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Iron uptake and physiological response of phytoplankton during a mesoscale Southern Ocean iron enrichment

Abstract—Iron supply is thought to regulate primary production in high nitrate, low chlorophyll (HNLC) regions of the sea in both the past and the present. A critical aspect of this relationship is acquisition of iron (Fe) by phytoplankton, which occurs through a complex series of extracellular reactions that are influenced by Fe chemistry and speciation. During the first in situ mesoscale Fe-enrichment experiment in the Southern Ocean (Southern Ocean iron release experiment [SOIREE]), we monitored the uptake of Fe by three size classes of plankton and their ensuing physiological response to the Fe enrichment. Rates of Fe uptake from both inorganic Fe (Fe′) and organic Fe complexes (FeL) were initially fast, indicative of Fe-limitation. After Fe enrichment phytoplankton down-regulated Fe uptake and optimized physiological performance, but by day 12 they had greatly increased their capacity to acquire Fe from FeL. The increase in Fe uptake from FeL coincided with a sixfold decrease in Fe′ that followed the production of Fe-binding organic ligands. Phytoplankton were able to use organically bound Fe at rates sufficient to maintain net growth for more than 42 d. Adaptation to such shifts in Fe chemistry may contribute to bloom longevity in these polar HNLC waters.

High nitrate, low chlorophyll (HNLC) waters account for 25% of the world surface ocean and have been the focus of recent investigations examining what factor(s) control primary productivity. Culture (Sunda and Huntsman 1997) and shipboard (Martin et al. 1989; Coale et al. 1996*a*) studies show that low Fe availability limits phytoplankton growth, a result that has been confirmed by in situ Fe enrichments in HNLC regions (Martin et al. 1994; Coale et al. 1996*b;* Boyd et al. 2000). The chemical complexities of Fe and its potential interactions with microbes in natural waters, however, may obfuscate the relationship between phytoplankton physiology and Fe concentrations (Geider 1999). Physiological adaptations of Fe-limited phytoplankton include variations in inorganic Fe uptake kinetics (Harrison and Morel 1986; Hudson and Morel 1990) and in their abilities to acquire Fe from organic Fe complexes (FeL) (Maldonado and Price 1999, 2001). However, the physiological responses of natural phytoplankton assemblages to shifts in Fe speciation in situ are unknown. Pronounced in situ changes in Fe speciation occurred during IronEx II in the Equatorial Pacific (Rue and Bruland 1997), but no concurrent measurements of algal Fe uptake physiology were made. Because Fe speciation strongly affects the ability of phytoplankton to acquire Fe (Geider 1999; Maldonado and Price 1999, 2001), changes in Fe speciation will undoubtedly determine the algal response to Fe enrichment.

Southern Ocean waters are characterized by a large reservoir of macronutrients and are thought to have a disproportionate influence on past and present global climate (Broecker and Henderson 1998; Sarmiento et al. 1998). The SOIREE (Boyd et al. 2000) was conducted in February 1999 to assess whether Fe supply controls phytoplankton production in polar waters (61°S 140°E). A 50 km^2 area of surface ocean was fertilized with inorganic Fe(II) labeled with SF_6 (sulfur hexafluoride) and with three subsequent additions of Fe on days 3, 5, and 7 of the experiment (Fig. 1A) (Boyd et al. 2000). The Fe enrichment resulted in significant changes in Fe speciation and dissolved Fe concentrations. Within 13 d, a pronounced accumulation of phytoplankton stocks, an increase in growth rate and photosynthetic competence of the dominant taxa, and a change in species composition were observed (Boyd et al. 2000). Moreover, SeaWiFS remote-sensing images of the Fe-fertilized patch showed that the bloom was still present 42 d after the onset of SOIREE and had spread over a ribbon-shaped area of 1,100 km^2 (Abraham et al. 2000). This observation raised fundamental questions regarding the mechanisms for the sustenance and longevity of this bloom. This paper investigates the physiological response of plankton to changes in Fe chemistry and speciation following this in situ Fe enrichment

(Boyd et al. 2000). We include data for five different physiological parameters that demonstrate the ability of phytoplankton to physiologically adapt to changes in Fe speciation and to access different Fe pools. Our results provide a physiological explanation for the observed bloom longevity from satellite imaging (Abraham et al. 2000).

