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Inducing phytoplankton iron limitation in iron-replete coastal waters with a strong chelating ligand

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Abstract

Dissolved iron (Fe) concentrations in the California coastal upwelling regime vary over two orders of magnitude (from <0.05 to >5 nM), which leads to a wide range in Fe effects on phytoplankton growth. Fe-addition experiments are appropriate to use to assess the biological role of Fe in low-Fe areas, but other methods are needed in Fe-replete regions. We present experiments that use additions of the exogenous siderophore desferrioxamine B (DFOB, obtained from a terrestrial actinomycete fungus) to sequester ambient Fe and to markedly decrease its availability to the biota. DFOB additions resulted in artificial Fe limitation of the phytoplankton community in high-Fe areas of the upwelling region. Results of these ''Fe-removal'' experiments mirror those of Fe-addition experiments in low-Fe, high-nutrient, low-chlorophyll (HNLC) waters. When DFOB is added to Fe-replete waters, changes in nutrient concentrations, biomass, and other biological parameters closely resemble those seen in Fe-limited controls in HNLC areas, while the controls without DFOB behave much like HNLC Fe-addition bottles. DFOB additions in high-Fe waters greatly reduced biological Fe uptake and, consequently, nitrate, silicic acid, and carbon-uptake rates as well as particulate production. Diatoms and other phytoplankton bloomed profusely in unamended controls but not in Fe-limited +DFOB bottles. Bacterial numbers and zooplankton grazing activity were also severely reduced in DFOB-addition bottles. These experiments demonstrate that artificially lowering Fe availability can induce limitation of autotrophic and heterotrophic plankton and can prevent utilization of the high ambient levels of upwelled nutrients along the California coast. Our results suggest that DFOB-bound Fe is highly unavailable to the plankton community, a result that offers researchers an important tool to use to probe the influence of Fe on biological community development in high-Fe regimes.

Recent work has given us a new perspective on the biological role of iron (Fe) in coastal upwelling regimes. Hutchins and Bruland (1998), Hutchins et al. (1998), and Franck et al. (unpubl. data) demonstrated that phytoplankton in parts of the California upwelling region are Fe limited. As in previous work in open-ocean, high-nutrient, low-chlorophyll (HNLC) waters, Fe additions along the nutrient-rich but Fe-depleted Big Sur coast of central California promoted the growth of large diatoms and allowed complete depletion of ambient nitrate. Fe also controlled major nutrient utilization ratios, bacterial production and abundance, and even grazing activity in these low-Fe coastal HNLC waters.

Not all areas along the California coast, however, are as Fe-limited as are the waters off of Big Sur. Hutchins et al. (1998) characterized the region as a complex regional mosaic of Fe limitation and attempted to describe the continuum of Fe availability in the region using a four-level model. In Type 1 Fe-replete areas, abundant shelf or riverine inputs supply large amounts of Fe, and primary and secondary productivity and nutrient biogeochemistry are unaffected by Fe additions. Type 2 regions are slightly Fe limited. Although sufficient Fe is present to allow for complete drawdown of nitrate and silicic acid at the typical nutrient-replete diatom molar ratio of 1 : 1 (Brzezinski 1985), addition of Fe in Type 2 waters allows for significantly greater growth rates and greater abundance of large diatom species. Type 3 waters are moderately Fe limited, with elevated silicic acid : nitrate ($H_2SiO_3 : NO_3^-$) molar drawdown ratios (~2–3), and they exhibit only modest nitrate utilization and diatom growth without added Fe. Silicic acid drawdown is relatively unaffected, and nanoplankton and bacteria are not severely Fe limited in Type 3 areas, however. Type 4 waters are extremely Fe limited and are characterized by very low nitrate uptake rates, reduced silicic acid utilization, high $H_2SiO_3 : NO_3^-$ utilization ratios, very low growth rates of diatoms and other eukaryotic phytoplankton, and low productivity by bacteria and higher trophic levels.

These studies demonstrate that Fe availability is intimately involved in shaping the development of biological communities in high-nutrient upwelled water along the California

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coast. Diatoms largely support the productive food webs in coastal upwelling areas and fuel their high export production fluxes and are also particularly vulnerable to Fe limitation. The dependence of diatoms on high Fe concentrations has now been well documented in both oceanic and coastal HNLC regions (Martin et al. 1991; Coale et al. 1996; Hutchins and Bruland 1998). High cellular Fe requirements have also been demonstrated in laboratory culture studies using coastal diatom species (Sunda et al. 1991; Sunda and Huntsman 1995).

Fe-addition experiments, such as those referenced above, provide strong evidence for Fe's biological importance in waters with low (~0.05 nM) to moderate (~1 nM) Fe concentrations, including Type 2, 3, and 4 regimes. In order to demonstrate the influence of Fe on community structure and biological productivity in Fe-replete Type 1 areas (>1 nM Fe), however, other techniques are needed. One such method is an "Fe-removal" experiment, wherein ambient Fe is sequestered by adding high-affinity ligands, which form Fe(III) chelates that are relatively unavailable to the phytoplankton community.

Wells et al. (1994) demonstrated that additions of a trihydroxamate siderophore produced by terrestrial actinomycete fungi, desferrioxamine B (DFOB), greatly reduces community Fe uptake in the equatorial Pacific HNLC area. DFOB (under its commercial trade name, Desferal) is used clinically to sequester excess Fe from human microbial pathogens (Weinberg 1989), and it performs a similar function by making Fe inaccessible to the biota when it is added to high-Fe seawater. The very high conditional stability constant with respect to inorganic Fe(III) (Fe′) of the Fe-DFOB complex in seawater (~10^{16} M^{-1}; Rue and Bruland 1995; Witter et al. unpubl. data) allows this siderophore to effectively complex any available dissolved Fe. Wells (1999) shows that DFOB additions prevent most biological Fe uptake in incubation experiments using coastal California waters.

We used DFOB in an Fe-removal experiment in a classic Type 1 regime along the coast of northern California. Dissolved Fe levels at this station (7.9 nM) were among the highest we have measured anywhere in the region. Despite these elevated Fe concentrations, our results demonstrate that addition of this exogenous chelator effectively shuts down biological Fe uptake and, consequently, community growth. The results of these experiments emphasize in a new way the vital role of Fe in supporting the prolific diatom blooms and high levels of biological productivity that characterize Type 1 areas along the California coast.

Methods

Near-surface seawater (10 m) was collected at an active upwelling center off of Cape Mendocino, California (38°42N, 123°36W) on 26 June 1997 using a trace-metal–clean Teflon pump system (Hutchins et al. 1998; Wells 1999). We occupied this station during a period of strong upwelling-favorable winds, which averaged 30 knots in the afternoon from the north/northwest. The relatively low sea surface temperature at this station (9.0°C) suggested that the sampled water had been recently upwelled. Indeed, nitrate levels (27 μM) and silicic acid concentrations (35 μM) were very high in the collected water. Abundant sediment-derived Fe supplies at this station also resulted in very high initial total dissolved Fe levels (7.9 nM), as measured by graphite furnace atomic absorption spectroscopy analysis of solvent-extracted acidified samples (Bruland et al. 1979). Mixed layer depth at this station was 80 m, but the 1% light level was only 40 m, suggesting possible light limitation of the phytoplankton community.

