended Fe concentrations. Sampling locations are shown in Fig. 1, and initial nutrient and biomass concentrations are shown in Table 1.

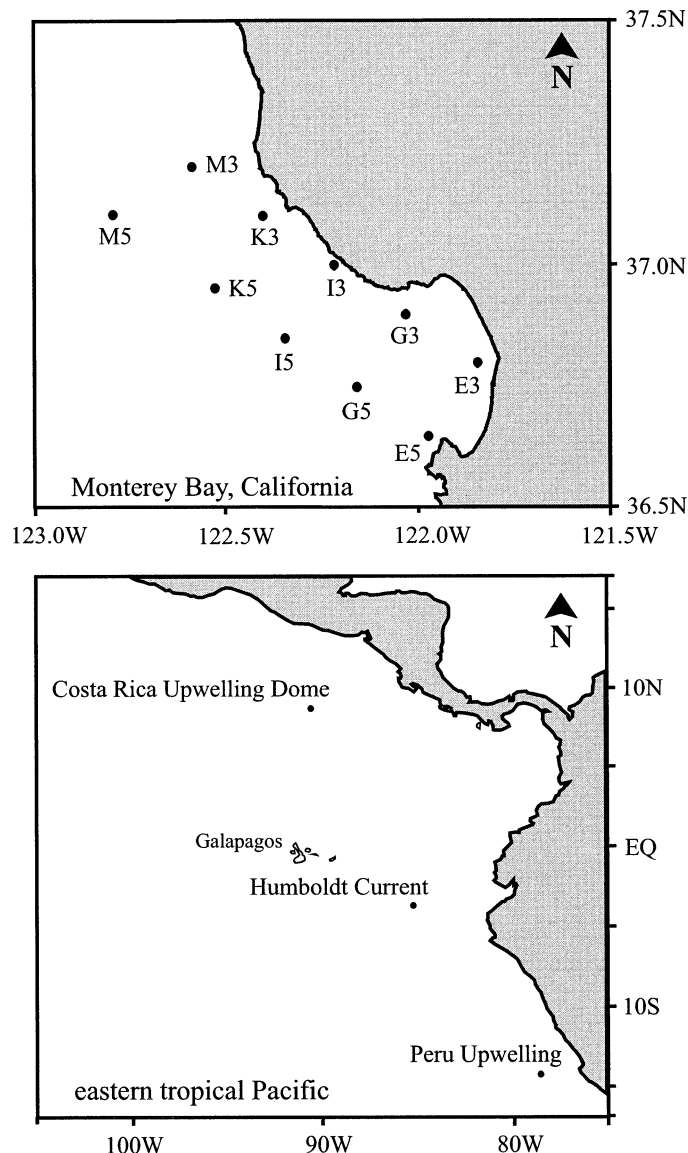


Fig. 1. Sampling locations in Monterey Bay (April 2000) and the eastern tropical Pacific (August–September 2000).

*Water collection and shipboard Fe enrichments*—Surface ( $\sim 5$  m) seawater from Monterey Bay was collected at 10 locations aboard the R/V *Pt. Sur* on 10 April 2000 using Niskin bottles. At each location, water was drained from Niskin bottles into acid-washed polypropylene carboys under low-light conditions and then mixed to resuspend cells. Size-fractionated biomass and uptake rates were measured immediately (see “Size-fractionated biomass and uptake rates” below).

Near-surface (5–15 m) seawater from the eastern tropical Pacific was collected at three locations aboard the R/V *Melville* between 30 August and 19 September 2000 using a trace-metal clean surface pumping system (Hutchins et al. 1998). Unfiltered seawater was collected in an acid-cleaned 50-liter polyethylene carboy using trace-metal clean methods in a class 100 laminar flow bench. The carboy was shaken to resuspend cells, and aliquots were dispensed into seven,

Table 1. Sampling locations and initial concentrations of nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ), silicic acid [ $\text{Si}(\text{OH})_4$ ], particulate organic carbon (POC), particulate organic nitrogen (PON), and biogenic silica (bSiO<sub>2</sub>) for 10 sites in Monterey Bay and three sites in the eastern tropical Pacific. Monterey Bay locations were sampled on 10 April 2000. The eastern tropical Pacific locations were sampled on 30 August 2000 (Costa Rica), 8 September 2000 (Humboldt), and 19 September 2000 (Peru).

	Latitude	Longitude	$\text{NO}_3^-$ ( $\mu\text{mol L}^{-1}$ )	$\text{PO}_4^{3-}$ ( $\mu\text{mol L}^{-1}$ )	$\text{Si}(\text{OH})_4$ ( $\mu\text{mol L}^{-1}$ )	POC ( $\mu\text{mol L}^{-1}$ )	PON ( $\mu\text{mol L}^{-1}$ )	bSiO <sub>2</sub> ( $\mu\text{mol L}^{-1}$ )
Monterey Bay								
E3	36.8°N	121.8°W	1.5	0.16	1.5			3.6
G3	36.9°N	122.0°W	6.9	0.84	8.4	28.5	3.2	4.6
I3	37.0°N	122.2°W	17.9	1.76	25.3			3.8
K3	37.1°N	122.4°W	13.6	1.23	17.4	16.5	1.8	4.5
M3	37.2°N	122.6°W	1.5	0.15	3.4	174.7	16.6	12.7
M5	37.1°N	122.8°W	0.9	0.14	2.5	160.5	15.6	14.5
K5	36.9°N	122.5°W	3.7	0.34	3.9	106.7	10.4	5.5
I5	36.9°N	122.3°W	1.1	0.20	1.3	88.2	7.5	3.5
G5	36.7°N	122.2°W	3.9	0.40	2.8	80.3	8.3	4.3
E5	36.6°N	122.0°W	4.5	0.42	6.2	89.4	11.1	4.3
Eastern tropical Pacific								
Costa Rica	08.7°N	090.6°W	4.4	0.9	7.2	25.5	3.7	0.1
Humboldt	03.8°S	085.3°W	11.0	0.6	6.3	12.9	1.3	0.7
Peru	14.3°S	078.4°W	3.2	0.7	4.3	16.4	1.6	0.4

10-liter acid-cleaned polycarbonate carboys. Three carboys with no added Fe were sealed immediately as controls, and three were amended with 2 nmol L<sup>-1</sup> Fe from a stock solution of FeCl<sub>3</sub> in 0.01 N Ultrex HCl and sealed. Fe additions were about a tenfold increase over ambient dissolved Fe concentrations, which ranged from ~0.1 nmol L<sup>-1</sup> (Costa Rica, Humboldt) to 0.3 nmol L<sup>-1</sup> (Peru) (Franck et al. 2003). Since bottle enrichments typically show a 1–3-d lag period before a response to trace-metal addition is observed, one carboy was used for initial (immediate) measurements of size-fractionated uptake rates and the three control and three +Fe carboys were incubated on deck until measurable differences in Si(OH)<sub>4</sub> concentrations were observed between +Fe and control carboys (~0.3  $\mu\text{mol L}^{-1}$ ). Incubators were maintained at surface temperatures with flowing seawater and 50% incident light with neutral density screening. Initial dissolved Fe concentrations in waters collected for the enrichment experiments were measured according to Bruland et al. (2001).

*Size-fractionated biomass and uptake rates*—Measurements of size-fractionated particulate organic carbon (POC), particulate organic nitrogen (PON), and biogenic silica (bSiO<sub>2</sub>) were made at all locations. Measurements of size-fractionated chlorophyll *a* (Chl *a*) were only made in eastern tropical Pacific waters, at two of the three locations. Biomass measurements were measured in triplicate at the eastern tropical Pacific locations. POC and PON were measured on the same particulate matter collected for C and N stable isotope measurements (see following paragraph). Samples for bSiO<sub>2</sub> (200–600 ml) were successively vacuum filtered (<150 mm Hg) through 10-, 5-, and 0.6- $\mu\text{m}$  polycarbonate filters by retaining and refiltering the filtrate. Filters were dried at 65°C for 3 d and analyzed in the laboratory as described by Brzezinski and Nelson (1995). Samples for Chl *a* (150–500 ml) were vacuum filtered (<150 mm Hg) onto 10-, 5-, and 0.6- $\mu\text{m}$  polycarbonate filters (Millipore), extracted in 10 ml of

90% acetone at –20°C for 24 h, and measured at sea on a Turner AU-10 fluorometer.

For size-fractionated C,  $\text{NO}_3^-$ , and Si(OH)<sub>4</sub> uptake measurements, initial or enrichment carboys were shaken and aliquots were dispensed into 250–500-ml acid-washed polycarbonate bottles: three per carboy for Si(OH)<sub>4</sub> and nine per carboy for C and  $\text{NO}_3^-$ . Small amounts of tracer stock solutions (288  $\mu\text{mol L}^{-1}$  H<sup>13</sup>CO<sub>3</sub><sup>-</sup>, 10% ambient [ $\text{NO}_3^-$ ] as <sup>15</sup>NO<sub>3</sub><sup>-</sup>, and 1,000 Bq <sup>32</sup>Si with <3 pmol L<sup>-1</sup> Si(OH)<sub>4</sub>) were then added to the bottles prior to incubation at sea. Tracer stock solutions were cleaned of potential trace-metal contamination in the laboratory as described by Price et al. (1989). Uptake rates of C,  $\text{NO}_3^-$ , and Si(OH)<sub>4</sub> were measured simultaneously in short-term (6–24 h) incubations in an on-deck flow-through incubator at ~50% incident light. Dual-label incubations with <sup>13</sup>C and <sup>15</sup>N were used to measure C and  $\text{NO}_3^-$  uptake rates on the same samples (Dauchez et al. 1995). Uptake rates were replicated in triplicate in each size fraction at the eastern tropical Pacific locations, both initially (in situ) and in the control and Fe addition carboys, but were not replicated at the Monterey Bay locations.

