Acquisition of iron bound to strong organic complexes, with different Fe binding groups and photochemical reactivities, by plankton communities in Fe-limited subantarctic waters

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[1] Though it is clear that plankton in oceanic regions access iron bound to strong organic ligands, the mechanism mediating the release of iron from these complexes remains unresolved. In this study, we aim to elucidate the mechanisms of organic iron acquisition by plankton in subantarctic waters. In particular, we investigated the importance of photochemistry in mediating the reductive dissociation of iron from organic complexes, using naturally occurring ligands, and model iron complexes, with different iron-binding groups and photoreactivities. Our results demonstrate that iron within the model ligands is available for uptake and growth by indigenous plankton, but that photolability of these complexes does not determine iron bioavailability. In contrast, light significantly enhances iron acquisition from the in situ ligands, suggesting that the in situ iron ligands are photolabile, and that photochemistry in surface waters may play a significant role in iron uptake from the dissolved organic iron pool by oceanic plankton.

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1. Introduction

[2] It is now apparent that insufficient iron (Fe) inputs limit phytoplankton growth and subsequently export of particulate organic carbon in 30% of the world's ocean [*Martin et al.*, 1994; *Coale et al.*, 1996; *Boyd et al.*, 2000; *Tsuda et al.*, 2003; *Boyd et al.*, 2004]. Of the three main Fe-limited regions (the equatorial and Subarctic Pacific, and the Southern Ocean), the Southern Ocean is believed to play a particularly important role in the global C cycle, by mediating the exchange of carbon between the atmosphere and the deep ocean [*Broecker and Henderson*, 1998; *Sarmiento et al.*, 1998]. In order to understand how Fe controls primary productivity in the Southern Ocean, it is essential to investigate how plankton in surface waters acquire this scarce micronutrient.

[3] The speciation of dissolved Fe in seawater is an important determinant of its bioavailability. The vast majority of dissolved Fe in surface ocean waters is bound to strong organic complexes of unknown origin [*Gledhill and van den Berg*, 1994; *Rue and Bruland*, 1995; *Wu and Luther*, 1995]. These complexes have extraordinarily high stability constants for Fe(III), and have been hypothesized to be siderophore-like molecules [*Rue and Bruland*, 1995; *Johnson et al.*, 1997]. Siderophores are Fe-specific, low molecular weight organic ligands, released by Fe-limited terrestrial and pathogenic bacteria and fungi to enhance Fe acquisition [*Raymond et al.*, 1984]. These complexing agents are essential for Fe acquisition by these microbes, which access the siderophore-bound Fe through a variety of dissociation mechanisms [*Byers and Arceneaux*, 1998]. Recently, organic Fe(III) ligands isolated from the California upwelling region have been shown to possess Fe-binding functional groups typical of siderophores [*Macrellis et al.*, 2001], further confirming the siderophore-like nature of the strong organic Fe(III) complexes in seawater.

[4] Similar to their terrestrial counterparts, many marine prokaryotes (heterotrophic and autotrophic bacteria) produce and/or excrete siderophores under Fe stress [*Wilhelm and Trick*, 1994; *Trick and Wilhelm*, 1995; *Granger and Price*, 1999; *Reid et al.*, 1993; *Haygood et al.*, 1993; *Martinez et al.*, 2000], and acquire siderophore-bound Fe to fulfill their Fe requirements [*Trick and Wilhelm*, 1995; *Granger and Price*, 1999]. It is believed that marine bacteria have high-affinity Fe acquisition systems specially designed to scavenge siderophore bound Fe, although the

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molecular mechanisms and genetic basis for such Fe uptake systems in marine prokayotes remains unresolved [*Granger and Price*, 1999; *Webb et al.*, 2001; *Armstrong et al.*, 2004].

[5] In contrast, only one study has thus far shown siderophore production by marine eukaryotic phytoplankton [*Trick et al.*, 1983]. Most models of Fe acquisition by eukaryotic phytoplankton emphasize the significance of inorganic Fe for uptake and growth, supporting the idea that the Fe transporters at the cell surface react with inorganic Fe species [*Hudson and Morel*, 1990; *Sunda and Huntsman*, 1995]. Only recently, the importance of organically bound Fe for the nutrition of marine eukaryotic phytoplankton has become apparent [*Hutchins et al.*, 1999; *Maldonado and Price*, 1999]. Eukaryotic phytoplankton have been shown to possess inducible reductases at the cell surface that mediate the reduction of organically bound Fe(III) and subsequent dissociation of Fe from the ligand [*Maldonado and Price*, 2001]. Once dissociation occurs, the cells internalize the inorganic Fe via inorganic Fe transporters at the cell surface. In addition to this enzymatic reductive mechanism, light has been shown to play an indirect role in Fe acquisition from marine siderophore-bound Fe, through the photooxidation of siderophores that bind Fe and the subsequent release of inorganic Fe [*Barbeau et al.*, 2001]. This light-mediated Fe dissociation from strong organic ligands may be disproportionally important in optically transparent waters of the open ocean, where light may penetrate down to the base of the surface mixed layer [*Barbeau et al.*, 2001].

[6] Though it is clear that bacteria (both heterotrophic and autotrophic) and eukaryotic phytoplankton in the open ocean are able to access Fe bound to strong organic ligands [*Hutchins et al.*, 1999; *Maldonado and Price*, 1999], the relative importance of photochemical [*Barbeau et al.*, 2001] versus biological [*Maldonado and Price*, 2001] reduction in mediating the release of Fe from strong organic complexes remains unresolved. In this study, using a variety of strong organic ligands with different photolabilities and chemical structures, we investigated the importance of photochemistry in mediating the reductive dissociation of Fe from strong organic ligands, in order to elucidate the mechanisms of Fe acquisition by plankton communities in the subantarctic Southern Ocean.

2. Materials and Methods

2.1. Study Area

[7] The FeCycle mesoscale project in the Southern Ocean was designed to investigate the biogeochemical cycling of Fe in unperturbed surface waters of a typical High Nutrient Low Chlorophyll (HNLC) oceanic region (P. W. Boyd et al., FeCycle: Attempting an iron biogeochemical budget from a mesoscale SF6 tracer experiment in unperturbed low iron waters, submitted to *Global Biogeochemical Cycles*, 2005) (hereinafter referred to as P. W. Boyd et al., submitted manuscript, 2005). Aboard the RV *Tangaroa*, from 28 January to 13 February 2003, we visited a suitable site in the HNLC subantarctic waters, located to the east of New Zealand in the vicinity of 46°30′S, 176°30′E (Boyd et al., submitted manuscript, 2005). This location was identified following a 3-day oceanographic survey prior to the FeCycle experiment. Once the experimental site was identified, a 47-km^2 surface water patch was labeled on 2 February 2003 with the chemically inert sulfur hexafluoride (SF_6) tracer, to ensure that a coherent body of water was the subject of the FeCycle study. However, no Fe was added to the FeCycle patch. The location and dimensions of the patch were tracked daily over the 10-day experiment using SF_6 underway mapping as described by Boyd et al. (submitted manuscript, 2005). Here 3 February 2003 was arbitrarily defined as Day 1 of FeCycle, and the last day was 12 February 2003 or Day 10 (R. M. L. McKay et al., The impact of phytoplankton on the biogeochemical cycling of iron in subantarctic waters southeast of New Zealand: Observations from the FeCycle study, submitted to *Global Biogeochemical Cycles*, 2005) (hereinafter referred to as McKay et al., submitted manuscript, 2005) (see also Boyd et al., submitted manuscript, 2005).

2.2. Water Collection

[8] Seawater samples for experiments were collected on 4, 6, and 9 February (at 0900 local time (LT), 0900 LT, and 0600 LT, respectively) at 5 m depth from the centre of the SF_6 labeled patch using a trace metal clean Teflon diaphragm pump system (see P. L. Croot et al., The effects of physical forcing on iron chemistry and speciation during the FeCycle experiment in the south west Pacific, submitted to *Global Biogeochemical Cycles*, 2005) (hereinafter referred to as Croot et al., submitted manuscript, 2005). The sampling depth corresponded to 30–40% of sea surface irradiance and was within the 45-m-deep surface mixed layer (Croot et al., submitted manuscript, 2005). The dissolved Fe concentration was consistently low throughout the mixed layer (<70 pmol L^{-1}) (Croot et al., submitted manuscript, 2005) typical of open ocean HNLC regions [*Johnson et al.*, 1997]. All experimental manipulations were carried out in a portable positive-pressure laboratory. Seawater used in the Fe uptake or grow-out experiments (see below) was not prescreened with netting to exclude grazers from the incubation bottles.

2.3. Biological and Chemical Analysis

[9] Samples for the determination of phytoplankton and bacterial biomass, as well as, phytoplankton species composition were taken immediately before the initiation of the experiments. Samples (25 mL) for microscopic phytoplankton enumeration and identification were preserved with 2% gluteraldehyde (final concentration). Size-fractionated (0.2–2, 2–20 and >20 μm) chlorophyll *a* (chl *a*) concentrations were determined by filtering 500 mL of seawater through a stack of Poretics® polycarbonate filters of varying porosity (0.2, 2 and 20 μm), separated with Millipore® drain disk filters. These polycarbonate filters were immediately frozen until further extraction. On board the vessel, the concentrations of chl *a* were determined fluorometrically with a Turner model 10 fluorometer [*Parsons et al.*, 1984] after extraction in 90% acetone for 24 hours at 0°C. For flow cytometric cell counts, an aliquot of seawater (200 mL) was filtered through a stack of Poretics® polycarbonate filters of varying porosity (0.2, 2 and 20 μm), separated with Millipore® drain disk filters. The cells collected on these

filters were then resuspended in 10 mL of 0.2-μm filtered seawater for analysis of *Synechococcus* and eukaryotic events using a flow cytometer [*Hall et al.*, 2004]. Heterotrophic bacteria were fluorescently labeled [*Hall et al.*, 2004] and counted using flow cytometry, a FACScan or FACSCalibur (15 mV air-cooled, argon-ion laser fixed at 488 nm) instrument (Becton Dickinson, Mountain View, California). The Fe status of the indigenous phytoplankton cells was established by measuring the photochemical quantum efficiency of photosystem II (F_v/F_m, where F_v is the difference between the maximum (F_m) and the minimum (F_o) chlorophyll fluorescence yield) in seawater samples before Fe additions, using fast repetition rate fluorometry (McKay et al., submitted manuscript, 2005).

2.4. Organically Bound Fe Complexes

[10] Six organic Fe complexes were chosen for this study: two synthetic metal chelators, ethylenediaminetetraacetic acid (EDTA) and diethyltriaminepentaacetic acid (DTPA), and four natural, strong organic Fe ligands: desferrioxamine B (DFB), rhodotorulic acid, (−) -gallocatechin, and phytic acid. Desferrioxamine B is a linear hexadentate trihydroxamic acid siderophore produced by species of the terrestrial bacteria *Streptomyces* and *Nocardia* [*Keller-Schierlein et al.*, 1965; *Mueller and Raymond*, 1984]. Rhodotorulic acid is a tetradentate linear dihydroxymate bacterial siderophore isolated from *Rhodotorula pilimanae* [*Atkin and Neilands*, 1968]. Gallocatechin, a major constituent of green tea, is a polyhydroxybenzene catechol derivative with a Fe:gallocatechin stoichiometry of 1:2 [*Jovanovic et al.*, 1998]. Phytic acid, an inositol hexaphosphoric acid, is a tridentate organic ligand, and a major phosphorus compound in plants [*Graf et al.*, 1987]. These Fe ligands were chosen to ensure a variety of Fe binding functionalities, including phosphate (phytic acid), hydroxymate (rhodotorulic acid, and DFB), catecholate (gallocatechin), and carboxylate groups (DTPA and EDTA). These model ligands (of the highest quality commercially available) were purchased from Sigma and were used without further purification.

