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Author(s)	Iwade, S.; Kuma, K.; Isoda, Y. et al.
Citation	Aquatic microbial ecology, 43(2), 177-191 https://doi.org/10.3354/ame043177
Issue Date	2006-06-27
Doc URL	https://hdl.handle.net/2115/53133
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Type	journal article
File Information	22_a043p177.pdf



Effect of high iron concentrations on iron uptake and growth of a coastal diatom *Chaetoceros sociale*

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ABSTRACT: The growth and iron uptake of the coastal marine diatom *Chaetoceros sociale* were experimentally measured in batch experiments at 10°C to which an acidic Fe(III) stock solution was added. The direct input of Fe(III) into the culture media induced the highest iron uptake rate (~ 3.4 to 4.2×10^{-16} mol Fe cell⁻¹ d⁻¹) by *C. sociale* during the first day of the incubation, resulting from the supply of bioavailable inorganic Fe(III) species at levels above its expected equilibrium value (~ 0.1 nmol l⁻¹) with solid amorphous Fe(III) hydroxide in seawater. The iron uptake rate during the first day of incubation in solid amorphous Fe(III) hydroxide medium aged for 1 d at 10°C was approximately 50 % lower than that in the direct Fe(III) input media. We used a modified approach in which further iron uptake by *C. sociale* from external iron in the direct Fe(III) input media was prevented by adding hydroxamate siderophore desferrioxamine B (DFB) during cultivation. After the addition of DFB, the highest growth rate (~ 0.5 to 0.6 d⁻¹) of *C. sociale* by intracellularly stored Fe in the direct Fe(III) input media was maintained for a few days since no iron uptake was observed after the DFB addition. The growth rate was independent of the amount of intracellularly stored Fe. However, the maximal cell yields appeared to be relatively dependent on the amount of intracellularly stored Fe, suggesting the presence of a critical concentration of intracellular Fe (minimum cellular Fe for growth) for phytoplankton growth ($\sim 1 \times 10^{-16}$ mol Fe cell⁻¹ for *C. sociale*). In the present study, maximal and minimal Fe quotas were 3.4 to 4.2×10^{-16} and $\sim 1 \times 10^{-16}$ mol Fe cell⁻¹ (the maximal/minimal Fe ratio of 3.4 to 4.2), respectively. The high iron uptake and storage capacity in *C. sociale* allows this species to accumulate excess iron at high concentrations of bioavailable inorganic Fe species and to support up to 1.8 to 2.1 cell divisions without any additional iron uptake. In addition, we attempted to model the effect of luxury uptake on growth, as biodilution of cellular Fe eventually decreases the Fe quota to a critical threshold.

KEY WORDS: Iron uptake · Growth rate · Luxury uptake · Bioavailable Fe · Inorganic Fe species · Coastal marine diatom · *Chaetoceros sociale*

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INTRODUCTION

Iron supply is thought to regulate primary production in the high-nitrate low-chlorophyll (HNLC) oceanic regions such as the equatorial Pacific Ocean (Martin et al. 1994, Coale et al. 1996), the polar South-

ern Ocean (Boyd et al. 2000) and the subarctic Pacific Ocean (Tsuda et al. 2003, Boyd et al. 2004) where iron fertilization mesoscale experiments were performed. A critical aspect of this relationship is the acquisition of iron by phytoplankton, which occurs through a complex series of extracellular reactions that are influ-

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enced by iron chemistry and speciation in seawater. The inorganic speciation of Fe(III) in seawater is dominated by its hydrolysis behavior and tendency to precipitate as particulate Fe(III) hydroxide. Recent studies of the Fe(III) hydroxide solubility in seawater suggest that the Fe(III) solubility is controlled by organic complexation (Kuma et al. 1996, Millero 1998, Liu & Millero 1999, 2002, Waite 2001, Tani et al. 2003, Chen & Wang 2004), which subsequently regulates dissolved iron concentrations in seawater (Kuma et al. 1996, 1998a, 2003, Johnson et al. 1997a,b, Archer & Johnson 2000, Nakabayashi et al. 2001). In a previous study (Kuma et al. 1996), the iron solubility limit of fresh solid amorphous $\text{Fe}(\text{OH})_3$ (am-Fe(III)) in ultraviolet (UV)-irradiated open-ocean waters (free of organic ligands) was 0.07 to 0.09 nmol l^{-1} ($\leq 0.1 \text{ nmol l}^{-1}$). In addition, Wu et al. (2001) also reported that the iron solubility limit for inorganic Fe(III) hydrolysis species ($\text{Fe}(\text{III})'$) in the UV-irradiated seawater was $\sim 0.08 \pm 0.03 \text{ nmol l}^{-1}$. Therefore, the equilibrium concentration of $\sim 0.1 \text{ nmol l}^{-1}$ only applies for iron in equilibrium with a fresh amorphous solid $\text{Fe}(\text{OH})_3$ and is a maximum limit on $\text{Fe}(\text{III})'$ in seawater. In addition, it has been reported that the complexation of iron with natural organic ligands increases the Fe(III) solubility in seawater (Hudson et al. 1992, Kuma et al. 1999, Liu & Millero 1999, 2002, Chen & Wang 2004).

In general, the iron uptake rate by phytoplankton is related to the computed equilibrium concentration of Fe^{3+} in seawater and is actually dependent on the concentration of dissolved inorganic Fe(III) species

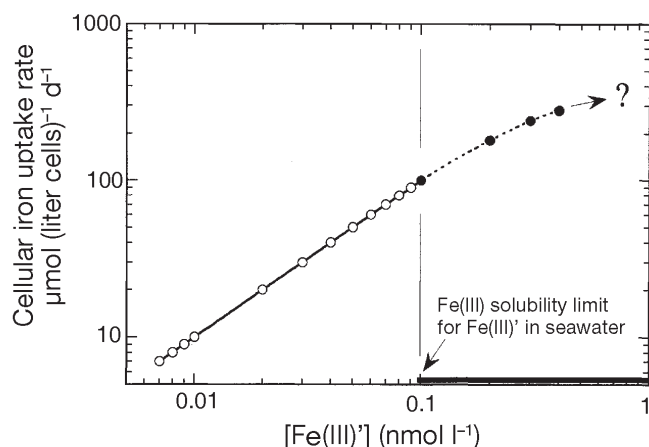


Fig. 1. Theoretical interrelationship between concentration of inorganic Fe(III) species ($[\text{Fe}(\text{III})']$) and cellular iron uptake rate by phytoplankton (Campbell 1995, Sunda 2001). The solid horizontal bar on the lower right indicates the region where amorphous Fe(III) hydroxide is observed to precipitate. $[\text{Fe}(\text{III})']$ should be in constant equilibrium with solid amorphous Fe(III) hydroxide in seawater. The Fe(III) solubility limit for $\text{Fe}(\text{III})'$ in seawater is likely to be approximately 0.1 nmol l^{-1} (Kuma et al. 1996, Wu et al. 2001). ?: unknown non-linear line for iron uptake rate over Fe(III) solubility limit

($[\text{Fe}(\text{III})']$, Fig. 1), which is proportional to $[\text{Fe}^{3+}]$ (Free-ion activity model (FIAM); Anderson & Morel 1982, Hudson & Morel 1990, Campbell 1995, Sunda & Huntsman 1995, Sunda 2001), although apparent exceptions to the FIAM exist; for example, specific transport ligands such as siderophores or lipophilic metal complexes that may be indirectly utilized by cells (Campbell 1995, Soria-Dengg & Horstmann 1995, Hutchins et al. 1999a,b, Maldonado & Price 1999, 2000, 2001, Sunda 2001), and a new model recently proposed by Shaked et al. (2005) for iron uptake by diatoms in which extracellular reduction of all Fe species is a necessary step for iron acquisition. Therefore, the iron uptake of phytoplankton is limited by the equilibrium concentration of $\text{Fe}(\text{III})'$ with particulate Fe(III) hydroxide in seawater (Fig. 1). In previous studies (Kuma et al. 1999, 2000), it has been suggested that the natural organic-Fe(III) complexes and acidic Fe(III) supplied by riverine input may play an important role in supplying bioavailable $\text{Fe}(\text{III})'$ above the equilibrium concentration of $\text{Fe}(\text{III})'$ in estuarine mixing systems and coastal waters. A high concentration of $\text{Fe}(\text{III})'$ represents the actual instantaneous availability of iron for uptake. Therefore, the direct input of concentrated acidic Fe(III) or Fe(II) stock solution into culture solution or natural seawater is assumed to enhance the concentration of bioavailable inorganic Fe (Fe' : $\text{Fe}(\text{III})'$ or $\text{Fe}(\text{II})'$) species above the equilibrium concentration in seawater and to induce the highest iron uptake rate and highest growth of phytoplankton. In mesoscale iron fertilization experiments, the addition of a highly concentrated acidic Fe(II) solution to the patch in HNLC oceanic regions promoted the rapid growth of large-size phytoplankton (Coale et al. 1996, Boyd et al. 2000) and coastal species (Tsuda et al. 2003). An increase in Fe' in marine environments is one of the most important mechanisms for providing bioavailable Fe' .