Following incubation, samples for total C and  $\text{NO}_3^-$  uptake rates were filtered directly onto precombusted (4 h at 500°C) Whatman GF/F filters (~0.7  $\mu\text{m}$ ). Samples for size-fractionated uptake rates were prefiltered through 10- or 5- $\mu\text{m}$  polycarbonate filters before collection on GF/F filters and uptake rates in the >5- and >10- $\mu\text{m}$  size fractions were calculated by difference. GF/F filters were placed in precombusted aluminum foil packets and stored at –20°C. In the laboratory, filters were dried for 3 d at 65°C, fumed with concentrated hydrochloric acid for 24 h to remove inorganic carbon, re-dried, and then assayed for atom percent <sup>13</sup>C and <sup>15</sup>N on a Europa Scientific 20–20 mass spectrometer. C and  $\text{NO}_3^-$  uptake rates were calculated as described by Dugdale and Goering (1967) and Dauchez et al. (1995). Samples for Si(OH)<sub>4</sub> uptake rates were size fractionated following incubation with <sup>32</sup>Si tracer by sequential vacuum filtration (<150 mm Hg)

Table 2. Comparison of average concentrations of chlorophyll *a* (Chl *a*), particulate organic carbon (POC), particulate organic nitrogen (PON), and biogenic silica (bSiO<sub>2</sub>), and the percentage of those concentrations occurring in the >5 μm cell fraction at 10 locations in Monterey Bay and three locations in the eastern tropical Pacific. In situ values are immediate measurements; control and 2 nmol L<sup>-1</sup> Fe values were measured in enrichment experiments after 3–6 d incubation. Monterey Bay values are averages of 10 locations, and eastern tropical Pacific values are averages of three replicates. Errors are standard errors. Monterey Bay Chl *a* values are from Wilkerson et al. (2000).

Location		Chl <i>a</i> (μg L <sup>-1</sup> )	>5 μm (%)	POC (μmol L <sup>-1</sup> )	>5 μm (%)	PON (μmol L <sup>-1</sup> )	>5 μm (%)	bSiO <sub>2</sub> (μmol L <sup>-1</sup> )	>5 μm (%)
Monterey Bay	In situ	2.16±1.88	50	97.3±15.0	73	9.8±1.4	64	6.4±1.2	81
Costa Rica	In situ			27.1±2.4	40	3.7±0.3	25	0.1±0.0	85
	Control			25.1±1.0	31	4.3±0.1	28	0.7±0.0	85
	2 nmol L <sup>-1</sup> Fe			32.8±3.1	50	5.3±0.1	50	1.6±0.3	78
Humboldt	In situ	0.47±0.05	49	16.0±0.6	22	1.3±0.1	17	0.7±0.1	62
	Control	0.72±0.08	61	12.8±1.0	37	2.0±0.0	43	1.3±0.0	87
	2 nmol L <sup>-1</sup> Fe	2.01±0.22	67	26.4±3.9	66	3.5±0.3	56	1.4±0.1	93
Peru	In situ	1.10±0.44	60	22.4±0.8	48	1.6±0.1	28	0.6±0.0	88
	Control	1.36±0.08	69	23.0±0.8	58	3.5±0.1	55	1.4±0.6	94
	2 nmol L <sup>-1</sup> Fe	1.78±0.07	70	24.3±2.4	63	3.8±0.2	61	3.2±0.1	97

through 5- and 0.6-μm polycarbonate filters (Monterey Bay) or 10-, 5-, and 0.6-μm polycarbonate filters (eastern tropical Pacific) by retaining and re-filtering the filtrate. Filters were then dried and assayed for <sup>32</sup>Si activity in the laboratory as described in Brzezinski and Phillips (1997). In cases where uptake rates were measured over periods <24 h, hourly rates of C and NO<sub>3</sub><sup>-</sup> uptake were extrapolated to daily rates by assuming linear uptake only during daylight hours. Si(OH)<sub>4</sub> uptake was assumed to be linear over the entire light–dark cycle (Martin-Jezequel et al. 2000).

## Results

Size classes are represented as 0.7–5, >5, and >10 μm in Figs. 2–7 rather than 0.7–5, 5–10, and >10 μm in order to make comparisons with previous studies on size-fractionated uptake easier, since many studies have used >5 μm as a cutoff for large cells. For the most part, the >5- and >10-μm size fractions were not very different, suggesting most cells were either <5 or >10 μm, which is consistent with previously published data on phytoplankton cell counts from these experiments (Franck et al. 2003).

*Size-fractionated biomass*—Size-fractionated biomass concentrations at all locations are shown in Table 2. Chlorophyll *a* (Chl *a*) concentrations were only measured at two locations in the HNLC waters of the eastern tropical Pacific: Humboldt and Peru (see Fig. 1 for locations). In situ Chl *a* concentrations at these sites were 0.47 ± 0.05 μg L<sup>-1</sup> and 1.10 ± 0.44 μg L<sup>-1</sup>, respectively. A relatively large fraction of this Chl *a* was in the >5-μm size fraction. Fe addition increased total Chl *a* only at the Humboldt location (2.8×). In both experiments, the proportion of Chl *a* in the large size fraction was high in the controls (>60%) and Fe addition did not alter these proportions.

Total POC and PON concentrations were relatively high at the Monterey Bay locations, with the majority (73% POC and 64% PON) attributable to the >5-μm size fraction on average (Table 2). The POC values ranged from 16.5 to 174.7 μmol L<sup>-1</sup> and the PON values from 1.8 to 16.6 μmol L<sup>-1</sup>. At the eastern tropical Pacific locations, in situ (initial

total POC and PON concentrations were at the low end of the range for Monterey Bay. Unlike the Monterey Bay locations, the small size fraction accounted for the majority of total POC and PON, with the larger fractions representing <50% and <30%, respectively. Fe addition increased total POC and PON concentrations nearly twofold relative to controls at the Humboldt current location but did not have as dramatic an effect on POC and PON concentrations at the other two locations in the eastern tropical Pacific. Fe addition increased the proportion of POC and PON attributable to the >5-μm size fraction at two locations, from ~40% to 56–66% at the Humboldt current site and from ~30% to 50% at the Costa Rica site.

Similar to total POC and PON concentrations, total bSiO<sub>2</sub> concentrations were high in Monterey Bay, ranging from 3.5 to 14.5 μmol L<sup>-1</sup>. Although the proportion of bSiO<sub>2</sub> attributable to the large (>5 μm) size fraction ranged widely, this size fraction accounted for the vast majority (81%) of total bSiO<sub>2</sub> on average (Table 2). In situ bSiO<sub>2</sub> concentrations at the eastern tropical Pacific locations were an order of magnitude lower than those in Monterey Bay, although a similar proportion was attributable to the >5-μm size fraction (~60–90%). Fe addition to eastern tropical Pacific waters doubled total bSiO<sub>2</sub> at the Costa Rica and Peru locations but had no effect on total bSiO<sub>2</sub> at the Humboldt location. At the Costa Rica location the increase in bSiO<sub>2</sub> after Fe addition was greater in the small size fraction (3×) than in the >5- and >10-μm size fractions (2×), and the end result of Fe addition in this experiment was to decrease the proportion of total bSiO<sub>2</sub> in the larger size fractions. In the Peru experiment, the increase in bSiO<sub>2</sub> after Fe addition was due entirely to increases in the >5- and >10-μm size fractions, since bSiO<sub>2</sub> in the 0.7–5-μm size fraction did not change.

*Size-fractionated C uptake*—Table 3 shows a comparison of size-dependent absolute C uptake rates ( $\rho$ , μmol C L<sup>-1</sup> d<sup>-1</sup>) and biomass-specific C uptake rates ( $V$ , d<sup>-1</sup>) at ambient Fe concentrations for the 10 Monterey Bay and three eastern tropical Pacific locations. Values of  $\rho$ C in surface waters ranged from 4.2 to 74.9 μmol C L<sup>-1</sup> d<sup>-1</sup> in Monterey Bay

Table 3. Comparison of in situ size-fractionated uptake rates of C, NO<sub>3</sub><sup>-</sup>, and Si(OH)<sub>4</sub> in Monterey Bay and the eastern tropical Pacific. Shown are absolute (ρC, ρNO<sub>3</sub><sup>-</sup>, and ρSi) and biomass-specific (VC, VNO<sub>3</sub><sup>-</sup>, and VSi) uptake rates. Values were measured at ambient Fe concentrations. Monterey Bay values include data from 10 locations, and eastern tropical Pacific values are averages of three replicates at each location. Errors are standard errors.

