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**Bioavailability of
organic iron**

C. S. Hassler and
V. Schoemann

Bioavailability of organically bound Fe to model phytoplankton of the Southern Ocean

C. S. Hassler¹ and V. Schoemann²

¹Centre for Australian Weather and Climate Research (CAWCR), a partnership between CSIRO and the Bureau of Meteorology, Castray Esplanade, Hobart, TAS, Australia

²Ecologie des Systèmes Aquatiques, Université Libre de Bruxelles, Campus de la Plaine, CP 221, Boulevard du Triomphe, 1050 Bruxelles, Belgium

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Correspondence to: C. S. Hassler (christel.hassler@csiro.au)

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Abstract

Iron (Fe) is known to be mostly bound to organic ligands and to limit primary productivity in the Southern Ocean. It is thus important to investigate the bioavailability of organically bound Fe. In this study, we used four keystone phytoplankton species of the Southern Ocean (*Phaeocystis* sp., *Chaetoceros* sp., *Fragilariopsis kerguelensis* and *Thalassiosira antarctica* Comber) to measure the influence of various organic ligands on Fe solubility and bioavailability. Based on the Fe:C ratios, the strength of Fe limitation was inversely related to the surface area to volume ratio, with the chain forming *Fragilariopsis* being the most Fe-limited strain. The ratio of extracellular to intracellular Fe is used to discuss the relative importance of diffusive supply and uptake to control Fe bioavailability. The effect of excess of organic ligands on Fe bioavailability cannot be solely explained by their effect on Fe solubility. For all strains studied, the bioavailability of Fe can be enhanced in presence of porphyrin, catechol and saccharides whereas it was decreased in presence of hydroxamate and organic amine. The enhancement of Fe bioavailability in presence of saccharides is presented as the result from both the formation of bioavailable (or chemically labile) organic form of Fe and the stabilisation of Fe within the dissolved phase. Given the ubiquitous presence of saccharides in the ocean, these compounds might represent an important factor to control the basal level of soluble and bioavailable Fe. Results show that the use of model phytoplankton is promising to improve mechanistic understanding of Fe bioavailability and primary productivity in HNLC regions of the ocean.

1 Introduction

The regulating role of ocean on the Earth's climate system has been widely recognised. During the last 15 years it has been demonstrated by microcosm experiments (e.g. de Baar et al., 1990) and large scale natural or in-situ Fe fertilisation experiments (e.g. de Baar et al., 2005) that Fe controls primary productivity, as well as the planktonic com-

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munity structure in more than 30% of the oceans (High-Nutrient, Low-Chlorophyll areas – HNLC). In HNLC regions of the ocean, the concentration of total dissolved Fe is low (e.g. 0.1–0.6 nmol L⁻¹ in the Southern Ocean, SO, de Baar and de Jong, 2001). Fe is usually present in different physical (particulate, colloidal, soluble) and chemical (inorganic and organic complexes) forms. A fraction (viz. the bioavailable fraction) of this Fe is expected to be accessible for phytoplankton growth, therefore controlling the plankton abundance and diversity (e.g. de Baar et al., 2005). Fe bioavailability is influenced by its chemical forms, biogeochemical cycling and the different uptake strategies of the phyto- and bacterio-plankton communities (Barbeau et al., 1996; Hutchins et al., 1999; Nodwell and Price, 2001; Strzepek et al., 2005; Maldonado et al., 2005). Because of highly dynamic and complex biogeochemical processes, Fe bioavailability is currently difficult to predict.

The members of the planktonic community are thought to be in competition for available Fe (Worms et al., 2006), when in short supply, possibly to the extent of sequestering it from others with their own ligands (e.g. Völker and Wolf-Gladrow, 1999). For example, large diatoms, have a lesser diffusive supply (Pahlow et al., 1997) of Fe and a possibly less efficient uptake strategy (e.g. surface reductase, Fe-EDTA reported as non-bioavailable, Shaked et al., 2005) than small cyanobacteria (e.g. siderophore excretion/recognition, Fe-EDTA reported as bioavailable, Hassler and Twiss, 2006). Therefore diatoms could be out-competed by cyanobacteria, when bioavailable Fe concentrations are limiting (e.g. Völker and Wolf-Gladrow, 1999). Distinguishing amongst different Fe uptake strategies is often not that simple. In fact, some neritic phytoplankton are able to specifically recognise Fe bound to siderophores (Trick et al., 1983) and some eukaryotic phytoplankton can excrete organic compounds that could interact with Fe chemistry or the microorganisms present (Boyé and van den Berg, 2000; Rijkenberg et al., 2008). Regeneration of Fe, through grazing, bacterial and viral activities, is another pathway that supplies the micronutrient to phytoplanktonic communities (Barbeau et al., 1996; Poorvin et al., 2004; Strzepek et al., 2005).

It has been shown that dissolved inorganic Fe concentrations are often insufficient

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to sustain the growth of planktonic organisms as observed in HNLC regions of the ocean (Price and Morel, 1998). Titration using the competitive ligand exchange adsorptive stripping voltammetry (CLEAdSV) revealed that most of the Fe (94–99.9%, e.g. Boyé et al., 2001) is associated with organic ligands. Even if some of these organic ligands have similar binding affinities for Fe as siderophore and porphyrin (Rue and Bruland, 1995; Witter et al., 2000), they still remain poorly characterised. The detection of weak and strong organic ligands is somewhat limited in the CLEAdSV technique because of defined analytical windows (depending on the competitive ligand added, e.g. $\log K_{\text{Fe(III)(TAC)}_2} = 10^{22.4} \text{ L mol}^{-1}$, Croot and Johansson, 2000) in which organic ligands bound to Fe can be detected. Most of the organic compounds in the open ocean would be biologically produced in situ. For example, siderophores are mainly bacterially produced (e.g. Völker and Wolf-Gladrow, 1999), carbohydrates such as exopolymeric substances are excreted by both prokaryotic and eukaryotic plankton (e.g. Hoagland et al., 1993; Mancuso et al., 2005), porphyrins are released upon grazing and cell lysis of plankton (Hutchins et al., 1999; Gledhill, 2007; Vong et al., 2007), some organic ligands could also be released because of viral activity (Poorvin et al., 2004). Whereas siderophores and porphyrins are known to directly affect Fe chemistry and bioavailability (e.g. Rue and Bruland 1995; Hutchins et al., 1999), little is known about the possible role of carbohydrates in the Fe oceanic cycle. A significant fraction (3–30%) of the dissolved organic carbon is present as carbohydrates (Wang et al., 2006). Acylated and acid saccharides are a significant reactive group (Wang et al., 2006; Panagiotopoulos et al., 2007) that has been shown to be important for carbon (Engel et al., 2004) and trace elements (Santschi et al., 2003) cycles. Saccharides could affect Fe cycling by chemical interactions (complexation, photo-degradation and/or effect on Fe solubility) or by biological processes. Indeed, saccharides can be a storage product for phytoplankton and a substrate for heterotrophic plankton. Both biological processes are known to be Fe responsive (e.g. Kirchman et al., 2000; van Oijen et al., 2005).

Because organic ligands are biologically produced and most of the Fe is bound to

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organic ligands, the organically bound Fe might well be a significant fraction controlling Fe bioavailability. In addition, organic complexation prevents or slows down precipitation, coagulation and settling in seawater (Chen et al., 2004; Tian et al., 2006).

