

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

Anonymous Referee #1

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This manuscript addresses observed differences in the apparent silicon isotope fractionation during spicule formation of marine sponges. The authors compare their new and relatively large dataset from the equatorial Atlantic Ocean to previously published data in an attempt to address the mechanisms influencing the wide range of silicon isotope compositions observed for sponges in the marine environment. The authors present an interesting and plausible argument correlating the apparent silicon isotope fractionation of marine sponges to their skeletal morphology, namely the degree of marine sponge spicule skeletal fusion. The data are interesting and are likely represent a substantial contribution to the scientific community, however the manuscript does have some major shortcomings, as discussed below. Therefore, my overall recommendation

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is that this manuscript be accepted for publication after moderate/major revision.

Moderate/Major revisions

The revisions described directly below are for specific sections that require more detail and better structural organization. However, the presentation of the manuscript has several (minor) imprecisions that become very distracting and unfortunately result in an unclear presentation of the scientific approach and the discussion of the data (see Problems with clarity). I have described these 'minor' revisions in a separate section below, but there are several points that need to be addressed to ensure a clear and precise message for the manuscript.

Section 3.2 – Much of this section would be better suited in the methods section, perhaps alongside the SEM work? For example, 'five levels of fusion defined here as F1, F2, F3, F4, and F5 (Table 1). In this section, it is unclear in the text what technique was employed to determine fusion stage. It is clear that SEM imagery was used, but how were the analysed samples chosen? Was it random? Were the analyses performed after the d30Si measurements had been finalized? Please explain.

In addition, the relationship between the fusion stage data and the measured d30Si of the spicules is really quite interesting, and is the basis for a major argument presented in this manuscript, however, this section falls short of describing the results. In particular, the principal 'results' presented in this section are contained in only one sentence (P5 L20-21), a sentence that is difficult to understand. I would suggest the authors revisit this section and provide a better description of how the d30Si of the spicules is strongly associated with different fusion stages.

I also think that figure 4 should be modified slightly. I noticed that the authors had included the data incorporated into making the boxplot for Fusion stage 5, but not the other stages. I actually appreciate the F5 data being presented like this and would prefer if all fusion stages (1-5) were presented in a similar manner.

Section 4.3

P 9 L3-23. — This paragraph is very difficult to follow. I would suggest the following modifications. (1) make a new paragraph starting at line 7 (Figure 6 shows the results...) (2) make a new paragraph starting at line 15 (In lopez-Acosta et al. (2016), ...), (3) change L18-19 to: 'A hypothesis is that...' (4) rephrase L18-19 — it is not clear as to why a Km of 10 uM was chosen, nor that the Low Km simulation was included in Figure 6. Also, what was the KMp hat Lopez-Acosta — why did you chose 10 uM?

P9 L26-31 –The argument presented here does not seem plausible or there is something missing in the text. How is efflux [rate] alone influenced by whether a bonding reaction is reversible or not? Can you provide some reasoning here? Even if the bonds are being created and/or destroyed simultaneously, would Si be removed from the organism? Further, the sentence on L29-31 is unclear. The Km of what organism? Or is this theoretical? Most organisms listed in the table have a Km that is much greater than 10 uM (i.e. 29.8-74.5 uM) therefore decreasing the Km to 10 uM doesn't seem likely, or am I missing something? Could you explain what you mean by 'the fractionation due to the efflux..'? Finally, please provide more information regarding the model that was presented as High E efflux and high Km. These are generally not very well described in the text.

Problems with clarity

P1 L7 – what anomalies? Anomalous compared to what? Up until this point no anomalies have been described. Please include a sentence to describe what you mean by anomalies.

P1 L8 – extremely light d30Si signatures? Compared to what? This was not described. Please clarify.

P1 L 10 – please clarify what you mean by spicule types. . .

P1 L 15 - molecular fossil what? The molecular fossil record? Please clarify.

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P1 L20 - Do you mean, Of the biomineralizing sponges? Please clarify.

P1/2 L21-2 – This sentence is unclear and I do not quite understand what the authors are trying to convey. This section needs to be developed a bit more and have a stronger link to the previous sentences so that I can understand why the authors wanted to include this information.

P2 L12 – Please provide more detail. Loose where within the skeletal framework? Can the authors please provide a better structural description here? Also what is meant by "...and they have a cellular organization." What kind of organization? Is it unique to each species? Also, the authors need to clarify that this sentence is discussing demosponge mega- and micro-scleres. As it is written, this is not clear in the text.