	C				NO <sub>3</sub> <sup>-</sup>				Si (OH) <sub>4</sub>			
	ρC (μmol L <sup>-1</sup> d <sup>-1</sup> )	ρC >5 μm (%)	VC (d <sup>-1</sup> )	VC >5 μm/VC <5 μm	ρNO <sub>3</sub> <sup>-</sup> (μmol L <sup>-1</sup> d <sup>-1</sup> )	ρNO <sub>3</sub> <sup>-</sup> >5 μm (%)	VNO <sub>3</sub> <sup>-</sup> (d <sup>-1</sup> )	VNO <sub>3</sub> <sup>-</sup> >5 μm/VNO <sub>3</sub> <sup>-</sup> <5 μm	ρSi (μmol L <sup>-1</sup> d <sup>-1</sup> )	ρSi >5 μm (%)	VSi (d <sup>-1</sup> )	VSi >5 μm/VSi <5 μm
Monterey Bay (average)	4.1-74.9 (33.6±8.5)	86-94 (92)	0.17-0.43 (0.33±0.03)	1.49-3.17 (2.23)	1.15-3.40 (2.08±0.26)	74-89 (81)	0.17-0.30 (0.23±0.02)	0.30-3.26 (1.11)	0.46-2.47 (1.54±0.26)	42-99 (98)	0.14-0.85 (0.59±0.11)	0.34-18.90 (2.55)
Costa Rica	8.0±0.4	43	0.24±0.01	0.22	0.42±0.05	34	0.17±0.02	0.85	1.10±0.03	5	2.05±0.25	0.05
Humboldt	1.9±0.2	27	0.15±0.01	0.26	0.18±0.00	39	0.17±0.00	0.91	0.17±0.02	95	0.25±0.01	3.31
Peru	2.6±0.2	42	0.15±0.01	0.68	0.30±0.03	59	0.33±0.05	0.76	0.26±0.02	95	0.30±0.01	3.01

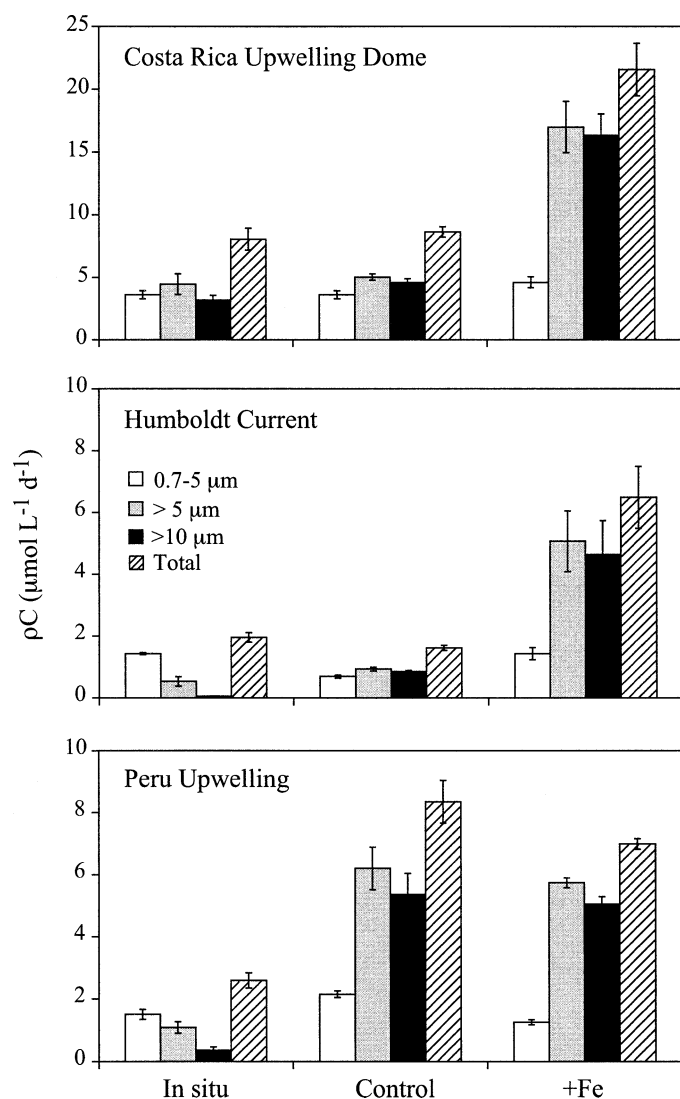


Fig. 2. Size-fractionated primary production (ρC) before and after Fe addition at three locations in the eastern tropical Pacific. In situ values were measured immediately after water collection. Control and +Fe values were measured after 3-6 d incubation in 10-liter carboys with no Fe addition (control) or with 2 nmol L<sup>-1</sup> Fe additions (+Fe). Error bars are standard errors of triplicate incubations.

and from 1.9 to 8.0 μmol C L<sup>-1</sup> d<sup>-1</sup> in the eastern tropical Pacific. In Monterey Bay, the large (>5 μm) size fraction dominated C uptake, accounting for 92% of ρC on average. At the eastern tropical Pacific locations, however, the small size fraction was most active with respect to C uptake, with the >5-μm size fraction accounting for only 27-43% of total C uptake. Biomass-specific C uptake rates (VC) in surface waters ranged from 0.17 to 0.43 d<sup>-1</sup> in Monterey Bay and from 0.15 to 0.24 d<sup>-1</sup> in the eastern tropical Pacific. In Monterey Bay, VC was about two times greater in the large size fraction than in the small size fraction on average, while at the eastern tropical Pacific locations, VC values in the >5-μm size fraction were only 22-68% of those observed in the small size fraction.

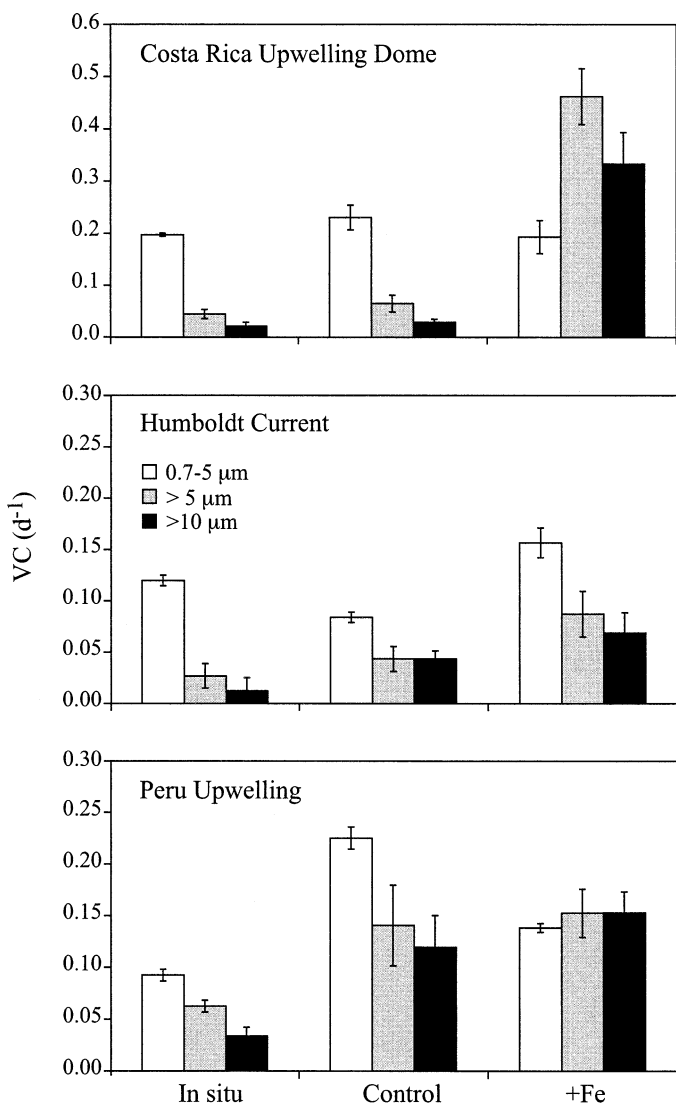


Fig. 3. Size-fractionated biomass-specific C uptake rates (VC) before and after Fe addition at three locations in the eastern tropical Pacific. In situ, control, and +Fe values and error bars are as described for Fig. 2.

Fe addition to the HNLC waters of the eastern tropical Pacific caused roughly a threefold increase in total  $\rho\text{C}$  relative to controls at two locations: the Costa Rica upwelling dome and Humboldt current upwelling regions (Fig. 2). Increases in C uptake were observed only in the large size fractions, so that Fe enrichment increased the percentage of  $\rho\text{C}$  attributable to the >5- $\mu\text{m}$  size fraction relative to controls from 58% in controls to 78% in Fe additions at both locations. The fractions of  $\rho\text{C}$  attributable to the >10- and >5- $\mu\text{m}$  size classes were similar at these locations, suggesting that the majority of cells and aggregates fixing carbon were >10  $\mu\text{m}$ . VC values in the >5- $\mu\text{m}$  size fraction also increased substantially after Fe addition at these two locations, by 5 $\times$  relative to controls at the Costa Rica upwelling dome and by 2 $\times$  at the Humboldt current upwelling region (Fig. 3). In the small size fraction, Fe addition increased VC values relative to controls at the Humboldt lo-

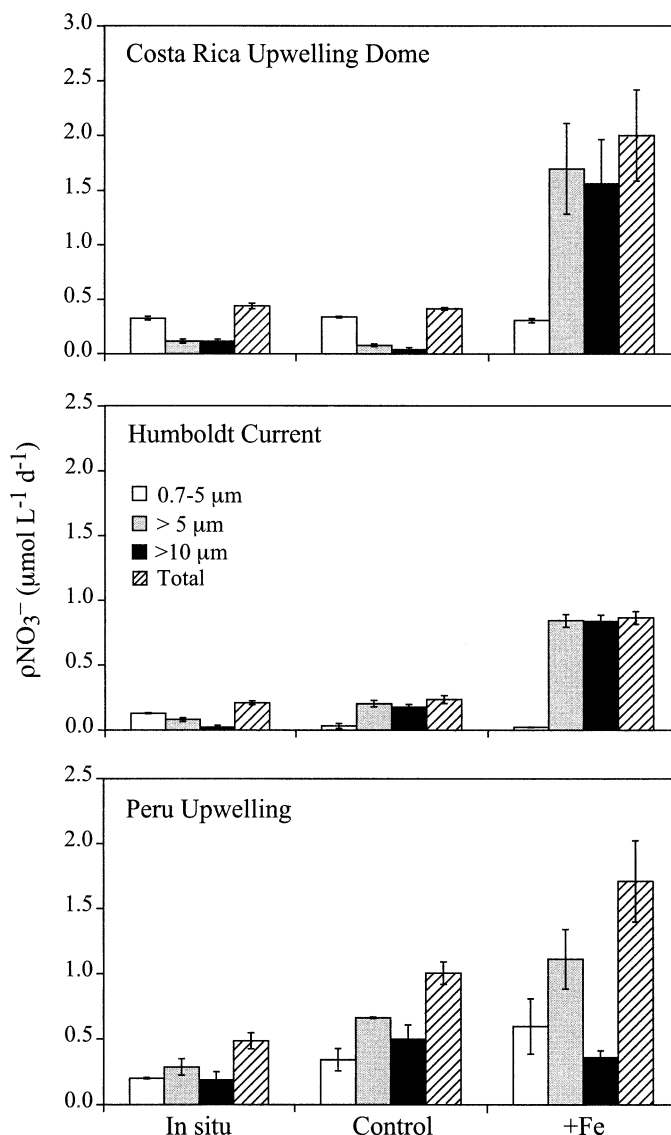


Fig. 4. Size-fractionated new production ( $\rho\text{NO}_3^-$ ) before and after Fe addition at three locations in the eastern tropical Pacific. In situ, control, and +Fe values and error bars are as described for Fig. 2.

cation by 2 $\times$  but appeared to decrease VC values to 61–84% of those in the controls at the Costa Rica and Peru locations.

