

## ***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 #2**

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This paper combined hydrogen isotope salinity data from alkenones (previously published) with hydrogen isotope salinity data from additional lipids (some previously published), also new temperature and nitrogen culture data for alkenones (C37). The data suggest that increased temperature may cause C37 2H-enrichment, confirms that higher growth rates (achieved through different media N levels) leads to increased C37 fractionation, and confirms that other lipid classes (not just C37) in 2 haptophyte groups also become 2H-enriched at higher salinity (but not phytol in 2 species). I especially appreciate the measurement of several different lipid classes. Alkenones have been the sole objective of many previous studies – but ignoring the other lipid classes restricts the potential for understanding the fractionation mechanisms (in haptophytes and other

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species). The isotopic responses of non-alkenone lipids to environmental variations in culture are inherently fascinating in their own right and add valuable insight into the innerworkings of microbes and their isotopes. Please, tell all your friends, measure the other lipids too – it is worth the instrument time. With that said, it would be great if the nutrient and temperature part could include other lipids besides just C37.

Despite the potential of the paper and the quality of the data, the flow of the paper is currently difficult to follow, and the discussion arguments seem like they are not fully thought out. I offer specific comments below that should hopefully help improve the manuscript, but suggest a major re-working of the structure and perhaps framing of the manuscript. I don't see why the authors want to combine the new temp/nutrient experiments with salinity data (maybe they are not enough for a stand-alone manuscript?) but as is, these aspects don't do a good job supporting one another in a comprehensible story. They seem disjointed and unrelated. One suggestion is to tell the reader why these two findings are combined in a single manuscript – how do they support each other, and what new insight can be gained from putting them both here? If it just doesn't work – maybe they should be separate. Finally, since the time this paper was submitted, a new D/H NADPH paper has been published. It might help streamline or motivate the discussion: [www.pnas.org/cgi/doi/10.1073/pnas.1818372116](http://www.pnas.org/cgi/doi/10.1073/pnas.1818372116)

Title - “metabolic pathways” should be replaced with “lipid biosynthesis pathways” or at least “lipid metabolism” because there are so many things associated with metabolism (but not directly related to lipid biosynthesis) that could potentially impact lipid isotope ratios (or not affect them at all). As it stands, your title doesn't capture the added contribution of various lipid classes that this paper has to offer, it would be great if it could. Additionally, why ignore the temp and nutrient data in the title?

Abstract - Line 27: Again, the word metabolism is too vague here. I think “location of lipid synthesis” would be more specific and thus more helpful for readers to follow your meaning. While the abstract successfully and clearly explains the results, it ends abruptly and the opportunity to add the “so what” part to your paper is lost. Are you

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excited about knowing a little bit more about the mechanism? Is it important that not all lipids respond equally to salinity. . . . what are the implications to the biogeosciences? I would pick a motivating point and wrap up the abstract with something that will make the reader want to read more.

Introduction - Line 12: Sorry if I am wrong about this, but would be worth checking if *C. tobin* is in Group 1. DOI:10.1371/journal.pgen.1005469 Page 2 Line 15: Sachs and Kawka 2015 is not an appropriate reference here as they don't experiment with salinity. Since you are including field studies (sachse et al. 2012) you might also mention studies that came out after 2012 (ie <http://dx.doi.org/10.1016/j.gca.2014.03.007> ).

Methods - It isn't mentioned anywhere that fatty acids were corrected for added H from methylation or sterol/phytol corrected for acetylation. I am assuming this was done? If it wasn't, please do so and update data/tables/graphs as necessary. Page 3 Line 21: Why are you calling the sterol/phytol fraction the polar fraction? Page 4 Line 6: Were the fatty acids extracted from the other half of the TLE? This is unclear Page 4 Line 9: Please provide the nutrient recipe(s) Page 5 Line 10-11. H<sub>2</sub> gas was only used to monitor machine accuracy? H<sub>2</sub> gas at beginning and end of sequence needs to be used to tie the Isodat software calculations as well, how else are you getting Isodat to correct?