P2 L15 – what do you mean by rays? Spines along one of the three axes?

P2 L16 – please clarify what you mean by secondary silica.

P2 L17-18 – It is unclear as to why you have included this sentence. Please provide context and improve the conclusion of this paragraph.

P2 L23 – please clarify what you mean by 'sensitive to their environment'. How does growth rate and immobility make sponges sensitive to their environment? This point is unclear.

P2 L 26 – this sentence needs to be reorganized – De La Rocha did not introduce the silicon isotopic composition of biogenic silica.

P3 L17 – the sentence '..by analyzing d30Si along the sponge skeleton?' is not clear, please rephrase.

P3 L18-19 – This sentence is not clear. What precisely is being investigated? Please rephrase.

P3 L22-25 – please rephrase this sentence, it is not clear.

P3 L26-27 – Please provide a list of the samples that were dried, preserved in ethanol and frozen. Please detail where exactly the samples were shipped in the UK.

P3 L28 – was the identification of the specimens to the major sponge classes carried out on subsamples that were preserved or were they fresh?

P3 L29-30 – what is the status of these identifications? Will the species ID be published as an appendix in this paper? Elsewhere? What journal?

P4 L7 – please rephrase the sentence 'If remaining, lithogenic material was removed by hand'. It is unclear.

P4 L7 – Please clarify where the subsample originates. Is it cleaned? Has the lithogenic material been removed? This is not clear.

P4 L13 - Please clarify and rephrase 'Reynolds et al. (2006) modification.'

P5 L7 – How was the D30Si calculated? There are no d30Si data for seawater presented in table A1.

P5 L24-28 – This paragraph could be improved. It is not very descriptive and there is no flow. It reads more like a set of bullet points with, in some cases, poor grammar. Please explain why this information is important, for example, why has 'particular attention been paid to samples with a D30Si larger than 5 permil'? Do all samples show a common feature or just the samples that have a D30Si larger than 5 permil? The information is not abundantly clear from the text and needs to be clarified.

P6 L3 – what studies? Please provide references.

P6 L5 – please define epsilon f. what does it mean?

P6 L26-27 – What do you mean by 'Despite the small range of temperature' – in this dataset? The Hendry and Robinson 2012 dataset? Please clarify.

P6 L29 - please explain what you mean by low concentration? What is the range?

C5

P7 L4-7 – where are these data compiled? Please provide a reference or an appendix. Also, the authors need to be cautious about using the Wille et al. 2010 data set since they did not measure the d30Si of seawater where their collected sponges resided. Their estimates for d30Si came from Cardinal et al. 2005. It would be a good idea to mention this in the text.

Minor revisions

Title: The authors do not provide any new information regarding the influence of biomineralisation on the silicon isotope composition of deep-sea sponges and I would recommend that they change the title to: "Silicon isotopes of deep-sea sponges: New insights from their skeletal structure"

Plurals: please check over text for plural usage. Often, words are incorrectly pluralized. Please correct throughout the manuscript. Here are a few examples from the abstract:

P1 L9 - change 'insights' to 'insight', change 'process' to 'process(es)'

P1 L10 - change 'isotopes' to 'isotope'

Definitions (e.g. Si, DSi, BSi ïĄď30Si, ïĄěf): Please define these abbreviated terms correctly, and once defined, continue to use them instead of their non-abbreviated form. Check throughout the text.

Fractionation: Throughout the text the authors use the word fractionation but often do not describe what is being fractionated (e.g. silicon isotopes). Sometimes the word silicon fractionation is used, when the authors presumably mean silicon isotope fractionation. Please check throughout the text and correct this oversight. I have included a few examples below:

P1 L4 – add 'silicon' to 'apparent isotopic fractionation'

P1 L7 – add 'silicon' to 'isotopic fractionation'

P3 L5 – add 'silicon isotope' to 'apparent fractionation factor'