To date, several studies have demonstrated the importance of organic ligands to control Fe bioavailability to natural phytoplankton communities from the SO (e.g. Hutchins et al., 1999; Maldonado et al., 2005; Schoemann et al., 2009). Whereas, work with natural phytoplankton is environmentally relevant, it provides little information on the processes controlling the bioavailability of organically bound Fe. In this study, the bioavailability of relevant forms of organically bound Fe was addressed at the species level using keystone phytoplankton species from the SO. Four strains were selected to represent various groups (diatoms and haptophytes) with likely differing Fe uptake strategies. Since size affects Fe diffusive supply and biological requirement (Sunda and Huntsman, 1995) and because the natural phytoplanktonic assemblage is variable in size, shape and morphological form, single cell, colonial or chain forming phytoplankton were chosen. For each strain, Fe bioavailability and carbon fixation were measured using short-term ^{55}Fe and ^{14}C bioaccumulation. The bioavailability of added inorganic Fe, as well as Fe bound to siderophores, porphyrin and saccharides, was studied in natural water collected from the SO. Results are discussed in the framework of the mechanistic role that organically bound Fe plays in controlling Fe solubility, bioavailability and primary productivity. Mechanistic understanding of Fe bioavailability is required before one proceeds to interpretation of the phytoplankton dynamics in response to Fe in the oceans.

2 Material and methods

2.1 Choice of microorganisms

Since Fe bioavailability depends both on the size and the phytoplankton species (e.g. Sunda and Huntsman, 1995; Völker and Wolf-Gladrow, 1999; Timmermans et al.,

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2001, 2004), relevant phytoplankton from the SO were selected accordingly from the CSIRO microalgae culture collection. *Phaeocystis* is known to recurrently bloom in the SO (Schoemann et al., 2005), it was therefore chosen to be representative of the haptophyte group. *Phaeocystis* sp. (CS 243, isolated from Antarctica, 68°47.5' S, 73°30.2' E) is mainly present as single cell (78%; $5.0 \pm 0.9 \mu\text{m}$ diameter, $69.2 \pm 32.8 \mu\text{m}^3$ biovolume as measured by microscopy) and small colonies (up to $100 \mu\text{m}$ diameter) in our culture medium. Although little is known on the mechanism of Fe transport in *Phaeocystis*, a complex interaction with the surrounding C-rich mucus from the colonies was recognised (Schoemann et al., 2001). Diatoms are an important group of phytoplankton present worldwide (Sarhou et al., 2005) and were selected to represent various taxa with variable size and morphological forms. *Chaetoceros* sp. (CS 624, isolated from Prydz Bay, Antarctica) is a small single cell diatom (Coulter: $3.7 \mu\text{m}$ spherical equivalent diameter, $29.8 \mu\text{m}^3$ biovolumes). *Thalassiosira antarctica* Comber (CCMP 982) is a larger diatom (Coulter: $16.4 \mu\text{m}$ spherical equivalent diameter, $1946.4 \mu\text{m}^3$ biovolumes), isolated from Oslo Fjord (59.5° N, 10.6° E, Norway). *Fragilariopsis kerguelensis* is a diatom (Inverted microscopy: $24.9 \pm 0.9 \mu\text{m}$ spherical equivalent diameter, $8150 \pm 841 \mu\text{m}^3$ cellular biovolume) forming long chains (average cell number per chain 28 ± 12 , $n=23$) isolated from Antarctica (53.5° S, 141.5° E, October 2006) by Kristen Karsh (CSIRO).

2.2 Growth media and culture condition

Cultures were maintained in exponential growth phase in an incubation cabinet (RUMED Model 1301) at 2°C with a 18 h:6 h light: dark cycle at photosynthetic saturating light levels. *Phaeocystis* was grown under $120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ and diatoms at $60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Light level was adjusted using neutral density filters. Filtered seawater ($0.2 \mu\text{m}$, Sartorius membrane filter cartridges Sartobran) collected during the ISPOL oceanographic voyage using trace metal clean techniques (68° S, 55° W, Lannuzel et al., 2008) was used as a culture media. Low dissolved Fe concentration of 0.29 nmol L^{-1} was measured according to de Jong et al. (2008) by isotopic dilution

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combined with multiple collector inductively coupled plasma mass spectrometry (ID-MC-ICP-MS) using nitrilotriacetic acid chelating resin for pre-concentration and matrix separation. The ISPOL water was spiked with chelexed macronutrients to reach final concentrations of $10 \mu\text{mol L}^{-1}$ Si, $10 \mu\text{mol L}^{-1}$ NO_3^- , and $1 \mu\text{mol L}^{-1}$ PO_4^{3-} , to avoid growth limitation by a nutrient other than Fe. *Fragilariopsis* was enriched with additional Si so as to reach a Si: NO_3^- of 6:1 (Kristen Karsh, personal communication) for optimal growth. Bacterial contamination of the culture was examined using epifluorescence microscope observation (DAPI staining, Porter and Feig, 1980). Cellular concentration, biovolume and derived average spherical diameter were measured by either using a Coulter counter (Multisizer Z3, except for *Fragilariopsis*) or inverted microscopy (Leitz Fluovert). Cellular volume (V), surface area (A) and surface to volume ratio were calculated according to Hillebrand et al. (1999) using microscopic measurements (Table 1). Calculated cellular biovolume were similar to the volume determined by Coulter counter for *Phaeocystis*, *Chaetoceros* and *Thalassiosira*.

2.3 Experimental solutions and ligands

The experimental media consisted of ISPOL filtered water (control treatment), ISPOL filtered water with 1 nmol L^{-1} Fe (Fe treatment) and ISPOL filtered water with 1 nmol L^{-1} Fe and 15 nmol L^{-1} of organic ligand. The organic ligands (L) were selected to represent the groups of hydroxamate (desferrioxamine B, DFB) and catecholate (Galocatechin, CAT) siderophores, porphyrins (protoporphyrin IX, PIX), organic amine (N,N'-Di(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid monohydrochloride hydrate, HBED) and saccharides (mono-saccharides: D-Galacturonic acid (GAL), D-Glucuronic acid (GLU), Alginic acid from brown algae (ALG); polysaccharides: Alginic acid from brown algae (ALG), dextran from *Leuconostoc* ssp. (DEX, 500 000 g/mole)). All organic ligands were from Sigma-Aldrich, except HEBD (Strem Chemical). Stock solutions of 1 mmol L^{-1} were prepared with ultra-pure water (Millipore Element, $18.2 \text{ M}\Omega\text{cm}$), except for PIX that was prepared in DMSO (JT Baker). Stock solutions were then diluted down to $15 \mu\text{mol L}^{-1}$ in milliQ water and analysed

for background Fe contamination by ICP-MS (Perkin Elmer Elan DRC II ICP-MS). When Fe contamination was detected (0.7 nmol L^{-1} for $15 \mu\text{mol L}^{-1}$ DFB, 16 nmol L^{-1} for $15 \mu\text{mol L}^{-1}$ PIX and 0.7 nmol L^{-1} for 15 nmol L^{-1} DEX), it was considered in the experimental setup (L equals 15 nmol L^{-1}) and calculations. Fe contamination was below detection limit (0.05 nmol L^{-1}) for the other ligands. Stock solutions were stored in the dark at 4°C or -18°C (for siderophores).

Since the ISPOL filtered water used was not UV photo-oxidised, it contains stable organic ligands that can react with the added Fe. In the control treatment, where the quantity of the added Fe (as ^{55}Fe) is negligible (5% of total dissolved Fe naturally present), the chemical speciation of Fe should represent its natural distribution (assuming equilibration within 24 h). Following the 1 nmol L^{-1} Fe addition (Fe treatment), weaker Fe-reactive organic ligands will be bound and probably saturated with Fe. The Fe treatment, therefore, corresponds to the amendment of highly reactive organically bound and possibly inorganic Fe.

