Limitation of phytoplankton production by Si and N in the western Atlantic Ocean

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ABSTRACT: Large areas of the western tropical Atlantic Ocean are influenced by the Amazon River plume, in which dissolved inorganic nitrogen (DIN) concentrations are low and nutrient N:P and N:Si ratios are lower than elemental ratios typical of phytoplankton. Accordingly, nutrient concentrations and ratios measured during winter (January to February 2001) and summer (July to August 2001) cruises in the area 2 to 14° N and 57 to 40° W suggest that DIN may limit phytoplankton growth at the majority of the 40 stations sampled. As diatoms are the dominant plankton in the Amazon plume-waters, short-term nutrient enrichment experiments were conducted to determine whether ambient N, P, or Si limited silica production rates (SiP). Results indicated that SiP were substrate limited both above and below the pycnocline at the majority of stations during both high (summer cruise) and low (winter cruise) discharge conditons. DIN concentrations also limited SiP in plumewater stations, but N additions depressed SiP at other stations, possibly using energy to take up the limiting nutrient. Finally, primary production rates were limited by ambient dissolved silicon concentrations at 9 of 13 summer stations. These results indicate the importance of dissolved silicon availability in controlling phytoplankton growth rates in a diatom-dominated river plume system.

KEY WORDS: Nutrient limitation · Nutrient enrichment · Tropical Atlantic Ocean · Amazon River plume · Silicon · Primary productivity · Diatoms

INTRODUCTION

The western Atlantic Ocean receives a tremendous discharge of dissolved and particulate matter-rich water from the Amazon River basin, in the order of 6×10^{12} m^3 annually. As a result, phytoplankton growth is supported in an extensive area of the tropical and equatorial surface ocean (Ryther et al. 1967, Muller-Karger et al. 1988). In the Amazon estuary and over the continental shelf, phytoplankton growth is light limited by suspended particles; farther down-plume, decrea-sing dissolved silicon concentrations indicate the development of diatom blooms as suspended particles settle (Milliman & Boyle 1975, De Master et al. 1986). Even farther down-plume, nutrient limitation occurs as plumewaters become diluted with the more oligo-

trophic seawater and/or by nutrient depletion from phytoplankton growth (De Master & Pope 1996, De Master et al. 1996, Smith & De Master 1996). It has been suggested that N availability limits net primary production rates (PP) on the Amazon shelf (e.g. De Master & Pope 1996) as a consequence of N:Si:P stoichiometric ratios that are low in N relative to ratios that are characteristic of phytoplankton and diatoms (~16:16:1; Redfield 1963, Brzezinski 1985). However, silica production rates (SiP) by diatoms can be substrate limited at very high dissolved silicon concentrations. For example, SiP can be limited by dissolved silicon concentrations as high as 4.7 µmol l^{-1} in the Mississippi River plume (Nelson & Dortch, 1996) and 10 µmol l^{-1} in upwelled coastal waters off Monterey Bay, California (Brzezinski et al. 1997). Although sub-

strate-limited SiP have been observed in many ocean systems, the extent to which dissolved silicon limits PP is unknown.

In this study, we evaluated the potential for limitation by major nutrient Si, N and P concentrations, and nutrient ratios at stations on the continental slope and further offshore in the western equatorial and tropical Atlantic Ocean. As diatoms are the dominant net phytoplankton in the Amazon River plume (e.g. Teixeira & Tundisi 1967), we determined the extent to which dissolved silicon and dissolved inorganic nitrogen (DIN) concentrations limit SiP. Finally, we determined the extent to which dissolved silicon concentrations limit net PP in surface waters with varying Amazon plumewater influence.

Study region

The Amazon river is characterized by low salinity waters with high dissolved silicon concentrations (e.g. Ryther et al. 1967, Froelich et al. 1978) and with mean riverine end-member concentrations of nitrate, phosphate, and dissolved silicon of 16, 0.7 and 144 µmol l^{-1}, respectively (DeMaster & Pope, 1996). On an annual basis, the discharge of the high nutrient Amazon plumewaters into the western Atlantic Ocean is at its highest in the spring and lowest in the winter; the mean monthly discharge at the Obidos gauging station was approximately a factor of 2 lower in November (1.15×10^5 m^3 s^{-1}) than in May (2.44×10^5 m^3 s^{-1}) during 1928 to 1996 (Global Runoff Data Centre 2002, www.grdc.sr.unh.edu). During June to January, the plumewaters become entrained into the northwestward flowing North Brazil Current, which subsequently enters a retroflection and flows to the east as the North Equatorial Counter current (Muller-Karger 1988). During spring (February and March) plumewaters flow to the northwest into the Caribbean Sea. In order to capture variability associated with the seasonal river plume influence, experiments were conducted during both high and low discharge seasons (January and February; July and August 2001, respectively).

MATERIALS AND METHODS

Sampling. Studies of phytoplankton dynamics and nutrient limitation were conducted in the western Atlantic Ocean at 20 stations in the area of 6 to 12° N and 41 to 57° W in the winter (January and February 2001) and at 20 stations in the area of 3 to 14° N and 42 to 57° W in the summer (July and August 2001) (Table 1, Fig. 1). Temperature, salinity, pressure and

Table 1. Station number, cast number and date of sampling during winter (January and February) and summer (July and August) 2001 cruises in the western Atlantic Ocean

	Winter			Summer	
Station	Cast	Date	Station	Cast	Date
20	4	30 Jan	27	3	27 Jul
21	6	31 Jan	29	3	28 Jul
22	5	1 Feb	30	4	29 Jul
23	5	2 Feb	31	4	30 Jul
24	8	3 Feb	32	5	31 Jul
25	9	4 Feb	33	5	1 Aug
27	8	5 Feb	35	5	2 Aug
29	8	6 Feb	38	1	3 Aug
31	6	7 Feb	39	4	4 Aug
37	7	9 Feb	41	4	5 Aug
39	10	10 Feb	43	3	6 Aug
41	37	12 Feb	44	3	7 Aug
41	9	11 Feb	46	3	8 Aug
42	4	13 Feb	48	3	9 Aug
44	7	14 Feb	49	4	10 Aug
46	7	15 Feb	50	4	11 Aug
48	4	16 Feb	52	4	12 Aug
49	8	17 Feb	53	7	13 Aug
51	7	18 Feb	53	19	14 Aug
51	23	19 Feb	55	5	15 Aug

