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Biogeosciences Discussions

Interactive comment on "Effects of alkalinity and salinity at low and high light intensity on hydrogen isotope fractionation of long-chain alkenones produced by *Emiliania huxleyi*" *by* Gabriella M. Weiss et al.

Anonymous Referee #1

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Weiss et al. (2017) conducted two experiments using batch cultures of the haptophyte Emiliania huxleyii (strain CCMP1516) to determine 1) how alkalinity (separate from salinity) might impact hydrogen isotope ratios in alkenones and 2) if high light conditions influence the previously reported salinity-fractionation relationship in alkenones. The results indicate that the alkenone hydrogen isotope salinity-fractionation relationship is robust (and similar to previously reported relationships) regardless of alkalinity and light level, which are useful and interesting findings. I recommend this manuscript for publication but request that the authors address the following issues: 1) more in-

formation about natural light levels, 2) separate alkalinity as the sole variable as part of the analysis, 3) improve the discussion about mechanisms (if possible include additional lipid isotope and lipid concentration data to support the lack of light effect), plus a handful of technical issues listed below.

High light growth conditions in the world's oceans: The idea that most alkenones are produced in the high light of surface waters is mentioned in several places (abstract line 5; intro p.3 line19; discussion section 4.2 line 30; conclusion line 26), but without references to support this claim. As satellite imagery clearly illustrates, major coccolithophore blooms occur in highly productive (mainly coastal) areas that are seasonally growth-limited and along the equator, and presumably bloom alkenones are produced in high light conditions near the surface (but surely also deeper in the water column where light is limited due to self-shading from bloom turbidity?). As is stands, the manuscript just states that high light alkenone production likely predominates. But to strengthen your argument and support your experimental design and data interpretation, the predominance of high light alkenone production should be illustrated with references that indicate 1) alkenones are mainly produced in high light at the surface, 2) how ocean surface water light level ranges compare to light ranges in your study, 3) how surface bloom productivity compares to non-bloom conditions/areas, and 4) that these "high light" bloom alkenones are indeed exported to the sediments (more so than "low light" alkenones). Because in vast areas of the ocean, it seems the primary source of alkenones comes from fairly deep in the water column where light levels are a fraction of surface values. For instance, this has been demonstrated at BATS see Fig 1 in Krumhardt et al. 2016 (doi:10.5194/bg-13-1163-2016), at ALOHA Table 3 Prahl et al. 2005 (doi:10.1016/j.dsr.2004.12.001) and further afield Ohkouchi et al. 1999 (DOI:Âă10.1029/1998GB900024). The vast areas of the ocean with potential deep-water/lowlight alkenone production should be addressed/acknowledged with clarification of how alkenones produced in non-saturating light conditions do or do not affect the alkenone dD paleosalinity proxy in some regions. The same references used in van der Meer (2015) (in quoted text below) are helpful, and should again be used here

– and if possible, supplemented with additional references: “E. huxleyi, for instance, is thought to thrive under high light conditions, at mixed layer depths generally <30 meter (Tyrrell and Merico, 2004; Harris et al., 2005). They outcompete other algal species that suffer from photoinhibition under these conditions, a process that is apparently absent in E. huxleyi (Nanninga and Tyrrell, 1996).”

Alkalinity: Fig 2c clearly shows that there is not a relationship between total alkalinity and fractionation (alpha). However, the discussion of the alkalinity results in the low light treatments is not entirely satisfying (p.6 lines 3-8), and it can be confusing when plots include data with many changing variables that are known to influence the isotopic composition of lipids. It would be useful (even if just in a supplement) to break up the different environmental parameters. Looking only at the low light cultures grown at salinity ∼35 (12 cultures, salinity range 34.5-35.4, alkalinity range 1.39-4.58, growth rate range 0.69-0.93, alpha range 0.795-0.806) (ie, excluding the cultures that have salinities of ∼26, ∼31, ∼37, or ∼42, nearly constant alkalinity ∼2.4, but growth rate range of 0.65-0.93, and alpha range of 0.776-0.824) . . . It is noteworthy that for these 12 cultures there is a correlation between total alkalinity and specific growth rate (= 0.05(0.01) * AT + 0.676(0.04), R2 = 0.51, p = 0.0056) in addition to a correlation between specific growth rate and fractionation (= 0.03(0.01) * + 0.776(0.01), R2 = 0.47, p = 0.0081) (figure attached as example). Although this is a very small range in growth rate (∼.7 to .9) compare to a previous study of the effect of growth rate on alkenones using a combination of chemostats and continuous cultures (Sachs and Kawka, 2015), it is striking (and probably noteworthy) a) how well they are correlated and b) that it is in the opposite sense of previous 2015 study. In the end, there is still not a significant relationship between alpha and total alkalinity – could it be that the minor growth rate effect here is overwriting any alkalinity effect? It is probable that you already considered all of this and decided it didn’t fit in the paper – but it would be useful for the interested reader to be able to reference in at least the supplement.

