

We thank the reviewers for their careful reading of the manuscript and their thoughtful comments. In response to their suggestions we have presented the different hypotheses in 3 different sections, and we think that this has made the manuscript much clearer. Below we detail the changes we have made.

### **Response to Reviewer #1:**

This short paper provides 18  $\delta^{56}\text{Fe}$  in chiton (marine molluscs living in the near shore coastal environment) that accumulate Fe biominerals in their radula's teeth. Different species have been analysed (including two from the same site) at 4 locations (2 in the Atlantic, 2 in the Pacific). The authors compare their values with published seawater data, even though they are far away from the chitons sampling time and location.

Data seem to be of good quality and the article is generally well written and illustrated. The authors acknowledge honestly several times that their study is preliminary. There exist indeed few data on  $\delta^{56}\text{Fe}$  in marine environments so any new dataset is welcome. This is why I would recommend publication.

1. I do however have a significant concern regarding the discussion when the authors try to interpret the differences in  $\delta^{56}\text{Fe}$  measured in these samples. They present three speculative hypotheses in an imbalanced way: e.g. the feed regime is preferred to explain the differences in two species from the same site whereas this hypothesis is not better supported by data from this paper or from previous studies. In the main text, the three hypotheses should be equally discussed (as in Fig.3). Organising the discussion into sub-sections would help. Abstract and conclusions should be modified accordingly. Some other points could be also clarified (e.g. seawater sites).

*We agree with the reviewer that in the original manuscript we emphasized the feeding hypothesis, although the other hypotheses discussed are equally possible. In the revised manuscript we have addressed this imbalance so that no particular pathway is favored.*

My detailed comments are listed below.

2. Title: add in brackets after "...in marine invertebrate (chitons, mollusca)..." to have more accurate information on the study which is very restricted to this type of invertebrate only.

*We agree with the reviewer that the proposed title is more accurate and it has been changed accordingly in the revised manuscript.*

3. Abstract and conclusions should present the three hypotheses to interpret the data.

*We agree with the reviewer that this would be informative. In the revised manuscript the different hypotheses are summarized in the abstract and conclusions (lines 280-284).*

4. P.5537 line25. Please provide a range of sample size: how much dry weight of radula and Fe have been processed for a single analysis?

*The amount of Fe in the analyses ranged from 30  $\mu\text{g}$  to 840  $\mu\text{g}$  and this is stated in the methods section of the revised manuscript (lines 87-88).*

5. P.5538. Specify whether Apex has been used with or without membrane.

*We used Apex without a membrane. This is mentioned in the Methods section of the revised manuscript (lines 101-102).*

6. P.5539 Line4: typo: delete one 'the'.

*This has been corrected in the revised manuscript.*

7. P5542, Line8: typo:  $2\sigma$  instead of  $\gamma\sigma$ .

*The reported values are in fact for  $2\sigma$ . The typo has been corrected in the revised manuscript.*

8. P 5542 and 5543 "Assuming that the isotopic difference between *T. lineata* and *M. muscosa* does indeed reflect their contrasting diets" is indeed highly speculative, so going a step forward, i.e. finding an explanation on why red algae would have a different isotopic signature than green algae, goes very far since no algae data were measured. Similarly P. 5543 can the authors provide a reference to support this statement: "relatively high Fe(II) concentrations in the eulittoral zone and low Fe(II) concentrations in the sublittoral zone."?

*We agree that this section is speculative but we think the discussion is worthwhile; it has long been known that differences in oxygen and carbon isotope exist between red and green algae (Anderson, T.F. and M.A., Arthur, 1983. *Stable Isotopes of Oxygen and Carbon and Their Application to Sedimentologic and Paleoenvironmental Problems*. In: *Stable Isotopes in Sedimentary Geology*, Arthur, M.A., T.F. Anderson, I.R. Kaplan, J. Veizer and L. Land (Eds.). SEPM, Short Course Notes Vol 10, Georgia, pp: 1-151.), These represent 'vital' kinetic fractionation effects and it is conceivable that such differences also may exist for Fe isotopes. This is mentioned in the revised manuscript (lines 208-211).*

*As for Fe(II) concentrations in the littoral and sublittoral zones, measurements in marine settings show a steep reduction in Fe(II) concentrations in the top 10 m of the water column, and this is attributed to light attenuation and the drop in photoreduction of Fe(III) to Fe(II) (e.g., Shaked, Y. (2008). *Iron redox dynamics in the surface waters of the Gulf of Aqaba, Red Sea*. *Geochimica et Cosmochimica**

*Acta*, 72(6), 1540-1554). However, we cannot be certain that this is also the case in the Washington site in our study. In the revised manuscript we have included additional citations and rewritten this section (Section 3.4) to make it more cautious.

