

## ***Interactive comment on “Validation of carbon isotope fractionation in algal lipids as a $PCO_2$ proxy using a natural $CO_2$ seep (Shikine Island, Japan)” by Caitlyn R. Witkowski et al.***

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We thank the reviewer for the comments, as well as for the recommendation for publication. Below, we respond to the specific/technical comments expressed by the reviewer (our response following the \*), which will improve the manuscript.

Page 3 Line 9. Suggest placing a reference to figure 1 here.

\*We will place a reference to Fig 1 here.

Page 3 Lines 15-19. Why were the currents and winds measured in 2014 and 2015 and not in 2016 when the samples were collected? How comparable is this for the

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‘normal’ situation in this region?

\*The currents and winds were studied in detail during the 2014-2015 expeditions (Agostini et al., 2015). Unpublished observational data suggest that this is normal for the region, based on visits to the site on a monthly basis for the past 5 years. Although 2016 was a particularly strong season for storms, this region experiences these kinds of storm activity annually. This annual storm season may explain why the samples collected in this study do not reflect the full  $PCO_2$  values observed on site.

Page 3 Line 25 Is the abbreviation SPM properly introduced?

\*We will add the appropriate introduction of SPM.

Page 4 Line 8. Remove ‘then’.

\*We will remove ‘then’.

Page 4 Lines 10-11. Change to ‘..... NBS-19), flushed with He, injected with 500  $\mu$ L of 85%  $H_3PO_4$ , and reacted for 1 h.’

\*We will change this accordingly.

Page 4 Lines 11-12. Change to ‘The headspace was measured and average values and standard deviation errors reported are based.....’

\*We will change this accordingly.

Page 4 Lines 16-17. Change to ‘.....using ultrasonication (5 times) with 2 ml dichloromethane (DCM): MeOH (9:1 v/v).’

\*We will change this accordingly.

Page 4 Lines 19-20. Change to ‘ .....and the organic matter the DCM layers were pooled and dried over  $Na_2SO_4$ .’

\*We will change this accordingly.

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Page 4 Line 20-21. Change to 'The resulting hydrolyzed TLEs were eluted over an alumina packed column and separated into apolar....'

\*We will change this accordingly.

Page 4 Line 22. Remove 'then'

\*We will remove 'then'.

Page 4 Line 23-24. Change to '.....prior to analyses by gas chromatography-with flame ionization detection(GC-FID),GC-mass spectrometry (GC-MS), and GC-isotope-ratio mass spectrometry (GC-IRMS).'

\*We will change this accordingly.

Page 4 Line 25. Would it not better to report that GC-FID was used for quantification and to check the signal to noise ratio?

\*We will change the phrasing of this to the reviewer's recommendation.

Page 4-Line 26. What are the ideal concentrations? What is the range?

\*Approximately 50 ng was injected on column.

Page 4 Line 28. Change to ' .... and He is used as carrier gas.'

\*We will change this accordingly.

Page 4 Lines 28-30. Suggest changing it to ' The GC oven was programmed from 70C to 130C at 20C/min and then to 320C at 4C/min at which it was held for 10 min. '

\*We will change this accordingly.

Page 4 Line 34. Replace ' C20 and C24' with 'the same'.

\*We will change this to 'the same'.

Page 5 Lines 6-9. Why include this information again? You have already given this

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information in the method section.

\*We used this paragraph as a summation of information that was spread across the site and materials subsections of the methods. Based on the reviewer's comments, we will remove this paragraph.

Page 5 Lines 5-11. If the SPM samples were not included in this study why mention them at all? See no reason for this and suggest removing all information related the SPM samples.

\*This information was included to let readers know that SPM was considered, though the clear waters did not yield enough material. We can minimize the reference to SPM to a single sentence within the manuscript.

Page 5 Lines 12-13. I cannot find the supplementary information anywhere so cannot comment on this figure.

\*Thank you for pointing this out. We will upload the supplementary material.

Page 5 Lines 17-20 and Fig 2. Not all compounds mentioned here are clearly labelled in Fig. 2. For completeness this should be corrected.

\*We have not specifically indicated all compounds as they crowd the chromatograms and are not part of the study. Our point was merely to show that the compounds investigated are abundant and that there are no large differences between sites.

Page 5 Line 23 and onward. Considering that only two (or three) sites are compared it is incorrect to talk about 'change' here (or shift in the next lines). It would be better to report the 'differences' between the sites or, as a couple of lines later, mention if the values are higher or lower if compared to....

\*We will change this phrasing to 'differences' from 'change'.