*Size-fractionated  $\text{NO}_3^-$  uptake*—Values of  $\rho\text{NO}_3^-$  in surface waters at the Monterey Bay stations ranged from 1.15 to 3.40  $\mu\text{mol NO}_3^- \text{L}^{-1} \text{d}^{-1}$  (Table 3). At the three eastern tropical Pacific locations, in situ  $\rho\text{NO}_3^-$  values in surface waters were about an order of magnitude lower, ranging from 0.18 to 0.42  $\mu\text{mol NO}_3^- \text{L}^{-1} \text{d}^{-1}$ . As with C uptake rates, cells in the large (>5  $\mu\text{m}$ ) size fraction dominated  $\text{NO}_3^-$  uptake in Monterey Bay, accounting for 81% of  $\rho\text{NO}_3^-$  on average. At the Peru upwelling location in the eastern tropical Pacific, cells in the >5- $\mu\text{m}$  size fraction also accounted for the majority of in situ  $\rho\text{NO}_3^-$ . At the other two locations in the eastern tropical Pacific, however, cells in the *small* size fraction dominated in situ  $\rho\text{NO}_3^-$ , with cells in the >5- $\mu\text{m}$  size

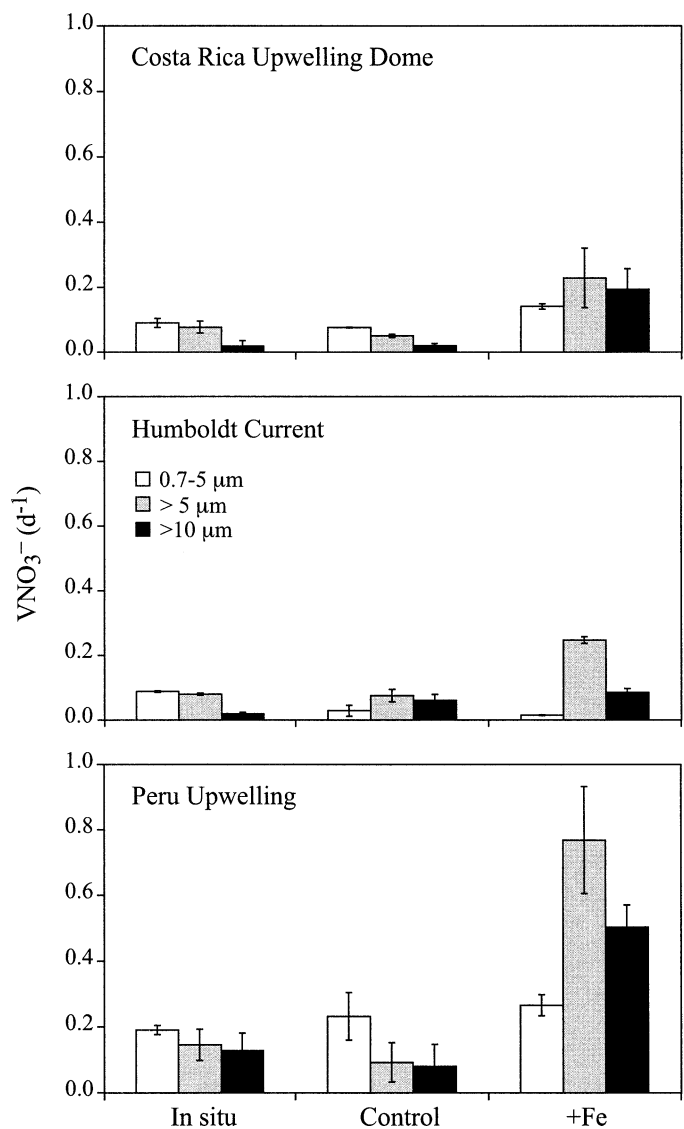


Fig. 5. Size-fractionated biomass-specific  $\text{NO}_3^-$  uptake rates ( $V\text{NO}_3^-$ ) before and after Fe addition at three locations in the eastern tropical Pacific. In situ, control, and +Fe values and error bars are as described for Fig. 2.

fraction representing only 34–39% of total  $\rho\text{NO}_3^-$  (Table 3). Values of  $V\text{NO}_3^-$  in surface waters ranged from 0.17 to 0.30  $\text{d}^{-1}$  in Monterey Bay and from 0.17 to 0.33  $\text{d}^{-1}$  at the eastern tropical Pacific locations.  $V\text{NO}_3^-$  appeared to be roughly equivalent in cells in the large and small size fractions regardless of ambient Fe concentrations in this study.

Fe addition to HNLC waters in the eastern tropical Pacific increased total  $\rho\text{NO}_3^-$  relative to controls at all locations: five times at the Costa Rica upwelling dome, four times at the Humboldt current, and almost two times at the Peru upwelling location (Fig. 4). In general, Fe addition stimulated  $\rho\text{NO}_3^-$  in the large size fractions but not in the small size fraction. At the Costa Rica and Humboldt current locations, Fe addition increased the proportion of  $\rho\text{NO}_3^-$  attributable to the  $>5\text{-}\mu\text{m}$  size fraction from 19% to 81% (Costa Rica) and from 87% to 98% (Humboldt). In the Peru experiment, how-

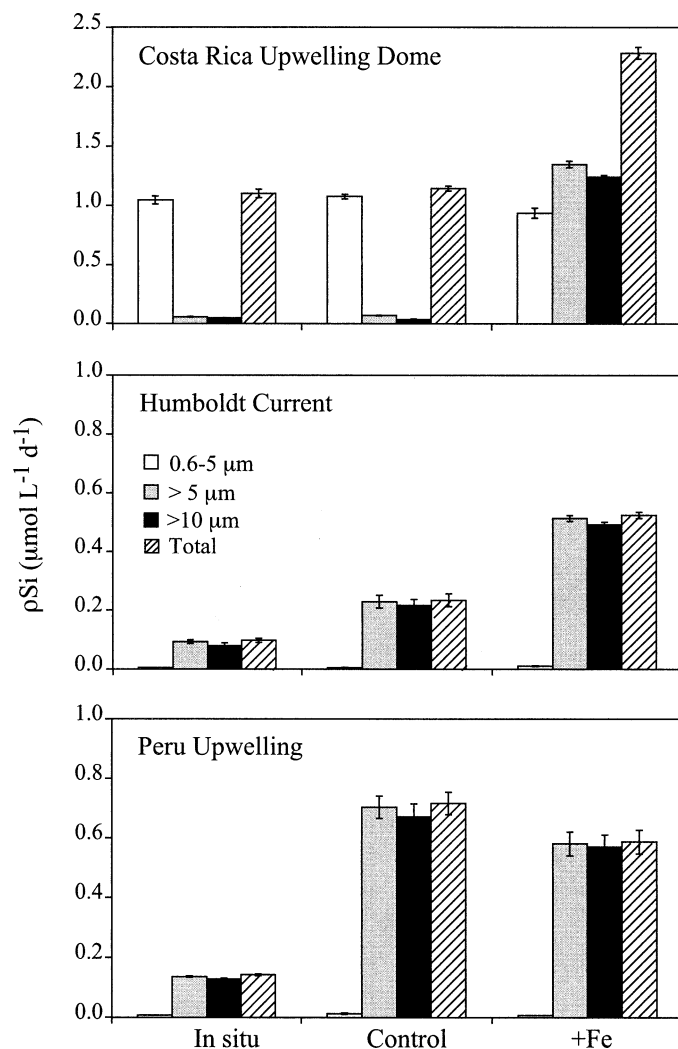


Fig. 6. Size-fractionated silica production ( $\rho\text{Si}$ ) before and after Fe addition at three locations in the eastern tropical Pacific. In situ, control, and +Fe values and error bars are as described for Fig. 2.

ever, Fe addition increased  $\rho\text{NO}_3^-$  in both the 0.7–5- and  $>5\text{-}\mu\text{m}$  size fractions and, so, did not change the percentage of  $\rho\text{NO}_3^-$  attributable to the  $>5\text{-}\mu\text{m}$  size fraction. The  $\rho\text{NO}_3^-$  values in the  $>5\text{-}$  and  $>10\text{-}\mu\text{m}$  size fractions were fairly different at the Peru location—as was the response to Fe in these two size fractions—suggesting the presence of a unique community of cells ranging in size from 5 to 10  $\mu\text{m}$ . Microscopic observations of preserved phytoplankton samples at this location show relatively large abundances of colonial flagellates (*Dinobryon* spp.) and *Chaetoceros* spp. cells within this size range (Franck et al. 2003). Fe addition also increased  $V\text{NO}_3^-$  at all three locations, and increases were greatest in the larger size fractions (Fig. 5).  $V\text{NO}_3^-$  values increased in the  $>5\text{-}\mu\text{m}$  size fraction by 4 $\times$  (Costa Rica), 3 $\times$  (Humboldt), and 8 $\times$  (Peru) and in the  $>10\text{-}\mu\text{m}$  size fraction by 7 $\times$  (Costa Rica) and 5 $\times$  (Peru). Only at the Costa Rica upwelling dome location did  $V\text{NO}_3^-$  increase in the small size fraction in response to Fe addition (2 $\times$ ). At the Humboldt current location, Fe addition appeared to de-