2.4 Fe bioaccumulation experiment

Radiolabelled Fe (^{55}Fe , PerkinElmer, $82.80 \text{ mCi mg}^{-1}$ as FeCl_3 in 0.5 mol L^{-1} HCl) was used in bioaccumulation experiments. Radioactive standard solutions of inorganic Fe ($0.7 \mu\text{mol L}^{-1}$ Fe and $0.3 \mu\text{mol L}^{-1}$ ^{55}Fe in 0.01 mol L^{-1} HCl) and organic Fe ($0.7 \mu\text{M}$ Fe, $0.3 \mu\text{M}$ ^{55}Fe , $15 \mu\text{M}$ L) were prepared one week in advance to reach equilibrium and stored in the dark at 4°C . Radioactive stock solutions were 1000-fold diluted into ISPOL filtered water (1.6 nCi mL^{-1}) and kept in the dark at 2°C for 24 h to reach equilibrium. In the control treatment, only 0.08 nCi mL^{-1} of ^{55}Fe were added corresponding to negligible Fe enrichment (5%). For each experimental solution, two mL were taken to determine the total initial ^{55}Fe content.

Following the equilibration phase, microorganisms were directly added into the radioactive experimental solution so as to reach a final biomass of $10\,000 \text{ cells mL}^{-1}$ for *Phaeocystis* sp. and *Chaetoceros* sp. and $1000 \text{ cells mL}^{-1}$ for *Thalassiosira antarctica* and *Fragilariopsis kerguelensis*. They were then incubated for 16 h at 2°C and con-

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stant light ($120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for *Phaeocystis* and $60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for diatoms). After 16 h incubation, the algal suspensions were transferred in the dark at 4°C and were immediately processed. Ten mL of the solution was successively filtered using a syringe with $0.2 \mu\text{m}$ and $0.02 \mu\text{m}$ filters (Anatop, Whatman) to determine dissolved and soluble Fe, respectively. The remainder of the sample was divided into two parts: one was gently filtered ($<5 \text{ mm Hg}$, $0.45 \mu\text{m}$, nitrocellulose, Sartorius) for the measurement of the total cellular Fe. Oxalate solution (Tang and Morel, 2006; Hassler and Schoemann, 2009) was directly added in a 1:10 v:v to the second portion of the sample. This solution was kept in the dark at 4°C for 20 min prior to being filtered ($<5 \text{ mm Hg}$, $0.45 \mu\text{m}$) for the determination of intracellular Fe. Following filtration, filters were rinsed 3-times with non-radioactive saline solution ($0.6 \text{ mol L}^{-1} \text{ NaCl}$, $2.38 \text{ mmol L}^{-1} \text{ NaHCO}_3$, Tang and Morel, 2006). This procedure was shown to be effective in removing extracellular Fe from *Chaetoceros* sp., *Phaeocystis* sp. and natural assemblages from the SO without biologically deleterious effects (Hassler and Schoemann, 2009). Radioactive Fe solutions and filters were collected in scintillation vials with 10 mL scintillation cocktail (Ultima Gold, Perkin Elmer), vortexed and measured using a liquid scintillation analyzer (Perkin Elmer Tri-Carb 2100TR). Counts per minutes were then converted into disintegration per minutes (dpm) using custom quench curves.

The uptake of radioactive Fe (dpm) was related to Fe concentrations using the sum of the dissolved Fe concentration measured in the ISPOL filtered water and the nominal Fe addition. Extracellular Fe concentrations were calculated as the difference between total cellular Fe and intracellular Fe. Experiments were done in duplicates.

Fe concentrations were normalised against incubation time and cell number and are expressed in $\text{amol Fe h}^{-1} \text{ cell}^{-1}$. Fe cellular concentrations were also normalised by cellular volume (V), surface area (A), and surface area to volume ratio (A/V).

An experiment was done without any microorganisms in order to measure the effect of organic ligand alone on Fe size fractionation (dissolved ($<0.2 \mu\text{m}$), soluble ($<0.02 \mu\text{m}$), and particulate ($>0.2 \mu\text{m}$) Fe). Loss of Fe due to adsorption on the wall of

the containers used was negligible (<5%) during the 24 h equilibration phase, as well as during the 16 h incubation.

2.5 Carbon uptake and Fe:C ratio

In parallel to Fe bioaccumulation experiments, carbon uptake was measured following 2 h and 16 h incubations in 50 to 100 mL samples. The 2 h incubation was conducted to measure the photosynthetic carbon fixation and to minimize radiocarbon losses by respiration whereas the 16 h incubation was done to compare with the longer incubation time needed with ^{55}Fe to achieve better sensitivity. Radioactive carbon was added at a final concentration of $100 \mu\text{Ci L}^{-1}$ sodium ^{14}C bicarbonate to $0.2 \mu\text{m}$ filtered water from ISPOL prior to the addition of microorganisms (see Fe bioaccumulation for details). The phytoplankton was then incubated in the same conditions as for Fe bioaccumulation. Cells were collected by gentle filtration (<5 mm Hg, 25-mm $0.8 \mu\text{m}$ Millipore polycarbonate filter) and rinsed with saline solution ($0.6 \text{ mol L}^{-1} \text{ NaCl}$, $2.38 \text{ mmol L}^{-1} \text{ NaHCO}_3$, Tang and Morel, 2006). Organic carbon fixation was differentiated using acidification ($100 \mu\text{l}$ $0.1 \text{ mol L}^{-1} \text{ HCl}$) of particulate material (see Schoemann et al., 2001). The ^{14}C radioactivity was determined by liquid scintillation with a Perkin Elmer (Tri-Carb 2100TR) analyser after the addition of the scintillation cocktail (EcoLume, MP Biomedicals). Experiments were done in duplicate. Fe:C ratio was calculated using the 2 h carbon uptake (or assimilation) and the total and intracellular iron uptake rates.

2.6 Precautions to avoid contamination and statistical test

No bacteria were observed by DAPI stain and epifluorescence microscopy (Porter and Feig, 1980) in the ISPOL filtered water, the cultures used or the experimental final biomass controls. All culture manipulation and solution preparation were done in a class-100 laminar flow bench following trace-metal-clean and sterility procedures. All containers and filters used, prior to radioactive data collection, made of polycarbonate (Nalgene, Millipore), were acid washed ($1 \text{ mol L}^{-1} \text{ HCl}$). Pipette tips were acid-cleaned

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with 6 mol L⁻¹ ultrapure HCl (Seastar). After acid cleaning, all material was thoroughly rinsed with ultra-pure water (Millipore, milli-Q Element).

The significance of the differences between treatments was assessed using a two-tailed paired student t-test at the 95% confidence level.

3 Results

3.1 Fe bioaccumulation – Fe enrichment

The addition of 1 nmol L⁻¹ Fe resulted in increased intracellular Fe uptake rates for all strains as well as the percent of extracellular Fe as compared to the control treatment, except for *Thalassiosira antarctica* (CONT, Fig. 1). Most of the cellular iron (>50%) is associated with extracellular binding sites (Fig. 1B). The extracellular to intracellular Fe pool ratio (Fe_{ext}:Fe_{int}), gives information on the cellular iron distribution. A Fe_{ext}:Fe_{int} ratio of 1 means that iron is equally distributed between the intracellular and extracellular pools (i.e. 50% Fe extracellular), whereas a Fe_{ext}:Fe_{int}>1 means that iron is predominantly associated with the extracellular fraction (i.e. Fe extracellular >50%). This ratio is nearly constant for all strains in the control treatment (*Chaetoceros*, 1.6; *Phaeocystis*, 1.6; *Thalassiosira*, 1.2; *Fragilariopsis*, 1.8). Except for *Thalassiosira* (0.9), this ratio increases for all strains after the addition of 1 nM Fe, attesting that higher increases are generally observed in the extracellular pool than in the intracellular pool as a result of Fe enrichment. The chain forming diatom *Fragilariopsis* has the highest ratio (6.9) followed by *Chaetoceros* (5.4) and *Phaeocystis* (3.5).