Page 6 Line 27– page 7 line 3. Here the possibility of a contribution of terrestrial derived cholesterol is discussed. I agree that this cannot be completely excluded but

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was wondering if the authors have some more information about the relative terrestrial contributions to the sediments in this region. Looking at fig 2, for instance, suggest that there is no substantial presence of terrestrial HMW n-alkanols. What about biomarkers present in the other fractions obtained?

\*As seen in this Fig 2, these samples lacked triterpenoids and long-chain alcohols typical of higher plants, suggesting lack of terrestrial input. Furthermore, our apolar fractions lacked long-chain n-alkanes associated with terrestrial input. Therefore, we think we can exclude this as a possible issue with cholesterol. This will be noted in the revised manuscript.

Page 7 Lines 15-25. I find this a bit of a confusing section, particularly in line with the information reported in the method section 2.1. As mentioned earlier I do not understand why the currents and winds were measured in 2014 and 2015 and not in 2016. It now seems that the conditions between the sampling seasons were completely abnormal. In addition, would it be possible to add a few references to information given in this section. I assume that the kind of impact this had on the corals etc must have been properly documented.

\*Based on unpublished observational data from part of the co-authors visiting the site on a monthly basis, the conditions covering 2014-2015 are typical of what they've witnessed over the past six years of research. This study on winds and currents was intensely time-consuming and was thus not repeated here. However, we will add weather station data to further support the normalness of currents and winds in this region of Japan (unfortunately, the only recorded data from this specific island is referenced here). As commented above, typhoons and strong tropical storms are common in this region and occur on an annual basis. Our June collected samples showed a lessened reconstructed value for PCO<sub>2</sub> than the PCO<sub>2</sub> measured at the site, which may be explained by this annual storm season which mixes the bay every year. Mixing during the storm season is a plausible explanation to why we observe a distinct difference between our two sampling seasons (one before the storm season and one after). Our

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sampling year (2016) happened to have particularly intense storms reaching land (see supporting figure attached here).

Page 8 Line 4. It should be 'Witkowski et al. (2018)'

\*This will be changed to Witkowski et al. (2018).

Page 8 Line 6. Change to '...however, has never been determined.'

\*This will be changed to '...however, has never been determined.'

Page 8 Line 17. Change to '.....sites for all three....'

\*This will be changed to 'for'.

Page 8 Line 23. Change to '...as it is the only....'

\*This will be changed to 'as it is the only'

References. Please check all references carefully. It should be 'Sinninghe Damsté, J. S.' and not 'Damste, J. S. S'.

\*We will check the references more carefully.

Figure 3 and 4. Currently the data in these figures is presented as line plots. However, considering that we are only dealing with samples from three discrete areas I feel that this is misrepresenting the results suggesting that there are trend between the three sites. Suggest removing the lines, showing the results as individual points.

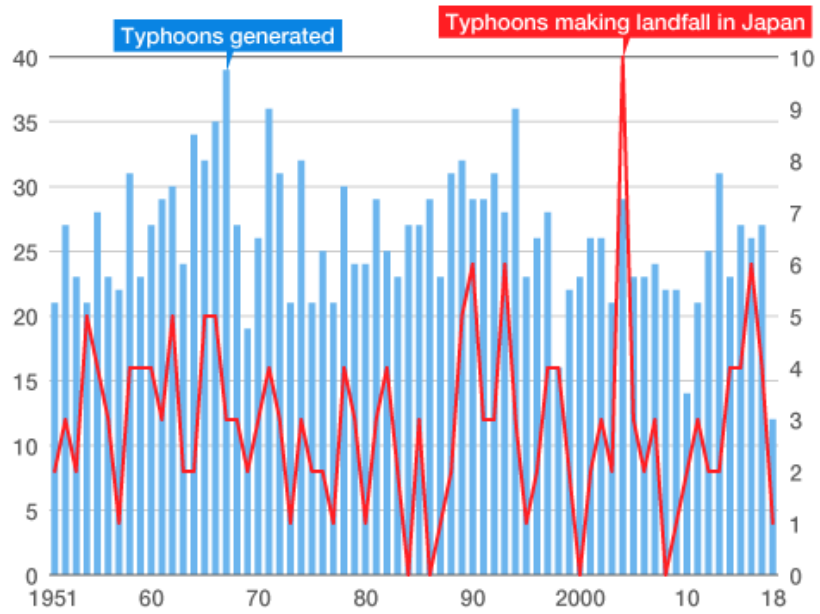
\*We will remove the lines and show the results as individual points.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2019-158>, 2019.

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## Typhoons by Year



Created by *Nippon.com* based on data from the Japan Meteorological Agency (as of July 2018).



Fig. 1.