2.5. Conditional Stability Constants of Organically Bound Fe Complexes

[11] Conditional stability constants for selected model ligands were analyzed by CLE-ACSV (competitive ligand equilibrium−adsorptive cathodic stripping voltammetry) using the competing ligand 2-(2-Thiazolylazo)-p-cresol (TAC) [*Croot and Johansson*, 2000] in artificial seawater, Aquil [*Price et al.*, 1989]. A Metrohm VA 663 Stand, with a hanging mercury drop electrode (HMDE), glassy carbon working electrode, Ag/AgCl/3M KCl reference electrode and Ecochemie μAutolab voltammeter was used. A EPPS buffer pH 7.9 was prepared according to *Croot and Johansson* [2000]. Iron-binding ligand titration proceeded as follows: 100 μL of 1M EPPS (pH 8) were added to 10 mL of Aquil, containing 15 nmol L^{-1} of the selected iron binding ligands (phytic acid, gallocatechin, DTPA, rhodotorulic acid and DFB), dispensed into a series of pre-cleaned Teflon bottles (60 mL). Iron was added to all but two of the bottles, yielding concentrations from 0 to 50 nmol L^{-1}. The added Fe was allowed to equilibrate with the selected ligands for 1 hour at laboratory temperature. At the end of this equilibration period, 10 μL of 10 mmol L^{-1} TAC was added to the sample and left to equilibrate overnight. The samples were then individually transferred to the Teflon cell cup of the electrode cell. After de-aeration of the sample for 3 min with dry nitrogen gas, the $Fe(TAC)_2$ complex was adsorbed while stirring the solution onto a fresh Hg drop at an accumulation potential of −0.40 V. After 1 min the stirrer was stopped and a differential pulse (DP) voltammogram was recorded from −0.38 V to −0.65 V at 0.0255 V s^{-1} (modulation amplitude: 0.05 V, interval time: 0.1 s and modulation time: 0.01 s). Samples were measured in the order of increasing Fe concentrations. All ligand titrations were analyzed with a single ligand model that was a nonlinear fit, a Langmuir adsorption isotherm [*Gerringa et al.*, 1995]. The theory of CLE-ACSV is described in detail by Croot et al. (submitted manuscript, 2005). Errors for the conditional stability constants were calculated using the NLREG software for nonlinear regression fits.

2.6. Photolability of Organically Bound Fe Complexes

[12] The photolability of the model organically bound Fe complexes was determined in the laboratory prior to the research cruise. Stock ligand solutions (2−10 mmol L^{-1}) were prepared in Milli-Q water, and were 0.2-μm-filter-sterilized after dissolution. The Fe was premixed with the ligand before addition to the synthetic ocean water, Aquil [*Price et al.*, 1989]. In the case of FeDFB, the Fe and the fungal siderophore were premixed as previously described [*Maldonado and Price*, 1999]. The seawater with the added Fe-ligand complex was allowed to equilibrate overnight. The final concentrations of Fe and ligand in seawater were 2 μmol L^{-1} and 20 μmol L^{-1}, respectively.

[13] The photolability of the model Fe ligand complexes was determined by measuring the light-dependent Fe(II) production rates, using the Fe(II) specific ligand, BPDS (batho-phenanthroline-di-sulphonate, extinction coefficient @ 535 nm = 22,140 mol L^{-1} cm^{-1} [*Blair and Diehl*, 1961]). The Fe-ligand enriched Aquil was dispensed in 50-mL trace metal cleaned polycarbonate bottles. Prior to the experiments, we determined spectrophotometrically that the polycarbonate bottles filter out most UV light (wavelengths < 350 nm, see section 4). At the beginning of the experiment 80 μmol L^{-1} BPDS was added to the Aquil containing the Fe-ligand complexes. The bottles were placed in a water bath equilibrated to ambient temperature (13°−14°C), and were either exposed to dark (control treatment) or natural sunlight. Every 1−2 hours an aliquot was removed from the bottles inside a dark, laminar flow hood, and the absorbance of Fe(II)BPDS was measured at 535 nm.

2.7. Rates of Fe Uptake From Organically Bound Fe

[14] The rates of Fe uptake by the indigenous plankton were determined three times during the cruise (on 4, 6, and 9 February of 2003), in 24−30 hours time course experiments using ^{55}Fe (79 mCi mg^{-1}, DuPont) [*Maldonado and Price*, 1999]. Seawater was dispensed in duplicate 2-L polycarbonate bottles that were capped and sealed with Parafilm, and

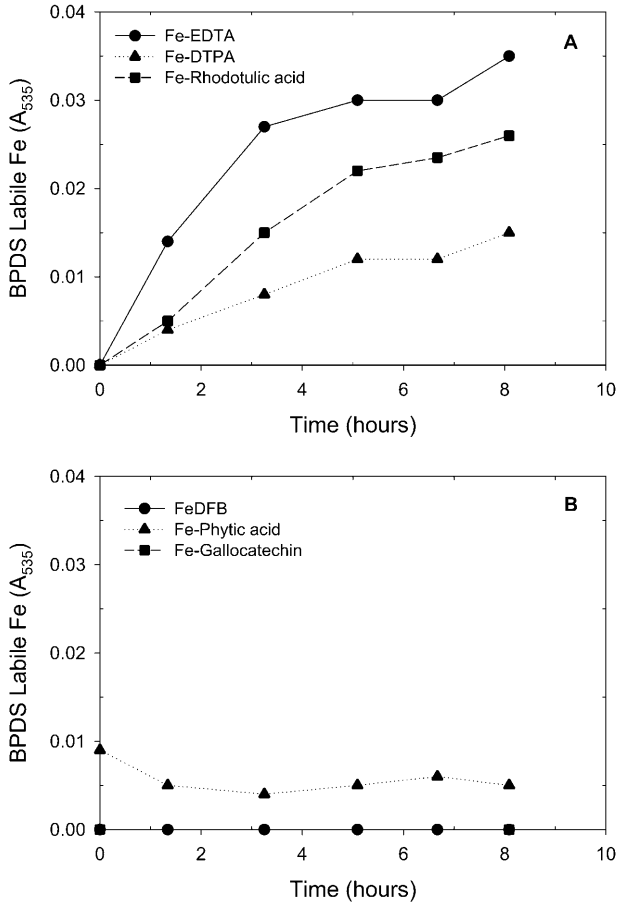

Figure 1. Kinetics of photochemically mediated Fe(III) reduction of a variety of organic Fe complexes, as determined from Fe(II)$BPDS_3$ formation (A_{535nm}). The reduction assay consisted of sterile, chelexed Aquil [*Price et al.*, 1989], enriched with 2 μmol L^{-1} Fe bound to 20 μmol L^{-1} L, and 80 μmol L^{-1} BPDS (see section 2). Duplicate bottles were placed in a water bath equilibrated to ambient temperature (13°–14°C), and were either exposed to dark (control) or natural sunlight. The reference treatment contained BPDS but no FeL. Absorbance of the reference treatment was subtracted from the absorbance of all control and experimental treatments. (a) Photolabile organically bound Fe complexes and (b) non-photolabile organically bound Fe complexes. Each data point represents the average (± range) of duplicate bottles. However, in all cases the range was smaller than the width of the symbol, and thus it is not apparent.

then placed in two plastic, Ziplock bags. Bottles were incubated in on-deck incubators at either in situ light levels (30–40% of sea surface irradiance) or in darkness (using heavy-duty black, plastic bags). In situ irradiance and temperature (~13.5°C) were controlled using neutral density screening and continuously flowing seawater (pumped from 5 m depth), respectively. Experiments were initiated by adding 2 nmol L^{-1} Fe bound to 20 nmol L^{-1} ligand. As described above, the Fe and organic ligand were premixed overnight before addition to seawater. An in situ ligand treatment was prepared by adding 2 nmol L^{-1} ^{55}Fe to natural seawater. To prevent precipitation of this inorganic Fe upon addition to seawater, the Fe was added as a FeEDTA complex (2 nmol L^{-1} Fe: 2.1 nmol L^{-1} EDTA). Given that the concentration of organic ligands in surface seawater during the FeCycle (1–2.7 nmol L^{-1} (Croot et al., submitted manuscript, 2005)) was comparable to that of added EDTA (2.1 nmol L^{-1} EDTA) and that the conditional stability constant of these natural ligands is orders of magnitude higher (log $K'_{Fe'L}$ = 11.7–12.4 M^{-1} (Croot et al., submitted manuscript, 2005)) than that of EDTA (log $K'_{Fe'L}$ = 7.3 M^{-1} [*Hudson et al.*, 1992]), we expect the added ^{55}Fe to equilibrate with in situ ligands within a couple of hours. Furthermore, the rates of Fe uptake in the in situ ligand treatments were linear for the duration of the experiment (see below), supporting our assumption that the equilibration of ^{55}Fe with the in situ ligands was achieved quickly after the Fe addition.

[15] During each experiment the bottles were sampled 3 times (approximately every 8 hours) inside a laminar flow hood. Approximately 500 mL were drawn from the bottles and filtered through a stack of Poretics® polycarbonate filters of 20, 2 and 0.2 μm porosity, separated with Millipore® drain disk filters. Before running dry, the filters were rinsed with 0.2 μm filtered Ti(III) citrate EDTA solution [*Hudson and Morel*, 1989] to dissolve any extracellular Fe, followed by a rinsed with 0.2 μm filtered seawater. Uptake rates were calculated by linear regression of particulate ^{55}Fe per ml against time.

[16] For the two larger size fractions (2–20 and >20 μm), volumetric Fe uptake rates were normalized to algal C biomass, using size-fractionated chl *a* and a C: chl *a* ratio of 60 g C: g chl *a* for S. Ocean HNLC waters in austral summer [*Gall et al.*, 2001]. For the picoplankton size fraction (0.2–2 μm), C biomass was calculated by summing the *Synechococcus*, heterotrophic bacterial, and the eukaryotic algal C biomass, determined by cell abundance (using flow cytometry) and published C conversion factors.

[17] The C conversion factors used for heterotrophic bacteria and *Synechococcus* were 12.4 fg C per cell [*Fukuda et al.*, 1998] and 250 fg C per cell [*Li et al.*, 1992], respectively. For eukaryotes in the 0.2–2 μm size fraction, a 220 fg C per μm^3 was used according to *Booth* [1988].

