Interactive comment on “Bioavailability of organically bound Fe to model phytoplankton of the Southern Ocean” by C. S. Hassler and V. Schoemann

C. S. Hassler and V. Schoemann

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We would like to thank the anonymous referee, D. Hutchins and A. Tagliabue for the effort they put in formulating comments that will improve this manuscript. They pointed some important points in the interpretation of our results and some important undiscussed areas that merit more attention. As per guidelines, a revised version of our manuscript will be shortly submitted on-line.

Reply to Anonymous Referee #1
Response to Specific Comments
Response regarding the design and interpretation of the Fe and C uptake experiments:
Regarding the use of short-term Fe and C uptake rates to infer the degree of Fe-limitation and/or Fe requirements of the strains examined: The authors agree that short term Fe:C uptake rates could be quite different than steady-state Fe:C. For this reason, any text that uses short-term Fe:C uptake rates to discuss iron limitation/requirement were removed. See also answer to David Hutchins below. In addition a statement concerning the dependency of Fe:C on the factor listed below by this reviewer (and David Hutchins) has been added in order to avoid misunderstanding that solely A/V ratio is important in defining Fe:C ratios. The text now reads: In this study, intracellular Fe to carbon uptake ratios are proportional to surface area to volume ratios (Fig. 4A), suggesting that A/V can be an important factor in determining their Fe limitation, as measured by Fe:C in the conditions of the experiment. However, other factors have previously been recognised to affect Fe:C ratio, such as the provenance of the species (neritic vs pelagic, e.g. Sunda and Huntsman, 1995; Maldonado and Price, 1996), growth irradiance (Sunda and Huntsman, 1997; Strzepek and Harrison, 2004), iron limitation and requirement (e.g. Maldonado and Price, 2001).

Regarding the dependence of Fe:C on the amount of Fe available in the growth medium: We agree that when Fe is added then short term uptake increase for Fe but remain unchanged for C. This results in an increase of Fe:C and this is now discussed in the text. However, for each treatment, iron chemistry should be constant and thus results can be compared between strains.

Regarding the dependence of Fe:C on the provenance of the species: We agree that the provenance of species might be important. Despite being originally isolated in coastal areas, both Chaetoceros and Phaeocystis are also observed in the open ocean. In addition, these two strains were maintained in open ocean Southern Ocean water for 2 years before these experiments were conducted. Therefore, biological properties, such as Fe requirement, related to their place of isolation should not be significant anymore. Thalassiosira and Fragilariopsis were maintained in the same Southern Ocean pelagic water for 1-year prior to experimentation. Finally, Fe:C ratio of 15-20
were already previously reported for oceanic iron limited strains (Twining et al., 2004; Hassler and Schoemann, 2009; Schoemann, Hassler et al., in prep.).

Regarding the dependence of Fe:C on the growth irradiance: All 4 strains were maintained at saturating light intensity. It is therefore assumed that those factors are not significant anymore. In addition, no difference in short-term Fe uptake was reported between 60 and 120 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \) for Phaeocystis (Schoemann et al., 2001).

Regarding consideration on the photolability of the Fe-ligand complex: The photolability of organic complexes is now being discussed as follow: Photodissociation of inorganic and organic forms of iron (e.g. Barbeau et al., 2001; Borer et al., 2005; Maldonado et al., 2005) was previously shown to improve its bioavailability. However, not all organic ligands are photolabile, amongst siderophores aquachelin is photosensitive (Barbeau et al., 2001) but DFB and CAT are photostable (Maldonado et al., 2005). Alginic acid dissociated when exposed to UV light (313 nm) but not when exposed to visible light (400nm; Kojima et al., 2001). Monosaccharides containing carboxylic group were photostable (Kojima et al., 2001). However, under 50% PAR light and temperature of 1-2°C, the presence of \( \mu \text{molar concentrations of mono-saccharides (Glucaric acid; Oztürk et al., 2004) induced an increased concentration of Fe(II) but no difference in H2O2 and organic peroxides concentrations. Under conditions of irradiance and temperature used in this study, using mathematical expression from Kuma et al., 1995, the rate of reduction would be negligible. This suggest that photolability of organically bound Fe would not be significant in the present study and thus cannot explain the enhanced Fe bioavailability measured.}

