

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 #1

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.

Moderate/Major revisions 1) Section 3.2 – Much of this section would be better suited in the methods section, per- haps alongside the SEM work? For example, ‘five levels

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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 $\delta^{30}\text{Si}$ measurements had been finalized? Please explain. In addition, the relationship between the fusion stage data and the measured $\delta^{30}\text{Si}$ 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 $\delta^{30}\text{Si}$ of the spicules is strongly associated with different fusion stages.

2) 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. (answer) In the corrected version section 3.2 is left at its original place. We do not think that it has to be in the method section because the degrees of fusion were discovered from the SEM images and so are more suited later in the manuscript. We have changed the first sentence of the section (L20-21 p5) to provide more details about the samples chosen for SEM analysis. Furthermore, the end of the section has been edited (L27-28 p5) to provide a better description of the relationship between $\delta^{30}\text{Si}$ spicule and the degree of fusion. Data were only incorporated into the F5 Box plot (figure 4) to show that the large error was caused by only 2 data points. Changes have been made to figure 4 to include data points of each box plot, and the significant relationship curve has been added to support the hypothesis linking the fractionation factor and the degree of fusion. Also figure 4 is now made of two plots with a) the box plot and b) the residual of the fusion stage against the published calibration to support our assertion that there is a positive relationship between the degree of fusion and the fractionation factor.

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3) 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 K_m of 10 μM was chosen, nor that the Low K_m simulation was included in Figure 6. Also, what was the K_{mp} hat Lopez-Acosta – why did you chose 10 μM ? (answer) The entire section 4.3 has been edited to make it easier to follow and also the recent sponge culture study, Lopez-Acosta et al., 2018, has been included. The low K_m simulation was already included in figure 6 and was/is referred to as Low K_m and the K_m value of the *T. citrina* species (Lopez-Acosta 2016) was/is given in table 2, K_m *T. citrina* = 29.84 μM . For the low K_m simulation, $K_m = 10 \mu\text{M}$ was chosen as a contrast with the published K_m value, and because it was the K_m value that fitted with the lower $\Delta^{30}\text{Si}$ limit of unfused sponges.

4) 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 K_m of what organism? Or is this theoretical? Most organisms listed in the table have a K_m that is much greater than 10 μM (i.e. 29.8-74.5 μM) therefore decreasing the K_m to 10 μM 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 K_m . These are generally not very well described in the text. (answer) The end of section 4.3 has been edited in order to present the efflux hypothesis with more details and explanations, now L18-30 p10.

Problems with clarity

5) 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

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mean by anomalies. (answer) The anomalies represent the fractionation factor that fall outside of the calibration curve. We would like to keep the phrase “anomalies in the isotopic fractionation” as we believe that it concisely describes these data.

6) P1 L8 – extremely light d30Si signatures? Compared to what? This was not described. Please clarify. (answer) “compared with previous studies” L9p1 has been added.

7) P1 L 10 – please clarify what you mean by spicule types. . . (answer) “Spicule types” has been chosen here to describe the spicules shape/fusion without being too technical because we would like to avoid too many taxonomic words in the abstract.

8) P1 L 15 – molecular fossil what? The molecular fossil record? Please clarify. (answer) Done L16p1

9) P1 L20 – Do you mean, Of the biomineralizing sponges? Please clarify. (answer) Done, now L21p1

10) 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. (answer) This sentence explains that the ratio of calcareous and siliceous sponges in the ocean has been changing with time due to the change in nutrient composition in the ocean. The end of the section has been changed to “but this ratio may have varied in the past due to changes in paleo-ocean chemistry (Montanez, 2002) because sponges rely on the ion chemistry of their surrounding water to build their skeleton“ L2-3p2

11) 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

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demosponge mega- and microscleres. As it is written, this is not clear in the text. (answer) L12-15p2 has been edited: “which compose their skeletal structure. Either Mega- and microscleres are loose, unfused, but joined by spongin (collagen protein) (Uriz, 2006). Demospongiae have a cellular organisation i.e composed of cells that form tissues, which themselves form organs, which form an organism.”

12) P2 L15 – what do you mean by rays? Spines along one of the three axes? (answer) Now L18p2 The spicules are described with number of axes in the line before. The rays describe one of the “branches”.

13) P2 L16 – please clarify what you mean by secondary silica. (answer) L20p2 “layer and or junction” has been added”.

