

Interactive comment on “Silicon isotopes of deep-sea sponges: new insights into biomineralisation and skeletal structure” by Lucie Cassarino et al.

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Response to Referee #2

First of all, thank you for the time taking to review the paper. On overall, I agree with the comments you have made, and I have re-worked the manuscript. I hope the corrected version and the detailed respond to your review will make it clearer. Your comments are listed, and our response/explanation will be written after it in the following paragraph.

1) One issue that could be easily improved is the presentation of the data. Currently, it is not possible to recreate the authors analysis because the spicule fusion degree isn't

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given in the data tables – this could be easily remedied, and it should be also possible to code the symbols in the figures so one can see where the different fusion levels fall. (answer) The data table A1 has been edited and the fusion degree of the spicules is now available. We have change figure 2 , which now has the fusion degree included, but we did not do this for figure 5 and 6 because figure 5 focuses on the comparison between Hexactinellids and Demosponges, and figure 6 is already very colourful due to the 8 ϵ f simulations. Adding additional colour-coding for the degree of fusion would make both figures unreadable.

2) More generally, because it was not possible to plot the data myself, I became a bit confused at parts, regarding what the takeaway message should be. Some samples fall off all the versions of the fractionation concentration regression: P5L20 makes clear that this is related to the degree of spicule fusion, which I interpreted from the introduction in general and P6L28 & P7L2 specifically to be related to the taxonomy of the samples, specifically whether they were hexactinellid or demosponges. But then P8L24 and the residual tests says this is not the case. It seems the deviation from the ‘average’ sponge is the most useful indicator, which is what the residual plots are showing – but in the end it’s unclear whether or not this is related to fusion degree or to the taxonomy. (answer) Previously L28p6 “a group of hexactinellid sponge” has been changed to “a group of sponge” now L8p7 to avoid confusion.

3) My feeling is that if Fig. 4 was altered to show some measure of deviation/residual, rather than absolute values, and the discussion altered to reflect this, things would be clearer. Otherwise surely the default interpretation of Fig. 4 is that different taxa like to live in different parts of the ocean? (answer) Figure 4 was only representing the samples identified from this study, which come from the deep-water masses of the equatorial Atlantic. This information has been added in the caption to avoid confusion. Furthermore, in the corrected version, figure 4b is added and shows the fractionation factor residual of the 5 degrees of fusion compared to the published calibration curve.

4) Similarly, I struggled to follow the rationale for the discussion in section 4.3 – it seems

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trivial if you have 5 (?) tunable parameters, you can make a model produce any magnitude of fractionation. Or have I missed the point here? I would recommend trying to emphasise the key point. (answer) Section 4.3 has been entirely edited and is now organised in 2 parts: first the Kmp is investigated and then the efflux. Kmp is first investigated due to the relationship between ϵ_f simulations results and the Michaelis-Menten parameters and then pushed to extreme value, $K_{mp}=10\mu\text{M}$, to see the effect on ϵ_f . Because ϵ_f of the dictional skeleton cannot be simulated by Kmp we then investigate the effect of the fractionation of the efflux. The section is now better structured to help the reader.

5) Finally, I wonder if the authors have given any thought to whether this difference in Si- isotope fractionating behaviour between different sponge types is something that could be exploited rather than avoided in a paleoceanographic context? (answer) At the end of section 4.3 we suggest that Si isotopes could potentially be used to investigate cellular uptake and silicification processes, see L29-30p10.

Minor comments - this is a non-exhaustive list of small typographic, etc. errors and some comments/questions.

6) P1L5: “ranges” (answer) Done, now L5p1

7) P1L15: “fossils” (answer) Now “fossil records” L16p1

8) P1L18: “and references therein” (answer) Done, now L19p1

9) P1L20: “Since sponges rely” or “because sponges rely” (answer) Done, now L2p2

10) P2L5: either “from” or “of”, not both (answer) Done, change to “made of”, now L5p2

11) P2L9: An approximate threshold size for distinguishing between micro- and megascleres would be useful. (answer) Size has been added “megascleres (up to and beyond 300 μm) and microscleres (up to 50 μm)”, now L9p2

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12) A reference to Jochum et al. (2017) might be appropriate here P2L29: (answer) Done, now L28p2

13) Phrasing is unclear, P2 Eqn 1: the permil is not necessary. (answer) We haven't changed it because $\times 1000$ is not included into the equation the permil symbol show that the delta value is express in permil unit.