It has been found that the addition of excess concentrations of the siderophore desferrioxamine B (DFB) essentially eliminated iron uptake in a picoplankton-dominated community by diminishing the concentration of bioavailable $\text{Fe}(\text{III})'$ (Wells et al. 1994) and regulated iron availability in coastal upwelling waters (Hutchins et al. 1999a, Wells 1999). DFB is a small trihydroxamate molecule that complexes inorganic Fe(III) with an extremely high conditional stability constant ($K'_{\text{FeL,Fe(III)'}} = [\text{Fe}(\text{III})\text{L}]/[\text{Fe}(\text{III})'][\text{L}] = 10^{16.5} \text{ M}^{-1}$; Hudson et al. 1992, Rue & Bruland 1995) in seawater. However, it has been suggested that marine diatoms, *Phaeodactylum tricornutum* (Soria-Dengg & Horstmann 1995) and iron-limited *Thalassiosira oceanica* (Maldonado & Price 2000, 2001), and phytoplankton communities in the subarctic Pacific (Maldonado & Price 1999) can utilize DFB-bound Fe(III) via a cell-

surface reduction process. In addition, Hutchins et al. (1999b) have reported that iron bound to a variety of siderophores is more available to prokaryotic picoplankton (cyanobacteria) than to eukaryotic phytoplankton that can efficiently assimilate porphyrin-complexed iron, suggesting that the 2 plankton groups exhibit fundamentally different iron-uptake strategies.

In the present study, we examined the effect of hydroxamate siderophore DFB addition and bioavailable Fe(III)' at levels above the equilibrium concentration with solid amorphous Fe(III) hydroxide in seawater on the iron uptake and growth dynamics of coastal diatom *Chaetoceros sociale*. The temporary high Fe(III)' concentration in medium was accomplished by adding acidic Fe(III) stock solution directly together with an inoculation of culture into the culture medium. In addition, the growth of *C. sociale* by intracellularly stored Fe was accomplished by the addition of DFB, which prevents further iron uptake from ambient extracellular Fe in the medium. In particular, we show that the high Fe(III)' concentration in seawater is remarkably bioavailable and induces the highest iron uptake and growth of coastal marine phytoplankton.

MATERIALS AND METHODS

Seawater was collected from a coastal region near Hokkaido, Japan, in the northern Japan Sea (salinity = 33.8), and was filtered through an acid-cleaned 0.22 µm Millipore cellulose membrane filter. The filtrate (100 ml) for Fe concentration analysis was buffered at pH 3.2 with a 10 mol l⁻¹ formic acid, 2.4 mol l⁻¹ ammonium formate buffer solution (0.5 ml 100 ml⁻¹ filtrate). The Fe concentration in buffered filtrate (≤2 nmol l⁻¹) was determined with an automated Fe analyzer (Kimoto Electric) by placing various concentrations on an 8-hydroxyquinoline chelating column and using luminol-hydrogen peroxide chemiluminescence (CL) detection in a closed flow-through system (Obata et al. 1993, 1997). The concentrations of NO₃+NO₂, PO₄, and Si(OH)₄ in the filtered seawater measured by a Technicon autoanalyzer were less than 0.5, 0.1, and 5 µmol l⁻¹, respectively, which are negligible compared with the concentrations added in the culture experiments (see below). DFB (sold commercially under the trade name Desferal) was purchased from Sigma and stored frozen in the dark until use. All bottles, flasks and tubes used in our experiments were acid-washed (soaked for at least 24 h in 1 or 3 mol l⁻¹ HCl solution: 1 mol l⁻¹ HCl for polycarbonate apparatuses) followed by repeated washing with Milli-Q water (Millipore, >18.0 MΩ cm⁻¹). All the preparation and sampling for experiments were performed in a Class 100 laminar flow cabinet to avoid inadvertent trace metal contamination.

Culture experiments. The filtered seawater was autoclaved for 20 min at 121°C (1.1 kg cm⁻² pressure), and the culture medium was prepared by adding modified f/2 nutrient (Guillard & Ryther 1962) without trace metals and EDTA to the autoclaved filtered seawater. The modified f/2 medium contained 880 µmol l⁻¹ nitrate, 38 µmol l⁻¹ phosphate, and 105 µmol l⁻¹ silicate. The chain-forming coastal marine diatom *Chaetoceros sociale* was grown in 1 l of the f/2 medium to which acidic ferric iron stock solution (25 µmol l⁻¹ Fe(III); FeNH₄(SO₄)₂ · 12H₂O in 5 mmol l⁻¹ HCl, pH 2.3) was added to make an iron concentration of 100 nmol l⁻¹. The pH of the seawater after the acidic iron addition was 7.98 to 8.03. Cells were grown at 10°C under 150 µmol photons m⁻² s⁻¹ fluorescent light (12:12 h, light:dark) to obtain the cell concentration of *C. sociale* (cell diameter: ~6 µm, height; ~12 µm in initial stationary growth phase) expected at the start of the following culture and iron uptake experiments.

Small amounts of acidic ferric iron stock solution (25 µmol l⁻¹ Fe(III)) and DFB solution were immediately mixed in an acid-cleaned 100 ml polycarbonate Erlenmeyer flask. Each DFB medium was prepared by adding 50 ml of autoclaved filtered seawater at 10°C to each premixed DFB-Fe(III) (molar ratios of 2.5:1, 5:1 and 10:1, pH 2.4~2.6) solution in the flask to determine which ratio of DFB:Fe(III) inhibited the growth of *Chaetoceros sociale* over at least 20 d in culture experiments. Solid am-Fe(III) medium was prepared by aging the seawater medium for 1 d after adding a small amount of acidic ferric iron stock solution to 50 ml of autoclaved filtered seawater (10°C). The final iron concentration was 100 nmol l⁻¹. A f/2 nutrient stock solution and then a small amount of culture (ca. 250 µl) in an initial stationary growth phase (Fe-limited cells) were added to each culture flask. All major nutrient stocks were passed through Chelex 100 ion-exchange resin to remove trace metals (Morel et al. 1979). The effect of direct Fe input was examined by adding a small amount of acidic ferric iron stock solution directly together with an inoculation of culture into the control culture medium (10°C). In addition, the effect of direct Fe(III) input and the growth by intracellular Fe were examined by the addition of DFB to a final concentration of 1 µmol l⁻¹ (DFB:Fe(III) = 10:1) after 2 min and 1, 3, 5 and 7 d cultivations in direct Fe(III) input medium to prevent further iron uptake by *C. sociale* from ambient external Fe (dissolved Fe(III) and solid Fe(III) hydroxide in the medium). DFB was also added to *C. sociale* cultivated for 1 d in 1 d aged am-Fe(III) culture. Control (without any added iron) medium (≤3 nmol Fe l⁻¹) was prepared to compare the growth rates and cell yields with those containing iron. *C. sociale* cell concentrations at the start of the culture experiments were approximately 1000 cells ml⁻¹. The

light, temperature, and nutrient conditions were the same as those of the stock culture described above. During the experiments, cell growth was monitored daily by triplicate cell counts (actual cells and not chains) done with an optical microscope. Culture experiments were conducted in triplicate.

Iron uptake experiments. Iron uptake experiments were conducted with *Chaetoceros sociale* grown in direct Fe(III) input and am-Fe(III) culture to which DFB was added just after 1 and/or 3 d cultivations. The long-term (3 h, 12 h, and/or daily for 1 to 7 d) iron uptake experiments were carried out in medium to which $^{59}\text{Fe(III)}$ was directly added. Incubations were done at 10°C under 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ fluorescent light.

The direct $^{59}\text{Fe(III)}$ input culture was prepared by adding a small volume of acidic radioactive $^{59}\text{Fe(III)}$ (New England Nuclear, NEZ-037) stock solution (pH 2.2–2.3) to 1 l of autoclaved filtered seawater (10°C), which already contained modified f/2 nutrient and the *Chaetoceros sociale* culture in initial stationary growth phase. The pH of the culture solution after acidic radioactive $^{59}\text{Fe(III)}$ addition was 7.98 to 8.05. Solid am- $^{59}\text{Fe(III)}$ culture was prepared by adding modified f/2 nutrient and the *C. sociale* culture (approximately 50 ml), after adding acidic stock $^{59}\text{Fe(III)}$ solution to 950 ml of autoclaved filtered seawater (10°C) and then aging for 1 d at 10°C. *C. sociale* cell concentration at the start of the iron uptake experiments was ca. 10 000 cells ml^{-1} . The nutrient and iron concentrations were the same as those in the culture experiments described above.

At each sample point (3 h, 12 h, and/or daily for 1 to 7 d) during the iron uptake experiment, a 50 ml aliquot of $^{59}\text{Fe(III)}$ culture solution was mixed with 20 ml of 0.175 mol l^{-1} Ti(III)–citrate–EDTA solution (0.175 mol l^{-1} TiCl_3 , $\text{Na}_3\text{citrate} \cdot 2\text{H}_2\text{O}$, and $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ in filtered seawater, followed by an adjustment of the pH to 8 with NaOH solution) in a glass beaker. The Ti(III) solution was used to rapidly dissolve freshly precipitated am-Fe(III) (Hudson & Morel 1989, 1990) and extracellularly adsorbed iron by reductive dissolution of Fe(III) without cellular damage to *Chaetoceros sociale*. After the mixture had stood for 10 min, it was gently vacuum-filtered through a quantitative filter paper (No. 5C, Advantec) that retains all precipitate $\geq 1 \mu\text{m}$. The filter was rinsed with 30 ml of 0.05 mol l^{-1} Ti(III)–citrate–EDTA solution. The drained filter was digested with 7 ml of conc. HNO_3 :conc. HClO_4 (1:1) and then diluted to 20 ml. The γ -activity of 4 ml of diluted sample in a counting vial was measured using a gamma counter (Aloka ARC-301 B), and the results were converted to amounts and rates of iron incorporation. In addition, iron uptake experiments for cell-free control media (direct $^{59}\text{Fe(III)}$ and am- $^{59}\text{Fe(III)}$) at 10°C

were conducted to ascertain the reductive dissolution of freshly precipitated and aged am-Fe(III) by the Ti(III) solution. In previous studies (Kuma & Matsunaga 1995, Kuma et al. 2000), freshly precipitated am-Fe(III) (aged for less than 1 d at 10°C) was almost completely dissolved by the reductive dissolution with Ti(III) treatment. However, aged am-Fe(III) produced in direct Fe(III) input cell-free media during aging above 1 d at 10°C was incompletely reductively dissolved by the Ti(III) solution because of the much slower dissolution of aged am-Fe(III). The dissolution rates of the am-Fe(III) phase decrease with aging time because of the conversion to more stable phases (Kuma & Matsunaga 1995). Therefore, iron uptake at each sample point during the iron uptake experiments was determined by subtracting the amount of insoluble iron in cell-free medium from the amount of iron uptake in medium containing cells. *C. sociale* cell concentrations at the start of the iron uptake experiments were approximately 10 000 cells ml^{-1} . During the experiments, cell growth in each culture was monitored by triplicate cell counts done with an optical microscope.