photosynthetically active radiation (PAR) were measured during the downcast of a Seabird™ SBE 9 unit with a PAR sensor, and derived parameters were calculated using Seasave v1.10 software. Salinity is reported on the practical salinity scale, which is a unit-less measure. Seawater was sampled at depths approximating 100, 50, 25, 10, 1 and 0.1% of surface irradiance, as estimated during the downcast of the CTD and rosette unit. The 100% light sample was taken approximately 1 m below the surface. Mixed layer depth was defined as the depth at which density changed ≥0.01 kg m^{-3} per meter for at least 3 consecutive meters. Water samples for nutrient and particle concentration analyses and rate measurements were collected with 10 l Niskin bottles. Nitrate, nitrite and phosphate concentrations were determined in duplicate on a Zelweger Analytics model QuikChem autoanalyzer using standard procedures and a distilled water baseline, with detection limits of 100 nmol l^{-1}, 50 nmol l^{-1} and 20 nmol l^{-1}, respectively. Dissolved reactive silicon concentrations (primarily in the form $Si(OH)_4$) were measured on 10 ml samples which were first filtered through 0.6 µm polycarbonate membranes, and were refrigerated for up to 2 d before further analysis using the acid-molybdate method of Strickland & Parsons (1972). As an indicator of diatom biomass, biogenic silica (bSi) concentrations were determined on 0.56 to 2.3 l of seawater filtered onto 0.6 µm polycarbonate membranes. Biogenic silica concentrations were determined by a 2 h digestion in a
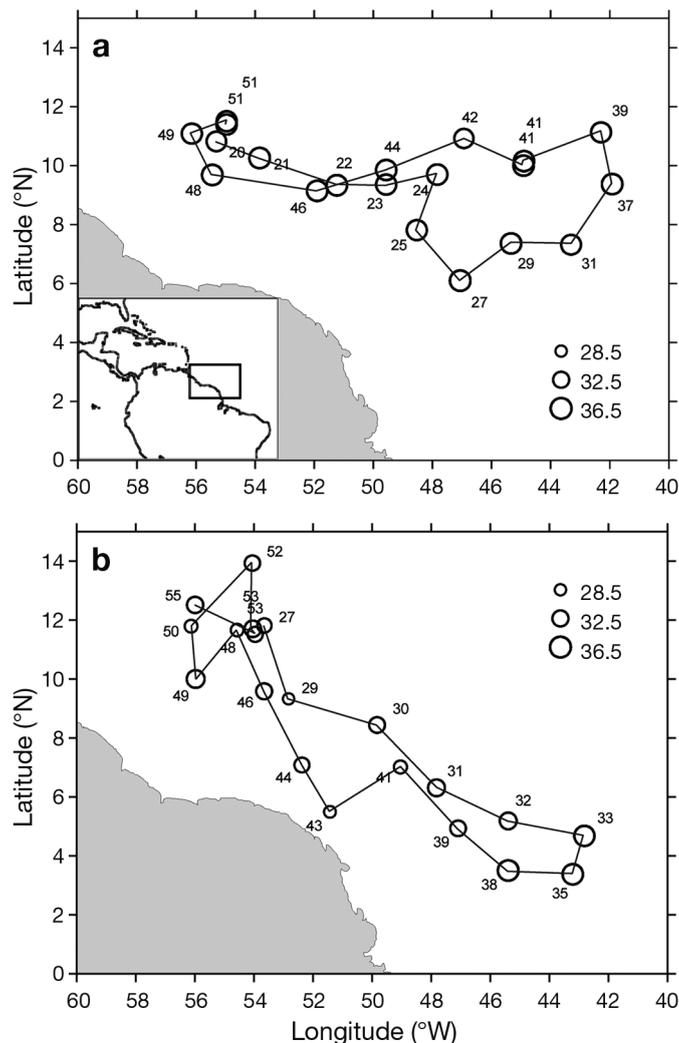
Fig. 1. Cruise track (——) and surface water salinities (○) at stations in the Western Atlantic Ocean where nutrient pools and limitation were studied during (a) January and February 2001 (b) July and August 2001. Circle diameters are proportional to surface water salinity (practical salinity scale). Inset map indicates the region in the larger image. Station numbers are labeled

0.2 N NaOH solution as in Shipe et al. (2006). High lithogenic silica concentrations can interfere with bSi measurement; therefore, concentrations were determined by subsequent dissolution in 2.5 mol l^{-1} hydrofluoric acid (HF) and were found to be low relative to bSi concentrations.

Microphytoplankton counts. The dominant taxonomic composition was determined at 5 stations during the summer cruise. Due to the highly variable biomass conditions sampled, multiple methods were used to collect samples for microphytoplankton species determination (all cells ≥10 μm) at 5 stations. At Stns 43 and 44, 10 l seawater samples from the 50% light depth

were filtered from the Niskin bottles directly onto 8 μm polycarbonate (PC) membranes. The samples were preserved in 0.2 μm filtered seawater with 1.7 weight percent acid formaldehyde, and cells were counted within 7 mo. Filters were visually inspected to ensure that cells had washed off into the fixative solution. Due to high biomass at Stns 27 and 39, 1.1 l seawater samples from the 50% light depth were filtered onto 1 μm PC membranes. The samples were preserved as above and cells were identified and counted within 2 wk. Counts were done on 10 random fields of view at a magnification of 200× in at least 4 aliquots from each sample in a Sedgwick-Rafter counting chamber. At Stn 41, a 202 μm mesh net with a 0.785 m^2 mouth opening was towed for 10 min from the ship's stern. As the net was completely clogged, many cells <202 μm were retained. Cells were rinsed with filtered seawater and cells ≥10 μm were identified and counted in 2 transects across a Palmer-Maloney counting chamber in duplicate aliquots. At all 5 stations, cyanobacterial symbionts were counted on 8 μm PC membranes through which 10 l Niskin bottles were drained. Epifluorescent microscopy with green excitation was used at 100× magnification to identify *Richelia intracellularis*, a cyanobacterium capable of nitrogen fixation.

Primary and silica production rates. PP and biogenic SiP were assessed by incorporation of radioactive tracers ^{14}C and ^{32}Si in polycarbonate bottles incubated for 4 to 5 h (normally beginning at 11:00 h) in on-deck water baths with flowing surface seawater. Neutral density screening was used to simulate the ambient light intensities. Net daytime PP was measured in triplicate light and darkened bottles using US Joint Global Ocean Flux Study core measurement protocols (Intergovernmental Oceanographic Commission 1996). Seawater was prefiltered through a 202 μm Nitex mesh to remove *Trichodesmium* spp. colonies and 0.03 μCi Na$_2$H^{14}CO$_3$ per ml of seawater was added to the 300 and 600 ml polycarbonate bottles used during the winter and summer cruises, respectively. At the end of the incubations, specific activities were measured on 250 μl sample aliquots and PP was terminated by filtration onto Whatman GF/F filters. Filters and filtration towers were rinsed with ~2 ml of 0.2 μm filtered seawater. Samples were acidified with 0.2 ml of 5% HCl, placed in vials for ~24 h, after which 5 ml of Packard Ultima Gold LLT scintillation cocktail was added to each vial. Activity was counted onboard ship using a Beckman LS 6500 scintillation counter.

Rates of SiP were measured in 280 ml polycarbonate bottles using ^{32}Si(OH)$_4$ as a tracer as described in Shipe et al. (2006). Seawater was not prefiltered, but visible grazers were removed. ^{32}Si tracer additions were 7.8×10^{-5} μCi per ml of seawater. Dissolved silicon concentrations were perturbed by less than 0.05 μmol l^{-1} by

[32]Si addition, due to the high specific activity of the tracer (23 000 Bq (µg Si)$^{-1}$). Sample activities were counted in Packard Ultima Gold XR using a Beckman LS 6000 liquid scintillation counter, and SiP were calculated using the logarithmic model of Brzezinski & Phillips (1997). We determined a 95% confidence interval for the variation in SiP using data from 16 sets of duplicate or triplicate samples throughout the cruises.

Nutrient addition experiments. Substrate limitation of SiP was assessed during both the winter (14 stations) and the summer (20 stations) cruises. Nitrogen limitation of SiP was evaluated during the summer (20 stations) cruise using additional experiments. These experiments were conducted in parallel with the measurement of PP and SiP at the 50 and 1% light depths. Silicon limitation of PP was assessed at the 50% light depth during the summer (12 stations) cruise. Nutrient stock solutions were prepared to final concentrations of 6 mmol l^{-1} Na$_2$SiO$_3$, 100 µmol l^{-1} NaNO$_3$, and 1.12 mmol l^{-1} NH$_4$Cl. The increase in dissolved silicon or NO$_3^-$ concentrations in experimental bottles was 15 µmol l^{-1}; the increase in NH$_4^+$ concentration was 1.0 µmol l^{-1} at summer stations 27 to 35 and 0.3 µmol l^{-1} at all other summer stations.

