

***Interactive comment on “The effect of temperature and salinity on the stable hydrogen isotopic composition of long chain alkenones produced by *Emiliana huxleyi* and *Gephyrocapsa oceanica*” by S. Schouten et al.***

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General Comments. The main point of this manuscript is to present new data showing that the hydrogen-isotopic fractionation between environmental water and alkenone lipids varies strongly as a function of salinity. This is highly significant, because alkenones can be isolated from old sedimentary rocks and might provide us with a new, rather sensitive paleosalinity proxy. While it would be even nicer to understand why (in biochemical terms) the fractionation varies with salinity, this is nevertheless an important first step that points the way to some very interesting future research. These are good experiments with high-quality data, and they definitely deserve to be

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published.

Specific Comments. 1. If D/H measurements of alkenones turn out to be useful, inevitably someone will repeat your culture experiments and get slightly different results. In that case, it will be very helpful to have described the culture conditions in as much detail as possible. For example, how large were the culture vessels? Were the cultures harvested during exponential or stationary phase of growth, and what were the specific growth rates (did growth rates vary as a function of salinity)? Were they bubbled with air or CO<sub>2</sub> and at what rate? Were they continuously shaken? 24 hours of light? How were they inoculated? Most of these details could probably go into some kind of online supplementary materials.

2. Your discussion of the data in terms of the slope and intercept of a  $dD(\text{lipid})$  vs  $dD(\text{water})$  plot was a little confusing to read (note: its correct, and I eventually understood it, it was just initially confusing). The math in the Sessions and Hayes (2005) analysis is only valid if there is a single, constant fractionation factor. Your discussion might be clearer if you simply said “The fractionation between lipid and water changes as a function of salinity, therefore the method of analysis described by Sessions does not apply”. In this case, the slope of the regression (ie in Figure 1b) has an entirely different meaning, and cannot be related to  $\alpha$ .

3. One fairly obvious possibility is that salinity affects the growth rate of the algae, and the fractionation is dependent on growth rate. I assume that you looked for this kind of relationship, and found none? If so, it would be worth stating this explicitly, and perhaps even add the growth-rate data to Table 1 so that we can decide for ourselves. If not, then I think it would be really important to try to test the relationship to growth rate experimentally, so that you can be sure you are really looking at a specific salinity effect and not just a general growth-rate effect. Obviously, the latter would not be nearly as useful as a paleosalinity proxy.

4. Your discussion about mechanisms for the salinity dependence of fractionation

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seems to imply that the effect must lie somewhere in the biosynthetic pathway for alkenones. There are two other possibilities that I think are worth mentioning. First, salinity could affect the dD value of acetate used as a biosynthetic precursor, in some unknown way (I doubt this is true, but it is at least possible). Second the dD value of water inside cells (or chloroplasts) might change as a function of salinity. There is a very nice recent paper in PNAS (Kreuzer-Martin et al, 2005, vol 102, no 48, pp17337-17341) showing that up to 70% of the water in E coli cells during exponential growth derives from respiration, rather than their culture medium. Apparently cells are not as rapidly flushed with water as we had all imagined, and it is quite possible that the large isotopic fractionation associated with photosynthesis could lead to cellular (or chloroplast) water that is D-enriched relative to the medium. Changing the salinity of culture water should have a big affect on osmoregulation and the residence time of water in a cell, and so might exert some control on the D/H ratio of alkenones in that way.

Technical Corrections Page 1682, line 22 - probably more correct to write “NADP+” rather than “NADP”. On this same line, why not just reference Yakir and DeNiro (1990), which is where that -171 permil value comes from.

Page 1683, line 29 - the correct author is “Sachse”, not “Saches”

Page 1684, line 9 - the correct first author name is “Englebrecht” (e instead of a); this needs to be corrected throughout the manuscript.

Page 1685, line 17 - the abbreviations ‘EA’ and ‘TC’ need to be explained.

Page 1687, line 6 and elsewhere - “intercept” not “intersect”. This needs to be corrected throughout the manuscript.

Page 1688, line 9 - “decrease in isotopic fractionation” is a little bit ambiguous in this context, because its unclear whether you mean the value of alpha is decreasing (which would be a bigger fractionation) or you mean the magnitude of the fractionation is decreasing (alpha getting bigger).

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Page 1689, line 5 - “species specific factor” is a little bit awkward. I suggest “factor that differs between species”, or something similar.

Page 1689, line 20-21 - I think you mean “a change of 1 psu in salinity”, not 1 per mill.

Page 1693, Table 1. There is a minus sign missing in column 5. Could you add the specific growth rate of each culture to the table?

Page 1694, Figures 1 and 2. These are so small that they are very hard to read (note that they probably get compressed somewhat when printed on American 8.5 x 11 inch paper). Please make them considerably bigger.

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