For all treatments (except in the case of extracellular Fe for *Fragilariopsis*), intracellular and extracellular Fe per cell follow the order: *Chaetoceros* sp. < *Phaeocystis* sp. < *Fragilariopsis kerguelensis* < *Thalassiosira antarctica*. A maximal variation in Fe_{int} and Fe_{ext} of 4.9-fold is observed for small single cells such as *Chaetoceros* and *Phaeocystis*, whereas it is 18.5-fold within all the strains studied (Fig. 1A). However, the variability of the cellular iron between strains was the smallest when iron was normalised against

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the surface area (Table 1, Supplement, see <http://www.biogeosciences-discuss.net/6/1677/2009/bgd-6-1677-2009-supplement.pdf>). In this case, the difference between all single cells decreased and a maximal variation of 6.5-fold for Fe_{int} and Fe_{ext} is observed in between diatoms and *Phaeocystis*, with a minimal variation between the three diatoms of 1.1-fold. Cellular Fe normalized against the surface area follows the order *Phaeocystis* > *Chaetoceros* \geq *Thalassiosira* \geq *Fragilariopsis*. A maximal variation between cellular Fe normalised against V and A/V of 70 and 7800-fold, respectively was observed for the four selected strains (Table 1, Supplement, see <http://www.biogeosciences-discuss.net/6/1677/2009/bgd-6-1677-2009-supplement.pdf>).

3.2 Fe bioaccumulation – excess of organic ligands

The addition of 1 nmol L^{-1} Fe with 15 nmol L^{-1} of various organic ligands resulted in different effects on the intracellular Fe uptake rates as compared to the intracellular Fe uptake rates after the addition of 1 nmol L^{-1} Fe without any amendment of organic ligand (Fig. 2A). The hydroxamate DFB and organic amine HBED strongly decreased Fe bioavailability, whereas no statistical effect (at the 0.05 level) was observed for the catecholate CAT (except for *Chaetoceros*) for all the strains studied (Fig. 2A). The addition of Fe bound to a tetrapyrrole (PIX) resulted either in no statistical change (*Thalassiosira*, level of 0.05) or in an increase (*Phaeocystis*, *Chaetoceros*, *Fragilariopsis*) in intracellular Fe uptake rates.

The presence of excess mono- (GAL, GLU) and poly-saccharides (ALG, DEX) generally resulted in increased intracellular and extracellular Fe uptake rates for *Chaetoceros*, *Phaeocystis* and *Fragilariopsis* (Fig. 2A and B). Except from a slight decrease of intracellular Fe uptake in the presence of DEX, the addition of saccharides had no significant effects on Fe uptake rates of *Thalassiosira*.

The organic ligands amendments have similar effects on intracellular and extracellular Fe (Fig. 2B), except for ALG for *Thalassiosira* and *Fragilariopsis* where a disproportionate increase of extracellular Fe was measured (see below). The $Fe_{ext}:Fe_{int}$ ratio was relatively constant for all treatments (except ALG for *Thalassiosira* and *Fragilari-*

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opsis). Most of the variation was found between strains. For each strain, $Fe_{ext}:Fe_{int}$ ratios were similar in presence and absence of added ligands. Ratios were 2.6 ± 0.8 for *Phaeocystis*, 4.2 ± 1.5 for *Chaetoceros*, 1.0 ± 0.2 for *Thalassiosira* (but 5.6 for ALG), 6.0 ± 1.7 for *Fragilariopsis* (but 25.3 for ALG).

5 3.3 Fe size distribution

The addition of ligands will affect Fe chemical speciation, but also its overall solubility. In this study, Fe solubility was assessed by discriminating between particulate ($>0.2 \mu m$), colloidal Fe ($0.02-0.2 \mu m$) and soluble Fe ($<0.02 \mu m$) following successive filtration of the Antarctic seawater (Fig. 3) in the absence of microorganism.

10 In the control treatment, most of the Fe stays in the dissolved phase ($>70\%$, $<0.2 \mu m$) with 38.5% soluble and 33.0% colloidal. The addition of 1 nmol L^{-1} Fe increased the concentration of particulate Fe from 28.5 to 55.7%. In the Fe treatment, both soluble (28.4%) and colloidal (15.8%) Fe decreased as compared to the control.

15 For an addition of 1 nmol L^{-1} Fe together with the organic ligands, particulate Fe remains lower than in the Fe treatment, except in presence of ALG. In presence of DFB and HEBD, Fe is fully relocated within the soluble phase. For the other ligands (except ALG), the soluble and colloidal Fe fraction is increased as compared to the Fe treatment, to a level close to what is observed in natural water (control). The aggregation of Fe in the presence of ALG could explain the disproportionately high and variable
20 extracellular Fe observed in the case of *Thalassiosira* and *Fragilariopsis* as abiotic particulate Fe can become significant as compared to the biotic Fe (Fig. 2B).

In presence of microorganisms, abiotic to biotic particulate Fe cannot be differentiated when total Fe uptake is being determined. However, similar trends in soluble and colloidal Fe were observed. For example, the addition of 1 nmol L^{-1} Fe resulted in a
25 decrease of both averaged colloidal (27% in CONT down to 16% in the Fe treatment) and soluble (35% in CONT down to 5% in the Fe treatment) Fe. The addition of DFB and HBED relocated the Fe (not already associated with phytoplankton) into the soluble fraction. In presence of the other ligands, both soluble and colloidal Fe, were higher

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than in the Fe treatments.

3.4 Carbon fixation

The carbon uptake rates per cell were contrasted for the various studied strains (e.g. variation of 126-fold for the 2h incubation) and follow the order: *Chaetoceros* (28.9±7.5 fmol C cell⁻¹ h⁻¹) < *Phaeocystis* (65.9±13.0 fmol C cell⁻¹ h⁻¹) < *Thalassiosira* (1059.8±121.0 fmol C cell⁻¹ h⁻¹) < *Fragilariopsis* (2828.5±597.0 fmol C cell⁻¹ h⁻¹). Normalisation of carbon uptake by cell volume gives the lowest variability in carbon uptake between the strains selected. For carbon uptake normalised per unit volume, only a two-fold variation was observed between all the diatoms.

This small variation shows that the large differences observed at the cellular level can largely be attributed to differences in cell volume. *Phaeocystis* has the highest C uptake per unit of cell volume (1.4 to 2.9-fold higher than for diatoms, Table 2, Supplement, see <http://www.biogeosciences-discuss.net/6/1677/2009/bgd-6-1677-2009-supplement.pdf>), which can be only partly due to the carbon allocated to the production of mucus in the colonies since most of the cells are isolated in the experimental conditions. The maximal inter-strain variation of carbon uptake normalised against A and A/V, were 8.4 and 58 700-fold, respectively (Table 2, Supplement, see <http://www.biogeosciences-discuss.net/6/1677/2009/bgd-6-1677-2009-supplement.pdf>).

The presence of 1 nmol L⁻¹ Fe or 1 nmol L⁻¹ Fe with organic ligands did not statistically affect carbon fixation. Carbon cellular uptake rate were similar following both 2 h and 16 h incubations.