14) P3L9: Reference to Fontorbe et al. (2016) and/or Fontorbe et al. (2017) might be appropriate here (answer) Reference Fontorbe et al., 2017 has been added, now L13p3

15) P3L11 "result" (answer) Done, now L14p3

16) P4L5: "to", not "in" (answer) change "to" by "into", now L10p4

17) P4L7: How do you know all the lithogenic material was removed? Could it be contaminating the samples? (answer) The sponge spicules analysed here are from live sponges. The potential lithogenic material is visible by eyes if remaining after the first steps of cleaning, and it is possible to remove it. Furthermore, further cleaning steps are done with high concentrated acid, which results in very clean spicules, so we are confident that the spicules are free of lithogenics. The sentence has been edited to "If remaining, lithogenic material was removed by hand before further cleaning steps. A subsample was taken and weighed before going through a final cleaning step", see L12-13p4

18) P4L12: "prior to isotopic", and "induced" (answer) Done, L18p4

19) P4L15: Has it been tested that the yield, including the washing step, is quantitative? (answer) Yes, the yield was tested and is now included in the method, see L23p4 "The yield recovery of Si is equivalent to 92.1%"

20) P4: Were any seawater standards analysed? (AHOLA, from Grasse et al. (2017)). (answer) We have analysed the ALOHA Deep water standard using this method and have added the results, see L1-3p5 " The new seawater standard ALOHA deep was

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analysed as an additional quality check, and yielded values within error of those obtained during an interlaboratory (Grasse et al., 2017): Aloha deep: $\delta^{30}\text{Si} = 1.08 \pm 0.12$ ‰ and $\delta^{29}\text{Si} = 0.58 \pm 0.12$ ‰ (2 s.d, n = 4).”

21) P4L21: “spectrometry” (answer) Done, Now L27p4

22) P5L20: The sentence starting “the $\delta^{30}\text{Si}$ _spicules. . .” is oddly phrases – it could just say ‘ $\delta^{30}\text{Si}$ _spicules and apparent fractionation both increase. . .’ (answer) The end of the paragraph has been changed to “ It is observed that $\delta^{30}\text{Si}$ Spicules and $\Delta^{30}\text{Si}$ show an enrichment in light isotopes with higher degree of spicule fusion (see figure 4).” Now L27-28p5

23) P6L5: Not sure it’s correct to say epsilon can result only from a biological model – epsilon is simply the permil expression of fractionation factor alpha – see Coplen 2011 “Guidelines and recommended terms for expression of stable- isotope-ratio and gas-ratio measurement results” (answer) We have changed the sentence to “Here $\Delta^{30}\text{Si}$ is defined by the difference between $\delta^{30}\text{Si}$ Spicules and $\delta^{30}\text{Si}$ DSi, which describes the observed apparent Si isotopic fractionation by sponges whereas ϵ_f is the result from the biological model from Wille et al. (2010) (equation 2)” (L12-14p6) to distinguish the observe and the calculated fractionation factor, which follow the previous published paper.

24) P6L7: I don’t see the relevance of mentioning Rayleigh fractionation here, these are samples from all over the ocean, not a single site evolving through time. (answer) This sentence is to emphasize that sponge Si isotopic fractionation does not follow the Raleigh type fractionation observed in diatoms.

25) P7L1: Or, more generally, that different taxa have different epsilons, K_m or V_{max} – worth mentioning here? (answer) The end of the paragraph has been changed but not the last sentences.