Hydrolytic precipitation of Fe(III) in seawater and dissolution of am-Fe(III) in seawater by adding DFB. The hydrolytic precipitation rate of Fe(III) in the 0.22 μm -filtered seawater was measured at 10 and 20°C by a simple filtration technique involving γ -activity measurement of ^{59}Fe previously reported by Kuma et al. (1996, 1998b). To examine the effect of aging time and temperature on the hydrolytic precipitation of Fe(III) in seawater, a small amount of acidic $^{59}\text{Fe(III)}$ stock solution was added, previously spiked with a small known amount of stable Fe(III), to 250 ml of the 0.22 μm -filtered seawater in acid-cleaned 250 ml Teflon bottles. In addition, the dissociative precipitation rate of premixed DFB-Fe(III) (10:1) complex added into the filtered seawater at 10°C was also measured by the same filtration method (Kuma et al. 2000). Briefly, a small amount of $^{59}\text{Fe(III)}$ stock solution and a solution of DFB were mixed in a precleaned 250 ml bottle (10°C, pH 2.4 to 2.6), followed by the addition of 250 ml of filtered seawater (10°C). The concentration of iron and pH in seawater solutions was kept constant (100 nmol l^{-1} , pH 7.98 to 8.05) in all experiments. In general, the addition of dissolved inorganic Fe(III) to seawater results in rapid hydrolytic precipitation of metastable Fe(III) hydroxide, which slowly converts to more stable solid phases (Kuma & Matsunaga 1995, Stumm & Morgan 1996). The bottles containing the seawater solution and radiolabelled Fe(III) were kept in an incubator (in the dark) at 10 or 20°C. While standing in the dark for 3 wk at 10 or 20°C, each 7.5 ml sample aliquot was filtered through a 0.025 μm Millipore cellulose membrane filter and acidified by the addition

of 10 µl of concentrated HCl to prevent adsorption of filtered Fe(III) on the wall of the collecting vial. The γ -activity of the 2.5 ml acidified sample filtrates was measured in 5 ml counting vials with a gamma counter. Finally, the 0.025 µm-filtered Fe(III) concentrations (Fe(III) hydroxide solubility) were calculated from the γ -activity (Kuma et al. 1996, 1998a).

Organic ligand (DFB)-promoted dissolution of solid am- $^{59}\text{Fe(III)}$ produced in the direct $^{59}\text{Fe(III)}$ input medium was monitored in the cell-free control and cell-containing culture medium at 10°C during the iron uptake experiments. Dissolution of am- $^{59}\text{Fe(III)}$ was measured on a daily basis using a simple filtration technique involving γ -activity measurement of ^{59}Fe following the same procedure as the hydrolytic precipitation experiments as described above.

RESULTS

Growth rate and cell yields

In the present study, direct Fe(III) input to the culture solution induced the highest growth rate (0.55 to 0.58 d⁻¹, Table 1, Fig. 2A) and the highest maximal cell yields (141 000 to 147 000 cells ml⁻¹, Table 1), showing values similar to those in the same medium in the previous study (Kuma et al. 2000). The relative order for growth rates and maximal cell yields on different media

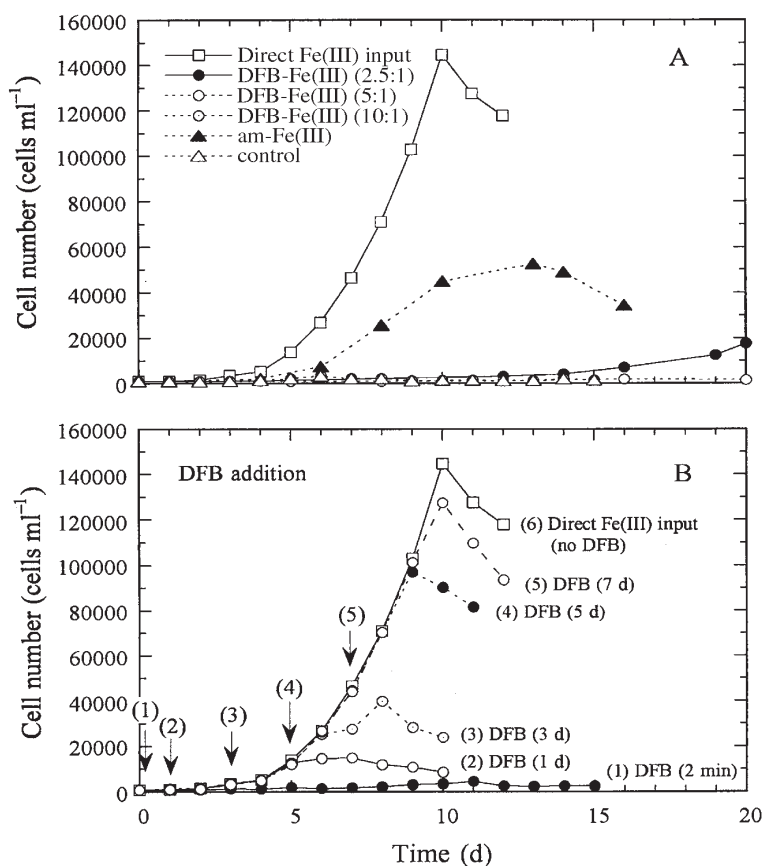


Fig. 2. *Chaetoceros sociale*. Change in cell numbers of cultures supplied with direct Fe(III) input, premixed DFB (desferrioxamine B)-Fe(III) complex and solid am-Fe(III) (amorphous Fe(OH)₃) (10°C). (A) Growth in direct Fe(III) input, premixed DFB-Fe(III) complex (2.5:1, 5:1 and 10:1), solid am-Fe(III) and control (no iron addition) media. (B) Growth in direct Fe(III) input medium without or with addition of DFB after 2 min and 1, 3, 5 and 7 d. Data on cell concentrations represent the mean (n = 3) for triplicate culture experiments

Table 1. *Chaetoceros sociale*. Growth rate and maximal cell yields (see Figs. 2B & 3) in direct Fe(III) input and 1 d aged am-Fe(III) (amorphous Fe(OH)₃) media (10°C) to which DFB (desferrioxamine B) was added after incubation for 1 or 3 d (DFB:Fe(III) = 10:1). N: not determined (range given for A and ± 1 SD for B)

Medium	Cell number at start (10 ³ cells ml ⁻¹)	Addition of DFB (1 µmol l ⁻¹)	Specific growth rate (µ, d ⁻¹)	Maximal cell yields [Cn] (×10 ³ cells ml ⁻¹)
(A) Growth rate experiment (n = 3, Fig. 2B)				
Direct Fe(III)	1	No addition	0.55–0.56 (0–10 d)	141–147
Direct Fe(III)	1	After 7 d incubation	0.57–0.58 (0–9 d)	124–130
Direct Fe(III)	1	After 5 d incubation	0.56–0.57 (0–8 d)	93–101
Direct Fe(III)	1	After 3 d incubation	0.55–0.57 (0–6 d)	39–40
Direct Fe(III)	1	After 1 d incubation	0.49–0.52 (0–5 d)	13–17
Direct Fe(III)	1	After 2 min incubation	N	N
(B) Iron uptake and growth rate experiment (n = 1, Fig. 3)				
Direct Fe(III)	10	No addition	0.50 ± 0.01 (0–5 d, r = 0.999)	126 ± 20
Direct Fe(III)	10	After 3 d incubation	0.47 ± 0.01 (0–4 d, r = 0.999)	66 ± 4.5
Direct Fe(III)	10	After 1 d incubation	0.46 ± 0.02 (0–3 d, r = 0.999)	52 ± 1.7
1 d am-Fe(III)	10	After 1 d incubation	0.45 ± 0.05 (0–2 d, r = 0.994)	43 ± 2.2

for 10 to 20 d cultivations was Direct Fe(III) input $\gg$ solid 1 d aged am-Fe(III) $\gg$ DFB-Fe(III) (2.5:1) $>$ DFB-Fe(III) (5:1 and 10:1) = control (Fig. 2A). No growth of *Chaetoceros sociale* was observed in DFB-Fe(III) (5:1 and 10:1) and control (no iron addition) media (Fig. 2A). Therefore, the effect of direct Fe(III) input on *C. sociale* growth and growth by intracellularly stored Fe were examined by the addition of DFB (final concentration of $1 \mu\text{mol l}^{-1}$, DFB:Fe(III) = 10:1) during cultivation to prevent iron uptake from ambient extracellular Fe. DFB was added to *C. sociale* cultivated for 2 min (DFB (2 min) medium), 1 d (DFB (1 d) medium), 3 d (DFB (3 d) medium), 5 d (DFB (5 d) medium) and 7 d (DFB (7 d) medium) in direct Fe(III) input culture medium (Fig. 2B). No growth was observed in the DFB (2 min) culture medium. However, the growth in other media continued for 3 to 4 d even after the addition of DFB with almost the same maximum growth rate (~ 0.5 to 0.6 d^{-1}) as that in direct Fe(III) input medium without any addition of DFB. Cell concentration in the DFB (1 d) medium increased up to approximately $15\,000 \text{ cells ml}^{-1}$, 15 times more than the initial cell concentration, after 5 to 6 d of cultivation after the addition of DFB (Fig. 2B). The relative order for maximal cell yields was direct Fe(III) input $>$ DFB (7 d) $>$ DFB (5 d) $>$ DFB (3 d) $>$ DFB (1 d) media, while the initial growth rates were almost the same in all media (Fig. 2B, Table 1).

