Response to Reviewer #1

General comments

In the introduction the authors mentioned quite extensively the anthropogenic impact on coastal seas, but it seems the data and consequently the results do not directly link to this topic. Although, they bring up interesting ideas (partly supported by literature) of possible links in the discussion.

We acknowledge the reviewer 1's comments. We believe our revision based on these comments will improve the quality of our manuscript. We reduced the descriptions of anthropogenic impacts because we cannot evaluate whether the variation is anthropogenic or not. We fully revised the discussion based on both of two reviewers' comments. At first, we found the duplicates in our data sets, and thus we removed 7 observations from the analyses. Secondly, we found the C:N ratio of all of our samples. The C:N ratio and the stages of *C. sinicus* were treated as the explanatory variables in the GLMs. Thirdly, we used the residuals of the GLMs for identifying the monthly and interannually variations. These revisions changed our results on the statistical analyses, in particular, the trend of the $\delta^{13}C$. The residuals of $\delta^{13}C$ in the GLM indicated that the $\delta^{13}C$ of *C. sinicus* was significantly decreased. Therefore, we revised our manuscript completely.

In my opinion, the objectives presented at the end of the introduction are a bit too vague and it would help if the authors could present some of their expectations (they kind of do in the abstract) regarding the isotope values and mechanisms behind. It seems that the authors talk a lot about anthropogenic impacts but their aims and data are a bit vague to the subject. Similarly, they talk about lower-trophic-level ecosystems but never bounce back on this idea with their data analysis and results and only briefly brings it back to the discussion. For this reason, the introduction and discussion felt a bit disconnected from the data and their results.

We acknowledge this comment. Aforementioned, we found new data, and revised the manuscript. As the reviewer suggested, our manuscript did not confirm that our detected interannual variations is anthropogenically impacted. Based on our revision, we detected the decline trends of $\delta^{13}C$. However, our observed parameters were very limited, and we considered the decline trends of $\delta^{13}C$ is exactly impacted by human activity. Therefore, we revised the aims of this study, and concept of this study the descriptions on the anthropogonic effect was reduced, and descriptions of local interests is increased.

The environmental data that have measured while sampling copepods are temperature, salinity and chlorophyll. The analysis they performed on this data (I have no complaints about it) are somehow *disconnected from context (and aims) of 1) showing copepods isotope ratio would record anthropogenic impacts (as written in the abstract) and 2) of a shift/change in lower trophic level. I would have expected other proxies for anthropogenic impact (e.g., nutrients concentrations) and trophic level estimation (i.e., isotope signal of POM) to support such hypotheses in relationship with copepod's isotope data. From the literature cited in discussion, it seems such data are available (at least POM isotope signal, Antonio et al 2012) which they could have used in some analyses to strengthen their says in discussion.*

We acknowledge this comment. Because this study is the result of long-term monitoring, the environmental data were very limited. Now, we reduced the descriptions on the anthropogenic effects: anthropogenic effects would be important, but we considered that our observations cannot be detected its effects. We cannot understand the meaning of the last sentence exactly. We collected stable isotope values of POM in two cruises nearby the observation area (April 2017 and May 2019). We added this data, and revised our discussion based on this data. On the basis of our stable isotope ratios of POM, the $\delta^{15}N$ of *C. sinicus* were considered to be reflected that of $\delta^{15}N$ of POM, but $\delta^{13}C$ of *C. sinicus* were not reflected the $\delta^{13}C$ of POM. The physiological processes of *C. sinicus* must be considered. However, the GLM approach indicated physiological processes are also controlled with the environmental parameters. Therefore, the residuals of $\delta^{13}C$ can be treated as the index of temporal variations. We considered that our revised discussion is more careful and stronger than the previous.

To me there is also a conceptual problem of using copepods to mirror anthropogenic changes due to their relatively short life cycle, mobile nature and fast tissue turnover. I would expect those to reflect seasonal variability in their food source and eventually anthropogenic activities. A direct link to such variables would thus be appreciated.

We do not agree with this comment. We considered that zooplankton including *C. sinicus* is better target to understand the environmental changes. Of course, as the reviewer 1 pointed, the seasonality of stable isotope ratio must be considered, but they moved with water masses, and sampling and measurements are easier than POM or fish. The collection of fish may be easy, but the environmental parameters cannot obtain. Besides, in Ohshimo et al. (2021), we evaluated the long-term variation of stable isotope ratios of fish, but to evaluate the trend, we must apply the time-series analysis because the time series data of fish contained auto-correlations. The stable isotope ratio of POM is considered as best to evaluate the trend because they directly reflected the environmental conditions. However, we need much water for measuring the stable isotope analysis of POM. in the coastal area, water environments were heterogenous, and so frequent samplings were necessary for understanding the temporal-spatial variations. The high-resolution samplings of stable isotope ratio of POM were quite difficult; therefore we considered the zooplankton observation is better for monitoring.