Differences between rates in control and nutrient enriched samples were considered significant if they fell outside of the 95% confidence interval as determined for replicate SiP rates (see 'Primary and silica production rates'). The ratio of the enriched rate to the control rate is hereafter referred to as the Enh factor, as in Brzezinski et al. (1997). At 5 stations during the winter cruise and 9 stations during the summer cruise, the Enh factor ranged from 1.0 (no difference) to 2.0. Mean ratios were 1.4 and 1.2 for winter and summer samples, respectively. A 95% confidence interval suggests that ratios of 0.5 to 1.9 during the winter and 0.7 to 1.4 during the summer are not distinguishable from the natural variation in SiP that was observed in the replicated samples. Biomass specific SiP is calculated as the SiP divided by the biogenic silica (bSi) concentration.

RESULTS

Hydrological conditions

Previous work indicates that freshwater fluxes from the Amazon River are more than twice as high in the summer than in the winter and that the river plume is entrained into the North Brazil Current as it flows to the northwest along the coastline (e.g. Muller-Karger et al. 1988, Bourles et al. 1999). Accordingly, surface salinities in the study region were much lower in the summer; at this time, the lowest salinities were observed at the coastal and northwestern stations (Fig. 1b).

At these stations, the upper mixed layer was 4 to 13 m deep in the summer and salinities <34 were present as deep as 20 m (data not shown). The 50% light depth was above the pycnocline during both cruises whereas the 1% light depth was in or near the pycnocline in the winter and below the pycnocline in the summer.

In accord with fresh, Si-rich, summertime Amazon river influence (e.g. Froelich et al. 1978), dissolved silicon concentrations in the mixed layer were uniformly low in the winter whereas dissolved silicon concentrations were often >5 µmol l^{-1} in the low salinity plumewaters (Fig. 2). Amazon river influence in these waters is further supported by high colored dissolved organic matter content at these stations (Del Vecchio & Subramaniam 2004). In contrast to summer conditions, near-surface nutrient concentrations were lower and showed no clear spatial trends during the winter cruise.

Vertical profiles of mean dissolved silicon concentrations also revealed seasonal river influence (Fig. 3). In
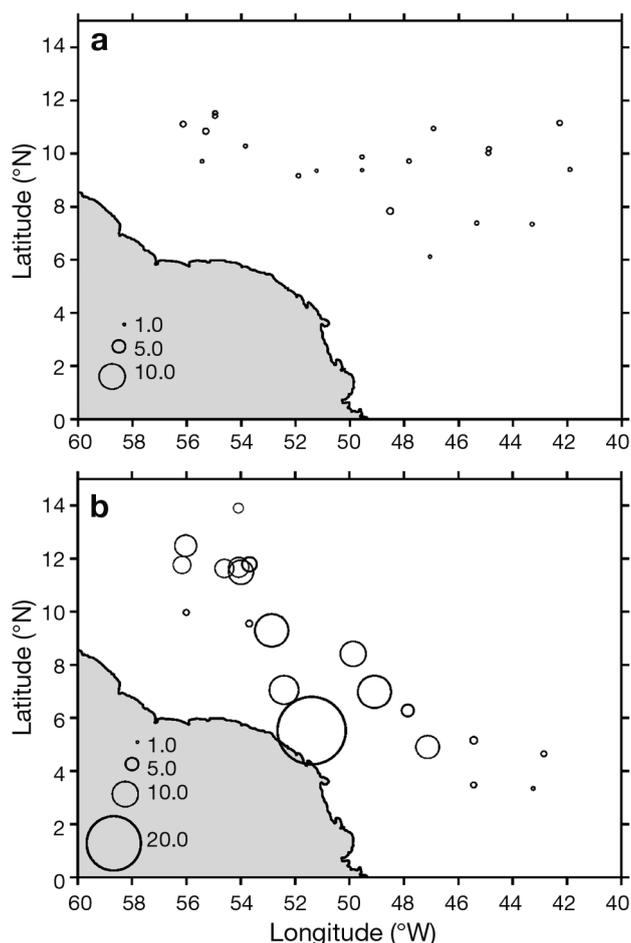

Fig. 2. Dissolved silicon concentrations (µmol l^{-1}) at the 50% light depth during (a) winter and (b) summer cruises in the western Atlantic Ocean. Concentrations are proportional to the diameter of the circle in the bubble plot

the winter, nutrient concentrations were low in the upper 50 m and increased with depth (Fig. 3a–c). At this time, mixed layer dissolved silicon concentrations ranged from 0.9 to 2.8 µmol l⁻¹, $NO_3^- + NO_2^-$ concentrations were generally undetectable (<0.10 µmol l⁻¹), and PO_4^{2-} concentrations ranged from <0.02 to 0.15 µmol l⁻¹. In contrast, summertime upper mixed layer dissolved silicon concentrations were highest, whereas $NO_3^- + NO_2^-$ and PO_4^{2-} concentrations were lowest in the mixed layer. At this time, dissolved silicon concentrations ranged from 0.5 to 26.5 µmol l⁻¹ at the surface and decreased to ~2 µmol l⁻¹ at ~50 m depth. Since $NO_3^- + NO_2^-$ concentrations were low or undetectable, mixed layer N:P and N:Si ratios were lower than the Redfield ratio (N:P of 16:1; Redfield 1967) and the typical diatom stoichiometric ratio (Si:N of ~1:1; Brzezinski 1985) during both cruises.

Phytoplankton assemblage

At 5 of the lower salinity summertime stations, phytoplankton assemblages were dominated by diatoms of the genera *Hemiaulus* spp. and *Skeletonema* spp. (Table 2). *Skeletonema* spp. accounted for 91 and 47% of the cells at Stns 44 and 43, respectively. These 2 stations were characterized by the highest integrated biogenic silica (bSi) concentrations, low surface salinities and high dissolved silicon concentrations. *Hemiaulus hauckii* contributed 96 and 91% of the phytoplankton at Stns 27 and 39, respectively. Further, approximately half of the *Hemiaulus* spp. (mostly *H. hauckii*) cells at Stn 27 and the majority of those at Stn 39 contained *Richelia intracellularis*. *Chaetoceros* spp. was the second most abundant taxa at all 5 stations. The assemblage at Stn 41 was not dominated by any one genus; *Skeletonema* spp.,
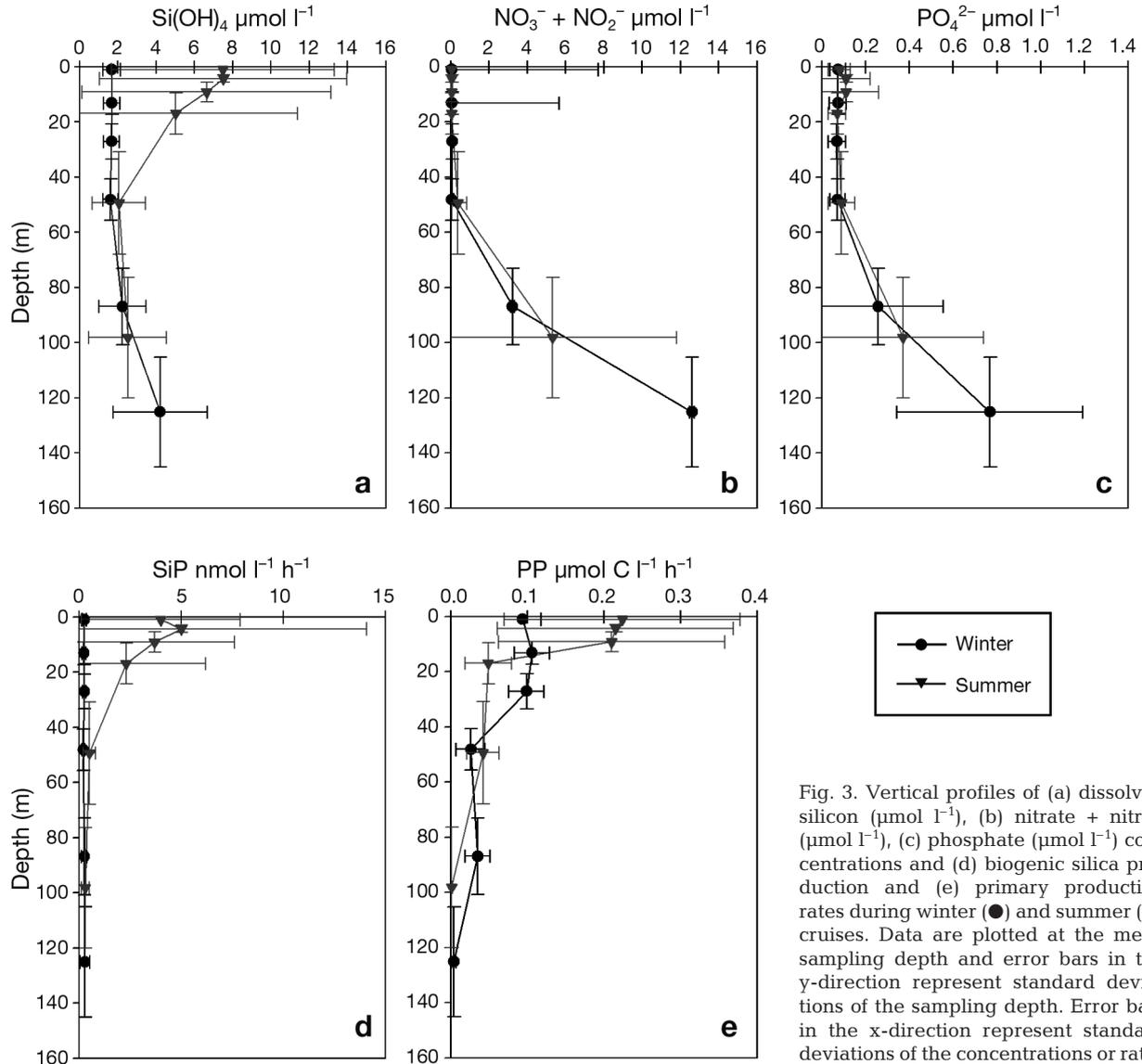

