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Biogeosciences Discussions

Interactive comment on "Variation in stable carbon and oxygen isotopes of individual benthic foraminifera: tracers for quantifying the vital effect" *by* T. Ishimura et al.

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My co-authors and I thank the anonymous referee #1 for the constructive comments, which helped to greatly improve the quality of our manuscript. We took into account all the suggestions/comments when revising our paper. Our replies are as follows:

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[Anonymous referee #1's comment] I have carefully read manuscript bg-2012-204, entitled 'Variation in stable carbon and oxygen isotopes of individual benthic foraminifera: tracers for quantifying the vital effect' by Ishimura and co-workers and recommend it for publication in Biogeosciences. However, there are a number of issues that need to

be addressed before acceptance, some of which may lead to substantial changes in the discussion and conclusions of this manuscript.

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[Anonymous referee #1's comment] Major issues: 1. Part of the inter-individual variability in δ18O and δ13C that is reported (Table 2, Figure 3) may be caused by in-sediment variability in δ13CDIC and, to a lesser extent, the δ18O of the pore water (Table 1). It may be that different individuals have calcified at different depths in the sediment and have thus utilzed DIC with different carbon isotope signatures. More inter-individual variability in isotopic composition may thus reflect a wider range in depth habitats. This possibility should be discussed in the manuscript.

[Reply] Thank you for your suggestions. We also think that part of the inter-individual variability in δ13C is caused by the isotopic variability of DIC in sediment pore water, which is caused by the decomposition of organic matter, and a wider range of depth habitats may result in large inter-individual variability in isotopic compositions. We will add this discussion to Section 3.3 of the revised manuscript.

On the other hand, even considering the isotopic variation in sediments owing to the decomposition of organic matter and the presence of a geothermal gradient, we cannot account for the extremely negative isotopic values and the large inter-individual isotopic variations in δ13C and δ18O. The δ13C values of most individuals were much lower than δ13C DIC values of pore water at the sediment depth of which they had been taken. Also, we could not explain the variability of δ18O because the decomposition of organic matter does not change the δ18O value of pore water. The δ18O values of pore water at each sediment-depth indicate almost homogeneous isotopic values (the magnitude of δ18O variation among different sediment depths is almost the same as the analytical error).

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[Anonymous referee #1's comment] Is there a relation between isotope composition and the sediment depth at which individuals from the same species were collected?

[Reply] Within the same species, there is no noticeable relationship between isotopic compositions and the sediment depth at which individuals are collected. We mentioned this point on p. 6198 as follows: 'No systematic difference was observed in isotopic values between living and dead individuals, and isotopic differences among shells collected from different depths were within the range of inter-individual isotopic deviations for each species.'

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[Anonymous referee #1's comment] 2. Variability in in-sediment depth habitat (within the sediment or water column) does not really count as a 'vital effect'. Rather, it is a shift in environmental conditions that produces variability in isotope (or element) composition. Although habitat-effects are sometimes regarded as part of the vital effect, it is better reserved for the effects of metabolism, photosynthesis by symbionts, etc. The claim that the results presented here show the magnitude of the vital effect on carbon and oxygen isotopic composition is somewhat idle and is better avoided. Statements such as are made in the final part of the Conclusions need to be omitted. Also remove 'vital effect' from the title.

[Reply] We agree completely with your comment about the "vital effect." We will change some occurrences of "vital effect" to "isotopic disequilibrium" (e.g., in the title and the Conclusion).

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[Anonymous referee #1's comment] 3. Section 3.2 suggests that the inter-individual variability in isotope composition can be used to reliably reconstruct δ13CDIC from foraminiferal samples 'from throughout the world'. Such a generalization cannot be made on the basis of the dataset presented here. The foraminiferal calcite's $\Delta\delta$13C

and intra-species variability therein, may well be different for populations from other depths/ areas or with DIC that has a δ13C outside the range found in the locations sampled here. Also adjust the Abstract accordingly.

[Reply] We agree that we should not generalize the characteristics of the inter-individual isotopic variation of benthic foraminifera observed in our study. We will change the sentence of 'from throughout the world' to 'in our studied sites.' We will adjust the abstract and conclusion as you suggested. However, in our study, we have compared the magnitude of inter-individual isotopic dispersions (Standard Deviation; SD) and $\Delta\delta$13C collected at the same sampling site, and then we calculated the relational equation between them. We did not compare between different sites. We would like to conclude that, 'Comparing the isotopic values of benthic foraminifera collected from the same sampling site, there is good correlation between the magnitude of inter-individual isotopic dispersions (SD) and the $\Delta\delta$13C of benthic foraminifera.' The relational equations of SD and $\Delta\delta$13C may not always be the same among different stations (depth/area/age). By studying various sites, we were able to clarify the detailed characteristics of the relationship between the magnitude of inter-individual isotopic dispersions (SD) and $\Delta\delta$13C of benthic foraminifera. We hope that we will be able to develop a more reliable paleo-δ13C proxy of bottom water.

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[Anonymous referee #1's comment] Minor issues: 1. The living-dead divide is made based on staining specimens with rose Bengal. This method, however, does not allow accurate identification of living individuals (e.g. Bernhard, 1988, JFR 18: 143; Bernhard et al., 2006, Paleoceanography 21). This should be mentioned and references to 'living' foraminifera throughout the text should be adjusted.

