

## Reviewer #1

The paper "Carbon and nitrogen dynamics in the coastal Japan Sea inferred from 15 years of measurements of stable isotope ratios of *Calanus sinicus*" have a interesting data and a good sampling effort. However, the authors do not have concise hypotheses and cannot clearly achieve the objective of the work. Also I see that many comments from the reviewers was not accepted or incorporated into the text by the authors. The work has a beautiful data but is a lot of information, which probably caused confusion and made the work difficult to write.

The methodological from collection to laboratory analysis not have predronization what make difficult the analyses. Why was not done carbon extraction from the Copepods exoskeleton?

The writing in all the text is very confusing, the paragraphs don't have a beginning, middle and end, which makes reading very difficult, losing focus and failing to understand numerous points. The introduction and discussion remain disconnected from the results. The discussion has a lot of information unrelated to the results and the form of writing makes it difficult to understand what the authors would like to highlight. I'm not a English native speaker but the text needs a revision. I have made some suggestions that were added in the attached PDF that can help the authors. Given all these observations, I suggest the rejection of this article, I hope the authors could reformulate the article and in the future publish this article.

We appreciate the reviewer's efforts on our manuscript. We revised our manuscript where it was hard to read which the reviewer pointed, and believe the revised manuscript is well improved. Most of the comment is informative, and help our revision. Some comments, however, are unclear to us. The most important point is that we had revised the previous manuscript following the reviews comments. We cannot accept a few comments, because we did not agree with the reviewers' opinion, and cannot re-exam. We described why we cannot agree with the comment. This is a fair action. The reviewer commented that our pretreatment is questionable. This is very important and was still weakness of our manuscript, but we had showed that the effect of lipid to the carbon isotope ratio of zooplankton can be removed using C:N ratio (Smyntek et al. 2007). The correction of lipid effects using statistical models are normal and not rejected (Logan et al. 2008) (L125–127). In addition, as the reviewer pointed out, the frozen preservation changed both of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, but we cannot find the evidence on preservation period. This indicated that our method and data sets are appropriate. The revised parts were marked with yellow color.

Smyntek, P. M., M. A. Teece, K. L. Schulz, and S. J. Thackeray (2007), A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models, *Limnol. Oceanogr.*, 52(5), 2135-2146,

doi:10.4319/lo.2007.52.5.2135.

Logan, J. M., T. D. Jardine, T. J. Miller, S. E. Bunn, R. A. Cunjak, and M. E. Lutcavage (2008), Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods, *Journal of Animal Ecology*, 77(4), 838-846, doi:10.1111/j.1365-2656.2008.01394.x.

Minor comment

L12: need some introductory phase

We acknowledge the comment. We added “Both nitrogen and carbon dynamics have changed in the Sea of Japan” (L13).

L16: Is possible prove or give support for this, or is just a speculation? Write present portabilities, not confirm some that you can't prove

We believe the reviewer is mistaken on this point. We had used “attribute” which contained meanings of “speculation”. We did not confirm some that we can't prove (L17).

L19: this phase is not good,

We are uncertain as to the meaning of the reviewer's comment. We revised as “>70% of variations in  $\delta^{13}\text{C}$  can be explained with”. We agreed that how to vary is important, but that needs words. Thus, we omitted here (L20–23).

L22: Varies how?

We added the sentence as “The  $\delta^{15}\text{N}$  values of female *C. sinicus* and stage V copepodites were the lowest and highest, respectively” (L24–25).

L24: You have a good data but poorly explored and write. Please show how interesting your work can be. Also need more to conclude the Abstract.

We appreciate to the comments. We revised the conclusion sentence as “These results suggest that the carbon isotope ratio in the secondary producer has linearly changed in the coastal Sea of Japan over the past 15 years. Moreover, the changes in carbon

dynamics of this area have been recorded and observed to impact the marine ecosystem, while the nitrogen dynamics have not been recorded despite the increasing nitrogenous nutrient inputs in this sea” (L26–29).

L28: This paragraph need be better write, present how this sea can be impacted and show why. We thank the comment. We revised the Introduction section. We clearly described as “The Sea of Japan is regarded as an oceanic microcosm of the changing global ocean (Chen et al., 2017); thus, the impacts of changing inorganic chemical environments on biological production can be clearly detected compared to those in other oceans.” in the middle of the second paragraph (L46–48).

L32: What could be the cause for these?

We added “owing to global warming and the increase in atmospheric carbon dioxide (L43–44)”

L34: And from waste discharge and industry?

We thanked sharing opinion. It maybe but not clearly described in the previous.

L41: Just form this source. This information was write in the first paragraph. From the two first paragraphs make one, than finish talk about the Stable isotopes

We move this sentence to the end of the first paragraph (L32–34). To combine the first tow paragraph is good, but it was too long.

