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***Interactive comment on* “Technical Note: Constraining stable carbon isotope values of microphytobenthos (C₃ photosynthesis) in the Arctic for application to food web studies” by L. E. Oxtoby et al.**

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Interactive comment on “Technical Note: Constraining stable carbon isotope values of microphytobenthos (C₃ photosynthesis) in the Arctic for application to food web studies” by L. E. Oxtoby et al.

Dear Reviewer 1,

We thank you for the time you spent reading our manuscript. Your comments will help us to greatly improve our work. We have itemized and addressed each of your

Full Screen / Esc

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Discussion Paper



comments below.

1) Comment: The manuscripts attempts to address the issue around the potential role of microphytobenthos in coastal food webs, a subject which is important not just for the Arctic but many global environments.

Author Response: We are pleased that the reviewer recognizes the importance of our research to Arctic food web ecology. To reiterate the importance of our research, we propose a modeling approach to constrain microphytobenthos (MPB) isotopic signatures in the Arctic in order to incorporate those end members into food web models. Modeling approaches in the Arctic are significant contributions in their own right in this region of the world ocean because of the physical and logistical challenges that limit site accessibility. Remote locations of field sites in the Arctic necessitate large research missions that involve many personnel, expensive ship time, high fuel costs, and extensive planning. Additionally, sampling can be limited by sea ice conditions, subsistence activities (i.e.whaling) and polar weather systems. In addition to our modeling approach, we also collected and analyzed (for our modeling approach) water samples that were collected as part of the Arctic Observing Network (AON) project research mission, an oceanography cruise to study physical and chemical oceanography in the Arctic Ocean on the Healy United States Coast Guard Cutter. It is important to note that we contribute a new dataset generated from analyses of novel samples collected from the Arctic and add to the growing body of isotopic measurements of Arctic bottom water DIC that will be of use for future food web and carbon cycling research efforts.

2) Comment: While this is a worthwhile exercise I feel the manuscript has several major flaws that need to be addressed before publication:

The authors present no information on previous studies addressing this issue (e.g. see Oakes, et al., 2005 Measuring carbon isotope ratios of microphytobenthos using compound-specific stable isotope analysis of phytol) and why their approach has merit.

Author Response: We have now included a discussion of the paper mentioned (Oakes

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10, C8750–C8755, 2014

Interactive
Comment

Full Screen / Esc

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Interactive Discussion

Discussion Paper



et al. 2005). In this discussion, we state more explicitly that our method to constrain the isotopic composition of MPB is one approach among many and complements those already established in the literature. Ideally, to produce a robust and confident estimate of MPB isotopic composition, one should use several proxy approaches (including Oakes et al. 2005). We have included the following paragraph to discuss the study conducted by Oakes et al. (2005):

“Robust measurements of the isotopic composition of MPB are difficult to perform due to the potential for contamination from additional organic matter sources such as microbial biomass, meiofauna, and detritus. Oakes et al. (2005) reviewed selected studies in which the isotopic composition of MPB may have been influenced by additional isotopic sources due to limitations of extraction techniques employed. To circumvent problems associated with sampling, Oakes et al. (2005) used a predictive model, as we have done in this manuscript, to determine $\delta^{13}\text{C}$ values from MPB. In our study region, predictive models are also especially useful because little is known about the spatial distribution of MPB, making sample collection difficult and, most always, opportunistic. Ideally, multiple approaches would be used to produce a confident estimate of MPB isotopic composition.”

3) Comment: The authors use $\delta^{13}\text{C}$ of dissolved inorganic carbon (DIC) to then model likely $\delta^{13}\text{C}$ of MPB, including certain key fatty acids. This involves several assumptions around a) growth rate, b) effect of cell geometry and c) fractionation due to biochemical pathways, all of which have large inherent variability, as has been shown in numerous studies.

Author Response: The referee has identified the central objective of our study, which was to conduct a series of sensitivity analyses or modeling exercises to identify the bounds for estimates of $\delta^{13}\text{C}$ values of total organic carbon (TOC) and fatty acids (FA) derived from MPB given variation in algal physiology. We will clarify the following points in the final version of the manuscript: a) Growth rate We modeled $\delta^{13}\text{C}$ values over a wide range of growth rates reported for Arctic phytoplankton (0.1-1.4 d⁻¹). In our paper,