Fig. 3. Vertical profiles of (a) dissolved silicon (µmol l⁻¹), (b) nitrate + nitrite (µmol l⁻¹), (c) phosphate (µmol l⁻¹) concentrations and (d) biogenic silica production and (e) primary production rates during winter (●) and summer (▼) cruises. Data are plotted at the mean sampling depth and error bars in the y-direction represent standard deviations of the sampling depth. Error bars in the x-direction represent standard deviations of the concentrations or rates

Table 2. Concentrations (cells l^{-1}) and % contribution of taxa to the ≥10 μm phytoplankton assemblage at the 50% light depth at 5 stations. Most of the unidentified centric diatoms were of the family Rhizosoleniaceae and most of the unidentified pennate diatoms were of the genus *Pseudo-nitzschia.* Only relative contributions are given for phytoplankton at 41, where sampling was not quantitative

Taxon	Stn 27 Cells l^{-1}	%	Stn39 Cells l^{-1}	%	Stn 41 Cells l^{-1}	%	Stn 43 Cells l^{-1}	%	Stn 44 Cells l^{-1}	%
Skeletonema spp.	0	0	0	0		29	15 800	47	46 500	91
Hemiaulus spp.	73 000	96	8400	91		15	1400	4.1	660	1.3
Asterionellopsis sp.	0	0	0	0		2.4	2400	7.0	700	1.4
Chaetoceros spp.	2100	2.6	460	6		21	8700	26	740	1.4
Thalassionema spp.	440	0.3	10	0.1		5.9	2000	5.9	660	1.3
Unidentified pennate	40	0	140	1.3		4.8	320	1.0	310	0.6
Unidentified centric	230	0.1	10	0.1		20	1600	8.9	160	0.5
Non-diatom	180	0.3	100	1.1		1.9	40	0	0	0
Richelia intracellularis	34 800		7000		71		0		4	

Chaetoceros spp., and unidentified centric diatoms, mostly from the family Rhizosoleniaceae, contributed almost equally to the assemblage. Although counts likely underestimated smaller cell abundances due to net usage at this station, the data clearly indicate the presence of the largest number of diatom taxa. The plankton assemblage at Stn 52 was dominated by an unidentified acantharian, but its abundance was not quantified. The different methods of sample collection for phytoplankton counts did not seem to account for the differences in communities observed.

Silica and primary production rates

There was no clear pattern in the distribution of SiP in the winter at either light depth, whereas the highest rates of SiP in the summer occurred at the river influenced near-shore and northerly stations (Fig. 4). At the 50% light depth, mean SiP were 0.2 and 5.2 nmol l^{-1} h^{-1} in the winter and summer, respectively. Diatom standing stock (estimated as bSi concentrations) during these 2 cruises was much higher in the summer (Shipe et al. 2006). Mean concentrations for the 20 stations were 3.9 ± 1.5 (SD) mmol m^{-2} in the winter and 11.9 ± 16.2 (SD) mmol m^{-2} in the summer, integrated from the surface to the 1% light depth. Mean biomass-specific rates were 0.0073 h^{-1} in the winter and 0.0378 h^{-1} in the summer, corresponding to doubling times of 95 and 18 h, respectively. Vertical profiles indicate that wintertime rates were similar at the 50 and 1% light depths (hereafter referred to as 'near-surface' and 'deep') (Fig. 3d). In contrast, deep SiP (0.5 nmol l^{-1} h^{-1}) were an order of magnitude lower than near-surface rates in the summer. Primary production rates (PP) were highest in the mixed layer, and mean near-surface rates were 0.106 and 0.215 μmol C l^{-1} h^{-1} during the winter and summer, respectively (Fig. 3e).

Table 3. Factor of increase in silica production (Enh) with 15 μmol l^{-1} dissolved silicon over controls at stations in the western tropical Atlantic Ocean in January and February 2001. All experimental rates were higher than controls (95% confidence interval). Water samples were collected and incubated at 50% (5 to 17 m) or 1% (66 to 144 m) light depths and the *in situ* dissolved silicon concentrations at these depths are given

Station	Enh 50% Light	1% Light	Dissolved silicon (μmol l^{-1}) 50% Light	1% Light
20	2.7	4.5	2.8	2.5
21	4.8	8.1	1.4	1.8
22	6.8	5.2	1.2	1.4
23	13.4	9.6	0.9	1.4
24	2.5	3.3	1.7	5.9
25	5.0	2.5	2.5	2.3
27	4.4	6.9	1.4	1.5
29	6.0	7.2	1.5	1.7
31	7.6	8.3	1.5	1.9
37	20	6.9	1.5	1.5
39	4.2	3.8	2.0	2.0
41	2.6	4.2	1.7	4.0
41	4.2	4.9	1.8	4.2
42	5.6	6.2	1.6	1.
44	6.1	4.9	1.5	1.6
46	4.4	2.1	1.6	3.5
48	6.1	8.7	1.3	1.3
49	4.9	7.4	2.2	2.0
51	4.7	6.4	1.8	1.0
51	6.4	4.2	1.8	1.8
Mean	6.1	5.8	1.7	2.3
SD	4.0	2.1	0.4	1.3