26) P7L10: “in different ways” (answer) Done, now L22p7

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- 27) P7L18: “from Hendry and Robinson” (answer) Done, now L30p7
- 28) P7L33: “main” (answer) Done, L15p8
- 29) P8L4: “impact the fractionation”. (answer) Done, L19p8 Also, a bit of skepticism about the ab initio calculations might be warranted.
- 30) P8L17/18: Is there an indication of uncertainty on the ‘thermal analysis’ – are 10% and 15% really different? (answer) The uncertainties on those values are not known, to our knowledge but to support it another experiment and reference is added, see L33p8 L2p9 “Furthermore, SDS-PAGE analysis of Hexactinellid Euplectella aspergilum has shown that the proteins of its axial filament have higher molecular weights than those isolated in demosponges (Weaver and Morse, 2003),”
- 31) P8L30 (and elsewhere): ‘Sponge fractionation’ would more correctly read as ‘fractionation of silicon isotopes by sponges’ or something similar. (answer) Done
- 32) P8L31: This sentence is missing a verb. (answer) The sentence has been changed to “ The Si isotopic fractionation by sponges is assumed to occur during Si uptake and during internal spicule formation with spicule formation being a function of Si influx and efflux from the sclerocyte (Milligan et al., 2004)”, see L13-14p9
- 33) P9L6: “described” (answer) Done, now L24p9
- 34) P9L24: “enzymes” (answer) Done, now L23p10
- 35) P9L28: “breaking of bonds” (answer) Done, now L22p10
- 36) Figures and Tables Figure 1: I guess the colours represent bathymetry – a color bar would be nice, and perhaps another panel showing a cross-section of dissolved silicon concentrations along the sampling transect. (answer) Colour bar for the bathymetry and D_{Si} cross section have been added.
- 37) Figure 2, 5 and 6: It would be convenient to show the different spicule types, perhaps color coded somehow, as in figure 7. (answer) The colour coding is mentioned

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and commented in general comments. I also notice that an outlier from Hendry and Robinson isn't shown – would recommend mentioning this somewhere to be clear. (answer) In fact, the core top samples from Hendry and Robinson (2012) are not included. This is now mentioned in figure 2 caption.

38) Figure 4: Why are the individual data points only shown for spicule fusion degree F5? (answer) Data points for all the fusion degree have been added. In the first version, only F5 was detailed to show why the error bars was so large. More generally, wouldn't it be more useful to plot the residuals as discussed in the main text? (answer) A residual test is now added, see figure 4b. Otherwise all this figure could be telling us is that sponges that produce highly fused spicules prefer to live in the deep sea/high Si concentration waters (and Alvarez et al. (2017) recently showed that different groups do seem to have distinct depth preferences). See also comments above. (answer) Figure 4a only show the samples with identified fusion degree from the deep water of the equatorial Atlantic indicating that there is a significant relationship between the Si isotopic fractionation and the degree of fusion. We have added the following to the caption of figure 4: " Data plotted here correspond to the samples from the equatorial Atlantic with identified fusion stage i.e. coloured diamond of b) and so occupy a very narrow range of DSi. b) $\Delta^{30}\text{Si}$ residual of samples with identified fusion stage compared to the published calibration (best fit 1)."

38) Figure 5: Would it make sense to plot the residuals for all data for each possible regression? i.e. then one could see where the new data sits with respect to the previously published data. (answer) Figure 4b has been added and show the residual test of the fusion degree compared to the previous published data. Also, it would good to see a justification for an expression of this form being chosen rather than e.g. linear fits, power laws, etc. (answer) The best fit equations are following the fractionation factor equation (equation 2), according to published methodology (e.g. Wille et al., 2010; Hendry & Robinson, 2012).

39) Figure 7 caption: Atlantic. (answer) Done

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40) Table 2: The recent data from López-Acosta et al. (2018) could be incorporated here. (answer) The recent data from Lopez-Acosta et al., 2018 are now included.

41) Figure A1: Could the global data compilation also be presented here? The temperature data of all data compilation is not available, the caption, 'ambient', not 'ambiant'. (answer) Done

42) Table A1: For this work to be most useful/reproducible, this table should include the class of fusion degree each sample has been assigned to. I would have like to plot the data myself but was unable to. (answer) The fusion degree are now included into table A1.

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