[Reply] As you suggested, we will mention that the "staining method does not allow accurate identification of living individuals." We will also refer to the previous reports about the problem of the rose-Bengal staining method. However, during the analyti-

cal procedures of “living” individuals in this study, we observed that foraminiferal soft tissues remained completely in the reaction tubes after the acid reaction of the calcite shell. [The analytical method (Ishimura et al, 2004) allows us to observe the entire reaction process under a microscope.]

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[Anonymous referee #1’s comment] 2. Section 3.3 invokes intracellular pH control as a source for inter-species (and perhaps also inter-individual) variability in calcitic carbon and oxygen isotopes. The inter- species variability in isotopes may be caused by the magnitude of pH control by different species (or by the inter-species variability in the pool of respired CO2 that participates in calcification). Please add this to the discussion.

[Reply] Thank you for your valuable suggestion. We will add your suggestion to the discussion in the revised manuscript.

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[Anonymous referee #1’s comment] 3. Language is sometimes ambiguous. E.g. Introduction, second page, line 5/6: the ‘isotopic composition of biogenic carbonate’ is not determined by ‘ambient isotopes’, but by the oxygen isotopic composition of seawater and carbon isotopic composition of dissolved inorganic carbon. Lines 10/11: ‘microhabitats’ are themselves not a ‘cause of the vital effect’, but rather, the vital effect may be caused by occupation of different microhabitats by different species/ individuals. Line 19: what are ‘details of the isotopic variations’? Line 21/22: what does a ‘clearer understanding’ mean? Please check the whole manuscript for such phrasings.

[Reply] We will find and revise the ambiguous sentences as you suggested.

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[Anonymous referee #1’s comment] 4. Final paragraph of the Introduction should be removed.

[Reply] We will remove the final paragraph of the Introduction.

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[Anonymous referee #1’s comment] 5. Were the foraminifera cleaned before isotope analysis? Could the data be contaminated by oxygen and carbon isotopes from the organic material? What could be the contribution of this source compared to the calcite?

[Reply] We cleaned all individual foraminifera with Mill-Q water before analysis, and we did not find any additional material on the shell under the microscope. We believe that there was no contribution of organic materials to the analyzed isotopic values because we did not find any noticeable isotopic differences between living and dead specimens.

In addition, we considered the possibility of CO2 gas generation through the reaction between acid and organic materials. For example: – We checked whether foraminiferal soft tissues react with acid. We did not find the generation of CO2 gas from soft tissue over several days’ observation. –Additionally, our analytical method (Ishimura et al., 2004, 2008) has a lower possibility of contamination of CO2 gas generated from organic matter than recent commercial analytical methods, because our reaction temperature (25 °C) is lower than that of recent commercial analytical systems (usually 50–90 °C).

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[Anonymous referee #1’s comment] 6. What is the saturation state of the bottom water/ pore waters with respect to calcite? Could there be any dissolution/ remineralization of the shells?

[Reply] Although we do not have data for the calcite saturation state, we carefully observed each sample under the microscope, and we did not find any dissolution/ remineralization in our analyzed samples. In addition, there is no evidence of the possibility of secondary calcification (the presence of authigenic calcite) in sediment.

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[Anonymous referee #1's comment] 7. Please use the terms 'inter-species' and 'intra-species' (or 'inter-indivdual') through- out the text.

[Reply] We will revise the manuscript and title as you suggested.

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[Anonymous referee #1's comment] 8. Does the dataset allow calculation of the number of individuals needed to accurately determine the ambient seawater δ18O/ δ13CDIC for species that calcify close to isotopic equilibrium (e.g. B. aculeata; Table 3)?

[Reply] It is difficult to calculate this in our dataset because the number of analyzed individuals is too low to examine the statistical calculation. We should analyze at least over 20–30 individuals for each species to calculate the number of individuals needed to accurately determine the ambient seawater δ18O/ δ13CDIC for species that calcify close to isotopic equilibrium.

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[Anonymous referee #1's comment] 9. I don't see the relevance of the water column δ18O and δ13CDIC in Table 1. Remove the '-' for the pore water temperatures. Statement at the end of Table 2 should be placed in the caption.

[Reply] We will revise the manuscript as you suggested.

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[Anonymous referee #1's comment] 10. Apparently, not all specimens analyzed were 'living' (rose Bengal-stained; Table 2). Is there a difference in isotopic composition between stained and non-stained individuals?

[Reply] As described in our manuscript, no systematic difference was observed in isotopic values between living and dead individuals, and isotopic differences among shells collected from different depths were within the range of inter-individual isotopic devia-

tions for each species.

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[Anonymous referee #1's comment] 11. It is very difficult to recognize the regression curves in Figure 4A. Differences between species are not (sufficiently) discussed in the manuscript. Is variability in morphology/ test thickness responsible for the different relations between weight and isotope composition?

[Reply] We will revise Figure 4A to make it easier to see. In this study, we focused the discussion only on the trend of relations between shell weight and isotopic compositions. We could not find any noticeable aspect related to the differences of morphology/ test thickness with regard to the regression curves in Figure 4A. In future studies, we will be able to clarify the isotopic signature depending on morphology / test thickness.

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We would like to thank you for the helpful comments and suggestions. We trust that the responses to your comments and questions are satisfactory.

Interactive comment on Biogeosciences Discuss., 9, 6191, 2012.