

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.

Anonymous Referee #1

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Witkoski et al. present a very interesting data-set that suggest that we may be able to use general algal biomarkers for reconstructing past PCO_2 . My impression of the MS is very positive although it is clear from the results that still more work needs to be done. It is very well organised and easy to read. The date is well presented and the interpretations are sound. I congratulate the authors on their effort. I think that the manuscript should definitely be published in BG and have no major critics. However, I do have a number of specific/technical comments (listed below) that I hope will help to improve the final revision of the MS.

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

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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 'normal' situation in this region?

Page 3 Line 25 Is the abbreviation SPM properly introduced?

Page 4 Line 8. Remove 'then'.

Page 4 Lines 10-11. Change to '..... NBS-19), flushed with He, injected with 500 μ L of 85% H₃PO₄, and reacted for 1 h.'

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

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

Page 4 Lines 19-20. Change to '.....and the organic matter the DCM layers were pooled and dried over Na₂SO₄.'

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

Page 4 Line 22. 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).'

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?

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

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

Page 4 Lines 28-30. Suggest changing it to ' The GC oven was programmed from

70°C to 130°C at 20°C/min and then to 320°C at 4°C/min at which it was held for 10 min. ' Page 4 Line 34. Replace ' C20 and C24' with 'the same'.

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

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.

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

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

Page 5 Line 23 and onwards. 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. . .

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 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?

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

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in this section. I assume that the kind of impact this had on the corals etc must have been properly documented.

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

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

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

Page 8 Line 23. Change 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'.

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.

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