



Interactive
Comment

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

Received and published: 26 August 2009

Reply to A. Tagliabue (interactive comment)

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 un-discussed areas that merit more attention. As per guidelines, a revised version of our manuscript will be shortly submitted on-line.

Response to comments:

1) Regarding growth conditions and physiological status: Cultures were maintained at

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



photosynthetic saturating light levels, that were defined by measuring Fv/Fm using the WaterPAM on cultures in the AQUIL growth medium (i.e. iron replete conditions). Light levels were adjusted to get optimal Fv/Fm around 0.6. All diatoms were grown at the same light level ($60 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and Phaeocystis at $120 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. In this case the short term (16h) incubation time used is not enough to induce any change in growth rates yet as being shown by the ^{14}C uptake experiments. Additional information on growth rates and physiological status of *Chaetoceros* and *Phaeocystis* has been added in the revised version of the MS. Please also refer to answers to both referees.

2) The definition of Fe limitation: Because short term uptake rate Fe:C ratio can be quite different than steady-state Fe:C, any direct link between Fe:C ratios in the MS to Fe requirement or limitation has been removed (see both referees comments above). However, the strains used in this study were iron limited. Iron concentration in the culture medium (and experimental water) was low, 0.29 nmole L⁻¹ (reported in section 2.2) and thus 1.29 nmole L⁻¹ after the addition of 1 nmole L⁻¹ Fe. Therefore Fe:C reported here were done alongside of those dissolved Fe concentrations. A study of iron acclimation using *Phaeocystis* and *Chaetoceros* (Hassler et al., in prep.) shows that both strains were iron limited in Southern Ocean waters with dissolved Fe concentrations of 0.2-0.3 nmole L⁻¹. In this case iron limitation was reported in iron uptake rates, growth rates, Fv/Fm and rapid light curves. It is then reasonable to assume that all strains here were iron limited given that: (i) iron dissolved concentration was even lower in the ISPOL water than in our other study, (ii) larger diatoms (*Thalassiosira* and *Fragilariaopsis*) have higher iron requirements than *Chaetoceros* (Timmermans et al. 2004) and (iii) all strains were grown for several generations (6-8 weeks) in ISPOL water to avoid persistence of luxury cellular iron and get enough time for the cell to adapt to iron-limited conditions. Finally, the observation that *Phaeocystis* was mainly under single cell state is also a good indication of iron-stress conditions (Becquevort et al. 2007). This information has been added in the text; please see answers to two referees. The argument that increase of iron bioavailability is related to an increase of

iron uptake, is justified by the fact that iron has first to be transported inside the cell to mediate metabolic reactions (e.g. electron transport chain) and mediate feedback biological regulation (e.g. transporter up-regulation). Usually phytoplankton needs more than 12-24h to alter metabolism or transport capacity in response to trace metals availability (e.g. Hassler et al., 2006). Therefore, biological up-regulation is not expected to occur during the short time scale of our experiments, and iron uptake is indeed related to its bioavailability.

3) Regarding extracellular Fe: We choose to use the term extracellular rather than adsorbed iron as this iron pool can be associated with membrane and/or extracellular mucus (e.g. in the case of *Phaeocystis* colonies, Fe can be adsorbed, complexed or precipitated in the mucus; Schoemann et al. 2001 and this can also be the case with chain forming diatom EPS). Extracellular pool is operationally defined as the exchangeable iron pool that is reversibly bound to the cells components in contact with the bulk solution, it can either be transported inside the cell or either can be lost back to solution. In the present study, we used the oxalate washing procedure that we optimised to discriminate between extracellular and intracellular iron pools for both cultures strains and natural plankton community (Hassler and Schoemann, 2009). The authors agree that, in theory, for identical transporters (affinities, turnover and concentrations) and extracellular binding sites concentration per surface unit, Fe should be more efficiently bound by cells with high SA: volume ratio. However, we know that iron biological requirements (Sarthou et al., 2005) and extracellular binding sites depend on biology. For example, more EPS would be expected around chain forming than single cell diatoms (Hoagland et al., 1993); and *Phaeocystis* colonies, surrounded by C polysaccharidic-rich mucus are especially efficient in accumulating Fe (Schoemann, et al., 2001). Finally uptake mechanisms between diatom and bacterioplankton are quite different as well (Volker and Wolf-Gladrow, 1999), resulting in variable iron bioavailabilities for identical iron chemistry (Hutchins et al., 1999).

4) Regarding the bioavailability of organically complexed Fe Bioavailability of organically

BGD

6, S1347–S1356, 2009

Interactive
Comment

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)

[Discussion Paper](#)



complexed Fe: If Cu is widely required for Fe uptake as reported by Maldonado et al. (2006) for diatoms, then it is expected that Cu bioavailability and chemistry could affect iron uptake. Synergistic or antagonistic effect of cationic (trace) metals on Fe uptake has been widely documented for bacteria and yeast since the 1990's (see e.g. Eide, 1997). However, we find the interactions between Fe and other trace metals beyond the scope of this paper.

