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Interactive comment on "Uptake of algal carbon and the synthesis of an "essential" fatty acid by *Uvigerina* ex. gr. *semiornata* (Foraminifera) within the Pakistan margin oxygen minimum zone: evidence from fatty acid biomarker and ^{13}C tracer experiments" *by* K. E. Larkin et al.

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Dear authors, Several of my earlier mentioned remarks (in my first referee report) are not adjusted in the current version of the manuscript.

Dear Reviewer. Thank you for your helpful comments. We apologise for not responding to the first review. We have taken these earlier points into account in the responses given below (comments 11-15).

Comment 1. p 257 line 3 how was this second question addressed? (Note - the second question is: 'Does this species utilise other food sources?').

Response 1: This question was addressed by the FA analyses. In lines 425-433 of the manuscript (Discussion) we suggest that 18:1(n-7) in the foraminiferal cells was derived from heterotrophic bacteria ingested from the sediment during the experiments. This was supported by the low 13C content of this fatty acid at the end of the five-day shipboard experiment. However, there was a substantial increase in the percentage of 13C in 18:1(n-7) at the endpoint of the in situ incubation (2.5 days). Another possibility therefore is that the foraminifera ingested bacteria that had already assimilated dissolved organic carbon derived from the 13C-labeled diatoms and had incorporated these atoms into other fatty acids that they synthesised de novo. In the Conclusions (manuscript lines 470-472), we conclude that 'increases in the bacterial biomarker fatty acid 18:1(n-7) supports the idea that U. ex. gr. semiornata also consumed some bacteria from the surrounding sediment.'

Comment 2. p 258, line 5: more details on the labelling technique are required: duration of the labelling? growth condition of the diatoms? did they go through the exponential growth phase? how dense were they when they were harvested? the incorporation of the label (13C) may differ substantionally according to the growth phase, see literature on that issue.

Response 2. We agree that information was lacking in this section, which has now been expanded to give a full account of the culturing of the labelled algae (lines 178-184). We also agree that when culturing algae under natural abundance levels of 12C and 13C, the incorporation of 13C varies over the growth cycle. However, we attempted to label the diatoms as highly as possible and excluded most 12C from the culture vessel and used 13C NaHCO3 as the carbon source for the algae.

Comment 3. p. 259, line 21: natural foraminifera: what is the origin of these specimens? same species? use the term control instead of natural throughout the text.

Response 3: We have changes 'natural' to 'control' throughout the text (manuscript lines 198. 215-217, 331, 335, 345, 367, 442)

Comment 4. p 260, line 21: -20°C is not the best for FA analyses, preferably -80°C should be used. This (the unstability of FA) should be stated in the discussion.

Response 4. Chloroform: Methanol often freezes in a -80°C freezer, expecially when it contains even small amounts of seawater and this would prevent extraction of the lipids from the foraminifera. Lipases contained within the foraminifera at -20°C could potentially be active and produce free fatty acids. However, this would not affect our analyses as lipids are broken down to free fatty acids and converted to fatty acid methyl esters during derivatisation.

Comment 5. part 3.1 very strange to report these observations in a study with biomarkers/trophic tracers. this raises the question whether the foraminifers were starved prior to the FA extractions? in other words, were you measuring the diatoms inside their tissue and not the assimilated carbon of the diatoms in the foraminifers? this is an extremely important issue for the entire study. - see also p 263 l 25: consumed differs from assimilated!

Response 5. Microscopic analyses are invaluable in conforming and supporting data generated by biomarker studies and green colouration of the cytoplasm of the foraminifera is good evidence that the cultured diatom food source was ingested by the foraminifera. Given that foraminifera are unicellular, we define as any material within the cytoplasm as assimilated. These points are made in lines 414-416,

Comment 6. p 264 line 1: 14:0 and 16:0 are NOT indicative for a particular source, they are omnipresent, they are not biomarkers, so they should not be discussed throughout the study.

Response 6. We agree that 14:0 and 16:0 are not useful biomarkers in studies where biological material is simply collected from nature. However, in controlled experimental

studies such as ours where we knew the composition of the food provided, the high amounts of 13C labelled 14:0 and 16:0 fatty acids in the diatoms (section 3.2) can be used as tracers of diatom uptake by the foraminifera. In fact, these saturated fatty acids are only mentioned twice (lines 330 and 358), and in both cases it is made clear that they are the dominant fatty acids in the diatom food source.

Comment 7. p 264, line 16: another ESSENTIAL point: how did diatoms build in the 13C? a figure of the 13C levels in their FA is absolutely necessary to understand the pattern in the consumer. It is missing on Fig 2, see also l 379. Compound-specific SIA is needed here.

Response 7. Labelling of the fatty acids is described in section 2.3.2. Overall, fatty acids were 90% labelled with 13C.

Comment 8. Legend fig 2: FA nomenclature should be mentioned in the Materials and methods and not in the legend.

Response 8: Agreed. Moved to manuscript lines 269-271

Comment 9. p 266 line 29 and following: you need to explain better what FA are typical for bacteria - what could trigger them to synthesise FA? The lab conditions?

Response 9. We consider this to be beyond the scope of the current paper.

Comment 10. common terminology for FA (eg EPA, DHA, ARA) would be appropriate - I regret that the already limited part on FA conversion is even shorter in this version of the manuscript (p. 267 l 15). It is an essential issue when using FA as biomarkers and you want to be critical towards the obtained data and results.

Response 10. The terminology we used for fatty acids, i.e 20:5(n-3) is standard in the literature (see lines 298-300) .

Additional points from the initial review

Comment 11. Abstract, lines 19-21. How can it provide evidence for the use of multiple

food sources if only diatoms were used as food in the experiments? Please rephrase.

Response 11: Lines 48-54 of the abstract revised as follows - 'In addition, levels of 20:4(n-6), a PUFA only present in low amounts in the diatom food, increased dramatically in the foraminifera during both the in situ and shipboard experiments, possibly because it was synthesised de novo. This 'essential fatty acid' is often abundant in benthic fauna yet its origins and function have remained unclear. If U. ex. gr. semiornata is capable of de novo synthesis of 20:4(n-6), then it represents a potentially major source of this dietary nutrient in benthic food webs'.

Comment 12. l 208-209: two to five days is not precise enough, note more precisely same remark for l 226 approx? 2.5 days

Response 12. (i) Manuscript line 207 changed to 'the two and five day incubation periods'. (ii) Line 220. The $\sim$ symbol deleted.

Comment 13. p. 260, line 251: only upper 1cm: why?

Response 13. Sentence modified as follows - 'For fatty acid analysis, foraminifera were extracted from the 0-1 cm layer of the sediment in which specimens of Uvigerina ex. gr. semiornata were concentrated.'

Comment 14. p. 260, line 252: for each time: but there is only 1 time interval for the in situ exp

Response 14. Manuscript lines 247-248 modified as follows - 'Two replicate cores were sampled for each of the two time points in the shipboard and the single time point in the in situ experiment.'

Comment 15. p. 260, line 255: first pooled and then 4 replicates? this is unclear? are these pseudoreplicates?

Response 15. Manuscript lines 250-251 modified as follows - ' Four batches of Uvigerina ex. gr. semiornata, each comprising 30 individuals , were picked from sediment

residues....'

Interactive comment on Biogeosciences Discuss., 11, 251, 2014.