3.5 Fe to carbon ratio

Both Fe and C uptake rates per cell follow the same order for *Chaetoceros*, *Phaeocystis* and *Thalassiosira*. *Fragilariopsis* has by far the highest carbon uptake rate but has the third highest Fe uptake rate. Because of a small difference of Fe uptake rates (e.g. 9-

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fold in control treatment, Fig. 1) as compared to carbon uptake rates (e.g. 126-fold in control treatment, Fig. 4) for the strains studied, the Fe to carbon ratio is also quite variable (20 to 25-fold, Table 2). Fe:C ratios were calculated both considering intracellular Fe only and considering total (intracellular + extracellular) Fe uptake. The Fe:C ratios are reported here for both control and 1 nmol L⁻¹ Fe addition treatments. Fe:C ratios are ranked as follows: *Chaetoceros* > *Phaeocystis* > *Thalassiosira* > *Fragilariopsis*. Because of a higher Fe uptake rates but identical carbon uptake rates in presence of 1 nmol L⁻¹ Fe as compared to the control treatment on the incubation time of the experiment, Fe:C were statistically higher for the Fe treatment (except for *Chaetoceros* considering Fe_{int}).

4 Discussion

4.1 Fe bioavailability to Antarctic phytoplankton

In this study, the bioavailability of organic forms of Fe is related to Fe intracellular uptake rates. The addition of Fe (1 nmol L⁻¹) resulted in increased bioavailability as attested by higher intracellular and extracellular Fe for all strains. The intracellular Fe uptake rates (0.4 to 3.3 × 10⁻¹⁸ mol cell⁻¹ h⁻¹) and the corresponding proportion of extracellular Fe as compared to total Fe taken up (55 to 65%) in the Antarctic seawater, are similar to those previously obtained (e.g. Hudson and Morel, 1989; Hutchins et al., 1999). The increase in iron cellular pools also suggested that both transport and non-specific Fe cellular sites are not saturated in the control treatment for all the studied strains (e.g. Wilkinson and Buffle, 2004).

In this study, intracellular and extracellular Fe pools were normalised in an attempt to identify parameters explaining the variability of Fe uptake for Antarctic phytoplankton. The least variability was observed when Fe pool was normalised per cellular surface area as compared to the normalisation per volume or per surface to volume ratio. For Fe normalised per surface area, the maximal difference was observed between *Phaeo-*

cystis and large diatoms, with both intracellular and extracellular Fe per surface area being the highest for *Phaeocystis*, suggesting a higher affinity for Fe binding for *Phaeocystis* than for diatoms.

4.2 Fe to carbon ratio for Antarctic phytoplankton

5 Fe to carbon ratio is usually higher for Fe-replete as compared to Fe-limited cells (e.g. Twining et al., 2004). Based on the Fe:C observed for the control treatment (filtered Antarctic seawater), variable Fe limitation was experienced for the studied strains, with *Fragilariopsis* being the most limited with the lowest Fe:C ratio. Previous studies reported Fe:C for Fe limited Southern Ocean natural phytoplankton between 1 and
10 20 $\mu\text{mol Fe}:\text{mol C}$ (Abraham et al., 2000; Twining et al., 2004; McKay et al., 2005), well in accordance to the Fe:C ratios observed in our study (Table 2).

In this study, intracellular Fe to carbon uptake ratios are proportional to surface area to volume ratios (Fig. 4A), suggesting that A/V is determining their Fe limitation, as measured by Fe:C in the conditions of the experiment. Since a Fe enrichment of
15 1 nmol L^{-1} increased Fe uptake (except for *Fragilariopsis*, see below), but did not affect C uptake following a 16-h incubation, linear relationship is maintained with little change in the slope but a higher intercept. Although higher Fe:C was previously reported for picoplankton as compared to larger phytoplankton in subantarctic waters (McKay et al.,
20 2005), further studies are required to verify the robustness of such relationship in the SO.

4.3 Limiting step for Fe bioavailability – ecological implications for Antarctic phytoplankton

For a given chemical composition, the following situations – diffusion limited transport, fast uptake processes, and low affinity for extracellular binding sites – all favoured a
25 lower ratio between the extracellular and intracellular Fe pools (Wilkinson and Buffle, 2004). Comparison of Fe and control treatments showed that in the presence of

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1 nmol L⁻¹ Fe, a larger increase of extracellular than intracellular Fe was observed for all strains except *Thalassiosira*, resulting in an increased Fe_{ext}:Fe_{int} ratio. This suggests that the diffusion of Fe does not limit Fe biological intracellular uptake in the control treatment for *Fragilariopsis*, *Chaetoceros* and *Phaeocystis*. Since the highest intracellular Fe uptake rates were measured for *Thalassiosira*, a fast Fe uptake could be the cause of the low Fe_{ext}:Fe_{int} ratio observed. In this case, *Thalassiosira antarctica* is able to take up Fe at the limit of its diffusive supply, it is, thus, expected to rapidly respond to environmental changes. Interestingly, chain-forming diatom *Fragilariopsis*, which is more prone to diffusion limitation (Pahlow et al., 1997), does not appear to be strongly diffusion limited here, because the Fe_{ext}:Fe_{int} ratio increases upon Fe enrichment. *Fragilariopsis* has the highest Fe_{ext}:Fe_{int}, which could result from either strong extracellular binding affinity or a small rate of intracellular Fe uptake. *Fragilariopsis* has the lowest Fe_{int}/A in this study. A 1 nmol L⁻¹ Fe enrichment resulted in the smallest increase in extracellular Fe as compared to the other tested species (probably as a result of lower diffusive supply, Pahlow et al., 1997) but also the smallest increase in intracellular Fe (which could result from a lowered Fe requirement in response to strong Fe limitation, Sunda and Huntsman, 1995). Under these circumstances, *Fragilariopsis* would slowly respond to changes in environmental conditions and long-term, sustained, Fe enrichment might be required for *Fragilariopsis* to bloom.

It has been recognised that large diatoms are responsive to large-scale in-situ (de Baar et al., 2005) and bottle (Takeda et al., 1998) iron artificial fertilisation. *Fragilariopsis* sp. and *Fragilariopsis kerguelensis* were reported as being dominant (and blooming) in naturally iron-enriched region of the ocean such as the Polar Front region (de Baar et al., 1997), continental margin-enriched region of the subarctic Pacific (Lam et al., 2006), and the Kerguelen region (Armand et al., 2008; Timmermans et al., 2008). In the Kerguelen region, *Thalassiosira antarctica* was dominating in all locations during the KEOPS study (Armand et al., 2008).

In contrast, with higher Fe:C ratios, *Chaetoceros* sp. and *Phaeocystis* sp., mainly present as solitary cells, did not appear strongly Fe-limited in our experiment. These

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strains should not be easily Fe-limited as a result of (i) high Fe diffusive supply because of their small size (as single cells), and higher surface to volume ratio (Pahlow et al., 1997), and (ii) lower Fe requirement as compared to the other studied strains (Coale et al., 2003; Timmermans et al., 2004). For these reasons, these strains are expected to be widespread and blooming in Fe-limited regions of the Southern Ocean. Small diatoms (e.g. *Chaetoceros* from this study) and *Phaeocystis* are found in most of the Southern Ocean (Sarhou et al., 2005; Schoemann et al., 2005).

Single species studies using water collected at variable location during the KEOPS study (Timmermans et al., 2008), showed that growth of the small diatom *Chaetoceros brevis* was not responsive to the natural iron enrichment observed in the deep water of the Kerguelen plateau (dissolved $[Fe]=0.35 \text{ nmol L}^{-1}$), whereas *Fragilariopsis kerguelensis* growth was strongly promoted in naturally iron-enriched water. In this case, an average cell number of 29 ± 6 per chain was reported (Timmermans et al., 2008), similar to what was observed in this study.