Materials and methods—Owing to the limited time available for hydrographic sampling in the Fe-fertilized waters during SOIREE (12–16 h d^{-1} were required for SF_6 mapping of the patch), it was not always possible to obtain concurrently water samples for all the physiological and/or chemical measurements presented here. We therefore compare parameters determined in samples collected on the same day but at different times and from different depths in the mixed layer (e.g., Fe speciation at 40 m, photosynthetic parameters at 60–70 m, Fe uptake rate measurements at 20 m, and diatom flavodoxin levels and sinking rates at 5 m). Comparison of samples from different times on the same day is justified by the relatively slow evolution of the SOIREE bloom (10–13 d, Boyd et al. 2000). In the case of samples taken from different depths, it should be noted that despite some vertical structure—due to thermal transients—within the mixed layer from days 6 to 13 of SOIREE (Boyd et al. 2000), physiological data (F_v/F_m) suggest that this vertical structure resulted in relatively small variations in physiolog-

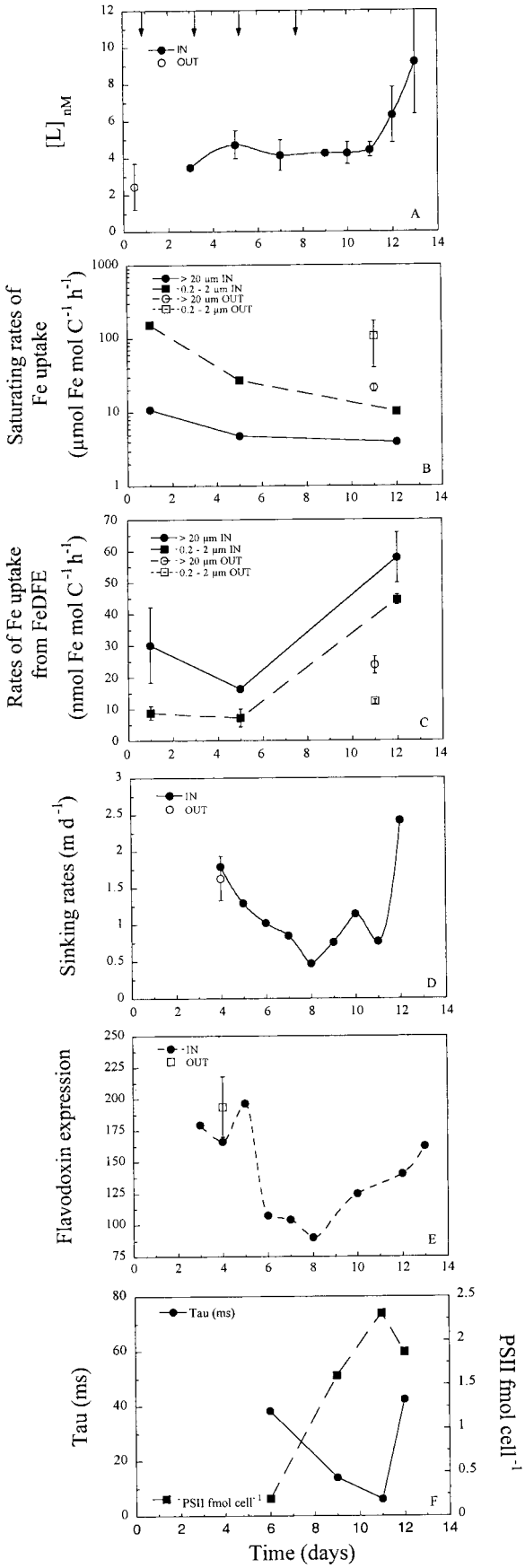

Fig. 1. Iron uptake and physiological parameters during the course of SOIREE. (A) Concentration of Fe-binding organic ligands (L, nM) at 40 m depth in the Fe-enriched patch, as measured using TAC (10 μM). The error bars represent the 95% confidence intervals. Preinfusion value, before day 1, is the mean ($\pm$ SD, $n = 3$) of outside the patch values throughout SOIREE (*see text for individual values*). The black arrows indicate the time of the Fe infusions. (B) Short-term saturating rates of Fe uptake (V_{max}, μmol Fe mol C^{-1} h^{-1}) for the >20-μm and the 0.2–2-μm plankton size-fractions inside and outside the patch. Error bars represent the range of the mean of uptake rates from duplicate incubation bottles; when absent, the range was less than the symbol width. (C) Short-term nonsaturating FeDFE uptake rates (nmol Fe mol C^{-1} h^{-1}) for the >20-μm and the 0.2–2-μm plankton size-fractions inside and outside the patch over time. Error bars represent the range of the mean of the uptake rates from duplicate incubation bottles; when absent, the range was less than the symbol width. (D) Diatoms (~90% of cells >20-μm size-fraction, mainly *Fragilariopsis kerguelensis*) sinking rates (m d^{-1}) inside the patch during the course of the experiment. The mean sinking rate outside the patch for four different days is plotted (mean $\pm$ SD, $n = 4$). The experimental error associated with these