2.8. Grow-Out Experiment in the Presence of Strong Organic Fe Ligands

[18] In the final Fe uptake experiment on 9 February (starts at 0600 LT), the phytoplankton and heterotrophic bacteria remained in the presence of the organic Fe complexes for two additional days (harvest at 1300 LT on 12 February) in order to investigate the ability of indigenous phytoplankton in HNLC waters to grow in the presence of the organic Fe complexes used in this study. At the end of the experiment, we measured chl *a* concentrations as a proxy for phytoplankton biomass and determined the physiological state of the phytoplankton by measuring the photochemical quantum efficiency of photosystem II (F_v/F_m, see above).

3. Results

3.1. Characterization of Selected Organic Ligands

[19] The photolability of the selected ligands varied greatly (Figure 1). Three of the ligands, DFB, phytic acid

Table 1. Conditional Stability Constants of Model FeL Complexes Measured in the Artificial Seawater Medium, Aquil [*Price et al.*, 1989] at pH 8, Literature Values for Comparisons and Concentrations of Inorganic Iron Species (Fe′) Calculated on the Basis of Our K′ Values and Concentration Used in Fe Uptake Experiment Containing 2 nM FeL and 18 nM of Free Model Ligand[a]

Ligand	$\log K'_{FeL,Fe'}$	$\log K'_{FeL,Fe3+}$ (Calculated From $K'_{Fe'L}$) Using $\log \alpha_{in} = 10$[b]	Literature Values for $\log K'_{FeL,Fe'}$ ($\log K'_{FeL,Fe3+}$)	[Fe′]
Phytic acid	10.8 ± 0.2	20.8	9.3 (22.3)[c]	1.76 pM
Gallo- catechin	12.6 ± 0.8	22.6	(27.0)[d]	0.027 pM
DTPA	12.2 ± 0.3	22.2		0.070 pM
Rhodotorulic acid	12.2 ± 0.2	22.2		0.070 pM
DFB	11.8 ± 0.2	21.8	8.6 (21.6)[c] >13 (23)[e] 16.5 (26.5)[e,f] > 13.4 (23.4)[g]	0.176 pM
EDTA			7.3 (17.3)[b] (16.38)[h]	1.44 nM

[a]Errors given for $\log K'_{Fe'L}$ were calculated for the non-linear regression of the Gerringa plot [*Gerringa et al.*, 1995] using NLREG software.
[b]In Aquil at pH 8 [*Hudson et al.*, 1992] $\log K'_{Fe'L}$ for EDTA using kinetic approach.
[c]In UV'd SW [*Witter et al.*, 2000].
[d]In 15 mM phosphate buffer at pH 7 from *Jovanovic et al.* [1998].
[e]In UV'd SW [*Rue and Bruland*, 1995].
[f]*Schwarzenbach and Schwarzenbach* [1963].
[g]In UV'd SW at pH 8 [*Croot and Johansson*, 2000].
[h]Calculated for SW (salinity 35, pH 8) using the EXCEL worksheet Ion pairing model for seawater, written by C. M. G. van den Berg (available at http://www.liv.ac.uk/%7Esn35/Documents/Useful links.html).

and (−) gallocatechin, were not photolabile (Figure 1b) judging from the lack of light-stimulated Fe(III) reduction. In contrast, DTPA, EDTA and rhodotorulic acid were photochemically reactive (Figure 1a). The photochemical production of Fe(II) was fastest for EDTA, and slowest for DTPA. The conditional stability constants of these model ligands for Fe were determined with CLE-ACSV measurements at seawater pH, and in general, compared well with those of previous studies (Table 1).

3.2. Plankton Standing Stocks, Size Fractions, and Functional Groups

[20] The chl *a* levels on 4, 6, and 9 February averaged 0.59 ± 0.07 μg chl *a* L^{-1}, slightly higher than those previously observed in these HNLC subantarctic waters [*Boyd et al.*, 1999]. The majority of chl *a* was allocated either in the 0.2–2 μm (experiment 1 (4 February) and experiment 3 (9 February)) or 2–20 μm size fractions (experiment 2, 6 February) (Table 2). The >20-μm size fraction accounted for ∼20% of the total chl *a* (Table 2).

[21] The total plankton C in the mixed layer was nearly constant throughout FeCycle study, with an average of 9.47 ± 1.8 μmol C L^{-1} (Table 2). In two out of the three sampling dates (experiment 1, 4 February and experiment 3, 9 February), most of the total C (45% and 34%) was allocated in the two smallest size fractions (0.2–2 and 2–20 μm, respectively). During the second experiment (6 February), however, most of the C was associated with the 2–20 μm size fraction (57%), and only 14% with the 0.2–2 μm size fraction.

[22] The two larger size fractions (2–20 and >20 μm) were dominated by eukaryotic phytoplankton (Table 2). On average, eukaryotic phytoplankton C accounted for 92 ± 8% and 96 ± 1% of the total C in the 2–20 μm and the >20 μm size fractions, respectively (Table 2). The 2–20 μm size fraction was dominated by small flagellates (<5 μm), while the largest size fraction (>20 μm) was equally partitioned between diatoms (*Pseudo-nitzschia cf seriata* (>50 μm), and *Cylindrotheca closterium* (∼20 μm)) and dinoflagellates (*Pyrophacus sp.* and *Alexandrium sp.*) [*Leblanc et al.*, 2005; McKay et al., submitted manuscript, 2005]. In contrast, the smallest size fraction was dominated by heterotrophic and autotrophic bacteria, with eukaryotic C biomass accounting for <2% of the total C biomass in this fraction. In two of the experiments (4 and 9 February), the total C in the 0.2–2 μm size fraction was partitioned equally between the heterotrophic bacteria (50 ± 10%) and *Synechococcus* (48 ± 10%). However, in one of the experiments (6 February), the smallest size fraction was dominated by *Synechococcus*, accounting for 79% of the total C in the 0.2–2 μm size fraction (Table 2).

3.3. Acquisition of Fe From Strong Organic Complexes

[23] All the Fe uptake experiments were replicated 3 times during FeCycle (on 4, 6, and 9 February). As the results from all three experiments were in close agreement, we report the mean and standard deviation for these three independent Fe uptake experiments (Figures 2 and 3, Table 3). The rates of Fe uptake, in the dark and in the light, were constant, resulting in linear tracer accumulation over the entire duration of the experiments (24–30 hours).

[24] In general, plankton within each size fraction were able to acquire Fe from all of the FeL complexes provided (Table 3). The volumetric rates of Fe uptake ranged from 0.012 to 3.0 pmol Fe L^{-1} h^{-1}. The bacterial size fraction

Table 2. Size-Fractionated Chlorophyll *a* Concentrations (μg chl *a* L^{-1}) and Particulate Organic Carbon Concentrations (μmol C L^{-1}) in Seawater Samples Collected at 5 m in Depth for the Fe Uptake Experiments[a]

Experiment	Fraction, μg chl*a* L^{-1}	Total C, μmol C L^{-1}	Percent Bacterial C	Percent *Synecoccus* C	Percent Eukaryotic C
1					
0.2–2 μm	0.26	4.98	57	41	2
2–20 μm	0.14	3.68	0	5	95
>20 μm	0.11	2.06	0	5	95
Total	0.51	10.72			
2					
0.2–2 μm	0.22	1.06	21	79	0
2–20 μm	0.28	4.22	0	17	83
>20μm	0.11	2.1	0	4	96
Total	0.61	7.38			
3					
0.2–2 μm	0.19	4.62	43	55	0
2–20 μm	0.30	3.64	0	2	98
>20 μm	0.15	2.06	0	3	97
Total	0.64	10.32			

[a]Experiment 1, 4 February; experiment 2, 6 February; and experiment 3, 9 February 2003. The particulate organic carbon concentrations in the 2–20 and >20 μm size fractions were calculated using size-fractionated chlorophyll a (chl *a*) and C:chl *a* ratio of 60 [*Gall et al.*, 2001]. For the picoplankton size fraction (0.2–2 μm), C biomass was calculated by summing the *Synechoccocus*, the heterotrophic bacterial, and the eukaryotic C biomass (determined by cell abundance using flow cytometry) and using published C conversion factors (see section 2).

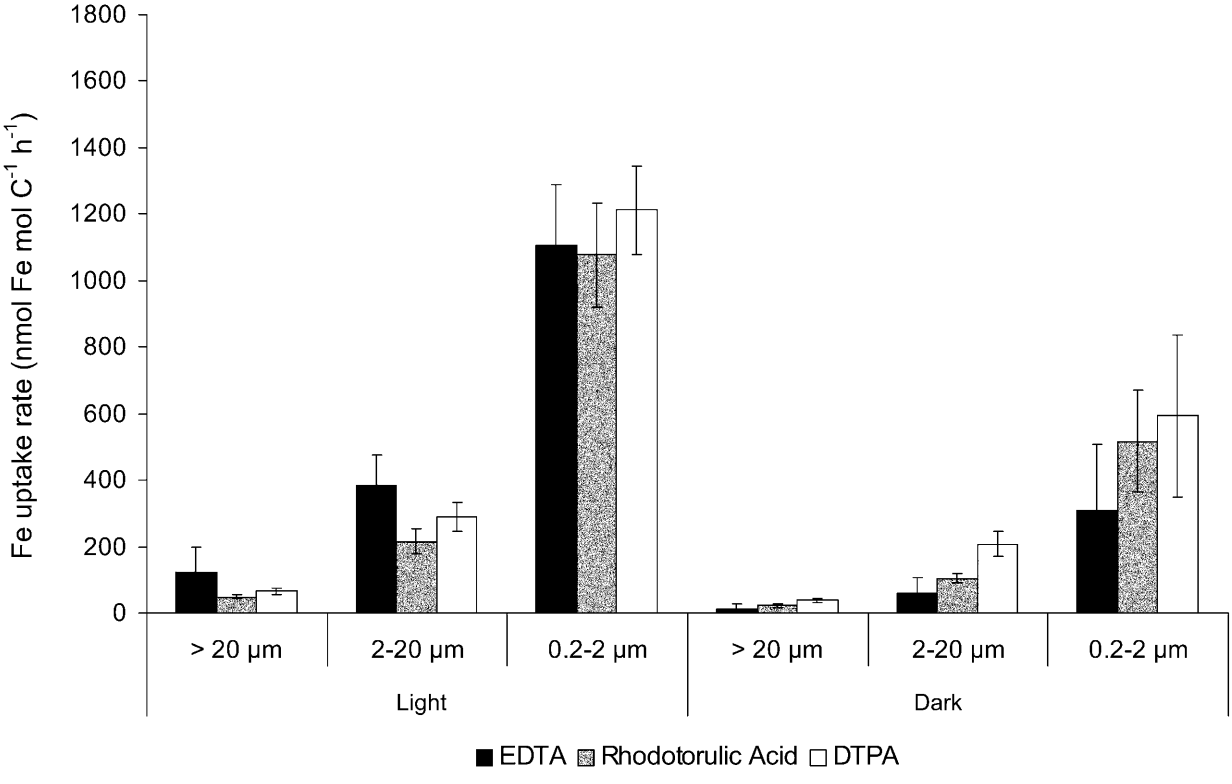

Figure 2. Carbon-normalized Fe uptake rates (nmol Fe mol C^{-1} h^{-1}) by size-fractionated plankton incubated at in situ light conditions or in complete darkness in the presence of various photolabile organic Fe complexes. Each short-term (24–30 hours) Fe uptake rate is the mean of three independent experiments on 4, 6, and 9 February 2003, and were determined in duplicate incubation bottles enriched with 2 nM ^{55}Fe complexed to 20 nM L. See color version of this figure at back of this issue.

