

## ***Interactive comment on “Effects of alkalinity and salinity at low and high light intensity on hydrogen isotope fractionation of long-chain alkenones produced by *Emiliana huxleyi*” by Gabriella M. Weiss et al.***

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Received and published: 6 October 2017

Anonymous Referee #3 Received and published: 26 September 2017 The study “Effects of alkalinity and salinity at low and high light intensity on hydrogen isotope fractionation of long-chain alkenones produced by *Emiliana huxleyi*” by Gabriella M. Weiss, Eva Y. Pfannerstill, Stefan Schouten, Jaap S. Sinninghe Damstè, and Marcel T.J. van der Meer is an important step forward in the quest to understand the environmental sensitivities of hydrogen isotope fractionation during lipid biosynthesis in unicellular photoautotrophs. The experimental design, measurements, analysis and interpreta-

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tions are all high quality, and I have no major criticisms of the work. However, I do have a few suggestions about ways that the manuscript could be improved, which I have outlined below. I recommend publication once the authors have had a chance to consider these, and the input from the other reviewers.

We would like to thank Anonymous Referee #3 for the constructive comments, which we will take into consideration and would like to address below as “Response:” following the original comment.

General comments: I found the discussion on mechanisms of hydrogen isotope fractionation to be well thought out and referenced, but I think that a slightly expanded discussion on the growth conditions that *E.hux* experiences in the environment, seasonality of lipid production, and effects of growth rate, light, and nutrients, etc. on alkenone production might be helpful to guide the discussion on alkenone H-isotope fractionation. This is a very well-studied organism after all, and we benefit from decades of research on these factors due to the work that has been done for understanding Uk37 and  $^{13}\text{C}/\text{pCO}_2$  applications. I suspect that these lessons could be applied to the present work a bit more than they are currently.

Response: Noted, we will incorporate them into our discussion.

In general the paper is careful to specify when discussing alkenones produced by *E.Hux* from those produced by other haptophytes, but there are a few cases where this isn't clear and I'd recommend clarifying these.

Response: We will clarify these.

The quality of the writing is fine for the most part, although I do have a few suggestions and typos that I've outlined below, so I would also just recommend here that it gets read again with this in mind. Detailed comments: P.2L1-10 - I would not refer to continental bodies of water as meteoric this way. Meteoric implies precipitation-derived and that  $\text{dD} = 8 \cdot \text{d}^{18}\text{O} + 10$ , and many continental bodies of water are enriched due to

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evaporation, making them non-meteoritic. Also, meteoric lakes and rivers are very fresh, making the statement about “low salinity” a little odd. I might rephrase this sentence to say something like “Therefore, most lakes and rivers that are fed by precipitation (i.e. meteoric waters) are characterized by a depleted isotopic signature. As these waters drain into the ocean and mix with seawater the result is to lower both the sea surface salinity as well as the water isotope value, as also occurs during direct precipitation on the ocean.”

Response: Thank you for the suggestion, we will rephrase in the revised manuscript.

P2.L17 - It is a little confusing here to use alpha without really defining it, especially here in this context since the sentence describes hydrogen isotope fractionation decreasing with increasing salinity, meaning an increase in the fractionation factor in this case. I think you don't need to define alpha right here anyway, so I might suggest leaving it until later, at the first actual required use. When the first use and definition do come, I'd also recommend including an equation at that point in line with the normal text because the definition of alpha is always application specific depending on the nature of the isotope system and product/substrate applications in any particular study.

Response: Noted, we will take this into account in a revised version.

P2.L21-27 – Leduc et al., EPSL, 2013 would be another reference that might be worth discussing here in example applications.

Response: Noted, we will include Leduc et al., 2013 in this discussion.

General - Throughout the entire manuscript, delta values (of all types) would be better used in the text with the word “value” (e.g. “d18Oforam values from the same region. . .”, as opposed to “d18Oforam from the same region. . .”)

Response: Noted and will be changed.

P2. – Acronyms should be defined at first use or not used in my opinion, so on this page “LGM” and “SPM”.

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Response: Noted and will be changed.

P2-3 – This paragraph might cite Nelson and Sachs, OG, 2014 in the discussion of field studies, and Wolhowe et al., Biogeosciences, 2009, in the discussion of growth phase. Also be sure to be clear about which observations/studies apply specifically to E.Hux and which don't.