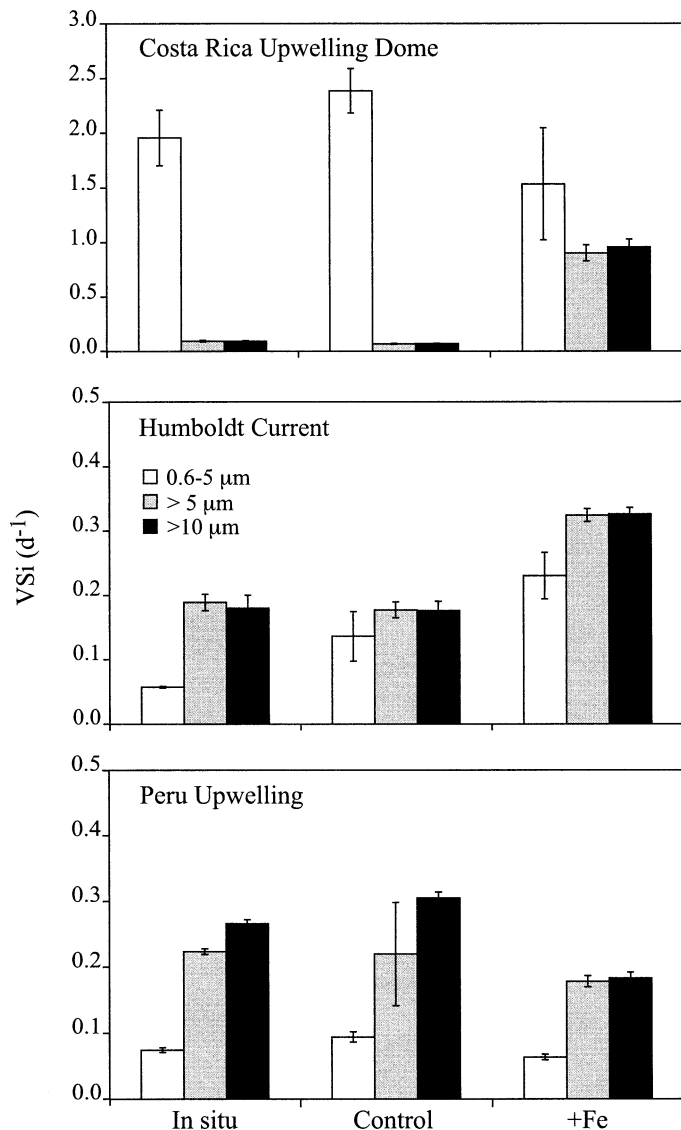


Fig. 7. Size-fractionated biomass-specific  $Si(OH)_4$  uptake rates ( $V_{Si}$ ) before and after Fe addition at three locations in the eastern tropical Pacific. In situ, control, and +Fe values and error bars are as described for Fig. 2.

crease the  $VNO_3^-$  value in the small size fraction to 50% of the control value.

**Size-fractionated  $Si(OH)_4$  uptake**—Values of  $\rho Si$  in surface waters at the Monterey Bay stations varied by an order of magnitude, ranging from 0.46 to 2.47  $\mu mol Si(OH)_4 L^{-1} d^{-1}$  (Table 3). In situ  $\rho Si$  values in surface waters at the three eastern tropical Pacific locations were much lower, ranging from 0.17 to 1.10  $\mu mol Si(OH)_4 L^{-1} d^{-1}$ . In Monterey Bay, the >5- $\mu m$  size fraction accounted for 98% of  $\rho Si$ , dominating  $Si(OH)_4$  uptake to an even greater extent than C (92%) and  $NO_3^-$  (81%) uptake (Table 3). At two locations in the eastern tropical Pacific—Humboldt and Peru—the percentage of  $\rho Si$  attributable to the >5- $\mu m$  size fraction was similar to the average for Monterey Bay (95%). At the Costa Rica location, however, only 5% of  $\rho Si$  was attributable to

the >5- $\mu m$  size fraction even though 85% of the siliceous biomass was in this size fraction (Tables 2 and 3). Unlike absolute uptake rates, the overall range of biomass-specific  $Si(OH)_4$  uptake rates ( $V_{Si}$ ) was higher in the eastern tropical Pacific (0.25–2.05  $d^{-1}$ ) than in Monterey Bay (0.14–0.85  $d^{-1}$ ).  $V_{Si}$  at the Costa Rica upwelling dome location was unusually high (2.05  $d^{-1}$ ), mainly because of the activity of the small size fraction, which had a 20-fold higher  $V_{Si}$  value than the >5- $\mu m$  size fraction. This  $V_{Si}$  value represents a surprisingly fast doubling time for a diatom assemblage (8 h) but is within physiological limits. Microscopic observations of diatoms at this location revealed a unique assemblage of abundant, small ( $\sim 5 \mu m$ ) solitary pennates. At the other two locations in the eastern tropical Pacific, where small pennates were not abundant, overall  $V_{Si}$  values were low and the values in the >5- $\mu m$  size fraction were three times faster than those in the small size fraction (Table 3). In Monterey Bay,  $V_{Si}$  values in the >5- $\mu m$  size fraction were two times faster than those in the small size fraction on average.

Fe addition to waters in the eastern tropical Pacific doubled total  $Si(OH)_4$  uptake relative to controls at the Costa Rica and Humboldt locations (Fig. 6). Fe addition appeared to decrease total  $\rho Si$  at the Peru location, however, by 18% relative to controls. Similar to C and  $NO_3^-$  uptake, the overall effect of Fe addition on  $Si(OH)_4$  uptake at the Fe-limited locations (Costa Rica and Humboldt) was to increase uptake rates only in the large size fractions. Fe addition at the Costa Rica location dramatically increased the proportion of  $\rho Si$  attributable to the >5- $\mu m$  size fraction, from 5% to 59%. The  $\rho Si$  values in the >5- and >10- $\mu m$  size fractions were similar in the eastern tropical Pacific experiments, suggesting that the majority of larger cells using  $Si(OH)_4$  were >10  $\mu m$ . Fe addition also had a large effect on size-dependent  $V_{Si}$  values in the larger size fractions at the Costa Rica and Humboldt locations, but again not at the Peru location (Fig. 7).  $V_{Si}$  values increased tenfold in the >5- and >10- $\mu m$  size fractions at the Costa Rica location and doubled in all size fractions at the Humboldt location. At the Peru location, Fe addition appeared to decrease  $V_{Si}$  to 60–80% of control values in all size fractions.

## Discussion

**Regional comparison of size-dependent biomass, production, and nutrient uptake rates**—Based on this study, phytoplankton communities in Monterey Bay and the eastern tropical Pacific appear to have a very different size structure. In Monterey Bay the vast majority of biomass was in the >5- $\mu m$  size fraction (Table 2), confirming previous studies documenting that phytoplankton community structure in highly productive waters is dominated by large phytoplankton, especially large diatoms (Owens et al. 1993; Chavez 1996; Wilkerson et al. 2000). Our results from HNLC waters in the eastern tropical Pacific also confirm previous studies demonstrating that the majority of POC and PON in this region resides in the <5- $\mu m$  size fraction (Chavez 1989; Chavez et al. 1990, 1991; Peña et al. 1990). Similar results have been reported from other HNLC regions (Boyd et al.

1996). Taken together, these studies suggest that despite high macronutrient concentrations, phytoplankton communities in HNLC regions are dominated by nanophytoplankton and picophytoplankton and, thus, are similar to oligotrophic, open-ocean regions. Although the majority of POC and PON resided in the small size fraction, the majority of biogenic silica resided in the large size fraction at both the Monterey Bay and the eastern tropical Pacific locations. Only one other study has measured size-fractionated biogenic silica (Quéguiner and Brzezinski 2002), but both preliminary studies demonstrate a remarkably similar percentage (~65–90%) of bSiO<sub>2</sub> attributable to larger phytoplankton fraction in three very dissimilar environments—the Southern Ocean, Monterey Bay, and the eastern tropical Pacific, suggesting that this percentage may be representative of much of the ocean.

Average values for primary production (33.6  $\mu\text{mol C L}^{-1} \text{d}^{-1}$ ) and new, nitrate-based production (2.1  $\mu\text{mol NO}_3^- \text{L}^{-1} \text{d}^{-1}$ ) at the Monterey Bay locations in this study fall within the range reported for other high-productivity upwelling regions. Previous studies report average new production values of 9.6  $\mu\text{mol NO}_3^- \text{L}^{-1} \text{d}^{-1}$  for upwelling regions off the coasts of Oregon and Washington (Kokkinakis and Wheeler 1987), 6.7  $\mu\text{mol NO}_3^- \text{L}^{-1} \text{d}^{-1}$  for the Benguela upwelling system (Probyn 1985), and 2.9  $\mu\text{mol NO}_3^- \text{L}^{-1} \text{d}^{-1}$  for the Peru upwelling region (Dugdale and Wilkerson 1986). A 2-yr study of C and NO<sub>3</sub><sup>-</sup> uptake rates in Monterey Bay surface waters by Wilkerson et al. (2000), however, showed much lower average values of primary production and new production than those reported here (6.32  $\mu\text{mol C L}^{-1} \text{d}^{-1}$  and 0.54  $\mu\text{mol NO}_3^- \text{L}^{-1} \text{d}^{-1}$ ). Values reported in this study most likely represent bloom or postbloom conditions in Monterey Bay. In situ fluorescence was high during our study, and large amounts of the chain-forming diatoms *Skeletonema costatum*, *Chaetoceros* spp., and *Pseudo-nitzschia* spp. were observed in water samples. In addition, the range of total silica production measured for Monterey Bay in this study is similar to previous values reported for bloom conditions in the bay (Brzezinski et al. 1997).

