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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.

T. Ishimura et al.

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My co-authors and I thank Dr. Fontanier 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:

[Dr. Fontanier's comment] The paper entitled “Variation in stable carbon and oxygen isotopes of individual benthic foraminifera: tracers for quantifying the vital effect” by Ishimura San et al. deals with the applicability of inter-individual $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ distributions (Standard Deviation within species) to reconstruct the bottom water isotopic signatures. This work is based on live and dead (assumed as modern)

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



foraminifera that were sampled at 4 deep- sea sites, off Japan. Different species and genera, with different individual weights (i.e. size), were analyzed individually and their isotope signatures were compared with bottom water $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{18}\text{O}_{\text{e.c.}}$. Within a species, lower $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values are recorded for smaller individuals. This is in agreement with other published works. Both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ vary between taxa, what is also consistent with other publications. Finally, the authors show that the average intra- individual $\delta^{13}\text{C}$ calculated for each species is correlated to the related Standard Deviation – when all species from a same area are plotted on the same graph, a simple equation ($a \times \text{SD}\delta^{13}\text{C}_{\text{foram}} + b = \delta^{13}\text{C}_{\text{foram}}$) can be determined by a linear regression. Then, the authors observe that when $\text{SD} = 0$, the $\delta^{13}\text{C}_{\text{DIC}}$ ($= b$) is close to the bottom water $\delta^{13}\text{C}_{\text{DIC}}$. Therefore, they propose that the average intra- individual $\delta^{13}\text{C}$ and the related Standard Deviation may be relevant and reliable proxy to calculate bottom water $\delta^{13}\text{C}_{\text{DIC}}$.

[Dr. Fontanier's comment] General comments This paper is well written and well illustrated. It is based on a large data set of isotopic measurements that should be published in a peer-review journal. Those high-quality analyses were done on single individuals belonging to taxa which are quite common along the Pacific margin. As a modest taxonomist, I would recommend the authors to provide an appendix with taxonomic references for all taxa which were analyzed in this study. SEM pictures for all taxa would be also necessary for readers who would like to use related taxa for their own investigations.

[Reply] We will add a taxonomic reference list and SEM pictures in the revised manuscript.

[Dr. Fontanier's comment] Now, when I deal with some interpretations proposed by the authors, I have got some concerns that should be clarified by the authors. For

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

instance, it seems that the authors have forgotten to use isotopes data of some species (*Nonionella globosa*, *Nonionella labradorica*) in figure 5 (in which interpretative linear regressions were drawn). According to me (I may be wrong!), adding those data (with low SD values) would change a large part of the interpretative story.

[Reply] Although we analyzed *Nonionella globosa* and *Nonionella labradorica*, there were not enough species analyzed (only two individuals) to discuss inter-individual isotopic variations. We listed the isotopic data merely as informative data (i.e., as “others”), but actually we did not discuss these isotopic data in our discussions. Therefore, we will remove the analytical data regarding *Nonionella globosa* and *Nonionella labradorica* from our revised manuscript.

[Dr. Fontanier’s comment] Moreover, in some cases, the authors have measured isotopes at a genus level (*Rutherfordoides* and *Stainforthia*) without considering the potential inter-specific variability. Such a point should be addressed somewhere.

[Reply] We did not discuss the isotopic data on a genus level. *Rutherfordoides* and *Stainforthia* did not consist of multiple species (not spp.) but rather single species. We will add the taxonomic name at the species level in the revised manuscript.

[Dr. Fontanier’s comment] Furthermore, the linear regressions which are proposed by the authors should be tested for their r-value and their p-level significance.

[Reply] We will add the p-value in the revised manuscript.

[Dr. Fontanier’s comment] Finally, I wonder whether this approach may be relevant and reliable in oligotrophic settings where only few fossilizing shallow infaunal species thrive.

BGD

9, C3380–C3393, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



[Reply] Although we are not able to apply our findings to oligotrophic settings right now, as you may know, we think that the inter-individual isotopic variations should be changed by the chemical conditions in sediment, the flux of organic matter, and so on. We will have to study the relationships between those aspects of various sediment settings (e.g., oligotrophic setting) in future work.