Results - It isn't clear until the Results section that all of the C<sub>37</sub> data built up in the introduction is actually from other studies. Maybe earlier you can clarify what exactly you are adding to previous C<sub>37</sub> data. The supplement table really helps to do this, perhaps it should be in the main part of the paper. Page 5 Line 26: since you used artificial seawater, can't you just measure your lab's water and estimate alpha with some reasonably big error bars - if you don't know the month it was collected, analyze samples from each month Page 6 Line 14: by "nutrients" don't you just mean "the effect of nitrogen limitation"? Please use more specific language Page 7 Line 4. I think this is supposed to be section 3.2 (not 3.1)

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Discussion - 4.1 – There are 3 issues. Firstly, it was claimed that this temperature part was of secondary interest earlier in the paper, and yet it is the leading discussion point. Either move this down or change the framing of the paper. Secondly, a tremendous amount of text was devoted to invoking abundance shifts in alkenone type to explain the temp trend but no graph (either data or schematic) is offered to support this interpretation. (Along those lines, it is always interesting to show how UK37 does in temperature experiments, even if just supplementary. It would be worth reporting how well this strain does at reconstructing temperature when grown in controlled temperature conditions.) Thirdly, the final sentence is confusing – how is invariable alkenone concentration evidence that growth rate didn't impact 2H/1H ratios? And do you mean total alkenone concentration? B/c most of this section eludes to alkenone abundance changes. Page 7 Line 20-21. How does it compare to the other microbe temp-D/H studies? (Dirghangi and Pagani 2013 <http://dx.doi.org/10.1016/j.orggeochem.2013.09.007> & <http://dx.doi.org/10.1016/j.gca.2013.05.023> and Zhang et al. 2009 doi:10.1016/j.orggeochem.2008.11.002 ) Page 7 Line 23: Please report somewhere the entire significant positive correlation (with slope, intercept, and their standard errors) for this and other relationships reported in this paper. Maybe just a table or on the graph would be fine if it fits.

4.2 – Line 25 a reference is missing here (Sachs and Kawka 2015) Same comment about section 4.1 apply regarding the framing of the paper. Both sections never really get around to the "so what" part and neither does the conclusion. Please, tell us what is the purpose of these sections – how do they add to the story and why are they important? It would make a little more sense if section 4.1 and 4.2 also included non alkenone data, but as is they really stick out.

4.3 – if you really want this to be the main point of your paper, you should address it first in your discussion Line 11 – "in" not "Impact of salinity "of" haptophyte lipids"

4.3.1 - Page 9 Line 21 – somewhere around here would be a good place to compare the lack of C<sub>16</sub>:0 EHUX correlation with the strong relationship found in Sachs et al.

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2016 Page 10 Line 1 – “values” is misspelled Page 10 – Lines 12-14. This is extremely misleading. Plenty of pyruvate is also made in the chloroplast (as the paper mentions later on). Furthermore, Acetyl-CoA is not known to pass organelle walls according to several plant biochem text books. DeNiro and Epstein is not an appropriate reference for this – instead you should check Lohr et al. 2012 (10.1016/j.plantsci.2011.07.018 ) and Hemmerlin et al. 2012 (10.1016/j.plipres.2011.12.001 ) even though they focus on sterols, it is clear that pyruvate can be made in the chloroplast. Certainly under some conditions algal pyruvate seems to be imported into the chloroplast (DOI:10.1371/journal.pgen.1006490) but it is incorrect to leave your statement as is. Page 11 Line 7. Incorrect information, actually the diatom sterol was highly affected by light intensity, strikingly in the opposite manner as phytol and the C14:0 fatty acid. This mistake, and the interpretation that depends on it needs to be fixed.

4.4 - This section would greatly benefit from some rearrangement and reworking to help the reader. It is difficult to follow. One way to improve this is add a brief outline of the points you want to make in the first paragraph before hitting on all of them. A schematic would also help. Are you suggesting anything new here or just reporting all the previously suggested hypotheses? There is no need to devote so much text to explaining these previous hypotheses, a short summary sentence for each should do. Isn't there something more unique you can add now that you have this extra data from the other lipids? Isn't it significant that several studies now have seen only a weak (or no) relationship with phytol? One of the main issues with the NADPH (OPP vs PS1) hypothesis is that NADPH isn't known to cross organelle walls. Is there an OPP pathway inside the chloroplast in haptophytes? If you want to rely so heavily on this explanation, some evidence (in the form of a citation) for 1) NADPH crossing the membrane or 2) OPP in the chloroplast is really needed here.

FIGURES - While the figures indicate in the caption where previous data is coming from, it would be helpful if this info was more visually accessible in the key, either next to species names if no regression is given (Fig 3 and 4), or, next to regressions that

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should be provided (full equations) (Fig 4). Some figures have regression lines some don't. Is there a purpose to this?

Fig. 4 - If a relationship isn't significant (phytol) don't add a regression line...or do something like make regression lines for significant regressions solid lines and not significant regressions dotted. C16:0 symbol colors and shape are too similar to phytol's.

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