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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.

Anonymous Referee #2

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Review of Heinzelmann et al. Seasonal changes in the D/H ratio of fatty acids of pelagic microorganisms in the coastal North Sea

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.

Specific comments:

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

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

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

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

Line 46. Reference

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

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.

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

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.

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?

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

Line 337, 339: provide references

Line 343: reference for diatom lipid profile

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

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).

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

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

Line 411: references beyond Heinzelmann 2015 are needed.

Tables and Figure:

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

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

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.

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?

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