Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2019-147-RC3, 2019


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.

**Anonymous Referee #3**

Received and published: 11 June 2019

The manuscript by Weiss et al. set up quite ambitious goals to address almost all factors affecting D/H fractionation in haptophyte lipids. For that purpose the authors included quite a bit previous published data. However, they were not mentioned until Section 3, Results. Through the Introduction and Method, as well as in figures, one can hardly tell what are new and what were previously published. In addition, the new and old dataset seem disjointed each other and it seemed to me that such a way of merging data helped to demonstrate a comprehensible story. Though the data presented are interesting and potentially valuable, the manuscript as written suffered many fatal deficiencies. It is very difficult to follow the flow of the manuscript. The arguments in the Discussion section were not well organized and demonstrated. I



would suggest resubmission after a complete overhaul.

It is a big headache to follow the Method section. I had to list the details of all different cultures on a piece of paper to sort out all different parameters. They were in such a mess: different media, seawater (artificial seawater and filtered seawater), growth temperature (E. huxleyi, E. glabana and R. lamellosa group set up at 15°C, T. lutea strain CCAP 927/14 culture at 20-23°C, and T. lutea strain CCAP 463 and NIES-2590 cultures at 10-35°C), light intensity (60, 100, and 180-220 $\mu$mol photons m-2 s-1), growth phases for collection (linear, exponentially, and stationary), measurements of growth rate (chlorophyll fluorescence, and daily cell counts), and even GC columns (leading to integrated C37 peaks or individual C37:2/C37:3 peaks). . ..It would be hard to imagine if anyone else could come up with a more complicated and confusing experiment design than this one. Such awful setup simply made it hard to isolate one single variable and the arguments based on such data less convincing.

I was curious why the authors did not give any description of methylation of fatty acids or acetylation of sterols, as they are essential to figure out how reliable their reported dD data of fatty acids and sterols. Neither did they present a GC-IRMS trace to demonstrate how well peaks were separated, as sterols often co-eluted. Those are essential to evaluate the data quality.

Another fundamental flaw was that not even a single growth curve was presented, given the fact harvest was taken in different growth phases, and temperature would impact the growth rate. In particular, the authors wanted to address the effect of nutrient replete (NR) and nutrient limited (NL) on lipid D/H fractionation. For this purpose, it would be essential to know how growth rate changed daily. When the authors stated NR or NL, only nitrate concentrations were given, but no phosphorous concentrations—this set of experiments were performed using filtered seawater but no information was available regarding N/P ratio in NR and NL cultures. Table 2 did not give the date for the presented division rate, Day 4 or D10âĂŤin fact the selection of the date seemed randomly. Indeed no one knows what happened between Day 1 to Day 4, or between

Day 4 and Day 10, as far as the status of culture is concerned. As a result we actually don't know when nutrient availability BEGAN to limit the growth rate! Presumably at the given light intensity and initial nitrate concentrations, there shall be no limit on growth rate solely by nitrogen availability at the onset. Then it would be essential to know when the rates in NR and NL cultures began to differ and what could cause the difference. These were batch cultures, not chemostatic cultures (Zhang et al., 2009; Organic Geochemistry). As a result, demonstration of growth rate during the log phase truly limited by nitrate availability would be the key. Without growth curves, one was not in a position to address the effect of growth rate. In fact, it would ideal for the authors to give the concentration of individual biomarkers per cell as we can tell if there are any strategic allocation of carbon source or energy during the different growth phases

The title set up two goals to address: 1) impact of metabolic pathways, and 2) salinity on the on the hydrogen isotope ratios of haptophyte lipids. However, the Discussion initiated with temperature effect, followed by nutrient effect. The title seemed misleading. I would hesitate to call them nutrient replete (NR) and nutrient-limited (NL) conditions as they merely differed in nitrate concentration by 0.6 mM, and growth rate by barely half. In Zhang et al. (2009, Organic Geochemistry, doi:10.1016/j.orggeochem.2008.11.002), NR and NL chemostatic cultures differed in nitrate concentrations by almost 70 times and cell division rates by 4.5 times. Even among such huge growth rate differences, fatty acids biosynthesized by acetogenic pathway did not show the difference in D/H fractionation.

In fact the data supporting for argument of temperature and growth rate effects seemed farfetched. Judged by Table 2, I would say the difference was rather small. If we choose dD of C37:3 as an example, the first line for growth rate at 0.14 could give -121-3= -124 ‰ the last line growth rate at 0.21 gave -130+6= -124‰ almost the same. At least such differences were rather small. The same could be found in Table 1 regarding the temperature effect. At least for the batch culture of T. lutea NIES 2590 with salinity varying from 15 to 30, all dD values of C37:3 could be rounded to -140‰ within stan-



dard deviationâĂŤthey were simply the same. It seemed to me that those data would hardly support the argument for the significant positive correlation with temperature, though the phenomena observed differed from reported in Zhang et al. (2009, OG). Maybe D/H fractionation in alkenones much less sensitive to temperature than fatty acids?

The data on salinity effect seemed more robust, at least for R. lamellose and I. galbana (Fig. 1), but it is questionable to say "The $\delta$2HC37 ratios from T. lutea (temperature and nutrient experiments) fit well with values noted for other Group II species I. galbana and R. lamellosa (Fig.3)." (Page 9, Lines 6-7). Such data varied a wide range at a given salinity which could be considered a substantially large standard deviation. Again, there are too many variables influencing D/H fractionation. As a result, such data should not be plotted in Fig. 3. On the other hand the authors should provide full linear equations for R. lamellose and I. galbana under different salinities as the relationship between slope and intercept could help reveal more information.

Section 4.4– Discussion This section needs an overhaul as it is very hard to follow the argument. I would strongly suggest add a schematic figure to demonstrate how biosynthetic pathways affect biomarker D/H fractionation. However, I don't think there were new discoveries here which deserves more than two full pages to elaborate already well known hypothesis. It is well known from previous algal culture experiments that different classes of biomarkers were characterized by substantially different D/H fractionation, in particular, among acetogetic, MVA and DOXP/MEP pathways. On the other hand, the current dataset could not provide sufficient evidence about the OPP pathway supplies a larger portion of NADPH for biosynthesis, as light intensities in the experiments were not low enough.

There are quite a few different families (species) of halophytes. Just wonder if alkenones might be biosynthesized in different organelle among different species. The authors cited (Rontani et al., 2006) to suggest that alkenones are synthesized from these shorter chain fatty acids by elongation and subsequent decarboxylation in the

chloroplast (Page 10, Lines 10-12), but then claimed “Chain elongation leading to long-chain alkenones does take place in the cytosol” (Page 14, Line 4). Previous studies did show different D/H fractionation in biomarkers biosynthesized among different organelle. Would it be possible for difference in alkenone D/H fraction among different families due to different organelle for synthesis of alkenones?

Technical corretions 1. Replace “metabolic” with “biosynthetic” as the paper only address about the biosynthesis of biomarkers. 2. Page 5, lines 8 and 12: “n-alkanes”, here “n” should be italic 3. Page 6, Line 22—-There was no Fig. 2c

---

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2019-147, 2019.