Interactive comment on “Fe(II) stability in seawater” by Mark J. Hopwood et al.

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Received and published: 21 January 2019

Two reviewers are thanked for detailed comments on the BGD text. Please find responses below (R:).

This manuscript addresses a topic that is relevant to the scope of Biogeosciences. There is a clear rationale for the work, the experiments have been carefully designed and executed, and the data analysis and interpretation are reasonable. However, I felt that the scope of the paper needs to be more accurately represented and that some details relating to the experiments and data analysis were missing. Overall, I believe that this manuscript should be published after revision.

SPECIFIC COMMENTS

1. The title of the manuscript is too broad, to the extent that I ĪňAnd it misleading. The manuscript does not directly address the issue of Fe(II) stability in seawater – there are no measurements of thermodynamic constants (which the word “stability” implies),
nor measurements or calculations of complex speciation, the underlying mechanisms are inferred or hypothesised rather than explicitly measured or tested, and the measurements are limited primarily to coastal seawater. This is all perfectly valid, but the manuscript really addresses iron redox speciation in coastal mesocosm experiments, and I would prefer to see a title more along these lines.

R: New title suggested: “Fe(II) stability in coastal seawater during experiments in Patagonia, Svalbard and Gran Canaria.” We accept that, to a chemist, ‘stability’ would imply thermodynamic stability, but in an environmental context -and considering how the term is used in prior Fe(II) work- it is challenging to find an alternative phrase within a limited title word count.

2. The assertions about “over-use of the “99%” statistic” (i.e. that “99% of DFe in the oceans is hypothesized to be present as Fe(III)-complexes” are subjective and I and this aspect of the Introduction to be overstated. It is true that “this observation explicitly or implicitly underpins the formulation of DFe in global marine biogeochemical models”, and that the influence of Fe redox speciation is often ignored. The authors also provide a nice summary of compelling evidence that Fe(II) is important in “two specificAc environments”. However, it does not automatically follow that the assumption that 99% of DFe is present as Fe(III)-complexes is invalid everywhere in the oceans, or that the “99% statistic” is “over-used”. To make this assertion objectively would require something like a meta-analysis of the literature to quantify the number of papers that make this claim, and the proportion of those that make this claim incorrectly. In my opinion, it would be better to just present the evidence and let the reader decide if they think this is an “over-used” statistic. I would suggest that the authors review the Introduction to remove or tone down subjective statements and ensure that any assertions are supported by an appropriate number of references.

R: Agreed, as the text is already quite long we have no desire to extent it further with an unnecessary literature review. Given that the key point is that all global biogeochemical models represent dissolved Fe basically as a dissolved Fe(III)-L species without
the complication of a redox cycle, we delete the line in question, ['Yet, as evidenced by over-use of the “99%” statistic, the presence of a fraction of DFe as Fe(II) in surface waters –exactly where most primary production occurs- is widely overlooked.]
And, as per other comments, this is replaced with a brief over-view of Fe(II) measurements in the deep ocean. ‘Fe(II) concentrations at depth are less well characterized, although there is extensive evidence of pM Fe(II) concentrations occurring throughout the pelagic water column suggesting that ‘dark’ Fe(II) production is a widespread phenomenon (Sarthou et al., 2011, Sedwick et al., 2014, Schallenberg et al., 2015).’

3. In the Introduction there is a strong focus on why Fe(II) is important, but the background about what is known in relation to the abundance and behaviour of Fe(II) in the ocean seems incomplete. For example, the growing body of work (including by some of the co-authors of this manuscript) around the influence of organic exudates from marine phytoplankton on Fe(II) oxidation kinetics is not mentioned in the Introduction, but this would seem critical to understanding much of the manuscript and its rationale. In addition, while there is a brief overview of Fe(II) dynamics in the photic zone and in suboxic zones, it would also be useful to briefly review reports of Fe(II) measurements in other parts of the ocean.