Substrate limitation of silica production

At ambient dissolved silicon concentrations, SiP were substrate limited at every station during the winter cruise and the majority of stations during the summer cruise (Tables 3 & 4). Enh factors ranged widely in both near-surface and deep samples. However, mean Enh factors for near-surface experiments were fairly
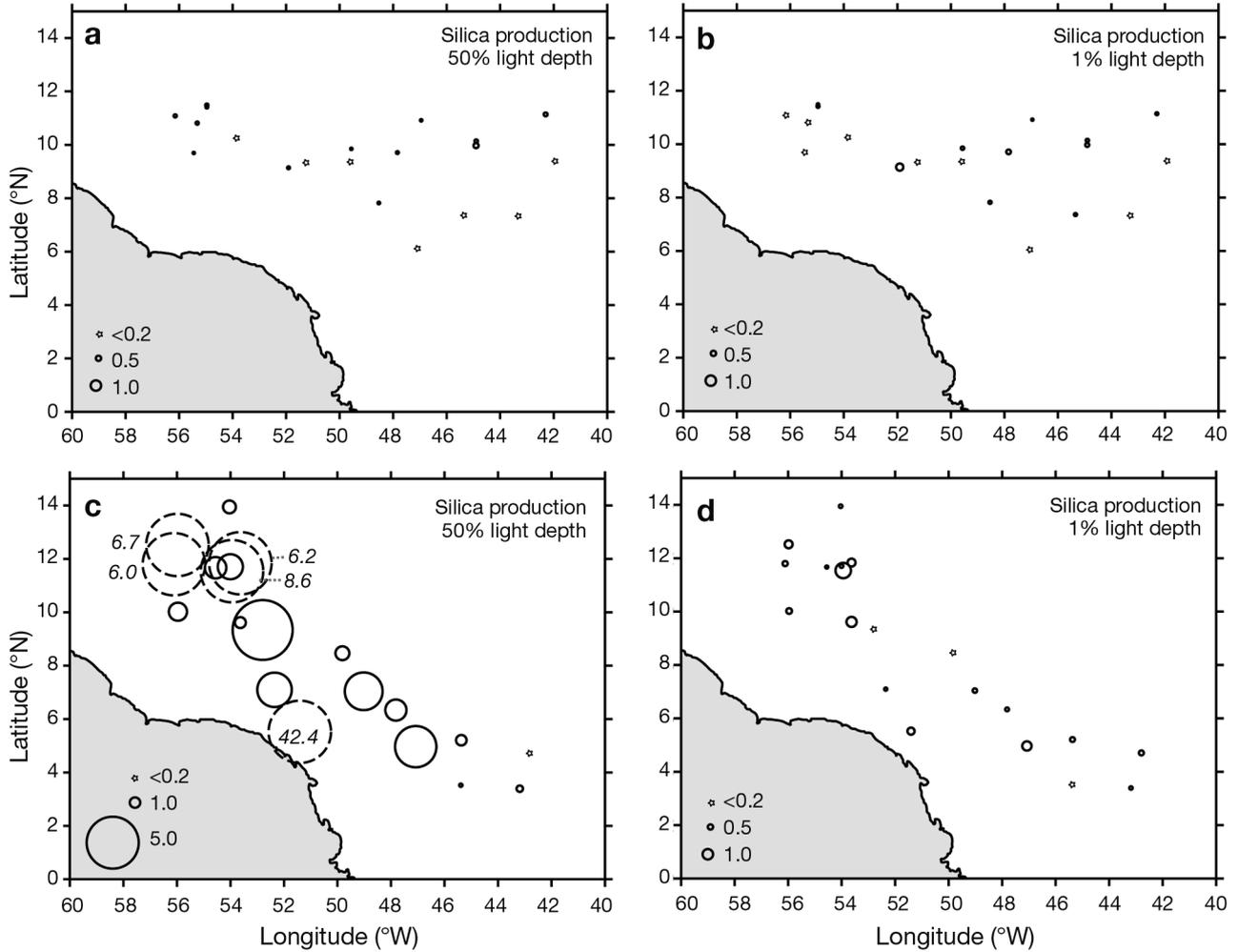
Fig. 4. Silica production rates (nmol Si l^{-1} h^{-1}) at the light depths of (a,c) 50% and (b,d) 1% of surface irradiance during (a,b) winter and (c,d) summer cruises. ☼: Rates >6.0 nmol Si l^{-1} h^{-1}. The rate is given in italics. Concentrations are proportional to diameter of the circle in the bubble plot. ☆: Values <0.2 nmol Si l^{-1} h^{-1}

similar during the 2 cruises; Enh factors were 6.1 in the winter and 5.3 in the summer. At 5 stations, SiP decreased with dissolved silicon enrichment by up to a factor of 5. There was no clear trend in the spatial distribution of the Enh factors in the winter (Fig. 5a,b). However, the highest Enh factors were observed at the more northerly stations in the summer. No changes or decreases in SiP with dissolved silicon addition were observed at either light depth at the more oceanic, low dissolved silicon, southerly Stns, 33, 35, 38 and 39 (Fig. 5c,d).

Dissolved silicon concentrations and the Enh factor were inversely correlated during both seasons (Fig.6). Higher Enh factors were observed in near surface samples in the winter but in the deeper samples in the summer because of low dissolved silicon concentrations at these depths. From these experiments, it is possible to derive half saturation constants (K_s) following the Michaelis-Menten model of uptake kinet-

ics of the form $V = (V_{max} + S)/(K_s \times S)$, where V is the uptake rate at substrate (nutrient) concentration S, and V_{max} is the maximum uptake rate. Nonlinear regression of a hyperbola of the form Enh factor = (K_s + [Si(OH)$_4$]) / [Si(OH)$_4$] resulted in K_s values of 8.5 μmol l^{-1} in the winter and 10.9 μmol l^{-1} in the summer.

Nitrogen limitation of silica production

The enrichment with either NO$_3^-$ or NH$_4^+$ resulted in changes in SiP relative to control samples at 15 of the 20 stations during the summer cruise (Table 4); SiP increased at 10 northerly stations and decreased at 5 southerly stations. However, N additions at higher ambient N concentrations in deep samples had less effect than in near-surface samples. The Enh factors in these experiments were generally smaller than those

Table 4. Factor of increase in silica production rate as in Table 3, but with additions of: dissolved silicon (dSi) (15 µmol l^{-1}), NO$_3^-$ (15 µmol l^{-1}), NH$_4^+$ (1 µmol l^{-1} at Stns 27 to 35, 0.3 µmol l^{-1} at Stns 38 to 55) at stations occupied during July and August 2001. *Experimental rates significantly different from controls (95% confidence interval). Water samples were collected and incubated at 50% (2 to 7 m) or 1% (15 to 80 m) light depths, and ambient nutrient concentrations are given. bd: Concentrations below the analytical detection limit. –: Experiment not conducted

Station	Enh						Nutrient concentrations			
	50% Light			1% Light			50% Light		1% Light	
	Si(OH)$_4$	NO$_3^-$	NH$_4^+$	dSi	NO$_3^-$	NH$_4^+$	dSi	NO$_3^-$ + NO$_2^-$	dSi	NO$_3^-$ + NO$_2^-$
27	1.4	0.8	6.8*	2.5*	–	–	5.4	0.13	3.9	0.21
29	1.8*	1.7*	126*	21.6*	–	–	18.8	bd	0.5	0.59
30	5.9*	1.6*	318*	184*	–	–	8.3	bd	bd	0.41
31	2.9*	0.3*	0.9	4.0*	0.8	–	4.1	bd	5.5	bd
32	3.4*	4.2*	6*	1.1	2.9*	2.8	3.1	bd	1.0	bd
33	0.3*	0.4*	1.2	0.2*	0.4*	0.7	1.6	bd	1.8	1.24
35	0.2*	0.9	1.2	0.4*	0.8	1.3	1.6	bd	1.6	bd
38	0.3*	0.5*	1.1	0.7	0.5*	0.6	1.3	bd	1.3	bd
39	0.6*	0.8	0.9	0.6*	3.4*	1.1	8.7	bd	4.8	0.60
41	57*	2.7*	2.6*	6.0*	1.5*	1.3	13.7	0.11	3.5	1.71
43	0.5*	0.4*	0.4*	4.0*	1.1	1.0	27.0	bd	2.6	0.10
44	2.0*	1.4	1.2	11.3*	1.1	1.3	11.2	bd	2.0	bd
46	5.8*	1.2	0.9	6.8*	0.9	0.8	2.9	bd	1.8	bd
48	3.3*	1.1	1.5*	14.1*	1.0	0.9	6.5	bd	1.3	bd
49	3.5*	0.9	0.9	10.3*	0.9	0.8	2.3	bd	1.5	0.04
50	1.9*	0.5*	0.4*	1.6*	0.8	0.8	6.2	bd	1.3	0.48
52	6.7*	0.8	1.1	–	1.3	1.2	2.0	bd	1.6	bd
53	4.1*	2.4*	1.3	10.1*	3.3*	0.7	7.6	bd	1.5	0.11
53	1.9*	0.8	0.9	8.1*	1.6*	0.9	9.5	bd	2.0	0.48
55	2.0*	0.9	1.0	9.8*	1.1	1.1	8.3	bd	1.5	bd
Mean	5.3	1.2	24	15.6	1.4	1.1	7.5	0.12	2.2	0.54
SD	12.4	0.9	75	41.2	0.9	0.5	6.5	0.01	1.3	0.51

observed with dissolved silicon additions. However, extremely high Enh factors were observed with the addition of 1.0 µmol l^{-1} NH$_4^+$ at Stns 29 and 30.

