

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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Reviewer 3 (Alex Sessions) The purpose of this manuscript is to demonstrate a seasonal shift in the D 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 D 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 is very interesting, and

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if it proves to be correct would be an exciting demonstration of the utility of lipid D 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 3µm 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.

First of all we want to thank the reviewer for the helpful comments. We see the problem with all fatty acids showing the same temporal pattern in depletion to enrichment and that therefore there is the possibility that the signal is due to changes in hydrogen isotopic fractionation in the phytoplankton and not so much the different input of photoautotrophs and heterotrophs. Unfortunately, we don't have any independent constraints on the relative abundance of heterotrophic bacteria versus autotrophic phytoplankton during our sampling period. However, as pointed out earlier Brandsma et al. 2012 studied this site earlier and reported algae, cyanobacteria and bacteria counts. They showed that in general the bacteria count was ten times the count of the algae/cyanobacteria and in general there were only changes in the number of algae and cyanobacteria cells with the two blooms and after the blooms the cell count dropped. For bacteria cells, the cell count was lowest during the spring bloom, increased directly after the bloom and stayed rather stable thereafter. Considering that we don't expect any drastic changes in this seasonal succession, we assume to have a similar situation during our sampling period. We are therefore quite confident that our signal is indeed due to changes in the relative input of photoautotrophs and heterotrophs. However we will add hypothesis that our isotopic signal due to changing fractionations by phytoplankton to the revised manuscript. As mentioned in the rebuttal to reviewer 1 we are aware that there are

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bacteria which will be smaller than the $0.7 \mu\text{m}$ and will therefore not be captured by the filters we used. Unfortunately, for lipid analysis the filters used have to be plastic free and at the time of sampling there were no suitable filters available with a smaller pore size. The clogging of the $3 \mu\text{m}$ and the rather low fatty acid yield of the $0.7 \mu\text{m}$ filters lead to the assumption that we captured the majority of the biomass in the water samples. Pitcher et al. 2011 (Limnology and Oceanography) also showed in their work a good signal for the small Thaumarchaeota in $3 \mu\text{m}$ filters. Of course we cannot rule out that we still have a discrimination towards bigger cells and that a portion of the smaller cells might not have been captured and therefore analysed. Unfortunately, at the time of sampling there was no methodological possibility to change those biases.

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.

Thanks for the comment and we will change the wording in order to be more precise

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

This will be changed in the manuscript.

L137-138: I agree that the $3\mu\text{m}$ 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.

The North Sea water samples were first filtered through a $3 \mu\text{m}$ and then through a $0.7 \mu\text{m}$ filter. For three random samples both filter sizes were extracted and the fatty acid fractions analysed on GC respectively GC/irMS. Unfortunately, for all three samples the $0.7 \mu\text{m}$ filters did not yield enough material for isotope analysis. Therefore, we

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assumed that the majority of the biomass did indeed end up in the 3 μm filters. We agree that there is a chance that the fatty acid results might be biased towards algae rather than bacteria and this will be noted in the revised manuscript.

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

The was the temperature set on the heater for refluxing. We therefore left the temperature out, and simply say that the mixture was refluxed.

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

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.

We thank the reviewer for the remark and we will shorten this part accordingly.

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 D 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.

Thanks for the comment. It is indeed true that it has been observed in some, but not all, culture experiments that the shortest-chain FA was the most depleted. We will add a comment concerning this observation to the revised manuscript.

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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...

When the photoautotrophic organisms are active, but not growing, while still producing sugars (similar to stationary phase). When using these sugars via for example the pentose phosphate pathway for the reduction of NADP to NADPH, the newly synthesized fatty acids would become more enriched in D.

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

We thank the reviewer for the comment and will mention in the manuscript that also the chain length might factor into the fact that the C14:0 is general the most depleted fatty acid.

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.

We appreciate the comment and agree with it. We will point out this possibility in the revised manuscript.

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

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We appreciate the remark and look forward to read the manuscript when it is published. The sentence will be deleted.

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

We actually meant here the ϵ values for chemoautotrophs summarized in figure 3 of Heinzelmann et al. 2015b here. As remarked with reviewer 2 we will add other references here.

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

Thanks for the note. Indeed we mean Figure 1 and this will be corrected in the manuscript.

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