Author Response to Reviewer Comments

Growth phase dependent hydrogen isotopic fractionation in alkenone-producing haptophytes

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Response to Reviewer 2

Reviewer 2 makes several excellent points illustrating how our work fits into the body of existing literature on paleosalinity reconstruction and algal physiology. The reviewer's primary concerns center around A) a perceived overlap in our overview of δD_{K37} paleosalinity applications with earlier work and B) and an "overselling" of the potential use of alkenone isotopic data as an indicator of nutrient stress. We believe that our introductory discussion is not redundant with the points made in previous papers, though the statements made about our advocacy of a paleo-physiological application are well-taken. I will address the reviewer's concerns one at a time below.

1) *The argument that Rohling (2007) already describes the uncertainties inherent in δD_{K37} paleosalinity methods, and that our discussion is thus redundant:*

   The discussion in Rohling (2007) is a very useful illustration of the limitations that are imposed on paleosalinity reconstructions by variation in marine $\delta D/\delta^{18}O$ and $\delta^{18}O/S$ relationships, and clear mention of it will be made in the revised manuscript. The biological sources of variability, however, which are the focal point of our discussion, are limited in Rohling's discussion to the parameterization of α as a function of salinity and are only addressed briefly.

   It is true that Rohling's approach is superior to the 'classical' $\delta D_{lipid} \rightarrow$ assumed α value $\rightarrow \delta D_{water} \rightarrow$ salinity approach that we describe in the introduction, but this strategy is still discussed in the literature, both contemporary to and following the publication of Rohling's work (Pahnke et al., 2007, Paleoceanography; Sachse and Sachs, 2008, GCA; van der Meer, 2008, EPSL). Furthermore, Rohling's method is still sensitive to changing physiological influence (the parameter 'C' in that work) – his discussion only addresses the effects of salinity described by Schouten et al. (2006), and thus would still be informed by our results. Additionally, Rohling's method provides relative salinity changes, not the absolute values that people do, and will continue to, strive to reconstruct.

2) *The argument that van der Meer et al. (2008) illustrate how to constrain haptophyte growth rate, and thus that our discussion of the uncertainties that Schouten et al's growth rate experiments imply for paleosalinity interpretations are unnecessary:*

   van der Meer et al. do not truly constrain growth rate in their Black Sea work. Their approach is to calculate a modern growth rate based on observations of alkenone δD, salinity, and the application of Schouten et al.'s $\alpha(\mu,S)$ functionality. This functional

relationship, furthermore, is shown by Zhang and Sachs (2009) to be less simple than first suggested. van der Meer et al. then apply this value throughout the core. They also state, correctly given the current consensus in the literature, that calculation of exponential growth rates by $\delta^{13}C_{alkenone}$ is impossible without extensive constraints on water chemistry.

The reviewer also implies that we are attempting to use $\delta D_{alkenone}$ as a way to constrain growth rate. This is not the case. We are comparing exponentially dividing cells to cells that are nutrient depleted (not rate limited) and have ceased division. This comparison is aimed to illuminate the physiological stress seen by cells at the termination of blooms relative to those experiencing balanced growth, and this is also the kind of distinction that we suggest could be made in the sediment record. As is the case in our response to Reviewer 1 (regarding the comparison of our data to that of Zhang and Sachs, 2009), this point is not merely semantic, but relates to the differences inherent in batch culture and chemostat experiments.

3) *The statement that the results of Zhang and Sachs (2009) warrant discussion:*

We agree. See the discussion to this effect in the Response to Reviewer 1. Discussion of this work will be made in the revised manuscript.

4) *The statement that the 'sales pitch' for a physiological application is excessive and that the data should stand on its own without the accompanying discussion of implications:*

This is a valid concern, voiced by both reviewers, and the language surrounding the potential paleo-physiological application has been toned down and reduced in length, accordingly. We would prefer not to remove it entirely, however, because the key message of our manuscript is that the physiological levers on $\delta D_{alkenone}$ are themselves a useful (and potentially the largest) signal, not simply noise in a hypothetical hydrologic proxy. We feel that some discussion of what the trends we observe might mean for the field is warranted, rather than just a simple presentation of numbers.

5) *The concern over the language used to describe values determined based on the assumed isotopic value of the G. oceanica culture medium:*

This is an excellent point, and the language in the revised manuscript will be changed accordingly.