9. Fig. 2. What are the distances to the chitons' sampling sites the locations of SW signatures d) and e)? Unclear also if 500km apply for a,b and c. See also my comment on table1 where this information could be provided.

*To clarify this we have added a reference in the text reporting the Bermuda SW sampling location [John, S. G. and Adkins, J. F.: The vertical distribution of iron stable isotopes in the North Atlantic near Bermuda, *Global Biogeochem. Cycles*, 26, GB2034, doi: 10.1029/2011GB004043 ]. We have also modified the figure caption as follows in the revised manuscript:*

*Blue squares are published surface seawater isotope analyses of dissolved Fe from locations as close to the chiton sampling sites as available data permits. Data reported by Rouxel and Auro, (2010) for three sites located off the north-eastern Atlantic coast of North America are (a) Vineyard Sound on Cape Cod, Massachusetts, USA (-0.82 ‰); (b) Waquoit Bay on Cape Cod, Massachusetts, USA (-0.55 ‰); (c) Connecticut River estuary in Long Island Sound, Connecticut, USA (+0.04 ‰). These three sites are located within less than 150 km distance from each other, on average about 500 km south of the chiton sampling site at Grand Manan Island, New Brunswick, CA). Data for the North Atlantic (d) (+0.3 ‰; sampled about 100 km southeast from Bermuda, John and Adkins, 2010; John and Adkins, 2012) are compared with the Bermuda chiton sampling site. The closest available coastal seawater Fe isotope data to compare with the Puget Sound chiton sampling site (Washington, USA) is from the San Pedro Basin (e) (0 ‰; John and Adkins, 2010), which is located off the Atlantic coast near Los Angeles (California, USA), about 1500 km south from Puget Sound.*

10. In Fig.3 Here, the three potential processes yielding to Fe isotopic fractionation are presented in an equal way. The legend correctly underline that those 3 mechanisms are not mutually exclusive. However, in the text, mechanism a (and in a less extent, b) is preferred and this is imbalanced. This is especially true in abstract and conclusion, where only hypothesis involving diet is provided.

*We agree with the reviewer that there is a certain imbalance in the discussion of the different fractionation pathways. In the revised manuscript this imbalance has been corrected by discussing separately the three individual mechanisms in separate subsections (Sections 3.2,3.3, 3.4).*

11. Text on Fig.3 is too small.

*This has been corrected in the revised manuscript.*

12. Table1. Provide exact locations: latitude and longitude of sampling sites.

*We agree with the reviewer that more information would be useful. Unfortunately, the exact sampling locations were not recorded when the samples were stored and archived at the Peabody Museum.*

13. Provide also locations and  $\delta^{56}\text{Fe}$  of seawater data (a,b,c,d,e) that are compared with chiton isotopic signatures.

*This has been included in the revised manuscript (see comment 9)*

14. Eventually four small maps of each site could be helpful.

*Again, we agree that this would be useful but the exact sampling locations were not recorded.*