Regarding modelling of the bioavailability of organically bound iron: The following text was added in the discussion: Global modelling results from Tagliabue et al. (2009) demonstrated the importance of considering the bioavailability of organically complexed Fe. This study reports that another type of organic ligand can affect iron bioavailability: the saccharides. These probably represent lower affinity ligand class (denominated as La or L2), that is defined as bioavailable in existing model (Tagliabue and Arrigo, 2006; Tagliabue et al., 2009). Given that saccharides are produced by a wide range of microorganisms (e.g. Decho, 1990; Hoagland et al., 1993), and are excreted or released upon lysis and grazing, their production and cycling should be considered to improve existing models. Based on current knowledge, we proposed a schematic representation of ligands release and cycling with subsequent effect on iron chemistry and bioavailability to planktonic organisms (Fig. 1, Supplement).

The figure itself is more discussed in the Supplementary material as it is slightly out of the scope of this MS. The following text was added to the Supplementary: In order to improve existing model used to predict the bioavailability of organically bound iron and associated global impact (Tagliabue and Arrigo, 2006; Tagliabue et al., 2009), one has not only to consider cycling and production pathways of organic ligands reacting with Fe. The variable bioavailabilities of strongly (L1 or Lb) or weakly (L2 or La) bound iron to eukaryotic and bacterioplankton need also to be included, with La being bioavailable to all plankton classes but Lb being mostly available to bacterioplankton only (see Hunter and Boyd, 2007). It is worth mentioning that for organically-complexed Fe to be bioavailable it does not necessarily imply that the complex itself is directly bioavailable,

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

but that the complex is labile or chemically reactive enough to be dissociated before being taken up (see van Leeuwen, 1999 and Morel et al., 2008). Finally, both La and Lb should be sensitive to light. We have summarised (Fig. 1) the potential impact that such ligands could have on iron bioavailability to bacterio- and phyto-plankton. At present this scheme is simplified as it only considers biological consumption of organic ligands by bacterioplankton but not by mixotrophic phytoplankton or protozoa.

Unfortunately we cannot submit any figure with our answer, so please have a look in the revised supplementary information for the new figure added.

References cited in replies to referees and interactive comment

Armand, L. K., Cornet-Barthaux, V., Mosseri, J., and Quéguiner, B.: Late summer diatom biomass and community structure on and around the naturally iron-fertilised Kerguelen Plateau in the Southern Ocean, Deep-Sea Res. II, 55, 653-676, 2008.

Barbeau, K., Moffett, J. W., Caron, D. A., Croot, P. L., and Erdner, D. L.: Role of protozoan grazing in relieving iron limitation of phytoplankton, Nature, 380, 61-64, 1996.

Barbeau, K., Rue, E. L., Bruland, K. W., and Butler A. : Photochemical cycling of iron in the surface oceanmediated by microbial iron(III)-binding ligands, Nature, 413, 409-413, 2001. Becquevort, S., Lancelot, C., and Schoemann, V.: The role of iron in the bacterial degradation of organic matter derived from *Phaeocystis Antarctica*, Biogeochemistry, 83, 119-135, DOI 10.1007/s10533-007-9079-1, 2007.

Borer, P. M., Sulzberger, B., Reichard, P., and Kraemer S. M.: Effect of siderophores on the light-induced dissolution of colloidal iron(III) (hydr)oxides, Mar. Chem, 93, 179-193, 2005.

Boye, M., Jun Nishioka, T. J., Croot, P.L., Laan, P., Timmermans, K. R., and de Baar, H. J. W.: Major deviations of iron complexation during 22 days of a mesoscale iron enrichment in the open Southern Ocean, Mar. Chem., 96, 257-271, 2005.

Boyd P. et al.: A mesoscale phytoplankton bloom in the polar Southern Ocean stimu-

lated by iron fertilization. *Nature*, 407, 695-702, 2000. Boyd, P. et al. Mesoscale iron enrichment experiments 1993-2005: Synthesis and Future Directions, *Science*, 315, 612-617, 2007.

Chen, M., Dei, R. C. H., Wang, W.-X., and Guo, L.: Marine diatom uptake of iron bound with natural colloids of different origins, *Mar. Chem.*, 81, 177-189, 2003.