Regarding the degree of Fe-limitation experienced by the culture at the time the short-term incubation is conducted: Two of the strains (Chaetoceros sp. and Phaeocystis sp.) used in this study were used to study iron bioavailability and physiological responses during acclimation to low iron concentration (Hassler et al., in preparation). These strains were grown under iron-replete condition (AQUIL) and iron-limited conditions (AQUIL low Fe and SO water, [Fe]dissolved = 0.3 nM). Under replete-Fe conditions,
light level was adjusted to get an Fv/Fm indicative of healthy cells (0.55-0.65). During acclimation to SO water the growth rate, the Fv/Fm and the cell size all decreased by 40-65%, 20-35% and 15-22%, respectively. In addition, Phaeocystis was forming large colonies (> 300 µm) in AQUIL and was mainly present as solitary cells in the SO, a sign that Phaeocystis was indeed iron-limited (Becquevort et al., 2007). The growth rate and Fv/Fm for Phaeocystis and Chaetoceros in SO water were 0.14 and 0.15 d-1 and 0.46 and 0.41, respectively. Here it was assumed that if smaller species were iron-limited then larger species, more prone to iron limitation, were also iron-limited. The text of the Methods of the MS has been changed accordingly.

Response regarding the design of the experiments:

Response to point 1 Regarding the lack of assessment of the physiological state of the cultures prior to measuring Fe and C uptake rates: Physiological measurements of Chaetoceros and Phaeocystis in Southern Ocean water with 0.3 nM Fe, showed that these strains were indeed iron-limited under growth conditions used here (see answer above).

Regarding the presence of natural ligands in the growth medium and report of their stability constants: We measured both stability constant and concentration of ligands from water collected during ISPOL using the methods described in Croot and Johansson (2000): [L] exceed [Fe] by a factor of 1.7 and their logarithmic conditional stability constant with Fe prime (log KFeprimeL) was 11.1 M-1 (Schoemann, Hassler, Lannuzel and De Jong, unpublished). This conditional stability constant show the presence of strong ligands during ISPOL (9/12, 30 m depth), but slightly weaker than previously reported data for surface waters in both coastal (e.g. 12.2 to 13.1 M-1, Tian et al., 2006; 11.4-12.2, Gerringa et al., 2008) and open Southern Ocean (e.g. 11.7 to 12.2 M-1, Boye et al., 2005). During ISPOL, as a result of the presence of organic ligands, the pool of labile Fe (potentially bioavailable) was 53% of the total Fe. However, these measurements were done in filtered seawater kept frozen but the experiments described here were performed using the same water kept in the dark at room temperature for
4 years. It is therefore expected that most of the organic ligands would have been altered, therefore most likely minimizing their impact on iron chemical speciation in our work.

Response to point 2 - possible differences on the Fe requirements of oceanic and coastal isolates: All these strains can be found both in coastal and oceanic areas. As pointed out above, these strains were kept in the same Southern Ocean pelagic water for at least 1 year prior to this set of experiments.

Response to point 3 - need to discuss the photolability of the ligands used: The authors agree that this point was missing in the MS. Some text was added in the discussion to address this point, as mentioned above.

Response to point 4 - Reason for different in illumination of Phaeocystis and possible impact on results: It was saturating light intensity (see above). In addition, this light difference had no effect on Fe uptake for Phaeocystis (Schoemann et al. 2001).

Response regarding the interpretation of the uptake experiments:

Response to point 1 - Given the number of factors that can affect Fe:C ratios, it is not possible to use this ratio to assess Fe limitation. The authors acknowledge that short term Fe:C cannot be directly related to Fe limitation or requirements; the text has been changed accordingly: Because steady-state Fe:C can be quite different that short term uptake Fe:C, the Fe:C ratio determined in this study were not used to discuss Fe biological requirement or strength of limitation.

Response to point 2

Regarding the fact that the Fe requirements of a species cannot be assessed solely on their short term Fe:C uptake ratios: As stated above the authors agree that short term uptake rates of Fe and C cannot be used to define degrees of Fe limitation or requirement and the text has been change, including the sentence cited above.

Regarding the use of Coale et al., 2003: The authors agree here. The reference to
Coale et al., 2003 was removed as it was unclear if Phaeocystis was in the colonial form in their study. Due to their lower A/V ratio, Phaeocystis in colonies are expected to have higher half-saturation constants for growth than solitary cells. It was previously observed (Becquevort et al., 2007) that Fe addition could have an effect on the morphotype dominance (colonies vs. single cells) of Phaeocystis antarctica with more solitary cells under low Fe conditions. Colonies are known to have a greater ability to adsorb iron than solitary cell (Schoemann et al., 2001) and mucus excretion being energy consuming it might have an effect on Fe requirement.