VC, VNO<sub>3</sub><sup>-</sup>, and VSi values were all greatest in the large (>5  $\mu\text{m}$ ) size fraction on average in Monterey Bay, suggesting larger cells were more active with respect to primary production, new production, and silica production, as well as more abundant. The fact that larger phytoplankton appear to be driving total C and NO<sub>3</sub><sup>-</sup> uptake rates in this region confirms previous reports on the importance of large cells to new and primary production in productive upwelling regions. During non-El Niño conditions in Monterey Bay, the >5- $\mu\text{m}$  size fraction has been shown to account for 78–97% of primary production and 87% of new production (Chavez 1996; Wilkerson et al. 2000). In the Benguela upwelling region, the >10- $\mu\text{m}$  size fraction accounted for 65% of total N assimilation (Probyn 1985), and in an upwelling region in the Indian Ocean, the >5- $\mu\text{m}$  size fraction accounted for 90% of total C and N assimilation (Owens et al. 1993). No previous studies have investigated the contribution of large cells to silica production.

As stated earlier, in situ primary production, new production, and silica production rates in the low-Fe HNLC waters of the eastern tropical Pacific were much lower than the average rates in Monterey Bay (Table 3). Rates in the >5-

$\mu\text{m}$  fraction in the eastern tropical Pacific were only a small percentage of average rates in the >5- $\mu\text{m}$  size fraction in Monterey Bay (1–5%  $\rho\text{C}$ , 3–6%  $\rho\text{NO}_3^-$ , and 2–11%  $\rho\text{Si}$ ), and the proportion of primary production and new production attributable to phytoplankton in the >5- $\mu\text{m}$  fraction was substantially lower. Specific uptake rates of C, NO<sub>3</sub><sup>-</sup>, and Si(OH)<sub>4</sub> were generally lower at the eastern tropical Pacific locations as well. Coupled with the fact that the small size fraction accounted for the majority of POC and PON at the eastern tropical Pacific locations, our results confirm that phytoplankton in the large size fraction are both less abundant and less productive in eastern tropical Pacific waters. A previous study by Chavez et al. (1991) reports primary production ( $\rho\text{C}$ ) rates of 1.0–1.5  $\mu\text{mol C L}^{-1} \text{d}^{-1}$  in the equatorial Pacific, with 72% attributable to the <5- $\mu\text{m}$  size fraction—remarkably similar to our results from the Humboldt current upwelling region (1.9  $\mu\text{mol C L}^{-1} \text{d}^{-1}$ , 27% >5  $\mu\text{m}$ ). Our study also confirms findings by Price et al. (1994) that the <3- $\mu\text{m}$  size fraction overwhelmingly dominates new production in the equatorial Pacific (85%), and our measurement of new production in the Peru upwelling region falls within the range of values reported for this area by Dugdale and Wilkerson (1986).

Previous studies of size-dependent C and NO<sub>3</sub><sup>-</sup> uptake in other HNLC regions are more varied. At Ocean Station P in the NE Subarctic Pacific, several studies report that the majority (>50%) of primary production was attributable to the <5- $\mu\text{m}$  size fraction, similar to our data from all three locations in the eastern tropical Pacific (Boyd et al. 1996; Schmidt and Hutchins 1999). In HNLC waters near the polar front in the Pacific sector of the Southern Ocean, however, Sambrotto and Mace (2000) report that the majority (>60%) of new production was attributable to the larger (>5  $\mu\text{m}$ ) size fractions, which is closer to our results from the high-Fe waters of Monterey Bay than to our results from the low-Fe waters of the eastern tropical Pacific (Table 3). As with the Sambrotto and Mace study, VNO<sub>3</sub><sup>-</sup> rates in the >5- $\mu\text{m}$  fraction in our study were similar to those in the small size fraction, irrespective of in situ dissolved Fe concentrations.

In contrast to size-dependent  $\rho\text{C}$  and  $\rho\text{NO}_3^-$ , silica production ( $\rho\text{Si}$ ) at ambient Fe concentrations was restricted almost entirely to the large size fraction at the Humboldt and Peru locations. This is consistent with our observations that the >5- $\mu\text{m}$  fraction accounted for the majority of siliceous biomass (Table 2), and that biomass-specific Si uptake rates were threefold higher in the large size fraction than in the small size fraction at these locations (Table 3). At the Costa Rica upwelling dome location, however, a substantial proportion (95%) of  $\rho\text{Si}$  was attributable to the small size fraction, despite the fact that this fraction accounted for ~15% of the total siliceous biomass. This suggests that smaller diatoms were especially active with respect to Si uptake at this location. The Costa Rica location had the highest overall biomass-specific Si uptake rates of any other location in this study (threefold higher than the average VSi value for bloom conditions in Monterey Bay), and VSi values in the small size fraction exceeded those in the large size fractions by 20 times. Biomass and uptake samples were size-fractionated in a similar manner, so differences in filtration methods cannot account for the dramatically higher VSi in the small cell

Table 4. Comparison of the percentage of  $\rho\text{Si}$  and  $\rho\text{NO}_3^-$  attributable to the large ( $>5\ \mu\text{m}$ ) size fraction, and of  $\text{Si}:\text{NO}_3^-$  uptake ratios in the small ( $0.7\text{--}5\ \mu\text{m}$ ) and large ( $>5\ \mu\text{m}$  and  $>10\ \mu\text{m}$ ) size fractions at the locations in Monterey Bay and the eastern tropical Pacific where size-fractionated uptake data were collected. Values were measured at ambient Fe concentrations. Errors are standard errors of averages for eight stations (Monterey Bay) or three replicates per station (eastern tropical Pacific).

	$\rho\text{Si} > 5\ (\%)$	$\rho\text{NO}_3^- > 5\ (\%)$	$\rho\text{Si}:\rho\text{NO}_3^- (<5\ \mu\text{m})$	$\rho\text{Si}:\rho\text{NO}_3^- (>5\ \mu\text{m})$	$\rho\text{Si}:\rho\text{NO}_3^- (>10\ \mu\text{m})$
Monterey Bay	98	81	$0.14\pm 0.04$	$0.95\pm 0.23$	
G3	98	87	0.22	2.04	
K3	98	89	0.25	1.57	
M3	99	78	0.02	0.93	
M5	99	74	0.02	0.91	
K5	98	85	0.14	1.07	
I5	82	87	0.40	0.26	
G5	98	81	0.07	0.81	
E5	42	75	0.04	0.01	
Costa Rica	5	34	$3.23\pm 0.04$	$0.50\pm 0.02$	$0.42\pm 0.02$
Humboldt	95	39	$0.04\pm 0.01$	$1.16\pm 0.02$	$3.29\pm 0.02$
Peru	95	59	$0.04\pm 0.01$	$0.47\pm 0.06$	$0.68\pm 0.06$

fraction at this location. Previously published phytoplankton counts from the Costa Rica enrichment experiment show a large number of small ( $\sim 5\ \mu\text{m}$ ) solitary pennate diatoms (Franck et al. 2003). It appears that these small diatoms were particularly active with respect to  $\text{Si}(\text{OH})_4$  uptake and that a major fraction of the larger diatoms were not actively taking up  $\text{Si}(\text{OH})_4$ . The  $\text{bSiO}_2:\text{PON}$  ratio in the  $>5\text{-}\mu\text{m}$  size fraction was very low (0.1), which suggests that the majority of cells in the large size fraction were not silicified.

*Contribution of diatoms to new production*—Very few data exist on the contribution of any one group of phytoplankton to total new production. A common assumption, however, is that most if not all  $\text{NO}_3^-$  uptake is attributable to larger diatoms, even when their abundance is relatively rare. It was our hypothesis that a comparison of size-fractionated  $\text{Si}(\text{OH})_4$  and  $\text{NO}_3^-$  uptake could test this assumption. Diatoms with sufficient macronutrients and micronutrients use  $\text{Si}(\text{OH})_4$  in a 1:1 ratio with  $\text{NO}_3^-$  in culture (Brzezinski 1985). Higher  $\text{Si}(\text{OH})_4:\text{NO}_3^-$  uptake ratios have been documented under Fe limitation (see following section), but it is rare for diatoms to have  $\text{Si}(\text{OH})_4:\text{NO}_3^-$  uptake ratios that are  $<1$ , with the exception of a few species that are only partially silicified. If larger diatoms are in fact responsible for the majority of new production, in situ  $\rho\text{Si}:\rho\text{NO}_3^-$  uptake ratios in the large size fraction should be  $\geq 1$ , and the percentages of both  $\rho\text{Si}$  and  $\rho\text{NO}_3^-$  attributable to the large size fraction should be large and approximately equal. Information on the contribution of large diatoms to  $\text{NO}_3^-$  uptake would be especially useful in models of global new production and C export, since environmental conditions (nutrients, light, Fe) which favor the growth of large diatoms are not necessarily the same as those that favor the growth of other  $\text{NO}_3^-$ -using phytoplankton such as *Phaeocystis* or *Synechococcus* spp.

