Author Response to Reviewer Comments

Growth phase dependent hydrogen isotopic fractionation in alkenone-producing haptophytes

M. D. Wolhowe, F. G. Prahl, I. Probert, and M. Maldonado

Response to Reviewer 1

Reviewer 1 makes several useful comments about suggested discussion that would lend further context to our manuscript and correctly points out some ambiguities in our presentation of error statistics. The reviewer's primary points of concern are: 1) the lack of comparison of our data, specifically the differences observed in lipid δD with nutrient stress, with the results of Zhang and Sachs (2009) and 2) an insufficient discussion of Schwab and Sachs (2009) with respect to our concerns about chromatographic recovery.

As for the first point, the reviewer is correct that Zhang and Sachs (2009) should indeed be referenced and discussed – I was made aware of this paper on the day I submitted our manuscript to BGD and have been looking forward to the opportunity to include a discussion of it after review. The temperature-dependant behavior that Zhang and Sachs observe in C_{16} fatty acids echoes the trend observed in our alkenone data, and this fact will be clearly noted in our revision. The more interesting comparison, however, is the apparent disagreement between our work and theirs over the direction of fractionation change upon exposure to 'nutrient stress'. Our data show an increase in fractionation when haptophyte cells cease division due to a lack of nutrients. A change of some sort in hydrogen isotopic composition with growth status is to be expected, due to the changes in the routing of D-depleted $NADPH^+$. As an example, consider the data of Chikaraishi et al. (2004, 2009), who showed isoprenoid synthetic products in a higher plant, such as phytol and sterols, to be significantly more D-depleted than acetogenic lipids. In a simplified model where $NADPH^+$ formation remains constant when division stops, but the resulting reducing power is shunted solely into acetogenic lipids (e.g. alkenones) rather than isoprenoid structural or metabolic components (e.g. membrane lipids, phytol), mass balance demands that the resulting acetogenic lipids be more depleted. Zhang and Sachs (2009) see this kind of chemical restructuring in their chemostat experiment, but, rather than the predicted isotopic depletion of the fatty acids, they observed approximately no change or a slight enrichment.

While this seems contradictory to our data, consider the hypothetical effect of a decrease or cessation of cell division on the pool of intracellular water. Zhang and Sachs (2009) cite Schmidt et al. (2003) to demonstrate that the intracellular water pool is D-enriched in photoautotrophs due to $NADPH^+$ synthesis. While increased division rates are commonly associated (in the literature) with an increased metabolic impact on the intracellular water pool, light is still striking the photosystems of cells in a stationary growth phase imposed by nutrient depletion. Depending on the organism and the suite of photoprotective mechanisms it employs, continued 'photosynthesis' may affect $NADPH^+$

production to a greater or lesser degree. If lipid synthesis serves as a primary energy shunt, as has been proposed for alkenone producers (Eltgroth et al., 2005), rather than the down-regulation of photosystem capacity, $NADPH^+$ production would still occur at a significant rate. If the net incorporation of extracellular water to form new cytoplasm were stopped, one would then expect the intracellular water to become progressively more D-enriched as lipid synthesis continued. Thus, one could imagine two potential processes related to growth status that would wield opposing effects on the δD of acetogenic lipids – a decrease in the synthesis of D-depleted isoprenoid products, which, as discussed above, could drive the acetogenic lipids to more negative δD values, and increased isolation of the intracellular water pool, which would drive them to more positive values. The fact, then, that Zhang and Sachs (2009) were observing A) different species of phytoplankton and B) the isotopic difference between cells grown under chemostat conditions with two different exponential growth rates, controlled by different rate-limiters, as opposed to exponential and fully stationary nutrient-depleted growth as were studied by means of our batch culture approach, means that their findings are not necessarily inconsistent with our own. A streamlined discussion to this effect will be added to our revised manuscript. These hypotheses are somewhat tangential to the main point of our manuscript, however, which is that the physiological 'complications' of a potential hydrologic proxy (specifically a growth-phase effect on the net hydrogen isotopic fractionation exhibited by C_{37} alkenones) may have their own paleoceanographic utility.

As for discussion of Schwab and Sachs' (2009) data on isotopic chromatographic effects, references are already made at several points in the paper. However, a more explicit statement regarding their quality assurance recommendations and our low recovery samples will be added to the revision.

With regards to the rest of the comments, for convenience I will respond to them following the reviewer's numbered list.

