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Biogeosciences Discussions

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 2

This ms seeks to show the applicability of lipid D/H as a biomarker for microbial metabolism in the environment by correlating lipid D/H with large metabolic shifts expected to be observed within a coastal plankton community over a bloom. The authors hypothesize that lipid water fractionation should shift to reflect the transition from an ecosystem dominated by photoautotrophy, during the spring bloom, to one where bacterial heterotrophy becomes progressively more important post-bloom. In addition to lipid D/H and water D/H measurements, the ms presents results from DNA sequencing of the bacterial community. The bloom scenario provides a very interesting test of the

usefulness of environmental lipid D/H for metabolism but the study hinges on showing the balance of autotrophic vs heterotrophic biomass in the system, which has not been clearly demonstrated. DNA sequencing data can not address the contribution of heterotrophic bacteria to the lipid pool relative to autotrophs, most of which are algae. Could any NPP, respiration measurements be used to show that the community shifts towards heterotrophy ? Additionally, the overall shift in lipid water fractionation is small (30 per mil increase in the weighted fractionation), and while it is in the direction that is consistent with a growing contribution of heterotrophy post bloom, the value of the most positive measurement (-170 permil) does not fall in the range of fractionation values that clearly correspond to heterotrophy (>-150, conservatively >-100 permil). Without knowing more about the contributions of autotrophy vs heterotrophy over the season, it does not seem possible to distinguish whether enriched plankton fatty acids reflect increases in heterotrophic contribution to lipids or a shift in the autotrophic population or its metabolism. Regarding shift in phytoplankton metabolism, how can the variation in the fractionation of the algal fatty acid from -240 to -180 permil be explained ? Since the relative importance of autotrophy versus heterotrophy has not been clearly demonstrated and the change in lipid water fractionation is relatively small, I believe that the conclusions are overstated and suggest that a significant revision be made before resubmission. We thank the reviewer for her/his comments and hope that we will be able to address them to her/his satisfaction.

Unfortunately our samples do not allow for any measurements of net primary production or respiration. However, as explained in the rebuttal to the comments of Reviewer 1 we are confident that this environment is indeed driven by a shift from photoautotrophy during the phytoplankton bloom towards heterotrophy following the phytoplankton bloom. This has been shown in a range of previous studies (as mentioned in the manuscript) over a time period of several decades. Additionally, Alderkamp et al. (2006) showed an increase in activity of the heterotrophic bacteria following the decline of the phytoplankton bloom in spring via MICRO-CARD-FISH. We are aware of the different isotopic ranges associated with the individual metabolism types. However, these

ranges were established for pure cultures grown under specific conditions exhibiting only one type of metabolism at the time. In the environment all microorganisms will contribute to the fatty acid pool making the isotopic signal of the fatty acids a reflection of the relative contribution of specific microorganisms to the individual fatty acid pools. Therefore, the mixed environment it can't be expected that the isotopic signals will fall into those quite clear ranges, but will rather fall into a range between those ranges. Changes in the relative contribution of different metabolism types will lead to a shift towards more negative or positive isotopic values while the values will stay in the same range (unless a complete change of metabolism with no input from other metabolism types will happen). Hence we don't think that the changes are too small to make a prediction about shifts in the community metabolism. However, we do agree that for future applications of the hydrogen isotopic composition of fatty acids as community metabolism indicator it might be helpful to look at both general and biomarker fatty acids, reflecting only one type of metabolism. Concerning the wide range of isotopic values of the C20:5 PUFA, as mentioned to reviewer 1 above the PUFA has multiple sources that might changes during the course of a bloom as well as potential "growth phase" changes of the organisms present during the course of a bloom also resulting in changes in hydrogen isotope fractionation.

Specific comments: Lines 29-30: add fractionation values for algal and C18:0 lipids

We will add the requested values to the manuscript.

Lines 30: there is > 50 per mil variability in C20:5 fatty acid, as much variability as in the weighted average : : :

We will add to the sentence to show that the C20:5 is mainly but not always highly depleted.

Line 36: there is no data that directly addresses the contribution of heterotrophs vs autotrophs to fatty acid pool

We will rephrase the sentence in question in the manuscript.

Line 37: can be a useful when combined with other measurements

Thanks for pointing this out, we added this to the sentence.

Line 46. Reference

A reference has been added to the manusript.

Line 58-59: there is a lot that needs to be verified regarding dD of NADPH ... suggest these are hypotheses

We will change this in the manuscript in order to point out that these are just hypotheses.

Line 63: not exactly sure what is meant by "integrated" core metabolism – how would you define this? Is there any need to differentiate between integrated and specific metabolisms? I guess authors are hinting at the fact that lipid D/H can not provide much information on specific microbial groups if lipids are not group specific. In any case, does integrated mean the amount of carbon or some element cycling through a particular metabolism or biomass from a microbial group using a metabolism?

Integrated metabolism means all different metabolism types in a community looked at as one.