On the other hand, we expect that the magnitude of the isotopic disequilibrium ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{18}\text{O}$) in species is also correlated with the inter-individual variation in various settings. This is because isotopic values are determined by:

1) Temperature (almost constant in deep sea)

2) $\delta^{13}\text{C}$ of DIC / $\delta^{18}\text{O}$ of H_2O of bottom water (homogeneous in deep sea)

+

3) $\delta^{13}\text{C}$ of DIC of pore water (heterogeneous in sediment, especially in microscale because of decompositions of organic particles)

4) Microhabitat effect, differences of intracellular pH, and many other factors, including the “vital effect” reported in previous studies (the magnitude of those influences should be different among individuals)

Factors 1 and 2 affect all foraminiferal isotopic compositions equally. The magnitudes of factors 3 and 4 are not always the same but are changeable on an individual level. Therefore, if the effects of factors 3 and 4 are increasing, not only the magnitude of $\Delta\delta^{13}\text{C}$ and $\Delta\delta^{18}\text{O}$ but also the inter-individual isotopic dispersions may increase. If we can collect multiple species having different inter-/intra-species isotopic compositions from the same sample, we expect that we may be able to estimate bottom water DIC by using the method presented in this study.

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[Dr. Fontanier’s comment] For instance, do the authors think that they may rebuild

BGD

9, C3380–C3393, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



bottom water $\delta^{13}\text{C}_{\text{DIC}}$ with long fossil records where only *Epistominella exigua* would be the taxon present along the overall archive? It sounds like a question picked up from the Pandora's Box...so?

[Reply] Our method to estimate $\delta^{13}\text{C}$ of bottom water presented in this study is based on the inter-individual isotopic variation of multiple species collected from the same sediment cores at three sites. If only one species was found in a sampling site, it is difficult to estimate $\delta^{13}\text{C}$ of bottom water by using our method presented here. If we are able to determine that the inter-individual isotopic variation (SD) of a species (e.g. *Epistominella exigua*) is almost constant in various sampling sites, we will be able to use them to estimate the $\delta^{13}\text{C}$ bottom water by using a generalized relational equation between SD and $\Delta\delta^{13}\text{C}$.

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[Dr. Fontanier's comment] More generally (and as a respond to the previous question), the authors focus their discussion on the applicability of isotopes to reconstruct bottom water signals. But, for most infaunal foraminiferal species, it seems that the (average) $\delta^{13}\text{C}$ is strongly constrained by in-sediment processes affecting pore water $\delta^{13}\text{C}_{\text{DIC}}$ (exported productivity, in-sediment organic matter mineralization, cold seeps...) (many publications as references). For instance, the authors should discuss the overall role of microhabitat on the specific (average value) $\delta^{13}\text{C}$, before dealing in detail with the inter- individual isotopic variations.

[Reply] We also thought that a main part of the inter-individual isotopic variability in $\delta^{13}\text{C}$ is caused by isotopic variability of DIC in sediment pore water. The microscale isotopic heterogeneities caused by the decomposition of organic matter and the wider range of depth habitats of foraminifera may result in large inter-individual variability in their isotopic compositions.

However, even considering the isotopic variation in sediments owing to the decomposition of organic matter and the presence of a geothermal gradient, we cannot account

BGD

9, C3380–C3393, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



for the extremely negative isotopic values and the large interindividual variation in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$. The $\delta^{13}\text{C}$ values of most individuals were much lower than $\delta^{13}\text{C}$ DIC values of pore water at the sediment depth of which they had been taken. Also we could not explain the variability of $\delta^{18}\text{O}$ because the decomposition of organic matter does not change $\delta^{18}\text{O}$ of pore water. The $\delta^{18}\text{O}$ values of pore water at each sediment-depth show almost homogeneous isotopic values (the magnitude of $\delta^{18}\text{O}$ variation among different sediment depths is almost the same as the analytical error). Therefore, as discussed in our manuscript, we concluded that the carbonate ion concentration effect seems to be a more reasonable explanation for the changes of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of benthic foraminifera in our study.

Meanwhile, most of the smaller species analyzed in this study were not studied in previous isotopic studies. Among thousands of calcareous foraminifera, we could analyze only a small number of species that have a larger shell. In other words, we could not see “the whole image of characteristics of the isotopic disequilibrium of the benthic foraminifera.” As discussed in our manuscript, the complex interactions between many factors and the isotopic composition of biogenic calcite make it difficult to discuss them separately. In this study, we focused on the inter-individual isotopic variations and the whole image of the characteristics of the isotopic signature of benthic foraminifera. Additional in situ biological observations and culture experiments of many kinds of benthic foraminifera should help to clarify the mechanisms responsible for large inter-individual isotopic variations, $\Delta\delta^{13}\text{C}$ and $\Delta^{18}\text{O}$. We expect also that the analytical method for microscale carbonate will be useful for this purpose.