measurements was <10%. (E) Flavodoxin levels, normalized to protein, in diatoms inside the patch. The mean flavodoxin value outside the patch for four different days is plotted (mean $\pm$ SD, $n = 4$). The mean flavodoxin value for days 6–8 inside the patch was significantly different from the overall mean for outside the patch (100 and 195, respectively; $P < 0.025$). (F) Maximum steady-state rate of photosynthetic electron transport (tau, ms) and cellular concentrations of the photosystem II reaction centers (PSII, fmol/cell) for the >20-μm size-fraction phytoplankton inside the patch. The experimental error associated with these measurements was <10%.

ical parameters (< 20%) with depth (*see* fig. 4, Boyd et al. 2000).

Iron speciation: Seawater samples were collected at 40 m depth using trace-metal clean GO-FLO sampling bottles deployed from a Kevlar line and were analyzed for dissolved Fe as described by Bruland et al. (1979) and for Fe speciation as described by Croot and Johansson (2000). The Fe speciation measurements were specific for Fe(III) and were performed using competitive ligand exchange—cathodic stripping voltammetry with the ligands TAC [2-(2-Thioazolylazo)-p-cresol] and 1N2N (1-Nitroso-2-Napthol) (Croot and Johansson 2000). All sample manipulations and analyses were performed inside a class-100 laminar airflow bench. The in situ inorganic Fe concentration ([Fe′]) was calculated as described (Croot and Johansson 2000) using the in situ [L], [DFe], and log K_{FeL}^{cond} and the quadratic equation for Fe′ derived from the Langmuir isotherm. The errors associated with the Fe′ values include full-cumulative error from DFe, L, and *K* estimates and represent 95% confidence intervals.

Fe uptake experiments: The ability of plankton to acquire Fe from both inorganic Fe (Fe′) and organic Fe pools was assessed by measuring uptake rates from inorganic Fe and Fe bound to the siderophore, desferrioxamine.

For the inorganic Fe uptake kinetic (V_{max} and K_m) experiments, ^{55}Fe (0.2, 2, 20, and 200 nM) was added bound to 10 μM EDTA (ethylenediaminetetraacetic acid) in order to keep Fe soluble. The ^{55}Fe additions of 0.2, 2, 20, and 200 nM resulted in [Fe′] of 7.7, 77, 771, 6,100 pM, respectively (calculated using MINEQL+, Schecher and McAvoy 1998). A Hanes-Woolf transformation of the uptake data was used to estimate the half-saturation constant for Fe uptake (K_m) and the maximum rate of Fe uptake (V_{max}) (Segel 1976). The data fit the Hanes-Woolf linear model with a mean $r^2 = 0.86 \pm 0.14$ ($n = 25$).