14) P2 L17-18 – It is unclear as to why you have included this sentence. Please provide context and improve the conclusion of this paragraph. (answer) The end of the paragraph has been edited, now L20-22p2 “One key feature that distinguishes between Demospongiae and Hexactinellida class is that Hexactinellida are characterised by a syncytial organisation, i.e. tissue composed of cells without individual plasma membrane (Leys and Lauzon, 1998; Maldonado and Riesgo, 2007).”

15) 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. (answer) This sentence has been edited, now at L26-28p2 “Because of their relatively low growth rate and their immobility, they are sensitive to change of their environment and because an individual sponge can live decades or centuries (Pansini and Pronzato, 1990; Leys and Lauzon, 1998) they can record information over long time periods (Jochum et al., 2017).”

16) P2 L 26 – this sentence needs to be reorganized – De La Rocha did not introduce the silicon isotopic composition of biogenic silica. (answer) Done, now L30p2 “The silicon isotopic composition of biogenic silica ($\delta^{30}\text{BSi}$) has been introduced to study the past nutrient utilisation by 30 De La Rocha et al. (1997) and since has been used

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to study the silicon cycle (e.g. Hendry et al., 2016; Fontorbe et al., 2017).”

17) P3 L17 – the sentence ‘..by analysing $\delta^{30}\text{Si}$ along the sponge skeleton?’ is not clear, please rephrase. (answer) Done, now L20p3 “Can we trace DSi concentration over time by analysing $\delta^{30}\text{Si}$ sections of sponge skeleton? “

18) P3 L18-19 – This sentence is not clear. What precisely is being investigated? Please rephrase. (answer) “ $\delta^{30}\text{Si}$ ” has been added now L23p3

19) P3 L22-25 – please rephrase this sentence, it is not clear. (answer) Done, now L25-28p3 “Sponge samples were collected by remotely operated vehicle (ROV) and seawaters using Niskin bottles attached to CTD rosette system at five stations, EBA, EBB, VEM, VAY and GRM between 298 m and 2985 m (figure 1) aboard the RRS James Cook on the TROPICS cruise (JC094), a West-East cross section in the equatorial Atlantic between 5°N and 15°N , from the 13th October to the 30th November 2013. ”

20) 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. (answer) “preserved in ethanol and frozen” has been deleted to not confuse the reader because none of the samples used in this study were preserved in ethanol or frozen (they were preserved by those means for other studies e.g. taxonomic identifications).

21) P3 L28 – was the identification of the specimens to the major sponge classes carried out on subsamples that were preserved or were they fresh? (answer) The major class identification was done on board (L3p4) based on analysis of the fresh sample.

22) 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? (answer) L5p4 “in a separate paper” is added. The paper has not been submitted yet so we cannot give more details about it.

23) P4 L7 – please rephrase the sentence ‘If remaining, lithogenic material was removed by hand’. It is unclear. (answer) The lithogenic material is visible by eye on living sponge spicules, we then remove it by hand if remaining. “before further cleaning steps” has been added L12p4 to precise that the spicule undergoes further cleaning steps.

24) P4 L7 – Please clarify where the subsample originates. Is it cleaned? Has the lithogenic material been removed? This is not clear. (answer) Done, L13p4 “A subsample was taken and weighed before going through a final cleaning step” was added.

25) P4 L13 – Please clarify and rephrase ‘Reynolds et al. (2006) modification.’ (answer) The MAGIC method is a common method used to analyse $\delta^{30}\text{Si}$ of seawater. The following sentence gives brief details of the method. This section has not been changed as other reviewers did not comment on it.

26) P5 L7 – How was the $\delta^{30}\text{Si}$ calculated? There are no $\delta^{30}\text{Si}$ data for seawater presented in table A1. (answer) In the corrected version $\delta^{30}\text{Si}$ of seawater have been added in table A1. It was not done in the first version because $\Delta^{30}\text{Si} = \delta^{30}\text{Si}_{\text{spicule}} - \delta^{30}\text{Si}_{\text{seawater}}$ is describe in the section 1.2 (now L9p3.)

27) 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 $\delta^{30}\text{Si}$ larger than 5 permil’? Do all samples show a common feature or just the samples that have a $\delta^{30}\text{Si}$ larger than 5 permil? The information is not abundantly clear from the text and needs to be clarified. (answer) This paragraph has been entirely edited, see L3-8p6.

