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1 Effect of aging time on the availability of freshly precipitated ferric
2 hydroxide to coastal marine diatoms

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31 marine diatom · *Chaetoceros sociale* · *Thalassiosira weissflogii*

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33 Running title: Bioavailability of ferric hydroxide to marine diatoms

1 **Abstract**

2 Cell growth and iron uptake of the coastal marine diatoms, *Chaetoceros*
3 *sociale* and *Thalassiosira weissflogii*, in the presence of short-aged amorphous ferric
4 hydroxide (am-Fe(III)) media, which were prepared by aging for 1 d, 3 d and 3 weeks
5 after adding a small amount of ferric iron acidic stock solution to autoclaved filtered
6 seawater, were experimentally measured in culture experiments at 10°C for *C. sociale*
7 and 20°C for *T. weissflogii*. The order of cell yields for both species was: 1-d aged
8 am-Fe(III) > 3-d aged am-Fe(III) >> 3-weeks aged am-Fe(III) media. The iron uptake
9 rates by *C. sociale* during 0–1 d in 1-d and 3-d aged am-Fe(III) media were about
10 two-third and one-fourth, respectively, lower than that in the direct Fe(III) input medium
11 containing *C. sociale* into which an acidic Fe(III) stock solution was added directly. The
12 longer aging time of am-Fe(III) in media results in reducing the supply of bioavailable
13 iron in media by the slower dissolution rate of am-Fe(III) with the longer aging time.
14 These results suggest that the chemical and structural changes of freshly precipitated
15 amorphous ferric hydroxide with short aging time affect their ability, such as iron
16 solubility and dissolution rate, to supply bioavailable iron for the phytoplankton growth.
17 The chemical and structural conversion of solid iron phases with time is one of the most
18 important processes in changing the supply of available iron to marine phytoplankton in
19 estuarine and coastal waters and in iron fertilization experiments.

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1 Introduction

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3 Iron is an essential micronutrient for phytoplankton growth, as an important
4 component of such biochemical processes as photosynthetic and respiratory electron
5 transport, nitrate and nitrite reduction, and a number of other biochemical reactions
6 (Weinberg 1989; Geider and Roche 1994). Acquisition of iron by phytoplankton occurs
7 through a complex series of extracellular reactions that are influenced by iron chemistry
8 and speciation in seawater. In oxic seawater, iron is present predominantly in the
9 insoluble (extremely low solubility) and thermodynamically stable 3+ oxidation state
10 (Morel and Hering 1993; Stumm and Morgan 1996; Waite 2001). The inorganic
11 speciation of Fe(III) in seawater is dominated by its hydrolysis behavior and ready
12 tendency to precipitate to particulate Fe(III) hydroxide. Recent studies of the Fe(III)
13 hydroxide solubility in seawater suggest that the Fe(III) solubility is controlled by
14 organic complexation (Kuma et al. 1996; Waite 2001; Liu and Millero 2002; Tani et al.
15 2003; Chen et al. 2004; Takata et al. 2004, 2005), which, subsequently, regulates
16 dissolved iron concentrations in seawater (Johnson et al. 1997a, b; Archer and Johnson
17 2000; Nakabayashi et al. 2001; Kuma et al. 2003; Rose and Waite 2004). There is also a
18 strong temperature effect here with high solubility at low temperatures (Liu and Millero
19 2002). In a previous study (Kuma et al. 1996), the iron solubility limit of fresh solid
20 amorphous Fe(OH)₃ in ultraviolet (UV)-irradiated open-ocean waters (free of organic
21 ligands) was 0.07–0.09 nmol L⁻¹ (≤0.1 nmol L⁻¹). In addition, Wu et al. (2001) also
22 reported that the iron solubility limit for inorganic Fe(III) hydrolysis species (Fe(III)') in
23 the UV-irradiated seawater was ~0.08±0.03 nmol L⁻¹. Therefore, the equilibrium
24 concentration of ~0.1 nmol L⁻¹ only applies for iron in equilibrium with a fresh
25 amorphous solid Fe(OH)₃ and is a maximum limit on Fe(III)' in seawater.

26 In general, the iron uptake rate by phytoplankton is primarily a function of the
27 equilibrium concentration of Fe³⁺ in seawater and is actually dependent on the
28 concentration of dissolved inorganic Fe(III) species ([Fe(III)']), which is proportional to
29 [Fe³⁺] (Free-ion activity model (FIAM)) (Anderson and Morel 1982; Hudson and Morel
30 1990; Campbell 1995; Sunda 2001), although apparent exceptions to the FIAM exist:
31 for example, specific transport ligands such as siderophores that may be directly or
32 indirectly utilized by cells (Maldonado and Price 2000, 2001; Sunda 2001). Therefore,
33 the iron uptake of phytoplankton is limited by the equilibrium concentration of Fe(III)'

1 with particulate Fe(III) hydroxide and the slow dissolution rate of particulate Fe(III) in
2 seawater if the dissolution rate of particulate Fe(III) is slow in relation to the further
3 demands of the phytoplankton. Phytoplankton growth is probably controlled by the
4 solubility and the dissolution rate of particulate Fe(III) hydroxide. In the previous
5 studies (Kuma et al. 1999, 2000), it has been suggested that the natural organic Fe(III)
6 complexes, such as fulvic-Fe(III) complex, and acidic Fe(III) supplied by riverine and
7 eolian inputs play an important role in supplying bioavailable Fe(III)', above the
8 equilibrium concentration of Fe(III)', in estuarine mixing systems and coastal waters
9 through its dissociation and hydrolytic precipitation at high pH of seawater and high
10 levels of seawater cations (Stumm and Morgan 1996). Recently, it has been reported
11 that small colloidal Fe (<0.2 μm size) and Fe bound with natural colloids were
12 biologically available depending on the colloidal geochemical characteristics (Nishioka
13 and Takeda 2000; Chen and Wang 2001; Chen et al. 2003; Wang and Dei 2003).
14 However, the crystalline ferric oxyhydroxides and ferric oxides, such as α -FeOOH
15 (goethite), β -FeOOH (akaganeite) and α -Fe₂O₃ (hematite) did not support autotrophic
16 phytoplankton growth at all (Wells et al. 1983; Rich and Morel 1990; Kuma and
17 Matsunaga 1995). Therefore, the thermodynamic stability of iron colloids may be
18 important in controlling the supply of bioavailable inorganic species of iron.

19 It is assumed that the chemical and structural changes of freshly precipitated
20 amorphous ferric hydroxide (am-Fe(III)) with time and water temperature in estuarine
21 and coastal waters affect their ability, such as iron solubility and dissolution rate of
22 am-Fe(III), to supply bioavailable iron. In the present study, we examined that the
23 chemical changes of freshly precipitated am-Fe(III) with aging time at 10 and 20°C will
24 affect their ability to supply iron for the growth of coastal marine diatoms *Chaetoceros*
25 *sociale* and *Thalassiosira weissflogii*. Solid am-Fe(III) forms used in our study are
26 short-aged amorphous ferric hydroxide at 10 and 20°C, which were prepared by aging
27 for 1 d, 3 d and 3 weeks after adding a small amount of ferric iron acidic stock solution
28 to autoclaved filtered seawater (10°C for *C. sociale* and 20°C for *T. weissflogii*).

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31 **Materials and methods**

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33 In the present study, seawater was collected from a coastal region near

1 Hokkaido, Japan, in the northern Japan Sea (salinity= 33.8) and was filtered through an
2 acid-cleaned 0.22- μm Millipore cellulosic membrane filter. The Fe concentration in the
3 filtered seawater was determined by an automated Fe analyzer (Kimoto Electric) with
4 use of a combination of chelating resin concentration and luminol-hydrogen peroxide
5 chemiluminescence (CL) detection in a closed flow-through system (Obata et al. 1993,
6 1997) after the filtered seawater was buffered at pH 3.2 with a 10 mol L⁻¹ formic
7 acid–2.4 mol L⁻¹ ammonium formate buffer solution (0.5 ml per 100-ml filtrate). The Fe
8 concentration was ≤ 2 nmol L⁻¹. The concentrations of NO₃+NO₂, PO₄, and Si(OH)₄ in
9 the filtered seawater measured by a Technicon autoanalyzer were less than 0.5, 0.1, and
10 5 $\mu\text{mol L}^{-1}$, respectively, which are negligible values compared with the concentrations
11 added in the culture experiments (*see below*). Desferrioxamine B (DFB, sold
12 commercially under the tradename Desferal), which is a strong Fe(III)-complexing
13 agent forming a 1:1 DFB-Fe(III) complex, was purchased from Sigma Chemical as a
14 fungal hydroxamate siderophore. The terrestrial fungal siderophore DFB is light
15 sensitive and should be stored at less than 0°C. It has been found that addition of excess
16 concentrations of the hydroxamate siderophore DFB essentially eliminated iron uptake
17 in picoplankton-dominated community by diminishing the concentration of bioavailable
18 Fe(III)^{*} (Wells et al. 1994) and regulated iron availability in coastal upwelling waters
19 (Wells 1999; Hutchins et al. 1999; Wells and Trick 2004). DFB is a small
20 trihydroxamate molecule that specially complexes inorganic Fe(III) with an extremely
21 high conditional stability constant ($K'_{\text{FeL,Fe(III)}} = [\text{Fe(III)L}]/[\text{Fe(III)}^*][\text{L}^*] = 10^{16.5} \text{ M}^{-1}$; Rue
22 and Bruland 1995) in seawater (Hudson et al. 1992). In the present study, the growth
23 with intracellular Fe stored by initial iron uptake of phytoplankton was measured by
24 addition of hydroxamate siderophore DFB to prevent further iron uptake from ambient
25 extracellular Fe in the media.