Croot, P. L., and Johansson, M.: Determination of iron speciation by cathodic stripping voltammetry in seawater using the competing ligand 2-(2-thiazolylazo)-p-cresol (TAC), *Electroanalysis*, 12, 565-576, 2000. Decho, A.W. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. In: Barnes M, editor. *Oceanogr Mar Biol Annu Rev*. Aberdeen: Aberdeen Univ Press, p. 73-153, 1990. Eide, D: Molecular biology of iron and zinc uptake in eukaryotes. *Current Opinion in Cell Biology*, 9, 573-577, 1997.

Gerringa , L. J. A., Rijkenberg, M. J. A., Wolterbeek , H. Th., Verburg, T. G., Boye, M., and de Baar, H. J. W.: Kinetic study reveals weak Fe-binding ligand, which affects the solubility of Fe in the Scheldt estuary, *Mar. Chem.*, 103, 30-45, 2007.

Gerringa, L. J. A., Blain, S., Laan, P., Sarthou, G., Veldhuis, M. J. W. , Brussaard, C. P. D., Viollier, E., and Timmermans, K. R.: Fe-binding dissolved organic ligands near the Kerguelen Archipelago in the Southern Ocean (Indian sector), *Deep Sea Res. II*, 55, 606-621, 2008.

Harrison, G. I., and Morel, F. M. M.: Response of the marine diatom *Thalassiosira weissflogii* to iron stress, *Limnol. Oceanogr.*, 31, 989-997, 1986.

Hassler, C. S., and Twiss, M. R.: Bioavailability of Iron Sensed by a Phytoplanktonic Fe-Bioreporter, *Environ. Sci. Technol.*, 40, 2544-2551, 2006. Hassler, C. S., and Schoemann, V.: Discriminating between intra- and extracellular metals using chemical extraction- the case of Fe. *Limnol. Oceanogr. Methods*, 7, 479-489, 2009. Hassler, C.S., Alasonati, E., Mancuso Nichols, C. A., and Slaveykova, V. I.: Exopolysaccharides

BGD

6, S1347–S1356, 2009

Interactive
Comment

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)

[Discussion Paper](#)



produced by bacteria isolated from the pelagic Southern Ocean – role in iron binding, chemical reactivity and bioavailability. Submitted to Mar. Chem, 2009.

Hoagland, K. D., Rosowski, J. R., Gretz, M. R., and Roemer, S. C.: Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology, *J. Phycol.*, 29, 537-566, 1993.

Hudson, R. J. M., and Morel, F. M. M.: Iron transport in marine phytoplankton: kinetics of cellular and medium coordination reactions, *Limnol. Oceanogr.*, 35, 1002-1020, 1990.

Hunter K. H., and Boyd, P. W.: Iron-binding ligands and their role in the ocean biogeochemistry of iron. *Environ. Chem.*, 4, 221-232, 2007.

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

Johnson, K. S., Coale, K. H., Elrod, V. A., and Tindale, N. W.: Iron photochemistry in seawater from the equatorial Pacific, *Mar. Chem.*, 46, 319-334, 1994.

Kojima, M., Takahashi, K., and Nakamura, K.: Cationic dye-sensitized degradation of sodium hyaluronate through photon induced electron transfer in the upper excited state. *Photochemistry and Photobiology*, 74, 369–377, 2001.

Kuma, K., Nakabayashi, S., and Matsunaga, K.: Photoreduction of Fe(III) by hydroxy-carboxilic acids in seawater, *Water Res.*, 29, 1559-1569, 1995.

Laglera, L. M., and van den Berg, C. M. G.: Evidence for geochemical control of iron by humic substances in seawater, *Limnol. Oceanogr.*, 54, 610-619, 2009.

Lannuzel, D., Schoemann, V., de Jong, J., Chou, L., Delille, B., Becquevort, S., and Tison, J. L.: Iron study during a time series in the western Weddell pack ice, *Mar. Chem.*, 108, 85-95, 2008.

Maldonado, M. T., A. E. Allen, J. C. Chong, K. Lin, D. Leus, N. Karpenko, and S. Harris.

BGD

6, S1347–S1356, 2009

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



Interactive
Comment

Maldonado, M. T., Strzepek, R. F., Sander, S., and Boyd, P. W.: Acquisition of iron bound to strong organic complexes, with different Fe binding groups and photochemical reactivities, by plankton communities in Fe-limited subantarctic waters, *Global Biogeochem. Cycles*, 19, GB4S23, doi:10.1029/2005GB002481, 2005.

Maldonado, M. T., and Price, N. M.: Influence of N substrate on Fe Requirements of Marine Centric Diatoms, *Mar. Ecol. Prog. Ser.*, 141, 161-172, 1996.

Mathot S., Smith, W. O. Jr, Carlson, C. A. and Garrison, D.L. Estimate of *Phaeocystis* sp. carbon biomass: methodological problems related to the mucilaginous nature of the colonial matrix. *J Phycol* 36:1049-1056, 2000.