The collected water was mixed in a 50-liter acid-washed carboy and was dispensed into acid-washed polycarbonate experimental containers. Two 4.5-liter bottles and one 10-liter carboy received additions of 100 nM DFOB in a clean hood. Two 4.5-liter bottles and one 10-liter carboy filled with the same water served as unamended controls. Equilibrium calculations indicate that the DFOB addition lowered dissolved inorganic Fe(III) concentrations (Fe′) to 10^{-16}–10^{-17} M, far below the levels required for even minimal phytoplankton growth (Sunda et al. 1991; Sunda and Huntsman 1995).

Our calculations indicate that the DFOB had a relatively minor effect on the speciation of other bioactive trace metals. For example, the DFOB would have chelated less than 10% of the dissolved zinc (Zn), and copper (Cu) was already strongly complexed by natural Cu-binding ligands that outcompete the DFOB for Cu. Wells (1999) saw virtually no initial effect related to DFOB additions on the biological uptake of Zn, manganese (Mn), and cobalt (Co).

Initial samples (T_0) were obtained from the 50-liter mixing carboy. All of the experimental bottles were incubated for 5 d on deck in a spectrally corrected flow-through incubator, as described in Hutchins et al. (1998) and Wells (1999). The replicate 4.5-liter control and +DFOB bottles were sampled on days 2, 4, and 5. Analyses on the 4.5-liter bottles included measurements of size-fractionated chlorophyll *a* (Chl *a,* >0.2 μm and >8.0 μm), particulate organic nitrogen (PON) and carbon (POC), nitrate and silicic acid, and algal pigments (by high-performance liquid chromatography), along with bacterial counts and phytoplankton floristics (by microscopic counts of Lugols-preserved samples). Only large phytoplankton taxa (diatoms and dinoflagellates) were counted, because nano- and picoplankton were difficult to enumerate accurately in the Lugols-preserved samples. Details of the methods are presented in Hutchins et al. (1998). All data are reported as the mean and range of duplicate bottles.

The effects on Fe uptake of DFOB addition were monitored in the 4.5-liter bottles using 24-h ^{55}Fe incubations. Initial ^{55}Fe-uptake rates were measured in the unamended collected water. At each time point, 300-ml subsamples were obtained (two control and two +DFOB) using clean techniques, and 2.22×10^6 Bq (3.67 nM) ^{55}Fe stock in 0.01 N Ultrex HCl was added. The ^{55}Fe-uptake bottles were then incubated for 1 d in the deckboard incubators. Size-fractionated intracellular ^{55}Fe uptake (0.2–1.0 μm, 1.0–8.0 μm, and >8.0 μm on polycarbonate filters) was measured by liquid scintillation counting of titanium-washed samples (Hudson and Morel 1989), as described in Schmidt and Hutchins (in press).

The two 10-liter carboys (control and +DFOB) were kept

sealed until the final time point on day 5, when incubation experiments were performed in order to determine the effect of DFOB addition on phytoplankton nutrient uptake kinetic parameters using $^{32}Si(OH)_4$ and $^{15}NO_3^-$. Kinetic experiments were performed by distributing water from each 10-liter carboy among 16 acid-washed 320–500-ml polycarbonate bottles. Eight bottles received additions of silicic acid (+0–20 μM Na_2SiO_3 + 6.66 × 10^4 Bq ^{32}Si), seven received additions of nitrate (+0–10 μM 99% $Na^{15}NO_3^-$), and one was used to examine productivity rates using labeled sodium bicarbonate (+240 μM 98% $H^{13}CO_3$). Incubation bottles were then returned to the incubator for 4–8 h, after which they were vacuum-filtered onto 0.6-μm polycarbonate filters (^{32}Si) or precombusted Whatman GF/F glass-fiber filters (^{15}N, ^{13}C). Filters from the ^{32}Si experiments were placed in the bottom of a 20-ml plastic scintillation vial. Those from the ^{15}N and ^{13}C studies were wrapped in precombusted aluminum foil. All filters were then frozen for transport. Back in the laboratory, the filters from the ^{32}Si experiments were thawed, loosely capped, and allowed to dry overnight. Then 2 ml of 2.5 M hydrofluoric acid (HF) was added to each vial in order to dissolve the particulate silica. After 2 h, scintillation cocktail was added, and the vials were shaken and placed in darkness for 2 h to allow chemoluminescence to subside. Each sample was then counted in triplicate, using a Beckman 500TA liquid scintillation counter, as described by Brzezinski and Phillips (1997). ^{15}N and ^{13}C stable isotope filters were dried in a dessicator and analyzed for N and C concentrations and for the atom % ^{15}N and ^{13}C, using a Europa Scientific Tracer Mass mass spectrometer. Uptake rate calculations followed the recommendations of Brzezinski and Phillips (1997). Particulate silicon samples (320 ml) were vacuum-filtered onto 0.6-μm polycarbonate filters, dried at 65°C for 3 d, and analyzed according to Brzezinski and Nelson (1995). Kinetic uptake parameters and associated errors were calculated from linear regressions of the Hanes–Woolf plots (Berges et al. 1994).

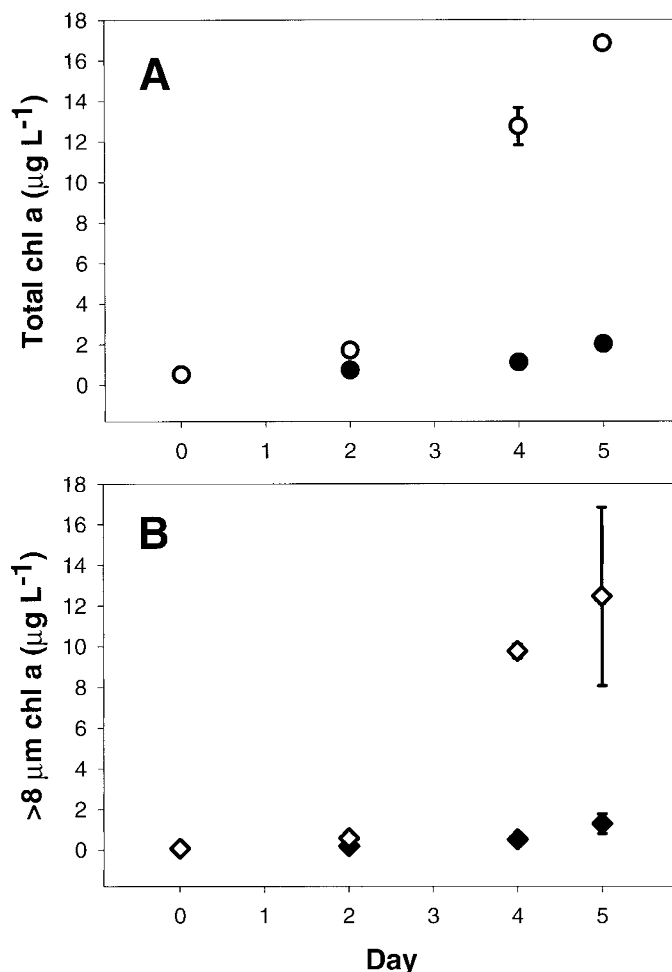

Fig. 1. Chlorophyll *a* concentrations in +DFOB bottles (filled circles) and unamended controls (open circles) for (A) the total phytoplankton community (>0.2 μm) and (B) the diatom-dominated large size class (>8.0 μm). Symbols and error bars represent the means and ranges of duplicate 4.5-liter bottles.