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28 Culture experiments

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30 All the preparation and sampling experiments were performed in a Class 100
31 laminar flow cabinet to avoid in advertent trace metal contamination. The polycarbonate
32 bottles for the diatom culture and uptake experiments were first soaked in acid and
33 rinsed with Milli-Q water (18.0 M Ω). The filtered seawater was autoclaved for 20 min

1 at 121°C (1.1 kg cm⁻² pressure), and the culture medium was prepared by adding
2 modified f/2 nutrient (Guillard and Ryther 1962) without trace metals and EDTA to the
3 autoclaved filtered seawater. The modified f/2 medium contained 880 µmol L⁻¹ nitrate,
4 38 µmol L⁻¹ phosphate, and 105 µmol L⁻¹ silicate. The large chain-forming coastal
5 marine diatoms *C. sociale* and *T. weissflogii* were grown in 1 liter of the f/2 media at 10
6 and 20°C, respectively, to which ferric iron stock solution (25 µmol L⁻¹ Fe(III);
7 FeNH₄(SO₄)₂·12H₂O in 5 mmol L⁻¹ HCl, pH 2.3) was added to make an iron
8 concentration of 100 nmol L⁻¹. Cells were grown under 150 µmol photons m⁻² s⁻¹
9 fluorescent light (12 h light: 12 h dark) to obtain the cell concentration expected at the
10 start of the following culture and iron uptake experiments.

11 Small amounts of ferric iron acidic stock solution and DFB solution were
12 immediately mixed in a precleaned 100-ml polycarbonate Erlenmeyer flask.
13 Desferrioxamine B medium was prepared by adding 50 ml of autoclaved filtered
14 seawater at 10°C for *C. sociale* (cell diameter: ~6 µm, height: ~12 µm) and at 20°C for
15 *T. weissflogii* (cell diameter: ~8 µm, height: ~13 µm) to premixed DFB-Fe(III) (10:1)
16 solution in the flask to ensure which DFB:Fe(III) (10:1) inhibit the growth of *C. sociale*
17 and *T. weissflogii* over at least 11–15 d in culture experiments. Solid amorphous ferric
18 hydroxide (am-Fe(III)) media were prepared by aging for 1 d, 3 d and 3 weeks after
19 adding a small amount of ferric iron acidic stock solution to 50 ml of autoclaved filtered
20 seawater (10°C for *C. sociale* and 20°C for *T. weissflogii*). The final iron concentration
21 was 100 nmol L⁻¹. An f/2 nutrient stock solution and then a small amount of culture (ca.
22 250 µl) in an initial stationary growth phase were added to each culture flask. All major
23 nutrient stocks were passed through Chelex 100 ion-exchange resin to remove trace
24 metals (Morel et al. 1979). The Fe concentration in the control culture medium (the
25 autoclaved filtered seawater which nutrient was added) was ≤3.5 nM. The effect of
26 direct Fe input was examined by adding a small amount of acidic ferric iron stock
27 solution directly together with an inoculation of culture into the control culture media.
28 In the previous study (Kuma et al. 2000), the high Fe(III)' concentration in direct Fe(III)
29 input medium was remarkably bioavailable and induces the highest iron uptake and
30 growth of coastal marine phytoplankton. In addition, the growth of phytoplankton by
31 intracellular Fe were examined by addition of DFB to final concentration of 1 µmol L⁻¹
32 (DFB:Fe(III)=10:1) after 1 d cultivation in direct Fe(III) input medium and in 1-d and
33 3-d aged am-Fe(III) media to prevent further iron uptake by phytoplankton from

1 ambient external Fe (dissolved Fe(III) and solid Fe(III) hydroxide in media). Moreover,
2 *T. weissflogii* was grown in 1-d aged am-Fe(III) media and direct Fe(III) input media
3 with iron concentrations of 10, 25 and 50 nmol L⁻¹ in addition to 100 nmol L⁻¹. Control
4 (without any added iron) media were prepared to compare the growth rates and cell
5 yields with those containing iron. Cell concentrations at the start of the culture
6 experiments were approximately 1,000 cells ml⁻¹. The light, temperature and nutrient
7 conditions were the same as those of the stock culture described above. During the
8 experiments, cell growth was monitored daily by triplicate cell counts done with an
9 optical microscope. Culture experiments were conducted in triplicate.

12 Iron uptake experiments

14 Iron uptake experiments were conducted with *C. sociale* grown in direct
15 Fe(III) input and 1-d and 3-d aged am-Fe(III) media which DFB was added just after 1
16 d cultivation. The long-term (daily for 1–7 d) iron uptake experiments were carried out
17 in media to which ⁵⁹Fe(III) was added. Incubations were done at 10°C under 150 μmol
18 photons m⁻² s⁻¹ fluorescent light (12 h light: 12 h dark).

19 The direct ⁵⁹Fe(III) input culture was prepared by adding a small amount of
20 premixed solution of acidic stock radioactive ⁵⁹Fe(III) (New England Nuclear Corp.,
21 NEZ-037) solution (7 μmol L⁻¹ Fe(III); ⁵⁹FeCl₃ in HCl, pH ~2.2–2.3) and ferric iron
22 acidic stock solution (25 μmol L⁻¹ Fe(III), pH 2.3) to 1000 ml of autoclaved filtered
23 seawater (10°C), which already contained modified f/2 nutrient and *C. sociale* culture in
24 initial stationary growth phase. The pH of culture solution after premixed acidic
25 radioactive ⁵⁹Fe(III) addition was 7.98–8.05. Solid aged am-⁵⁹Fe(III) cultures were
26 prepared by adding modified f/2 nutrient and *C. sociale* culture (approximately 50 ml),
27 after adding premixed ⁵⁹Fe(III) solution to 950 ml of autoclaved filtered seawater
28 (10°C) and then aging for 1 and 3 d at 10°C. *C. sociale* cell concentration at the start of
29 the iron uptake experiments were ca. 10,000 cells ml⁻¹. The final iron concentration was
30 100 nmol L⁻¹. The nutrient concentrations were the same as those in the culture
31 experiments described above.

32 At each sample point (daily for 1–7 d) during the long-term iron uptake
33 experiment, a 50-ml aliquot was mixed with 20 ml of 0.175 mol L⁻¹

1 Ti(III)–citrate–EDTA solution ($0.175 \text{ mol L}^{-1} \text{ TiCl}_3$, $\text{Na}_3\text{citrate}\cdot 2\text{H}_2\text{O}$, and
2 $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$ in filtered seawater, followed by an adjustment of the pH to 8 with
3 NaOH solution) as demonstrated by Hudson and Morel (1989, 1990). The Ti(III)
4 solution was used to rapidly dissolve freshly precipitated am-Fe(III) and extracellularly
5 adsorbed iron by reductive dissolution of Fe(III) without cellular damage to *C. sociale*.
6 After the mixture was allowed to stand for 10 min, it was gently vacuum filtered
7 through a quantitative filter paper (No. 5C, Advantec) that retains all precipitate $\geq 1 \mu\text{m}$
8 in size. The filter was rinsed with 30 ml of 0.05 mol L^{-1} Ti(III) solution which was
9 prepared by diluting 0.175 mol L^{-1} Ti(III)–citrate–EDTA solution with filtered seawater.
10 The drained filter was digested with 7 ml of conc. HNO_3 : conc. HClO_4 (1:1) and then
11 diluted to 20 ml. The γ -activity of 4 ml of diluted sample in a counting vial was
12 measured using a gamma counter (Aloka ARC-301B), and the results were converted to
13 amounts and rates of iron incorporation. In addition, iron uptake experiments for
14 cell-free control media (direct $^{59}\text{Fe(III)}$ and 1-d and 3-d aged am- $^{59}\text{Fe(III)}$) at 10°C were
15 conducted to ascertain the reductive dissolution of freshly precipitated and aged
16 am-Fe(III) by the Ti(III) solution. In previous studies (Kuma and Matsunaga 1995;
17 Kuma et al. 2000), freshly precipitated am-Fe(III) (aged for less than 1 d at 10°C) was
18 almost completely dissolved by the reductive dissolution with Ti(III) treatment.
19 However, aged am-Fe(III) produced in direct Fe(III) input cell-free media during aging
20 above 1 d at 10°C was incompletely reductively dissolved by Ti(III) solution in the
21 present study and even by new oxalate reagent wash technique (Tovar-Sanchez et al.
22 2003) because of the much slower dissolution of aged am-Fe(III). The dissolution rates
23 of am-Fe(III) phase decrease with aging time because of the slow conversion to more
24 stable phases (Kuma and Matsunaga 1995). Therefore, iron uptake at each sample point
25 during iron uptake experiments was determined by subtracting the amount of insoluble
26 iron in cell-free medium from the amount of iron uptake in medium containing cells.
27 During the experiments, cell growth at each culture was monitored by triplicate cell
28 counts done with an optical microscope.

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31 Hydrolytic precipitation of Fe(III) in seawater

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33 Hydrolytic precipitation rate of Fe(III) in the $0.22\text{-}\mu\text{m}$ -filtered seawater was

1 measured at 10°C and 20°C by a simple filtration technique involving γ -activity
2 measurement of ^{59}Fe previously reported by Kuma et al. (1996, 1998a, 1998b). To
3 examine the effect of aging time and temperature (10 and 20°C) on hydrolytic
4 precipitation of Fe(III) in seawater, a small amount of $^{59}\text{Fe(III)}$ stock solution,
5 previously spiked with a small known amount of stable Fe(III), was added to 250 ml of
6 the 0.22- μm -filtered seawater in acid-cleaned 250-ml Teflon bottles (10 and 20°C). The
7 final iron concentration was 100 nmol L⁻¹. In general, the addition of dissolved
8 inorganic Fe(III) to seawater results in rapid hydrolytic precipitation of metastable
9 Fe(III) hydroxide, which slowly converts to more stable solid phases (Kuma and
10 Matsunaga 1995; Stumm and Morgan 1996). The bottles containing the seawater
11 solution and radiolabelled Fe(III) were kept in an incubator (dark condition) at 10 and
12 20°C. During standing in the dark for 3 weeks at 10 and 20°C, each 7.5-ml sample
13 aliquot was filtered through a 0.025- μm Millipore cellulosic membrane filter and
14 acidified by addition of 10 μl of concentrated HCl to prevent adsorption of filtered
15 Fe(III) on the wall of the collecting vial. The γ -activity of the 2.5-ml acidified sample
16 filtrates were measured in 5-ml counting vials with a gamma counter. Finally, the
17 0.025- μm -filtered Fe(III) concentrations (Fe(III) hydroxide solubility) were calculated
18 from the γ -activity (Kuma et al. 1996, 1998a).

