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## ***Interactive comment on “Growth phase dependent hydrogen isotopic fractionation in alkenone-producing haptophytes” by M. D. Wolhowe et al.***

### **Anonymous Referee #2**

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This manuscript describes cultivation experiments of two haptophyte algae whereby the hydrogen isotopic fractionation patterns of alkenones are measured. The authors find differences in hydrogen isotopic composition of alkenones for haptophytes cultivated under different growth conditions. The authors conclude that this phenomenon may be used to constrain the effects of growth rate on the UK37 paleothermometer. Compound specific hydrogen isotopes is a rapidly expanding field and recently much attention has been paid to the delta D of alkenones as a potential tool to trace delta D of water and/or salinity. Cultivation experiments such as those performed here are very useful in gaining some idea of the fundamental controls on the hydrogen isotopic composition of alkenones in the natural environment. Hence, the data provide a valu-

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able contribution and thus they should be published. Unfortunately, the study was not perfect, i.e. the  $\delta D$  of the *G. oceanica* medium could not be determined and the recovery of the C37:3 was sometimes too low to allow  $\alpha$ C37:3-C37:2 calculations. I also have some comments on the way the data are presented and how the manuscript is generally written. Basically the observations are reasonably simple and straightforward and thus the manuscript could be written short, sweet and concise. Instead it is sometimes long and excessive and sometimes addresses issues not always relevant for the study. The authors are extremely focused on selling the  $\delta D$  as a tool to correct growth rate-induced changes in the UK37. I think there is no need make this sale pitch as the data are interesting on their own and this study does not make clear to me how  $\delta D$  could be used in this way without running into several problems (see below). Rather the authors should discuss their finding with regard to those reported by others, what potential causes are for the observed differences.

**Introduction:** The introduction is excessive and provides not an overview of the state of the art but rather a discussion on why the  $\delta D$  of alkenones should be sensitive to growth rate and is not suitable for  $\delta D$  of water and/or salinity. A part of the discussion presented here was already presented by Rohling (2007, *Paleoceanography*, doi:10.1029/2007PA001437) in which the uncertainties in salinity estimates using  $\delta D$  were quantified. In addition, van der Meer et al. (2008, *EPSL*) provide a discussion on how to constrain the effects of growth rate on  $\delta D$  by measuring the  $\delta^{13}C$  of alkenones, which is also very sensitive to growth rate. They also do not discuss the recent findings of Zhang et al. (2009, *Org. Geochem.*) on the effects of temperature and growth rate on the hydrogen isotopic fractionation of algal lipids. I suggest that the authors revise the introduction to give a brief overview on the state of the art of  $\delta D$  of algal lipids, culture results, preliminary applications and the need to know more about the fundamental controls.

**Methods:** It is a great pity of course that the  $\delta D$  for the medium in which *G. oceanica* is not known. I agree that this uncertainty is unlikely to change the main picture of

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overall fractionation but nevertheless the alfa is estimated and simply not known. I feel this should be made clear throughout the manuscript, figures and tables, i.e. stating “estimated alfa values” rather than “alfa values”. Otherwise the reader might assume that the alfa value has been accurately determined.

Discussion: 4.1 Another problem with this study arises in this section. The authors nicely separate the two isomers and seem to find an effect of increasing difference between C37:2 and C37:3. However, the authors discuss, in a quite complicated fashion, that this may not be true because the recovery is low for the C37:3. The fact that recovery is low should already be enough the discard this data and the simple fact that the mass balance does not fit. Hence they do not have accurately measured data to determine if the difference between C37:2 and C37:3 is changing with growth rate. Rather, they infer using several assumptions that it is unlikely that growth rate has an effect. In addition, these alfa’s are significantly different from those reported in the literature. I find this all in all not a strong basis to extrapolate their finding and simply state (line 5, p. 4185) that “the alfaC37:3-C37:2 remains constant”. Nevertheless, despite the weak support this conclusion forms the basis for further discussions in sections 4.2 and 4.3. This important finding, and core result for their further discussion, would benefit from repeat experiments in which recovery of the C37:3 was sufficiently high to allow accurate measurements.

4.2 Lines: 16-29. Very speculative with no evidence. You have basically just a few points so how can you conclude this ? Line 15, p. 4187: I am confused. Not only nutrient stress but also temperature is an important factor ? This is not obvious from the rest of the paper where a great emphasis is put on growth phase. Your data seems to differ not only from Schouten et al. (2006) but also from Zhang and Sachs (2009). Please discuss this and how this would implicate the generality of your finding. Could the different culture conditions (chemostat vs batch cultures) be one reason for the different findings ?

4.3 The premise for using delta D for constraining growth rate effects on UK37 is clear

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but I have no idea how this should work in practice. Suppose I measure a delta D of -260 per mill for a C37:2 alkenone in an open ocean setting. How in practical terms could I know then if the UK37 was affected by growth rate and how could I constrain this effect ? How would I know alfaC37:2-water and would this be accurately enough to estimate the impact of the UK37 ? I am afraid you would run in the same problems as the authors have described in the introduction for salinity or delta D water estimates ,i.e. one assumes some consistent relationship between alfa and growth phase, a single source (E. huxleyi differs strongly from G. oceanica in alfa), consistent UK37 changes with growth phase (not always observed here), etc.

I also wonder why the authors did not discuss an alternative, as partly discussed in van der Meer et al. (2008), i.e. using the del 13C to constrain growth rate effects on the delta D (or UK37) ? Like delta D it has also been shown that 13C of alkenones is quite sensitive to growth rate affects in N-limited chemostats (eg Popp et al.,1998, GCA). What is the benefit of using delta D over using del 13C ? Could a combination be used ?

Minor comments:

General: The authors excessively use italics for certain words in order to get their points across and even use exclamation marks. This gave me the impression that I was being lectured on hydrogen isotopes rather than reading on some nice experimental work. Please rewrite these parts .

Line 5, p. 4169: I presume you want to say here:”Based on Schouten et al. (2006), the range in alfa that is associated. . .”. Schouten et al did not quantify the natural variation in alfa for a range of growth rates.

Heading 4.1; what are ‘unsaturation-specific results’ ? I suggest to rephrase the heading Lines 1, p. 4186:. What is a ‘real biosynthetic change’ ? A change in UK37 is also a ‘real’ biosynthetic change in rates of production.

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Table 1.: The reported values of water has too many significant numbers.

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