For all the pure phytoplankton cultures studied here (except *Fragillariopsis*) $Fe_{\text{ext}}:Fe_{\text{int}}$ is directly related to the A/V (Fig. 4B). Both $Fe:C$ and $Fe_{\text{ext}}:Fe_{\text{int}}$ suggest that single cells with $A/V \geq 1.0$ are well adapted to Fe-limited conditions prevailing in the Southern Ocean, which could be explained by a high iron diffusive flux (Pahlow et al., 1997). In this study, diatoms with $A/V < 0.5$ showed either a fast iron intracellular uptake (*Thalassiosira*) or high $Fe_{\text{ext}}:Fe_{\text{int}}$ (*Fragilariopsis*), suggesting variable strategies to survive under low iron concentrations. Since both $Fe_{\text{ext}}:Fe_{\text{int}}$ and $Fe:C$ respond to A/V, implications related to the maximum diffusive supply of Fe (Pahlow et al., 1997) would be important in predicting Fe limitation and its impact on C cycling or export. However, more studies are required to verify the robustness of these relationships for other phytoplankton from the Southern Ocean.

4.4 Importance of organic ligands in controlling Fe bioavailability to Antarctic phytoplankton

Based on conventional thermodynamic chemistry and models used to describe biological uptake (e.g. Wilkinson and Buffle, 2004; Shaked et al., 2005), complexation of Fe with ligands should result in a decrease in Fe biological uptake if non- or partially bioavailable complexes are formed. In such case, labile forms of Fe might contribute to the bioavailable pool of Fe (van Leeuwen, 1999). Unchanged biouptake should be observed if the Fe complex is fully bioavailable, or if the inorganic Fe is either enough to sustain the biological requirement or not significantly decreased (Morel et al., 2008). Bioavailability can thus be described as a function of both chemical lability/reactivity (dependant upon possible dissociation within the diffusive layer around the microorganism) and specific and non-specific biological transport pathways (carrier, surface reductase, siderophore receptor/transporter, etc.). The presence of organic complexes that can be specifically recognised by micro-organisms (e.g. siderophores), results in an increased apparent bioavailability of Fe for this specific class of plankton. Each ligand affect Fe bioaccumulation (a surrogate for its bioavailability) in a similar way for all the strains studied here (except CAT for *Chaetoceros* sp.). In general, the presence of ligands affects both extracellular and intracellular Fe in the same way, which is not surprising given the need for Fe to react with the biological surface prior to its transport inside the microorganism.

The least responsive strain to the presence of organic ligands is *Thalassiosira antarctica*, whereas the most responsive strain is *Chaetoceros* sp. The fact that Fe bioavailability to *Thalassiosira* is poorly enhanced by the presence of organic ligands (see below), is likely the result from its ability to rapidly take up Fe, at the limit of its diffusive supply. In this case, labile complexes may contribute to the bioavailable pool of Fe (van Leeuwen, 1999). In order to enhance Fe bioaccumulation, the complex needs to be taken up directly or to be specifically bioavailable (such as siderophores).

Both DFB and HBED, are strong ligands for Fe with $\log K_{\text{Fe(III)L}}$ of 41.8 L mol^{-1} for

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HEBD and 44.1 L mol^{-1} for DFB (NIST, ver. 8.0, stability constant corrected for a ionic strength of zero, using the Davies equation). The log of the conditional stability constant for Fe(III)DFB ($\log K_{\text{Fe(III)DFB}}^{\text{cond}}$) previously measured using voltammetric technique varied from 21.6 to 26.5 L mol^{-1} in UV-treated seawater (e.g. Rue and Bruland, 1995; Maldonado et al., 2005). In this study, these strong ligands out-compete ligands naturally present relocating all the Fe into the soluble fraction and decreased Fe bioavailability for all the model strains. Gallocatechin (CAT), is a catechol siderophore with strong binding affinity for Fe ($\log K_{\text{Fe(III)CAT}}^{\text{cond}}=22.6 \text{ L mol}^{-1}$ in UV-treated seawater, Maldonado et al., 2005), which partially relocates Fe between the soluble and colloidal phases. Surprisingly, it did not significantly diminish Fe bioavailability for any of the strains studied here. Indeed, an important enhancement of Fe bioavailability was observed for *Chaetoceros* sp., suggesting that this diatom was able to recognise Fe bound to this catecholate. Contrasting response to in-situ ligands, DFB and CAT has already been observed in the subantarctic region by Maldonado et al. (2005). In that study, the presence of 2 nmol L^{-1} Fe and 20 nmol L^{-1} CAT increased the average Fe bioaccumulation for all plankton size fractions ($0.2\text{--}2$, $2\text{--}20$ and $>20 \mu\text{m}$) as compared with the addition of 2 nmol L^{-1} Fe (buffered with EDTA) to ligands naturally present, whereas a decreased Fe bioaccumulation was observed for 2 nmol L^{-1} Fe and 20 nmol L^{-1} DFB addition. In addition, Hutchins et al. (1999) reported a smaller decrease in Fe bioaccumulation to eukaryotic phytoplankton in the presence of catecholate (enterobactin) as compared with hydroxamates (ferrioxamine and ferrichrome).

The protoporphyrin IX (PIX) is also a strong ligand for Fe ($\log K_{\text{Fe(III)PIX}}^{\text{cond}}=22.0 \text{ L mol}^{-1}$, Rue and Bruland, 1995) resulting in a partial Fe relocation within the soluble and colloidal phases. The presence of 1 nmol L^{-1} Fe and 15 nmol L^{-1} PIX resulted in an enhanced Fe bioaccumulation as compared to the 1 nmol L^{-1} Fe enrichment for the four phytoplankton studied, in accordance with what was previously reported for polar eukaryotic phytoplankton (Hutchins et al., 1999).

This study focussed on other type of organic ligands, (poly)saccharides, probably weaker than siderophores or porphyrins, where little information of Fe complex for-

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mation is known. Polysaccharides constitute a significant fraction of storage carbohydrates and exopolymeric substances (EPS; Mancuso et al., 2005; Wang et al., 2006). Storage carbohydrates and EPS are produced by prokaryotes as well as eukaryotic plankton (Hoagland et al., 1993; Freire-Nordi et al., 2005; Mancuso et al., 2005). For example, diatoms are known to produce EPS involved in substrate attachment, chain formation and nutrient uptake (Hoagland et al., 1993). These substances can be released upon active secretion (e.g. EPS) or cell lysis (e.g. storage products) at concentrations much higher than dissolved Fe (generally 0.1–0.6 nmol L⁻¹ in the SO, de Baar and de Jong, 2001) or reported siderophores or porphyrins concentrations in natural water (McCormack et al., 2003; Gledhill, 2007; Vong et al., 2007). Dissolved sugar concentrations of 50–4000 nmol L⁻¹ were reported in the SO (Panagiotopoulos and Sempéré, 2005, and references therein). Although polymeric substances found in the ocean are complex, polyfunctional, polydisperse and still remain poorly characterised, uronic acids are often pointed out (here represented by GAL, GLU and ALG). Uronic acids represent a significant fraction of bacterial EPS produced in the Southern Ocean (Mancuso et al., 2005), can be excreted by Antarctic haptophytes (Janse et al., 1996) and are a structural component of the cell wall of some phytoplankton (e.g. Davies et al., 2003). In addition, uronic acids have carboxylic groups known to be reactive with transition metals (e.g. Davies et al., 2003; Fe(III) has a strong affinity for oxygenated ligands, e.g. Morel and Hering, 1983). EPS have been shown to bind transition metals (e.g. Freire-Nordi et al., 2005) and have also been suggested as being important for Fe in the Southern Ocean (Mancuso et al., 2005). Both coccolithophores and diatoms were shown to excrete organic compounds able to alter Fe chemical lability and its bioavailability (Boyé and van den Berg, 2000; Rijkenberg et al., 2008). Indeed, the addition of 1.2 μmol L⁻¹ hydroxycarboxylic acid (glucaric acid) to iron, further stimulate the phytoplankton from Antarctic winter ice edge (Öztürk et al., 2004). Together with some experiments conducted on natural community in the Southern Ocean (Schoemann et al., 2009), this study, is the first, to our knowledge, to demonstrate that saccharides can stabilise Fe within the soluble and colloidal phases, as well as quantitatively enhance