Morel, F. M. M., Kustka, A. B., and Shaked, Y.: The role of unchelated Fe in the iron nutrition of phytoplankton, *Limnol. Oceanogr.*, 53, 400-404, 2008.

Nodwell, L. M., and Price, N. M.: Direct use of inorganic colloidal iron by marine mixotrophic phytoplankton, *Limnol. Oceanogr.*, 46, 765-777, 2001.

Porter K.G. and Feig Y.S The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* 25:943-948, 1980.

Rich, H. W., and Morel, F. M. M.: Availability of well-defined iron colloids to the marine diatom *Thalassiosira weissflogii*, *Limnol. Oceanogr.*, 35, 652-662, 1990.

Sarthou, G., Timmermans, K. R., Blain, S., and Treguer, P.: Growth physiology and fate of diatoms in the ocean: a review, *J. Sea Res.*, 53, 25-42, 2005.

Schoemann, V., Wollast, R., Chou, L., and Lancelot, C.: Effects of photosynthesis on the accumulation of Mn and Fe by *Phaeocystis* colonies, *Limnol. Oceanogr.*, 46, 1065-1076, 2001.

Schmidt, M. A., and Hutchins, D. A.: Size-fractionated biological iron and carbon uptake

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



along a coastal to offshore transect in the NE Pacific, Deep-Sea Res. II, 46, 2487-2503, 1999.

Strzepek, R. F., and Harrison P. J.: Photosynthetic architecture differs in coastal and oceanic diatoms. *Nature*, 431, 689-692, 2004.

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

Sunda, W. G., Huntsman, S. A. Interrelated influence of iron, light and cell size on marine phytoplankton growth, *Nature*, 390, 389-392, 1997.

Tagliabue, A., and K. R. Arrigo. Processes governing the supply of iron to phytoplankton in stratified seas, *J. Geophys. Res.*, 111, doi:10.1029/2005JC003363, 2006.

Tagliabue, A., L. Bopp, O. Aumont, and K. R. Arrigo. The influence of light and temperature on the marine iron cycle: From theoretical to global modelling, *Global Biogeochem. Cycles*, 23, GB2017, doi:10.1029/2008GB003214., 2009.

Tian, F., Frew, R. D., Sander, S., Hunter, K. A., and Ellwood, M. J.: Organic iron(III) speciation in surface transects across a frontal zone: the Chatham Rise, New Zealand, *Mar. Freshwater Res.*, 57, 533-544, 2006.

Timmermans, K. R., van der Wagt, B., and de Baar, H. J. W.: Growth rates, half-saturation constants, and silicate, nitrate, and phosphate depletion in relation to iron availability of four large, open-ocean diatoms from the Southern Ocean, *Limnol. Oceanogr.*, 49, 2141-2151, 2004. van Leeuwen, H. P.: Metal Speciation Dynamics and Bioavailability: Inert and Labile Complexes, *Environ. Sci. Technol.*, 33, 3743-3748, 1999.

Tovar-Sanchez, A., Sanudo-Wilhelmy, S. A., Garcia-Vargas, M., Weaver, R. S., Popels, L. C. and Hutchins, D. A.: A trace metal clean reagent to remove surface-bound iron from marine phytoplankton, *Mar. Chem.*, 82, 91-99, 2003.

BGD

6, S1347–S1356, 2009

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



Twining, B. S., Baines, S. B., Fisher, N. S., and Landry, M. R.: Cellular iron contents of plankton during the Southern Ocean Iron Experiment (SOFeX), Deep-Sea Res. I, 51, 1827-1850, 2004.

Utermöhl H. Zur Vervelkommnung der quantitativen Phytoplankton-Methodik. Mitt Int Verein Theor Angew Limnol 9:1-38, 1958.

van Leeuwen, H. P.: Metal Speciation Dynamics and Bioavailability: Inert and Labile Complexes, Environ. Sci. Technol., 33, 3743-3748, 1999.

Völker, C., and Wolf-Gladrow, D. A.: Physical limits on iron uptake mediated by siderophores or surface reductases, Mar. Chem., 65, 227-244, 1999.

Wells, M. L., Price, N. M., Bruland, K. W.: Iron limitation and the cyanobacterium *Synechococcus* in equatorial Pacific waters, Limnol. Oceanogr., 39, 1481-1486, 1994.

Zhang, W., and Wang, W.-X.: Colloidal organic carbon and trace metal (Cd, Fe, and Zn) releases by diatom exudation and copepod grazing, J. Exp. Mar. Biol. Ecol., 307, 17-34, 2004.

Interactive comment on Biogeosciences Discuss., 6, 1677, 2009.

BGD

6, S1347–S1356, 2009

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