A comparison of the percentage of  $\rho\text{Si}$  versus the percentage of  $\rho\text{NO}_3^-$  attributable to phytoplankton in the large ( $>5\ \mu\text{m}$ ) size fraction and of  $\rho\text{Si}:\rho\text{NO}_3^-$  ratios in the large and small size fractions is shown in Table 4. These data suggest that  $\text{NO}_3^-$  uptake by large diatoms dominated new production at the Monterey Bay locations, where phyto-

plankton in the large size fraction accounted for a similarly large proportion of  $\rho\text{Si}$  and  $\rho\text{NO}_3^-$  (81–98%), and  $\rho\text{Si}:\rho\text{NO}_3^-$  ratios in the  $>5\text{-}\mu\text{m}$  fraction were  $>0.8$  at all but two locations. Such was not the case for our experiments in the eastern tropical Pacific. Based on ratios of  $\rho\text{Si}:\rho\text{NO}_3^-$  in the large size fractions, diatoms were probably responsible for the majority of new production at the Humboldt location and possibly the Peru location as well. As mentioned previously, however, a much smaller percentage of total  $\rho\text{NO}_3^-$  was attributable to larger phytoplankton at these locations relative to Monterey Bay (Table 4). Approximately 40–60% of total new production at the Humboldt and Peru sites remained in the small size fraction, and our results suggest that this new production was not attributable to diatoms. Ratios of  $\rho\text{Si}:\rho\text{NO}_3^-$  in the small size fraction at these locations were  $\ll 1$ , and the percentage of  $\rho\text{NO}_3^-$  attributable to the small size fraction was substantially greater than for  $\rho\text{Si}$  (40–60% vs. 5%). This would imply that some fraction of  $\text{NO}_3^-$  uptake at these two locations was due to smaller, nonsiliceous phytoplankton or bacterioplankton. Studies in eastern tropical Pacific waters have documented large numbers of *Synechococcus* spp. cells (Price et al. 1994; Raphe Kudela pers. comm.), which are known to use  $\text{NO}_3^-$ . Previous studies have shown that bacterioplankton can also have relatively high  $\text{NO}_3^-$  uptake rates in some regions (e.g., Kirchman et al. 1992). What effect new production by smaller cells would have on C export is uncertain, although it may enhance nitrogen retention in surface waters, potentially contributing to the high- $\text{NO}_3^-$ , low- $\text{Si}(\text{OH})_4$  conditions observed in the equatorial Pacific (Dugdale and Wilkerson 1998).

The data in Table 4 suggest that a completely different community structure was contributing to new production at the Costa Rica upwelling dome location compared with the Humboldt and Peru upwelling locations. Ratios of  $\rho\text{Si}:\rho\text{NO}_3^-$  in the small size fraction were  $>3$ , but ratios were very low (0.5) in both the larger size fractions, suggesting that most of the smaller cells taking up  $\text{NO}_3^-$  were diatoms while the larger cells taking up  $\text{NO}_3^-$  were not. The difference between the percentages of  $\rho\text{NO}_3^-$  and  $\rho\text{Si}$  attributable to the large size fractions suggests that almost a third of new production ( $\sim 30\%$ ) was due to larger, nonsiliceous phyto-

plankton. One possibility could be autotrophic nanoflagellates such as *Phaeocystis* spp., since microscopic counts revealed relatively high concentrations of unidentified nanoflagellates at this site (Franck et al. 2003). Based on a comparison of size-dependent  $\text{Si(OH)}_4$  and  $\text{NO}_3^-$  uptake rates measured in this study, it appears that significant new production in HNLC regions can be attributable to the smaller size fractions, and cells other than diatoms can contribute a substantial proportion of new production in both the smaller and larger size fractions.

*Effect of Fe addition on production and nutrient uptake rates in the eastern tropical Pacific*—Fe addition to HNLC waters in the eastern tropical Pacific increased rates of new production (total  $\rho\text{NO}_3^-$ ) by two to four times at all locations, increased rates of primary production (total  $\rho\text{C}$ ) by three times, and increased diatom silica production (total  $\rho\text{Si}$ ) by two times at all locations except the Peru upwelling site (Figs. 2, 4, and 6). Such a strong response confirms reports that Fe availability is regulating rates of primary production, nutrient uptake, and diatom growth in the equatorial Pacific region (Coale et al. 1996). In this study, Fe availability appeared to regulate production only in the large ( $>5$  and  $>10$   $\mu\text{m}$ ) size fractions, since rates of production in the small ( $0.7$ – $5$   $\mu\text{m}$ ) size fraction did not respond to Fe addition. Fe addition also increased the proportion of organic matter and biogenic silica in the large size fractions (Table 2). Increased rates of new production—especially when coupled with increased production of biomass in size classes with faster sinking rates—should lead to increased C export. It remains unclear, however, whether Fe availability regulates C export in HNLC regions.

In addition to increasing production and biomass in the larger size fractions, Fe addition also increased biomass-specific uptake rates for  $\text{NO}_3^-$  ( $\text{VNO}_3^-$ ) in the  $>5$ - $\mu\text{m}$  size fractions relative to controls at all locations in the eastern tropical Pacific, as well as biomass-specific C (VC) and  $\text{Si(OH)}_4$  (VSi) uptake rates in the large size fractions at the Costa Rica and Humboldt locations (Figs. 3, 5, and 7). The fact that Fe addition stimulated *biomass-specific* uptake rates in the large size fractions suggests that the observed increases in production by larger cells were not just a result of increased biomass but also the result of an increased capacity for C and nutrient [ $\text{NO}_3^-$  and  $\text{Si(OH)}_4$ ] uptake, and/or faster growth rates. In general, phytoplankton in the large size fractions initially had biomass-specific uptake rates that were equal to or greater than those cells in the small size fraction at ambient Fe concentrations. Once Fe availability increased at these locations, specific uptake rates in the larger size fractions typically exceeded those in the small size fraction, especially for  $\text{NO}_3^-$  and C uptake. Thus it appears that physiological as well as ecological advantages (i.e., slower growing grazers) favor larger cells at high Fe concentrations.

At the Costa Rica location, phytoplankton in the small size fraction had dramatically higher biomass-specific C and  $\text{Si(OH)}_4$  uptake rates at ambient Fe than phytoplankton in the larger size fractions, and this uptake capacity remained largely unaffected by Fe addition (Figs. 3 and 7). The small size fraction dominated total  $\text{Si(OH)}_4$  uptake at ambient Fe, with  $\sim 90\%$  of  $\rho\text{Si}$  attributable to the  $0.7$ – $5$ - $\mu\text{m}$  size fraction

(Fig. 6). Fe addition reduced this proportion to  $\sim 50\%$  by increasing biomass and  $\text{Si(OH)}_4$  uptake capacity in the large size fraction. This suggests that Fe availability may be regulating the dominant diatom taxa with respect to  $\text{Si(OH)}_4$  production at this location.

The study site in the Peru upwelling region appears to be something of an anomaly in its response to Fe addition when compared with the other two locations in the eastern tropical Pacific. Total new production and  $\text{VNO}_3^-$  in the larger size fractions increased after Fe addition, but primary production, silica production, and biomass-specific C and Si uptake rates actually appeared to decrease in response to Fe addition (Figs. 2–7). Several factors may have contributed to these trends. The fact that  $\text{bSiO}_2$  concentrations, as well as  $\rho\text{NO}_3^-$  and  $\text{VNO}_3^-$  values, increased substantially after Fe addition suggests that contamination of the controls was unlikely. The initial dissolved Fe concentration at this location was relatively high ( $\sim 0.3$   $\text{nmol L}^{-1}$ ; Eden Rue pers. comm.), and it is probable that the Peru experiment was not severely Fe limited.

Release from light limitation may have also stimulated uptake rates in the controls. This is consistent with the higher rates in the controls relative to in situ rates at the Peru site compared with other sites (Figs. 2–7). The mixed layer was 60 m at the Peru site, making it likely that the initial waters collected for this experiment were light limited, or more likely Fe and light colimited, since light limitation increases Fe requirements in phytoplankton (Sunda and Huntsman 1997). Chl *a* per cell was higher in the Fe additions than in the controls in this experiment by 1.5 times, implying that cells in the controls were somewhat chlorotic because of Fe limitation. However, the increase in uptake rate in controls was often as large as or, in some cases, larger than in the Fe addition treatments, indicating uptake was responding to other factors in addition to Fe. Under these conditions our experimental design was not able to separate the effects of Fe from potential confounding factors. We were able to observe a clear effect of Fe on both  $\rho\text{NO}_3^-$  and  $\text{VNO}_3^-$  (Figs. 4 and 5). Previous enrichment experiments have shown  $\text{NO}_3^-$  uptake rates to be very sensitive to Fe availability, increasing after Fe addition when other parameters, such as Chl *a* concentration and  $\text{Si(OH)}_4$  uptake, do not (Franck et al. 2000, 2003; Firme et al. 2003).

Overall, the Fe-enrichment experiments performed in this study confirm previous reports that Fe-stimulated increases in C and  $\text{NO}_3^-$  uptake are mainly restricted to the large size fractions (e.g., Price et al. 1994; Boyd et al. 1996). Price et al. (1994) show a 75% increase in  $\text{VNO}_3^-$  in the  $>3$ - $\mu\text{m}$  size fraction, from  $0.02$   $\text{d}^{-1}$  in situ to  $1.13$   $\text{d}^{-1}$  after Fe addition, but no increase in the  $<3$ - $\mu\text{m}$  size fraction. Similarly, increases in primary productivity were greatest in cells in the  $>3$ - $\mu\text{m}$  size fraction. In Fe-enrichment experiments conducted in the northeast subarctic Pacific, Boyd et al. (1996) show Fe-stimulated increases in  $\text{VNO}_3^-$  and phytoplankton growth rates were confined to the  $>5$ - $\mu\text{m}$  size fraction, which resulted in an increase in the percentage of primary production attributable to phytoplankton in the  $>5$ - $\mu\text{m}$  size fraction from  $<30\%$  to  $>80\%$ .