Silicon limitation of primary productivity

Near-surface PP was limited by dissolved silicon concentrations at 9 of 13 summertime stations, despite ambient dissolved silicon concentrations of 2.0 to 27.0 µmol l^{-1} (Table 5). At these 9 stations, experimental addition of 15 µmol l^{-1} of dissolved silicon to experimental samples resulted in Enh factors of 1.19 to 2.04. The mean Enh factor, 1.39, corresponded to a 39% increase in PP. There was no clear relationship between ambient dissolved silicon concentrations and the increase in PP in these experiments (linear regression; r = 0.025, n = 13). At Stn 48, experimental PP was also significantly different from control rates, but it decreased by 26% with dissolved silicon enrichment. Due to high variance among triplicate PP samples at 3 stations (30, 41 and 49), experimental PP was not significantly different from control rates. There was no clear trend in the horizontal distribution of the changes in PP with dissolved silicon enrichments (not shown).

DISCUSSION

Substrate limitation of silica production

Silica production is important in the western Atlantic Ocean, as diatoms are the dominant primary producers in the Amazon plumewaters (e.g. Teixeira & Tundisi 1967, Milliman & Boyle 1975). When suspended sediment concentrations in plumewaters fall below 10 to 20 mg l^{-1}, the uptake of dissolved silicon begins and silica-mineralizing plankton are responsible for increases in primary productivity (DeMaster et al. 1986).

The observed increases in SiP in response to experimental dissolved silicon enrichments in both near-surface and deep waters indicate widespread substrate limitation of SiP in the western tropical Atlantic Ocean. This limitation occurred not only during low-discharge, oligotrophic winter conditions but also in plumewaters sampled during the summer at dissolved silicon concentrations as high as 18.8 µmol l^{-1}. Assuming that 15 µmol l^{-1} dissolved silicon additions resulted in saturating concentrations for silica production, Enh factors of 1.7 to 57 corresponded to assemblages that were taking up dissolved silicon at 2 to 69% of their maximum rate. These results suggest that silica
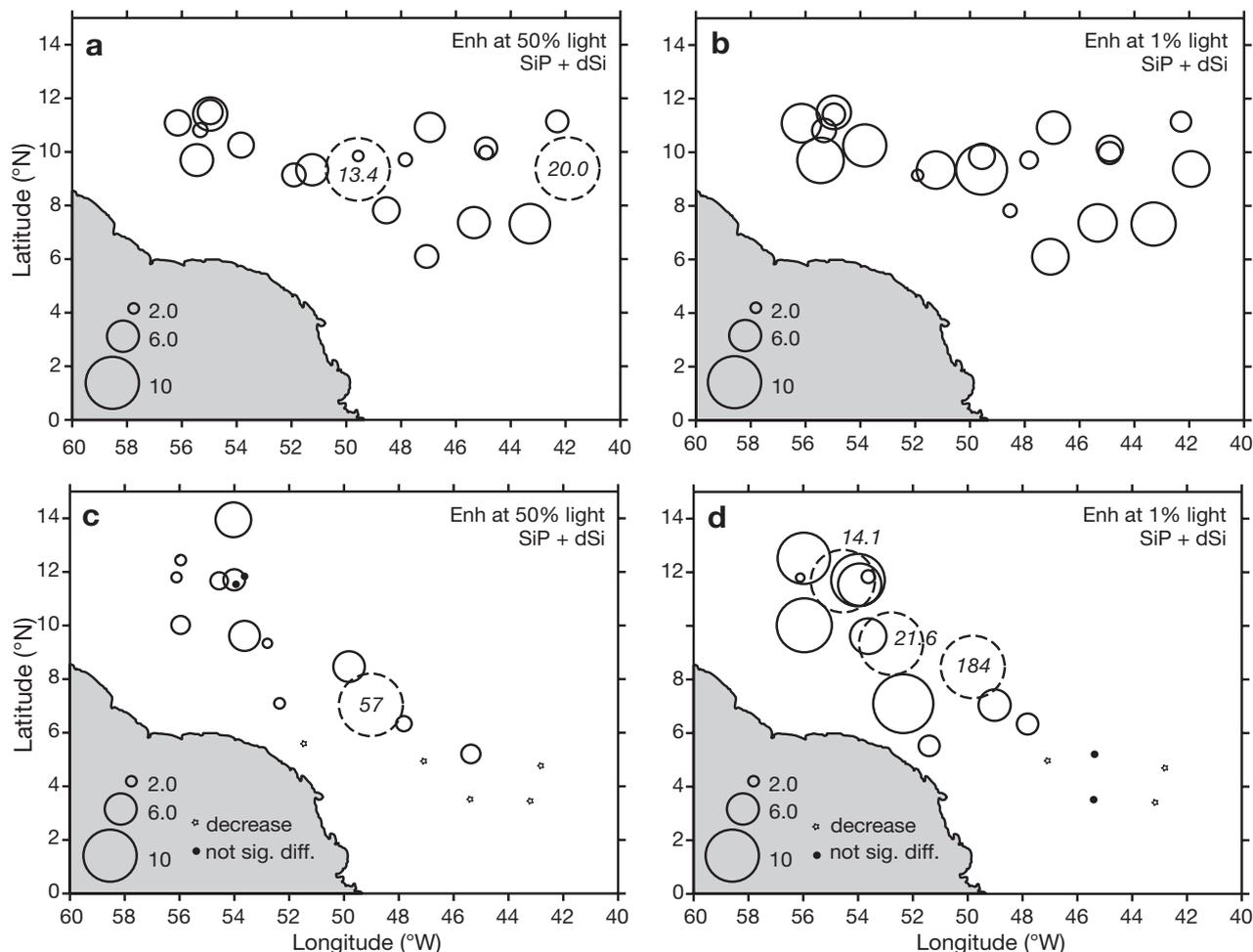
Fig. 5. Enh values (ratio of silica production rate, SiP, with 15 mmol l^{-1} dissolved silicon to control rate) at the (a,c) 50% light depth and (b,d) 1% light depth during (a,b) winter and (c,d) summer cruises. ○: Stations where Enh was greater than the 95% CI. ●: Stations where Enh was not outside of the 95% CI. ⬭: Values >10. The Enh value is given in italics. ✰: decrease in Si production with nutrient addition. dSi: dissolved silicon. Values are proportional to the diameter of the circle in the bubble plot

production in this region commonly occurs under Si limited conditions, a state that De La Rocha & Passow (2004) suggest can be of adaptive advantage, as Si-starved cells recover from nutrient limitation more quickly than N-starved cells.