Results

Plankton growth and biomass—Initial phytoplankton biomass in the collected water was quite low (Fig. 1). Because the water was very freshly upwelled, biological community development was just beginning. Light limitation may have also been a factor, since the mixed layer was much deeper than the euphotic zone at this station. With increased light availability in the deckboard incubator and existing high nutrient concentrations, conditions in the experimental bottles were ideal for phytoplankton growth.

Little difference was observed between the control and +DFOB bottles until after the day 2 time point. After this apparent lag period, however, autotrophs in controls began rapid exponential growth, while Chl *a* biomass in +DFOB bottles increased only slightly. Addition of 100 nM DFOB resulted in dramatic decreases in final concentrations of both total (>0.2 μm, Fig. 1A) and large size fraction (>8.0 μm, Fig. 1B) Chl *a*, relative to controls. Five-day Chl *a* net specific growth rates in controls were 0.70 d^{-1} (>0.2 μm) and 1.0 d^{-1} (>8.0 μm) but were only 0.27 d^{-1} (>0.2 μm) and 0.56 d^{-1} (>8.0 μm) in the +DFOB bottles.

Taxon-specific phytoplankton accessory pigments showed the same trend as Chl *a* (Fig. 2). Fucoxanthin and chlorophyll *c* (Chl *c*) are primarily found in the diatoms that dominate in this system, and both increased greatly in control bottles after a 2-d lag period (Fig. 2A). By comparison, fucoxanthin and Chl *c* concentrations in +DFOB bottles barely increased over the course of the 5-d incubation. Net specific growth rates for fucoxanthin in controls and +DFOB bottles were 0.88 d^{-1} and 0.37 d^{-1}, respectively, and Chl *c* net specific growth rates were 0.98 d^{-1} (controls) and 0.41 d^{-1} (+DFOB). These results are corroborated by the dramatic 44-fold increase in biogenic silica (BSi) concentrations in the 10-liter control carboy, compared with only a sevenfold increase in the 10-liter +DFOB carboy (Table 1).

Most of the pigments characteristic of minor algal taxa also increased in controls but showed little or no growth in +DFOB bottles. These included alloxanthin (cryptophytes, Fig. 2B), peridinin (dinoflagellates, Fig. 2C), 19-butanoyloxyfucoxanthin (pelagophytes, Fig. 2C), lutein (chlorophytes, Fig. 2C), and zeaxanthin (*Synechococcus* and *Prochlo-*

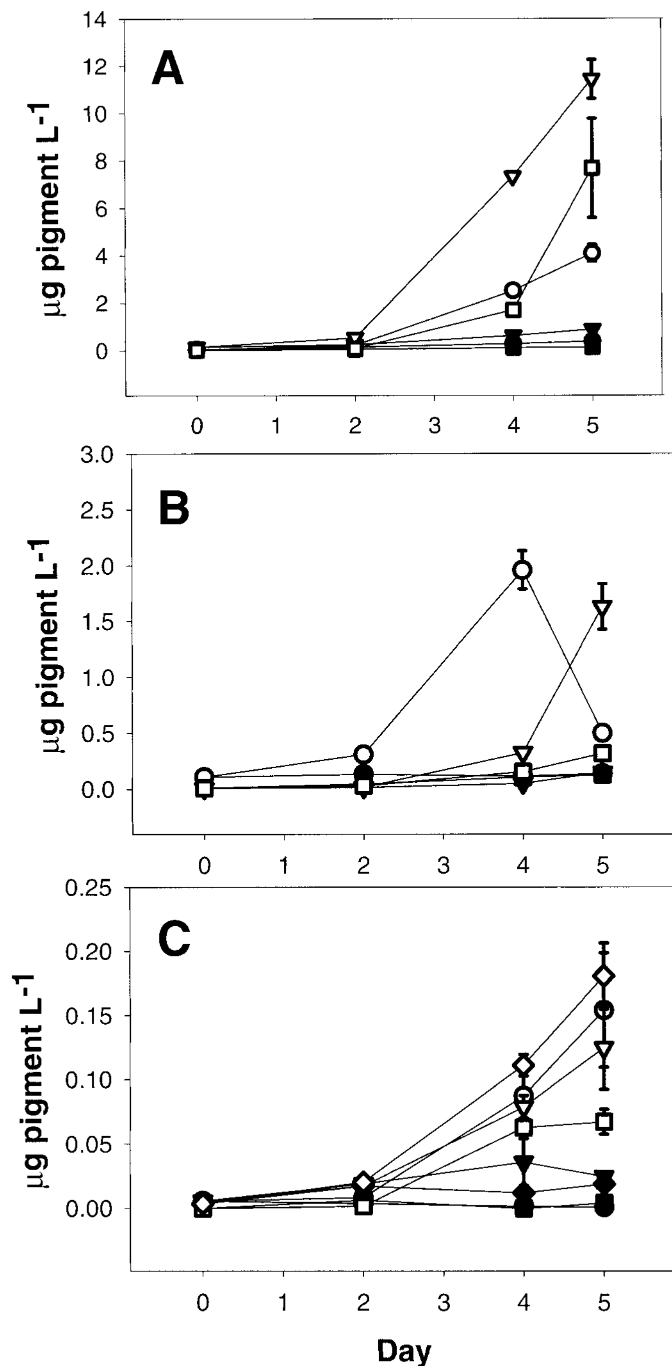

Fig. 2. Taxon-specific phytoplankton accessory pigment concentrations in the +DFOB bottles (filled symbols) and unamended controls (open symbols). Symbols and error bars as in Fig. 1. (A) Fucoxanthin (diatoms, triangles), chlorophyll *c* (primarily diatoms, circles), and combined phaeophorbides (produced by grazer-mediated degradation of chlorophyll, squares). (B) Chlorophyll *b* (*Prochlorococcus* and chlorophytes, circles), alloxanthin (cryptophytes, triangles), and 19-hexanoyloxyfucoxanthin (prymnesiophytes, squares). (C) Peridinin (dinoflagellates, circles), 19-butanoyloxyfucoxanthin (pelagophytes, triangles), lutein (chlorophytes, squares), and zeaxanthin (*Prochlorococcus* and *Synechococcus,* diamonds).

rococcus, Fig. 2C). Chlorophyll *b* (Chl *b*) (*Prochlorococcus* and chlorophytes) had increased greatly in controls by day 4, but concentrations decreased at the final time point (although they were still higher than final levels in the +DFOB bottles) (Fig. 2B). The only taxon-specific pigment that showed little difference between treatments throughout the 5-d incubation was 19-hexanoyloxyfucoxanthin (prymnesiophytes, Fig. 2B).