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21 **Results**

22

23 Growth rate and cell yields for *C. sociale* and *T. weissflogii*

24

25 Fig. 1 presents the results of the culture experiments for *C. sociale* (10°C) and
26 *T. weissflogii* (20°C) in direct Fe(III) input and DFB-Fe(III) (10:1) media. In the present
27 study, direct Fe(III) input to the culture solution induced the highest growth rate and the
28 highest maximal cell yields (0.52 d⁻¹ and 150,000 cells ml⁻¹ for *C. sociale* and
29 0.63–0.64 d⁻¹ and 170,000 cells ml⁻¹ for *T. weissflogii*, respectively, Table 1), similar
30 value to that in the same medium for *C. sociale* in the previous study (Kuma et al. 2000).
31 However, there was no growth in DFB-Fe(III) (10:1) medium for both species.
32 Therefore, the effect of direct Fe(III) input, aging time of particulate am-Fe(III) and the
33 growth by intracellularly stored Fe were examined by addition of DFB (final

1 concentration of $1 \mu\text{mol L}^{-1}$ (DFB:Fe(III)=10:1)) during cultivation to prevent iron
2 uptake from ambient extracellular Fe. Desferrioxamine B was added to *C. sociale* and *T.*
3 *weissflogii* cultivated for 1 d in direct Fe(III) input medium (direct Fe(III)-DFB) and 1-d
4 and 3-d aged am-Fe(III) media (1-d aged am-Fe(III)-DFB; 3-d aged am-Fe(III)-DFB).

5 For the culture experiment of *C. sociale* without any addition of DFB, the
6 relative order for maximal cell yields on different media for 8–12 days cultivation was
7 direct Fe(III) input > 1-d aged am-Fe(III) > 3-d aged am-Fe(III) >> 3-weeks aged
8 am-Fe(III) > control (no iron) although the initial growth rates in all media except for
9 control were almost same with $0.45\text{--}0.53 \text{ d}^{-1}$ (Fig. 2a, Table 1). These results then
10 suggest that the time history of exposure to iron is very important in assessing the
11 growth rate. The growth in direct Fe(III)-DFB, 1-d aged am-Fe(III)-DFB and 3-d aged
12 am-Fe(III)-DFB media (Fig. 2b) continued for 5–8 d even after addition of DFB with
13 almost same maximum growth rate ($\sim 0.42\text{--}0.49 \text{ d}^{-1}$) as those in all Fe(III) media
14 without any addition of DFB (Table 1). Cell concentration in the direct Fe(III)-DFB
15 medium increased up to approximately $40,000 \text{ cells ml}^{-1}$, 40 times more than initial cell
16 concentration, by 8 d cultivation after addition of DFB. The relative order for maximal
17 cell yields was direct Fe(III)-DFB > 1-d aged am-Fe(III)-DFB > 3-d aged
18 am-Fe(III)-DFB media, while the initial growth rates were almost the same in all media
19 (Fig. 2b).

20 For the culture experiment of *T. weissflogii*, the maximal cell yields and the
21 growth rates were the same in all media, except for 3-w aged am-Fe(III) and control
22 media, without any addition of DFB (Fig. 3a, Table 1). However, the growth in direct
23 Fe(III)-DFB, 1-d aged am-Fe(III)-DFB and 3-d aged am-Fe(III)-DFB media continued
24 for 4–5 d even after addition of DFB with the almost same maximum growth rate
25 ($\sim 0.57\text{--}0.61 \text{ d}^{-1}$) as those in all Fe(III) media without addition of DFB but with different
26 maximal cell yields (Fig. 3b, Table 1). The similar maximal growth rates but different
27 cell yields suggest that the length of the exponential growth phase differed between the
28 iron treatments. The order of maximal cell yields after addition of DFB was direct
29 Fe(III)-DFB > 1-d aged am-Fe(III)-DFB > 3-d aged am-Fe(III)-DFB media, the same
30 order as that for *C. sociale* (Table 1). The maximal cell yields in direct Fe(III) input
31 media with lower iron concentrations ($10, 25$ and 50 nmol L^{-1}) than 100 nmol L^{-1} were
32 higher than those in 1-d aged am-Fe(III) media with same iron concentrations (Fig. 3c,
33 Table 1) although the maximal cell yields and the growth rates were the same in direct

1 Fe(III) input, 1-d aged am-Fe(III) and 3-d aged am-Fe(III) media with iron
2 concentration of 100 nmol L⁻¹ (Fig. 3a).

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5 Iron uptake and growth rate of *C. sociale*

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7 The long-term iron uptake rates by *C. sociale* and the growth in direct Fe(III)
8 input medium and 1-d and 3-d aged am-Fe(III) media, into which DFB were added after
9 1 d cultivation, were measured during cultivation for 7 d at 10°C (Fig. 4). The cellular
10 iron uptake (nmol L⁻¹) by *C. sociale* in all media was prevented by addition of DFB and
11 remained nearly constant for 4 d after DFB treatment (Fig. 4a). However, the iron
12 uptakes after 5 d decreased gradually with decreasing in cell concentrations during 5–7
13 d. The iron uptake rate during 0–1 d in 3-d aged am-Fe(III) medium was approximately
14 one-fourth lower than that in direct Fe(III) input medium and two-fifth lower than that
15 in 1-d aged am-Fe(III) medium. The order of mean iron uptake for 4 d after addition of
16 DFB was direct Fe(III)-DFB > 1-d aged am-Fe(III)-DFB > 3-d aged am-Fe(III)-DFB
17 (Fig. 4a, Table 2).

18 Cell concentrations in media, which were prevented from further iron uptake
19 from ambient extracellular Fe by addition of DFB, increased logarithmically for 1–2 d
20 even after addition of DFB (Fig. 4b). The initial growth rates (0.44±0.04–0.47±0.01 d⁻¹)
21 in DFB-added media were nearly the same as those (0.42–0.53 d⁻¹) in all media with
22 and without addition of DFB (Table 1). However, the maximal cell yields in DFB-added
23 media were one-fourth to one-half lower than that in direct Fe(III) input medium
24 without any addition of DFB. The order of maximal cell yields was: direct Fe(III) input
25 (no DFB) > direct Fe(III)-DFB > 1-d aged am-Fe(III)-DFB > 3-d aged am-Fe(III)-DFB.

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28 Hydrolytic precipitation of Fe(III) at 10 and 20°C

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30 Figure 5 presents the results of the Fe(III) hydrolytic precipitation rates of
31 Fe³⁺ in filtered seawater at 10 and 20°C. The hydrolytic precipitation rates of Fe³⁺ in
32 filtered seawater were extremely fast, resulting in extremely low 0.025 µm filterable
33 (dissolved) Fe concentrations within short aging times. For example, the dissolved Fe

1 concentrations with 1 d aging were 6.2 and 1.9 nmol L⁻¹ at 10 and 20°C, respectively.
2 The dissolved Fe would include small colloidal and soluble Fe species and the colloidal
3 species may be removed rapidly through particle/particle interactions (Honeyman and
4 Santschi 1991). In addition, the hydrolytic precipitation rate of Fe³⁺ at 10°C was slower
5 than that at 20°C. The dissolved Fe concentrations at 10°C decreased gradually 1.98 to
6 0.74 nmol L⁻¹ during aging for 1 to 3 weeks, while those at 20°C were nearly constant
7 within the range 0.26 to 0.36 nmol L⁻¹ during 1–3 weeks. The long term dissolved
8 (soluble) iron concentrations of ~0.3 nmol L⁻¹ are most likely due to the presence of
9 soluble iron organic species as mentioned above.

12 Discussion

14 Effect of aging time on bioavailability of freshly precipitated ferric hydroxide

16 Direct Fe(III) input medium promoted the maximal cell yields and growth rate
17 (Fig. 1). However, there was no growth in premixed DFB-Fe(III) complex (10:1) media.
18 In general, iron uptake rate by marine eukaryotic phytoplankton is related to the
19 concentration of kinetically labile inorganic Fe species ([Fe²⁺]: [Fe(II)] and [Fe(III)]),
20 which is proportional to [Fe²⁺] and [Fe³⁺], respectively, and independent of the
21 concentration of Fe chelated to organic ligands such as EDTA and DFB (Campbell 1995,
22 Sunda 2001) although the iron uptake rates from DFB-Fe complex by iron-limited
23 phytoplankton are more correlated to the concentrations of DFB-Fe complex than those
24 of Fe²⁺ (Maldonado and Price 2000, 2001). The equilibrium calculation in premixed
25 DFB-Fe(III) (10:1; Fe(III)=100 nmol L⁻¹) medium indicates that bioavailable Fe(III)
26 would be limited to ~10⁻¹⁷ mol L⁻¹ ($K'_{FeL,Fe(III)} = 10^{16.5}$ M⁻¹, Rue and Bruland 1995,
27 Croot and Johansson 2000), or several orders of magnitude below the levels needed to
28 support phytoplankton growth. In the present study, the direct input of Fe(III) into the
29 culture media containing phytoplankton induced the highest radiolabelled iron uptake
30 rate ($[D_{radio}]/[C_{DFB}] = \sim 3 \times 10^{-16}$ mol cell⁻¹ d⁻¹, Table 2) by *C. sociale* during the first day
31 of incubation in the long-term iron uptake experiment (Fig. 4a), resulting from the high
32 supply of bioavailable Fe(III). In addition, the highest iron uptake rate (~3x10⁻¹⁷ mol
33 cell⁻¹ h⁻¹) were also observed at short-term iron uptake experiment in the direct Fe(III)

1 input medium in our previous study (Kuma et al. 2000). Extremely high concentration
2 of Fe(III)' represents the actual instantaneous availability of iron for uptake. Therefore,
3 the direct input of concentrated acidic Fe(III) stock solution into culture media enhances
4 the concentration of bioavailable Fe(III)' above the equilibrium concentration with solid
5 amorphous Fe(III) hydroxide in seawater and induces the highest iron uptake rate and
6 highest cell yields of phytoplankton (Figs 2, 3 and 4).