For the organically bound Fe uptake measurements, desferrioxamine (DF) was used as a model organic Fe complex. This compound was chosen because it has Fe-binding functional groups and a conditional stability constant similar to the organic chelators naturally present in the sea and also to alterobactin, a siderophore produced by an open-ocean bacterium (Reid et al. 1993; Rue and Bruland 1995). For these experiments 2 nM ^{55}Fe was bound to 20 nM desferrioxamine B or E (DFB/DFE) as described previously (Maldonado and Price 1999). The [FeDF] complex mimicked the in situ conditions on day 13 with respect to [Fe] (1.05 ± 0.17 nM Fe) and [L] (9.2 nM ± 2.8 nM L). Note that as the rates of Fe uptake from FeDFB and FeDFE were virtually identical, only rates of Fe uptake from FeDFE are plotted in Fig. 1C.

Seawater collected from 20 m depth inside (day 1, 5, and 12) or outside the patch (day 9 and 12) was incubated on deck at in situ temperature and ambient irradiances for 24 h with additions of ^{55}Fe and either the synthetic chelator EDTA or the siderophore DFB or DFE. Every 6 h, samples from duplicate bottles were collected on polycarbonate filters of varying porosity (0.2, 2, and 20 μm) and were rinsed with Ti reagent to dissolve ferric oxides adsorbed to the cell surfaces (Hudson and Morel 1989). Uptake rates were calculated by linear regression of particulate Fe per ml against time. Even though the Fe uptake experiments in the surrounding HNLC waters were only performed on days 9 and 12, we assume that they represent initial conditions, given that oceanographic properties remained constant outside the fertilized patch (Boyd et al. 2000).

For the two major algal size-fractions (2–20 and > 20 μm), volumetric Fe uptake rates (nmol Fe L^{-1}) were normalized to C biomass, using size-fractionated chlorophyll *a* (Chl *a*) and C : Chl *a* ratios of 90, 60, and 45 for days 1, 5, and 12, respectively (Boyd et al. 2000; Gall et al. 2001). These algal size-fractions (2–20 and >20 μm) were dominated by haptophytes and autotrophic nanoflagellates, and by the large pennate diatom *Fragilariopsis kerguelensis*, respectively (Gall et al. 2001). Given the similarity in the Fe uptake rates for both of these algal size-fractions (2–20 and >20 μm), here we only report the latter.

For the picoplankton size-fraction (0.2–2 μm), C biomass was calculated by summing the picophytoplankton and heterotrophic bacterial C biomass, determined by cell abundance and using published C conversion factors (Hall and Safi 2001). The C biomass in the 0.2–2-μm size-fraction was partitioned 1 : 1 (55% picophytoplankton [all were eukaryotic] and 45% heterotrophic bacteria, Gall et al. 2001; Hall and Safi 2001).

Photosynthetic parameters: Fifteen liters of seawater were collected from the base of the mixed layer at 60–70 m depth. Phytoplankton were concentrated by gentle filtration onto polycarbonate filters of 20-μm porosity. Cells were then resuspended in 4 mL of 0.2-μm filtered seawater. The concentrate was divided in half for photosynthesis-irradiance (P-E) curves and oxygen flash yield (PSU-O_2) measurements. Photosynthesis-irradiance curves were determined at 4°C using a thermostated Hansatech oxygen electrode system (Model DW1), with a quartz-halogen lamp (Fibre-Lite, Cole-Palmer), and various neutral density screening. Changes in dissolved oxygen concentration were measured in 10 steps from 0 to 2,630 μmol photons m^{-2} s^{-1}. Incubations were short to reduce photoinhibition and were conducted at identical times each day to minimize diel effects. A hyperbolic tangent function fitted the P-E data. Oxygen flash yields were performed as described (Falkowski et al. 1981), except that a single xenon flash lamp (Oriel Corporation) was used to provide saturating light flashes. We used PSU-O_2 measurements to derive cellular concentrations of photosystem II reaction centers (fmol PSII $cell^{-1}$). The maximum steady-state rate of photosynthetic electron transport (τ, tau) was calculated by multiplying the reciprocal of PSU-O_2 by the chlorophyll-specific maximum photosynthetic rate (P^B_{max}) (Myers and Graham 1971).

Flavodoxin: Relative flavodoxin abundance in diatoms was quantified (LaRoche et al. 1996) in samples collected from the ship's pumped nontoxic seawater system at 5 m depth. Particulate material from 20 to 40 liters was concentrated by filtration and centrifugation and was then stored in liquid nitrogen until analysis. Flavodoxin abundance is normalized to protein concentrations and reported in relative units.