Iron uptake and growth rate

The long-term radiolabelled iron uptake rates by *Chaetoceros sociale* and the growth in direct Fe(III) input and am-Fe(III) media, into which DFB was added after 1 and/or 3 d cultivations, were measured during cultivation for 7 d at 10°C (Fig. 3). The cellular iron uptake (nmol l^{-1}) by *C. sociale* in all media was prevented by the addition of DFB and remained nearly constant for 2 to 4 d after DFB treatment (Fig. 3A). However, after 5 d the cells reached stationary phase and thus iron uptake rates decreased (Fig. 3). In the direct Fe(III) input medium, the iron uptake rate decreased gradually with cultivation time for 0 to 3 d before DFB addition and the highest rate

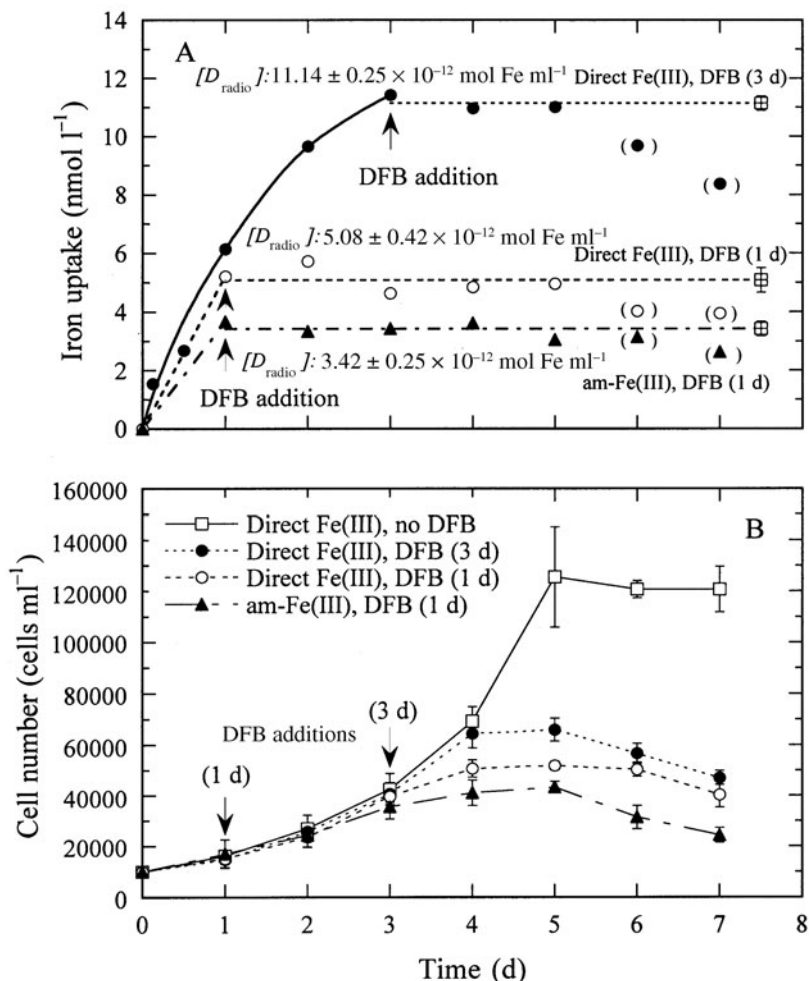


Fig. 3. *Chaetoceros sociale*. Long-term iron uptake and growth in direct Fe(III) input media and 1 d aged am-Fe(III) medium without or with addition of DFB after 1 and/or 3 d cultivations. (A) Iron uptake rates by *C. sociale* in direct Fe(III) input media with addition of DFB after 1 and 3 d and in 1 d aged am-Fe(III) medium with addition of DFB after 1 d. Radiolabelled iron uptake [D_{radio}] represents mean ± 1 SD for values from 1 to 5 d in the DFB (1 d) media and from 3 to 5 d in the DFB (3 d) medium. (B) Growth in direct Fe(III) input media and 1 d aged am-Fe(III) medium without or with addition of DFB after 1 and/or 3 d. Data on cell concentrations represent mean ± 1 SD ($n = 3$) for triplicate cell counts

was observed during the initial iron uptake during 0 to 1 d. The iron uptake rate during 0 to 1 d in 1 d aged am-Fe(III) medium was approximately one-third lower than those during 0 to 1 d and almost the same as that during 1 to 2 d in direct Fe(III) input medium. The order of iron uptake was: DFB (3 d) $>$ DFB (1 d) $>$ am-Fe(III)-DFB (1 d) (Fig. 3A, Tables 1 & 2). If cells are under identical steady state growth conditions both before and after addition of radiolabelled Fe, it is necessary to allow the phytoplankton to go through 8 successive transfers in order to measure intracellular Fe quotas by radioactive ^{59}Fe uptake, so that the Fe within the cells was 100% radiolabelled. The initial cultures in the iron uptake experiments had a cell density of $10\,000 \text{ cells ml}^{-1}$. The growth rate was

Table 2. *Chaetoceros sociale*. Iron uptake (see Fig. 3) in direct Fe(III) and 1 d aged am-Fe(III) media (10°C) to which DFB was added after incubation for 1 or 3 d. Values of $[Q]$ for the direct Fe(III) input medium (DFB (1 d)) and 1 d aged am-Fe(III) medium (DFB (1 d)) are also the corrected iron uptake rate with units of $\text{mol Fe cell}^{-1} \text{d}^{-1}$

Medium	Radiolabelled Fe uptake (for 1, 2 or 3 d) $[D_{\text{radio}}]$ ($\times 10^{-12} \text{ mol Fe ml}^{-1}$)	Cell density when DFB was added $[C_{\text{DFB}}]$ ($\times 10^3 \text{ cells ml}^{-1}$)	Maximal cell yields $[Cn]$ ($\times 10^3 \text{ cells ml}^{-1}$)	Cellular Fe (Fe quota: $[Q]$) $[D_{\text{radio}}] / [C_{\text{DFB}}]$ ($\times 10^{-16} \text{ mol Fe cell}^{-1}$)	Critical cellular Fe $[Q_{\text{cri}}]$ $[D_{\text{radio}}] / [Cn]$
Iron uptake and growth rate experiment (cell density at start: 10 000, $n = 1$, Fig. 3)					
Direct Fe(III)-DFB-3 d	6.14 (1 d ^a)	14.8 $\pm$ 1.7 (1 d ^a)	66 $\pm$ 4.5	~4.15 ^a	~0.93 ^a
	9.66 (2 d ^a)	25.9 $\pm$ 2.8 (2 d ^a)	66 $\pm$ 4.5	~3.73 ^a	~1.46 ^a
	11.14 $\pm$ 0.25 (3 d)	40.7 $\pm$ 4.5 (3 d)	66 $\pm$ 4.5	2.73 $\pm$ 0.07	1.69 $\pm$ 0.16
Direct Fe(III)-DFB-1 d	5.08 $\pm$ 0.42 (1 d)	15.2 $\pm$ 3.4	52 $\pm$ 1.7	3.35 $\pm$ 0.27	0.98 $\pm$ 0.11
1 d am-Fe(III)-DFB-1 d	3.42 $\pm$ 0.25 (1 d)	17 $\pm$ 5.6	43 $\pm$ 2.2	2.01 $\pm$ 0.14	0.80 $\pm$ 0.10

^aValues were obtained by incubation for 1 and 2 d before DFB addition on Day 3

approximately 1 division d^{-1} (Fig. 3B). Thus, after 1 d, the cell density will increase to 20 000 cells ml^{-1} and cells will be 50 % radiolabelled. In the next cell division, the Fe within the cells will be 75 % radiolabelled and the cell density will reach 40 000 cells ml^{-1} . Therefore, if the cells are collected before undergoing 8 divisions, the intracellular Fe concentrations which were measured with radioactive Fe need to be corrected for the non-radioactive (cold) Fe in the cells at the beginning of the experiment. However, the iron uptake experiments in the present study were started with Fe-limited cells, which are documented to have higher iron uptake rates than Fe replete cells under excessive Fe(III)' conditions. Sunda & Huntsman (1995) reported that relative ratios of Fe replete and limiting quotas in oceanic and coastal phytoplankton are roughly 10 to 20 in a long-term iron uptake experiment. For example, if Fe limited cells contained 10 amol Fe cell^{-1} before the addition of excessive Fe', and the steady state quota (Q) with excessive Fe' is 100 to 200 amol Fe cell^{-1} , with a specific growth rate (μ) of 0.7 d^{-1} , then the steady state uptake rate under the excessive Fe' conditions is $\mu \times Q = 70$ to 140 amol Fe $\text{cell}^{-1} \text{d}^{-1}$. Thus, after 1 d, the Fe within the cells would be nearly 100 % (~93 to 97 %) radiolabelled.