Regarding the normalization of the data: Fe uptake rates per surface area are now presented in Fig. 1. Since D. Hutchins found the normalization per cell more meaningful, therefore normalization per cell was also kept in Fig. 1.

Regarding normalization of the data, specific comment to Fragilariopsis: We agree with this, but we considered that only the cell surface in direct contact with the external medium did participate to the uptake process. This argument justifies our consideration of the chain for normalization per surface area and volume. However, we agree that presenting both normalization per chain and cell could benefit further comparison with Fragilariopsis of variable chain's sizes. For this reason, we added both value of A, V and A/V (Table 1) considering single cells and chains.

Response to comments related to the Abstract: 1) the word keystone was removed 2) The text has been changed to reflect this comment. The use of the word can does not suggest and homogenous effect.

Response to comments related to the Introduction:
1) This statement is now removed considering the comments from David Hutchins. 3) This statement has been removed.

Response to comments related to the Results and Discussion:
4) The level of statistical significance is now reported in the text and trend of C uptake
following 16h (as compared to C uptake following 2h) are now shown for the control treatment. In addition, Fe:C ratio calculated using the C uptake rate measured after 16h is now presented in the text. Data on Phaeocystis are unfortunately not available due to mishandle of the sample. The modified section of the text now reads: No statistical difference ($p > 0.05$) was observed between carbon cellular uptake rate following both 2 h and 16 h incubations for the three tested diatoms. In the control treatment, carbon uptake rate following 16h was between 1.4-fold smaller to 1.2-fold higher than the uptake rate measured following 2 h incubation. An experimental problem prevented us to measure carbon uptake following 16 h incubation for Phaeocystis. Similar results were obtained with Fe:C uptake ratio using the carbon uptake rate measured following 16 h incubation. For the control treatment, the short-term Fe:C ratios calculated using the 16 h carbon uptake data were 27.7 $\mu$mol:mol for Chaetoceros, 3.2 $\mu$mol:mol for Thalassiosira, and 0.7 $\mu$mol:mol for Fragilariopsis. Following a 1 nmol L-1 Fe addition, the Fe:C uptake ratio increased for Chaetoceros (46.4 $\mu$mol:mol), Thalassiosira (14.9 $\mu$mol:mol) and Fragilariopsis (1.6 $\mu$mol:mol). Standard deviations are not shown here but are shown in the revised text.

5) Reference to Twining et al. 2004 is now added in the text.

6) We agree that the observation from SOIREE should be included in the revised version of the MS, but this does not refute our argumentation. Several other parameters need to be fulfilled to generate biological response to Fe enrichments such as the original plankton composition and physical oceanography (see Boyd et al., 2007). The following sentence was added: It is to be noted that four iron infusions were enough during the SOIREE experiment to induce a shift in phytoplankton community towards Fragilariopsis kerguelensis after 6 days (Boyd et al., 2000).

Regarding the assumption that the extracellular Fe is associated with extracellular binding sites, rather than passively sorbed to the cell surface: The extracellular Fe represent Fe bound to extracellular binding sites but also iron sorbed (i.e. not a surface complexation per se) to the surface of the cell. To our knowledge no simple procedure would
allow the differentiation between these two fractions of Fe. The use of low iron and water with low level of natural organic matter (as it is the case here) might minimise colloidal or oxide Fe sorption to the cell surface. However, our inability to distinguish these two iron adsorptive pools does not change the interpretation of Fe_{ext}:Fe_{int} ratio, for very low Fe_{ext}:Fe_{int} ratio, the cell is able to efficiently transport extracellular iron and so on. It is indeed the iron present in the surrounding of the microorganisms (adsorbed and bound) that matters in defining the gradient of Fe concentration inside/outside the cell and the pool of iron that can physically react with transporters, both parameters are important to define biological uptake. A sentence has been added in the Method section to make this point clear: The extracellular iron could be either associated to binding sites or simply adsorbed to the surface of the cell.

7) This is now clarified in the text: where (such as siderophores) was replaced by (e.g. a specific recognition from the cell such as the one for Fe-siderophore complexes).

8) polar has been removed from the text

Response to Technical corrections:

All technical corrections were done, except point 3, where we do not agree: abundance refers to the total number of plankton cells whereas diversity refers to the plankton taxonomical diversity.

References for all authors comments


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


Tagliabue, A., and K. R. Arrigo. Processes governing the supply of iron to phytoplank-


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