Sinking rate determinations: Sinking rates of phytoplankton >22 μm collected at 5 m depth were measured daily using Chl *a* as a biomass indicator and the modified SETCOL method (*see* Waite et al. 1992, and references therein). One-liter seawater samples were gently concentrated using a cylindrical 22-μm sieve inserted into the sample chamber such that the filtrate seeped into the cylinder center for removal.

Calculations: Intracellular Fe : C ratios for the >20-μm size-fraction phytoplankton based on rates of Fe uptake from Fe bound to strong organic complexes on day 12 and in situ growth rates: The rates of Fe uptake from organic complexes (mean Fe uptake rates from FeDFB and FeDFE for >20-μm size-fraction on day 12 = 55 ± 4 nmol Fe mol C^{-1} h^{-1}) yielded intracellular Fe : C ratios of 6.6 μmol Fe mol C^{-1} (6.6 μmol Fe mol C^{-1} = 55 ± 4.0 nmol Fe mol C^{-1} h^{-1} × 24 h d^{-1} ÷ 0.19 d^{-1} [the reported SOIREE algal growth rate on day 12 = 0.19 ± 0.020 d^{-1}, Boyd et al. 2000]). Total biological Fe demand to sustain the bloom for six weeks: The mean total volumetric FeDFB/FeDFE uptake rates on day 12 were 21 ± 1.0 × 10^{-14} mol Fe L^{-1} h^{-1}, and were used to calculate the total biological Fe demand to sustain the bloom for 6 weeks as observed from SeaWiFS (Abraham et al. 2000). The calculated biological Fe demand is 0.21 nM (0.21 nM = 21 × 10^{-14} mol Fe L^{-1} h^{-1} × 24 h d^{-1} × 42 d).

Results and discussion—The mixed layer ambient concentration of dissolved Fe ([DFe]) was 0.08 ± 0.03 nM (n = 10, Bowie et al. 2001) and was titrated with excess of Fe-binding organic ligands (3.6 ± 0.49, 2.7 ± 0.4, and 1.2 ± 0.11 nM [L] in water samples collected outside the fertilized patch in days 3, 7, and 11, respectively). The concentration of ligands in the iron-fertilized waters increased slightly between day 1 and day 10 (Fig. 1A). The collective, conditional stability constant of the organic ligands for Fe complexation ranged from log $K^{cond}_{Fe(III)L}$ = 20.8 ± 0.130 to 23.8 ± 3.91 (n = 13), similar to that reported in IronExII (Rue and Bruland 1997). Despite excess L, [DFe] decreased rapidly during SOIREE after infusions 1, 2, and 3 (Bowie et al. 2001). On the day immediately following each of these three Fe infusions, [DFe] had dropped to 0.27 ± 0.16 nM Fe (n = 112, Bowie et al. 2001) because of abiotic particle scavenging and horizontal dispersion of the enriched patch (Abraham et al. 2000; Bowie et al. 2001). After infusion four (days 7–8), a similar trend of a decrease in [DFe] was observed on day 10; however, 3 d later the concentration of [DFe] stabilized and remained relatively high (0.74 ± 0.43 nM Fe, n = 99, Bowie et al. 2001) compared to the ambient, unfertilized waters. Concurrently, a dramatic increase in [L] was observed after day 10 inside the patch (Fig. 1A), prolonging the residence time of dissolved Fe (DFe) and buffering all inorganic Fe (Fe′). The complexation of DFe by L resulted in an approximately sixfold decrease in [Fe′] inside the patch after infusion four, from 6.8 ± 1.0 to 1.1 ± 0.82 pM Fe′, on days 9 and 13, respectively.