Substrate-limited SiP has been demonstrated or suggested in several other high-Si systems, e.g. Monterey Bay (Brzezinski et al. 1997), Southern Ocean (Franck et al. 2000), as well as in river plumewaters. However, half saturation constants for Si uptake observed in this study (8.5 µmol l^{-1} in the winter and 10.9 µmol l^{-1} in the summer) are much higher than any previously observed in marine waters (e.g. as high as 5.3 µmol l^{-1} in the Mississippi plume; Nelson & Dortch 1996). In the Mississippi river plume Si limitation occurs during the spring (higher discharge) at salinities >20, and both N and Si limitation occur in the summer (Dortch & Whitledge 1992). In that study,

DIN, dissolved silicon and PO$_4^{2-}$ were considered limiting below concentrations of 1, 2 and 0.2 µmol l^{-1}, respectively. Interestingly, *Skeletonema costatum* dominated the Mississippi plumewater at stations where SiP was not substrate limited. This is in contrast to our observation of Si limitation in waters dominated by *S. costatum*, suggesting flexibility in the nutrient affinity of this species.

It is important to draw a distinction between the limitation of SiP and the limitation of PP because diatoms can continue to double at maximum rates during silica limitation by reducing frustule Si content (Paasche 1973, Olsen & Paasche 1986). Based on the severity of Si-uptake limitation indicated by these experiments, C fixation is likely limited by dissolved silicon concentrations as well. Experiments that directly assess the extent to which PP is Si limited are discussed in the following section.

Table 5. Mean primary production rates (PP) in control bottles and in experimental bottles with 15 µmol l^{-1} dissolved silicon additions (+dSi) at stations occupied in July and August 2001. The factor of change (Enh) in PP at each station is given. *Experimental rates significantly different from controls (95% confidence interval). Water samples were collected at the 50% light (2 to 7 m) depth, and the *in situ* dissolved silicon concentration at that depth is given

Station	PP (µmol C l^{-1}h^{-1}) Control	+ dSi	Enh	Dissolved silicon (µmol l^{-1})
29	0.158	0.198	1.26*	18.8
30	0.142	0.119	0.84	8.3
31	0.083	0.112	1.35*	4.1
39	0.311	0.427	1.37*	8.7
41	0.156	0.178	1.14	13.7
43	0.298	0.435	1.46*	27.0
46	0.137	0.197	1.44*	2.9
48	0.198	0.146	0.74*	6.5
4	0.137	0.143	1.05	2.3
50	0.187	0.227	1.21*	6.2
52	0.125	0.256	2.04*	2.0
53	0.103	0.123	1.19*	7.6
55	0.111	0.133	1.20*	8.3

Relationship between Si and C production rates

Previous studies have shown that Si availability can dramatically affect phytoplankton bloom dynamics. For example, depletion of dissolved silicon relative to other macronutrients in coastal systems leads to the decline of phytoplankton blooms (i.e. DelAmo et al. 1997, Kristiansen et al. 2001). However, the observed PP increases with dissolved silicon enrichments are the first direct evidence of silicon limitation of net PP, to our knowledge. As there is not a strong relationship between *in situ* natural rates of PP and dissolved silicon concentrations when data from all stations are considered (not shown), our results indicate that dissolved silicon concentrations were one of likely several factors which limited PP in the region. The absence of strong relationships between Si and C production rates observed throughout the study is at least in part due to photosynthetic contributions of other non-siliceous phytoplankton, including picophytoplankton, that were not quantified in this study.

Although the addition of dissolved silicon produced an increase in both SiP and PP at 7 of 13 stations, the increases were at C:Si ratios lower than expected for diatoms, possibly due to limitation by another nutrient. Further, at some stations, SiP and PP changes were not in the same direction; i.e. there was no significant change in PP, whereas SiP increased (3 stations), or, SiP decreased, whereas PP increased (or vice versa) (3 stations). At stations at which both SiP
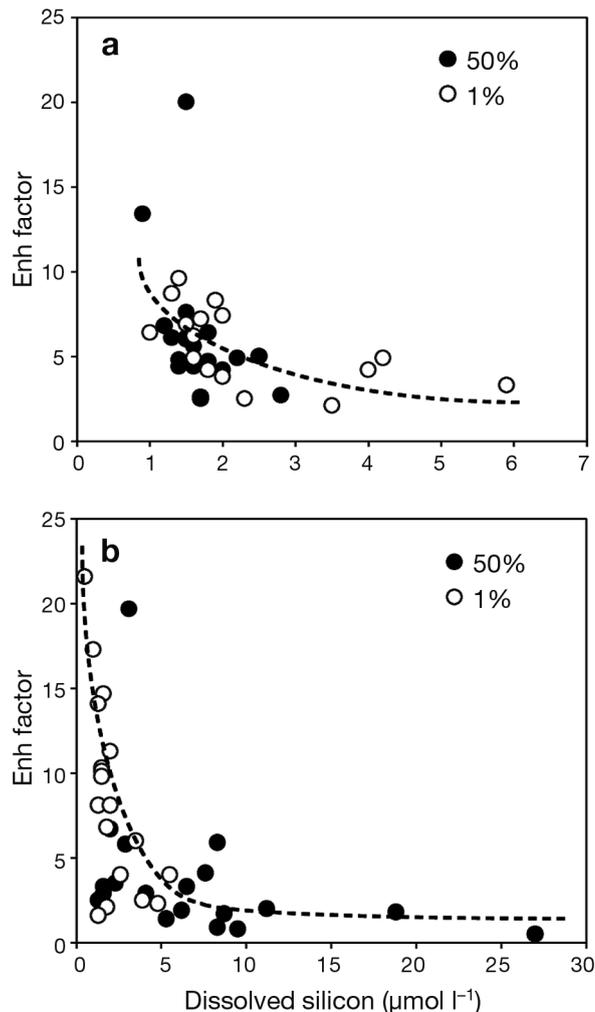

Fig. 6. Relationship between Enh value and *in situ* dissolved silicon concentrations during (a) winter and (b) summer cruises. (---------) Nonlinear regressions: Enh factor = $(K_s + [Si(OH)_4]) / [Si(OH)_4]$ and K_s values are (a) 8.5 µmol l^{-1} and (b) 10.9 µmol l^{-1}. Two outliers — (below detection,184) and (13.7, 57) — are not plotted on the summer graph

and PP increased (or SiP increased but PP did not change) with dissolved silicon addition, the excess of Si uptake relative to C may have been a result of storage of dissolved silicon in internal nutrient pools. At the majority of stations, the dissolved silicon stored during the 4 h incubation would be <35% of the bSi content of the cells (published internal pools vary widely; review by Martin-Jézéquel et al. 2000). Alternatively, diatoms in the experimental samples may have been more silicified than those in control samples, as diatoms can grow under silicon limitation by reducing Si content (e.g. Brzezinski et al. 1990). At stations where dissolved silicon enrichment led to decreases in SiP and increases in PP, or vice versa, there may have been a partitioning of energy for

only one of these processes, to the detriment of the other. Decreases in nutrient uptake rates with experimental nutrient enrichment have been previously reported by Falkowski & Stone (1975).