6) *The argument that our low-recovery samples should be discarded:*

The limitations of our 'calculated' values for the log-phase unsaturation-specific isolates will be more explicitly stated in the revised manuscript. We feel, however, that the mathematical argument we have presented is fairly straightforward and by no means founded on circular reasoning – if isotopic mass balance is maintained, knowledge of δD_{K37s} and $\delta D_{K37:2}$ necessarily defines $\delta D_{K37:3}$. While potentially

unconvincing on its own due to the reliance on accurate $\delta D_{K37:2}$ values, we feel that the reconstruction is lent weight by the agreement of our final derived $\alpha_{K37:3\text{-}K37:2}$ values with those from other literature (Schwab and Sachs, 2009) that include field samples whose origin clearly lie in a disparate species under disparate conditions. Given time and funding limitations, we feel that our existing data should be presented, warts and all, as the reader may always exercise his or her discretion with regards to accepting our mathematical interpretation. We agree that there would be merit in re-running the experiments, but we also feel that the exercise would not fundamentally alter our conclusions that the existing data is meaningful and should not go to waste.

7) *The concern over our comparison of the trends shown by the G. oceanica and E. huxleyi samples (sec 4.2 lines 16-26).*

A re-examination of the data from the UBC *E. huxleyi* cultures has allowed for a slight re-working of this section since the time of submission. The resultant discussion should now make much more sense. Note also that we do not "conclude" anything here, we simply state that the data suggests a trend, and that more work is clearly called for to quantify the trend as it may have significant value for refined $U_{37}^{K'}$ proxy development.

8) *The concern over Sec. 4.2 in general, centering around the practical concerns in applying $\delta D_{alkenone}$ as an indicator of stress without knowledge of water isotopic composition and the potential overlap of this proposed technique with $\delta^{13}C_{alkenone}$ growth-rate estimates:*

As stated above in response #2, there is a significant distinction to be made in this case between growth rate and growth phase. The real-world analog of our batch-culture experiments would be locations where export of alkenones was dominated by blooms that would generate large sinking fluxes after pronounced starvation of the constituent haptophytes. This conceptualization will be made much more explicitly clear in the revised manuscript.

Our data indicate that the isotopic shifts that these conditions could impart would be large, in open ocean settings, relative to the variability in δD_{water}. This is stated explicitly in the manuscript. Thus, we believe the proposed technique could be used fairly simply in a qualitative sense. However, the reviewer is right in stating that knowing the isotopic composition of the water is necessary for a quantitative assessment, as we propose. It should be possible to use the initial $U_{37}^{K'}$ temperature estimate and an initial estimate of water composition (say from $\delta^{18}O_{calcite}$) to arrive at a final value for a "stress corrected SST". The effects of temperature proxy biasing of the scale we are discussing (~1°C) on values of δD_{water} reconstructed from $\delta^{18}O_{calcite}$ are small (~3‰) relative to the changes we see between growth phases (~20‰). Thus, an approximation based on the initial SST estimate may be all that is required. For more exact calculation, it may be possible to use an iterative approach, but,

without a robust functional form for the relationship between $\alpha_{K37\text{-}water}$, T, and $U_{37}^{K'}$, we felt that including a discussion of this in the manuscript was overly speculative and premature.

Again, we simply want to state that this is the sort of analysis that may be possible, given further work to quantify A) the form of the $\alpha_{K37\text{-}water}$, T, and $U_{37}^{K'}$ relationship and B) the similarity/dissimilarity of the relationship between different alkenone producers. The impression that this section made on the reviewer is taken to heart, however, and, given than we do not have the space or data to make a full discussion of this sort, the language will be pared down and made into more of a forward-looking 'suggestion' and less of a 'statement'.

Concerning the determination of growth rate via $\delta^{13}C_{alkenone}$, while growth rate can provide information about nutrient stress, as stated above in response #2, this measure is not redundant with our proposed use of $\delta D_{alkenone}$, and $U_{37}^{K'}$ does not respond in the same way to changes in growth rate and growth phase. Furthermore, van der Meer (2008) correctly states that $\delta^{13}C_{alkenone}$ is not an effective way to constrain growth rate down core, given the lack of necessary information about water chemistry.

9) *Displeasure with use of italics and exclamation points:*

A point well made; the offending emphases will be removed in the revision.

10) *Confusion over the reference to Schouten et al. (2006) on line 5 of page 5:*

The reviewer is correct; the language will be revised to make this more clear.

11) *Concern over the number of significant figures on reported isotopic values for water:*

Changes to the use of error statistics throughout the paper (see response to Reviewer 1) should correct this issue.