Since Fe speciation—organic Fe (FeL) and inorganic Fe (Fe′) pools—changed significantly during SOIREE, our experiments investigating the rates of Fe uptake from inorganic Fe and Fe bound to strong organic ligands (DFE/DFB) provided insights into the ability of plankton to access these different forms of Fe. Inorganic forms of Fe are generally thought to be more bioavailable than organic forms because of their rapid reaction kinetics with Fe transporters of phytoplankton (Hudson and Morel 1990). However, when inorganic Fe concentrations are insufficient, Fe-limited phytoplankton are able to access organically bound Fe (Maldonado and Price 2001). The fastest rates of inorganic Fe uptake (V_{max}) were measured in the unfertilized waters and within the patch on day 1 (Fig. 1B). These rates were determined using high concentrations of Fe and thus represent maximal, saturated rates that are a direct function of the numbers of inorganic Fe transporters of the phytoplankton (Hudson and Morel 1990), and an inverse function of the cellular Fe status (Harrison and Morel 1986). The similarity between the rates from SOIREE (V_{max} = 10.9–153 μmol Fe mol C^{-1} h^{-1}) and those of Fe-limited phytoplankton in Subarctic Pacific HNLC waters (Maldonado and Price 1999) and controlled laboratory experiments (Maldonado and Price 2001) provides further evidence that the community was Fe-limited at the onset of SOIREE (Boyd et al. 2000). After infusion one, V_{max} gradually declined to 1/10 of its initial rate (Fig. 1B).

Iron uptake from strong organic complexes (FeDF) on day 1 was also similar inside and outside the patch and remarkably fast (Fig. 1C). Within 5 d the FeDF uptake rate declined twofold, indicating that Fe fertilization had partially alleviated the Fe stress. The fastest rates of Fe uptake from FeDF were observed in the Fe-enriched waters on day 12, around three times greater than those of plankton outside the patch (Fig. 1C). Such an increase in uptake from FeDF suggests a physiological adaptation of the phytoplankton to the fourfold higher [L] (Fig. 1A) and concurrent sixfold lower [Fe′]. Under these conditions, virtually all the Fe was strongly bound to L, which plankton can access by extracellular reduction (Allnutt and Bonner 1987; Maldonado and Price 1999, 2001) or by specific uptake of FeL (Granger and Price 1999). Since fast rates of Fe uptake from FeDF are the hallmark of Fe-stressed plankton (Granger and Price 1999; Maldonado and Price 2001), these results suggest that Fe was limiting initially as expected, but also after day 12 when [Fe′] had fallen to ~3 pM. Although [Fe′] was the lowest before the first Fe infusion (<1 pM), the rates of Fe uptake for FeDF were faster on day 12 than on day 1. This result may reflect the complexity of the mechanism of Fe acquisition from FeL by phytoplankton, which is determined by the [FeL], the free [L], the density of plasmalemma ferric reductases, and phytoplankton abundance (Maldonado and Price 2001). Although community composition changed during SOIREE, favoring diatoms over picophytoplankton and prymnesiophytes (Hall and Safi 2001; Gall et al. 2001), at present there is no published evidence suggesting that diatoms have a greater ability to acquire Fe from FeL than other types of plankton. Surprisingly, V_{max}, which is faster in Fe-limited than in Fe-replete phytoplankton cultures (Harrison and Morel 1986), was not enhanced on day 12. If biological reduction is the limiting step in the acquisition of Fe from FeL and is mediated by plasmalemma ferric reductases (Maldonado and Price 2001), then the number of inorganic Fe

transporters at the cell membrane may be restricted by the finite space available for transport sites (Hudson and Morel 1990) and reductases in the cell membrane.

The alleviation of algal Fe-limitation after Fe enrichment and its subsequent recurrence by days 12–13 is apparent from other measures of phytoplankton physiological performance. Sinking rates of diatoms, which have been reported to increase under Fe stress in lab cultures (Muggli et al. 1996), were roughly four times faster on days 1 and 12 than on day 8 (Fig. 1D). Flavodoxin concentration in diatoms, a proxy for Fe stress (LaRoche et al. 1996), was also the lowest between days 6 and 10, and the highest on days 3 and 13 (Fig. 1E). Although, based on a limited data set, temporal changes in tau, the rate of photosynthetic electron transport, and PSII, the concentration of photosystem II reaction centers, also followed a similar pattern (Fig. 1F). The maximum steady-state rate of tau was fastest on days 9–11 but was significantly slower by day 12. Similarly, the cellular concentration of Fe-rich PSII increased following Fe enrichment but decreased by day 12 (Fig. 1F). Further support for the recurrence of Fe stress by day 12 is provided by Fe : C uptake ratios, which approximate the cellular Fe content of the phytoplankton. The average ratio inside the patch was 2.7 $\pm$ 1.4 μmol Fe mol C^{-1} (Abraham et al. 2000) and the lowest ratio was measured on day 12 in the >20-μm sized phytoplankton (< 1 μmol Fe mol C^{-1}). Theoretical calculations and empirical observations indicate that values less than ~2 μmol Fe mol C^{-1} are severely rate limiting (Sunda and Huntsman 1997).

