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

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Anonymous Referee #4 Received and published: 17 October 2017 The paper by Weiss et al. presents new data from a laboratory experiment aiming to clarify whether and how strong salinity and light intensity affect the hydrogen isotope fractionation during alkenone biosynthesis. Such results pave the way towards an application of algal lipid biomarker hydrogen isotope ratios as a paleosalinity proxy. While similar experiments have been conducted before and salinity and light intensity have been found to affect the hydrogen isotope fractionation, results from the current study test in particular the

effect of alkalinity (which can change independently of salinity) on the isotope fractionation. It therefore adds to the understanding of how representative the previous findings from laboratory cultures are for the natural environment. The study finds that alkalinity does not affect the isotope fractionation and finds similar relationships between isotope fractionation and salinity as observed in previous studies. They also find that changes in light intensity do not change the relationship between salinity and isotope fractionation. These results provide a more robust base to use alkenone D/H ratios as a paleosalinity proxy and may therefore help to identify the actual cellular mechanism responsible for the observed changes in fractionation. While not representing groundbreaking new insights, the study adds to the growing body of literature on this subject. The study is well designed and interpretations are supported by the data. I believe this study should be published after some minor changes. In particular I suggest some clarification of statistical data treatment and a few more detailed descriptions of the experimental setup.

We would like to thank anonymous referee #4 for their constructive comments, which we take into consideration and will address them as “Response:” following the original comment.

General comments: In the study a non calcifying strain of e.hux was used. The authors discuss this to some degree, but a bit more detailed discussion, on how representative these results would be for the natural marine environment, where mostly calcifying strains produce the alkenones, should be part of the discussion.

Response: We will address this in more detail in a revised version. Please also have a look at the reply to comments from Alex Sessions for more specific details.

It appears that the statistical data treatment was done using the three replicate data points as individual datapoints – I think it would make more sense to calculate the mean of the replicates and present the standard error of the mean for each treatment. This applies to the actual slope and intercept calculations as well as for the figures and

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the estimation of the error of the actual regressions (i.e. the shaded area around the regression lines in the figures), see also below.

Response: Yes, we could combine the data points for the statistical analyses, but we kept them as individual data points because they were separate culture flasks (3 flasks for each variable) with slightly different growth rates. We did average the duplicate isotope measurements.

The figures could need some more explanation, in the text but also the figure captions. See detailed comments below. Detailed comments: P6 line 30-31: Can you separate this sentence into 2? It conveys important information, but sounds a bit awkward.

Response: Yes, we will rephrase this.

P7 line 3-4: Can you mention by how much the intercepts from the other studies vary? I believe it would be instructive to present the data from the current study and previous data from the literature in one graph, see comment below (Table 2).

Response: Yes, we can add a graph to supplementary material to help visualize these differences.

P7 line 9: Header for this section does only mention salinity but the second half of the paragraph deals with light intensity. Either separate the paragraph into 2 or mention light intensity in the headline.

Response: We will change the heading of this section.

P7 line 14: In the cited studies not only alkenones, fatty acids and sterols were analyzed, also alkanes and isoprenoids if I remember correctly. I think it would be important to mention that in all these compound classes similar salinity effects have been observed. This is important to identify the underlying mechanism.

Response: Yes, Sachse and Sachs (2008) also measured phytene and diploptene. We will include this.

P8 line 20-21: Interesting hypothesis. Would this hold some advantage for the cell, i.e. using more OPP derived NADPH under higher salinity? Or could this be the result of less water exchange (extracellular with intracellular)?

Response: Danevčič and Stopar (2011) found a more active pentose phosphate cycle at high salinity in Vibrio sp., and also that intracellular production of L-proline, an osmoregulating amino acid, increased. The advantage of up regulating the OPP derived NADPH would be tied to this increase in L-proline, "the production of L-proline, therefore, increases the ratio of intracellular NADP/NADPH, which regulates carbon flux through the oxidative pentose phosphate pathway." (Danevčič and Stopar, 2011). L-proline increases to help continue growth and biosynthesis at higher salinities in Vibrio sp. We think this could also be happening in E. huxleyi.

Page 8 in general: This is a good summary of the hypotheses being discussed for the observed salinity-fractionation relationship. Except a few points (see above) these have all been proposed in previous papers which have identified the salinity-fractionation dependency. This could be mentioned more explicitly. I suggest to give credit to these papers here, for example in the section about osmolytes the first papers proposing this idea as a factor for the observed change in fractionation, should be cited.

Response: We will add references for the discussions already proposed in previous papers.

Figure 1a: Can you briefly explain, why the culture media water dD values at salinity of 35 are so different from the rest?

Response: The difference has to do with the way the media were made, as the media were created separately for the alkalinity/salinity and high light experiments.

Figure 1b: I suggest to use the same scale on the x and y axis as in a) Figure 2: also here I suggest to use the same scaling of the x and y axis (at least for salinity). I think that statistically it would make more sense to use the mean of the replicates and their

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standard deviation for the plots and also to estimate the error of the regression line (standard error of the mean).

Response: We will fix the figures but prefer to keep the statistical analyses on the individual points as mentioned above.

Figure 2c: Can you briefly explain the alpha variability at an alkalinity of 2.5?

Response: Yes, this is the salinity effect observed in previous culture studies, when alkalinity was constant but salinity was varied.

Figure 3: also here, I suggest to sue the same axis scaling (both for alpha and growth rate and salinity). Clearly, and this is the main point of the paper, salinity has a much stronger effect on isotope fractionation compared to growth rate and this would be easily visible in the graphs, when the same axis scaling is used. Also, if a regression line is plotted through the data, you imply a statistically significant correlation. Is that so in all cases, and if so, then you should present the statistical parameters (p value). If it is not statistically significant, no line should be plotted through the data.

Response: Noted, we will fix these figures.

Table 2: I think it would be useful to see these data compared to the data from the current study in a graph.

Response: We can plot the data to give a more visual representation of the comparisons and add this to supplementary material.

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