7 The stable oxidation state of iron in oxic seawater is Fe(III), which has an
8 extremely low solubility and the hydrolytic precipitation rate of Fe³⁺ in seawater was
9 extremely fast (Fig. 5). The equilibrium concentration of Fe(III)' with particulate Fe(III)
10 hydroxide in seawater is approximately 0.1 nmol L⁻¹ (Kuma et al. 1996, Wu et al. 2001),
11 which limits the iron uptake of phytoplankton if the dissolution rate of particulate
12 Fe(III) is slow in relation to the further demands of the phytoplankton. For the culture
13 experiment of *C. sociale*, the longer aging time in solid am-Fe(III) media tends to be the
14 lower maximal cell yields with a order of direct Fe(III) input > 1-d aged am-Fe(III) >
15 3-d aged am-Fe(III) > 3-w aged am-Fe(III) media with the same initial growth rates (Fig.
16 2a, Table 1). In addition, the relative order for maximal cell yields in media (direct
17 Fe(III)-DFB > 1-d aged am-Fe(III)-DFB > 3-d aged am-Fe(III)-DFB) by addition of
18 DFB after 1 d cultivation was the same as that for media without any addition of DFB
19 (Fig. 2b, Table 1). However, there is no difference among direct Fe(III) input, 1-d aged
20 Fe(III) and 3-d am-Fe(III) media with iron concentration of 100 nmol L⁻¹ for the
21 maximal cell yields and growth rates of *T. weissflogii* (Fig. 3a) although the lower
22 maximal cell yields in solid am-Fe(III) media than direct Fe(III) medium were clearly
23 observed in the culture experiments with addition of DFB after 1 d cultivation (Fig. 3b)
24 and with lower iron concentrations such as 10, 25 and 50 nmol L⁻¹ (Fig. 3c). It is likely
25 that the dissolution rate of particulate Fe(III) with 100 nmol L⁻¹ in culture media is
26 sufficiently fast to accomplish the maximal cell yields and highest growth rate of *T.*
27 *weissflogii*, Therefore, the lower concentration and the longer aging time of particulate
28 Fe(III) in media result in the slower dissolution rate of particulate Fe(III), reducing the
29 supply of bioavailable Fe(III)' in media.

30
31
32 Growth by intracellularly stored Fe for *C. sociale*

33

1 In the present study, the highest specific growth rates of *C. sociale* and *T.*
2 *weisflogii* were maintained for 2–7 d even after addition of DFB, which prevented
3 further iron uptake from ambient extracellular Fe in culture media (Figs 2b, 3b, 4b). The
4 growth rate of *C. sociale* was independent of the amount of intracellularly stored Fe
5 (Fig. 4, Table 1, 2). If cells are under identical steady state growth conditions both
6 before and after addition of radiolabelled Fe, it is necessary to allow the phytoplankton
7 to go through 8 successive transfers in order to measure intracellular Fe quotas by
8 radioactive ⁵⁹Fe uptake, so that all the Fe within the cells is in isotopic equilibrium with
9 the ⁵⁹Fe. The initial cultures in the iron uptake experiments had a cell density of 10,000
10 cells ml⁻¹. The growth rate was approximately 1 division per day (Fig. 4b). Thus, after 1
11 day, the cells density will increase to 20,000 cells ml⁻¹ and cells will be 50%
12 radiolabelled. In the next cell division, the Fe within the cells will be 75% radiolabelled
13 and the cell density will reach 40,000 cells ml⁻¹. Therefore, if the cells are collected
14 before undergoing 8 divisions, the intracellular Fe concentrations, which were measured
15 with radioactive Fe, need to be corrected for the non-radioactive (cold) Fe in the cells at
16 the beginning of the experiment. The corrected intracellularly stored Fe (Fe quota [*Q*];
17 mol Fe cell⁻¹) was calculated by dividing the amount of radiolabelled Fe uptake
18 ($[D_{radio}]$: mol Fe ml⁻¹) for 1 d by cell density ($[C_{DFB}]$: cells ml⁻¹) when DFB was added
19 for the Fe uptake measurements and percentage of intracellular radiolabelled Fe
20 ($[P]$: %) (Fig. 4, Table 2):

21

$$22 \quad [Q] = [D_{radio}] / [C_{DFB}] / [P] / 100 \quad (1)$$

23

24 Values of corrected Fe uptake ($[D_{radio}] / [P] / 100$) and Fe quota [*Q*] for the direct
25 Fe(III) input medium (DFB (1 d)) and 1-d and 3-d aged am-Fe(III) media (DFB (1 d))
26 are also the iron uptake rates with units of mol Fe ml⁻¹ d⁻¹ and mol Fe cell⁻¹ d⁻¹,
27 respectively (Table 2). The corrected cellular Fe [*Q*] in culture media ranged
28 3.1–9.8x10⁻¹⁶ mol Fe cell⁻¹ and the highest value was found in the direct Fe(III) input
29 medium (DFB (1 d)) (Table 2). However, the maximal cell yields ($[Cn]$; cells ml⁻¹)
30 appeared to be relatively dependent on the amount of intracellular Fe, suggesting the
31 presence of a critical concentration of intracellular Fe (minimum cellular Fe on growth)
32 on phytoplankton growth (Fig. 4). The critical intracellular Fe ($[Q_{cri}]$: mol Fe cell⁻¹) is
33 calculated by multiplying [*Q*] by $[C_{DFB}]$ and then by dividing by $[Cn]$ (Table 2):

$$[Q_{cri}] = [Q] \cdot [C_{DFB}] / [Cn] \quad (2)$$

1
 2
 3
 4 by assuming that 1) the cells reached stationary phase because of low Fe availability,
 5 and not because of either N, P, or Si became limiting, and 2) the Fe uptake was
 6 corrected for the additional cold Fe in the cells before the Fe uptake rate measurements
 7 were made. However, the iron uptake experiments in the present study were started with
 8 Fe-limited cells, which are documented to have higher iron uptake rates than Fe replete
 9 cells (Sunda & Huntsman 1995), under the excessive Fe(III)' conditions. Thus, after 1
 10 day, the parameter $[P]$ would be nearly 100% rather than 50%. Therefore, the value of
 11 $[Q]$ was simply calculated by dividing the amount of radiolabelled Fe uptake ($[D_{radio}]$)
 12 for 1 d by cell density ($[C_{DFB}]$) when DFB was added for the Fe uptake measurements
 13 (Fig. 4, Table 2). Values of $[D_{radio}]$ and $[Q]$ are also the iron uptake rates with units of
 14 mol Fe ml⁻¹ d⁻¹ and mol Fe cell⁻¹ d⁻¹, respectively. Figure 6 presents a plot of the daily
 15 intracellular Fe per cell in direct Fe(III)-DFB, 1-d aged am-Fe(III)-DFB and 3-d aged
 16 am-Fe(III)-DFB media after addition of DFB. The daily intracellular Fe values in direct
 17 Fe(III)-DFB and 1-d aged am-Fe(III)-DFB media tend to be nearly constant ($\sim 1 \times 10^{-16}$
 18 mol Fe cell⁻¹) after 3 d, probably indicating the critical cellular Fe. The highest
 19 intracellular Fe ($[Q] = \sim 3.4 \times 10^{-16}$ mol Fe cell⁻¹, Fig. 6, Table 2) of *C. sociale* in the
 20 present study was approximately one-half lower than the Fe quota values
 21 ($[Q] = \sim 5-9 \times 10^{-16}$ mol Fe cell⁻¹) of *T. weissflogii* in the Fe sufficient media reported by
 22 Sunda & Huntsman (1995) and Maldonado & Price (1996). The critical concentration of
 23 intracellular Fe ($[Q_{cri}]$) on phytoplankton growth was simply calculated by dividing
 24 $[D_{radio}]$ by $[Cn]$ (minimum cellular Fe on growth: $[Q_{cri}] = [D_{radio}] / [Cn] = [Q] \times [C_{DFB}] / [Cn] =$
 25 $\sim 0.5-1 \times 10^{-16}$ mol Fe cell⁻¹ for *C. sociale*, Table 2). The critical cellular Fe values are
 26 also one-half lower than the Fe quota values ($\sim 0.7-2 \times 10^{-16}$ mol Fe cell⁻¹) for *T.*
 27 *weissflogii* in the Fe deficient media (Hudson & Morel 1990; Sunda & Huntsman 1995;
 28 Maldonado & Price 1996). The lower values of $[Q]$ and $[Q_{cri}]$ for *C. sociale* are
 29 probably due to one-half smaller cell volume of *C. sociale* than *T. weissflogii*. However,
 30 values of $[Q]$ and $[Q_{cri}]$ for 3-d aged am-Fe(III) medium were unreliable because of low
 31 iron uptake and incomplete dissolution of aged am-Fe(III) by Ti(III) treatment (Kuma
 32 and Matsunaga 1995; Kuma et al. 2000). The Ti(III) solution (Hudson and Morel 1989,
 33 1990) and the oxalate reagent (Tovar-Sanchez et al. 2003) treatments may overestimate

1 the intracellular Fe in field samples where aged amorphous ferric hydroxide and
2 crystalline ferric oxyhydroxides may be present.