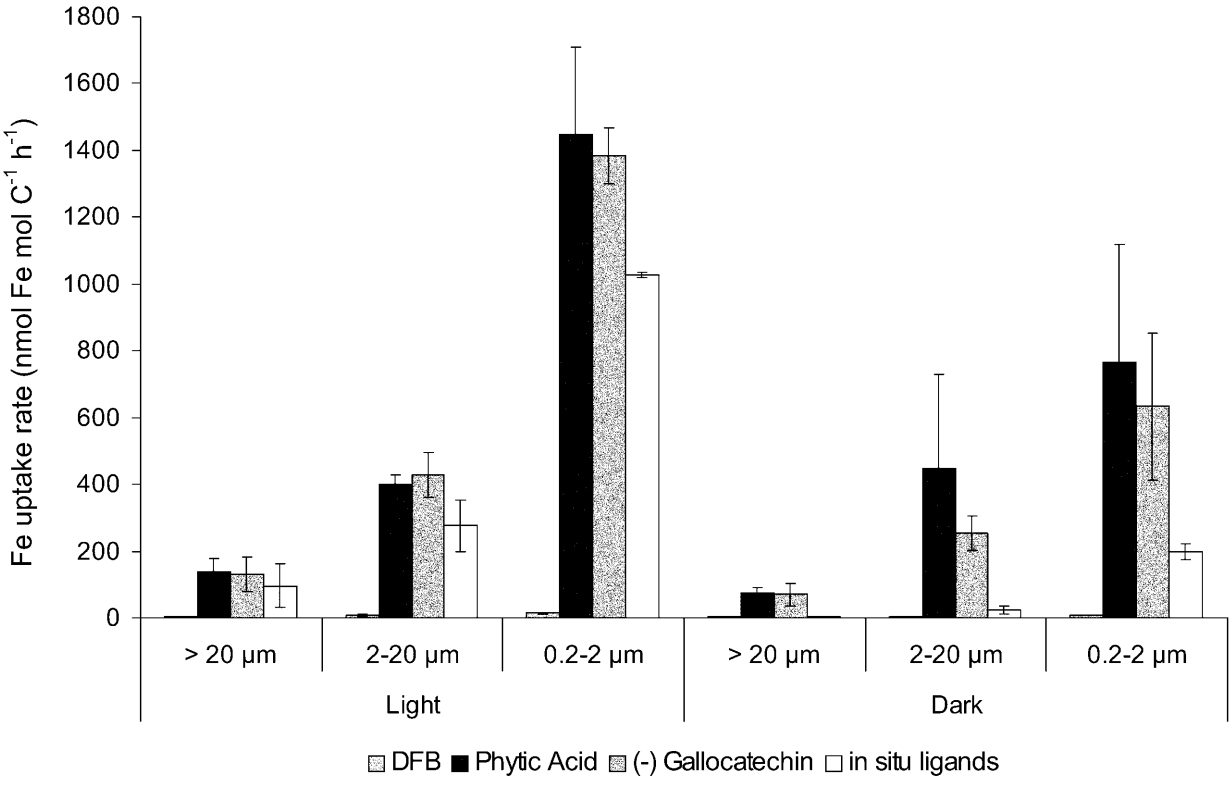

Figure 3. Carbon-normalized Fe uptake rates (nmol Fe mol C^{-1} h^{-1}) by size-fractionated plankton incubated at in situ light conditions or in complete darkness in the presence of various non-photolabile organic Fe complexes, and the in situ ligands. Each short-term (24–30 hours) Fe uptake rate is the mean of three independent experiments on 4, 6, and 9 February 2003, and was determined in duplicate incubation bottles enriched with 2 nmol L^{-1} ^{55}Fe complexed to 20 nmol L^{-1} L. The in situ ligand treatment was prepared by adding 2 nM ^{55}Fe, but no L. However, to prevent precipitation of this inorganic Fe upon addition to seawater, the Fe was added as a FeEDTA complex (2 nM Fe: 2.1 nM EDTA). See color version of this figure at back of this issue.

(0.2–2 μm) exhibited the fastest volumetric rates of Fe uptake, regardless of the Fe chelate added to seawater. After the volumetric rates were normalized to C biomass, the Fe uptake rates in the bacteria size fraction (0.2–2 μm) were still faster than those in either of the eukaryotic phytoplankton size fractions (2–20 μm and >20 μm). The slowest C-specific Fe uptake rates were measured for the >20 μm phytoplankton (Table 3). For a specific ligand-treatment, the Fe uptake rates were 3- (DFB, dark) to 57-fold (in situ ligands, dark) faster for the 0.2–2 μm size fraction than for the >20 μm size fraction.

[25] The order of preference of the organically bound Fe complexes as an Fe source for the plankton was similar for all size fractions. Iron bound to phytic acid was the preferred Fe complex for Fe acquisition, followed by gallocatechin, then EDTA or DTPA, rhodotorulic acid, in situ ligands and DFB (Table 3). Thus the rates of Fe uptake were indeed faster for the non-photolabile (phytic acid and gallocatechin; Figure 3) than for the photolabile ligands (EDTA, DTPA, and rhodotorulic acid; Figure 2 and Table 3). The exception to this trend was FeDFB; the rates of Fe uptake in the presence of this non-photolabile siderophore were the slowest of all the uptake experiments (Figure 3, Table 3).

[26] Regardless of the photolability of the Fe chelate, light enhanced the rates of Fe uptake from all Fe complexes. The C-specific Fe uptake rates were ~2 times (1.77 ± 0.36) faster in the presence of light than in darkness for all ligands, except EDTA and the in situ ligands (Figures 2 and 3, Table 3). The light enhancement for the EDTA and the in situ ligand treatment was even more pronounced, such that the rates of Fe uptake were on average 6.5 and 15 times faster in the light than in the dark treatment, respectively (Figures 2 and 3, Table 3).

3.4. Grow-Out Experiment in the Presence of Strong Organic Fe Ligands

[27] After 3 days of growth in the presence of organic Fe complexes, the photosynthetic competence (F_v/F_m) and biomass (μg chl *a* L^{-1}, Figure 4) of the indigenous phytoplankton had increased relative to their initial conditions for all ligands except DFB. The average F_v/F_m value for all the treatments, except for DFB, was 0.3 ± 0.01 at the end of the experiment, higher than both the initial value (0.19 ± 0.02), and the typical F_v/F_m values for this Fe-limited HNLC oceanic region [*Boyd et al.*, 1999]. In terms of biomass (μg chl *a* L^{-1}), the 2–20 and >20 μm size fractions responded

Table 3. Volumetric and Carbon-Normalized Fe Uptake Rates by Size-Fractionated Plankton Incubated at in Situ Light Conditions or in Complete Darkness in the Presence of Various Fe Complexing Ligands[a]

	EDTA	Rhodotorulic Acid	DTPA	DFB	Phytic Acid	(-) Gallocatechin	in Situ Ligands
				C-Normalized Fe Uptake Rate, nmol Fe mol C^{-1} h^{-1}			
Light							
>20 μm	122.4 ± 76	48.92 ± 6.71	66.67 ± 10.4	3.38 ± 0.34	137.4 ± 42.6	130.9 ± 50.9	95.49 ± 65.1
2–20 μm	384.97 ± 89.6	215.87 ± 38.4	290.55 ± 44	7.44 ± 2.5	398.8 ± 29.7	426.68 ± 67.1	277.43 ± 77.31
0.2–2 μm	1106.90 ± 180	1077.2 ± 157.3	1212.2 ± 133.8	15.56 ± 1.7	1445.7 ± 264.9	1383.9 ± 85	1025.78 ± 7.16
Dark							
> 20 μm	12.89 ± 12.9	23.14 ± 5.9	37.97 ± 6.2	2.47 ± 0.72	75.0 ± 15.8	69.69 ± 33.1	3.48 ± 1.27
2–20 μm	57.53 ± 50.8	104.01 ± 14.4	206.6 ± 37.9	5.10 ± 0.31	448.36 ± 279.6	253.47 ± 51.4	22.27 ± 11.7
0.2–2 μm	309.7 ± 196.0	517.35 ± 153.8	593.56 ± 243.5	8.24 ± 1.26	766.6 ± 351.8	632.52 ± 220	198.28 ± 22.2
				Volumetric Fe Uptake Rate, pmol Fe L^{-1} h^{-1}			
Light							
> 20 μm	0.58 ± 0.34	0.23 ± 0.04	0.318 ± 0.056	0.016 ± 0.002	0.65 ± 0.19	0.62 ± 0.22	0.45 ± 0.32
2–20 μm	1.47 ± 0.26	0.83 ± 0.13	1.11 ± 0.114	0.028 ± 0.008	1.54 ± 0.23	1.65 ± 0.32	1.01 ± 0.28
0.2–2 μm	2.29 ± 0.35	2.23 ± 0.31	2.51 ± 0.26	0.032 ± 0.003	3.0 ± 0.57	2.87 ± 0.21	2.11 ± 0.01
Dark							
>20 μm	0.06 ± 0.06	0.11 ± 0.027	0.18 ± 0.03	0.012 ± 0.003	0.36 ± 0.085	0.33 ± 0.15	0.017 ± 0.005
2–20 μm	0.1 ± 0.03	0.4 ± 0.07	0.8 ± 0.19	0.02 ± 0.002	1.69 ± 0.97	0.98 ± 0.23	0.09 ± 0.05
0.2–2 μm	0.65 ± 0.42	1.07 ± 0.322	1.23 ± 0.51	0.017 ± 0.003	1.59 ± 0.75	1.31 ± 0.47	0.41 ± 0.05

[a]Each short-term (24–30 hours) Fe uptake rate is the mean of three independent experiments on 4, 6, and 9 February 2003 and was determined in duplicate incubation bottles enriched with 2 nmol L^{-1} ^{55}Fe complexed to 20 nmol L^{-1} L. The in situ ligand treatment was prepared by adding 2 nmol L^{-1} ^{55}Fe, but no L. However, to prevent precipitation of this inorganic Fe upon addition to seawater, the Fe was added as a FeEDTA complex (2 nmol L^{-1} Fe: 2.1 nmol L^{-1} EDTA).

similarly to the additions of Fe complexes (Figure 4). The response of the phytoplankton > 20 μm was moderate, and on average μg chl *a* L^{-1} increased by approximately threefold. The greatest enhancement of biomass for the >20 μm phytoplankton was observed in the EDTA treatment, where chl *a* increased by 4.5-fold relative to the initial stocks. The phytoplankton biomass in the 0.2–2 and 2–20 μm fractions increased, on average, by 2.5-fold relative to the initials. Regardless of the size fraction, the phytoplankton showed the smallest increases in bio-

