Authors reply to the reviews on:

Fractionation of iron species and iron isotopes in the Baltic Sea euphotic zone

We gratefully acknowledge the efforts of the two reviewers to provide two in depth reviews of our manuscript, which lead to the much improved revised version presented here.

Reviewer 1:

This paper presents a study of the spatial and temporal distribution of iron species in coastal brackish water systems. The topic is interesting. By using a multiple speciation technique the authors have been able to examine different iron species in the water column, based on physical and chemical properties. I think that the results have the potential to make a scientifically important paper. I recommend some minor changes to improve the MS. It would also be informative and strengthen this paper greatly if some comparison with previous studies of iron speciation in coastal waters was made.

1- Is it really necessary to use the stations codes such as C3, BY31 BY15? The name of the studied zones or their acronyms are good enough to follow the study sites.

Response:

This is now changed.

2- While the water is waiting for cross filter filtration (5 to 24 hr, did you expect any changes in Fe speciation? According to Chin et al,. (1998) gels re-form from soluble and colloidal precursors after previous gels are removed by filtration, upon filtration new colloids form as a consequence of re-equilibration in the system. It means that in the filtrated samples, in time, colloid aggregation will start and samples will contain particulates again. The authors should discuss this point in the discussion section.

Response:

The 5-24h delay before the ultrafiltration was started may have caused aggregation of the colloidal phase, and also soluble and colloidal precursors. According to Chin et al. (1998), this may cause the sample to contain particles again. Thus, there is a risk that some of the SFe measurements in this study are slightly underestimated, while CFe could be overestimates.

3- The description of the method in the MS is insufficient in some points to understand the method performed. I would recommend a slightly more detailed description of the sample preparations. For example: How the authors determined TFe, and DFe? Was total Fe determined from un-filtrated samples? If so can ICP-MS technique determine all Fe in the un-filtrated samples, or only the acid soluble portion of it? When the TFe and DFe samples acidified? Readers need to know the cleanliness of the acid used during extractions of DGT and to acidify the TFe and DFe samples. Either acid blanks or preferably method blanks should be presented in the MS. Did the authors perform any "accuracy and precision" tests by using certified samples or spikes prepared in the lab? These points should be clear for the reader of an iron study.

<u>Response:</u>
This is now discussed more in detail than in the methods section:

"Total Fe (TFe) is defined as the sum of particulate Fe (PFe, total Fe collected on a filter divided by filtered volume) and dissolved Fe (DFe)." (page 8)

"After sampling, DGT devices were disassembled in the laboratory and the gels were eluted in 5ml of 5M qHNO_3 (single sub-boiling quartz distilled, AR grade nitric acid, Merck). Prior to analysis, water samples were diluted 4-fold with 0.16 M qHNO_3 (of the same quality as for the DGTs) in MilliQ water. The resulting Fe blank from HNO_3 was routinely tested and has not been found to represent a significant contribution to the overall blank levels." (page 6)

"Limit of detection for Fe was typically ~2 nM (100ppt) for water samples Rodushkin and Ruth (1997)." (page 8)

4- The authors assumed that the diffusive boundary of DGT unit are 0.23 mm (page 8 line 6) Is there other supporting data for this assumptions regarding the water movement/turbulence in the deployment sites, besides citation of Warnken et al., (2006)?

<u>Response:</u>
Unfortunately, there are no measurements from this study to estimate the DBL. An additional reference was inserted.

“DGT concentrations ($[Fe]_{DGT}$) were calculated assuming a 0.23 mm diffusive boundary layer (DBL), which is reasonable to expect in moderate to well mixed waters (Warnken et al., 2006;Garmo et al., 2006)” (page 7)

5-Did authors observe any biofouling on the active windows of DGT units, especially due to long deployment time (14 days to two months) (page 8, line 1). Biofouling may occur in all waters., and organisms can cover the active diffusive windows of DGT. This can cause two detrimental effects: a) changes in the diffusive properties due to clogging b) organisms may adsorb/uptake the studied element (Webb and Keough, 2002).Therefore, DGT technique with long deployment may underestimate the real DGT labile fraction. The likelihood of biofouling on the DGT windows should be considered while deciding the deployment time, and the authors should discuss this point when they interpret the DGT data.