Milligan & Morel (2002) provide evidence that the cycles of Si and C may be linked by the use of biogenic silica as a proton acceptor for the activity of diatom carbonic anhydrase. If so, higher SiP could provide the opportunity for greater PP rates. Our results show that increased Si availability and higher SiP often were associated with higher C fixation but that the 2 rates are not consistently linked. If biogenic silica does provide a buffering capacity as suggested, our prediction may be complicated by surface area or a threshold biogenic silica concentration above which enough silica was available to provide the buffering capacity needed.
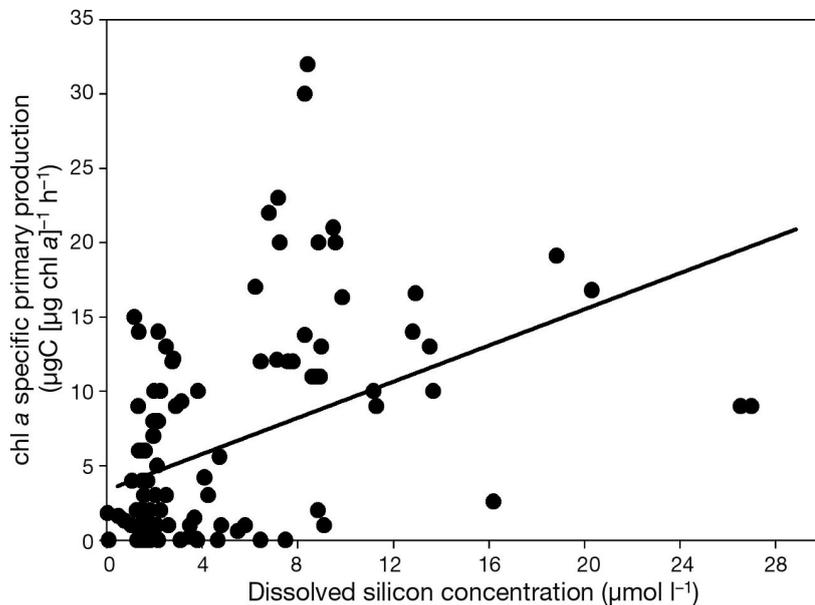
A statistically significant positive correlation between dissolved silicon concentrations and the corresponding chl a normalized PP (μg C [μg chl a]$^{-1}$ h^{-1}) indicates that dissolved silicon availability may have controlled the phytoplankton assimilation efficiency during the summer cruise (Fig. 7; r = 0.68, p = 0.012, n = 120). This relationship is similar to a diatom bloom in the Hudson River plume that depleted dissolved silicon relative to NO_3^- over a period of days (Malone et al. 1980). Likewise, the same authors found that the quantity of dissolved silicon depleted was negatively correlated with assimilation numbers of netplankton. Analysis of our data indicate weakly negative or statistically insignificant correlations between chl a normalized PP and PO_4^{2-} and $NO_3^- + NO_2^-$ concentrations, light availability, salinity and temperature during both cruises (data not shown). Thus, of the physical and nutrient factors measured, dissolved silicon availability appeared to have the greatest effect on C assimilation rates in the summer.

Nutrient control of phytoplankton production

Previous studies of phytoplankton growth and limitation in the Amazon River plume have focused on continental shelf and nearshore waters. In this region, nutrient, particle and pigment distributions indicate that phytoplankton growth over the continental shelf is often light limited by suspended particle loads (Milliman & Boyle 1975, Edmond et al. 1981, De Master et al. 1986). Farther offshore, where Amazon plume salinities are >32, phytoplankton growth is limited by low nutrient availability (Smith & De Master 1996). Comparison of nutrient availability in sur-

Fig. 7. Relationship between dissolved silicon concentration and chl a specific primary production rate at all 6 depths during the summer cruise

Table 6. Percent of stations at which nutrient concentrations, molar nutrient ratios or enrichment experiments suggest limitation by the nutrients N, P and Si at the 50% (2 to 7 m) light depth. For nutrient concentrations, N limitation if $NO_3^- + NO_2^- \leq 1.0$ μmol l^{-1}, P limitation if $PO_4^{-2} \leq 0.2$ μmol l^{-1}, Si limitation if dissolved silicon ≤ 2.0 μmol l^{-1}. For nutrient ratios, N limitation if N:P ≤ 10 and N:Si < 1, P limitation if N:P ≥ 30 and Si:P > 16, Si limitation if N:Si > 1 and Si:P < 3. n: Number of stations at which the analysis was done. –: Experiment not conducted

| | Winter | | | | | | Summer | | | | | |
| | N | | P | | Si | | N | | P | | Si | |
	n	%	n	%	n	%	n	%	n	%	n	%
Nutrient concentrations	20	100	20	100	20	80	20	100	20	85	20	20
Nutrient ratios	17	100	20	0	20	0	18	100	20	0	20	0
Enrichment of PP rate	–	–	–	–	–	–	–	–	–	–	13	77
Enrichment of SiP rate	–	–	–	–	20	100	20	35	–	–	20	70

face waters to the cellular composition of plankton implies that phytoplankton production would rarely be limited by dissolved silicon concentrations (De Master & Pope 1996).

Indicators of nutrient limitation of phytoplankton growth include (1) nutrient concentrations relative to the half-saturation constant (K_s) and (2) nutrient ratios relative to typical phytoplankton or diatom composition (Redfield 1967, Brzezinski 1985). We consider N limiting if $NO_3^- + NO_2^- \leq 1.0$ µmol l^{-1} (e.g. Goldman & Glibert 1983), P limiting if $PO_4^{-2} \leq 0.2$ µmol l^{-1} (e.g. Tilman & Kilham 1976) and Si limiting if dissolved silicon ≤ 2.0 µmol l^{-1} (e.g. Martin-Jézéquel, 2000). Thus, N and P would limit growth at more stations than Si concentrations (summary in Table 6). Comparison of nutrient ratios to Redfield or Brzezinski ratios of 16Si:16N:1P, implies N limitation at all stations during both cruises. Despite these predictions, nutrient enrichment experiments indicated Si limitation of both SiP and PP. As previously discussed for C and Si, uptake ratios during experiments were often not in Redfield or Brzezinski proportions. For example, in the winter, there was enough $NO_3^- + NO_2^-$ and PO_4^{2-} available to support SiP at a ratio of 16Si:16N:1P during the 4 to 5 h incubation at only 5 and 9 of the 20 stations for N and P, respectively. In wintertime Si enrichment experiments, there was never enough $NO_3^- + NO_2^-$ or PO_4^{2-} for uptake in Redfield proportions. Again, this decoupling of Si from N and P cycles may be due to internal pools of dissolved silicon, which can contribute greatly to total cellular Si content. It is possible that high rates of nitrogen fixation at some of the summer stations (Capone et al. 2004) could supply the N for balanced growth if 50% of it was released as dissolved organic nitrogen (a reasonable quantity based on Glibert & Bronk 1994). Alternately, dissolved organic nitrogen sources may have directly or indirectly supplied the needed N (Berman & Bronk 2003). The P necessary for balanced growth was likely supplied by rapid recycling (turnover times ~15 min) and/or dissolved organic phosphorous pools (K. Bjorkman pers. comm.). The decoupling of the Si cycle from the cycles of N and P emphasizes the flexibility of the elemental ratios in phytoplankton assemblages.

Perhaps counterintuitively, nitrogen limitation could have been the cause of both the increases and decreases in SiP that were observed when N was added in experimental manipulations. Increases in SiP in response to N addition may have been the result of the release of N limitation in some fraction of the phytoplankton assemblage, which allowed SiP to proceed. N limitation could also result in decreased SiP, as in previous studies, in which uptake and assimilation of non-limiting nutrients is depressed during surge uptake of the limiting nutrient (Falkowski & Stone 1975,

Conway et al. 1976). A more complete understanding of nutrient limitation of natural phytoplankton assemblages would benefit from time-course and nutrient enrichment studies that trace the uptake of many nutrients simultaneously. Further, in our effort to focus on silicon productivity and limitation, the role of the photosynthetic cyanobacterium *Trichodesmium* spp. was not evaluated in these experiments. *Trichodesmium* spp. did not contribute to SiP (data not shown) but did contribute significantly to total PP rates during these cruises (unpublished results), and they contributed 8 to 47% of total PP during spring and autumn cruises in this region (Carpenter et al. 2004).

In conclusion, SiP is substrate limited in an extensive area of both oligotrophic open ocean waters and Amazon plumewaters in the western equatorial and tropical Atlantic Ocean. Despite conditions that indicate limitation by other nutrients, most of the diatom productivity in this region takes place under Si limitation. Furthermore, we provide the first evidence that net PP can be limited across a range of dissolved silicon concentrations, which may also occur in other coastal and nutrient-rich systems where diatoms form a significant proportion of natural phytoplankton assemblages.

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