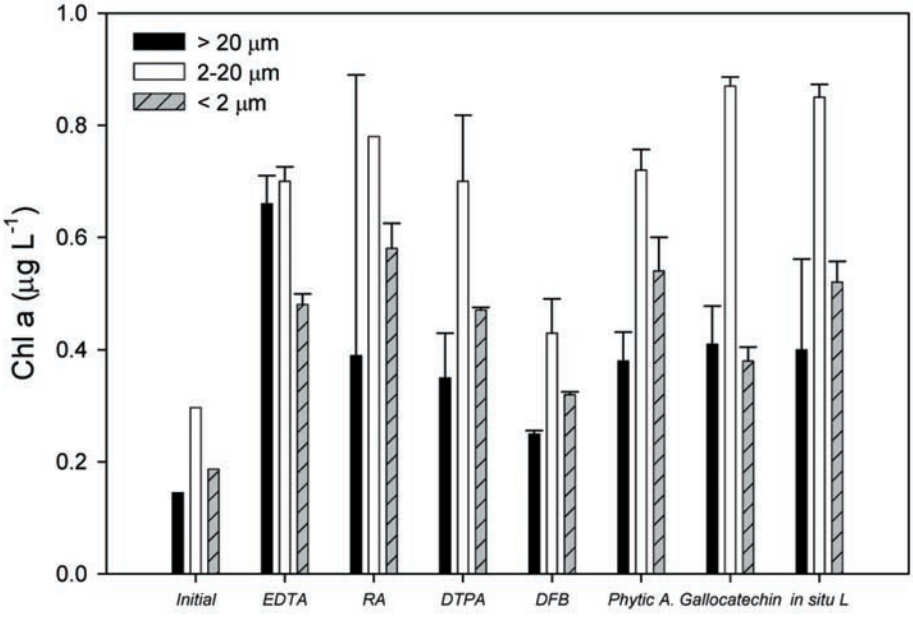

Figure 4. Size-fractionated chlorophyll *a* concentrations (μg chl *a* L^{-1}) at the beginning (0600 LT on 9 February 2003) and at the end (1300 LT on 12 February 2003) of the grow-out experiment (see section 2). The experiment was initiated by amending duplicate bottles with various FeL complexes, at concentrations of 2 nmol L^{-1} Fe and 20 nmol L^{-1} L. The bottles were immediately incubated at in situ light and temperature conditions. Each bar represents the average (± range) of duplicate bottles.

mass in the DFB treatment, with increases in chl *a* ranging from 1.45- to 1.72-fold (Figure 4). Although chl *a* concentrations increased slightly in the DFB treatment, F_v/F_m did not change (0.19 ± 0.02). We thus believe that the small increase in chl *a* levels in the DFB treatment was most likely due to an inadvertent "bottle effect," such as a decrease in grazing pressure.

4. Discussion

4.1. General Trends in Fe Acquisition

[28] The slowest volumetric rates of Fe uptake were observed in the presence of the fungal siderophore DFB, and the fastest in the presence of phytic acid. Neither of these Fe complexes is photolabile. As previously observed [*Tortell et al.*, 1996; *Maldonado and Price*, 1999], the bacterial size fraction was largely responsible for the majority of the total community Fe uptake, accounting for ~57% of the total volumetric Fe uptake rates in all of our experiments. The 2–20 μm and >20 μm fractions accounted for the remaining ~30% and 13%, respectively. The fast volumetric rates of Fe uptake by the bacteria were partly explained by their high C biomass, accounting for 45% of the total C biomass in two of the experiments and 15% of the total in the remaining experiment.

[29] When the volumetric rates were normalized to C biomass, the rates of Fe uptake were still fastest for the 0.2–2 μm size fraction and slowest for the >20 μm fraction. Within a specific ligand and treatment, the difference in C-specific Fe uptake rates between the largest and the smallest size fractions ranged from 3.3- to 57-fold, implying that the bacteria had a more efficient mechanism for acquisition of organically bound Fe, per unit C biomass. This may also reflect the higher Fe requirement of marine heterotrophic [*Tortell et al.*, 1996] and autotrophic bacteria [*Sañudo-Wilhelmy et al.*, 2001; *Brand*, 1991] relative to eukaryotic phytoplankton [*Brand*, 1991; *Sunda and Hunstman*, 1995]. Uptake rates for the eukaryotic phytoplankton ranged between 2.5 and 140 nmol Fe mol C^{-1} h^{-1}, and were in agreement with those measured in previous investigations of acquisition of Fe bound to strong organic ligands by Fe-limited phytoplankton in HNLC waters, including the subarctic NE Pacific (~88 nmol Fe mol C^{-1} h^{-1} [*Maldonado and Price*, 1999]) and the polar Southern Ocean (50 nmol Fe mol C^{-1} h^{-1} [*Maldonado et al.*, 2001]).

4.2. Fe Acquisition by the Bacterial Plankton Size Fraction (0.2–2 μm)

[30] In general, the total C biomass in the bacterial size fraction (0.2–2 μm) was partitioned equally between the heterotrophic and the autotrophic (*Synechococcus*) bacteria. Therefore the uptake of Fe in this size fraction reflects the ability of both functional groups to acquire organically bound Fe. This size fraction achieved the fastest rates of Fe uptake among all size fractions, ranging from 8 to 1400 nmol Fe mol C^{-1} h^{-1}. The fastest rates of Fe uptake by the bacteria (in the presence of phytic acid and gallocatechin) were 10 times faster than those measured previously with FeDFB in the subarctic NE Pacific [*Maldonado and Price*, 1999], and may thus reflect the difference in bioavailability of specific Fe siderophores/chelators. These fast Fe uptake rates from organically bound Fe do not indicate whether the bacterioplankton are accessing organic Fe directly or simply utilizing the inorganic Fe that is liberated from these complexes.

[31] Previous laboratory studies suggest that inorganic Fe acquisition by marine heterotrophic bacteria is not upregulated under Fe stress, and it is extremely inefficient compared to organic Fe acquisition [*Granger and Price*, 1999]. Iron limited heterotrophic bacteria, like those in the Southern Ocean [*Pakulski et al.*, 1996; *Arrieta et al.*, 2004], acquire Fe-siderophore complexes at very fast rates by either internalizing the Fe-siderophore complex or by an exchange reaction of the Fe between the Fe-siderophore and a transport ligand at the cell surface (see below) [*Granger and Price*, 1999]. When the Fe-siderophore is internalized as a complex, marine bacteria, like terrestrial and pathogenic bacteria [*Byers and Arceneaux*, 1998], are believed to mediate the dissociation of Fe from the siderophore intracellularly or in the periplasmic space. Physiological data indicates that similar siderophore-mediated high-affinity Fe transport systems occur in *Synechococcus* [*Wilhelm and Trick*, 1994; *Trick and Wilhelm*, 1995; *Webb et al.*, 2001]. It thus seems most likely that the rates of Fe uptake by heterotrophic and autotrophic bacteria (0.2–2 μm size fraction) in the Southern Ocean reflect their ability to acquire Fe bound to strong organic ligands directly.

4.3. Fe Acquisition by the Eukaryotic Plankton Size Fractions (2–20 and >20 μm)

[32] Thus far, eukaryotic phytoplankton have not been shown to internalize intact organic Fe complexes, and it is believed that these organisms possess Fe transporters at the cell surface that react specifically with inorganic Fe species [*Hudson and Morel*, 1990; *Sunda and Huntsman*, 1995]. However, when the concentrations of inorganic Fe are insufficient for growth, Fe-limited eukaryotic phytoplankton are able to access Fe bound within organic Fe complexes [*Soria-Dengg and Horstmann*, 1995; *Maldonado and Price*, 1999, 2001]. For our experiments, we calculated the rate of inorganic Fe supply to the cell surface in order to determine whether eukaryotic phytoplankton during FeCycle could meet their Fe uptake demand without accessing the Fe bound within the strong organic Fe complexes. Using DFB and EDTA as model ligands, we calculated the supply rate of inorganic Fe (Fe′) in seawater at the concentration of FeL added in the uptake experiments (Fe′ supply rate = $k'_d \times [FeL]$; where k'_d for FeEDTA = 1×10^{-6} s^{-1}, and k'_d for FeDFB = 63.25×10^{-12} s^{-1} [*Hudson et al.*, 1992; *Rue and Bruland*, 1995]). Our calculation indicates that the rate of inorganic Fe supplied by thermodynamic dissociation of Fe from FeDFB and FeEDTA is 0.00045 pmol Fe L^{-1} h^{-1} and 7.2 pmol Fe L^{-1} h^{-1}, respectively. In the case of FeEDTA, this rate of inorganic Fe dissociation is sufficient to supply inorganic Fe for the rates of Fe uptake by the eukaryotic phytoplankton (0.06–1.47 pmol Fe L^{-1} h^{-1}, Table 3). However, in the case of DFB, the inorganic Fe supply, as a result of thermodynamic dissociation, is too

slow to account for the measured rates of Fe uptake (0.012 and 0.03 pmol Fe L^{-1} h^{-1}, Table 3). Thus, in our Fe uptake experiments with the strongest organic Fe complexes, which have very slow dissociation rate constants, eukaryotic phytoplankton appear to access organically bound Fe.

[33] Two mechanisms have been proposed to allow phytoplankton to access the Fe within organic Fe complexes. Photoreduction and biologically mediated Fe(III) reduction enhance the rate of dissociation of Fe from strong organic complexes. These reduction processes may result in higher concentrations of inorganic Fe in the vicinity of the cell, and thus faster uptake rates. The results of the present study suggest that photochemistry is not the main mechanism mediating Fe acquisition from model Fe organic ligands by plankton in the Southern Ocean. The C-specific rates of Fe uptake for all size fractions were faster for the non-photolabile (phytic acid and gallocatechin) than for the photolabile Fe complexes (DTPA, EDTA, and rhodotorulic acid). The exception was FeDFB; the rates of Fe uptake in the presence of this non-photolabile siderophore were the slowest among all ligands. The order of preference of the organically bound Fe complexes was similar for all size fractions. Iron bound to the non-photolabile Fe complex phytic acid was the preferred FeL for Fe acquisition, followed by gallocatechin, EDTA or DTPA, rhodotorulic acid, and DFB. These results strongly suggest that photolability of the model Fe-complexes is not the most important mechanism liberating Fe from these organic Fe complexes.

[34] While the rates of Fe uptake from all ligands were faster in the presence of light than in darkness, we believe that this artifactual effect was due to the physiological state of the plankton in the dark treatment. The cells in this treatment were placed in the dark continuously for 24–30 hours. Under this condition, most likely, the growth rates of the phytoplankton declined slightly, their Fe demand was lower, and thus their Fe uptake rates slowed down. The observation that the rates of Fe uptake for all ligands, except EDTA, were ~2 times faster in the presence of light than in its absence, regardless of their photolability, further supports the effect of darkness on cell physiology. We thus believe that the light enhancement observed in the presence of the non-photolabile ligands (DFB, phytic acid, and gallocatechin), on average a twofold increase, was exclusively due to their physiological state in the dark treatment. Any additional light enhancement over and above this twofold increase may be due to photoreduction of the Fe complex. For example, in the EDTA treatment, the rates of Fe uptake were on average 6.5 times faster in the light than in the dark, respectively. Thus, in the presence of FeEDTA (a highly photolabile Fe complex), light-mediated reduction of organically bound Fe(III) may be an important pathway to increase the inorganic Fe pool available for Fe uptake [*Anderson and Morel*, 1982].