Response:

Some text is added to the methods section:

“Biofouling may occur during long term deployment of DGTs in water were the bioproductivity is high. This may change the diffusion length, and the area of the filter exposed to solution. Organisms might also affect the concentration gradient by sorbing metal ions. Several workers have found biofuoling during long term deployments in coastal areas (Munksgaard and Parry, 2003;Dunn et al., 2003).Visible biofouling was limited to absent during all sampling occasions in this study, suggesting this to be a minor problem in the present study.” (page 14)

Page 10 line 20: “Jasco FP-777 spectrophotometer” you mean spectrofluorometer?

Response:

Corrected.

Page 14 line 5: “Probably, Fe-oxyhydroxides can act as P scavengers, and remove P from the euphotic zone” Referance is needed here.

Response:

The sentence is changed to

“Possibly, Fe-oxyhydroxides can act as P scavengers, and remove P from the euphotic zone, in line with findings of Gunnars et al. (2002).” (page 21)

Page 14 line 15-20: I am not sure that the estimated amount of sedimented Fe from May to August (ca 50 _mol d-1 m-2) is correct. My quick calculation gives about 80_mol d-1 m-2. Please check the estimation.

Response:

This is not an estimated number, sediment traps were used and Fe content measured. The numbers discussed are the actual collected amount of Fe in the sediment traps.

Page 15, lines 13-15: The authors wrote that “: : :the intercept of the regression line on the Fe/Ti axis shows a value of 17, clearly above average crust”. While in the Fig 6b, the intercept of the regression line on the Fe/Ti axis show a value about 11. Please check the intercept.

Response:

Corrected, the figure was wrong.

Page 16, lines 2-3: Who concluded? Stolpe and Hassellöv or Gustaffsson et al., (2000), please check this lines.

Response:

Corrected.

Page 20 lines 2-4: Something is missing in that sentence. Please re-formulate it. Page 26, Lines-1-5: Is it correct to compare the Fe uptake with Fe concentrations? Uptake can be compared with rate of changes in Fe concentration. Additionally , could the authors cite the calculated Fe uptake rates for Baltic phytoplankton? Why did you consider only SFe as a bioavailable fraction? There are reports that shows colloidal Fe can be bioavailable too.

Response:

Since SFe alone is enough to sustain cyanobacterial growth, there is no point to discuss colloidal Fe, even though it probably to some extent is available to phytoplankton.

In figure 11 A and C "_56 Fe" appears as "d56Fe", please correct them

Response:

Corected.

There are several places where the text is either difficult to read or small corrections are required. In conclusion I consider that the paper is acceptable for publication with minor revisions.

Response:

We have gone through the text to make it more readable.

References:

Chin, W.-C., Orellana, M. V. & Verdugo, P. Nature 391, 568–572 (1998)

Webb J.A. and Keough, M.J Mar. Pollut. Bull. 44, (2002).

Reviewer 2:

The authors present a study of the speciation and isotope composition of Fe in the euphotic zone of the Baltic sea, during spring and summer. The prospect of using Fe-isotopic and Fe-speciation techniques to investigate particulate and colloidal Fe cycling in coastal waters is interesting and this topic is principally well suited for publication in Biogeosciences. That said, I'm not convinced that the authors provided compelling interpretations of the Fe-isotope data, especially regarding the distinction between various Fe-sources and the importance of water-column cycling. Although those Fe-isotope data are worthwhile of publication, I unfortunately found too many erroneous statements or reasoning that precluded me to recommend publication at this point. Hence, I don't think this paper should be accepted, unless major clarifications and revisions are being made. I think the paper could be largely improved if the data were presented with more careful considerations of alternate interpretation and proper credit to other publications.

Specific comments:

1) First, the text needs careful editing for grammar and spelling. In several places, the text is really difficult to read - and understand.

Response:

We have edited the text to make it more readable.

2) The introduction is too long and way too technical for what it has to say. It does not present the paper in a concise way nor give a clear overview of the sites selected for this study. More importantly, it does not give a justification and perspective for using Fe-isotopes, which is rather a new tracer. The sentence L14, p6494 "This will lead to an enrichment of heavy Fe in the aggregated fraction" will not resonate in mostreader mind, unless a presentation (even succinct) of the Fe-isotope systematics is given upfront.