3 At high iron concentrations, phytoplankton can often accumulate an excess of
4 iron than that needed to support maximum growth. Such luxury uptake at high iron
5 concentrations was observed in a culture experiment of oceanic and coastal eukaryotic
6 algae (Sunda and Huntsman 1995, 1997; Sunda 2001). We will discuss the luxury iron
7 uptake elsewhere with supporting data by additional culture experiments. In the present
8 study, the highest iron uptake by *C. sociale* was observed during the first day of
9 incubation in the direct Fe(III) input medium and was 1.5 to 4 times higher than those in
10 1-d and 3-d aged am-Fe(III) media, respectively (Fig. 4a). The iron uptake rate by
11 eukaryotic phytoplankton is generally dependent on the concentration of dissolved
12 Fe(III)' (Anderson & Morel 1982; Campbell 1995). In solid am-Fe(III) media, therefore,
13 the higher iron uptake rate by phytoplankton would have been accomplished by the
14 shorter aged precipitated amorphous ferric oxide with the higher Fe(III)' dissolution rate
15 because of the larger surface area. The Fe(III)' dissolution rate of amorphous phase
16 decrease rapidly with aging time because of the conversion to more stable phases
17 (Crosby et al. 1983; Wells et al. 1983, 1991; Kuma and Matsunaga 1995).

18
19
20 Conversion to more stable am-Fe(III) phases with less bioavailability

21
22 The hydrolytic precipitation rate of Fe³⁺ in seawater was fast, resulting in an
23 extremely low 0.025-µm filterable (dissolved) Fe concentration with short aging time
24 (Fig. 5). In general, the freshly precipitated am-Fe(III), in which very fine particles with
25 a large surface area and structural disorder occur, consists of aggregates of hydrated
26 ferric ions that have a very low thermodynamic stability. Freshly precipitated am-Fe(III)
27 in solution undergoes continuous chemical changes with time (loss of water and
28 increased crystallization) that are not easily quantified. An increase in the
29 thermodynamic stability of am-Fe(III) substantially decrease the solubility and lability
30 of the solid am-Fe(III) phase and thereby decrease iron availability to phytoplankton
31 (Wells et al. 1983, 1991; Rich and Morel 1990; Kuma and Matsunaga 1995). In addition,
32 it has been reported that small colloidal iron particle fraction (< 0.2 µm size) was the
33 most dynamic size fraction during the growth of the diatom *Chaetoceros* sp. in

1 laboratory culture experiments (Nishioka and Takeda 2000). The actual particulate solid
 2 ferric hydroxide, oxyhydroxide, or oxide phase regulates the solubility of dissolved
 3 inorganic Fe species [Fe(III)] and its influence on biological availability of iron. In the
 4 present study, the solubility measurements of am-Fe(III) in seawater indicated a rapid
 5 decrease of dissolved Fe concentrations with aging time (Fig. 5), suggesting that
 6 am-Fe(III) in seawater solution changes to larger and more stable particles with aging
 7 time (Crosby et al. 1983; Pankow 1991). In addition, lower temperature retarded the
 8 conversion to larger particles. In a previous study (Kuma and Matsunaga, 1995),
 9 proton-promoted dissolution rates of am-Fe(III) decreased rapidly with aging time.
 10 These above results suggest that the bioavailability of am-Fe(III) produced in culture
 11 solution decreases rapidly with time during culture experiments, resulting from a
 12 decreased dissolution rate of am-Fe(III) with time.

13 To interpret the temporal decrease in dissolved Fe concentrations with aging
 14 time in the Fe(III) hydrolytic precipitation experiment at 10°C (Fig. 5), we present a
 15 model assuming a first-order transfer reaction with a rate constant, k_1 , from dissolved Fe
 16 to particulate Fe at an early period of rapid Fe(III) hydrolytic precipitation and a back
 17 reaction with a rate constant, k_2 , from unstable fine particulate Fe to dissolved Fe in the
 18 slow conversion process to more stable particulate Fe with time. Here, a
 19 time-developing model between dissolved Fe and particulate Fe concentrations is given
 20 by

$$21 \quad \frac{dD_{Fe}}{dt} = -k_1 D_{Fe} + k_2 P_{Fe} \quad (3)$$

$$22 \quad \frac{dP_{Fe}}{dt} = k_1 D_{Fe} - k_2 P_{Fe} \quad (4)$$

23
 24 where D_{Fe} and P_{Fe} are dissolved Fe and particulate Fe concentrations, respectively. We
 25 can roughly estimate $k_1 = 30 \text{ d}^{-1}$ from a rapid decrease in dissolved Fe concentration at
 26 an early stage (less than 3 h) of Fe(III) hydrolytic precipitation experiments (Fig. 5)
 27 because of a first-order reaction of hydrolytic precipitation at $k_1 D_{Fe} \gg k_2 P_{Fe}$. The
 28 initial values of D_{Fe} and P_{Fe} at $t=0$ are 100 and 0 nmol L^{-1} , respectively. In our model,
 29 however, a back reaction rate constant (k_2) is the unknown parameter so that the
 30 following three types of k_2 were considered as follows:
 31

$$32 \quad \text{(i) Constant value:} \quad k_2 = C_1$$

- 1 (ii) Exponential function: $k_2(t) = C_1 \exp(-t/C_2)$
 2 (iii) Hyperbolic function: $k_2(t) = C_1 / (C_2 + t)$
 3

4 Here, the time-dependent $k_2(t)$ in (ii) and (iii) types means that a part of unstable fine
 5 particulate Fe converts to more stable particulate Fe with time. The constant values of
 6 C_1 and C_2 were determined by fitting procedure in order to search for best fitting of
 7 temporal decrease in the dissolved Fe concentration (D_{Fe}) in the Fe(III) hydrolytic
 8 precipitation experiment at 10°C (Fig. 5). The calculated D_{Fe} changes with time, which
 9 is the most similar to the measured D_{Fe} change, were given at $C_1 = 1 \text{ d}^{-1}$ in the type (i),
 10 $(C_1, C_2) = (8 \text{ d}^{-1}, 2)$ in the type (ii) and $(C_1, C_2) = (5, 1.5 \text{ d}^{-1})$ in the type (iii).

11 In case of type (i) with constant k_2 , the equilibrium state between dissolved Fe
 12 and particulate Fe is rapidly performed at an early stage and then the temporal decrease
 13 in the dissolved Fe concentration in the Fe(III) hydrolytic precipitation experiment can
 14 not be reproduced at all (Fig. 7-(i)). In comparison between types (ii) and (iii) with
 15 time-dependent $k_2(t)$, it is found that the calculated D_{Fe} change using hyperbolic
 16 function (type (iii)) is much better consistent with the temporal measured D_{Fe} change in
 17 the hydrolytic precipitation experiment. It is assumed that a true back reaction rate
 18 constant is k_2^* , i.e., $C_1 = k_2^*$ in the types (ii) and (iii) so that temporal change in $k_2(t)$ is
 19 due to the chemical conversion process from unstable fine particulate Fe to stable
 20 particulate Fe phase. Therefore, the term of $k_2(t)P_{Fe}$ in equation (4) is interpreted by
 21 $k_2^*[\exp(-t/C_2)P_{Fe}]$ in type (ii) and $k_2^*[P_{Fe} / (C_2 + t)]$ in type (iii).

22 First, we consider the differential equation with an exponential solution of
 23 $[\exp(-t/C_2)P_{Fe}]$, which is easily inferred as follows:

24
 25
$$dP_{Fe}/dt = -CP_{Fe} \quad (5)$$

26
 27 where P_{Fe} is unstable fine particulate Fe concentration and C is constant. This equation
 28 form implies that unstable fine particulate Fe converts to stable particulate Fe with a rate
 29 constant at any time and continuously decrease. In other words, all of unstable fine
 30 particulate Fe finally converts to stable particulate Fe and the equilibrium state between
 31 dissolved Fe and particulate Fe is never performed (Fig. 7-(ii)). On the other hand, the
 32 differential equation with a hyperbolic solution of $[P_{Fe} / (C_2 + t)]$ is that temporal
 33 decrease in P_{Fe} is proportional to the square of P_{Fe} as follows:

1
2
$$dP_{Fe}/dt = -CP_{Fe}^2 \quad (6)$$

3

4 This equation can infer an interesting conversion process from unstable fine particulate
5 Fe with large surface area to stable particulate Fe phase (Pankow 1991) such that stable
6 particulate Fe rapidly grow at high P_{Fe} and its growth is slow with decreasing P_{Fe} .
7 Therefore, a small amount of unstable fine particulate Fe can remain for a long time and
8 the equilibrium concentration of dissolved Fe with stable particulate Fe will be
9 performed (Fig. 7-(iii)). Mayer (1982) and Hunter and Leonard (1988) have presented
10 theoretical grounds for expecting second-order particle number kinetics from Brownian
11 aggregation to translate into pseudo-second order removal of riverine dissolved iron
12 ($<0.5 \mu\text{m}$) after mixing with seawater, remarkably consistent with second-order kinetics
13 with respect to the unstable fine particulate Fe concentration [removal of dissolved iron
14 ($<0.025 \mu\text{m}$)] in seawater [Eqn. (6)] in the present study. In estuarine mixing system, the
15 fresh iron precipitates with higher bioavailability would be formed through the
16 dissociative hydrolytic precipitation of natural dissolved organic Fe complexes, such as
17 fulvic-Fe(III) complex, supplied by riverine input at both high pH and high
18 concentrations of cations in seawater (Stumm & Morgan 1996; Kuma et al. 1999).
19 Additionally, in mesoscale iron fertilization experiments (Martin et al. 1994; Coale et al.
20 1996; Boyd et al. 2000, 2004; Tsuda et al. 2003), high concentrations of freshly
21 precipitated am-Fe(III) would have been caused by the supply of concentrated acidic
22 Fe(II) solution to HNLC oceanic regions. The fresh iron precipitates would convert to
23 more stable iron precipitates with less bioavailability with time in seawater.