R: An extensive discussion of the role of organics on Fe(II) ‘stability’ is included in the discussion (as per the original text). We now also include a few lines of introduction to this subject in the Introduction. ‘Fe(II) speciation in seawater and the potential role of ligands in Fe(II) biogeochemistry is however still uncertain. Organic Fe(II) ligands, akin to Fe(III) ligands in seawater but likely with different binding constant ranges and functional groups (Boukhalfa and Crumbliss 2002), are widely speculated to affect the oxidation rate of Fe(II) in seawater (Santana-Casiano et al., 2000, Rose and Waite 2003, Gonzalez et al 2014). Yet characterizing the concentration and properties of organic Fe(II) ligands in natural waters using titration approaches, as successfully adapted to determine Fe(III)-speciation, has proven challenging (Statham et al., 2012) due to practical difficulties in stabilizing Fe(II) concentrations without unduly affecting its...
speciation. Never-the-less a broad range of cellular exudates have been demonstrated to positively affect Fe(II) concentrations in seawater, either via enhancing Fe(II) formation rates or retarding its oxidation rate (Rijkenberg et al., 2006, Santana-Casiano et al., 2014, Lee et al., 2017).

4. Analysis of Fe(II) data was based on an assumption of pseudo-first order kinetics, but there are no details on whether this assumption was tested or verified.

R: This is indeed assumed here, as elsewhere in manuscripts on the same topic, but also demonstrated to be a reasonable assumption with the linearity of the ln[Fe(II)] vs time response for each experiment where data is presented (these values are already included in the datasheet). This is clarified in the main text . . . ‘correlation coefficients are noted for each linear regression’. . .

5. I think it is highly problematic to exclude discussion of the Mesomed Fe(II) results from the manuscript because “Fe(II) concentrations were universally < 0.2 nM” (p. 3, lines 8-9). Given that you are arguing that Fe(II) is widespread and overlooked, excluding presentation of results from one set of mesocosms because Fe(II) was not measurable in those conditions could be perceived as cherry picking data. Again, I think this would be less of an issue if the scope of the manuscript as suggested by the title and Introduction was revised. If this is recast to make it clear that this is a study of Fe(II) dynamics in a discrete set of mesocosm experiments, then I think it is a good idea to mention the Mesomed experiments in this way without a detailed presentation of results. However, I think it is also important not to overlook these results in the discussion when generalising about Fe(II) behaviour.

R: 0.2 nM was the detection limit. So it isn’t the case that we excluded results, not a single [Fe(II)] for any of the Med experiments was above the detection limit of 0.2 nM. This may simply reflect the high temperature of the Med experiments (20°C) and similarly unfavorable pH/Salinity for Fe(II) measurements; the half-life of Fe(II) under in-situ Med conditions was sufficiently short that it would be practically impossible to
measure in situ Fe(II) concentrations with a dual-loop FIA system as multiple Fe(II) half-lives occur as sample water is flowing into the FIA. There isn’t therefore any insight to be gained from the Med work. The text is changed slightly to address this ‘Fe(II) concentrations were universally below detection <0.2 nM.’

6. The discussion about processes contributing to Fe(II) formation lacks mention of superoxide-mediated Fe reduction or other biological ferrireductase processes. This would seem remiss given that recent publications have suggested extracellular superoxide production may well be ubiquitous (e.g. Diaz et al., 2013, Widespread production of extracellular superoxide by heterotrophic bacteria, Science 340: 1223-1226) and is likely to influence Fe speciation (e.g. Rose, 2012, The influence of extracellular superoxide on iron redox chemistry and bioavailability to aquatic microorganisms, Frontiers in Microbiology 3:124).