28) P6 L3 – what studies? Please provide references (answer) Done, see L11p6

29) P6 L5 – please define epsilon f. what does it mean? (answer) Sentence changed to “Here $\Delta^{30}\text{Si}$ is defined by the difference between $\delta^{30}\text{Si}_{\text{Spicules}}$ and $\delta^{30}\text{Si}_{\text{DSi}}$, which

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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)” see L12-13p6

30) 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. (answer) Sentence changed, see L6-7p7 “Despite the small range of seawater temperature in this study, our data show no relationship between $\Delta^{30}\text{Si}$ and temperature (figure A1 in appendix).”

31) P6 L29 – please explain what you mean by low concentration? What is the range? (answer) The range was added, see L9p7

32) 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 $\delta^{30}\text{Si}$ of seawater where their collected sponges resided. Their estimates for $\delta^{30}\text{Si}$ came from Cardinal et al. 2005. It would be a good idea to mention this in the text. (answer) The data compiled for the residual a) are from the data presented in each paper from Hendry and Robison, 2012; Hendry et al., 2010; Wille et al., 2010. They are the same data presented in figure 2. The data from this study are detailed in table A1 with the fusion degree in the corrected version, which allow the reader to reproduce the residual. Because table A1 is very large, we decide to not add the data from previously published papers as they are already available. Wille et al., 2010 did not measure the $\delta^{30}\text{Si}$ of seawater but due to the conservative nature of $\delta^{30}\text{Si}$ in deep water masses the fractionation factor calculated in Willes et al., 2010 is valid.

Minor revisions

33) 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” (answer) We would like to keep the original title

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because despite that the study did not investigate the direct influence of the biomineralization on the silicon isotope but the relationship between the degree of fusion and the K_{mp} (half saturation constant of polymerisation) implies that there is.

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:

34) P1 L9 – change ‘insights’ to ‘insight’, change ‘process’ to ‘process(es)’ (answer) Done, now L9p1

35) P1 L10 – change ‘isotopes’ to ‘isotope’ (answer) Done, L10p1

36) Definitions (e.g. Si, DSi, BSi \bar{A} \bar{I} d³⁰Si, \bar{A} \bar{I} e \bar{E} g \bar{f}): Please define these abbreviated terms correctly, and once defined, continue to use them instead of their non-abbreviated form. Check throughout the text. (answer) Done 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,

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P9 L30, P10 L2, P10 L9, Figure 2 caption, Figure 4 caption, Figure 6 caption, Figure 7 caption, Table A1. . .

(answer) Because the paper is focused only on the silicon isotopic fractionation the “silicon isotopic fractionation” term was reduced in the previous version to have lighter sentence to read. This has been changed in the corrected version.

37) P1 L1 – change to “The silicon isotope composition ($\delta^{30}\text{Si}$) of deep-sea sponges’ skeletal elements – spicules – reflect the . . .” (answer) Done, L1p1

38) P1 L18 – change to (Strehlow et al., 2010 and references therein) (answer) Done, L19p1

39) P2 L5 – Please change sentence to “. . .spicules through the incorporation and deposition of hydrated amorphous silica ($\text{SiO}_2\text{-nH}_2\text{O}$), otherwise known as bio-silica.” (answer) Sentence changed to “produce their spicules made of bio-silica (amorphous silica)” L4-5p2

40) P2 L10-11 – These two sentences would be better merged. (answer) New paragraph started L11p2 and the sentences have been merged.

41) P2 L16 – change “loose” to “loosely attached” (answer) We decided to keep “loose” to distinguish between cases where the spicules are attached to each other with secondary silica

42) 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. (answer) Done, now L23p2

48) P2 L21 – remove ‘may be’ and replace with ‘are. (answer) Now L25p2 has not been changed because this subject has been solely highlighted in one paper and the study used one area rather than a compilation to extend their standing Si stock to a global scale.

49) P2 L 22 – remove ‘immobility’ and replace with ‘inability to move’. (answer) Done, now L26/27p2

50) P2 L28 – please write ‘approximately’. (answer) Done, now L32p2

51) 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 $\delta^{29}\text{Si}$ or $\delta^{30}\text{Si}$ using the permil (‰) scale. . .’. (answer) This has been changed to : “Silicon isotopic abundances in samples (SMP) are expressed as $\delta^{29}\text{Si}$ or $\delta^{30}\text{Si}$ with the abundance ratio, $^{29}\text{Si}/^{28}\text{Si}$ or $^{30}\text{Si}/^{28}\text{Si}$ respectively and measured relative to the reference standard (NBS28). The results presented in this study are expressed as permil to be consistent with the International Union of Pure and Applied Chemistry (IUPAC) nomenclature. i.e.” (L33p2 to 2p3).