24 This is the first confirmation that the maximal cell yields and iron uptake rate
25 of phytoplankton are probably controlled by the solubility and the dissolution rate of
26 am-Fe(III), which decrease with increasing aging time even at 10 and 20°C. The iron
27 nutrition by phytoplankton is strongly related to the dissolution of particulate and
28 colloidal Fe. The conversion from unstable fine am-Fe(III) to stable large am-Fe(III)
29 with time is one of the most important processes in changing the supply of bioavailable
30 Fe(III) to marine phytoplankton in estuarine and coastal waters and in iron fertilization
31 experiments. In addition, our results suggest that the growth rate of phytoplankton is
32 independent of the amount of intracellularly stored Fe, above a critical concentration of
33 intracellular Fe on the growth, and the maximal cell yields are controlled by a critical

1 concentration.

2
3
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5
6 Figure captions

7
8 Fig. 1. Cell numbers of *C. sociale* at 10°C (a) and *T. weissflogii* at 20°C (b) cultures
9 supplied with direct Fe(III) input and premixed DEB-Fe(III) complex (10:1) with iron
10 concentration of 100 nmol L⁻¹. Data on cell concentrations represent mean (n=3) for
11 triplicate culture experiments.

12
13 Fig. 2. Growth of *C. sociale* in direct Fe(III) input medium and solid 1-d, 3-d and 3-w
14 aged am-Fe(III) media (100 nmol Fe L⁻¹, 10°C) and control (without any addition of Fe)
15 without (a) or with (b) addition of DFB after 1 d cultivation. Data on cell concentrations
16 represent mean (n=3) for triplicate culture experiments.

17
18 Fig. 3. Growth of *T. weissflogii* in direct Fe(III) input medium and solid 1-d, 3-d and
19 3-w aged am-Fe(III) media (100 nmol Fe L⁻¹, 20°C) and control (without any addition
20 of Fe) without (a) or with (b) addition of DFB after 1 d cultivation and in direct
21 Fe(III) input and 1-d aged am-Fe(III) media with iron concentration of 10, 25 and 50
22 nmol L⁻¹ at 20°C (20°C). Data on cell concentrations represent mean (n=3) for triplicate
23 culture experiments.

24
25 Fig. 4. Long-term iron uptake and growth of *C. sociale* in direct Fe(III) input media and
26 1-d and 3-d aged am-Fe(III) media without or with addition of DFB after 1 d cultivation.
27 (a) Iron amount accumulated by *C. sociale* in direct Fe(III) input medium and 1-d and
28 3-d aged am-Fe(III) media with addition of DFB after 1 d cultivation. Each point with
29 ±1 SD at 7.5 d and line are mean cellular iron ($[D_{radio}]$) for 4 d after addition of DFB.
30 (b) Growth of *C. sociale* in direct Fe(III) input media and 1-d and 3-d aged am-Fe(III)
31 media without or with addition of DFB after 1 d cultivation

32
33 Fig. 5. Hydrolytic precipitation rate of Fe³⁺ in seawater with 100 nmol Fe L⁻¹ at 10°C

1 (○) and 20°C (●).

2

3 Fig. 6. The daily intracellular Fe per cell in direct Fe(III)-DFB, 1-d aged
4 am-Fe(III)-DFB and 3-d aged am-Fe(III)-DFB media after addition of DFB (dotted line:
5 critical cellular Fe: $[Q_{\text{cri}}] = \sim 1 \times 10^{-16}$ mol Fe cell⁻¹).

6

7 Fig. 7. The hydrolytic precipitation rate models of Fe³⁺ in seawater with 100 nmol Fe
8 L⁻¹ at 10°C: (i) Constant value ($k_2 = C_1$); (ii) Exponential function [$k_2(t) = C_1$
9 $\exp(-t/C_2)$]; (iii) Hyperbolic function [$k_2(t) = C_1/(C_2 + t)$] (thick line: hydrolytic
10 precipitation rate model; open circle: observed dissolved Fe concentration).

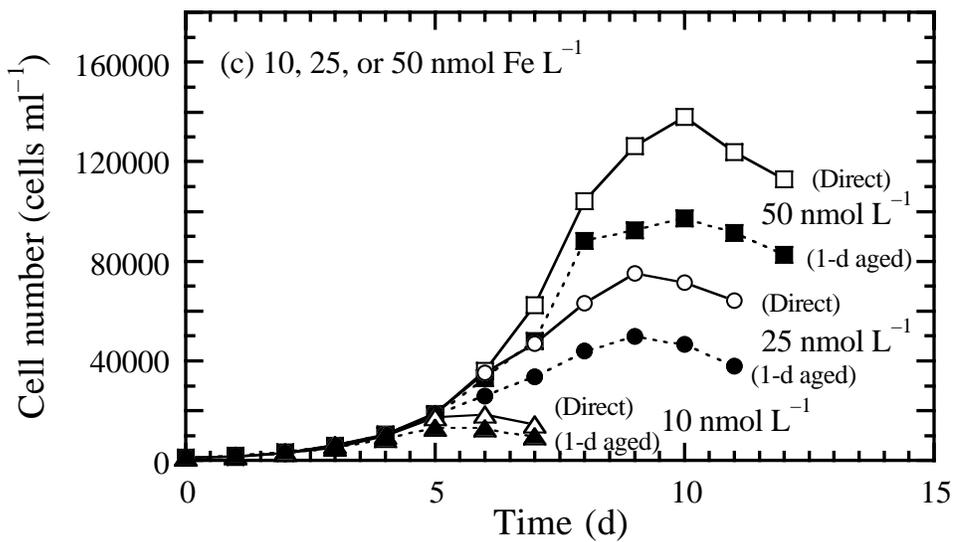
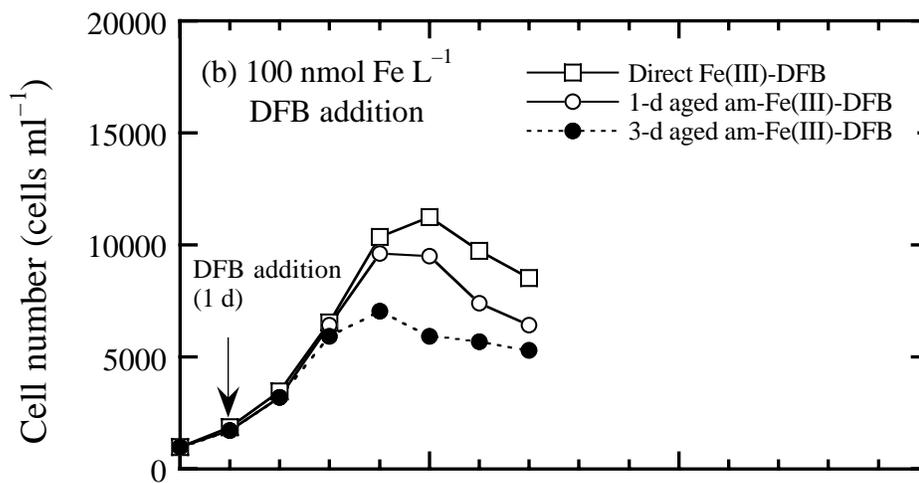
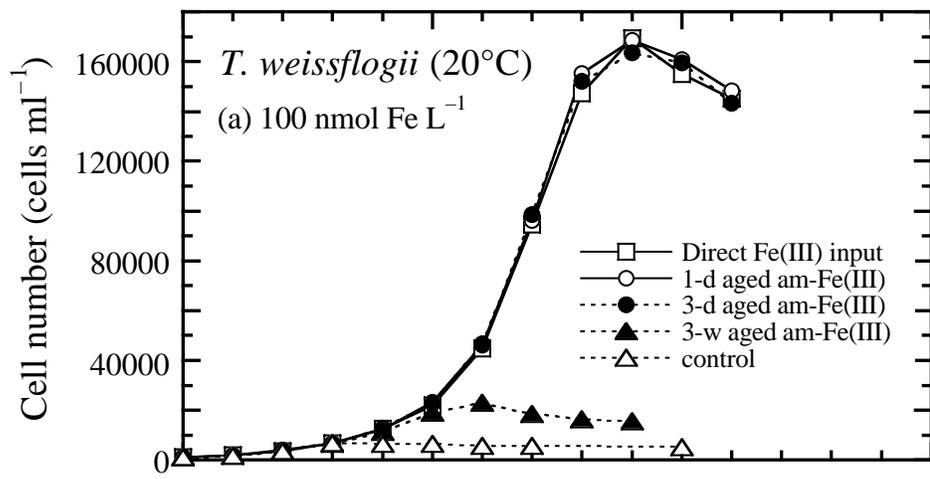