[35] Given that photolability of the different model organic Fe complexes did not control the bioavailability of Fe for eukaryotic plankton in the Southern Ocean, it seems likely that Fe acquisition of organically bound Fe was mediated by biological reduction at the cell surface. Unfortunately, rates of biological reduction of Fe were not determined during FeCycle. However, indirect evidence can be used to support the role of biological Fe reduction for acquisition of Fe from strong organic Fe complexes. Using previous field (NE subarctic Pacific) Fe(III) bioreduction rate data (13.6 μmol Fe mol C^{-1} h^{-1} at 0.1 μmol L^{-1} FeDF [*Maldonado and Price*, 1999]), we are able to compare the rates of supply of Fe by biological reduction and the rates of Fe uptake measured here. Note that the reduction rates of Fe(III) bound to weak (NTA, EDTA, and DTPA) and strong organic complexes (DFB and DFE) only vary by a factor of 2, like the reduction potentials of these Fe complexes [*Maldonado and Price*, 2001]. The reduction potentials of all the ligands used in the present study are between that of DFB (−468 mV [*Boukhalfa and Crumbliss*, 2002]) and that of EDTA (+94 mV [*Soria-Dengg and Horstmann*, 1995]), and thus the reduction rates of FeDF measured previously are probably applicable to all ligands used in our experiments. For this calculation we assumed that the Fe(III)DF reduction rates by plankton in the subarctic Pacific Ocean (summer waters T ~ 8°–12°C) are equivalent to those by plankton in the subantarctic Southern Ocean (summer waters T ~ 12°C), that the rates scale linearly with substrate concentration (from 2 to 100 nmol L^{-1} FeL [*Maldonado and Price*, 2001]), and that the average plankton C biomass in the 2–20 μm and >20 μm fractions is 3.5 and 2 μmol C L^{-1}, respectively (Table 2). Under these assumptions, the rates of Fe supply resulting from biologically mediated Fe reduction of organically bound Fe complexes would be 0.95 and 0.54 pmol Fe L^{-1} h^{-1} for the 2–20 μm and >20 μm size fraction, respectively. These supply rates are in close agreement with the rates of Fe uptake we measured in the presence of various Fe complexes. The rates of Fe uptake for the 2–20 μm fraction ranged from 0.028 to 1.65 pmol Fe L^{-1} h^{-1}, while those for the >20 μm fraction ranged from 0.016 to 0.62 pmol Fe L^{-1} h^{-1}. These calculations and comparisons suggest that extracellular enzymatic reduction of Fe(III) may be an important mechanism for Fe acquisition of organically bound Fe by eukaryotic phytoplankton in the Southern Ocean. Once the Fe(II) is reduced, the affinity of the ligand for the reduced Fe(II) will determine the rate of Fe dissociation, and thus Fe uptake. According to the affinity constants of the ligands for Fe(II), Fe dissociation will be faster for the gallocatechin ($\log K_{Fe(II)L} = 8.79$ M^{-1}, calculated using the equation: $E = E^o - 0.05916 * \log(K_{Fe(III)}/K_{Fe(II)})$, and E and $K_{Fe(III)L}$ values from *Jovanovic et al.* [1998]) than for the rhodotorulic acid and DFB complexes ($\log K_{Fe(II)L}$ = 10.6 M^{-1} and 10.3 M^{-1}, respectively [*Boukhalfa and Crumbliss*, 2002; *Cooper et al.*, 1978]). This trend is consistent with that observed for the rates of Fe uptake in the presence of these ligands during our experiments.

[36] Alternatively, if the uptake of organically bound Fe by phytoplankton and bacteria is mediated by the exchange of Fe(III) between ligands in seawater and specific Fe transporters at the cell surface, the denticity of the ligands (the number of donor groups from a given ligand attached to the same central Fe atom) will partially determine the bioavailability of the organically complexed Fe [*Boukhalfa and Crumbliss*, 2002]. According to the denticity of the ligand, Fe bound to DFB, a hexadentate siderophore, and

rhodotorulic acid (tetradentate) will be less available for exchange than other ligands of lower denticity, such as phytic acid (tridentate) and gallocatechin (bidentate). Indeed, in support of the role of denticity, the rates of Fe uptake from Fe bound to strong organic complexes were the fastest for phytic acid and gallocatechin, followed by rhodotorulic acid and DFB.

4.4. Bioavailability of Fe Bound to In Situ Organic Ligands

[37] This study shows that plankton in the subantarctic HNLC waters are able to access Fe bound to the in situ ligands in the dark, albeit at significantly slower rates than that bound to the model ligands (EDTA, DTPA, gallocatechin, rhodotorulic acid, and phytic acid). Interestingly, in the dark, the rates of algal Fe uptake by the >20 μm size fraction from the in situ Fe-ligand treatment were identical to those in the FeDFB treatment, suggesting that these in situ organic Fe-complexes may be similar to FeDFB. Indeed, the conditional stability constants of FeDFB (Table 1) and of the surface in situ ligands (Croot et al., submitted manuscript, 2005), during the FeCycle were indistinguishable (log $K'_{FeL,\ Fe'}$ = 11.8 ± 0.2 versus 11.8 ± 0.3, respectively). In contrast to FeDFB, however, these in situ Fe-ligands seem to be extremely photolabile, as light enhanced the rates of Fe uptake by 15-fold, on average, for all size fractions. This was the greatest light enhancement effect observed in all the Fe uptake experiments. The importance of light in enhancing Fe uptake from the in situ Fe-ligands suggests that some naturally occurring organic ligands are photolabile, and that light in the photic zone partially increases the rates of Fe uptake from the dissolved organic Fe pool. These findings are in agreement with those of recent studies on the photochemical degradation of organic iron complexes in coastal waters, which results in an increase of dissolved inorganic Fe concentrations [*Powell and Wilson-Finelli*, 2003].

[38] Two main mechanisms have been proposed to mediate photochemical reactions of organically bound Fe in the sea. The first mechanism is indirect, and involves chromophoric dissolved organic matter (CDOM) as a source of photochemical radicals (i.e. H_2O_2) for Fe(III) reduction. However, low CDOM concentrations and sunlight in Antarctic waters limit surface H_2O_2 levels [*Sarthou et al.*, 1997], and thus this indirect photoinduced Fe(III) reduction mechanism is not believed to be significant in HNLC regions of the Southern Ocean [*Rijkenberg et al.*, 2005]. The second photochemical reaction mechanism is direct, mediating a light-induced ligand-to-metal charge transfer reaction, and resulting in the reduction of Fe(III) and the partial oxidation of the ligand [*Barbeau et al.*, 2001, 2003]. This direct photochemical mechanism is most likely important in oligotrophic and/or Fe-limited regions, where CDOM concentrations are low and the vast majority of the dissolved Fe is bound to strong organic complexes.

[39] Even though little is know about the origin and nature of in situ Fe(III) ligands in seawater, these ligands have functional groups [*Macrellis et al.*, 2001], and Fe(III) conditional stability constants typical of siderophores [*Rue and Bruland*, 1995]. A recent study on the photolability of Fe(III) siderophore complexes produced by marine heterotrophic bacteria (Fe-aquachelin) indicates that the photolysis of Fe(III) aquachelin complexes leads to the formation of a lower affinity Fe(III) ligand and the reduction of Fe(III) [*Barbeau et al.*, 2001]. This photochemical Fe(III) reduction readily occurs in polycarbonate bottles exposed to natural sunlight, and increases the bioavailability of organically bound Fe to indigenous plankton in the Atlantic Ocean [*Barbeau et al.*, 2001]. As in the work of *Barbeau et al.* [2001], we used polycarbonate bottles, which filter out most UV wavelengths (<350 nm). Our results thus suggest that the photochemical reactions of the in situ Fe-ligands are mainly mediated by the visible portion of the light spectrum (400–700 nm) and to a lesser degree by UVA (320–400 nm). Given that during austral summer in these subantarctic waters, the visible portion of the light spectrum (FeCycle 1% PAR at 50 m) and UVA penetrate deeper (FeCycle 1% UVA at ~34 m) than UVB (280–320 nm, FeCycle 1% UVB at ~19 m) (Croot et al., submitted manuscript, 2005), wavelengths >350 nm would be more effective in mediating photochemical reactions of ferrated in situ ligands in the euphotic zone.

[40] The photochemical reaction in the Fe-aquachelin complex is mediated by the α-hydroxy acid moiety [*Barbeau et al.*, 2001, 2003], a functional group which is common in the marine siderophores characterized to date [*Reid et al.*, 1993; *Haygood et al.*, 1993; *Martinez et al.*, 2000]. Indeed, most of the siderophores produced by open ocean marine bacteria are mixed-moeity ligands, containing hydroxymate and catechol functional groups, as well as the α-hydroxy carboxylate moiety [*Reid et al.*, 1993; *Haygood et al.*, 1993; *Martinez et al.*, 2000; *Barbeau et al.*, 2001, 2003]. However, the α-hydroxy carboxylate moiety is the one that imparts photoreactivity to the ferrated, mixed, marine bacterial siderophores studied to date [*Barbeau et al.*, 2003]. The in situ Fe(III) ligands in the Southern Ocean may well be mixed-moeity siderophores, with hydroxymate or catecholate functional groups and the photoreactive α-hydroxy carboxylate moeity. The photolability of these in situ Fe-ligands may thus account for the considerably faster rates of Fe uptake in the light than in the dark. Interestingly, the concentrations of strong natural ligands during the FeCycle were slightly lower at shallow depths (~1 versus 2 nmol L^{-1} (Croot et al., submitted manuscript, 2005)), perhaps indicating photodegradation of these ligands in the upper water column.

5. Conclusion

[41] At present we cannot unambiguously establish the mechanism of Fe acquisition of organically bound Fe by phytoplankton and bacteria at the FeCycle site in the Southern Ocean. However, the data presented here do suggest that photolability of model organic Fe complexes does not necessarily determine the Fe bioavailability to this indigenous plankton community. It seems that bioreduction of organically bound Fe may be an important mechanism to enhance the dissociation of Fe from the model ligands and therefore, the rates of Fe uptake by eukaryotic phytoplankton. However, other mechanisms, such as exchange of Fe(III) between ligands in seawater and specific Fe trans-

porters at the cell surface, deserve further examination. In contrast, light significantly increases Fe uptake from the in situ Fe-ligands, suggesting that the ferrated in situ ligands are photolabile, and may contain a photoreactive α-hydroxy carboxylate moiety. Thus photochemistry in surface waters of this subantarctic region may play a significant role in Fe uptake by plankton from the dissolved organic Fe pool.

[42] **Acknowledgments.** We especially thank Julie Hall and Karl Safi for cell abundances of heterotrophic bacteria, picoeukaryotic phytoplankton, and *Synechococcus*. Philippe Tortell provided insightful comments during the preparation of the manuscript. We are grateful to the captain and crew of the R/V *Tangaroa* for help during seawater sampling. This work was supported by the National Sciences and Research Council of Canada (NSERC) and by the Center for Environmental BioInorganic Chemistry (CEBIC, funded by National Science Foundation) in Princeton.

