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Interactive comment on "Seasonal changes in the D/H ratio of fatty acids of pelagic microorganisms in the coastal North Sea" *by* Sandra Mariam Heinzelmann et al.

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The purpose of this manuscript is to demonstrate a seasonal shift in the δD values of marine fatty acids; such a pattern has not previously been reported. This manuscript is concise and well-written, and was a pleasure to read; the relative brevity belies a significant amount of effort that must have gone into the analysis of samples. The data all appear to be of high quality.

The main conclusion is that the δD value of average fatty acids gets more negative during the spring bloom; authors attribute this pattern to an increase in autotrophic contributions to particulate biomass during the spring, and greater heterotrophic input later in the summer. The attribution of this pattern to changing auto/heterotrophic inputs

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is very interesting, and if it proves to be correct would be an exciting demonstration of the utility of lipid δD values as ecological tracers. So I think the work is definitely significant. However, I don't think the case is as firmly settled as the current manuscript makes it appear. The main issue I have is that all of the fatty acids – including 20:5, which is a putative algal marker – show the same temporal pattern of depletion then enrichment over the year. Plus the use of 3um filters should preferentially collect algae rather than bacteria. So it seems entirely possible that the observed pattern is due (mainly or entirely) to changing fractionations by phytoplankton, rather than changing relative inputs from bacteria and algae. Perhaps the authors can think of some way to rule this hypothesis out; if not, I think it has to be presented as an alternative hypothesis that could explain the data.

Other minor comments:

L56-57; its the hydrogen supplied by NADPH (not the whole molecule) that matters, and its the reduction of NADP+ to NADPH that matters, not its biosynthesis. I'm sure the authors know this, just be precise in the wording.

L91: 'comprised of' rather than 'formed by'

L137-138: I agree that the 3um filters can trap some bacteria as they get loaded, but still your sample will be strongly biased towards algae rather than bacteria. You might want to point this out, because the seasonal signal you observed could be even larger in a sample that more effectively traps bacterial biomass. Collecting bigger samples on smaller pore-size filters would also allow to test your assumption that bacterial lipids are D-enriched.

L147. Boiling point of methanol is around 65C, so how did you reflux something at 140C in methanol? Would have to be under pressure...?

L183: I believe the internal standard (squalane) is allowing you to test accuracy, rather than precision. Precision is simply the reproducibility of unknown values.

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L261-275. This is the one place where I thought the manuscript could be a bit shorter. The patterns of changing abundance are shown clearly in the figure, and not every detail needs to be recounted here in the text. Just point out the most important trends.

L309-311. I believe a similar trend (shortest-chain FA are most D-depleted) has been observed in many pure cultures and single (macro)organisms as well. Here it would be worth comparing the trend you see to some previously published results. Later, when you try to attribute C18:x to greater heterotrophic input, I would be cautious: the fact that it is D-enriched relative to 14:0 might be true even in algae, and would not necessarily require heterotrophic input. You could also point out that a similar pattern (increasing δD with chain length, except for PUFA's) has been observed in other environmental samples, like Maggie's Yellowstone paper and Ashley Jones' Santa Barbara paper.

L347. I agree this is theoretically possible, but the timing is weird, because its in the summer (not fall). Why would plankton switch to more storage products in the middle of summer, when they have the most sunlight. Even if they are nutrient-limited they can still make sugars...

L354-355 This is where I'd be cautious; similar pattern seen in many individual organisms, I think.

L375. The statement that "C18:0 will be mainly derived from heterotrophic bacteria" is not supported by table S6, which just lists the relative abundance of fatty acids. If algal inputs are 3 orders of magnitude greater than bacteria, then the bulk C18:0 will still be mainly from the algae, even though bacteria have a higher relative abundance. Note that I am not saying it is not possible for C18 to be mainly bacterial, just that the data presented does not prove that point.

L377-378. I am currently working on a manuscript with Dave Valentine that compiles published fractionations for a variety of chemoautotrophs, and shows that they are not statistically significantly different from photoautotrophs. The first few organisms that

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were studied, that yielded such huge depletions, turn out to be anomalous. So I would probably just delete this sentence.

L380: You are comparing epsilon values (from current study) to the δD values from Sandra's earlier culture work. The epsilon values from her study for chemoautotrophs are -217 to -275‰ (numbers taken from the abstract).

L393. I think you mean Figure 1 here? Fig 5 does not show data for the summed fatty acids.

Nice job!

Alex Sessions

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