The effects of DFOB additions on phytoplankton growth were also evident from the microscopic cell counts (Table 2). The diatom genera *Chaetoceros, Thalassiosira,* and *Navicula* were numerically dominant in all experimental bottles, but final cell numbers were at least an order of magnitude higher in controls. The same trend was observed for all eight of the rarer diatom genera found in the bottles. Differences in cell numbers between +DFOB and control bottles were significant for all diatom species (*t*-test, $p \leq 0.04$). Final cell numbers of all diatom species combined were nearly 22 times higher in controls than in +DFOB bottles (Table 2), a significant difference at the $p = 0.004$ level.

Dinoflagellates were scarce at the final time point, but they were significantly more abundant ($p = 0.03$) in +DFOB bottles than in controls (Table 2). Although these data seem inconsistent with the small increases in peridinin observed in controls relative to +DFOB bottles (Fig. 2B), we were unable to distinguish between heterotrophic and autotrophic dinoflagellates in the Lugols-stained samples. It is possible that heterotrophic species could have been equally or more abundant in +DFOB bottles, because protozoa do not require dissolved Fe sources but can obtain Fe from their prey (Chase and Price 1997). At the same time, autotrophic dinoflagellates that depend on dissolved Fe could have been more numerous in controls, consistent with the pigment data.

Effects of DFOB on biological production and biomass were not confined to the phytoplankton community. After a 2-d lag period, heterotrophic bacterial numbers also increased exponentially in controls (relative to almost static levels in the +DFOB bottles), thereby closely paralleling the trends in autotrophic biomass (Fig. 3). Final numbers of heterotrophic bacteria were ~6.5 times higher ($p = 0.01$) in controls (Table 2). Combined phaeophorbide pigments, which are indicators of grazing activity (Strom 1993; Head and Harris 1994), also reached much higher levels in controls than in +DFOB bottles (Fig. 2A). We did not measure grazer numbers in these experiments, but this accumulation of Chl *a*-degradation products suggests that protozoan and/or metazoan zooplankton were far more active in controls than in +DFOB bottles.

Iron uptake—Figure 4 presents the effects of DFOB on uptake of ^{55}Fe in picoplankton (0.2–1.0 μm), nanoplankton (1.0–8.0 μm), and microplankton (>8.0 μm) size classes. Initial molar Fe uptake rates in the unamended water just prior to addition of DFOB were undetectable for the 0.2–1.0-μm size class; were 1.07×10^{-14} moles ml^{-1} h^{-1} for the 1.0–8.0-μm size class; and were 3.56×10^{-15} moles ml^{-1} h^{-1} for the >8.0-μm size class. These uptake rates are similar to those observed by Schmidt and Hutchins (in press) in nearshore waters of the subarctic Pacific. Because Fe con-

Table 1. Nitrate and silicic acid concentrations and uptake kinetic parameters, including V_{max} (maximum biomass-specific uptake rate) and K_s (half-saturation constant); total community carbon uptake rate (ρ); and particulate concentrations including BSi (biogenic silica), PON (particulate organic nitrogen), and POC (particulate organic carbon) for both 4.5-liter and 10-liter experimental bottles. Data from the experiment carried out in 10-liter carboys are presented for both initial and final timepoints, as single samples only. Errors for kinetic constants are standard errors. Data from the experiment in 4.5-liter bottles are presented for the final timepoint only; values and errors are the mean and range of two replicates. ND, no data; *, ambient nutrient concentrations too high for K_s determinations.

Experiment	Silicon				Nitrogen				Carbon	
	$Si(OH)_4$ (μM)	BSi (μM)	V_{max} (hr^{-1})	K_s (μM)	NO_3^- (μM)	PON (μmol N $liter^{-1}$)	V_{max} (hr^{-1})	K_s (μM)	POC (μmol C $liter^{-1}$)	ρ (μmol C $liter^{-1}$ h^{-1})
Initial (10 liter)	35.0	0.4	ND	ND	27.0	1.0	ND	ND	7.3	0.065
Control final (10 liter)	13.9	15.3	0.060 (0.011)	15.6 (4.1)	0.2	20.2	0.072 (0.009)	15.3 (0.4)	164	6.2
+DFOB final (10 liter)	34.2	2.6	0.034 (0.001)	*	23.7	5.2	0.033 (0.010)	*	31.3	0.88
Control final (4.5 liter)	3.9	ND	ND	ND	0.4	17.7 (0.2)	ND	ND	178 (1.0)	1.9 (0.36)
+DFOB final (4.5 liter)	29.4	ND	ND	ND	23.5	2.3 (0.7)	ND	ND	19.1 (4.3)	0.34 (0.03)

centrations were measured only at the initial time point, molar Fe uptake rates were calculated only for this sample. Throughout the rest of the incubation, uptake rates are presented as dpm ^{55}Fe ml^{-1} h^{-1}, giving an estimate of relative uptake rates between treatments and size classes (Fig. 4).

Nanoplankton dominated Fe uptake in the freshly collected water, and only for this 1.0–8.0-μm size class did DFOB addition result in substantially lowered Fe uptake at the next time point (when compared with initial values) (Fig. 4B). For the smaller and larger size classes, the very low initial Fe uptake rates simply failed to increase substantially during the 5-d incubation in DFOB-addition bottles.

DFOB addition had the smallest effect on picoplankton Fe uptake (Fig. 4A), producing uptake rates in controls that were similar to or only slightly higher than those in DFOB bottles. This may partly be the result of the ability of prokaryotic picoplankton to utilize exogenous siderophores (Hutchins et al. unpubl. data), since they produce chelators with similar functional groups (Wilhelm 1995; Butler 1998). However, uptake rates for the 0.2–1.0-μm size class (mostly heterotrophic bacteria and cyanobacteria) were negligible even without DFOB additions. This is consistent with the dominance of Fe uptake by large cells, which has been observed in other coastal waters, such as shelf regions of the subarctic Pacific (Schmidt and Hutchins in press).

Fe uptake by nanoplankton (Fig. 4B) and the diatom-dominated microplankton size class (Fig. 4C) was strongly inhibited by the presence of DFOB. Fe-uptake rates for both size classes were very low and invariant in +DFOB bottles. Nanoplankton uptake in controls peaked on day 4 (Fig. 4B) and fell below initial levels at the final time point, possibly because these small taxa were outcompeted by the developing diatom bloom. The diatom-dominated large size class was responsible for nearly all the Fe uptake at the final time

Table 2. Plankton counts in cells ml^{-1} for the final timepoint (Day 5) in the desferrioxamine B addition experiment carried out in 4.5-liter bottles. Reported values are the means and ranges of duplicate bottles.