L44: Talk more about the biology of this copepod specie, how they can be a node in the trophic web, what can influence it's, and than who changes in this population can made a cascade effect.

We added the biological aspects on the *C. sinicus* (L54–L65).

L65: Understand or describe? I expected more than this objective loose here, what you expected found?

We fully revised the final paragraph in the Introduction. (L66–72).

L77: Stations with different characteristics? This can influence directly the plankton, how you available this?

We described the characters of four stations (L81–83): “Based on a previous study (Kodama et al., 2018a), the TB and IB stations were located in a cold-water area, and the NN and WB stations were located in a warm water area. Additionally, the TB station was located near the mouths of two rivers (the Sho and Oyabe rivers; Fig. 1), and the WB station was in an area of restricted circulation.”

L89: How long samples stay frozen? Was know that long times in this condition can change the isotopic signal.

Some samples were stocked over a decade (L91–92). We added. However, we could not the evidence on the frozen period and isotopic changes. We agree that some zooplankton species are not preserved in frozen at -20°C. In our samples, the euphausiids were not kept their forms, but *C. sinicus* were kept their forms clearly. In our knowledge, the difference of preservation method significantly impacts on the stable isotope ratios, and frozen samples are not same with the raw samples. In the ship-board observations, however, it is very hard to bring the raw samples to on-shore laboratory.

L116: Not continuous clear about the number of the samples collected and samples analyzed. what kind of sample?

We revised as “subsample” (L114 and 118). We checked the text from start to end, and revised.

L127: For carbon analysis, the samples of organisms (because of carapace) are not soaked in HCL to remove carbonates and exoskeletons? This can alter the C values?

This is an important point. We did not soak HCl. We could not find evidence that HCl soak are necessary for zooplankton isotope ratio. Instead, we soaked the picked *C. sinicus* into 3% NaCl aqueous solution in order to wash the bodies (we need to remove diatoms or other zooplankton organisms). We added this description (L111–112). The rinse by carbonate free water is an alternative method to remove the carbonate.

L140: Need be done the residual analyses to confirm this.

We thank this comment. We checked the dispersion values, and those of both the models were  $<1$ , suggesting gaussian distribution is appropriate. We added the dispersion values in the results (L194–195).

L168: This work have too much results, and the writing format make difficult follow the author's reasoning to understand what he wants to show. I suggest focusing on what you want to respond to with this work and being more objective, part of the results could be supplementary material to facilitate the reader in this study. All most part of the M&M and results is confusing and difficult to follow.

This is an important suggestion. We also are worried the descriptions of results. We considered that the simple comparisons among the areas or stages are not important. We moved them as the supplemental materials.

L179: Describe the complete results df and f?

We added (L168 and L170).

L254: All the paragraphs need follow the structure and need be connected with the next idea to be presented. in the format in which it is written, it is an agglomeration of information that does not bring the attention and recognition that its results deserve.

We thank sharing the opinion. We fully revised and simplified the discussion part based on this suggestion (L218–279).

## Reviewer #2

- 1) The annual trend of GLM residuals is compared with the annual trend of small pelagic fishes shown by Ohshimo et al. (2021) (lines 54,286,319) but the cited year trend of small pelagic fishes was estimated by the MARSS model without including environmental variables and fish size. Therefore, it is not suitable to compare with the trend estimated in this study. On the other hand, Ohshimo et al. (2021) also conducted a GLM analysis that considered environmental variables similar to the present study, and the linear year effect in that model for d13C was estimated to be about -0.05 per mil/year. Although it is difficult to compare with Ohshimo et al. (2021) due to the difference in the significant digits, it seems that the slope shown by Ohshimo et al. (2021) is not necessarily larger than the slope shown in this study, considering the uncertainty estimated in this study.

We appreciate to this comment. In Ohshimo et al. (2021), the uncertainty was not presented: in their MARSS model, the standard error of slope cannot be calculated. Considering the 95% confidential intervals (mean  $\pm$  1.96SE), 0.5‰ year<sup>-1</sup> is within our estimated interannual variation of d13C. Therefore, we revised the manuscript as “within the ranges”. We revised the discussion at L256–263.

- 2) The sampling method and the stable isotope ratio analysis of POM should be described in different sections for sampling and analysis. Specifically, information of sampling (when and where the samples were derived) should be described in 2.1, and methodology of stable isotope ratio analysis for POM should be described in 2.2. Also, the results of stable isotope ratios of POM (lines 157-159) are easier to understand if they are included in 3.2. The abbreviation of POM should be described where it first appears (line 87).

We appreciated to this comment, too. We added the descriptions of samplings (L103–106), and isotope analysis (pretreatment, L131–133). The results of POM were described in section 3.2 (L172–175).