References

Anderson, M. A., and F. M. M. Morel (1982), The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira weissflogii*, *Limnol. Oceanogr.*, *27*(5), 789–813.

Armstrong, E., J. Granger, E. L. Mann, and N. M. Price (2004), Outer-membrane siderophore receptors of heterotrophic oceanic bacteria, *Limnol. Oceanogr.*, *49*(2), 579–587.

Arrieta, J. M., M. G. Weinbauer, C. Lute, and G. J. Herndl (2004), Response of bacterioplankton to iron fertilization in the Southern Ocean, *Limnol. Oceanogr.*, *49*(3), 799–808.

Atkin, C. L., and J. B. Neilands (1968), Rhodotorulic acid, a dike-topiperazine dihydroxamic acid with growth-factor activity: I. Isolation and characterization, *Biochemistry*, *7*, 3734–3739.

Barbeau, K., E. L. Rue, K. W. Bruland, and A. Butler (2001), Photochemical cycling of iron in the surface ocean mediated by microbial iron (III)-binding ligands, *Nature*, *413*, 409–413.

Barbeau, K., E. L. Rue, C. G. Trick, K. W. Bruland, and A. Butler (2003), Photochemical reactivity of siderophores produced by marine heterotrophic bacteria and cyanobacteria based on characteristic Fe (III) binding groups, *Limnol. Oceanogr.*, *48*(3), 1069–1078.

Blair, D., and H. Diehl (1961), Bathophenanthrolinedisulphonic acid and bathocuproinedisulphonic acid, water soluble reagents for iron and copper, *Talanta*, *7*, 163–174.

Booth, B. (1988), Size classes and major taxonomic groups at two locations in the subarctic Pacific Ocean in May and August 1984, *Mar. Biol.*, *97*, 275–286.

Boukhalfa, H., and A. L. Crumbliss (2002), Chemical aspects of siderophore mediated iron transport, *BioMetals*, *15*, 325–339.

Boyd, P. W., J. LaRoche, M. Gall, R. Frew, and R. M. L. McKay (1999), The role of iron, light, and silicate in controlling algal biomass in subAntarctic waters SE of New Zealand, *J. Geophys. Res.*, *104*(C6), 13,395–13,408.

Boyd, P. W., et al. (2000), A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization, *Nature*, *407*, 695–702.

Boyd, P. W., et al. (2004), The decline and fate of an iron-induced subarctic phytoplankton bloom, *Nature*, *428*, 549–553.

Brand, L. E. (1991), Minimum iron requirements of marine phytoplankton and the implications for the biogeochemical control of new production, *Limnol. Oceanogr.*, *36*(8), 1756–1771.

Broecker, W. S., and G. M. Henderson (1998), The sequence of events surrounding Termination II and their implications for the causes of glacial-interglacial CO_2 changes, *Paleoceanography*, *13*, 352–364.

Byers, B. R., and J. E. L. Arceneaux (1998), Microbial iron transport: Iron acquisition by pathogenic microorganisms, *Iron Transp. Storage Microorg. Plants Animals*, *35*, 37–66.

Coale, K. H., et al. (1996), A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean, *Nature*, *383*, 495–501.

Cooper, S. R., J. V. McArdle, and K. N. Raymond (1978), Siderophore electrochemistry: Relation to intracellular iron release mechanism, *Proc. Natl. Acad. Sci. U.S.A.*, *75*(8), 3551–3554.

Croot, P. L., and M. Johansson (2000), Determination of iron speciation by cathodic stripping voltammetry in seawater using the competing ligand 2-(2-Thiazolylazo)-p-cresol (TAC), *Electroanalysis*, *12*(8), 565–576.

Fukuda, R., H. Ogawa, T. Nagata, and I. Koike (1998), Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments, *Appl. Environ. Microbiol.*., *64*, 3352–3358.

Gall, M., P. W. Boyd, J. Hall, K. Safi, and H. Chang (2001), Phytoplankton processes: Part 1. Community structure during the Southern Ocean Iron RElease Experiment (SOIREE), *Deep Sea Res., Part II*, *48*, 2551–2570.

Gerringa, L. J. A., P. M. J. Herman, and T. C. W. Poortvliet (1995), Comparison of the linear Van den Berg/Ruzic transformation and a non-linear fit of the Langmuir isotherm applied to Cu speciation data in the estuarine environment, *Mar. Chem.*, *48*, 131–142.

Gledhill, M., and C. M. G. van den Berg (1994), Determination of complexation of iron (III) with natural organic complexing ligands in seawater using cathodic stripping voltammetry, *Mar. Chem.*, *47*, 41–54.

Graf, E., K. L. Empson, and J. W. Eaton (1987), Phytic acid: A natural antioxidant, *J. Biol. Chem.*, *262*(24), 11,647–11,650.

Granger, J., and N. M. Price (1999), The importance of siderophores in iron nutrition of heterotrophic marine bacteria, *Limnol. Oceanogr.*, *44*(3), 541–555.

Hall, J., K. Safi, and A. Cumming (2004), Role of microzooplankton grazers in the subtropical and subantarctic waters to the east of New Zealand, *N. Z. J. Mar. Freshwater Res.*, *38*, 91–101.

Haygood, M. G., P. D. Holt, and A. Butler (1993), Aerobactin production by a planktonic marine *Vibrio sp.*, *Limnol. Oceanogr.*, *38*(5), 1091–1097.

Hudson, R., and F. Morel (1989), Distinguishing between extra- and intracellular iron in marine phytoplankton, *Limnol. Oceanogr.*, *34*(6), 1113–1120.

Hudson, R. J. M., and F. M. M. Morel (1990), Iron transport in marine phytoplankton: Kinetics of cellular and medium coordination reactions, *Limnol. Oceanogr.*, *35*(5), 1002–1020.

Hudson, R. J. M., D. T. Covault, and F. M. M. Morel (1992), Investigations of iron coordination and redox reactions in seawater using ^{59}Fe radiometry and ion-pair solvent extraction of amphiphilic iron complexes, *Mar. Chem.*, *38*, 209–235.

Hutchins, D. A., A. E. Witter, A. Butler, and G. W. Luther (1999), Competition among marine phytoplankton for different chelated iron species, *Nature*, *400*, 858–861.

Johnson, K. S., R. M. Gordon, and K. H. Coale (1997), What controls dissolved iron concentrations in the world ocean?, *Mar. Chem.*, *57*, 137–161.

Jovanovic, S. V., M. G. Simic, S. Steenken, and Y. Hara (1998), Iron complexes of gallacatechins: Antioxidant action or iron regulation?, *J. Chem. Soc. Perkin Trans.*, *2*, 2365–2369.

Keller-Schierlein, W., V. Mertens, V. Prelog, and A. Walser (1965), Stoffwechseklprodukte von Actinomyceten. Die Ferrioxamine A1, A2 und D2, *Helv. Chim. Acta*, *48*, 710–722.

Leblanc, K., C. E. Hare, P. W. Boyd, K. W. Bruland, B. Sohst, S. Pickmere, M. C. Lohan, K. Buck, M. Ellwood, and D. A. Hutchins (2005), Fe and Zn effects on the Si cycle and diatom community structure in two contrasting high and low-silicate HNLC areas, *Deep Sea Res.*, in press.

Li, W. K. W., P. N. Dickie, B. D. Irwin, and A. M. Wood (1992), Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea, *Deep Sea Res.*, *39*, 501–519.

Macrellis, H. M., C. G. Trick, E. L. Rue, G. Smith, and K. W. Bruland (2001), Collection and detection of natural iron-binding ligands from seawater, *Mar. Chem.*, *76*, 175–187.

Maldonado, M. T., and N. M. Price (1999), Utilization of iron bound to strong organic ligands by plankton communities in the subarctic Pacific Ocean, *Deep Sea Res., Part II*, *46*, 2447–2473.

Maldonado, M. T., and N. M. Price (2001), Reduction and transport of organically bound iron by *Thalassiosira oceanica* (Bacillariophyceae), *J. Phycol.*, *37*, 298–309.

Maldonado, M. T., P. W. Boyd, R. Strzepek, J. L. LaRoche, A. Waite, P. L. Croot, and N. M. Price (2001), Phytoplankton physiological responses to changing iron chemistry during a mesoscale Southern Ocean iron-enrichment, *Limnol. Oceanogr.*, *46*(7), 1802–1808.

Martin, J. H., et al. (1994), Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean, *Nature*, *371*, 123–129.

Martinez, J. S., G. P. Zhong, P. D. Holt, H.-T. Jung, C. J. Carrano, M. G. Haygood, and A. Butler (2000), Self-assembling amphiphilic siderophores from marine bacteria, *Science*, *287*, 245–247.

Mueller, G., and K. N. Raymond (1984), Specificity and mechanism of ferrioxamine-mediated iron transport in *Streptomyces pilosus*, *J. Bacteriol.*, *160*, 304–312.

Pakulski, J. D., R. B. Coffin, C. A. Kelley, S. L. Holder, R. Downer, P. Aas, M. M. Lyons, and W. H. Jeffrey (1996), Iron stimulation of Antarctic bacteria, *Nature*, *383*, 133–134.

Parsons, T. R., Y. Maita, and C. M. Lalli (1984), *A Manual of Chemical and Biological Methods for Seawater Analysis*, Elsevier, New York.

Powell, R. T., and A. Wilson-Finelli (2003), Photochemical degradation of organic iron complexing ligands in seawater, *Aquat. Sci.*, *65*, 367–374.

Price, N. M., G. Harrison, J. Hering, R. J. Hudson, P. M. V. Nirel, B. Palenick, and F. M. M. Morel (1989), Preparation and chemistry of the artificial algal culture medium Aquil, *Biol. Oceanogr.*, *6*, 443–461.

Raymond, K. N., G. Muller, and B. F. Matzanke (1984), Complexation of iron by siderophores, *Top. Curr. Chem.*, *123*, 49–102.

Reid, R. T., D. H. Live, D. J. Faulkner, and A. Butler (1993), A siderophore from a marine bacterium with an exceptional ferric ion affinity constant, *Nature*, *366*, 455–458.

Rijkenberg, M. J. A., A. C. Fischer, J. J. Kroon, L. J. A. Gerringa, K. R. Timmermans, H. T. Wolterbeek, and H. J. W. de Baar (2005), The influence of UV irradiation on the photoreduction of iron in the Southern Ocean, *Mar. Chem.*, *93*, 119–129.

Rue, E. L., and K. W. Bruland (1995), Complexation of Fe (III) by natural organic ligands in the central North Pacific as determined by a new competitive ligand equilibration/absorptive cathodic stripping voltammetric method, *Mar. Chem.*, *50*, 117–138.

Sañudo-Wilhelmy, S. A., A. B. Kustka, C. J. Gobler, D. A. Hutchins, M. Yang, K. Lwiza, J. Burns, D. G. Capone, J. A. Raven, and E. J. Carpenter (2001), Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean, *Nature*, *411*, 66–69.