52) P3 L3 – Please include Wille et al. 2010 and Hendry and Robinson 2012 here along with other references. (answer) Done, now L6p3

53) P3 L18 – replace ‘issues’ with ‘questions’ and ‘are going to’ with ‘will’ (answer) Done, now L21p3

54) P3 L22 – ‘a remotely operated vehicle’ and ‘seawater was sampled using’ (answer) Done, now L25p3

55) P3 L25 – please change to ‘. . .as smaller individuals encrusted on other organisms. . .’ (answer) Done, now L1p4

56) P4 L3 – remove ‘taken and’, replace ‘in’ with ‘into’ (answer) Done, now L8p4

57) P4 L5 - replace ‘in’ with ‘into’ (answer) Done, now L10p4

58) P4 L7 - replace ‘in’ with ‘with’ (answer) Done, now L12p4

59) 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). (answer) We decided to keep the cleaning procedure at the end of the para-

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graph. Now, L16p4

60) P4 L22 – please add ‘at the University of Bristol’ ‘after Bristol Isotope Group facilities’ (answer) Done, L28p4

61) P4 L22 – change sentence to: ‘. . .were repeated at least twice.’ and add the word ‘methods’ after ‘Mg doping’ (answer) Done, L28p4

62) P5 L 23 – please add ‘marine’ or ‘deep-sea’ P6 L8 – remove ‘have’ (answer) deep-sea has been added, now L2p6

63) P6 L9 – change to ‘ which suggests that silicon isotope fractionation in marine sponges is like to be controlled by a mechanism of Si uptake.’ (answer) “marine sponges” has been added but we kept “mainly”. Now L17p6

64) 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. (answer) This sentence has been kept as “which supports DSi concentration being the main factor controlling Si isotopic fractionation” (L6p7).

65) P7 L21 – please move this information up to L13. (answer) Done, now L25p7

66) P7 L29 – remove ‘the fact’ (answer) Done, now L9p8

67) 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. (answer) This sentence introduces the reader to this new section that focus on the spicule composition, so this information is a key point. This sentence is a reminder like that reader does not need to return to the beginning of the paper to understand the rest of the paragraph. “and organic molecules (Uriz et al., 2003)” has been added at the end of the sentence. Now L21p8

68) P8 L7 – remove ‘The’ as in “The biosilicification’ (answer) Done, now L23p8

69) P8 L 21 – change to ‘sponge E. aspergillum is comprised of small spicules that are

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embedded in a silica matrix surrounding a larger (answer) Done, now L5p9

70) P8 L26 – change to ‘. . .solely a result of the differences in organic composition’ (answer) Done, now L9-10p9

71) P8 L 30 – change to ‘. . .Si isotopes by sponges, epsilon f (see equation 2). (answer) Done, now L13p9

72) P8 L32 – please provide a reference for your definition of efflux (Milligan? Wille? Other?). (answer) “(Wille et al., 2010, and references therein)” has been added, now L16p9

73) 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 maximum polymerisation rates.’ (answer) The end of the paragraph has been changed to “To date, only Reincke and Barthel (1997); Maldonado et al. (2011); López-Acosta et al. (2016) and López-Acosta et al. (2018) have cultured sponges to investigate the 15 Michaelis-Menten enzyme kinetics of sponges. In this section, ϵf has been modelled using Km,P and Vmax,p values from these four sponge culture experiments and are summarised in table 2.” L16-19p9

74) P9 L24 – change to ‘Biosilicification in sponges results in the condensation. . .’. Enzyme should be plural. (answer) Done, now L18p10

75) Figure 2 caption – please add the abbreviated terms d30Si, D30Si and Si(OH)4 to the figure caption. (answer) Done, “a) Silicon isotopic composition of the spicules ($\delta^{30}\text{Si}_{\text{Spicules}}$) and b) deep sea sponges apparent Si isotopic fractionation ($\Delta^{30}\text{Si}$) against DSi” has been added.

76) Figure 7 – please clarify in the caption that these data are only from the current study. (answer) Done, “from this study” has been added.

77) Table 2 – please define the parameters listed in the table in the table caption. Capitalize the first letter of ‘reference’ (answer) Done, “with Vmax,p the the maximum

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polymerisation rates and K_m , P the half saturation constant of polymerisation.” Has been added.

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