In contrast to changes in V_{max} (Fig. 1B), the half-saturation constant for Fe uptake (K_m) varied only twofold and was similar inside and outside the patch and for the three size-classes of plankton (Fig. 2). The calculated K_ms (3.3 $\pm$ 0.60, 2.6 $\pm$ 1.3, and 4.8 $\pm$ 1.4 nM Fe′ for the >20-, 2–20-, and 0.2–2-μm size-fractions, respectively) are comparable to those of temperate oceanic (1.2 nM Fe′, Maldonado and Price 2001) and coastal diatoms (3.1 nM Fe′, Hudson and Morel 1990). The similarity between K_m values for plankton from different oceanic regimes and of various taxa, size, and/or Fe nutrition suggests that K_m may simply be a function of the apparent kinetic constants for the formation and the dissociation of the Fe transporter-inorganic Fe complex and of the Fe internalization reactions (Hudson and Morel 1990; Morel et al. 1991). Such high K_m (~3.5 nM Fe′) relative to ambient [Fe′] (~ 1.1–6.8 pM) ensures that the Fe uptake system remains undersaturated at in situ [Fe′]. The remarkable convergence of the measured kinetic coefficients for Fe uptake by plankton from a range of oceanic regimes, derived from both lab culture and field populations, will facilitate the incorporation of detailed phytoplankton physiology in mathematical models of oceanic Fe biogeochemistry.

The half-saturation constants of Fe for phytoplankton growth ($K\mu$) (as opposed to uptake K_m) determined from shipboard experiments are 0.11 nM DFe in Pacific waters south of the Polar front (K. Coale, pers. comm.) and 0.12 nM DFe near the Equator (Coale et al. 1996*a*). Assuming that 99% of DFe in seawater is bound to strong organic ligands (Rue and Bruland 1995), these $K\mu$ values correspond to ~0.0012 nM Fe′. Thus, the half-saturation constant of Fe uptake for phytoplankton (K_m = ~4.0 nM Fe′, *see above*)

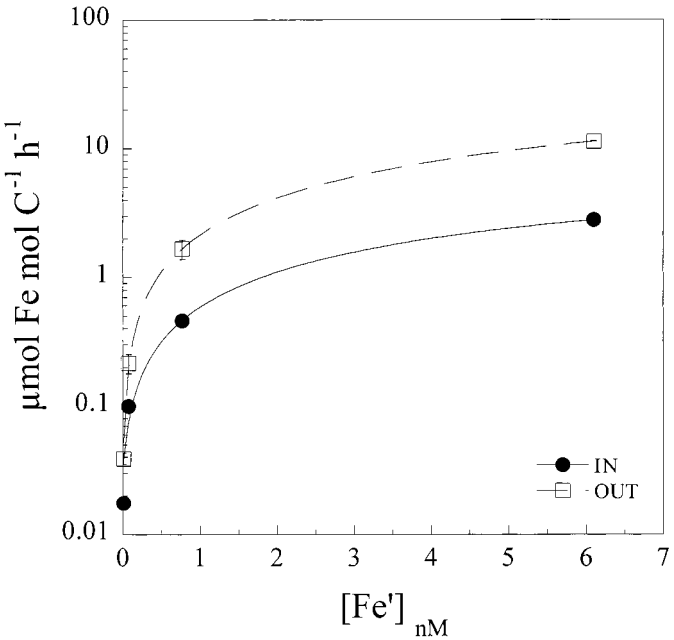

Fig. 2. Two inorganic Fe uptake kinetic curves shown as examples. Carbon-normalized Fe uptake rates (μmol Fe mol C^{-1} h^{-1}, mean $\pm$ range of uptake rates from duplicate incubation bottles) as a function of [Fe′] (nM), on day 12 for >20-μm size-fractions inside and outside the patch. Throughout the SOIREE experiment the mean K_ms were 3.3 $\pm$ 0.60, 2.6 $\pm$ 1.3, and 4.8 $\pm$ 1.4 nM Fe′ (n = 25) for the >20-, 2–20-, and 0.2–2-μm size-fraction plankton, respectively, and were indistinguishable outside and inside the patch.