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we reported a 2.5 ‰ increase in $\delta^{13}\text{C}$ values of TOC and FA over the growth range for the taxa most likely to be dominant in Arctic MPB, *P. tricornutum*. Results of the effect of growth rate on $\delta^{13}\text{C}$ values are included on p. 18161 Lines 3-7. We omitted growth rate results from all algal taxa and did not include those for *P. tricornutum* in Table 1 in order to avoid redundancy and to be succinct. We would be very willing to expand our discussion of the effect of growth rate on $\delta^{13}\text{C}$ MPB values for the other algal taxa and include corresponding columns of data in Table 1. b) Cell geometry We selected a diverse group of algal taxa with varying cell geometries (a haptophyte, a cyanobacterium, a pennate and a centric diatom species) to demonstrate the effects of cell geometry on isotopic fractionation. We relied on previous research conducted by Popp et al. (1998) describing empirically-derived relationships between photosynthetic fractionation factors, growth rate, and carbon dioxide availability for these algal taxa. We observed a mean difference of ~ 4 ‰ between the most geometrically distinct taxa included in our study, the pennate and centric diatoms, using a fixed growth rate of $\mu = 0.1 \text{ d}^{-1}$. These results are reported on p. 18160 Lines 20-24 and included in Table 1. c) Biochemical pathway To investigate the influence of biochemical pathway on stable carbon isotopic fractionation in MPB, we included a cyanobacterium, *Synechococcus* sp. Cyanobacteria are unique in that they carry out photosynthesis and respiration in a single compartment in contrast to eukaryotes in which processes of photosynthesis and respiration occur within distinct organelles. Cyanobacteria differ from eukaryotic species in the extent to which they fractionate carbon isotopes during photosynthesis to produce biomass, and, more specifically, lipids (Sakata et al. 1997). In a controlled laboratory environment, *Synechococcus* sp. did not respond to changes in availability or demand of aqueous carbon dioxide ($[\text{CO}_2]_{\text{aq}}$) (Popp et al. 1998), so we used a fixed fractionation factor to model $\delta^{13}\text{C}_{\text{TOC}}$. Mean $\delta^{13}\text{C}_{\text{TOC}}$ (-16.2 ± 0.4 ‰ for *Synechococcus* sp. was distinctly higher than those for the eukaryotic species. Again, we presented results for *Synechococcus* sp. on p. 18160 Line 23, but omitted the results from Table 1 to distill the results of our calculations represent the most biology likely to be dominant in Arctic MPB, *P. tricornutum* (Vetrov and Romankevich 2004 and refer-

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ences therein). We would be willing to include an additional column of data in Table 1 to include our results for *Synechococcus* sp. and to address the effect of biochemistry (to describe it in broad terms) in more depth in our discussion. It is evident that differences in physiology, which may result from reliance on unique biochemical pathways, influence the isotopic composition of organic matter derived from MPB. 4) Comment: The authors make no comparison with actual measurements of samples collected at the same time.

Author Response: The referee is correct in pointing out that we were unable to compare modeled values to actual measurements from the same locations. This study describes the development of a method and its implications for food web modeling in the Arctic marine environment. It requires further validation through collection of bottom water DIC in concert with MPB samples for isotopic analyses. Sample collection will present many challenges in the Arctic given the nature of field sampling and the patchy distribution of MPB, but may be possible in the estuarine or nearshore environments which are more easily accessed in the Beaufort region (e.g. Matheke and Horner 1974, Dunton et al. 2012). Our samples were collected on an oceanographic research expedition in which no ship time or gear was available for sediment sampling. A validation study would be the next step to corroborate the results of the modeling exercise. However, we have collected and analyzed a large number of surface sediments from the Bering Sea shelf domain and would be willing to include a table showing the data from those analyses in our revised manuscript. (See more details in response to comment number 3 from the other reviewer and below).

5) Comment: Thus, I was left wondering how to assess how good or otherwise their technique might be. I feel that for this manuscript to go forward there needs to be some way of assessing how good it is as variation of a few per mil are important when trying to infer between contributions from pelagic and benthic algae.

Author Response: Although, due to logistical constraints, we were unable to collect sediment samples in concert with bottom water DIC at our study sites in the Beaufort

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10, C8750–C8755, 2014

Interactive
Comment

Full Screen / Esc

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Interactive Discussion

Discussion Paper



and Chukchi Seas, we have extensive spatial and temporal coverage of sediment samples collected in the sub-Arctic Bering Sea. We have analyzed these samples for stable carbon isotopic composition of sediment TOC and FA (Oxtoby et al, unpublished). We would be willing to include a table containing this data to provide added context and discussion of the $\delta^{13}\text{C}$ values generated by this study. For example, the mean $\delta^{13}\text{C}$ of TOC and corresponding FAs from sediments in the Bering Sea are both relatively low and are not consistent with a substantial source from ice derived organic matter.

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BGD

10, C8750–C8755, 2014

Interactive
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Interactive Discussion

Discussion Paper

C8755