Mechanisms: It is welcome that some effort is put forth to explain the cellular mecha-

nisms of the salinity response at both high and low light. However, this part of the discussion seems confused and needs some work by improving the organization, offering introductory or concluding summaries to help identify your main points, supporting hypotheses with literature, perhaps a schematic outlining your favorite mechanism (which one is most likely here and why?), and the issues below. A little bit of reorganization could help since it seems like the discussion tries to deal with the high light and low light salinity response in both section 4.3 and 4.4. One way to improve this is if section 4.3 can just concentrated on salinity (regardless of light), then address why a different response at high light was expected, and then finally address why there was not a strong light effect here (but more on this below). The first paragraph of section 4.3 is very long, and the main point of it is lost in the length. Maybe at the start let the reader know how many mechanisms you will cover, then at the end summarize which one seems most likely. Can you rule any out with the results from your experiment? A lot of attention is given to metabolically reduced NADPH, such as that generated through OPP. However, there are a few things to consider. NADPH can’t cross organelle membranes – so p.8 lines 17-21 doesn’t make sense, unless the complete OPP pathway (and final steps to the alkenone synthesis pathway?) were present in this hypothetical closed compartment. Alternatively you might invoke the import of alkenone precursors (fatty acids? Pyruvate?) used to build alkenones that reflect proportional changes in photosynthetically vs metabolically reduced NADPH pools (and would have to introduce them around p.7 line18).

Lack of light effect: p.9 line3 states “we do not see a clear relationship between hydrogen isotope fractionation and light intensity.” But there is no mention of this lack of relationship in the results, where should the reader go to visualize this lack of relationship? Do you mean between the 24 low light cultures compare to the 14 high light cultures in this study? There aren’t really cultures in the high light with the same growth rate and salinity as the cultures in the low light (although 3 HL cultures at S=35.9 and 12 LL cultures at S=34.5-35.4 actually do show a (very small) significant increase in alpha at the higher light level – however, that could in theory be due to the slightly saltier HL media

or the slightly lower HL growth rate rather than the higher light). More importantly, I am not convinced that there is no light effect with only 2 light levels (75 and 600), especially considering the previously observed non-linear relationship between high light in strain RCC1238 and the fact that different strains have shown different responses (van der Meer et al. 2015). Is it possible that both the 75 and 600 light levels are above the range where a response would be detected for this strain? After all, growth rate is lower in the higher light cultures. Even though (it seems) the goal of the paper is not to characterize the light effect (but to test if the salinity effect holds at high light), this part of the discussion should consider the option that if batch cultures were grown at additional light levels, then a light effect in this strain might be apparent. p.9 line 4 – "At higher light intensities, we expect a larger pool of photosynthetically derived NADPH inside the cell" – yes of course, but another thing to consider is this doesn't necessarily mean that this NADPH is available for alkenone synthesis. At high light levels cells might be working hard to dump this extra reductive power (into alkenones? or other molecules types – is there literature on algae at different light levels you can turn to?), along with reducing light harvesting capacity, which might be a reason for the lack of light effect (if there is truly a lack of light effect). It seems like cells have many different options for dealing with high light situations. Were alkenone (and other lipid) concentrations measured? Were there any large concentration differences at different light levels indicating strategies for dumping excess NADPH? p.9 line 9 – why do you think that transhydrogenase activity is increased at high light? Can you provide a reference to demonstrate that this is a response to high light in haptophytes or even just eukaryotic (or even prokaryotic) algae? Rokitta et al. (2012) doi:10.1371/journal.pone.0052212 might be helpful here. Regarding the last paragraph of section 4.4 – is there any chance hydrogen isotopes were measured on other lipids from these cultures (fatty acids, brassicasterol, phytol. . .except probably not phytol if you didn't saponify)? This information could be extremely helpful for illuminating cellular changes in response to environmental variables, and the interplay between different pools of cellular hydrogen, as it was for Sachs et al. (2017) that found different responses to different fatty acids,

