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Biogeosciences Discussions

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

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Anonymous Referee #1 – 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 Sand 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, 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:

RESPONSE: We would like to thank anonymous referee 1 for their thoughtful feedback on our manuscript and address their major concerns as "RESPONSE: " following the original comment.

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 mu would be independent of light intensity, growth would continually slow if grown in batch). At this light level, it's 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?

RESPONSE: Referee #1 inquired about growth rate information (points 1 and 2), which we provide now as Figs. 1 and 2 in this comment. For the temperature experiment, we only have the chlorophyll fluorescence data, but for the nutrient experiment, we have cell count data. Growth rates for both experiments follow the traditional pattern of exponential to stationary growth reported in previous haptophyte culture experiments. N was the limiting nutrient here in our nutrient experiments, leading to slower growth for the N-reduced relative to N-replete batches.

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?

RESPONSE: We agree that a Rayleigh fractionation mechanism might not be the best way to explain the data from the temperature experiment. van der Meer et al. (2013) explained the offset between the two alkenones (Dd2H) in this manner. Both the Dd2H vs UK'37 (Fig. 3a) and the UK'37 vs temperature (Fig. 3b) suggest that temperature likely does have some effect on 2H ratios of alkenones. When we plot our data on top of the van der Meer et al. (2013) compilation of Dd2H vs UK'37, we observe much more scatter / outliers, especially from our temperature experiment, suggesting that temperature alone cannot explain this. The effects of desaturation might be dampening / overwriting the temperature correlation with 2H ratios, especially at the extreme high and low ends of the temperature range. The situation is further complicated by the fact that the C37:3 is synthesized from a pool of C37:2 which is also still being synthesized, so not a fixed source. The influence of temperature on 2H ratios of alkenones is still unclear, and as mentioned, other compounds show the opposite of what we report here. However, we highlight here that temperature likely exhibits an effect on hydrogen isotope ratios of alkenones and this effect does not appear to be uniform across experiments. Ultimately, this issue would greatly benefit from testing in a chemostat culture to remove any effects associated with growth phase and growth rate. Analysis of a suite of compounds from such a chemostat could also help to understand these biosynthetic differences in further detail.

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.

RESPONSE: In our nutrient experiment, there was greater accumulation of alkenones during stationary phase under both nutrient concentrations, and a previous study showed a greater concentration of alkenones per cell in stationary and decline phases for other Group II species I. galbana and R. lamellosa (Chivall et al., 2014, GCA). These previous growth phase experiments measuring 2H ratios of alkenones (Chivall et al., 2014) showed a decrease in sensitivity to salinity during stationary and death phases, relative to exponential growth. There was depletion in 2H (lower alpha, more fractionation) associated with longer growth in I. galbana, but no difference was seen between phases for R. lamellosa (potentially as a result of cell clumping). We also noted a depletion in 2H with longer growth, similar to I. galbana. Thus, while accumulation of alkenones might be different, the fractionation response to growth phase appears to be similar.

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

RESPONSE: We are not suggesting that desaturation from 18:0 to 18:1 is salinity dependent. Instead we hypothesize that the hydrogen isotope fractionation related to this desaturation step might be large enough to mask the salinity effect. We will clarify this in a revised version.

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.

RESPONSE: Eltgroth et al. (2005) suggest that PULCA are associated with the chloroplast, but also the endoplasmic reticulum. Sawada and Shiraiwa (2004) report alkenones are found in the ER and the coccolith producing vesicle. Eltgroth et al. (2005) suggest that their chloroplast fraction likely included ER components. Haptophytes have a peripheral ER (Andersen, 2004). A peripheral ER has a connection with the cytosol (English et al., 2009), thus our main argument remains true. If alkenones are indeed present / stored in the ER, (as well as other organelles), they could be associated with the chloroplast, but they are also connected to the cytosol. Furthermore, both Eltgroth et al.(2005) and Sawada and Shiraiwa (2004) show where alkenones are accumulating, not where they are produced. It is possible that alkenones are produced elsewhere and then stored in lipid bodies in various locations in the cell. Additionally, if alkenones are synthesized from fatty acids, this might occur immediately in the ER where fatty acid elongation and desaturation takes place (Jónasdóttir, 2019), or potentially at a later stage from excess fatty acids which have been exported to the cytosol. With respect to phytol, Sachs et al. (2016) do not show a significant change in 2H with

salinity, similar to what we observe here. We strive to clarify our argument in a revised manuscript by emphasizing the E.R. in the discussion, which has a connection to the cytosol compared to the chloroplast, which is closed.