1) *The δD values of individual alkenones as a potential proxy for stress in paleoceanographic studies should be better justified…*

   A discussion of the range of variability that changing δD_{water} values could impart on $\delta D_{alkenone}$ is already made in the introduction (Sec. 1.1), and in Sec 4.3 it is stated that these variations are small relative to the shift that nutrient depletion appears to impose. The key point we are attempting to make in this section is that if nutrient depletion is a particularly large lever on $\delta D_{alkenone}$ values, then the measure's utility as a stress proxy is strongly suggested. Further analytical work is clearly warranted to investigate this potential possibility for $U_{37}^{K'}$ proxy development. The language 'selling' the idea of the stress proxy has not been eliminated, but has been toned down appropriately.

   We do not, as the reviewer states, suggest that the sedimentary record is derived from exponentially dividing cells. In fact, we state the opposite, and refer to molecular (Prahl et al., 2006; Conte et al., 1995) and, potentially, isotopic (Englebrecht and

Sachs, 2005) evidence in support this belief. We believe that the bloom / starve / die / sink dynamic of many natural marine settings makes batch cultures, rather than chemostats, a particularly useful experimental analogy in this regard.

2) *The authors did not clearly address the Uk'37 difference of 0.15 between their experiment and Prahl et al (1988)…*

Unfortunately, no explanation is forthcoming. We have consistently, however, seen lower than expected $U_{37}^{K'}$ values for a given growth temperature with this culture stock under all conditions. Given space considerations, we wanted to avoid discussion of data outside the scope of the experiments under consideration. If desired, however, we could either add more language to this effect, or remove the discussion entirely to avoid confusion. However, we believe this phenomenon has not been deleterious to our primary use of the data, the demonstration of the growth-phase effect.

3) *The authors should justify the error produced by their mass-balance calculation of δD values of individual alkenones…*

These sources of error are included in the mass balance calculations in Sec. 4.1 and 4.2. All uncertainties in these calculated quantities are determined following the full form of the standard error propagation formula:

$$\Delta f = \sqrt{\left(\Delta a \frac{\partial f}{\partial a}\right)^2 + \left(\Delta b \frac{\partial f}{\partial b}\right)^2 + ... \left(\Delta n \frac{\partial f}{\partial n}\right)^2}$$

where *a*, *b*…*n* represent all variables used in the calculation of the term *f*, in this case including $U_{37}^{K'}$, $\alpha_{K37:3\text{-}K37:2}$, and δD_{K37s}.

4) *The authors claimed that the measured enriched D/H ratios of K37:3 is an analytical artifact of the argentation chromatography…*

There was nothing systematic in the experimental execution – two samples came out poorly, and, unfortunately, they both happened to belong to the same experimental group.

5) *The authors stated (with respect to G. oceanica culture water)…*

The range that is given, within which δD_{water} values for these cultures would not shift the calculated fractionation factors outside a window of ±0.005, is +2 to -8, not +2 to -18 as stated in the review. Furthermore, it is the trend in the data that is important – our point in the discussion is to demonstrate the effect of nutrient depletion on hydrogen isotopic fractionation, and to emphasize that further work to 'flesh out' the exact quantification across a range of conditions is justified.

6) *The authors state "the growth-phase effect on αk37-water is not haptophyte species-specific"…*

This is why the term "similar" is used rather than "statistically identical". Again, it is the presence and direction of this effect that are being described, and further work to fully quantify the relative response of the two species is called for in the discussion given the potential significance of this prospect for refined $U_{37}^{K'}$ proxy development..

7) *The authors should cite one or more studies on pg. 5…*

Citations are already made to this effect.

8) *Apparent calculation errors:*

A: Good catch. This has been corrected.

B: Actually, both the initial calculations and the reviewer's version are incorrect. Full propagation of error gives $\partial S/\partial(\delta D_{K37}) = 0.316$. Uncertainties in δD_{K37} of 3 and 5‰, then, give uncertainties in S of 1.0 to 1.6. This information will be changed accordingly in the revised manuscript. This level of uncertainty is still far superior to a realistic calculation of paleosalinity, as it assumes perfect meteoric water line and "ocean main sequence" relationships. Rohling (2007, Paleoceanography), in a presentation complimentary to out own, provides a useful discussion of the uncertainties inherent in these relationships.

C: Error statistics have now been more clearly discussed. Standard errors of the mean are now used on individual δD values, and the more-conservative mean standard deviation of the standards for each "type" of measurement over the course of the sample runs is used in the propagation-of-error determinations for calculated fractionation factors. Incidentally, a re-calculation of the data with more up to date values for laboratory working standards has improved the assessed measurement uncertainties to a small degree.

D: Again, good catch. This has been corrected.

E: These values have all been adjusted following the recalculation described above, and should be internally consistent when the revision is completed.