Lines 61-81: Osburn et al were the first to explore the environmental applicability of lipid D/H using hot spring systems where a wide range of metabolic groups are physically separated. Lipid D/H over the course of a phytoplankton bloom is another interesting environmental test that extend lipid D/H to more widespread systems– I think there is no need to bring up the relative diversity of the systems. And if so, a reference is needed to compare the diversity of hot springs with plankton communities.

We thank the reviewer for her/his remark. We will shorten this part as pointed out.

Line 85: again the idea of general metabolism is very vague – in this context it would be the balance of autotrophy vs heterotrophy?

We will make this more clear in the revised manuscript.

Lines 99-109: Do the bacteria synthesize fatty acids de novo or utilize the lipids of the autotrophs? If there is research here it should be mentioned in the intro and included in the interpretation of the results.

For our study site it is to the best of our knowledge not known if the heterotrophic bacteria do synthesise the fatty acids de novo or if they utilize the lipids derived from the autotrophs.

Line 329: does it make sense to be so precise with the fractionation values given the error of D/H is a few per mil?

We thank the reviewer for the remark, however it is unclear to us what the reviewer exactly has in mind. The precision of the analysis is approximately 3 ‰ however our measurement error is much lower. We agree that reporting hydrogen isotope values with decimal places might not be meaningful.

Line 335 -336: need to acknowledge significant variability in C20:5 PUFA, yes it's one of the most depleted, but it also gets relatively enriched, within the range of fractionation expressed by heterotrophs

Concerning the wide range of isotopic values of the C20:5 PUFA, as mentioned to reviewer 1 above the PUFA has multiple sources that might changes during the course of a bloom as well as potential "growth phase" changes of the organisms present during the course of a bloom also resulting in changes in hydrogen isotope fractionation. Additionally, according to published data the ε values of the C20:5 PUFA fall within the range associated with photoautotrophic growth (-150 to -250 ‰. Nevertheless, we will mention the variability in the manuscript.

Line 337, 339: provide references

We added the required references.

Line 343: reference for diatom lipid profile

The references are in Table S6.

Line 348: variations in the dD of NADPH varying with different pathways of production remains a hypothesis

It indeed remains a hypothesis at the moment. We will make that clear in the text.

Line 374: If 18:0 is mainly derived from heterotrophic bacteria, wouldn't the fractionation be expected to more positive (> - 150 per mil)? Many of the 18:0 values are below -190 per mil, and there is also significant variability between points (e.g. Dec through March).

While we assume that the C18:0 will be mainly produced by heterotrophic bacteria, diatoms and Phaeocysitis produce this fatty acid in minor amounts. This might as well contribute to the relatively low isotopic values.

Line 380: I believe there is some data from Valentine 2002, an H2 +CO2 acetogen?

Here we meant the summary for all data published in previous papers but summarised in Heinzelmann et al. 2015b. However, we will add the Valentine paper also as reference.

Line 403-404: Please address the possibility of a shift in the photoautrophic population as explaining the 30 per mil shift upwards

Data published by Brandsma et al. 2012 show changes in the total cell number of bacteria and phytoplankton during their sampling period. Although there are changes in the phytoplankton population from Phaeocystis dominated to diatom/cyanobacteria dominated, we think that the shift in the isotopic values is rather due to changes in the relative contribution of heterotrophic bacteria versus photoautotrophic phytoplankton. At the moment published isotopic data shows that changes in the metabolism

leads to bigger shifts in the isotopic composition of fatty acids while different organisms expressing the same metabolism show similar isotopic values.

Line 411: references beyond Heinzelmann 2015 are needed.

We will add additional references, like Zhang et al. 2009a, here.

Tables and Figure: Table 1. Possible to include relative abundance of differenty fatty acids (parentheses after isotope values)?

The relative abundance of the different fatty acids is in Table S4. Adding it to Table 1 might make it quite packed and more difficult to keep the overview.

Figure 1. include C20:5 pufa as “calibrating” data for autotrophy

Due to the multiple sources with different isotopic values for this fatty acid, the changing contributions during the season the isotopic values of the C20:5 PUFA might not be suited as calibrating data for autotrophy. PUFA sources and potential reasons for its changing isotopic composition should be studied further before it can be used as “autotrophy” endmember. However, as discussed above, it would be nice to be able to include biomarker fatty acids for the different metabolisms to compare with the general fatty acids reflecting the entire community.

Fig 2: Do you have total cell counts? If so, maybe you can use this to get a rough estimate of the heterotroph cell abundance.

Unfortunately, as discussed above we don’t have total cell counts of the heterotrophic bacteria.

Figure 4,5: add in bar highlighting bloom data, the most obvious trends in fractionation are the dips in C16:0 data, one corresponding to the bloom, and another dip in October. The fractionation goes as low as the bloom fractionation but is unrelated to photoautotrophy?

As mentioned already in our rebuttal to the comments of reviewer 1, we will add indi-

cations for the different season to the graphs.

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