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[Dr. Fontanier's comment] They could do so for the 1208-m depth station where they have pore water data. Indeed, the $\Delta\delta^{13}\text{C}$ of some taxa is sometimes proposed as relevant proxies of environmental parameters (redox conditions in the sediment, exported organic matter flux) that are partly disconnected from bottom water signature.

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

[Reply] Yes. The inter-individual isotopic variations will be affected and changed by the environmental parameters of a microhabitat (e.g., redox condition / decompositions of organic matter). Further studies will be needed to discuss the effect of sediment conditions on the inter-individual isotopic variations.

[Dr. Fontanier's comment] Specific and technical Comments: p. 6194, line 10. The Nankai Trough and the Sagami Bay are not located in “marginal seas”, are they? The authors should correct this sentence.

[Reply] We will change this sentence in the revised manuscript (“marginal seas” → “continental margin”)

[Dr. Fontanier's comment] p. 6194, line 23. “Multicorer” is better than “multiple corers”.

[Reply] We will use the word “multicorer” in the revised manuscript, as you suggested.

[Dr. Fontanier's comment] p. 6195, lines 7-10. This paragraph is unclear and should be reformulated. For instance, did the authors use either ethanol or formaldehyde (with Rose Bengal solution) to store sediments before sieving?

[Reply] We did not use ethanol and formaldehyde but filtered seawater for the rose Bengal solution.

[Dr. Fontanier's comment] p. 6195, line 11. The authors should precise that they have also analyzed *N. labradorica* and *N. globosa*.

[Reply] As mentioned above, we will remove those data from the revised manuscript.

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



[Dr. Fontanier's comment] p. 6195, lines 11-19. As explained in my comments for the figure 2, many taxa that are analyzed in this draft are complex in terms of taxonomic identification. For instance, *Nonionella labradorica* (name used in this paper) is generally described as *Nonionellina labradorica* (in most “Japanese” papers that I know). What is the difference between both taxa according to the authors? *Nonionella globosa* is very close to *Nonionella stella*, isn't it? But few taxonomic plates exist as relevant illustration of what a *Nonionella globosa* looks like. Furthermore, *Rutherfordoides* and *Stainforthia* are both tricky genera the species of which may be easily confused. Maybe the authors should be more precise concerning the related species? *Rutherfordoides cornuta*? *Rutherfordoides rotundata*? *Stainforthia fusiformis*? (. . .) *Globobulimina* presents different species that are very close in terms of morphology (*Globobulimina affinis*, *Globobulimina auriculata*, *Globobulimina hoeglundi*. . .). (. . .) Finally, the authors should add an appendix with taxonomic references for all taxa which were analyzed in this study. If possible, they should precise the species of *Rutherfordoides* and *Stainforthia*. SEM pictures for all taxa are necessary for readers who would like to use related taxa for their own investigations.

[Reply] As mentioned above, we will remove the isotopic data on *N. labradorica* and *N. globosa* from the revised manuscript. We will add the photograph and SEM pictures and the taxonomic reference list in the revised manuscript.

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[Dr. Fontanier's comment] p. 6197, line 21. “Authigenic” instead of “authentic”.

[Reply] Thank you for pointing out our error. We will correct this misspelling in the revised manuscript.

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[Dr. Fontanier's comment] p. 6197, lines 22-25. Can the authors provide the data (with values and graphs) confirming that “interspecies differences in average isotopic values

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

were not due to the reduced sample size?”?

[Reply] We already provide those isotopic values in Table 2, and we showed that “The average single-shell isotopic values approximately corresponded to the average values from five shells analyzed together”. The isotopic values of five shells are within the average isotopic values with SD of single-shell analysis. In addition, the reliability of isotopic analysis for samples over 0.2 μg CaCO_3 has already been demonstrated in Ishimura et al. (2004,2008). We believe that “the interspecies differences in average isotopic values were not due to the reduced sample size.”