Fig. 2

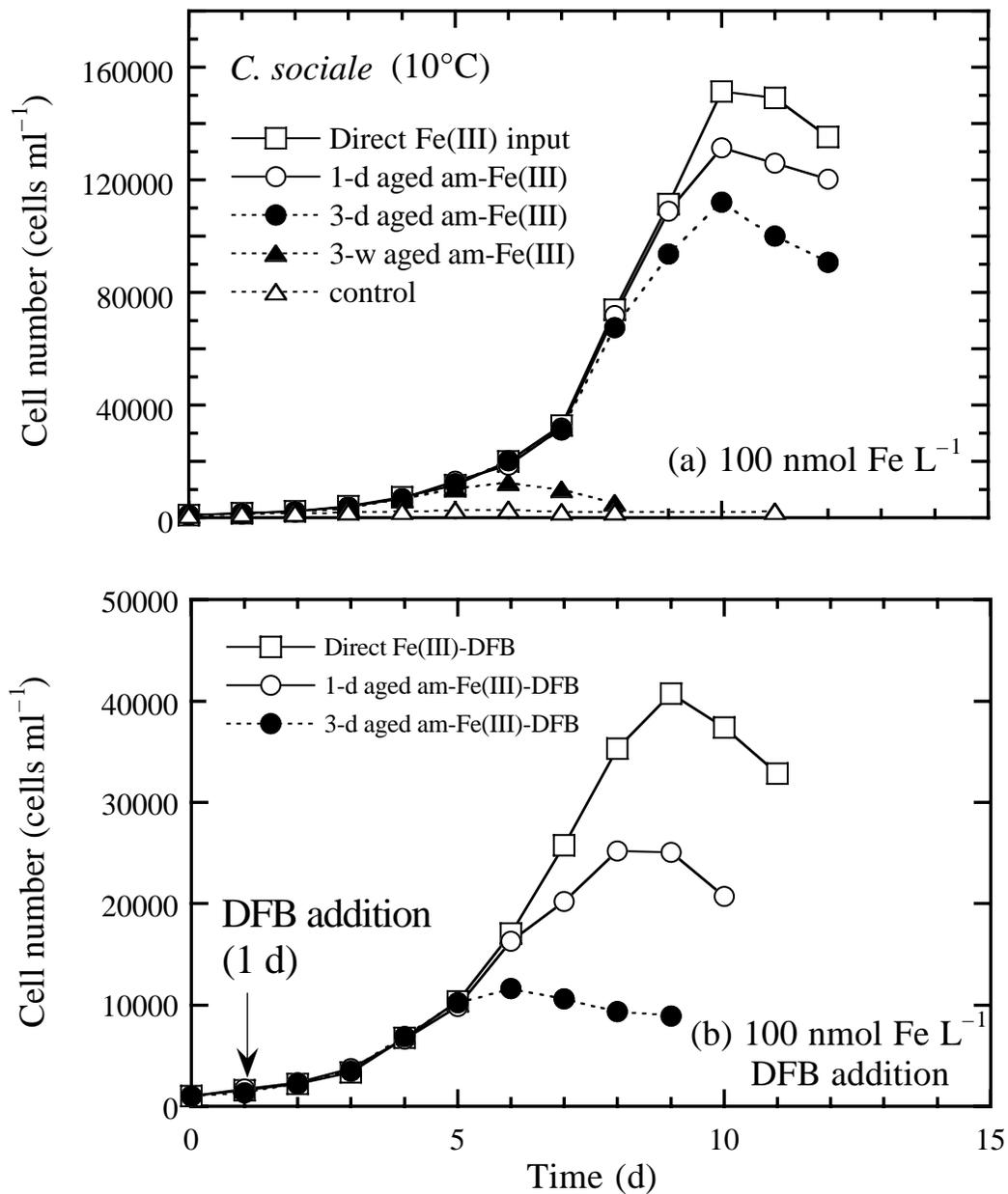


Fig. 4

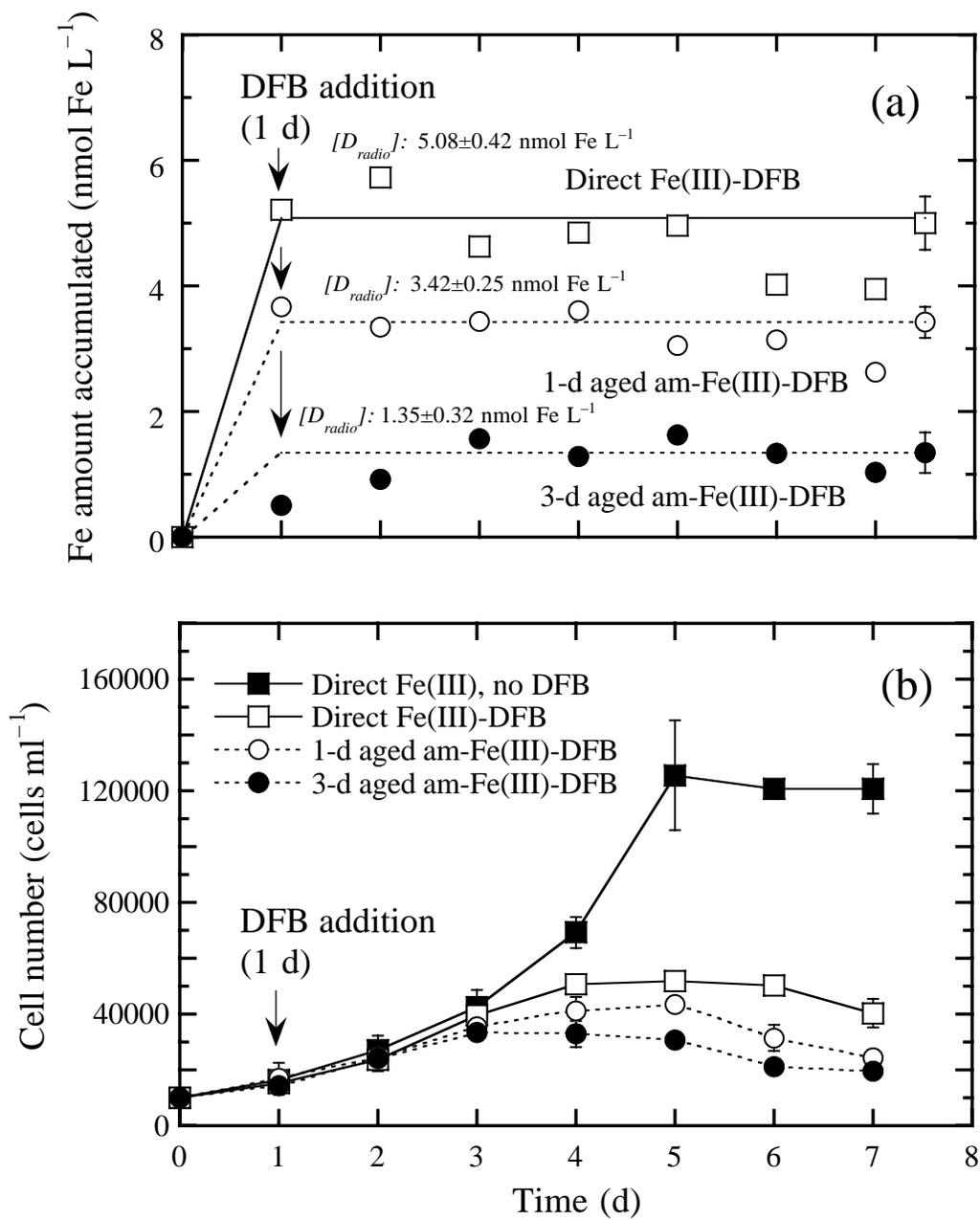


Fig. 5

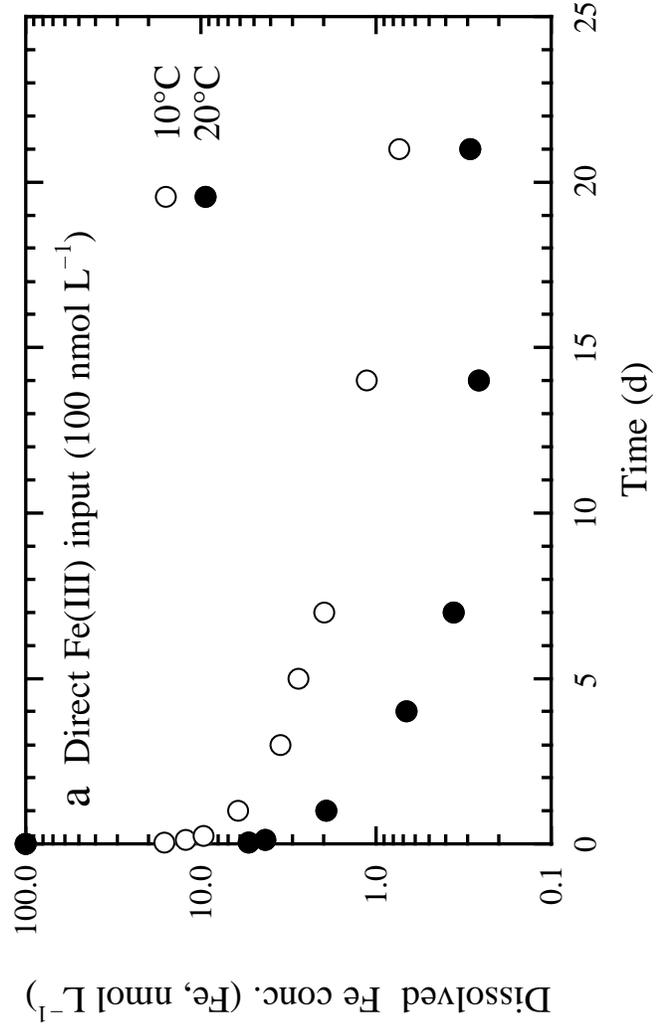
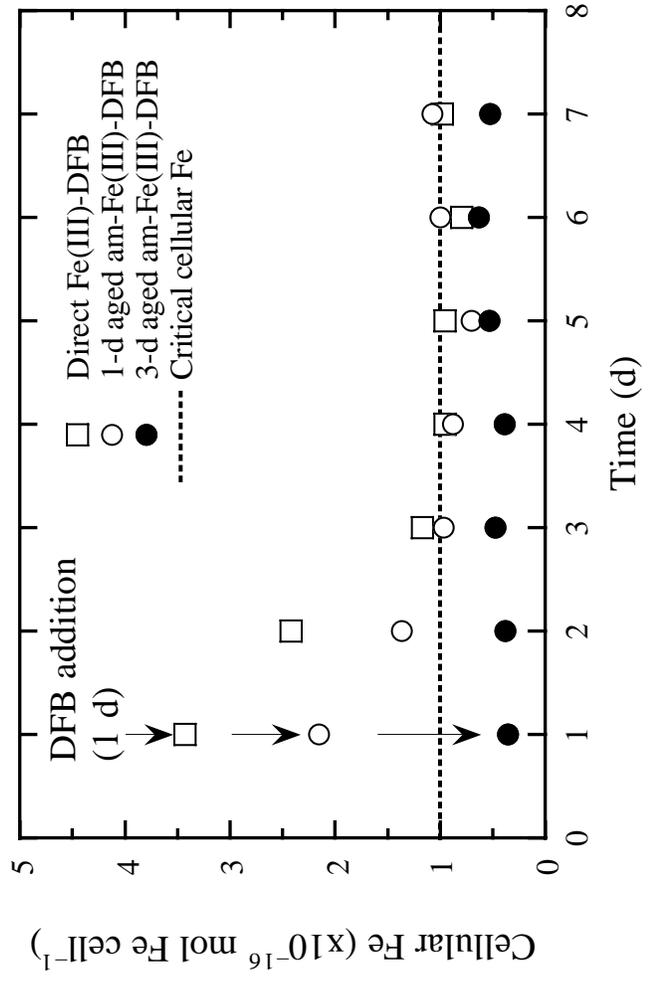