Response:

Introduction is shortened and revised. The mentioned sentence is removed. Text about Fe isotopes is also extended.

"Measurements of stable Fe isotope fractionation has provided a new tracer which can give valuable insights into the sources of Fe and Fe biogeochemical cycles in marine and terrestrial environment (e.g. Beard et al., 2003;Bergquist and Boyle, 2006;de Jong et al., 2007). In particular, significant fractionation of Fe isotopes has been demonstrated during oxidation and reduction reactions, suggesting that Fe isotopes are useful tracers of Fe redox cycling (e.g. Staubwasser et al., 2006;Rouxel et al., 2008)." (page 3)

3) The description of the analytical methods is insufficient and, in some case, poses questions about the reliability of the data. I understand that dissolved Fe concentrations (<0.22um) in the water samples is determined by ICP-MS without any matrix separation. Using a dilution factor of only 4, matrix effects are certainly problematic even with the use of internal standards. Please give the limit of determination and analytical precision on certified water standards with this technique. The presentation of the Fe-isotope technique is also insufficient. The reference to Ingri et al., 2006 does not give a clear idea about the overall external precision of the d56Fe measurements. For example, precisions of 0.01 per mil (2STD) reported by Ingri et al. is probably related to internal precisions of a single measurement, not the external precision of the method. Please explain how the errors bars in

Figures 6 and 11 have been obtained. Also, please report the d56Fe values and other ancillary data in the form of tables.

Response:

Some text is changed and added to the MS:

"Limit of detection for Fe was typically ~2 nM (100ppt) for water samples Rodushkin and Ruth (1997)." (page 8)

"The external reproducibility expressed as one standard deviation is generally better than 50 ppm. Therefore, changes in d-values at the 0.1‰ level can be readily distinguished (Malinovsky et al., 2003)." (page 9)

Figure captions for figure 6 and 11 are revised. "Error bars indicate instrumental precision ±2 STD of the mean."

4) A major issue of this paper is that the variations of d56Fe values in particulate Fe in the Baltic sea is quite small (between -0.1 to 0.2 per mil) compared to the overall variations of d56Fe in rivers (between -0.9 to +0.4 per mil, see reference below). Hence, I'm absolutely not convinced by the interpretation of Fe-isotope signatures as reflecting oxidation of dissolved Fe(II) in the euphotic zone. Basically, everything could be explained by the input of riverine Fe without the need of water-column redox processes. What do we know about the variability of Fe-isotope composition of freshwater source to the Baltic Sea?

Fantle, M.S. and DePaolo, D.J., 2004. Iron isotopic fractionation during continental weathering. Earth Planet. Sci. Lett., 228, 547-562.
Escoube, R., Rouxel, O., Sholkovitz, E. and Donard, O., 2009. Iron Isotope Systematics in Estuaries:The case of the North River, Massachusetts (USA). Geochim. Cosmochim. Acta.(73),4045–4059. Bergquist and Boyle, 2006 (reference cited).

Response:

We believe that also small changes in PFe isotopic composition indicate significant changes in the water geochemistry. For instance Fig 11a: in just three weeks time (31/3-21/4) d56Fe increases ~0.35 ‰. This is small compared to the overall variations in world rivers, but the trend is clear and is most likely not a sign of changed input from rivers. If river water would have been the source of Fe, it would have been obvious from other ancillary parameters (see discussion in the manuscript, pages 17-18).

Of course, the composition of the fresh water source to the Baltic Sea would have been useful, but such data is missing, except for data from the Kalix river (Ingri et al., 2006).

5) The authors assume throughout the paper that average continental crust d56Fe values is 0 per mil (relative to IRMM-14). This is not true. Beard et al.2003 and many other studies (none cited!) have dealt with the variations of d56Fe in crustal rocks and the accepted d56Fe value for the bulk crust is about 0.09 per mil +/- 0.05 relative to IRMM-14. This distinction is really important considering the small range of d56Fe values discussed in this paper. Hence, "positive" d56Fe at around 0.2 per mil as presented in Figure 6 are only 0.1 per mil heavier than the bulk crust. Considering the analytical precision and pential variability of d56Fe in various crustal materials, it is very speculative to explain such "positive" values solely by the occurrence of Fe-oxyhydroxides.