Plankton taxa	+Desferrioxamine B	Control
Diatoms		
Chaetoceros	611 ± 341	6,870 ± 952
Thalassiosira	261 ± 26	7,570 ± 525
Navicula	210 ± 181	3,960 ± 600
Leptocylindrus	0	212 ± 24
Coscinodiscus	7 ± 7	292 ± 14
Asterionellopsis kariana	0	181 ± 95
Asterionellopsis glacialis	26 ± 26	527 ± 418
Cylindrotheca	0	73 ± 18
Thalassionema	0	56 ± 32
Skeletonema	0	111 ± 40
Stephanopyxis	0	28 ± 19
Grammatophora	0	83 ± 29
Total diatoms (all species)	889 ± 581	19,500 ± 2,524
Total dinoflagellates (all species)	308 ± 24	115 ± 17
Heterotrophic bacteria (all species)	$(0.70 \pm 0.17) \times 10^6$	$(4.53 \pm 0.68) \times 10^6$

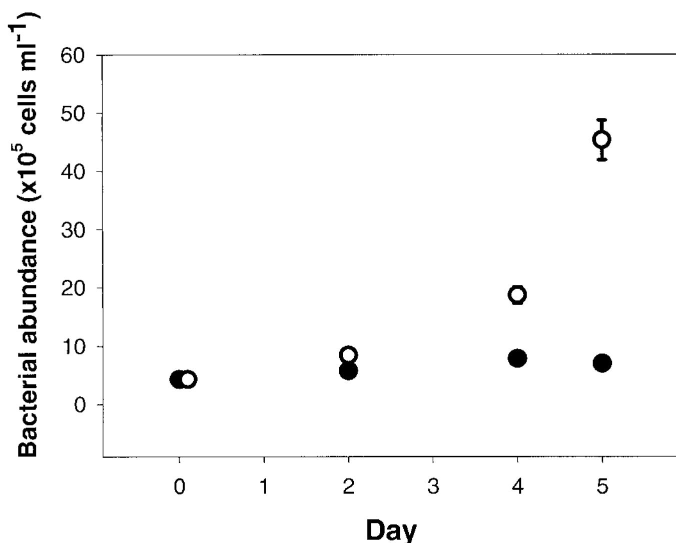

Fig. 3. Bacterial abundance in the +DFOB bottles (filled circles) and unamended controls (open circles). Error bars are included on all data points as in Fig. 1, but in many cases are smaller than the symbols.

point in controls but was able to obtain very little Fe in the +DFOB bottles (Fig. 4C).

Major nutrient biogeochemistry—DFOB inhibition of Fe uptake was strongly reflected in major nutrient dynamics as well. Phytoplankton in the controls were able to draw down the high initial nitrate concentrations to near-zero levels, using 26.7 μM NO_3^- over the 5-d incubation, whereas only 5.2 μM was used in +DFOB bottles (Fig. 5A, Table 1). DFOB additions also greatly reduced utilization of silicic acid, with only 5.6 μM H_2SiO_3 being utilized by diatoms in the +DFOB bottles, as compared with 31 μM that was used in controls (Fig. 5B, Table 1).

Final PON levels were 7.7 times greater in the controls than in +DFOB treatments for the 4.5-liter bottles (Fig. 6A, Table 1) and were 3.9 times higher in the 10-liter controls (Table 1). Final BSis were 5.9 times higher in controls (10-liter carboys, Table 1). POC was 5.2 times (10-liter carboys, Table 1) to 9.3 times (4.5-liter bottles, Fig. 6B, Table 1) higher in controls at the final time point. Carbon fixation rates increased in both treatments over the course of the incubation, but final rates were 7.0 times (10-liter carboys) and 5.6 times (4.5-liter bottles) higher in controls (Table 1).

DFOB addition had a dramatic effect on the kinetics of silicic acid and nitrate use. V_{max} values were two times lower for H_2SiO_3 and 2.3 times lower for NO_3^- in 10-liter carboys with DFOB additions (Table 1). Very similar effects of Fe on nitrate and silicic acid V_{max} values were observed in enrichment experiments conducted in a Type 4 HNLC area along the California coast (Franck et al. unpubl. data). In these experiments, Fe addition increased V_{max} of the phytoplankton community for nitrate and silicic acid 2.4 times compared with the low-Fe controls. These results suggest that Fe availability may possibly affect the number of silicon transporters in diatom cell walls. Because so little nitrate and silicic acid was used by phytoplankton in the +DFOB bot-

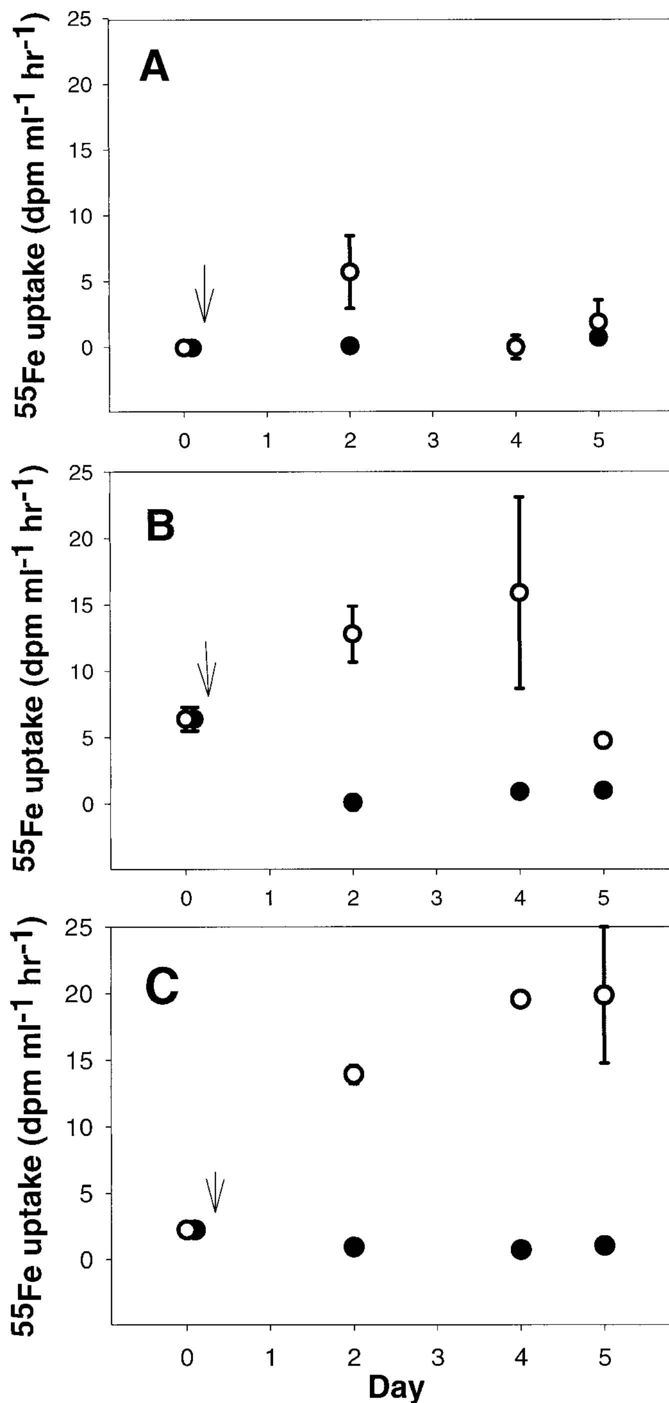

Fig. 4. Size-fractionated biological ^{55}Fe uptake in 24-h incubations of 300-ml subsamples taken at each time point from the 4.5-liter bottles. Shown are uptake rates in +DFOB bottles (filled circles) and unamended controls (open circles) for (A) picoplankton (0.2–1.0-μm size class), (B) nanoplankton (1.0–8.0-μm size class), and (C) microplankton, mostly diatoms (>8.0-μm size class). Initial samples were taken before addition of the DFOB; arrows indicate when DFOB was added. Symbols and error bars as in Fig. 1.