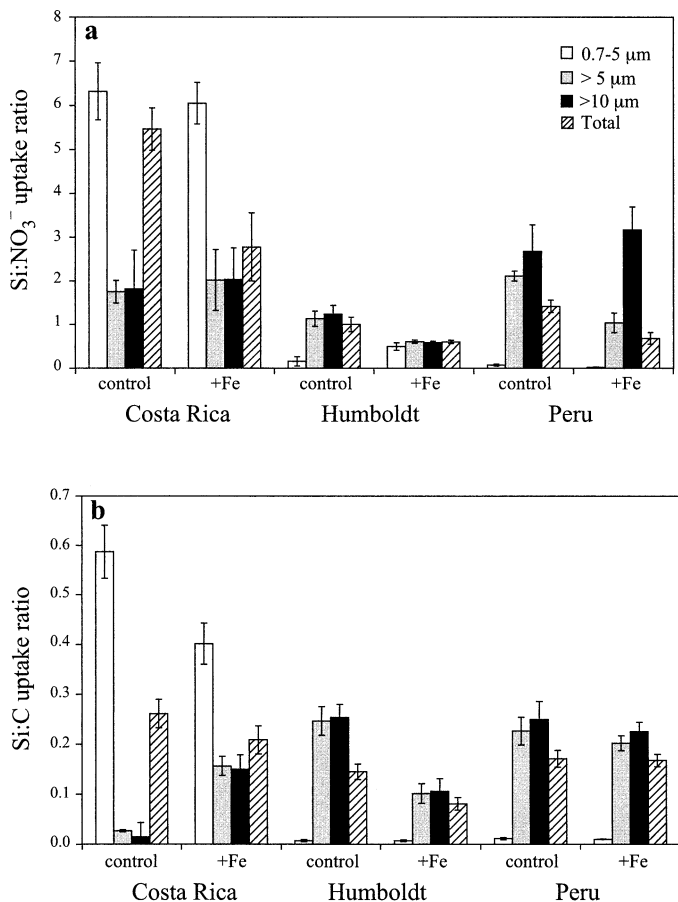


Fig. 8. (a) Size-fractionated Si:NO<sub>3</sub><sup>-</sup> uptake ratios and (b) size-fractionated Si:C uptake ratios before and after Fe addition at three locations in the eastern tropical Pacific. Error bars are standard errors of triplicate incubations.

*Effect of Fe on size-dependent Si:NO<sub>3</sub><sup>-</sup> and Si:C ratios in the eastern tropical Pacific*—Previous Fe-enrichment experiments have documented higher Si:NO<sub>3</sub><sup>-</sup> and Si:C uptake ratios, draw down ratios, and biomass ratios in Fe-limited versus Fe-replete phytoplankton (e.g., Hutchins et al. 1998, 2002a; De La Rocha et al. 2000; Franck et al. 2000; Firme et al. 2003). Some studies have suggested that these ratios are higher in Fe-limited phytoplankton because Fe limitation depresses C and NO<sub>3</sub><sup>-</sup> uptake to a greater extent than Si(OH)<sub>4</sub> uptake (Franck et al. 2000, 2003; Firme et al. 2003). In this study, Fe addition substantially decreased overall ρSi:ρNO<sub>3</sub><sup>-</sup> uptake ratios at all three locations in the eastern tropical Pacific (Fig. 8a). Fe addition also decreased overall ρSi:ρC uptake ratios in the enrichments at the Costa Rica and Humboldt current locations (Fig. 8b). Decreases in ρSi:ρNO<sub>3</sub><sup>-</sup> and ρSi:ρC uptake ratios after Fe addition at the Costa Rica and Humboldt locations were a result of greater Fe-stimulated increases in ρNO<sub>3</sub><sup>-</sup> and ρC relative to increases in ρSi. At the Peru location, ρSi:ρNO<sub>3</sub><sup>-</sup> uptake ratios decreased after Fe addition because ρNO<sub>3</sub><sup>-</sup> increased while ρSi and ρC did not. This could be due to a shift in phytoplankton species composition or to the fact that the resident phytoplankton assemblage was only partially Fe stressed. As mentioned earlier, NO<sub>3</sub><sup>-</sup> uptake rates are more responsive to Fe addition

than Si(OH)<sub>4</sub> uptake rates (Firme et al. 2003; Franck et al. 2003).

In general, decreases in the overall Si:NO<sub>3</sub><sup>-</sup> uptake ratios after Fe addition appear to be driven by either a decrease in the ρSi:ρNO<sub>3</sub><sup>-</sup> uptake ratio in the large size fractions—such as at the Humboldt and Peru locations—or an increase in the relative contribution of large size fractions with inherently low ρSi:ρNO<sub>3</sub><sup>-</sup> uptake ratios—such as at the Costa Rica upwelling dome (Fig. 8a). An important question requiring further research is whether higher Si:NO<sub>3</sub><sup>-</sup> and Si:C uptake ratios in mixed phytoplankton assemblages result in more silicified diatoms. Increased Si:NO<sub>3</sub><sup>-</sup> and Si:C uptake ratios in this study may indicate thicker frustules produced as a result of some environmental condition (e.g., Fe and/or light limitation), a switch from NO<sub>3</sub><sup>-</sup> to other N sources, less organic matter per cell (as opposed to more silica per cell), or a diatom assemblage rich in heavily silicified species. A new technique used to quantify the elemental composition of individual diatom cells demonstrated that silicon per cell was tightly constrained relative to four non-structural elements (Mn, Fe, Zn, and Ni; Twining et al. 2003), which suggests that silicon per cell might be under genetic control. It has been well documented that nitrogen per cell can vary widely with environmental conditions in autotrophs (e.g., Gisselson et al. 2001).

The small size fraction at the Costa Rica upwelling dome site exhibited Si:C and Si:NO<sub>3</sub><sup>-</sup> uptake ratios in both controls and +Fe treatments that were considerably higher than observed at other sites and that are severalfold higher than the average ratios for nutrient-replete diatoms (Brzezinski 1985). This trend was largely driven by very high ρSi relative to ρC and ρN in both the control and +Fe treatments (Figs. 2, 4, and 6). This implies that the small pennate diatoms growing at this site were highly silicified and remained largely so even after the addition of Fe. Whether these diatoms are inherently highly silicified or were producing thick frustules in response to some other environmental stress (Claquin et al. 2002) cannot be determined from our data.

This study demonstrates that Fe availability can cause changes in Si:NO<sub>3</sub><sup>-</sup> and Si:C uptake ratios in addition to changes in productivity and nutrient uptake capacity in larger phytoplankton and implies that Fe availability has a significant effect on regional biogeochemistry in the eastern tropical Pacific. As stated earlier, healthy diatoms in culture use Si(OH)<sub>4</sub> in approximately a 1:1 ratio with NO<sub>3</sub><sup>-</sup> (Brzezinski 1985). The eastern equatorial Pacific is one HNLC region where Si(OH)<sub>4</sub> draw down can become decoupled from NO<sub>3</sub><sup>-</sup> draw down, leading to high-NO<sub>3</sub><sup>-</sup>, low-Si(OH)<sub>4</sub> conditions (Dugdale and Wilkerson 1998). Several studies have suggested that productivity in the eastern tropical Pacific is solely regulated by Fe availability, but in high-NO<sub>3</sub><sup>-</sup>, low-Si(OH)<sub>4</sub> waters, productivity may in fact be regulated by both Fe and Si(OH)<sub>4</sub> availability (Franck et al. 2000; Hutchins et al. 2002b). There is no question that higher ρSi:ρNO<sub>3</sub><sup>-</sup> uptake ratios in Fe-limited phytoplankton can contribute to the high-NO<sub>3</sub><sup>-</sup>, low-Si(OH)<sub>4</sub> conditions observed in the eastern equatorial Pacific. In fact, higher Si:NO<sub>3</sub><sup>-</sup> uptake ratios may be more important in establishing such conditions than differential silicon export from the euphotic zone—the previously proposed “silica pump” hypothesis

(Dugdale and Wilkerson 1998). Similar arguments have been made for high- $\text{NO}_3^-$ , low- $\text{Si}(\text{OH})_4$  waters off central California (Bruland et al. 2001; Firme et al. 2003). This is an important distinction because if changes in  $\rho\text{Si}:\rho\text{NO}_3^-$  uptake ratios are causing high- $\text{NO}_3^-$ , low- $\text{Si}(\text{OH})_4$  conditions, then increased Fe availability in these regions would prevent the decoupling of  $\text{Si}(\text{OH})_4$  and  $\text{NO}_3^-$  draw down and potentially lead to complete  $\text{NO}_3^-$  use.

This study provides further evidence that Fe availability regulates community size structure in the eastern tropical Pacific. Results from our Fe-enrichment experiments show that Fe-stimulated increases in primary production and new production are due almost entirely to large ( $>5\ \mu\text{m}$ ) phytoplankton, despite the fact that new and primary production is dominated by small cells at ambient Fe concentrations. Increases in silica production after Fe addition also appear to be restricted to increased production by larger diatoms. The Fe-stimulated increases in primary production, new production, and silica production in larger cells observed in our experiments appear to result from a combination of increased growth as well as increased uptake capacity for C,  $\text{NO}_3^-$ , and  $\text{Si}(\text{OH})_4$ . This study also provides further evidence that Fe availability can alter regional biogeochemistry in HNLC waters through effects on  $\text{Si}:\text{NO}_3^-$  and  $\text{Si}:\text{C}$  uptake ratios and demonstrates that it is mostly larger phytoplankton driving the observed changes in these ratios. Together, these results show that Fe availability plays an important part in the ecology and biogeochemistry of marine ecosystems.

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