Cell concentrations in DFB-added media increased logarithmically for 1 to 2 d even after addition of DFB (Fig. 3B). The growth rates (0.45 ± 0.05 to $0.47 \pm 0.01 \text{ d}^{-1}$) in DFB-added medium were the same as that ($0.50 \pm 0.01 \text{ d}^{-1}$) in direct Fe(III) input medium without addition of DFB (Table 1). However, the maximal cell yields in DFB-added medium were one-third to one-half lower than that in direct Fe(III) input medium without any addition of DFB. The order of maximal cell yields was: direct Fe(III) input (no DFB) > DFB (3 d) > DFB (1 d) > am-Fe(III)-DFB (1 d).

Hydrolytic precipitation of Fe(III) and organic ligand-promoted dissolution of am-Fe(III) by addition of DFB

The hydrolytic precipitation rates of Fe^{3+} in filtered seawater were extremely fast, resulting in extremely low 0.025 μm filterable (dissolved) Fe concentrations within short aging times (Fig. 4A). For example, the dissolved Fe concentrations with 1 d aging were 6.2 and 1.9 nmol l^{-1} at 10 and 20°C, respectively (initial Fe added: 100 nmol l^{-1}). In addition, the hydrolytic precipitation rate of Fe^{3+} at 10°C was slower than that at 20°C. The dissolved Fe concentrations at 10°C decreased gradually (1.98 to 0.74 nmol l^{-1}) during aging for 1 to 3 wk, while those at 20°C were nearly constant (within the range 0.26 to 0.36 nmol l^{-1}) during 1 to 3 wk. Total (unfiltered) Fe concentrations decreased gradually with aging time over 3 wk up to ~50 to 60 nmol l^{-1} because of the sorption of hydrolytic precipitated Fe (particulate Fe) on the wall of Teflon bottles (data not shown). The long-term dissolved (soluble) iron concentrations of ~0.3 nmol l^{-1} are most likely due to the presence of soluble iron organic species as mentioned above. In contrast, precipitation (loss from solution) from DFB-Fe(III) (10:1) complex in seawater was not observed during aging for 10 d at 10°C (Fig. 4B). In addition, Fig. 4B presents the organic ligand (DFB)-promoted dissolution rates of solid am-Fe(III) produced in direct Fe(III) input media (DFB (1 d) and DFB (3 d)), which were aged for 1 and 3 d at 10°C during the iron uptake experiments. There was almost no difference between the cell-free control and the cell-containing medium. The rates of DFB-promoted dissolution were relatively fast for 1 d after the addition of DFB and then became slower with time. DFB-promoted dissolution rate of 1 d aged am-Fe(III) was faster than that of 3 d aged am-Fe(III).

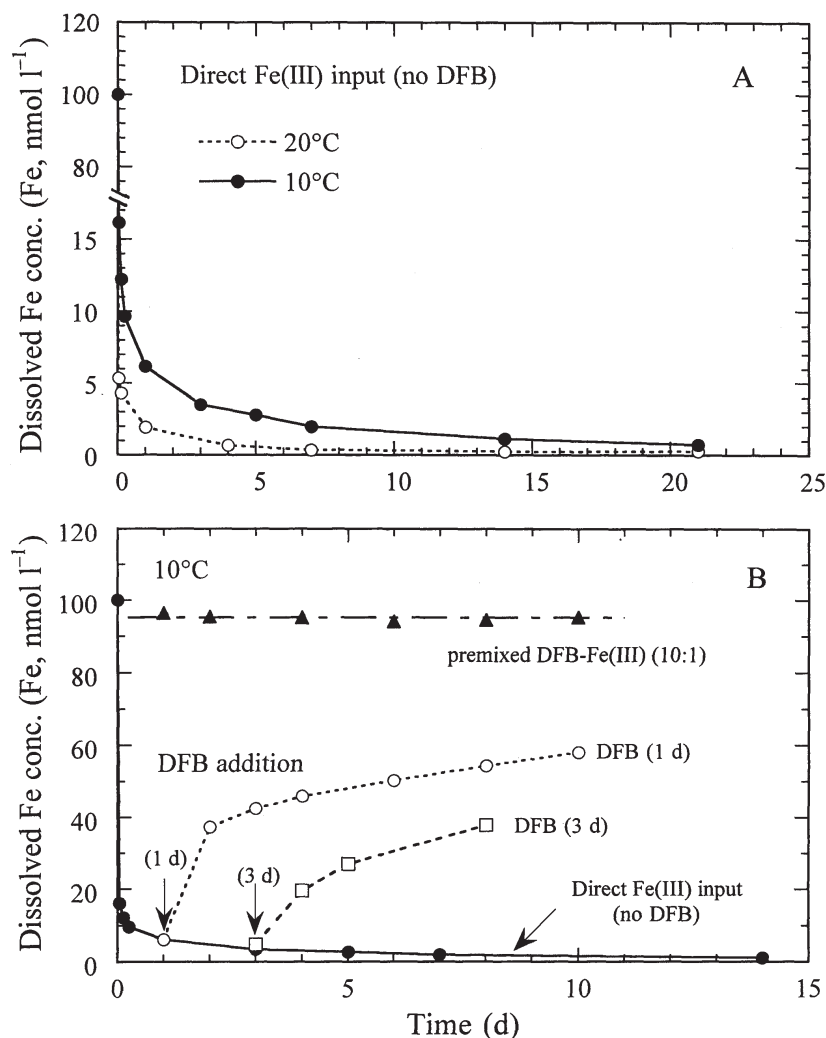


Fig. 4. Time-course of hydrolytic precipitation of Fe^{3+} , Fe(III) dissociative precipitation of premixed DFB- Fe(III) complex and organic ligand (DFB)-promoted dissolution of solid aged am- Fe(III) . (A) Hydrolytic precipitation rate of Fe^{3+} in seawater at 10°C and 20°C. (B) Fe(III) dissociative precipitation of premixed DFB- Fe(III) (10:1) complex in seawater (10°C) and DFB-promoted dissolution of solid 1 and 3 d aged am- Fe(III) in culture media (10°C)

DISCUSSION

Biological effect of high bioavailable Fe level

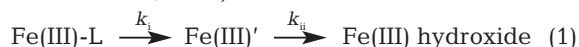
No growth was observed in premixed DFB- Fe(III) (5:1 and 10:1) complex and control media (Fig. 2A) and in DFB (2 min) culture medium (Fig. 2B). Therefore, cells inoculated into the culture and iron uptake experiments were iron-limited. The cells collected at an initial stationary growth phase (~9 to 10 d cultivation in the direct Fe(III) input medium) were iron-limited because of the rapid decrease in bioavailability of hydrolytic precipitated Fe with time in the medium (Kuma & Matsunaga 1995).

Direct Fe(III) input medium promoted the maximal cell yields and growth rate (Fig. 2A). In general, iron uptake rate by marine eukaryotic phytoplankton is related to the concentration of kinetically labile inorganic Fe species (Fe' : $\text{Fe(II)}'$ and $\text{Fe(III)}'$), which is proportional to $[\text{Fe}^{2+}]$ and $[\text{Fe}^{3+}]$, respectively (Fig. 1), and independent of the concentration of Fe chelated to organic ligands such as EDTA and DFB (Campbell 1995, Sunda & Huntsman 1995, Sunda 2001) although the iron uptake rates from DFB-Fe complex by iron-limited phytoplankton are more strongly correlated to the concentrations of the DFB-Fe complex than those of Fe' (Maldonado & Price 2000, 2001). The stable oxidation state of iron in oxic seawater is Fe(III) , which has an extremely low solubility. The equilibrium concentration of $\text{Fe(III)}'$ with particulate Fe(III) hydroxide in seawater is approximately 0.1 nmol l^{-1} (Kuma et al. 1996, Wu et al. 2001), which limits the iron uptake of phytoplankton. In fact, the maximal cell yields in 1 d aged am- Fe(III) medium was two-thirds lower than the direct Fe(III) input medium (Fig. 2A). In addition, equilibrium calculations in premixed DFB- Fe(III) (5:1 and 10:1, $\text{Fe(III)} = 100 \text{ nmol l}^{-1}$) media indicate that bioavailable $\text{Fe(III)}'$ would be limited to $\sim 10^{-17} \text{ mol l}^{-1}$ ($K'_{\text{FeL,Fe(III)}} = 10^{16.5} \text{ M}^{-1} = [\text{Fe(III)L}]/[\text{Fe(III)}][\text{L}]$, Rue & Bruland 1995, Croot & Johansson 2000), or several orders of magnitude below the levels needed to support phytoplankton growth. In the present study, the

direct input of Fe(III) into the culture media containing phytoplankton induced the highest radiolabelled iron uptake rate ($\sim 3.4 \times 10^{-16} \text{ mol Fe cell}^{-1} \text{ d}^{-1}$, Fig. 3A, Table 2) by *Chaetoceros sociale* during the first day of incubation in the long-term iron uptake experiment, resulting from the high supply of $\text{Fe(III)}'$. In addition, the highest radiolabelled iron uptake rate ($\sim 3 \times 10^{-17} \text{ mol Fe cell}^{-1} \text{ h}^{-1} \times 24 \text{ h} = \sim 7 \times 10^{-16} \text{ mol Fe cell}^{-1} \text{ d}^{-1}$) was also observed during the short-term iron uptake experiment with *C. sociale* in the direct Fe(III) input medium in our previous study (Kuma et al. 2000). The short-term iron uptake rate exceeded the long-term uptake rate (Fig. 3A), probably suggesting that the inoculated cells were iron-limited

before the start of culture and iron uptake experiments in the present study. In addition, the long-term cellular radiolabelled iron uptake rate by *C. sociale* (cell diameter: ~7 to 10 μm , smaller than that [~12 μm] for *Thalassiosira weissflogii* normalized to equivalent spherical cell surface area was 1 to 2 $\times 10^{-6}$ mol Fe m^{-2} d^{-1} , nearly consistent with rates for coastal and oceanic phytoplankton (*T. weissflogii*, *T. pseudonana*, *T. oceanica* and *Prorocentrum minimum*) at the high Fe' concentrations in previous studies (Sunda & Huntsman 1995, 1997). Therefore, the direct input of concentrated acidic Fe(III) stock solution into culture media temporarily enhances the concentration of Fe(III)' above the equilibrium concentration (~0.1 nmol l^{-1}) with solid amorphous Fe(III) hydroxide in seawater and induces the highest iron uptake and growth rates of phytoplankton, although the hydrolytic precipitation rate of Fe^{3+} in seawater was extremely fast (Fig. 4A).