## **Response to Reviewer #2:**

I have now reviewed the manuscript (bg-2014-110) titled ‘Iron isotope fractionation in marine invertebrates in near shore environments’ by Emmanuel et al. The submitted study presents for the first time the natural variability in the stable isotope composition of iron ( $\delta^{56}\text{Fe}$ ) measured in biomineralized magnetites extracted from several species of modern chitons. These were acquired from the museum collections, and sampled some 100 years ago in areas of the south and north Pacific, as well as the sub-tropical and the north Atlantic ocean. The main objectives of this study were (i) to explore  $\delta^{56}\text{Fe}$  variability in modern marine chitons and its possible species-dependence, and (ii) to constrain the isotope offsets (i.e. fractionation factors) between the magnetite hosted Fe and the published  $\delta^{56}\text{Fe}$  signatures of modern seawater. The ultimate goal was then to investigate whether the  $\delta^{56}\text{Fe}$  of chiton’s magnetite could be possibly used as a natural proxy, or a recording phase, for the  $\delta^{56}\text{Fe}$  signature of the ambient ocean basically only the local scale (i.e. basin scale) phenomena. In addition, this extremely short residence time of Fe might also have some implications, or cause possible complications, for the presented comparisons of modern seawater  $\delta^{56}\text{Fe}$  values relative to  $\delta^{56}\text{Fe}$  data measured in ‘modern’ chitons, as these were collected way back in the early 1900’s (See Page 5537, Lines 1 to 2) when the local seawater might have had distinct  $\delta^{56}\text{Fe}$  signatures compared to those published in recent papers from the early 2000’s (see data in Fig. 2; Page 5553, and References herein, e.g. Lacan et al. 2008, 2010; John and Adkins, 2010).

1. Page 5543 (Lines 27 to 29), and Page 5544 (Lines 1 to 12): Here you mention that “In addition to magnetite ( $\text{Fe}_3\text{O}_4$ ), chiton radula contain other Fe minerals, including goethite, lepidocrocite, ferrihydrite”, and also that during the ontogenesis of chitons “the ferrihydrite precursor is transformed to magnetite”. This is an important information and a factor that, in my view, might be quite relevant for the interpretation of the observed  $\delta^{56}\text{Fe}$  variability in your ‘bulk magnetite’ samples. As from the theoretical calculations, as well as experimental data, it is now well know

that there is different ‘equilibrium Fe-isotope fractionation’ for different Fe-bearing minerals, relative to the aqueous Fe species, i.e. Fe(II)aq. Specifically, published data indicate that at temperatures of 20C the representative Fe isotope fractionation factor ( $\delta^{56}\text{Fe}$ ), between dissolved Fe(II) and mineral-hosted Fe is as follows: about -1.5 per mil for magnetite; -1 per mil for goethite; and up to -2.5 per mil for ferrihydrite (see data in Wu et al. 2011; ES&T, Vol. 45, p. 1847-1852; and Friedrich et al. 2014, GCA, Vol. 139, p. 383-398). Thus, in theory, it is also possible that the observed large variability in your ‘bulk magnetite’ samples could be partly controlled by different mineralogy of your samples. For example, the extremely light  $\delta^{56}\text{Fe}$  signatures (up to -2 per mil) measured in your ‘Mopalia muscosa’ samples could be due to higher contribution of the isotopically lighter ‘ferrihydrite precursor’ phase in your bulk ‘magnetite’ sample. This possible effect of mineralogy on your bulk ‘magnetite’  $\delta^{56}\text{Fe}$  data could be tested by X-ray diffraction (XRD) analysis of your samples, if enough material is left for such analysis. Hence, if realistic, I strongly recommend that the authors would provide and discuss also ‘mineralogical data’ in the revised version of the MS to further evaluate the role of such mineralogy-controlled effects on their  $\delta^{56}\text{Fe}$  values. Alternatively, if the limited sample size will render such additional XRD analysis impossible, the authors should at least mention and discuss in their revised MS also the ‘mineralogy-controlled’ scenario, outlined above, as possible explanation for their  $\delta^{56}\text{Fe}$  variations observed in chitons.

*The reviewer makes an import point: mineralogy could well play a role in controlling the isotopic composition. Unfortunately, all the material was used for the Fe isotope analyses and mineralogical analyses were not carried out on our samples. However, this is certainly worth exploring in future studies, and in the revised manuscript we include a subsection (Section 3.2) that discusses the potential influence of mineralogy on isotopic composition, including the references cited by the reviewer.*

2. Technical Corrections Page 5535 (Line 9): The reference of Brantley et al. 2004, mentioned here, is not included in the Reference list (see Page 5546), so please correct this.

*This error has been corrected in the revised manuscript.*