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Discussions

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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We would like to thank Dr. Sessions for the comments on our manuscript, which we will take into consideration and would like to address as "Response:" following the original comment.

My only general comment is to question why the authors chose to describe culture conditions in terms of alkalinity rather than pH. With [DIC] fixed by equilibrium with atmospheric PCO2, alkalinity and pH are in a sense interchangeable (fixing one uniquely

determines the other). Thus the same experiments could be described in terms of either parameter. Alkalinity is probably more popular among oceanographers, but pH is much more widely used among biologists. And I might argue that there is some reason to think that cellular H isotope fractionation depends more on the concentration of H+ (i.e., pH) than on the ability to consume H+ (i.e., alkalinity). So my suggestion is to at least consider describing the first series of experiments as a pH series, rather than an alkalinity series. Or maybe there is a way to gracefully do both.

Response: We understand that in the natural environment alkalinity and pH are linked, however, in our experiment, we kept pH constant (7.9 $\pm$ 0.07) and only changed the alkalinity by adding NaHCO3 and Na2CO3 to increase and concentrated HCl to decrease total alkalinity in our original media.

I was curious why a non-calcifying strain of E. hux was chosen. Perhaps it simplifies controlling alkalinity? In any case, it would be worth a few sentences of explanation about why you chose this strain, and how it might relate to strains that are prevalent in the oceans. Is it likely to be representative of strains that produce alkenones in most marine sediments?

Response: A non-calcifying strain was chosen because we wanted to avoid changes in the total alkalinity of the media caused by the organisms themselves, which has been shown to be the case in the natural environment by Hooligan et al., 1993 (Global Biogeochemical Cycles 7). We agree that a calcifying strain might be more representative of the natural environment, but in order to get a handle on whether alkalinity has an effect on hydrogen isotope fractionation or not, we chose a non-calcifying strain that has been used in previous studies (M'Boule et al., 2014). Furthermore, all previous culture experiments were done with non-calcifying strains, therefore, by using a non-calcifying strain we would better compare our results with previous results. We will add this to a revised version of the manuscript.

Section 2.1. Please tell us how you measured (or calculated) alkalinity?

Response: Alkalinity was measured by titration with 0.1 M HCl, and calculated using Gran plots. (G. Gran: Determination of the equivalence point in potentiometric titrations. Part II, Analyst 1952, 77, 661). We will add this to the manuscript.

Page 6, line 25. You say that you performed a statistical comparison, and then that "This showed a strong similarity between slopes..". What does strong similarity mean in statistical terms? They are indistinguishable? Given that the slopes differ between experiments by more than a factor of 2, this is probably more a statement about variability between experiments rather than a constant slope. Seems like the discussion of this 'similarity' could be a bit more nuanced. Differences of a factor of _2 would still make a huge difference in reconstructing seawater salinity, even if they are statistically indistinguishable.

Response: True. We were referring to the fact that the slopes were not statistically different because we wanted to examine the data as a whole data set, but we realize that this might not actually be useful for reconstructing salinity because of the differences mentioned above. Furthermore, we believe that the individual experiments themselves (i.e., conducted by different people in different labs using different techniques) do play a large role in the observed differences between slopes, as well as different strains of E. huxleyi that were utilized.

Page 7, line 6. The differences in intercepts amount to a range of nearly 78‰Ź That does not seem (to me, at least) plausible to explain solely by interlaboratory differences. Maybe modify the text to say that "part of" the differences could possibly be attributed to this.

Response: We will fix this.

Page 7, lines 20-35. The term "photosynthetically-derived NADPH" struck me as a little odd, especially in contrast to the more precise "pentose-phosphate pathway". Photosynthesis both produces (in photosystem I of the light reactions) and consumes (in CO2 fixation of the Calvin cycle reactions) NADPH. It would thus be more precise to

refer to NADPH from the "light reactions of photosynthesis", or to "ferredoxin-NADP+ reductase (FNR) in photosystem 1", etc.

Response: We will fix this.

Page 7, line 30-33. I like this explanation, a lot. It is the best one I have heard yet.

Response: Thanks.

Page 9, line 4. "At higher light intensities, we expect a larger pool of photosynthetically derived NADPH inside the cell," Do you have direct evidence (either your own, or from a reference) to support this? Photosynthesis is pretty tightly regulated, so my expectation would be that as soon as NADPH levels start to creep up, photons are shunted to nonphotochemical quenching instead of to the photosystems and NADP reduction. In which case, NADPH levels might not depend on light levels. There should be papers about this in the biochemical literature.

Response: Due to balancing between ATP and NADPH production and consumption within the cell (Walker et al., 2014, Plant Physiology 165), NADPH formation dominates at high light levels, whereas ATP synthesis dominates at lower light levels (Beardall et al., 2003 in Photosynthesis in Algae), leading to the idea of a larger pool of photosynthetically derived NADPH inside the cell. We will add these references to our discussion of this topic.

Page 9, lines 5-10. Larger pool of reduced NADPH could also mean a longer lifetime, and greater D/H exchange.

Response: Yes, true. We agree.

Table 1. Can you at least include the initial alkalinity and/or pH for the high-light experiments? It is not essential, just seems weird not to report them given the emphasis on that variable of the rest of the paper.

Response: Alkalinity and pH were not measured for the high light experiments, since

they were performed separately and not originally considered to be discussed alongside the alkalinity/salinity experiment results, but we used filtered North Sea water, so the alkalinity is presumably around 2.3 (Brasse et al., 1999. Journal of Sea Research 42.)

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