

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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This study focuses on the relationship between salinity, alkalinity, and light intensity with H isotope fractionation. The result is to show again that salinity is an important factor, but that alkalinity and light intensity are not. I would characterize this as an important, incremental advance in our understanding of this proxy. The result is not Earth-shaking, but it is an important step forward.

The results seem quite clear and unambiguous, and the data analysis and interpreta-

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tion is convincing. This is a nice study, with little to complain about. 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.

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?

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

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.

Page 7, line 6. The differences in intercepts amount to a range of nearly 78%. That does not seem (to me, at least) plausible to explain solely by interlaboratory differences.

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Maybe modify the text to say that "part of" the differences could possibly be attributed to 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.

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

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 non-photochemical 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.

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

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.

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