Sarmiento, J. L., T. M. C. Hughes, R. J. Stouffer, and S. Manabe (1998), Simulated response of the ocean carbon cycle to anthropogenic climate warming, *Nature*, *393*, 245–249.

Sarthou, G., C. Jeandel, L. Brisset, D. Amouroux, T. Besson, and O. F. X. Donard (1997), Fe and H_2O_2 distributions in the upper water column in the Indian sector of the Southern Ocean, *Earth Planet. Sci. Lett.*, *147*, 83–92.

Schwarzenbach, G., and K. Schwarzenbach (1963), Hydroxamatkomplexe: 1. Die stabilitat der eisen (III)-komplexe einfacher hydroxamsauren und des ferrioxamins B, *Helv. Chim. Acta*, *46*, 1390.

Soria-Dengg, S., and U. Horstmann (1995), Ferroxiamines B and E as iron source for the marine diatom *Phaeodactylum tricornutum*, *Mar. Ecol. Prog. Ser.*, *127*, 269–277.

Sunda, W. G., and S. A. Huntsman (1995), Iron uptake and growth limitation in oceanic and coastal phytoplankton, *Mar. Chem.*, *50*, 189–206.

Tortell, P. D., M. T. Maldonado, and N. M. Price (1996), The role of heterotrophic bacteria in iron-limited ocean ecosystems, *Nature*, *383*, 330–332.

Trick, C. G., and S. W. Wilhelm (1995), Physiological changes in the coastal marine cyanobacterium *Synechococcus sp.* PCC 7002 exposed to low ferric ion levels, *Mar. Chem.*, *50*, 207–217.

Trick, C., et al. (1983), Examination of hydroxamate-siderophore production by neritic eukaryotic marine phytoplankton, *Mar. Biol.*, *75*, 9–17.

Tsuda, A., et al. (2003), A mesoscale iron enrichment in the western subarctic Pacific induces a large centric diatom bloom, *Science*, *300*, 958–961.

Webb, E. A., J. W. Moffett, and J. B. Waterbury (2001), Iron stress in open-ocean cyanobacteria (*Synechococcus*, *Trichodesmium* and *Crocosphaera* spp.): Identification of the *IdiA* protein, *Appl. Environ. Microbiol.*, *67*, 5444–5452.

Wilhelm, S. W., and C. G. Trick (1994), Iron-limited growth of cyanobacteria: Multiple siderophore production is a common response, *Limnol. Oceanogr.*, *39*(8), 1979–1984.

Witter, A. E., D. A. Hutchins, A. Butler, and G. W. Luther III (2000), Determination of conditional stability constants and kinetic constants for strong model Fe-binding ligands in seawater, *Mar. Chem.*, *69*, 1–17.

Wu, J., and G. W. Luther (1995), Complexation of Fe (III) by natural organic ligands in the northwest Atlantic Ocean by a competitive ligand equilibrium method and a kinetic approach, *Mar. Chem.*, *50*, 159–177.

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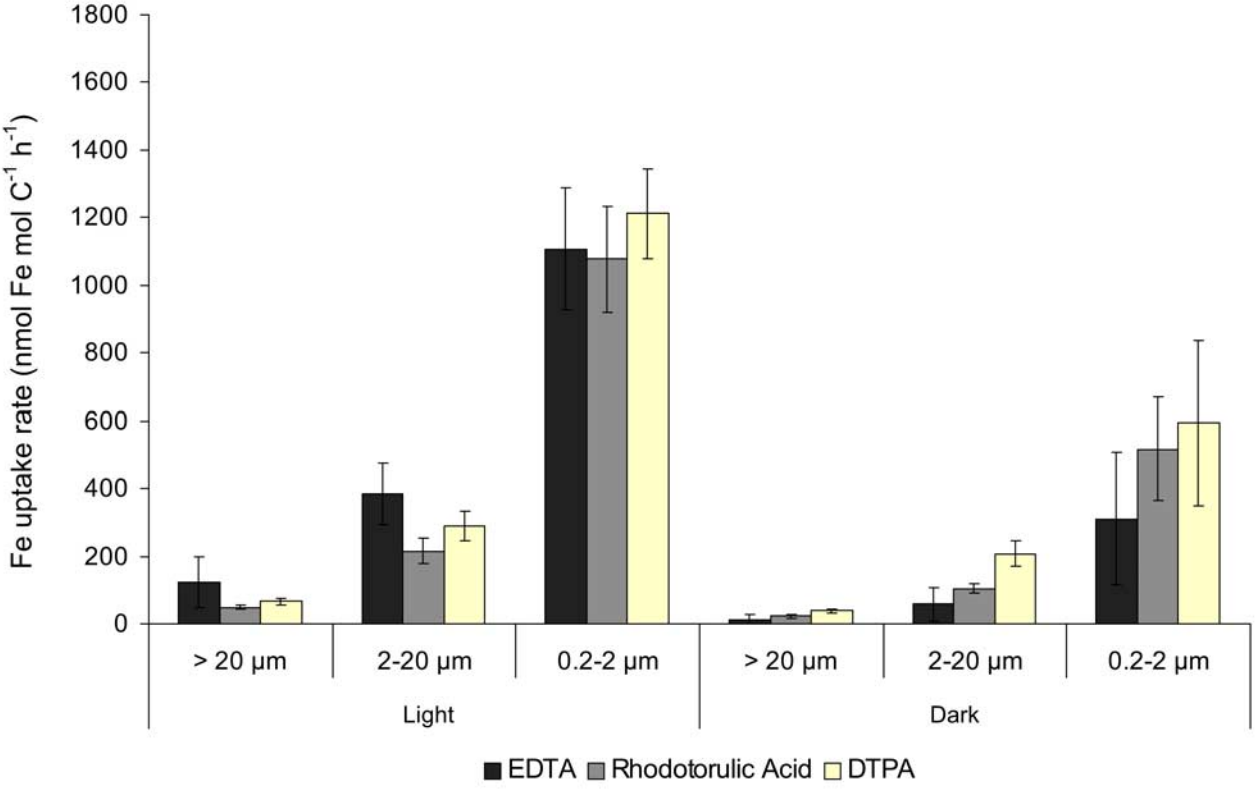

Figure 2. Carbon-normalized Fe uptake rates (nmol Fe mol C^{-1} h^{-1}) by size-fractionated plankton incubated at in situ light conditions or in complete darkness in the presence of various photolabile organic Fe complexes. Each short-term (24–30 hours) Fe uptake rate is the mean of three independent experiments on 4, 6, and 9 February 2003, and were determined in duplicate incubation bottles enriched with 2 nM ^{55}Fe complexed to 20 nM L.

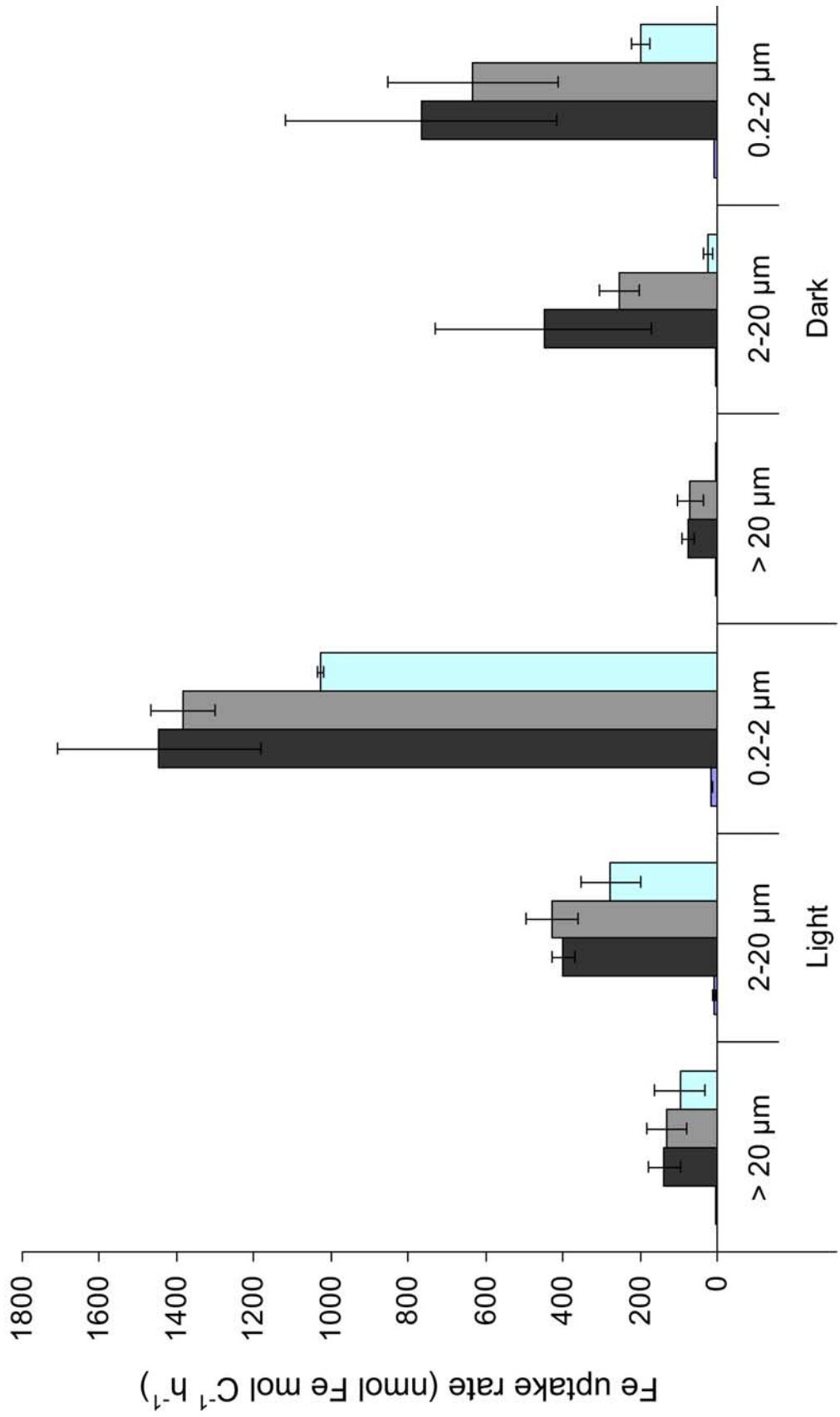

Figure 3. Carbon-normalized Fe uptake rates (nmol Fe mol C^{-1} h^{-1}) by size-fractionated plankton incubated at in situ light conditions or in complete darkness in the presence of various non-photolabile organic Fe complexes, and the in situ ligands. Each short-term (24–30 hours) Fe uptake rate is the mean of three independent experiments on 4, 6, and 9 February 2003, and was determined in duplicate incubation bottles enriched with 2 nmol L^{-1} ^{55}Fe complexed to 20 nmol L^{-1} L. The in situ ligand treatment was prepared by adding 2 nM ^{55}Fe, but no L. However, to prevent precipitation of this inorganic Fe upon addition to seawater, the Fe was added as a FeEDTA complex (2 nM Fe: 2.1 nM EDTA).