[Dr. Fontanier’s comment] p. 6199, lines 13-16. If I look carefully at the table 2 and at the figure 3, a part of the large inter-individual deviation for *Brizalina pacifica* (MR) and *Stainforthia* sp. is pulled by measurements performed on very small and dead individuals. Can you trust those values as reliable for primary calcite signals? Don’t the authors think that a part of very low $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ signatures recorded for both taxa may be influenced by secondary calcite (more or less related to cold seeps, for instance)?

[Reply] We believe that the determined isotopic values are original isotopic values of primary calcite. We cleaned all individual specimens using Milli-Q water, and we did not observe any evidence of added errors from foraminiferal sampling (e.g., addition of authentic carbonate, staining by rose Bengal, etc.) or any systematic analytical errors (e.g., leakage of air, isotopic fractionation).

In addition, if we consider the possibility of the addition of secondary calcite to the foraminiferal shell, we could not explain the large isotopic shift of $\delta^{18}\text{O}$. The $\delta^{18}\text{O}$ values of pore water at each sediment-depth show constant isotopic values (the magnitudes of $\delta^{18}\text{O}$ variation among different sediment depths are almost the same as the analytical error).

BGD

9, C3380–C3393, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

Interactive
Comment

[Dr. Fontanier's comment] Moreover, the authors have worked with *Stainforthia* at the genus rank (i.e. without discrimination between different species). In other words, a part of “inter-individual” variability may be related to “inter-species” variability. Right or wrong? If so, related values should be considered with utmost cares and not used for calibration (and regression).

[Reply] All the *Stainforthia* sp. analyzed in this study belong to a single species. Therefore, it is not a problem to use isotopic data of *Stainforthia* sp. as “inter-individual isotopic variation” in this study. (We will change “*Stainforthia* sp.” to “*Stainforthia fusiformis*” in the revised manuscript.)

[Dr. Fontanier's comment] Authors' interpretations on the relationship between the $\delta^{18}\text{O}_{\text{e.c.}}$ and foraminiferal signatures are certainly right. However, I don't think that “species with low inter- individual deviations in (carbon) isotopic composition are more suitable as direct proxies of the bottom water environment”. Indeed, for 90% of taxa there is clear shifts compared to the equilibrium line (bottom water signature) (Fig. 3) . . . and it is well-known that those shifts (>1 permille in this study) are not constant for one species and varies in function of in-sediment parameters (organic matter mineralization in the sediment, pore-water oxygenation, alkalinity, methane seepages. . .) (McCorkle et al., 1990, 1997; Schmiedl et al., 2004; Fontanier et al., 2006, . . .). That's why the $\delta^{13}\text{C}$ of some taxa is sometimes proposed as relevant proxies of environmental parameters (redox conditions in the sediment, exported organic matter flux) that are either partly or totally disconnected from bottom water signature.

[Reply] One of the important findings in this study is that species with low inter-individual deviations in isotopic composition are “more” suitable as direct proxies of the bottom water environment “in the same sampling site and in the same sediment depth.” We surmise that the ranges of inter-individual isotopic dispersions of certain

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

species are not always the same among different environmental conditions (redox condition, flux of organic matter, bottom water chemistry, etc.). We have to evaluate the reliability of our results in various environments to make reliable bottom water proxies in future studies.

[Dr. Fontanier's comment] p. 6199-6200. The paragraph 3.2 and the related illustrations (Figure 5 and Table 3) are slightly confusing. To be honest, I have got some doubts on the related conclusions (i.e. the applicability of inter-individual distributions (SD within species) to reconstruct the bottom water $\delta^{13}\text{CDIC}$). Why? (1) I don't trust in the isotopes values related to either *Stainforthia* or *Rutherfordoides* without specific determination. If you consider *Uvigerinids* for instance, signatures are totally different between *U. mediterranea*, *U. peregrina* and *U. elongatastriata* and they belong all to *Uvigerina* genus. (2) The authors have forgotten to add isotopes data of *Nonionella globosa*, *Nonionella labradorica* and *Takayanagia delicata* for the Sea of Okthosk in the figure 5. Am I right? If you add those average values (and related SD), it seems that mathematical regressions are suddenly much less convincing for this station. I may be wrong but the authors should discuss this point! (3) If the authors want to draw a convincing regression line, they should have provided the same quality of data for each species at each site (equal number of measurements per species at one site, only living foraminifera). It is not the case in the present study. (4) Coefficients of Determination (R^2) are high but it does not mean that the correlation coefficient (r) is statistically significant. The authors should calculate the r -value and the p -level of significance.