is 3–4 orders of magnitude greater than that for growth (expressed as inorganic Fe concentration or Fe′, K_μ = ~ 0.0012 nM Fe′) providing the first field confirmation of the relationship between $K\mu$ and K_m reported for phytoplankton lab cultures ($K_m \sim 10^3 \times K\mu$, Harrison and Morel 1986; Morel et al. 1991). The difference in magnitude between $K\mu$ and K_m results from physiological adaptation that enables phytoplankton to grow optimally at low Fe by modulating both their intracellular Fe requirements and maximum short-term Fe uptake rates in response to ambient Fe levels (Morel et al. 1991).

The status of the SOIREE bloom on days 12 to 13 is puzzling, because evidence from five independent proxies (Fig. 1 and Fe : C uptake ratios) suggested that the phytoplankton were Fe stressed at this time even though total dissolved Fe concentrations remained relatively high compared to the ambient, unfertilized waters. Despite this apparent Fe stress, instantaneous rates of production were high (Boyd et al. 2000) and the bloom continued for more than 40 d (Abraham et al. 2000; Boyd et al. 2000) so limitation of algal growth by Fe was not prohibitive. The onset of algal Fe stress appears to have resulted from low [Fe′] in spite of high concentrations of DFe and seems to have been mediated by high [L]. Thus, the mechanisms that permit sustained algal growth for the following 42 d are not immediately obvious. The Fe uptake data, however, show enhanced rates of Fe uptake from FeL in response to high [FeL] on day 12. These rates, while slower than predicted if Fe were supplied

in an inorganic form (Hudson and Morel 1990; Maldonado and Price 2001), are sufficient to maintain intracellular Fe stores more than twofold higher than minimal algal requirements (Sunda and Huntsman 1997), allowing phytoplankton to grow at 0.19 d^{-1} (Boyd et al. 2000) (*see Methods calculations*), and the bloom to persist for 42 d (*see below*).

If the conditions observed inside the patch on day 13 persisted—i.e., high [FeL], slow abiotic loss rates of dissolved Fe (Abraham et al. 2000; Bowie et al. 2001), and moderate ability of phytoplankton to take up Fe from FeL and thus grow—the estimated biological dissolved Fe demand to sustain the bloom for 42 d is only 0.21 nM (*see Methods calculations*). This concentration is well below that remaining in the patch center at the end of the site-occupation (1.1 nM [DFe]) and is comparable to the calculated peak DFe concentration expected 42 d after our departure (0.20 nM [DFe]; Abraham et al. 2000). These data provide a physiological mechanistic explanation for the longevity of the SOIREE bloom observed from remote sensing (Abraham et al. 2000). In addition, lateral advection of phytoplankton as the patch increased in size, was the greatest algal loss term during SOIREE (Abraham et al. 2000), and is thought to be the most important physical mechanism accounting for the longevity of the bloom, as it minimizes cell aggregation and self-shading (Boyd et al. 2000; Abraham et al. 2000). Paradoxically, the ability of phytoplankton to acquire Fe from FeL at moderate, rather than maximal, rates may be partially responsible for the maintenance of the SOIREE bloom. Uptake of Fe at faster rates might result in rapid growth rates and the collapse and decline of the bloom due to nutrient limitation (Abraham et al. 2000), phytoplankton aggregation, and/or self-shading at elevated biomass levels (Boyd et al. 2000). Thus, physiological adaptation to changes in Fe speciation and the ability of phytoplankton to access different Fe pools have major implications for understanding the bloom dynamics in HNLC Southern Ocean waters. Important questions concerning the nature and production of the Fe-binding ligands remain to be answered.

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