R: As noted, we do not specifically investigate the mechanism of apparent Fe(II) stability, but the potential role of O2- is certainly of interest in light of the Rijkenberg 2006 work highlighted by another reviewer. A paragraph addressing this point of interest is added at the end of the discussion. A ubiquitous ‘background’ production of radicals in the deep ocean by bacteria would indeed be interesting as a potential driver of trace element redox chemistry, but we note it is incredibly challenging to make reliable measurements of trace species under dark pelagic (i.e. below the photic zone) conditions and thus very speculative to comment on the potential significance of O2-/Fe cycling on a grand scale; ‘Apart from the influence of organic Fe(II) ligands on Fe(II) stability arising from the slower oxidation rates of some organically complexed Fe(II) species, Fe(II) binding organics may also have a role in the generation of superoxide (O2-) which is speculated to be a dominant mechanism for the formation of Fe(II) in the dark. Experiments with 65-130 nM of protoporphyrin IX demonstrated increased formation of Fe(II) in the dark with both increasing porphyrin concentration and increasing irradiation of seawater prior to the onset of darkness (Rijkenberg et al., 2006). Whilst the rates of this process are challenging to investigate at the sub-nanomolar
porphyrin and Fe(II) concentrations expected throughout most of the ocean, the dark formation of Fe(II) mediated by ROS interactions with Fe(II)-organic complexes could potentially be important in both the diurnal cycling of Fe in the surface ocean and the non-photochemical formation of Fe(II) in the dark of the ocean's interior (Rose 2012). From a mechanistic perspective, it is difficult to establish from the experiments herein whether apparent Fe(II) stability arises from reduced oxidation rates due to Fe(II) complexation, or dark Fe(II) formation via a mechanism, such as that proposed for superoxide, which involves Fe(II)-organic complexes.'

7. The organisation of different aspects of the manuscript needs to be reviewed to ensure material is presented in the correct location. For example, the ï¬µrst paragraph of section 3.1 is discussion, not results. The second paragraph of section 3.1 is methods, not results.

R: Text shifted accordingly. A greater number of subtitles are now used to separate the method/results/discussion of each component.

Details about measurement of hydrogen peroxide concentrations are n provided in the methods section at all, but rather addressed only by the statement “as per Hopwood, 2018” in the results section.

R: A text mainly concerning H2O2 in the mesocosms is also under review for BGS, the two are linked as companion manuscripts and thus we did not want to include unnecessary detail in this already long manuscript.

8. P. 1, line 14. I suggest changing “exclusively” to “almost exclusively” or “primarily”. It is not strictly correct to say that dissolved Fe speciation is assumed to consist exclusively of Fe(III)-L, as Fe’ is generally also considered (although minor).

R: ‘Almost exclusively’ used where applicable.

9. P. 2, lines 31-32. The argument that “the potentially widespread presence of Fe(II)” implies that “redox cycling is an important feature of marine Fe biogeochemistry” is
a circular argument. The three cited papers do not show that Fe(II) is potentially widespread – they show that Fe(II) is persistent in certain speciﬁc environments and locations studied in this paper. I don’t mean to be overly critical about this – I think Fe(II) is important and possibly overlooked – but I think it’s important to be objective and precise.

R: Rephrased . . . ‘raise interest in the role of redox cycling in the marine biogeochemical Fe cycle.’

10. P. 17, lines 9-11 and 19-21. This hypothesis is not plausible, in my opinion. A difference in rate constants between different Fe(II) concentrations could be related to a difference in chemical mechanism, but should be completely independent of calibration. Also, there are several studies of Fe(II) oxidation kinetics in seawater that have been conducted at low nanomolar concentrations such that there is a coherent mechanistic understanding (and ability to predict) Fe(II) rate constants from the low nanomolar range right through to the micromolar range.

R: Yes there are multiple studies providing excellent formulas for the calculation of the rate constant with varying T/S/pH/O2. Constructing spreadsheets from different formulations does produce small changes in the calculated value of K (or t1/2) which are systematic. These are however minor. Here we opted to use a single, already published, formulation to determine K and we agree that under these conditions (T/pH/O2)-which are generally well covered by experimental rate constant data,- there is low uncertainty in the value of K. But we thought it was important to raise, and eliminate the suggestion nevertheless.

TECHNICAL CORRECTIONS 11. P. 1, line 20. I suggest changing “retarded relative to” to “less than”. Rates can be fast or slow, but rate constants are large or small.

R: changed.

12. P. 1, line 25. Please add a qualiﬁcation to this sentence explaining under what
conditions your work challenges these assumptions (e.g. in coastal surface waters?).
R: ‘in coastal seawater’ added.

