

Interactive comment on “Impact of metabolic pathways and salinity on the hydrogen isotope ratios of haptophyte lipids” by Gabriella M. Weiss et al.

Anonymous Referee #1

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The manuscript by Weiss et al. reports new isotopic compositional data for alkenones, fatty acids, a sterol, and phytol for three different alkenone producers grown under S- and nutrient- experimental conditions. The primary novel contributions the data makes are the interesting responses of alkenone dD to both T and growth phase, in opposition to what has been reported in the literature for other alkenone-producers. They also further document the "salinity effect" on hydrogen isotope fractionation in a number of lipids produced under varying experimental conditions. While the new data is well worth reporting, and may tell very interesting stories about the potential mechanisms behind the temperature, growth rate/phase, and salinity effects, the manuscript as it currently stands has several flaws in its arguments. The data quality, and overall writing,

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are worthy of publication - the well-documented experimental section is particularly appreciated. However, major revisions to the overall argumentative thread of the paper will be necessary before this can be vetted.

In no particular order, here are some concerns with the paper that need to be addressed:

1) We really need the *T. lutea* growth rates for the temp experiments. It is true that, depending on culture density, you may have divergence of chlorophyll fluo. growth rate from cell count growth rate, due to shading, but it is still better than nothing. Given that the temperature effect is completely the opposite of what has been seen before (note that in addition to the somewhat-indeterminate alkenone work, the negatively-sloped temperature effect is also seen in other lipids by Zhang et al. 2009, Organic Geochem), and given the growth rate effects shown here and elsewhere, an attempt should be made at least to constrain how much temp-dependent rate change may impact (counteract?) this curve. The note about the approximately-identical per-volume alkenone concentrations is potentially useful, but only if the cultures were all inoculated at exactly the same density, took off identically with identical lag phases, etc. F curves would be more useful.

2) We also need to see growth curves for the nutrient experiment (f-based or otherwise). "Day 4 and day 10" doesn't give enough info about the status of the culture. This is particularly important because the lower growth rate in the N-limited culture implies that this experiment was truly RATE limited during 'log' phase by N availability. If this is true, it means the culture should have had a constantly-decreasing growth rate as N drawdown occurred, not a single log-linear rate. Make sure the reader is clear on how these cultures were limited and how growth proceeded - i.e. the difference between N being the limiting nutrient in the Redfield sense (determines maximum culture density as opposed to, say, P or vitamins or something) vs N being the rate limiter (growth is limited by the kinetics of N uptake, instantaneous μ would be independent of light intensity, growth would continually slow if grown in batch). At this light level, it's

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hard to envision a batch culture that was rate limited by N at its outset, but still could be grown dense enough to get good alkenone isotope measurements, unless these were truly massive experiments. Give us more information. Do we have final nutrient concentrations or any sense of their evolution over the growth curve?

3) Back to the temperature effect, this 'opposite' effect could be VERY useful for determining the mechanism of the temp. effect, as it would seem to indicate it has to be something more subtle than rate dependence on growth temp., or shifting metabolite into structural vs storage products at different levels of stress, ect. It has to be something that COULD vary strain to strain and has an (apparently?) linear response. However, it does not seem likely from the data that it's related to relative abundance of K37:2 and K37:3. Not only is it not at all clear from Figure 1 that the slopes or intercepts of the isolated 2's and 3's values are significantly different from each other (given their variance at a given temp), but it is unclear from the discussion how the authors are suggesting this 'indirect' effect manifests. If they are invoking a Rayleigh-type mechanism (it sounds like they are) where the negative-offset 3's get progressively heavier as they pull from a progressively heavier pool of remaining 2's, then there shouldn't be any 'switchover' of which compound is heavier or lighter, and K37:3 should get heavier, not lighter, as desaturation becomes more complete at low temperatures. I'd like to see a conceptual model with some rough ballpark numbers explaining the theoretical mass balance between 2s, 3s, and 'waste' hydrogen. If the overall slope of integrated K37s is due to the removal of isotopically heavy hydrogen as a 'loss' term from the desaturation, how would one explain the temp effects observed in saturated lipids (16:0 in Zhang et al) and the difference in the signs of the slopes observed for K37s here and by Wolhowe?