Response:

Beard et al. (2003) is now added to the manuscript, as well as the proper crustal average relative the IRMM-14. It is true that the variation in δ^{56}Fe shown in figure 6 is quite small. But there is a significant difference between the highest and the lowest value. There is a very good fit between δ^{56}Fe and Fe/Ti, which we believe should not be neglected.

From our data, we cannot tell for sure that positive δ^{56}Fe is caused by formation of Fe-oxyhydroxides. It is a suggestion, that to some extent fit into a previous proposed model (Ingri et al., 2006).

6) I'm puzzled by the comparison between Fe/Ti and d56Fe values. Although a correlation is actually observed in Figure 6 (please, report error bars for both Fe/Ti and d56Fe values in this diagram), a two-component mixing line should be observed between d56Fe and Ti/Fe, not

Fe/Ti. On the other hand, in figure 11, the authors report a range of d56Fe values between -0.1 and -0.25 while Fe/Ti remains practically unchanged near crustal values. Those two conflicting observations are not sufficiently discussed in the paper. Also, while Al can be also affected by organic compounds, such as Fe, it would have been interested to also report Al/Fe ratios together with Ti/Fe ratios.

Response:

Sorry to say, the analytical error for the Fe and Ti measurements were not measured. Measurements are well above the LOD, though. We have used normalization of Fe to Ti (or Al) to evaluate the influence of detrital (lithogenic) Fe:

"Although the relationship between Al and Ti appears to be almost linear for all filters collected, there are indications from our data that Al does not at all times have a conservative behaviour. Al may be associated with the humic fraction (Ingri et al., 2006;Warren and Haack, 2001), and since this fraction may also be a carrier for Fe, Al can neither be regarded to always behave conservative nor to only represent the lithogenic phase. Therefore, Fe/Ti ratios are used throughout this paper as an indicator if Fe has a terrestrial origin. The constantly high Fe/Ti ratio (> 23, w/w, tab. 3) exceeds the average crust ratio of 10.2 (Rudnick et al., 2003). If the suspended Ti represents lithogenic, detrital particles, then the excess Fe/Ti (above the crustal average) represents non-detrital particles and a strong enrichment of Fe the suspended matter. The Fe/Ti ratios in PFe were much higher in the Bothnian Sea compared to the Landsort Deep which again indicates a more prominent riverine input."
(page 15-16)

A table showing the Fe/Al together with Fe/Ti and δ^{56}Fe values is now inserted into the MS (table 4).

About the non-existent correlation between Fe/Ti ratios and the δ^{56}Fe value at the Landsort Deep:

" Further, a correlation between the Fe/Ti ratios and the δ^{56}Fe value, as for the Bothnian Sea is not apparent (fig. 11b) and one probable reason for this is that the suspended material in the surface water of the Landsort Deep station is less homogeneous and more affected by the processes occurring in the suboxic zone."

(Page 19)

7) P6511, the references related to the importance of redox cycling in generating light Fe-isotope fluxes from coastal sediments are not appropriate. Severmann et al., 2006 a mixing between river-borne colloids or particles and Fe-oxyhydroxides derived from initially reported Fe-isotope compositions of several margin sediment porewaters and suggested the importance of diagenetic reactions in producing isotopically light benthic Fe fluxes. This study was also confirmed by Staubwasser et al., 2006 and Rouxel et al.,2008. Staubwasser, M., Blanckenburg, F.v. and Schoenberg, R., 2006. Iron isotopes in the early marine diagenetic iron cycle. Geology, 34, 629–632. Severmann, S., Johnson, C.M., Beard, B.L. and McManus, J., 2006. The effect of early diagenesis on the Fe isotope compositions of porewaters and authigenic minerals in continental margin sediments.

Geochimica et Cosmochimica Acta, 70, 2006-2022. Rouxel, O., Sholkovitz, E., Charette, M. and Edwards, K., 2008. Iron Isotope Fractionation in Subterranean Estuaries. Geochem. Cosmochim. Acta, 72, 3413-3430.