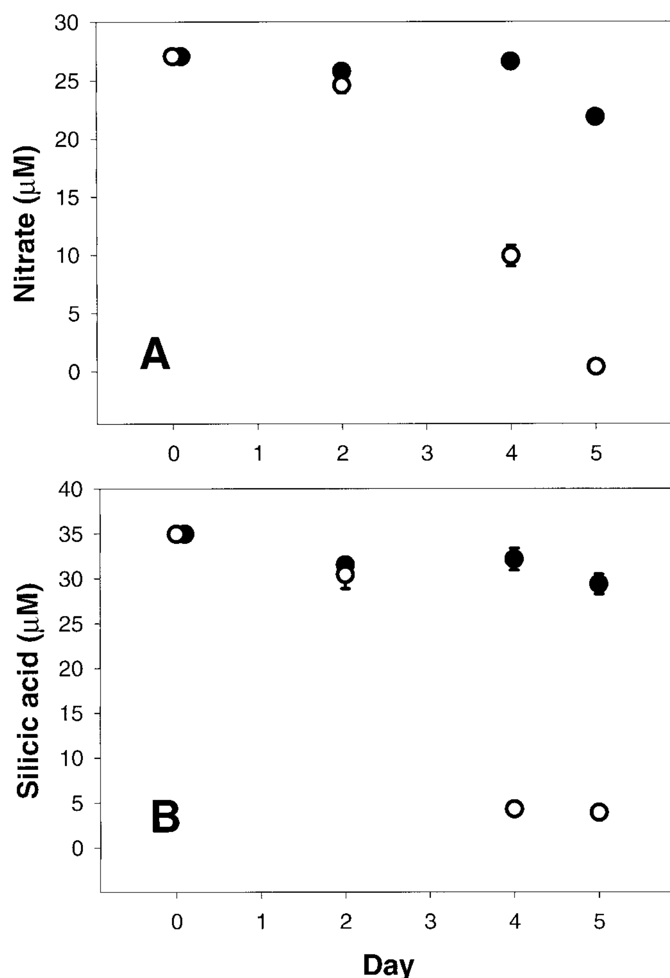

Fig. 5. Nutrient drawdown by phytoplankton in +DFOB bottles (filled circles) and unamended controls (open circles). (A) Nitrate. (B) Silicic acid. Error bars are included on all data points as in Fig. 1 but in many cases are smaller than the symbols.

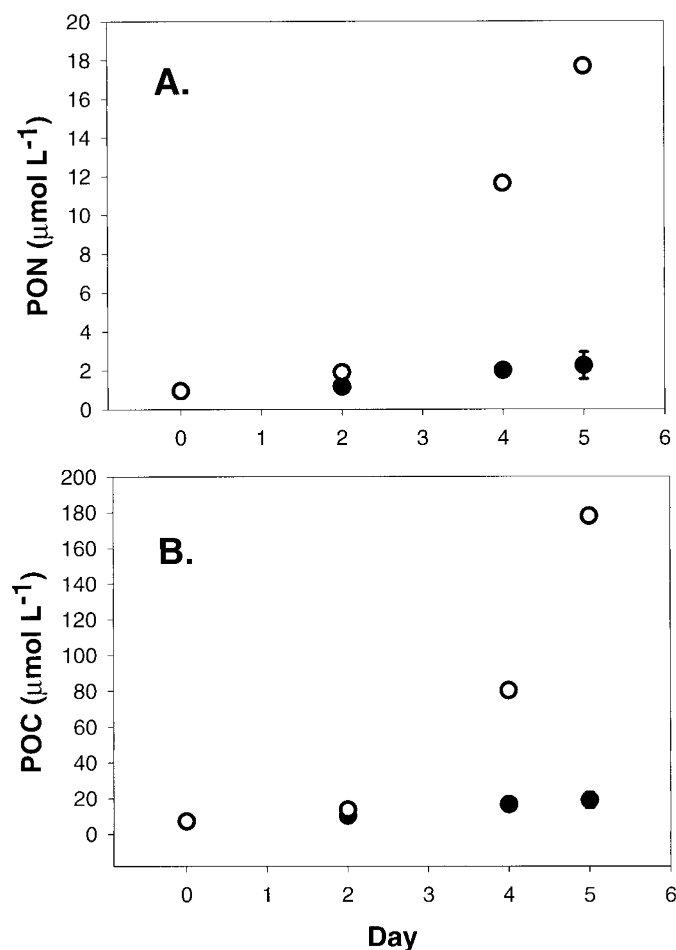

Fig. 6. Particulate organic nitrogen and carbon concentrations in the +DFOB bottles (filled circles) and unamended controls (open circles). (A) Particulate organic nitrogen (PON). (B) Particulate organic carbon (POC). Error bars are included on all data points as in Fig. 1 but in many cases are smaller than the size of the symbols.

tles over the 5-d incubation, we were unable to calculate final half-saturation constants (K_s) for these samples as uptake was saturated at ambient concentrations. No published data are available on the effect of Fe on phytoplankton nitrate K_s values. However, previous work using ^{32}Si shows that Fe has little effect on the K_s for silicon uptake in diatoms, suggesting that Fe availability does not alter the affinity of the transport system for silicic acid (De la Rocha et al. unpubl. data; Franck et al. unpubl. data).

^{32}Si and ^{15}N measurements of silicic acid and nitrate total community uptake rates at the end of the incubations (day 5) showed that the community in the control bottles had become substrate limited (data not shown). Nitrate concentrations were depleted to 0.4 μM in the 4.5-liter controls and to 0.2 μM in the 10-liter control, resulting in specific-uptake rates that were <10% of V_{max}. Silicic acid concentration in the 10-liter control carboy was depleted to less than the K_s for diatom silicic-acid uptake (Brzezinski et al. 1997), lowering specific-uptake rates to less than one-half of V_{max}. In contrast, substrate limitation of nitrate and silicic-acid uptake was not apparent in the 10-liter +DFOB carboy, in which ambient nitrate and silicic acid concentrations remained >20 μM and in which final specific-uptake rates were somewhat higher than in the substrate-limited controls (not shown).