4) Back to the nutrient experiments, it seems like a major point that the exponential- to stationary-phase effect appears to be reverse of what's been observed previously. No discussion is made of this, however.

5) On page 9, there is discussion of how desaturation of 18:0 to 18:1 could counteract

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the salinity effect. Are you suggesting that the 18:0 to 18:1 ratio is salinity dependent? Because the depletion from desaturation would occur under all conditions.

6) Lastly, and most importantly, the big "sell" of the paper is the determination that lipids synthesized in the chloroplast don't experience salinity effects, and lipids synthesized (or completed) in the cytosol do. However, there are a couple of problems with the authors' argument that this is the case. First of all, the only lipid that A) clearly does not exhibit a significant slope vs salinity at the same time as B) appearing statistically distinct from the slopes of the OTHER lipids measured in the same organism is phytol from *E. huxleyi*. *I. galbana* phytol, while apparently not being significantly correlated with salinity, does not to the eye, at least, appear to exhibit a slope that is statistically distinct from that of, say, brassicasterol. It's lack of slope appears to be driven by a single data point. *R. lam* phytol, of course, DOES correlate with S. I would like a more consistent demonstration/argument that we can say lipids built in the chloroplast show a distinct response from cytosolic products. Adding to this ambiguity is the discussion of the alkenones. On page 10, the authors state that alkenones are synthesized in the chloroplast. On page 14, they state that alkenones are made in the cytosol. The former statement seems most consistent with previous work - note the work of Eltgroth et al., who show alkenones building up as lipid bodies in the chloroplast. If this is true, it undermines the cytosol-vs-chloroplast-salinity-effect argument. If they are produced in the cytosol, they help the argument, but there's no evidence or citation provided to this effect.

In response to the specific questions for assessment:

Does the paper address relevant scientific questions within the scope of BG? Yes.

Does the paper present novel concepts, ideas, tools, or data? Yes.

Are substantial conclusions reached? Yes.

Are the scientific methods and assumptions valid and clearly outlined? The methods

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are good, but there are some concerns about the reasoning, as discussed above.

Are the results sufficient to support the interpretations and conclusions? It is currently slightly unclear whether they are or are not. As stated, no, but better explanation of the authors' reasoning may help here.

Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)? Mostly, but as noted above some additional info about the cultures would be appreciated.

Do the authors give proper credit to related work and clearly indicate their own new/original contribution? It is at times difficult to keep track of what data comes from wholly new experiments, what data was collected in the course of other studies, and what data is NEW data but collected in the COURSE of those other studies. Credit is given, yes, but organization of the various "classes" of data could be better to avoid the appearance of 'double publishing'.

Does the title clearly reflect the contents of the paper? Yes

Does the abstract provide a concise and complete summary? Yes.

Is the overall presentation well structured and clear? The logical flow of the discussion needs work. It should be clear from the outset what the problem is and the facts presented in a clear progression to lead the reader to the conclusion. No parts are wholly inappropriate, but for example the discussion of 1) NADPH sources, then 2) the discussion of H-water exchange, then 3) the discussion of osmolytes and then 4) the (paraphrased) statement "as you can see NADPH sources are what control things" makes it seem as though 2 and 3 were just inserted after the fact without any consequence to the narrative. Transition sentences between sections/paragraphs would help.

Is the language fluent and precise? Yes. This is refreshingly polished for a review manuscript!

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Are mathematical formulae, symbols, abbreviations, and units correctly defined and used? Yes.

Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated?

We really don't need both dD vs _____ and alpha vs _____ plots, at BEST they show the same thing, and if they don't it's because dD water is varied and dD plots are useless.

Are the number and quality of references appropriate? Yes.

Is the amount and quality of supplementary material appropriate? Mostly. I sure would like some growth data.

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