[Reply] 1) We did not discuss the isotopic data on a genus level. *Rutherfordoides* and *Stainforthia* did not consist of multiple species (not spp.) but rather single species. 2) As mentioned above, we will remove the data on *Nonionella globosa* and *Nonionella labradorica* from the revised manuscript. We have plotted the data of *Takayanagia delicata* for the Sea of Okthosk in the figures. 3) We provided the same quality and

C3390

BGD

9, C3380–C3393, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



quantity of data at each site (an almost equal number of measurements per species at one site). 4) We will add the p-values in the revised manuscript.

[Dr. Fontanier's comment] p. 6200-6201. Now again, and as explained above, the lower $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ recorded for the smaller and dead individuals belonging to *Brizalina pacifica* (MR) and *Stainforthia* sp. might be related to secondary calcite. Have the authors investigate the possibility of authigenic carbonate precipitation that may be related to cold seeps?

[Reply] We did not find any additional carbonate on the shell during microscopic observation of foraminiferal shells and sediments. If the authigenic carbonate affected the isotopic compositions of the foraminiferal shell, we would not be able to explain the variability of $\delta^{18}\text{O}$ (negative shift larger than the $\delta^{18}\text{O}$ variation of pore water). For $\delta^{13}\text{C}$, as you suggested, we might consider the possibility of authigenic carbonate precipitation, which may be related to cold seeps. In our studied site, no evidence of methane release is found. Also, the statement, 'No systematic difference was observed in isotopic values between living and dead individuals' supports the idea of a lower possibility of the addition of authigenic calcite to the foraminiferal shell.

[Dr. Fontanier's comment] Figure 1. According to the caption, the location of both stations A and B in the related map seems wrong and should be checked.

[Reply] We checked them and found that those stations are not wrong. Because Fig. 1 seems to be misleading, we will revise it.

[Dr. Fontanier's comment] Figure 2. *Rutherfordoides* sp., *Globobulimina affinis* (from Sagami Bay), *Bulimina aculeata* (from Nankai Trough), *Stainforthia* sp., *Nonionella globosa* and *Nonionella labradorica* (from Okhotsk Sea) should be pictured. Indeed, most

BGD

9, C3380–C3393, 2012

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper



of these species/genus are quite complex in terms of taxonomic identification. Therefore, some complementary illustrations would be very useful for readers interested in the related study areas. Moreover, SEM pictures for all taxa (with different views) would be more relevant than normal photographs. Please, don't use "sp." in italics for *Rutherfordoides* sp.

[Reply] We will add the photograph and SEM pictures in the revised manuscript. We will correct the *Rutherfordoides* "sp." in Table 2c.

[Dr. Fontanier's comment] Figure 3. Figure 4. Where are data for *N. globosa* and *N. labradorica*?

[Reply] As mentioned above, we will remove those data from the revised manuscript.

[Dr. Fontanier's comment] Figure 4. "R2" is not sufficient. "r" is required with the p-level of statistical significance.

[Reply] We will add the p-values in the revised manuscript.

[Dr. Fontanier's comment] Table 1. The presentation of sediment intervals used for pore water analyses is awkward. For instance, what is the meaning of "2"? Is it the sediment interval 1-3 cm? or the sediment interval 0-2 cm? What is the meaning of bottom water?

[Reply] Sediment intervals are ± 0.5 cm (e.g., "2" means "2 $\pm$ 0.5 cm"). Bottom water means the bottom water taken from the multicorer, about 5–10 cm above the sea floor. We will add these explanations in the revised manuscript.

[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)[Discussion Paper](#)

[Dr. Fontanier's comment] Table 2a-c. Please, precise in the caption the meaning of "cmbfs", and also the meaning of "*" used for *B. aculeata*.

[Reply] We will add the following explanation to the caption: "cmbfs denotes cm below the sea floor." We did not use "*" for *B. aculeata* but rather for *Cassidulina norvangi* in our manuscript. (Results of *Cassidulina norvangi* reported in a previous study (Ishimura et al., 2004) are denoted by an asterisk.)

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.

BGD

9, C3380–C3393, 2012

Interactive
Comment

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Interactive Discussion

Discussion Paper

C3393