Our results and interpretations also suggest that the natural organic-Fe(III) complexes (Fe(III)-L) and acidic Fe(III) supplied by riverine input may play an important role in supplying Fe(III)' in estuarine mixing systems and coastal waters (Stumm & Morgan 1996, Kuma et al. 1999, 2000) as follows:



In addition, temporary high concentrations of Fe(II)' and Fe(III)' in seawater would have been caused by the supply of concentrated acidic Fe(II) solution (approximately 0.5 mol l^{-1} Fe(II) at about pH 2) to HNLC oceanic regions in mesoscale iron fertilization experiments (Martin et al. 1994, Coale et al. 1996, Boyd et al. 2000, Tsuda et al. 2003). In oxic seawater, the oxidation rate of Fe(II) is strongly dependent upon pH and temperature (Millero et al. 1987). In addition, a longer half-life can be expected when there is organic complexation (Santana-Casiano et al. 2000) and/or lower temperature (Kuma et al. 1995, Croot et al. 2001, Croot & Laan 2002). For example, the half-lives of Fe(II) in seawater are approximately 10, 7, and 5 min at 5, 10, and 15°C, respectively (Kuma et al. 1995). In addition, the oxidative hydrolytic precipitation of Fe(II) in seawater is given by:



Eqs. (1) & (2) are termed consecutive reactions where the rate constants of individual steps are such that all steps must be considered. Eqs. (1) & (2) consider the time-dependent concentrations of the intermediate products, and the final product (Brezonik 1994). The concentrations of the intermediate product (Fe(III)') reach a maximum at some intermediate time and would be temporarily above the solubility equilibrium concentration of Fe(III)' with solid Fe(III)

hydroxide in seawater. The maximum concentrations of Fe(III)' depend on the relative sizes of k_i and k_{ii} in Eq. (1) and k_{iii} and k_{iv} in Eq. (2).

Growth by intracellularly stored Fe

In the present study, the highest specific growth rate of *Chaetoceros sociale* was maintained for 2 to 4 d even after the addition of DFB, which prevented further iron uptake from ambient extracellular Fe in the culture media (Figs. 2B & 3B). The growth rate was relatively independent of the amount of intracellularly stored Fe (Fig. 3, Tables 1 & 2). The value of intracellular Fe ([Q]) was simply calculated by dividing the amount of radiolabelled Fe uptake ($[D_{\text{radio}}]$) for 1 or 3 d by cell density ($[C_{\text{DFB}}]$) when DFB was added for the Fe uptake measurements (Fig. 3, Table 2). Values of $[D_{\text{radio}}]$ and [Q] for the direct Fe(III) input and 1 d aged am-Fe(III) media (DFB (1 d)) are also the iron uptake rates with units of mol Fe ml^{-1} d^{-1} and mol Fe cell^{-1} d^{-1} , respectively. The intracellular Fe ([Q]) in culture media ranged from 2 to 3.4 $\times 10^{-16}$ mol Fe cell^{-1} (Table 2). The critical concentration of intracellular Fe ($[Q_{\text{cri}}]$) for phytoplankton growth was simply calculated by dividing $[D_{\text{radio}}]$ by maximal cell yields ($[Cn]$) (minimum cellular Fe on growth: $[Q_{\text{cri}}] = [D_{\text{radio}}] / [Cn] = \sim 1 \times 10^{-16}$ mol Fe cell^{-1} except for the value (1.7 $\times 10^{-16}$ mol Fe cell^{-1}) obtained in the direct Fe(III) input (DFB (3 d)) medium, Table 2). The higher $[Q_{\text{cri}}]$ for the direct Fe(III) (DFB (3 d)) medium is probably due to the overestimate of $[D_{\text{radio}}]$ because of the incomplete reductive dissolution of 3 d aged hydrolytic precipitated am-Fe(III) by Ti(III) treatment (Kuma & Matsunaga 1995, Yoshida et al. 2005).

At high iron concentrations, phytoplankton can often accumulate an excess of iron than that needed to support maximum growth. Such luxury uptake at high iron concentrations was observed in a culture experiment of oceanic and coastal eukaryotic algae (Sunda & Huntsman 1995, 1997, Sunda 2001). In the present study, the higher iron uptake rates ($\sim 3.4 \times 10^{-16}$ and 4.2×10^{-16} mol Fe cell^{-1} d^{-1}) by *Chaetoceros sociale* were observed during the first day of incubation in the direct Fe(III) input media and were 1.5 to 2 times higher than that in 1 d aged am-Fe(III) medium (Fig. 3A, Table 2). The iron uptake rate by eukaryotic phytoplankton is generally dependent on the concentration of dissolved Fe(III)' (Anderson & Morel 1982, Campbell 1995) although a new model for iron uptake through the extracellular reduction of all Fe species was recently proposed by Shaked et al. (2005). Therefore, the higher iron uptake rates would have been accomplished at a high iron concentration of bioavailable Fe(III)' above the equilibrium concentration

($\sim 0.1 \text{ nmol l}^{-1}$) with solid amorphous Fe(III) hydroxide in seawater during the initial incubation after the direct input of Fe(III) into the culture media. Sunda & Huntsman (1995, 1997) reported that the maximum iron accumulation in an oceanic coccolithophore and in coastal dinoflagellates was 2 to 3 times the amounts needed to support maximum growth rates, but substantially higher luxury uptake was observed in diatoms. In the present study, maximum iron accumulation (higher intracellular Fe: $3.4 \text{ to } 4.2 \times 10^{-16} \text{ mol Fe cell}^{-1}$) in the coastal diatom *C. sociale* was 3 to 5 times the amounts (minimum intracellular Fe on growth [Q_{cri}]: $\sim 1 \times 10^{-16} \text{ mol Fe cell}^{-1}$) needed to support maximal cell yields (Fig. 3, Table 2). The high iron uptake and storage capacity in *C. sociale* allows this species to accumulate excess iron at a high concentration of Fe(III)' and to support up to 2.3 cell divisions without any additional iron uptake (Fig. 3B, initial cell density: $10\,000 \text{ cells ml}^{-1}$ at 0 d). According to previous work on minimal [Q_{minimal}] and maximal [Q_{maximal}] intracellular Fe levels (Maldonado & Price 1996), phytoplankton isolated from oceanic and coastal waters have a ratio ($[Q_{\text{maximal}}]/[Q_{\text{minimal}}]$) of 15 ± 9.9 and 9.33 ± 7.54 , respectively. If [Q_{minimal}] is equal to [Q_{cri}], the ratios of 9 to 15 would support 3.2 to 3.9 cell divisions. In the present study, growth of *C. sociale* after the addition of DFB at 1, 3 and 5 d cultivations in direct Fe(III) input media (Fig. 2B, initial cell density: $1000 \text{ cells ml}^{-1}$ at 0 d; intracellular Fe was not measured) continued for 3 to 4 d up to 3.0 to 3.5 cell divisions ($\log_2 ([Cn]/[C_{\text{DFB}}])$). These values were remarkably consistent with those calculated from [$Q_{\text{maximal}}]/[Q_{\text{minimal}}$] ratios (9 to 10) of phytoplankton isolated from coastal waters (Maldonado & Price 1996). In addition, the iron uptake rate may have been affected by cell density when iron was directly added to the culture solution, probably resulting in the higher iron uptake rate with the lower cell density (Figs. 2B & 3, Table 1). However, according to the minimum Fe quota [Q_{min}] required for maximum growth rates (μ_{max}) and the maintenance Fe quota [Q_{maint}] (= [Q_{cri}] in the present study) extrapolated to where growth rate is zero, since cell yields are affected by DFB, DFB must cause the growth rate to decrease once the growth rate becomes dependent upon cellular Fe, where [Q] < [Q_{min}] but > [Q_{maint}]. In fact, a slight decrease in the growth rates ($\leq \mu_{\text{max}}$) was observed before reaching the maximal cell yields [Cn] after the addition of DFB (Figs. 2B & 3B), indicating the existence of [Q_{min}] (> [Q_{maint}]) and [Q_{maint}] (= [Q_{cri}]).

Sunda (2001) concluded that diatoms can accumulate excess iron during a period of high availability, which can then be drawn upon later to support high growth rates during blooms. The enhanced capacity for iron storage may be an important factor in allowing phytoplankton to out-compete other species during

episodic blooms. Such luxury uptake can be especially beneficial where iron concentrations are spatially and temporally variable, such as in many coastal environments, coastal and oceanic upwelling regimes, and oceanic systems receiving episodic eolian iron inputs. It has been reported that the oceanic diatom had a much lower growth requirement for cellular iron than its coastal congener, but the surface area-normalized iron uptake rates of the 2 species were similar (Sunda et al. 1991, Sunda & Huntsman 1995, 1997). The ability of the oceanic species to outgrow its neritic congener at low iron concentrations was due to its unusually low cellular iron requirement. In the present study, therefore, the oceanic species may have a lower critical concentration of intracellular Fe ($\text{mol Fe (mol C)}^{-1}$) for growth than the coastal species. The supply of high concentrated acidic iron solution to iron-limited HNLC regions in iron fertilization experiments may induce luxury iron uptake by coastal and/or large phytoplankton species at high concentrations of bioavailable Fe'.