13. P. 2, line 8. Ligands are not necessarily small or organic. Perhaps could change this to “Organic ligands (L) capable of complexing Fe(III) can...”
R: ‘organic’ is added to the prior sentence to clarify. We define ligands as ‘small’ and ‘organic’ when referring to filtered DFe in seawater. The supporting references demonstrate that these ligands are organic.

14. P. 2, lines 24-26. 14. This sentence seems like it belongs in the next paragraph... I can’t see how this relates to the presence of Fe(II) in suboxic or photic zones.
R: lines now separated.

15. P. 2, line 28. “There is a paucity of Fe(II) data...” – what sort of Fe(II) data?
R: amended ‘pelagic Fe(II) concentration’

16. P. 2, line 29. What do you mean by “kinetic availability”? Do you mean kinetic lability?
R: Yes, but specifically in the context of cellular uptake. The kinetic lability of Fe(II) makes its uptake (theoretically) less energetically costly than Fe(III) uptake. (We now use ‘lability’ to avoid ambiguity). But this is an over-simplistic argument because cellular-uptake systems may be specifically designed to bind Fe(III) at the cell surface, an argument we don’t wish to raise here, hence we simply use the Sunda reference to state that it is theoretically more favorable for a cell to uptake Fe(II) than Fe(III) from a simple energetic perspective.

17. P. 2, lines 34-35. “as evidenced by over-use of the 99% statistic” – what evidence? No citations are provided and this is not tested robustly, as stated in point 2 above.
R: Rephrased to refer exclusively to the use for a formulation based on this assumption
in global biogeochemical models (as above).

18. P. 3, lines 6-10. Following on from point 5 above, I ï ŋ And it confusing that some Mesomed results are included in the results, but no details are provided in the methods about these experiments, other than these couple of sentences. I think you need to treat this dataset in a similar way to the other mesososm results, namely describe the method details in full, and fully account for the Mesomed results in your discussion.

R: Rephrased for clarity, there are no results, all data was below detection due to the challenge of measuring Fe(II) using the setup herein under warm conditions due to the shorter half-life of Fe(II).

19. Tables 1A and 1B. It would make more sense to me to label these Table 1 and Table 2, as they show quite separate information. Furthermore, it would be useful to provide coordinates for the mesocosm locations in Table 1.

R: Done. Now provided.

20. P. 6, line 4. Can you provide any information about the spectral quality of the lighting?

R: We can provide the exact manufacturer’s description which includes the wavelength distribution of the lamps.

21. P. 6, line 25. Should this be “trace metal clean low density polyethylene” rather than “trace metal low density polyethylene”? 22. P. 7, line 22. Change “as described by (Paulino et al., 2013)” to “as described by Paulino et al. (2013)”.

R: Yes, amended.

23. P. 9, equation 1. Please define precisely the meaning of Vaddition and Vmesocosm.

R: Moved into methods and defined.
24. P. 11, lines 6-7. The sentence “Before presenting...” is redundant and could be removed – this is self-evident to the reader.
R: Removed.

25. P. 11, lines 10-11. Where the correlations statistically signifi cant? 
R: For one experiment yes, for the other no. (Test / p-values added in text).

26. P. 12. Please define the meaning of the error bars on Figure 3.
R: Error bars defined (always standard deviation of 3 measurements).

27. P. 13, line 1. Does “highest resolution” refer to temporal resolution? Please clarify.
R: Clarified, yes, “highest temporal resolution over the experiment duration”.

28. P. 13, lines 14-16. Is linear regression meaningful for these data? Why use linear regression in this case?
R: This section didn’t add much value to the text and following comments from both reviewers the figure and corresponding paragraph are removed.

29. P. 14, lines 2-5. What do the +/- symbols represent here? 3
R: Standard deviations (now explicitly stated when used and when referring to mean +/- SD for a dataset, n is specified in the text).

30. P. 14, line 4. Change “measurements was” to “measurements were”. 31. P. 14, line 21. There is no section 3.3.
R: Amended.

32. P. 15. Figure 5 is unreadable as it is too small. 33. P. 15, line 11. Should this refer to Fig. 5(c) rather than Fig. 5(b)?
R: Re-structured so the figure is clearer when displayed in word.