phytol, and a sterol, in a diatom grown under constant salinity, temperature, and growth rate at a range of light levels. In other words, just because there wasn't a light effect in the combined C37:2 and C37:3 values doesn't mean that the unique alkenones are not reacting to light differently, or that the hydrogen isotope ratios of other cellular lipids (and lipid precursors) are not reacting to light. In this sense, measuring hydrogen isotopes in all possible lipids is a powerful tool for helping to understand these cellular mechanisms and has tremendous value beyond validating paleoproxies. In the end – I am not sure you need to devote an entire section of the discussion to explaining the lack of light effect with only two light levels (and only one lipid measurement) presented. It would be great if this section of the paper could be extended with concentration data or additional lipid isotopes if that is available.

Technical comments: Abstract: First sentence should read "Over the last decade, hydrogen isotope ratios of long chained. . .." Line 18. It would be helpful to add "at low light" (or something along those lines) to the end of the sentence ". . .and independently assess the effects of salinity and alkalinity".

Intro: p.1 line 28: add the word "and" between UK37 and LDI p.2 line 30: SPM has not yet been defined. p.3 line 2: growth phase should include also the Wolhowe et al. (2009) reference. p.3 line 7: "created media by evaporation" . . . some culture studies did not use this method: note that salt was added to ultrapure water in Sachs et al. 2017. p. 3 line 13. How would this work? Please explain why the capacity (of environmental water) to take up H+ would, in theory, impact the hydrogen isotope composition of internal cell water and fractionation during synthesis.

Methods: How was total alkalinity determined? How was salinity determined? What was the equation to calculate growth rate based on how many cell count readings over how many days? What was the size of the growth vessel for batch cultures and how many liters of culture were maintained inside? Were growth chambers swirled to prevent sedimentation? How many grams of Al2O3 were used and how many ml of solvents for purification? What were the FID and IRMS instrument settings (column, oven

temp, reactor temp, gas flow, etc)? Were external standards used to correct/calibrate reported Isodat values referenced to H2 for each run? (you could cite a previous paper with this info if the settings were the same). p.5 line 10: “difference” (not “different”)

Results: Several findings that are brought up in the discussion are not mentioned in the results (but should be) including: constant alpha over a range of alkalinities, the weak negative correlation of growth rate and fractionation for both experiments, the lack of light effect. Why not report the uncertainty on the slope and intercept of the least squares linear regressions? If you are using R for your stats, then that is easy to get using R’s “summary(lm(x∼y))”. Although only very slightly biased in this case, it is probably more responsible to report R’s Adjusted R-squared as opposed to the Multiple R-squared. In the first 2 lines of the results the ranges are reported with different dashes (“25 – 35” and then “26-42”) p.5 line 20 – “in contrast to previous culture studies” . . . this isn’t true – salt was added to fresh water in Sachs et al. 2017.

Discussion: p.6 line 19 – incorrect reference – Sachs and Kawka 2015 did not find a correlation between growth rate and salinity since growth rate was held constant using continuous cultures – this reference would be more appropriate in the proceeding sentence since they did find a correlation between growth rate and fractionation. p.7 line 6. – missing the word “to” – after the words “could be due” p. 7 lines 15-17 this doesn’t make much sense – please rephrase – are you trying to say that cell water is the same as extracellular water isotopically and NADPH is more important? p.7 line 22 – missing important reference (Luo, Y.-H., Sternberg, L., Suda, S., Kumazawa, S., Mitsui, A., 1991. Extremely low D/H ratios of photoproduced hydrogen by cyanobacteria. Plant and Cell Physiology 32, 897–900) after “Photosynthetic production causes NADPH to be depleted by ∼600‰ in D when compared to intracellular water” p.7 line 24 – missing the letter “s” in “OPP pathway cause_” p.7 line 32 – after the statement “A similar mechanism could be present in E. huxleyi, causing the metabolically reduced NADPH pool to increase relative to other pools, and possibly become a more important source of NADPH for biosynthesis” you might qualify that with something like “if the