Discussion

Upon first approximation, the results of our DFOB-addition experiments can be described as the "mirror image" of those produced by an Fe-addition experiment. Our controls behaved very similarly to +Fe bottles in HNLC Fe-addition experiments, and parameters in our +DFOB bottles closely resembled those of Fe-limited controls in Type 4 low-Fe areas (Hutchins et al. 1998). Chl *a* in our controls (both total and large size fraction) followed the pattern usually observed in HNLC +Fe bottles, even to the extent of exhibiting a 2-d apparent lag period before the onset of rapid biomass increases (Fig. 1). The fact that Fe was already plentiful in our collected water suggests that these typical lag periods in HNLC experiments may be the result of a low abundance of large diatoms in initial water samples and do not represent

a real extended physiological delay that occurs after Fe is added.

Concentrations of chl *a* and other pigments in the +DFOB bottles also showed minor increases, as are usually observed in HNLC Fe-addition controls (Hutchins and Bruland 1998). Most previously published Fe-addition work suggests that this effect is the result of grazer exclusion (e.g., Buma et al. 1991). We suggest that another likely explanation for the small amount of growth in our +DFOB bottles was an ability of the phytoplankton to access small amounts of Fe from the DFOB complex (see below). Similarly, growth in HNLC control bottles may be at least partly related to trace Fe contamination, which can occur with even the most scrupulous clean techniques.

In addition, as in HNLC Fe additions, diatoms flourished in our Fe-replete controls but did poorly in the Fe-limited +DFOB bottles (Fig. 2A, Table 2). These results underscore the dependence of diatoms on abundant Fe supplies and demonstrate that high levels of ambient Fe are needed to support the normally high production rates by large diatoms in Type 1 areas. DFOB additions also effectively Fe limited most other phytoplankton taxa, including both eukaryotes and prokaryotes, with the possible exception of prymnesiophytes (Fig. 2B). This could be the result of extremely low Fe requirements for prymnesiophytes; for instance, an oceanic coccolithophore can sustain maximum growth rates at the low ambient Fe concentrations in the subarctic Pacific HNLC area (Muggli and Harrison 1997). Alternatively, prymnesiophytes may have an ability to somehow effectively access DFOB-bound Fe. The 19-hexanoyloxyfucoxanthin pigment data suggested low growth rates in both treatments, however, and prymnesiophytes could have been limited by some factor unrelated to Fe availability, or they may have simply been outcompeted by other phytoplankton in both treatments.

The results of Wells (1999) support the idea that the inhibition of phytoplankton community growth that we observed with DFOB was due to Fe limitation and not to some toxic effect of the chelator. Community Fe uptake in both our experiments (Fig. 4) and those of Wells was severely reduced, but initial carbon fixation rates of the dominant large cells (mostly diatoms) were unaffected by DFOB additions (Wells 1999). Wells found that carbon fixation by smaller cells was depressed temporarily, but this effect was short-lived (~6 h). Although inhibition of sensitive open-ocean species of phytoplankton by ethylenediaminetetraacetic acid (EDTA) has been reported (Muggli and Harrison 1996), the levels of EDTA that they found to be deleterious were 1,000 times higher (100 μM) than the concentration of DFOB we used in our experiments with more robust coastal phytoplankton. No toxic effects of DFOB have been noted in the several other phytoplankton studies that have used this chelator (Wells et al. 1994; Soria-Dengg and Horstmann 1995; Maldonado and Price in press).

DFOB-induced low-Fe conditions also adversely affected bacteria (Fig. 3). Once again, these results mirror those of Hutchins et al. (1998) in Type 4 Big Sur waters, with bacterial parameters in our +DFOB bottles resembling those in Fe-limited Big Sur controls and with Big Sur +Fe bottles looking much like the −DFOB controls. This is not, however, strong evidence for direct bacterial Fe limitation, such as has been reported for the Southern Ocean (Pakulski et al. 1996), or even for indirect bacterial Fe limitation, such as the reduced carbon growth efficiencies reported for laboratory cultures grown at low Fe levels (Tortell et al. 1996). Reduced bacterial growth in +DFOB bottles (and Big Sur no-Fe controls) could be the result of carbon limitation, since phytoplankton production was also very low in these samples. Like bacterial productivity in the experiments of Hutchins et al. (1998), bacterial numbers in the incubations presented here closely followed phytoplankton biomass, even down to the 2-d lag period. This suggests that bacterial growth was more closely tied to phytoplankton-derived carbon supplies rather than being directly dependent on Fe availability.

Grazing also appeared to be linked to Fe availability. Concentrations of grazer-produced phaeophorbide pigments were much lower in +DFOB bottles (Fig. 2A), just as they were in the Fe-limited Big Sur controls of Hutchins et al. (1998). Again, these results should not be taken as evidence of direct Fe limitation of grazers. Nevertheless, the data presented here and in Hutchins et al. (1998) provide strong evidence that zooplankton can dramatically increase grazing rates to take advantage of Fe-induced diatom blooms. These increases can occur without net immigration (our enclosed shipboard experiments preclude this) and without biomass increases by macrozooplankton, since their reproductive cycles are much longer than our 5-d experiments. It is apparent that Fe limitation of autotrophs, whether natural (such as in Type 4 HNLC waters) or artificial (such as when DFOB is added to Type 1 Fe-replete waters), has major trophic consequences. Bacteria, zooplankton, and all larger metazoans ultimately depend on phytoplankton-derived carbon and energy sources that are not available without adequate Fe supplies.

Nitrate utilization also reflected the trends seen in Fe-addition experiments, with major drawdown (Fig. 5A) and high uptake rates (Table 1) occurring only in the controls that had not received DFOB additions. Phytoplankton in the +DFOB bottles, on the other hand, were able to use very little of the ambient nitrate, as is usually the case in Fe-limited controls in HNLC experiments. PON and POC production also followed this same pattern, closely resembling an "inverse" Fe-limitation experiment. Rates of autotrophic carbon fixation in the +DFOB bottles were much lower by the end of the incubation (Table 1), thus reproducing the effects of Fe limitation in HNLC regions along the California coast (Franck et al. unpubl. data).

One caveat to the mirror image analogy is related to silicic-acid utilization. In Fe-limited HNLC control bottles, silicic-acid–uptake rate and drawdown are usually less affected than are nitrate uptake and drawdown, leading to $H_2SiO_3:NO_3^-$ molar utilization ratios that are ~ two to three times higher than in +Fe bottles (Hutchins and Bruland 1998; Hutchins et al. 1998; Takeda 1998; Franck et al. unpubl. data). In our Fe-limited +DFOB bottles, diatoms used much less silicic acid than is usually the case in HNLC controls (Fig. 5B). Although there were major differences in the magnitude of nutrient utilization between the control and +DFOB treatments, $H_2SiO_3:NO_3^-$ molar drawdown ratios did not show a consistent trend between treatments. Com-

munity $H_2SiO_3:NO_3^-$ drawdown ratios were 0.2 (+DFOB 10-liter carboys), 1.6 (+DFOB 4.5-liter bottles), 0.8 (control 10-liter carboys), and 1.2 (control 4.5-liter bottles). Molar uptake ratios of C : N : Si from the tracer experiments were 19 : 0.13 : 1 in control 10-liter carboys at the end of the experiment, concordant with the near exhaustion of nitrate in those samples. In contrast, the C : N : Si molar uptake ratio on day 5 in +DFOB carboys (8 : 1.4 : 1) was essentially at Redfield proportions.