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its bioavailability to selected eukaryotic phytoplankton from the Southern Ocean. It is unlikely that Fe bound to saccharides resulted in species-specific bioavailability, similar to what has been described for siderophores. Since similar enhancement of Fe bioavailability was observed for small uronic acids and larger polysaccharides (dextran), it seems that the enhanced bioavailability does not result from direct uptake of the Fe bound to the saccharides. Finally, since no bacteria were observed in the cultures used in this study (DAPI stain), bacterial degradation cannot account for the enhanced bioavailability observed. In this case, the increased Fe bioavailability in presence of saccharides, could result from a simultaneous Fe stabilisation within the dissolved phase (Chen et al., 2004; Tian et al., 2006, this study), and the formation of chemically labile Fe forms that are rapidly exchangeable with the bioavailable pool (possibly inorganic, Morel et al., 2008). The study from Öztürk et al. (2004) reported an enhanced Fe(III) photoreduction by primary and possibly secondary photochemical processes in presence of glucaric acid, suggesting that reduction processes and formation of bioavailable and labile Fe(II) species are critical to increase Fe bioavailability.

Although strong organic ligands for iron binding in seawater (reported by CLEAdSV) are reported with stability constants close to what is observed for porphyrins and siderophores (Rue and Bruland, 1995; Croot and Johansson, 2000; Witter et al., 2000; Boyé et al., 2001; Maldonado et al., 2005), their nature is still uncharacterised. The saccharides systematically occur at higher concentrations than that of the dissolved Fe (e.g. Rue and Bruland, 1995; Croot and Johansson, 2000; Boyé et al., 2001), and at levels higher than reported for siderophores and porphyrins (McCormack et al., 2003; Gledhill, 2007; Vong et al., 2007). If saccharides accounted for part of this Fe organic complexation, or if they were able to significantly outcompete other strong organic ligands reported for Fe, then Fe bioavailability could be enhanced as a result of the ubiquitous presence of saccharides in natural water. However, study demonstrating the ability of saccharides to form organic complex with iron that falls within the detection windows of the CLEAdSV techniques, are still missing.

As mentioned above, the chain-forming *Fragilariopsis kerguelensis* used in the study

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would produce EPS (Hoagland et al., 1993) and showed the highest extracellular Fe per surface area for diatoms in unenriched Antarctic seawater. In addition, *Phaeocystis*, known to produce EPS for their extracellular mucus (Janse et al., 1996), had the highest extracellular Fe per unit of surface area in this study, suggesting that EPS might in fact favour Fe complexation and adsorption to biological surfaces (Schoemann et al., 2001). Preferential binding of EPS has already been demonstrated for other transition metals (e.g. Davies et al., 2003; Freire-Nordi et al., 2005).

5 Conclusions

Given the ubiquitous presence of saccharides in the ocean, these compounds might represent an important factor to control the basal level of soluble and bioavailable Fe. The importance of saccharides on iron bioavailability in the ocean needs thus, to be further investigated, especially with natural plankton communities. In addition, further studies are required to understand the mechanism by which saccharides can promote iron bioavailability to phytoplankton.

Results presented here demonstrate that the use of model phytoplankton is promising to improve mechanistic understanding of Fe bioavailability (in presence of natural or model organic ligands) and of primary productivity in HNLC regions of the ocean. This study also brings considerations about the control that Fe might have on the species distribution observed in the SO.

The differential effect that organic ligands have on the solubility and the bioavailability of Fe, shows that no direct link exist between soluble and bioavailable Fe. In fact, colloidal Fe seems to be an important pool to sustain Fe bioavailability. Bioavailability of organic colloidal Fe to phytoplankton has already been demonstrated in previous studies (e.g. Nodwell and Price, 2001; Wang and Dei, 2003).

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Table 1. Cellular volume (V), surface area (A) and surface area to volume ratio calculated according to Hillebrand et al. (1999) for: *Chaetoceros*, *Thalassiosira* present as single cells only, *Phaeocystis* mainly occurring as single cells (78%) and *Fragilariopsis* chains of 28±12 cells (n=23).

| Strain | Volume (μm^3)±SD | Surface area (μm^2)±SD | A/V (μm^{-1})±SD |
|------------------------------------|-------------------------------|-------------------------------------|-------------------------------|
| <i>Phaeocystis</i> sp. | 69±33 | 79±27 | 1.25±0.24 |
| <i>Chaetoceros</i> sp. | 31±5 | 62±9 | 2.01±0.07 |
| <i>Thalassiosira antarctica</i> | 1747±353 | 801±112 | 0.47±0.04 |
| <i>Fragilariopsis kerguelensis</i> | 218 194±91 589 | 25 871±9969 | 0.12±0.01 |

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Table 2. Iron to carbon ratio ($\mu\text{mol}:\text{mol}$) for the four strains studied: *Phaeocystis* sp., *Chaetoceros* sp., *Thalassiosira antarctica* Comber, and *Fragilariopsis kerguelensis*. Mean values ($n=4$) are given with standard deviation for the control (C) and addition of 1 nmol L^{-1} Fe (Fe) experimental treatments. Ratios are calculated considering either total cellular Fe (Fe_{tot}) or intracellular Fe (Fe_{int}) uptake rates and short term (2 h) carbon uptake rates. The statistical difference in the Fe:C ratios between the two experimental treatments is shown in italic (p value of paired t-test).

| Treatment | Parameter | <i>Phaeocystis</i> | <i>Chaetoceros</i> | <i>Thalassiosira</i> | <i>Fragilariopsis</i> |
|-----------|-----------------------------------|--------------------------------|--------------------------------|-----------------------------------|-----------------------------------|
| CONT | $\text{Fe}_{\text{int}}:\text{C}$ | 14.7 ± 1.0 <i>0.0001</i> | 19.1 ± 0.8 <i>0.3122</i> | 3.5 ± 0.2 <i><0.0001</i> | 0.8 ± 0.1 <i>0.0010</i> |
| | $\text{Fe}_{\text{tot}}:\text{C}$ | 23.4 ± 1.9 <i>0.0037</i> | 49.0 ± 1.2 <i>0.0050</i> | 7.8 ± 0.3 <i><0.0001</i> | 2.2 ± 0.2 <i><0.0001</i> |
| Fe | $\text{Fe}_{\text{int}}:\text{C}$ | 21.4 ± 1.2 | 23.5 ± 8.1 | 11.0 ± 0.2 | 1.0 ± 0.1 |
| | $\text{Fe}_{\text{tot}}:\text{C}$ | 74.8 ± 4.0 | 154.6 ± 32.6 | 20.8 ± 0.9 | 8.1 ± 0.6 |

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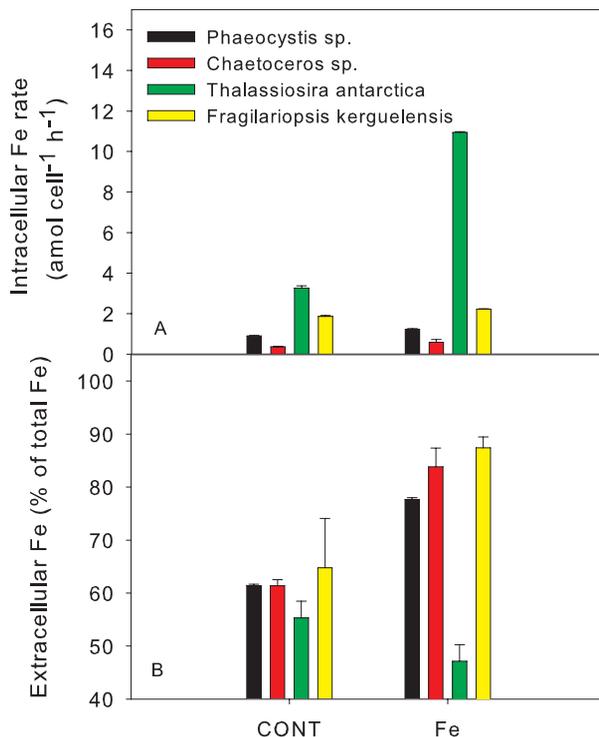


Fig. 1. Intracellular Fe uptake rates (A) and percentage of extracellular Fe (B) for *Phaeocystis* sp., *Chaetoceros* sp., *Thalassiosira antarctica* and *Fragilariopsis kerguelensis* in ISPOL filtered water (CONT treatment) and with 1 nmol L⁻¹ Fe enriched ISPOL seawater (Fe treatment). Both intracellular and extracellular Fe are expressed on a cellular basis. Error bars represent half of data interval (n=2).