Fig. 6



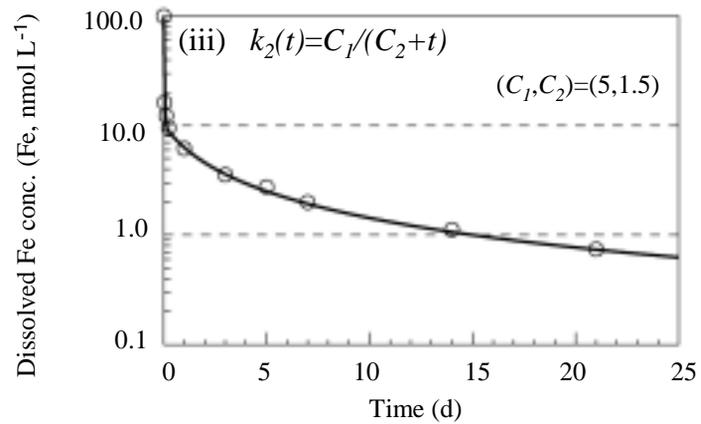
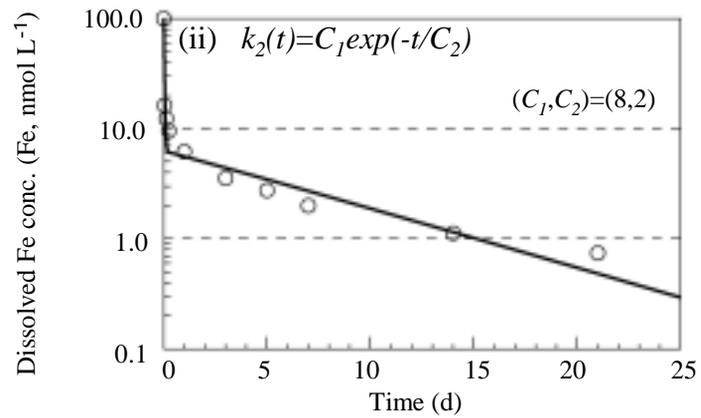
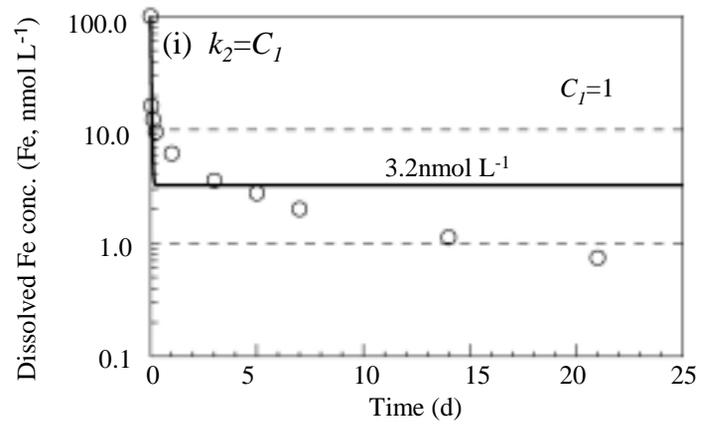


Table 1. Growth rate and maximal cell yields of *Chaetoceros sociale* (10°C) and *Thalassiosira weissflogii* (20°C) in direct Fe(III) input and 1-d, 3-d and 3-weeks aged am-Fe(III) media which DFB was added after incubation for 1 day or not (Not determined–N). *Values are from our previous study (Iwade *et al.*, submitted).

Medium	Cell number at start (cells ml ⁻¹)	Addition of DFB (1 µmol l ⁻¹) (DFB : Fe(III) = 10 : 1)	Specific growth rate (µ, d ⁻¹) (range for n = 3 or ± 1 SD for n = 1)	Maximal cell yields [Cn] (cells ml ⁻¹)
A. <i>Chaetoceros sociale</i> (100 nmol L⁻¹ Fe concentration, 10°C)				
(1) Growth rate experiment (n = 3, Figs 1a and 2)				
Direct Fe(III) input	1000	no addition	0.51–0.53 (0–10 d)	146,000–155,000
1-d aged am-Fe(III)	1000	no addition	0.52–0.53 (0–9 d)	129,000–133,000
3-d aged am-Fe(III)	1000	no addition	0.50–0.53 (0–7 d)	108,000–117,000
3-w aged am-Fe(III)	1000	no addition	0.45–0.49 (0–5 d)	129,000–133,000
Direct Fe(III) input	1000	DFB (after 1 d incubation)	0.46–0.47 (0–8 d)	39,300–41,100
1-d aged am-Fe(III)	1000	DFB (after 1 d incubation)	0.42–0.47 (0–7 d)	24,400–25,900
3-d aged am-Fe(III)	1000	DFB (after 1 d incubation)	0.42–0.49 (0–5 d)	11,100–12,600
(2) Iron uptake and growth rate experiment (n = 1, Fig. 4)				
Direct Fe(III) input	10000	no addition	*0.50±0.01 (05 d, r=0.999)	*126,000±20,000
Direct Fe(III) input	10000	DFB (after 1 d incubation)	*0.47±0.01 (0–3 d, r=0.999)	*52,000±2,000
1-d aged am-Fe(III)	10000	DFB (after 1 d incubation)	*0.45±0.05 (0–3 d, r=0.994)	*43,000±2,000
3-d aged am-Fe(III)	10000	DFB (after 1 d incubation)	0.44±0.04 (0–3 d, r=0.996)	33,000±1,000
B–1 <i>Thalassiosira weissflogii</i> (100 nmol L⁻¹ Fe concentration, 20°C)				
(1) Growth rate experiment (n = 3, Figs 1b, 3a and 3b)				
Direct Fe(III) input	1000	no addition	0.63–0.64 (0–8 d)	169,000–170,000
1-d aged am-Fe(III)	1000	no addition	0.63–0.64 (0–8 d)	160,000–176,000
3-d aged am-Fe(III)	1000	no addition	0.63–0.65 (0–8 d)	157,000–169,000
3-w aged am-Fe(III)	1000	no addition	0.52–0.63 (0–4 d)	21,500–25,600
Direct Fe(III) input	1000	DFB (after 1 d incubation)	0.59–0.60 (0–4 d)	10,700–11,500
1-d aged am-Fe(III)	1000	DFB (after 1 d incubation)	0.57–0.61 (0–4 d)	9,300–10,000
3-d aged am-Fe(III)	1000	DFB (after 1 d incubation)	0.58–0.61 (0–4 d)	6,700–7,400

B-2 *Thalassiosira weissflogii* (10, 25 and 50 nmol L⁻¹ concentration, 20°C)

(1) Growth rate experiment (n=3, Fig. 3c)

Direct Fe(III) (50nM) 1000	no addition	0.59–0.63 (0–8 d)	134,000–140,000
Direct Fe(III) (25nM) 1000	no addition	0.59–0.61 (0–6 d)	70,400–78,100
Direct Fe(III) (10nM) 1000	no addition	0.60–0.62 (0–4 d)	18,100–19,300
1-d aged am- (50nM) 1000	no addition	0.56–0.60 (0–6 d)	93,000–104,000
1-d aged am- (25nM) 1000	no addition	0.59–0.60 (0–5 d)	47,000–51,900
1-d aged am- (10nM) 1000	no addition	0.60 (0–2 d)	12,600–13,700

Table 2. Iron uptake by *Chaetoceros sociale* in direct Fe(III) and 1-d and 3-d aged am-Fe(III) media (10°C) which DFB was added after incubation for 1 day. Values of $[Q]$ for the direct Fe(III) input medium and 1-d and 3-d aged am-Fe(III) medium are also the iron uptake rate with unit of mol Fe cell⁻¹ d⁻¹. Values of $[Q]$ and $[Q_{cri}]$ in parenthesis were corrected by the percentage of intracellular radiolabelled Fe ($[P]$ in parenthesis). *Values for the 3-d aged am-Fe(III) medium is unreliable because of low iron uptake and incomplete dissolution of aged am-Fe(III) treatment.

	Radiolabelled Fe uptake (for 1 d)	Cell density when DFB was added	Maximal cell yields	% of radiolabelled cellular Fe	Cellular Fe (Fe quota: $[Q]$)	Critical cellular Fe $[Q_{cri}]$
	$[D_{radio}]$	$[C_{DFB}]$	$[Cn]$	$[P]$	$[D_{radio}]/[C_{DFB}]/[P]/100$	$[Q] \times [C_{DFB}]/[Cn]$
	$\times 10^{-12}$ mol Fe ml ⁻¹	cells ml ⁻¹	cells ml ⁻¹	%	$\times 10^{-16}$ mol Fe cell ⁻¹	
Medium						
Iron uptake and growth rate experiment (cell density at start: 10,000, n=1, Fig. 4)						
Direct Fe(III)-DFB	5.08±0.42	15,200±3,400	52,000±1,700	100% (34.2%)	~3.4 (~9.8)	~0.98 (~2.86)
1-d am-Fe(III)-DFB	3.42±0.25	17,000±5,600	43,000±2,200	100% (41.2%)	~2.0 (~4.9)	~0.80 (~1.93)
3-d am-Fe(III)-DFB	1.35±0.32*	14,400±3,300	33,000±1,000	100% (30.6%)	~0.9* (~3.1*)	~0.41* (~1.34*)