Dissolution rate and bioavailability of aged am-Fe(III)

The hydrolytic precipitation rate of Fe^{3+} in seawater was fast, resulting in an extremely low $0.025 \mu\text{m}$ filterable (dissolved) Fe concentration with short aging time (Fig. 4A). In general, the freshly precipitated am-Fe(III), in which very fine particles with a large surface area and structural disorder occurs, consists of aggregates of hydrated ferric ions that have a very low thermodynamic stability. In the present study, some of the fine particles (polymeric ferric oxyhydroxide species) in the freshly precipitated am-Fe(III) may pass through a $0.025 \mu\text{m}$ filter. Freshly precipitated am-Fe(III) undergoes continuous chemical changes with time (loss of water and increased crystallization) that are not easily quantified. An increase in the thermodynamic stability of am-Fe(III) substantially decreases the solubility and lability of the solid am-Fe(III) phase and thereby decreases iron availability to phytoplankton (Wells et al. 1983, 1991, Kuma & Matsunaga 1995). The actual particulate solid hydroxide or oxide phase regulates the solubility of dissolved inorganic Fe species [Fe(III)']. In the present study, the solubility measurements of am-Fe(III) in seawater indicated a rapid decrease of dissolved Fe concentrations with aging time (Fig. 4A), suggesting that am-Fe(III) in seawater solution changes to larger and more stable particles with aging time (Crosby et al. 1983). In addition, lower temperature retarded the conversion to larger particles.

In a previous study (Kuma & Matsunaga, 1995), proton-promoted dissolution rates of am-Fe(III) decreased rapidly with aging time. In the present study, organic

ligand (DFB)-promoted dissolution rate of 3 d aged am-Fe(III) was slower than that of 1 d aged am-Fe(III) because of the conversion to more stable phases in culture solution with time during culture experiments (Fig. 4B). Organic ligands tend to become adsorbed specifically and to form surface complexes with the Fe(III) Lewis acid centers of the hydrous oxide surface. They also usually form complexes with Fe(III) in solution. Complex formation in solution increases the solubility. The enhancement of the dissolution rate by a ligand in surface-controlled reaction implies that surface complex formation facilitates the release of ions from the surface to the adjacent solution (Stumm 1992). In addition, there was no growth in DFB-Fe(III) (10:1) medium at 10°C (Fig. 2A) and Fe(III) dissociative precipitation of premixed DFB-Fe(III) (10:1) complex in seawater (10°C) was not observed for 10 d (Fig. 4B). The rate of DFB-Fe(III) dissociation is quite slow ($K'_{\text{FeL,Fe(III)'}} = k_{\text{formation}}/k_{\text{dissociation}} = [\text{Fe(III)L}]/[\text{Fe(III)'}][\text{L}] = 10^{16.5} \text{ M}^{-1}$). Excess ligand in this experiment (DFB-Fe(III) (10:1)) would rapidly recomplex any liberated Fe(III)'. Thus, the steady state concentration of Fe(III)' is several orders of magnitude below the expected range of oxyhydroxide formation, which should preclude the formation of a colloidal precipitate. Therefore, the dissolution of am-Fe(III) by the addition of DFB during incubation in the present study prevented further iron uptake by *Chaetoceros sociale* from ambient external iron in the culture media. These above results suggest that the bioavailability of am-Fe(III) produced in culture solution decreases rapidly with time during culture experiments, resulting from a decreased dissolution rate of am-Fe(III) with time.

Simple model for phytoplankton growth by intracellularly stored Fe in direct Fe(III) input media

The results of iron uptake and growth rate experiments indicated that the direct Fe(III) input to the culture media induced the highest Fe uptake rate ($\sim 3.4 \times 10^{-16} \text{ mol Fe cell}^{-1} \text{ d}^{-1}$) by *Chaetoceros sociale* during the first day of incubation. The highest specific growth rate ($\sim 0.5 \text{ d}^{-1}$) of *C. sociale* was maintained for a few days even after the addition of DFB. The duration of cell division yields and the maximum cell yields appeared to be dependent on the amount of intracellularly stored Fe, suggesting the presence of a critical concentration of intracellular Fe on phytoplankton growth ($[Q_{\text{cri}}] = \sim 1 \times 10^{-16} \text{ mol Fe cell}^{-1}$ for *C. sociale*). In this model, we simply assumed that $[Q_{\text{cri}}]$ in the present study is equal to the minimum Fe quota $[Q_{\text{min}}]$ required for μ_{max} and the maintenance Fe quota $[Q_{\text{maint}}]$ extrapolated to where growth rate is zero ($[Q_{\text{cri}}] = [Q_{\text{min}}] = [Q_{\text{maint}}]$), although a slight decrease in the

growth rates ($\leq \mu_{\text{max}}$) was observed before reaching the maximal cell yields after the addition of DFB (Figs. 2B & 3B) and $[Q_{\text{min}}]$ is generally $> [Q_{\text{maint}}]$. In addition, the decrease in cell concentrations after maximal cell yields (Figs. 2B & 3B) is presumably due to a continuous release of non-bioavailable Fe from cells and a temporal change from dissolved Fe to the particulate Fe phase. These model assumptions result in maximal cell yields being reached without a decrease in growth rates ($\leq \mu_{\text{max}}$) after DFB addition, very similar to the experimental results of phytoplankton growth in the present study. To understand the effect of iron on phytoplankton growth in the present study, we constructed a preliminary iron-based ecosystem model in the direct Fe input media by determining some parameters (i.e. phytoplankton growth, transfer rate from dissolved Fe to particulate Fe, excretion rate of cellular Fe from dead cell).

To interpret the temporal decrease in dissolved Fe concentrations with aging time in the Fe(III) hydrolytic precipitation experiment (Fig. 4A), we present a model assuming a first-order transfer reaction with a rate constant, k_1 , from dissolved Fe to particulate Fe at an early period of rapid Fe(III) hydrolytic precipitation and a back reaction with a rate constant, k_2 , from unstable fine particulate Fe to dissolved Fe in the slow conversion process to more stable particulate Fe with time. Hence a reciprocal of k_2 is defined as an experimental progress time t as follows:

$$k_2(t) = c_1/(c_2 + t) \quad (3)$$

where c_1 and c_2 are unknown constant values. In our previous study (Yoshida et al. 2006), we estimated the constant values of c_1 and c_2 using a fitting procedure in order to search for the best fit to the temporal change of dissolved Fe concentration (D_{Fe}) (Fig. 4A). In our model, D_{Fe} change calculated with $c_1 = 5.0 \text{ d}$ and $c_2 = 1.5 \text{ d}$ is well consistent with the measured D_{Fe} change with time in the culture experiment at 10°C (Fig. 5, Yoshida et al. 2006). Here, a time-developing model between D_{Fe} and particulate Fe concentrations (P_{Fe}) is given by:

$$dD_{\text{Fe}}/dt = -k_1 D_{\text{Fe}} + k_2(t) P_{\text{Fe}} \quad (4)$$

$$dP_{\text{Fe}}/dt = k_1 D_{\text{Fe}} - k_2(t) P_{\text{Fe}} \quad (5)$$

We can roughly estimate $k_1 = 30 \text{ d}^{-1}$ from a rapid decrease in D_{Fe} (100 nmol l^{-1} ($t = 0$) to 16 nmol l^{-1} ($t = 1 \text{ h}$) with $k_1 = 44 \text{ d}^{-1}$ and to 12 nmol l^{-1} ($t = 3 \text{ h}$) with $k_1 = 17 \text{ d}^{-1}$) at an early stage of the Fe(III) hydrolytic precipitation experiment (Fig. 4A) because of a first-order reaction of hydrolytic precipitation at $k_1 D_{\text{Fe}} \gg k_2 P_{\text{Fe}}$. Initial values of D_{Fe} and P_{Fe} at $t = 0 \text{ d}$ in the direct Fe input media are 100 and 0 nmol l^{-1} , respectively.

We constructed a simple iron-based ecosystem model using the total amount of intracellular Fe (Da) in