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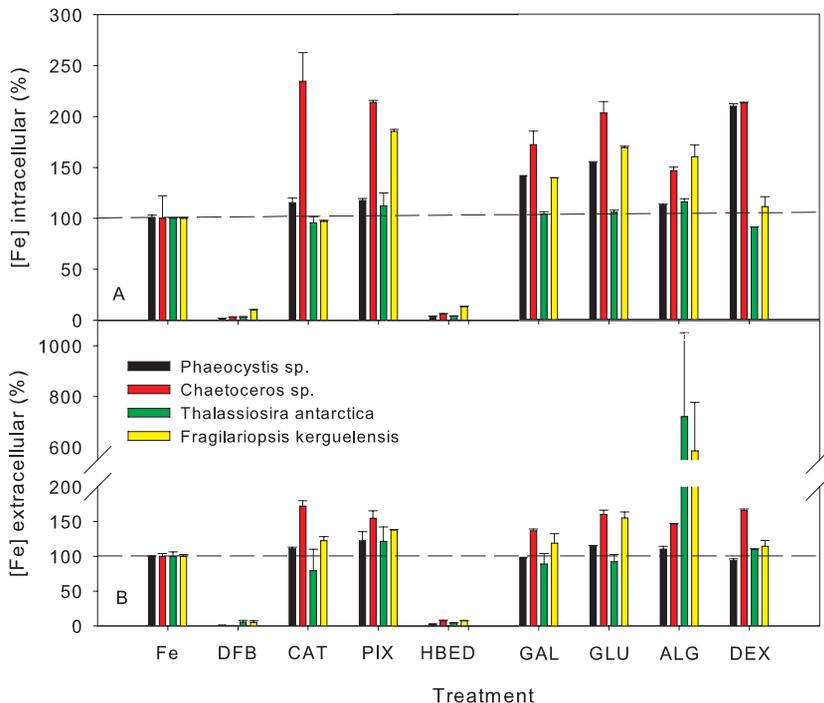
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Fig. 2. Intracellular Fe uptake rates **(A)** and extracellular Fe **(B)** for *Phaeocystis* sp., *Chaetoceros* sp., *Thalassiosira antarctica* and *Fragilariopsis kerguelensis* in filtered Antarctic seawater enriched with 1 nmol L^{-1} Fe (Fe treatment) and 15 nmol L^{-1} organic ligands. The organic ligands studied were desferrioxamine B (DFB), Gallocatechin (CAT), protoporphyrin IX (PIX), N,N'-Di(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid monohydrochloride hydrate (HBED), D-Galacturonic acid (GAL), D-Glucuronic acid (GLU), Alginic acid from brown algae (ALG), and dextran from *Leuconostoc* ssp. (DEX). Both intracellular and extracellular Fe were expressed in % of the Fe treatment (without any addition of organic ligands) on a cellular basis. Dash line represents results for the Fe treatment (100%). Error bars represent half of data interval ($n=2$).

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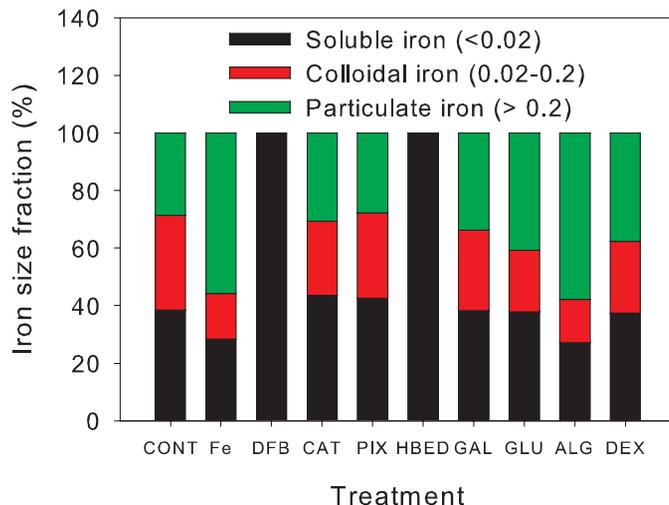


Fig. 3. Average Fe size distribution following successive filtration on 0.2 and 0.02 μm of the seawater in absence of micro-organisms. Colloidal Fe is defined as $<0.2\text{--}0.02\ \mu\text{m}$ and soluble Fe as $<0.02\ \mu\text{m}$. Experimental treatments are filtered Antarctic seawater without (CONT) and with an Fe enrichment ($1\ \text{nmol L}^{-1}$, Fe); an Fe enrichment together with $15\ \text{nmol L}^{-1}$ organic ligands: desferrioxamine B (DFB), Gallocatechin (CAT), protoporphyrin IX (PIX), N,N'-Di(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid monohydrochloride hydrate (HBED), D-Galacturonic acid (GAL), D-Glucuronic acid (GLU), Alginic acid (ALG) and dextran (DEX).

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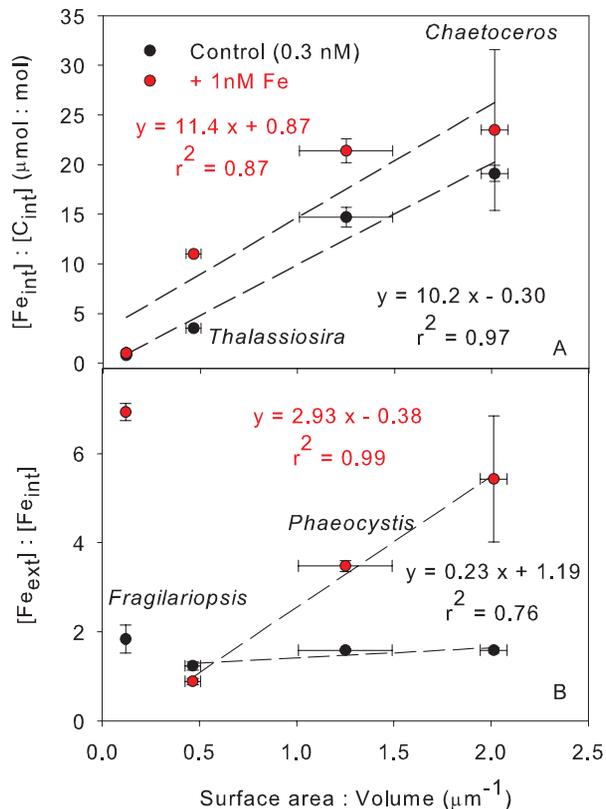
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Fig. 4. Influence of the cellular surface area to volume ratio (A/V) on the Fe to carbon ratio (Fe:C, **A**) and the cellular Fe distribution ($[Fe]_{\text{ext}}:[Fe]_{\text{int}}$, **B**). Error bars represent standard deviation ($n \geq 4$). Results for linear regression are shown in the figure.

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