Response: Noted and will include both references in the discussion as well as better clarify which species we are referring to.

P3.L7 – Reference needed for “the culture experiments”

Response: Will correct.

P3.L9 – change “on” to “by”

Response: Will correct.

P3.L17 – Reference needed for “most of the culture studies”

Response: Will correct.

P3.L29 – Not necessarily here, but somewhere in the paper there should be a discussion about why a non-calcifying strain was selected.

Response: Noted, see comment to Referee A. Sessions.

P4.L1-5 – Somewhere in here it would be good to state the pH of the cultures too.

Response: Noted, see comment to Referee A. Sessions.

P5.L.19 - change “and therefore” to “and were therefore”

Response: Will correct.

P5.L20 - provide reference when making a comparison to “previous studies”

Response: Will correct.

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P5.L21 - remind us here, as well as in the caption for figure 1, why those dDwater values are so high in that one group of samples. Maybe different colors for the modified alkalinity samples in the figure?

Response: Will correct.

P5.L24 - alpha values should also be discussed in the text using the word "value" so change "a37" to "a37 values".

Response: Will correct.

Section 3 - Results sound better when consistently described in the past tense in my opinion (e.g. P5.L24-25 as: "A strong linear relationship between  $\delta_{C37}$  values and salinity was observed in both experiments). Either way, be consistent about tense use throughout.

Response: Will correct.

P6.L10-15 - It would be useful here to provide a reference to what surface ocean light levels are and how these dissipate with depth.

Response: Will correct.

P6.L15 - "statistically similar" – be quantitative

Response: Will correct.

P6.L23-25 - Which individual C37 alkenone from the Sachs16 reference is being used to compare to the mixed C37 alkenone  $\delta_{D}$  values reported in the other studies?

Response: We used a weighted mean average to compare the separated C37 alkenones measured by Sachs et al., 2016 to the integrated alkenones in our experiments and the previous experiments of E. huxleyi.

P6.L24 - Chivall14 used a coastal producer, no? The equation is also not listed in table 2. Should this reference be omitted from this list?

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Response: Yes. Will correct.

P6.L30 - I think that this issue of production depth/light exposure could benefit from a slightly expanded summary and literature survey. This gets to one of my general comments at the beginning. It might also be useful to comment on where in the ocean one might expect to find light levels that might cause a large H-isotope effect (i.e.  $<100 \mu\text{mol}$ , based on the van der Meer, GCA, 2015 paper).

Response: Will correct. See comment to Anonymous Referee #1.

Section 4.2 - Describe the statistical similarities in slopes and differences in intercepts in quantitative terms. What thresholds were applied? Response: Will correct.

P7.L6 - change "due differences" to "due to differences"

Response: Will correct.

P7.L6 - As written, "different sources of hydrogen" is probably not the best language. I gather that the implication invokes biochemical mechanisms relating to the routing of hydrogen during biosynthesis, but the way it is currently makes it sound like they are accessing different source water, which is probably not the intention.

Response: Will correct.

P7.L5-9 - what about chemostats vs. batch cultures? That probably deserves a mention somewhere in here in comparing to Sachs16.

Response: Will correct.

P7.L13-15 - Nelson and Sachs, GCA, 2014 would be worth including in this list of references

Response: Will correct.

P7.L14 – I would specify "algal" or "unicellular" photoautotrophs, or include references to alpha-salinity relationships in plants (Aichner et al., OG, 2017; Ladd and Sachs, OG,

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2012).

Response: Will add these references.

P8.L22 - change "by OPP" to "by the OPP"

Response: Will correct.

Figure 1 - I suggest labeling the panels directly on the graphs to allow them to be read without looking at the caption. . . . I also would personally prefer if the graphs were the same width, and were aligned with each other. I'd also use the same x-axis scale for both, and would note the y-axis scale differences in the caption. . . State clearly that the gray shaded areas are confidence intervals (they are, right?) and provide the threshold that was used to define these in the caption Figure 2 - The font in the axis labels, as well as the plotted symbols look like they were compressed vertically. Can this be fixed so they don't look squished like this? . . . . label the experimental design directly on panels a and b, or consider adding this information using a legend to indicate symbol color. . . . see my comment about confidence intervals as related to figure 1 above. Figure 3 - See my comment about confidence intervals as related to figure 1 above.

Response: We will fix these issues with the figures.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-311>, 2017.