OPP pathway is present in the same compartment as alkenone production” (since NADPH doesn’t cross organelle membranes). p.8 line 2 – the better reference here is Luo et al. 1991 (provided above)

Figures: Please specify what the gray polygons are (95% confidence intervals?). Why no error bars displaying analytical uncertainty on alpha or dD? Fig.2c is useful for showing the (lack of) relationship between alkalinity and alpha – in this fashion, perhaps an additional figure that displays the (lack of) relationship between light and alpha could also help. In Fig.2c, does it make sense to include the regression line and confidence intervals here? – might be more useful to use a dashed line or some other visual aid to highlight how 2c is different than 2a and 2b rather than (or in addition to) leaving out the regression statistics. Fig. 1 might not be necessary, (useful to show that culture water did not have a relationship b/t salinity and dDwater but that is a big figure for a very minor point). Why are a handful of cultures around S=35 15‰ D-enriched relative to the other cultures in the Fig.1a?

References: p.10 line 12: Is there an extra “.”? Check this and other references for common mistakes (superscripts missing in some locations, middle initials incorrect in some locations, missing italics for species names in many locations, too many words in title are capitalized in at least one reference).

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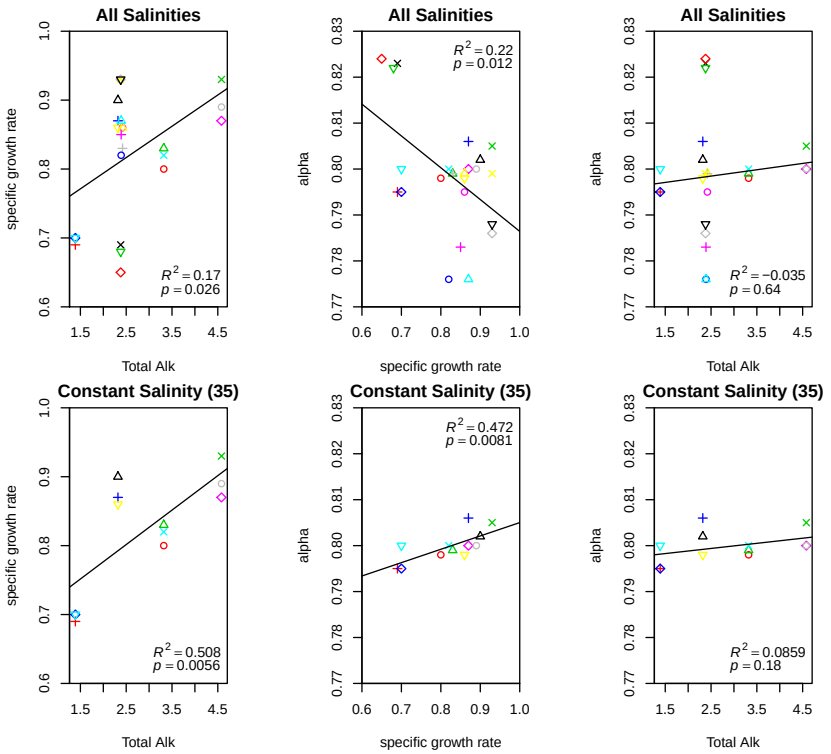
All Salinities
specific growth rate
Total Alk
$R^2 = 0.17$
$p = 0.026$
All Salinities
alpha
specific growth rate
$R^2 = 0.22$
$p = 0.012$
All Salinities
alpha
Total Alk
$R^2 = -0.035$
$p = 0.64$
Constant Salinity (35)
specific growth rate
Total Alk
$R^2 = 0.508$
$p = 0.0056$
Constant Salinity (35)
alpha
specific growth rate
$R^2 = 0.472$
$p = 0.0081$
Constant Salinity (35)
alpha
Total Alk
$R^2 = 0.0859$
$p = 0.18$

Fig. 1.