It is possible that chelating ambient Fe to a strong ligand like DFOB produces a different physiological effect on diatom silicic-acid uptake than does lack of ambient Fe. We suggest, however, that it is more likely that this effect is simply due to extremely severe Fe limitation of diatoms by the relatively high levels of DFOB we added in our experiments. Hutchins et al. (1998) have suggested that the highest $H_2SiO_3:NO_3^-$ utilization ratios are probably found in moderately Fe-limited Type 3 waters, where some limited diatom growth is possible. Our DFOB additions may have shut down diatom growth to such an extent that little silicic-acid uptake could take place (final diatom numbers in +DFOB bottles were <5% of those in controls, Table 2). Additions of lower amounts of DFOB might better simulate natural slightly or moderately Fe-limited conditions, including characteristic higher $H_2SiO_3:NO_3^-$ utilization ratios.

Our results and those of Wells et al. (1994) and Wells (1999) unequivocally demonstrate that DFOB additions can virtually halt community Fe uptake and, thus, plankton growth and nutrient drawdown. This seems to be at odds with the results of Soria-Dengg and Horstmann (1995), which suggests that DFOB-bound Fe is accessible to cultures of the marine diatom *Phaeodactylum tricornutum,* as is Fe chelated to the related fungal siderophore desferrioxamine E (DFOE). Similarly, new work by Maldonado and Price (in press) suggests that both of these siderophores can supply Fe to natural phytoplankton communities in the northeast subarctic Pacific.

Both studies invoke a reductive mechanism by which phytoplankton, especially diatoms, can access DFOB-bound Fe. Soria-Dengg and Horstmann (1995) used a method based on ''trapping'' ferrous Fe produced by biological reduction, using the Fe(II) chelator bathophenanthroline-disulfonic acid (BPDS). Lack of uptake from ferrioxamine complexes in the presence of BPDS was interpreted to mean that complexed Fe(III) needed to be reduced before uptake. Maldonado and Price (in press) used a chemiluminescence method to measure Fe(II) production, but their studies were based on ligand and Fe concentrations that were three to four orders of magnitude higher than ambient levels and used phytoplankton communities concentrated ~1,700 times above natural cell densities and analyses carried out at pH 6.6. These two methods resulted in opposing conclusions about biologically mediated Fe reduction from the two fungal siderophores. Soria-Dengg and Horstmann (1995) concluded that DFOB-bound Fe undergoes reductive uptake, but they found no evidence of a similar mechanism for DFOE. Maldonado and Price (in press), on the other hand, found that phytoplankton could sometimes reduce Fe from the DFOE complex but not from the DFOB complex. A complete mechanistic understanding of reductive Fe uptake from ferrioxamine complexes may have to await further methodological developments.

Maldonado and Price (in press) showed that some radiolabeled Fe can be taken up from ferrioxamine complexes by phytoplankton; however, examination of their data suggests that the amount of Fe obtained from this source was typically too small to support growth. In one experiment, Maldonado and Price (in press) found that phytoplankton (>3-μm size class) took up an average of only 0.2 μmol Fe : mol C d^{-1} from the DFOB complex, even though, out of methodological necessity, they added ~20 times more Fe (2 nM Fe with 10 nM DFOB) than is typically found in the subarctic Pacific HNLC area (~0.1 nM, Martin and Gordon 1988). In separate experiments, they found that the Fe : C ratio of this large size class averaged about 3.7 μmol : mol. It is apparent that the uptake rates they measured from DFOB-bound Fe could supply only about 5% of the amount needed to support unrestricted phytoplankton growth rates of ~1 division d^{-1}. Studies using pure laboratory cultures of oceanic diatoms show that their Fe : C ratios are even higher (4–5 μmol Fe : mol C, Sunda et al. 1991; Sunda and Huntsman 1995), which suggests that DFOB complexes could supply even less of their total Fe requirement. C-normalized Fe-uptake rates were similarly very low for DFOE-bound Fe in the Maldonado and Price study (in press). In keeping with this observation, neither phytoplankton growth rates nor biomass were stimulated by the Fe added in their incubations.

Diatoms and other phytoplankton taxa may very well be able to obtain Fe from organic complexes by a reductive mechanism. However, siderophore-bound Fe is probably among the least available types they are likely to encounter. Hutchins et al. (unpubl. data) found that eukaryotic phytoplankton can take up small amounts of Fe that are bound to DFOB and to other trihydroxamate siderophores, such as ferrichrome. However, Fe-uptake rates from these sources are much lower than those observed from other strong organic ligands with similar conditional stability constants (Witter et al. unpubl. data), such as porphyrins and Fe-binding proteins. Pyrrole-type ligands, such as porphyrins, are derived from a variety of common marine sources, such as algal pigments and electron-transport proteins, and recent ^{15}N nuclear magnetic resonance measurements suggest that they are probably a ubiquitous component of marine dissolved organic matter (McCarthy et al. 1997). We suggest that they are probably much more likely than siderophores to serve as significant Fe sources for phytoplankton in nature.

The term ''bioavailable'' is only meaningful in a relative sense. As Hutchins (1995) noted, some portion of the Fe added in almost any chemical form can eventually be assimilated by the biota. Our experiments suggest that on a relative scale of bioavailability to eukaryotic phytoplankton, siderophores such as DFOB probably rank near the bottom.

The results of our California experiments demonstrate that DFOB is a promising tool to use to shed light on the biological role of Fe in high-Fe Type 1 areas. DFOB additions can effectively Fe-limit primary producers, even when ambient Fe is abundant. The effects of DFOB-induced phytoplankton Fe limitation then rebound throughout the system,

with large consequences for major nutrient biogeochemistry and heterotrophic production.

As suggested by Wells (1999), carefully fine-tuned DFOB additions, at a range of concentrations, could achieve an Fe-limitation gradient that mimics the large natural heterogeneity in Fe availability that is observed throughout the California coastal upwelling region. Our experiments simulated severely Fe-limited Type 4 conditions by adding high levels (100 nM) of the siderophore. Phytoplankton could be slightly Fe-limited by adding lower amounts of DFOB (thereby possibly closely reproducing observed natural responses in Type 2 or 3 regimes, including limitation of diatoms but not of other taxa and/or elevated $H_2SiO_3:NO_3^-$ drawdown ratios). Imaginative application of exogenous siderophore-addition methodology should give us a better picture of the importance of Fe to the plankton community and carbon export in both high- and low-Fe waters.

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