Response:

This is now corrected accordingly to the comment.

“The authors hypothesized that the low initial values were originating from water diffusing up from the basin margin sediments after suboxic early-diagenetic remineralization, since such a process commonly generate low δ^{56}Fe values, similar to what has been described in several previous studies (Rouxel et al., 2008;Severmann et al., 2006;Staubwasser et al., 2006). This hypothesis follows the findings of fractionation of Fe during redox processes, both by abiotoc processes (Bullen et al., 2001) and microbially mediated (Balci et al., 2006).” (page 19)

8) P6506, L20, the statement that the aggregation of colloidal Fe during estuarine mixing should remove heavy isotopes is misleading. In fact, Bergquist and Boyle (2006) reported river water-seawater mixing experiments and found that precipitated colloids have d56Fe values up to 0.2 per mil heavier than than dissolve Fe. Since this value is close to the *analytical precision, those results should be used with caution. More recently, Escoube et al., 2009 (see reference above) reported dissolved and particulate Fe-isotope composition along the North River estuary and didn't observe any significant Fe-isotope fractionation during colloid flocculation.*

Response

The section is now slightly changed and text is added about Escoube et al (2009):

"River water-seawater mixing experiments by Bergquist and Boyle (2006) indicated that aggregated Fe was enriched in heavy isotopes. Hence, aggregation and sedimentation of the oxyhydroxide fraction during estuarine mixing may remove heavy isotopes from surface suspended matter, resulting in a shift towards a more negative isotopic signature in the remaining suspended phase. However, here the salinity gradient is weak (S = 4.7) and thus significant flocculation and removal of Fe-oxyhydroxides may not to be expected. Apparently this phase is dominating to such an extent that it masks the isotopic signature from the Fe-C colloids. In contrast, during mixing experiments Stolpe and Hassellöv (2007) concluded that most of the removal of Fe colloids already takes place at salinities below 2.5‰. While Fe-oxyhydroxides may be aggregated at the present salinity, our data suggest that they may still be present as suspended matter due to a relatively slow sedimentation. Findings in the Kalix river estuary (Gustafsson et al., 2000) indicate that the low presence of "sinkers", i.e. detrital particles, inhibited sedimentation even though aggregation took place trough the salinity gradient and the situation could be similar for the Bothnian Sea. In contrast with our results, Escoube et al. (2009) found no significant isotopic fractionation of Fe during estuarine mixing. This clearly indicates that there is a need of more studies on the topic of Fe isotopic fractionation during land to sea transport."

(page 16)

Still, the data from Bergquist and Boyle (2006) clearly indicate a heavier flocculent compared to the river water end member.

References cited in this reply:

Balci, N., Bullen, T. D., Witte-Lien, K., Shanks, W. C., Motelica, M., and Mandernack, K. W.: Iron isotope fractionation during microbially stimulated Fe(II) oxidation and Fe(III) precipitation, Geochimica et Cosmochimica Acta, 70, 622-639, 2006.

Beard, B. L., Johnson, C. M., Skulan, J. L., Nealson, K. H., Cox, L., and Sun, H.: Application of fe isotopes to tracing the geochemical and biological cycling of Fe, Chemical Geology, 195, 87-117, 2003.

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Escoube, R., Rouxel, O. J., Sholkovitz, E., and Donard, O. F. X.: Iron isotope systematics in estuaries: The case of North river, Massachusetts (USA), Geochimica et Cosmochimica Acta, 73, 4045-4059, 2009.

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Rodushkin, I., and Ruth, T.: Determination of trace metals in estuarine and sea-water reference materials by high resolution inductively coupled plasma mass spectrometry, Journal of Analytical Atomic Spectrometry, 12, 1181 - 1185, 1997.

Rouxel, O., Sholkovitz, E., Charette, M., and Edwards, K. J.: Iron isotope fractionation in subterranean estuaries, Geochimica et Cosmochimica Acta, 72, 3413-3430, 2008.

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Warnken, K. W., Zhang, H., and Davison, W.: Accuracy of the diffusive gradients in thin-films technique: Diffusive boundary layer and effective sampling area considerations, Anal. Chem., 78, 3780-3787, 2006.

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