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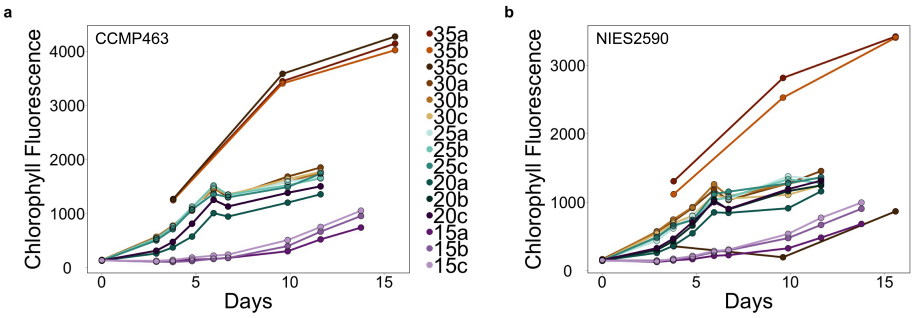

Fig. 1. Growth curves for the Temperature experiment based on chlorophyll fluorescence.

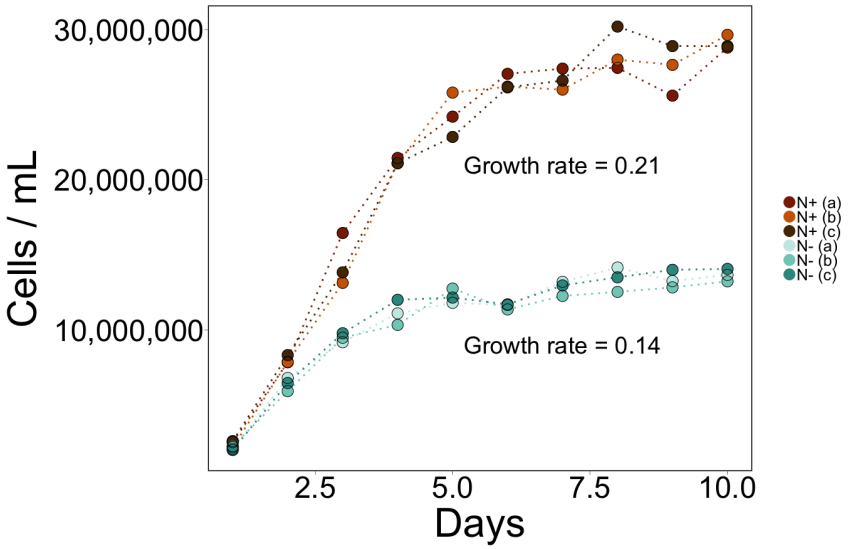

Fig. 2. Cell counts and growth rates for the nutrient experiment.

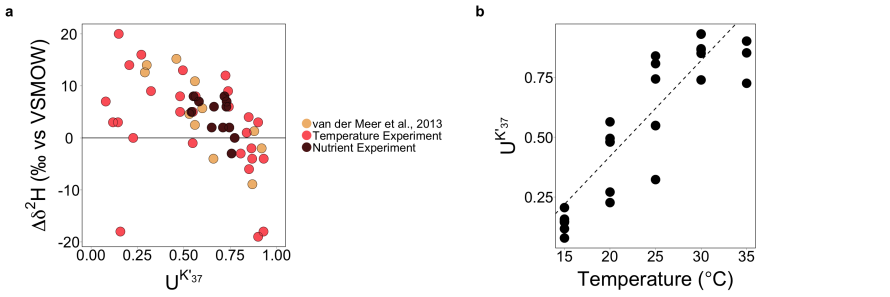

Fig. 3. (a) Difference between 2H ratios of C37:3 and C37:2 vs UK'37. (b) UK'37 vs temperature for the temperature experiment.