- P3 L7 add 'silicon isotope' to 'fractionation factor'
- P3 L16 chose to use Si or silicon (see section on definitions below)
- P3 L16 add 'silicon isotope' to 'fractionation'
- P3 L18 add 'silicon' to 'isotopic fractionation'
- P5 L8- add 'silicon isotope' to 'fractionation'
- P5 L21 add 'silicon isotope' to 'fractionation'
- P6 L5 add 'apparent silicon isotope' to 'fractionation'
- P6 L7 add 'model' to 'Rayleigh-type fractionation' also, change Raleigh to Rayleigh.
- P6 L9 add 'isotope' to 'Si fractionation'
- P6 L16 add 'silicon isotope' to 'fractionation'
- P6 L20 add 'silicon isotope' to 'fractionation' (three times)
- P6 L20 add 'isotope' to 'Si fractionation'
- P6 L29 add 'silicon isotope' to 'fractionation'
- P7 L20 add 'isotope' to 'fractionation of Si'
- P7 L4, P7 L27, P7 L31, P8 L3, P8 L5, P8 L19, P8 L24, P8 L25, P8 L30, P9 L3, P9 L8, P9 L9, P9 L20, P9 L21, P9 L30, P10 L2, P10 L9, Figure 2 caption, Figure 4 caption, Figure 6 caption, Figure 7 caption, Table A1...
- P1 L1 change to "The silicon isotope composition (d30Si) of deep-sea sponges' skeletal elements spicules reflect the ..."
- P1 L18 change to (Strehlow et al., 2010 and references therein)
- P2 L5 Please change sentence to "....spicules through the incorporation and deposition of hydrated amorphous silica (SiO2-nH20), otherwise known as bio-silica."

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- P2 L10-11 These two sentences would be better merged.
- P2 L16 change "loose" to "loosely attached"
- P2 L19 please clarify what type of sponges (deep-sea/marine) I think that you mean 'roused' and not 'aroused'. also, it is the 'marine' silicon cycle.
- P2 L21 remove 'may be' and replace with 'are'
- P2 L 22 remove 'immobility' and replace with 'inability to move'.
- P2 L28 please write 'approximately'
- P2 L29 you need to include a statement about how the silicon isotope composition is expressed as permil... for example: '...are reported using delta notation as either d29Si or d30Si using the permil (\emptyset L') scale..."
- P3 L3 Please include Wille et al. 2010 and Hendry and Robinson 2012 here along with other references.
- P3 L18 replace 'issues' with 'questions' and 'are going to' with 'will'
- P3 L22 'a remotely operated vehicle' and 'seawater was sampled using'
- P3 L25 please change to '...as smaller individuals encrusted on other organisms...'
- P4 L3 remove 'taken and', replace 'in' with 'into'
- P4 L5 replace 'in' with 'into'
- P4 L7 replace 'in' with 'with'
- P4 L11 please state the following sentence earlier in the paragraph: 'The cleaning procedure followed the technique in Hendry et al. (2010) and Hendry and Robinson (2012).
- P4 L22 please add 'at the University of Bristol' 'after Bristol Isotope Group facilities'

P4 L22 – change sentence to: '...were repeated at least twice..' and add the word 'methods' after 'Mg doping'

P5 L 23 - please add 'marine' or 'deep-sea'

P6 L8 - remove 'have'

P6 L9 – change to 'which suggests that silicon isotope fractionation in marine sponges is like to be controlled by a mechanism of Si uptake.'

P6 L24-26 –remove 'concentration, supports Dsi concentration being the main factor controlling silicon isotope fractionation'. There still could be other factors such as pressure, salinity, etc.

P7 L21 – please move this information up to L13.

P7 L29 - remove 'the fact'

P8 L6-7 – 'A spicule is composed of hydrated amorphous silica (SiO2...' was already defined on P2 L5. The purpose of this sentence is unclear, please rephrase.

P8 L7 - remove 'The' as in "The biosilicification'

P8 L 21 - change to 'sponge E. aspergillum is comprised of small spicules that are embedded in a silica matrix surrounding a larger

P8 L26 - change to '...solely a result of the differences in organic composition'

P8 L 30 – change to '...Si isotopes by sponges, epsilon f (see equation 2).

P8 L32 – please provide a reference for your definition of efflux (Milligan? Wille? Other?).

P9 L1-2 – please consider changing to 'values from the aforementioned studies in four different laboratory-based sponge culture experiments (summarized in Table 2).'. Remove 'and with KmP and Vmaxp, the maxium polymersation rates.'

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P9 L24 – change to 'Biosilicification in sponges results in the condensation...'. Enzyme should be plural.

Figure 2 caption – please add the abbreviated terms d30Si, D30Si and Si(OH)4 to the figure caption.

Figure 7 – please clarify in the caption that these data are only from the current study.

Table 2 – please define the parameters listed in the table in the table caption. Capitalize the first letter of 'reference'

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