Review Ladd, Dubois & Schubert: Interplay of temperature, productivity [...] lipid biomarkers

The authors measured the hydrogen isotopic composition (d2H) of lipid biomarkers, in particular short-chain fatty acids (C14-C18) and brassicasterol, from particulate organic matter filtered on a monthly basis from the surface water of two Swiss lakes, over the course of the algal 'growing season' of 2015. They combined these measurements with estimates of productivity using 13C labeling and data of community assemblage, and other environmental data like temperature and trophic status of the two lakes - one eutrophic and the other oligotrophic. This study gives useful insights in the hydrogen isotopic fractionation during biosynthesis of these lipids through time, the factors that influence this fractionation. The study is a welcome expansion of similar efforts performed on algal cultures, and aids in the assessment under what conditions biomarker d2H can potentially be used as a sedimentary proxy for past hydrological changes, and/or what the limitations are. The study is set up and executed properly, and the paper is well written. I have, however, some remarks, comments and questions I like the authors to address.

* Page4 Line26. I need to assume the carboys were made of clear plastic to allow photosynthesis?
* P8L15 increased from April to July? Or levels were low from April to July? Write more clearly.
* P9L24-25 "When analyzing.. Table 2)". Unclear sentence, rewrite.
* P10L31-34 "The slope.... " Not clear, rewrite.
* P11L30-33. This part appears out of place and fits better within the next section
* P12. Section 4.1.3. Lipid production rate. - I suggest to rename this section to 'trophic conditions' or 'nutrient availability', which is a primary environmental factor similar to temperature and light - with all three bearing on productivity and related fractionation. At the moment the discussion appears a bit mixed, nutrient availability and growth rate are somewhat used interchangeably.
* About the source of the fatty acids: The authors appear to only consider algae, or at any case aquatic organisms, as their source. However, fatty acids may also come from terrigenous sources, and this potential source may change over time. For example, surface runoff during early spring may bring in relatively large amounts of terrestrial organic matter at a time that lake primary productivity is still low.
* The non-existent correlation between fractionation factor and growth rate is likely due to the surprising low growth observed at day 220 at lake Greifen (why so much lower than at day 180, do the authors have an explanation?), and with just 5 measurements over the entire period this is bound to give bad statistics. I therefore wonder if there is no other information available about algal productivity, possibly the data from the long-term monitoring program at EAWAG could be used? Have the authors considered a more simple method of estimating productivity like chlorophyll concentration? How dependable and reproducible is the labeling-incubation method? What if the productivity data point at day 220 (and even the concentration of FA) from lake Greifen is compromised - would a higher rate at day 220 suddenly result in a good correlation?
* To what extent do turnover time and export of dead organic matter (or lack thereof) may have an influence on the bulk hydrogen isotopic compositions measured? How much algal biomass is taken up by heterotrophs and recycled, thereby partially

keeping the original isotopic signature? How much particulate OM is alive? In other words, how much 'memory' does the system have over the season leading to attenuation of the isotopic signal? If there is such attenuation, then the instantaneous productivity at a given point in time, especially at a later stage when it is going down, may be ever more unrelated to the accumulated particulate OM and lipid stock. Note that lake temperature has a large inertia thus will automatically correlate well with any other parameter with a slow response time.

* On page 14, the authors argue against a large contribution of heterotrophic bacteria based on low abundances of iso- and anteiso fatty acids. However, a large amount of heterotrophic biomass might be planktonic and not bacterial, while also not all heterotrophic bacteria will produce exactly those biomarkers - the majority will still predominantly produce C16:0 FA. It is not clear from the text to what extent the presented algal community data reflects only phototrophic algae (it is presented as such), or if these data are more inclusive to all microbial life (in which case heterotrophic plankton is surprisingly absent).
* P13L32. I would be very careful assuming that all heterotrophs have more enriched fatty acids than phototrophs based on only one study.
* P15L11-15. It is very well possible, or even likely, that the different lakes (with quite different trophic status) host different diatom species (or even non-diatoms, who knows..) making brassicasterol. Zhang et al has shown that different species making the same lipid may fractionate quite differently. This may also explain the large difference in fractionation of brassicasterol in the two different lakes.
* It would be useful to plot temperature through the season - not one based on five own measurements, but those from EAWAG or a similar service.