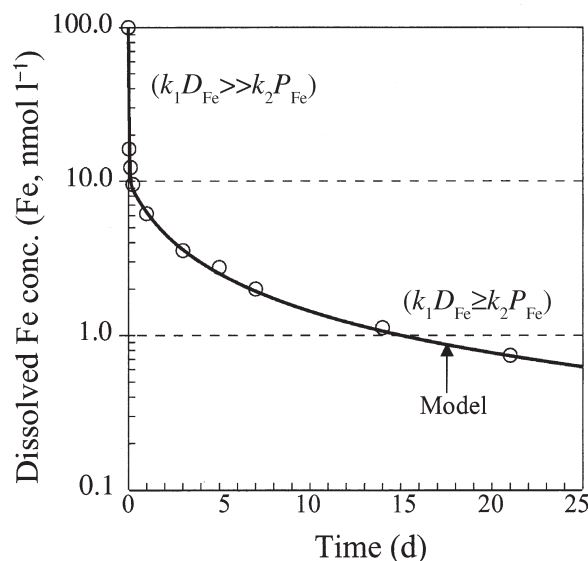


Fig. 5. Hydrolytic precipitation rate model of Fe^{3+} in seawater with $100 \text{ nmol Fe l}^{-1}$ at 10°C (thick line: hydrolytic precipitation rate model; open circle: observed dissolved Fe concentration). The model assumed a first-order transfer reaction with a rate constant, k_1 , from dissolved Fe (D_{Fe}) to particulate Fe (P_{Fe}) at an early period of rapid Fe(III) hydrolytic precipitation and a back reaction with a rate constant, k_2 , from unstable fine particulate Fe to dissolved Fe in the slow conversion process to more stable particulate Fe with time (Yoshida et al. 2006)

phytoplankton and D_{Fe} and P_{Fe} in seawater to represent the results of culture experiment (10°C) in the present study. This system is represented by the following ordinary differential equations. The equations for D_{Fe} in seawater and Da in phytoplankton can be written as follows:

$$dD_{\text{Fe}}/dt = -\sigma Da - k_1 D_{\text{Fe}} + k_2 P_{\text{Fe}} \quad (6)$$

$$dDa/dt = \sigma Da \quad (7)$$

where $Da = D_{\text{radio}}$ in the present study (Fig. 3A, Table 2). The function of σ denotes the iron uptake rate by phytoplankton, which is only a function of D_{Fe} and not limited by light and other nutrients. That is,

$$\sigma = \sigma_{\text{max}} M \quad (8)$$

where σ_{max} is the maximum iron uptake rate by phytoplankton and M will show classic saturation kinetics with increased external concentration of D_{Fe} , as expressed by the Michaelis-Menten equation as follows:

$$M = D_{\text{Fe}}/(D_{\text{Fe}} + K) \quad (9)$$

where K is the half-saturation constant for D_{Fe} . In the present study, we used a K value of 1.1 nmol l^{-1} for the large chain-forming diatom *Chaetoceros dictyota* determined by Timmermans et al. (2001). We assume that the amount of Fe released from the phytoplankton cell after

the maximal cell yields is proportional to the total cellular Fe. The value of 0.1 d^{-1} was roughly estimated as the excretion rate of cellular Fe from the decreasing rate of cell number after the maximal cell yields (Fig. 3B). In our model, we ignored the mortality of phytoplankton and the excretion of cellular Fe during the exponential growth phase before the maximal cell yields.

To examine the cellular Fe concentration cell^{-1} , we counted the model's phytoplankton cell number (Cn) following:

$$dCn/dt = \sigma_c Cn \quad (10)$$

where σ_c is a growth rate of phytoplankton. Simply, we consider that σ_c is the same as σ_{max} . The value of $\sigma_{\text{max}} = \sigma_c$ can be a typical apparent growth rate (0.5 d^{-1}) measured during culture experiments in the direct Fe(III) input media (Figs. 2B & 3B, Table 1). In our model, we simply used the growth rate ($\sim 0.5 \text{ d}^{-1}$) observed in the iron uptake and growth rate experiments (Fig. 3B, Table 1).

Although the highest σ by phytoplankton was observed at the initial stage in the long-term iron uptake experiments in the direct Fe(III) input media (Fig. 3A), the maximum uptake rate at t close to 0 d cannot be determined. Therefore, we estimated a model's initial value of Fe uptake with 3.9 nmol l^{-1} at $t = 0.5 \text{ d}$ in the culture experiments in the direct Fe(III) input media (no DFB, DFB (1 d) and DFB (3 d)) from reverse calculation using a simple exponential growth rate with $\sigma_c = 0.5 \text{ d}^{-1}$ and the experimental value of Fe uptake with $Da = 5 \text{ nmol l}^{-1}$ at $t = 1 \text{ d}$ (Fig. 3A). In the case of 1 d aged am- Fe(III) medium (DFB (1 d)), a value of 2.3 nmol l^{-1} at $t = 0.5 \text{ d}$ was estimated from the value of Fe uptake with $Da = 3 \text{ nmol l}^{-1}$ at $t = 1 \text{ d}$ (Fig. 3A). In addition, a cell number of $Cn = 12840 \text{ cells ml}^{-1}$ at $t = 0.5 \text{ d}$ was estimated from an exponential growth rate (0.5 d^{-1}) and $Cn = 10000 \text{ cells ml}^{-1}$ at $t = 0 \text{ d}$. The D_{Fe} and P_{Fe} values of 7.8 and 92.2 nmol l^{-1} , respectively, at $t = 0.5 \text{ d}$ were easily calculated from time-developing model equations (Eqs. 4 & 5) (Fig. 5). The growth conditions for Da and Cn are as follows: (1) $\sigma_{\text{max}} = 0.0 \text{ d}^{-1}$, no further iron uptake from external iron by phytoplankton after addition of DFB and (2) $\sigma_c = 0.0 \text{ d}^{-1}$, no growth below a critical concentration of intracellular Fe (total amount of intracellular Fe cell^{-1} : Da/Cn) on phytoplankton growth. We could simulate temporal changes of Da and Cn using a value of $[Da/Cn] = [Q_{\text{cri}}] = 1 \times 10^{-16} \text{ mol Fe cell}^{-1}$ defined as a critical concentration of intracellular Fe cell^{-1} ($[Q_{\text{cri}}]$, Table 2).

Fig. 6 presents the changes in Da , Da/Cn and Cn with time, resulting from the simulation of our model. The phytoplankton growth rates and maximal cell yields in the direct Fe(III) input media (no DFB, DFB (1 d) and DFB (3 d)) and 1 d aged am- Fe(III) medium (DFB (1 d)) obtained from our model (Fig. 6C) are similar to those observed in the culture experiments

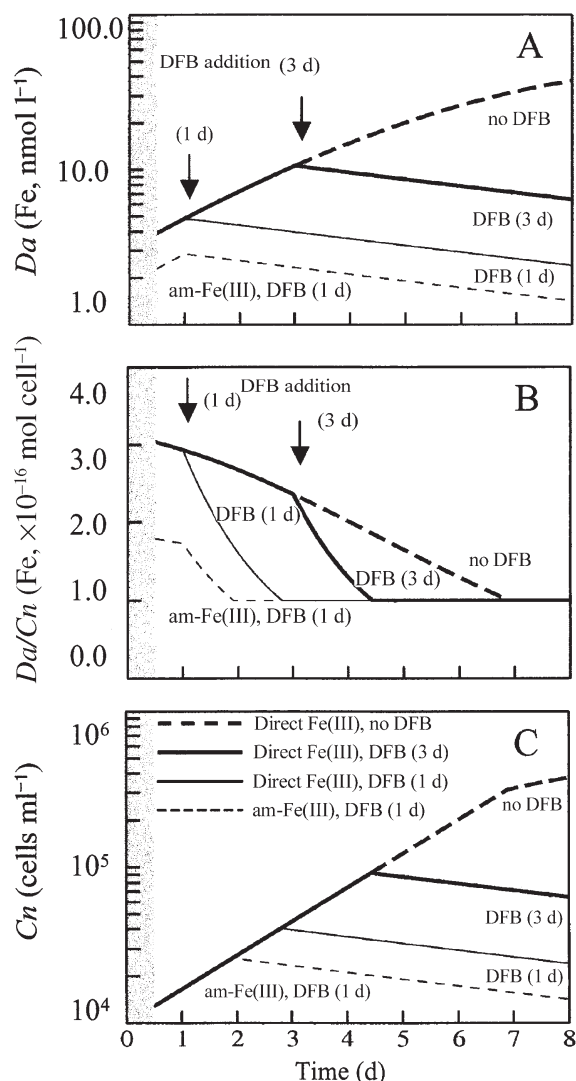


Fig. 6. Time-course models of (A) Fe uptake (Da), (B) intracellular Fe concentration cell⁻¹ (Da/Cn) and (C) cell number (Cn) in the direct Fe(III) input media (no DFB, DFB 1 d) and DFB (3 d) and 1 d aged am-Fe(III) medium (DFB 1 d) during the cultivation. Maximum uptake rate at $t = 0$ d cannot be determined and is indicated by the stippled area on the left of each panel

(Figs. 2B & 3B). The similarity suggests that the growth rate of phytoplankton is independent of the amount of intracellularly stored Fe, above a critical concentration of intracellular Fe on the growth, and the maximal cell yields ($[Cn]$) are controlled by a critical intracellular Fe ($[Da/Cn] = [Q_{crit}]$). However, maximum cell concentration (approximately 300 000 cells ml⁻¹ at 7 d, Fig. 6C) in the direct Fe(III) input medium (no DFB) obtained from our model is remarkably different from that in the culture experiment ($126\,000 \pm 20\,000$ cells ml⁻¹ at 5 d, Fig. 3B and Table 1) probably because of the rapid decrease in bioavailability of hydrolytic precipitated Fe with time in the medium (Kuma & Matsunaga 1995)

and/or the change of chemical environment in the culture solution during cultivation experiments.

Our results suggest that the high iron uptake and storage capacity in phytoplankton allows them to accumulate excess iron at a high concentration of bioavailable Fe' (Fe(II)' and Fe(III)') in estuarine mixing systems and coastal waters and in mesoscale iron fertilization experiments. The high supply of Fe' in marine environments is one of the most important mechanisms for providing bioavailable Fe' into these environments. The luxury iron uptake and the presence of critical intracellular Fe concentration per cell on phytoplankton growth are important factors in supporting high growth rates during blooms. DFB treatment during incubation in the Fe input media is a unique technique for measuring the iron uptake and storage capacity in phytoplankton by preventing further iron uptake from external iron in the culture media.

Acknowledgements. We are grateful to the anonymous reviewers for their constructive and helpful comments on this work. This study was partially supported by grants for Environmental Research (No. 023039) from the Sumitomo Foundation and for Scientific Research (No. 17651001) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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*Editorial responsibility: Paul J. Harrison,
Kowloon, Hong Kong, SAR*

*Submitted: June 10, 2005; Accepted: February 27, 2006
Proofs received from author(s): June 12, 2006*