I also think the structure of the results could be improved by following the objective(s) and the methods (in the methods it is clear which equation the authors used to test their questions, but the questions are unclear in intro and do not appear in results). Results should be restructured to mirror the objectives and analyses for instance from the temporal aspect (long term and seasonal) and spatial (comparing stations) so it doesn't read as a report or a textbook. As an example, 3.3. currently named 'Generalized linear models' should absolutely be changed (and maybe split in different sections) to something more informative such as 'temporal trends' or 'seasonal patterns in copepods isotopes'... the idea is to better connect aims with the statistical analyses the authors chose and to the results they found. Again, I would recommend the author to consider some kind of data analyses more directly related to anthropogenic impacts and to trophic level estimation to better support their say in discussion and the general thematic of the introduction.

We agree with the reviewer's comment. We firstly compose the complex model, but we removed the model in the final version. The model can contain temporal trend (interannual and monthly variations) as well as the environmental parameters. We did not apply the temporal variations directly in the model to avoid the collinearity with the environmental parameters. To overcome this problem, we calculated the residuals, and show their temporal variations. We found that residuals of $\delta^{13}C$ are significantly decreased in these 15 years. These suggested that $\delta^{13}C$ of *C. sinicus* is decreasing. We added this in the revised manuscript.

The discussion is well documented and the author explored different explanation they bring up. However, some statements would need a bit more in depth connexion with the literature hey cite and also with the data. It seems sometimes some statements are disconnected from the results.

We carefully revised the discussion.

A last remark, I am not myself a native speaker but the manuscript was not so smooth to read through. Even though the manuscript has been revised by professional editors, one being a native speaker, it reads odd in some parts and there is a lot of repetitive sentences around one same idea/say that could *be condensed for a better reading flow (see examples in detailed comments).*

We are sorry for that. We are not native speakers, and so we usually depended on the revised sentences by the natives. We revised based on the detailed comments. If our revisions are insufficient, our manuscript will be checked again in the next revision.

Detailed comments

Abstract & introduction

L 12-13. Move 'during the last half century et the beginning of the sentence.

We deleted this sentence (L12).

L 19. high d13C values in copepods were associated...

We deleted this sentence (L16).

L 27-31. These three sentences read odd and feel a bit repetitive... human activities is repeated three times over two lines.

We shortened the first sentence as "Coastal ocean ecosystems are important for human activities and have been greatly changed as a result of those activities." (L26–27)

L37-46. This selection of lines should be a paragraph all together, talking about stable isotopes. Lines 45-46 are redundant with lines 37-38.

We revised as suggested (L34–42).

L 47. 'in this study' this should be the beginning of a paragraph in itself talking about Calanus.

We revised as suggested (L43).

L 65. The aim of the study should be clearer and it would be great that the authors give some expectations regarding the results. For instance, their expectations of isotope changes in copepods regarding anthropogenic activities.

We appreciate and agree with this comment. We revised our aims with the leading sentences (L56–60). We did not describe anthropogenic impacts, and only described as "identify the long-term variations", because the previous studies pointed that stable isotope ratios of carbon and nitrogen in food of small pelagic fish in the Japan Sea with chemical environments.

L 66. The spatiotemporal variations of lower-trophic levels ... variation of what ? chemistry ? food quality ? trophic interactions ? food web structure ?

We deleted this sentence (L60).

Material and methods

L 84. Remove 'a temperature below'

We revised as suggested (L76).

L 100-103. Unclear how copepods were collected, pooled. 94 nets but 274 dried samples ? Please clarify this section.

We revised the sentences to clarify the meaning (L94–99).

L 103-104. At some stations [...] data from the same station. This sentence should be in data analysis section.

We revised as suggested (L115–116).

L 113-114. An example of sentence to rewrite. It is confusing the way it is currently written, especially mentioning twice the 'database' that was not introduce before. Maybe something like this could be an option: 'The amounts of carbon and nitrogen in each sample were also measured from which the C/N ratio of C. sinicus was calculated, however, this information was missing for 69 samples.

We revised. We re-searched the raw data, and finally found. Therefore, we can show the C/N of all subsamples. Based on the C/N ratio, we calculated the lipid free d13C values based on Smyntek et al. 2007, and used the C/N ratio as the explanatory variables in the GLM (L108-109).

L 116. Cite which environmental parameters.

We revised as suggested (L117).

In the 2.3. you mention what each equation is meant to test. Please keep the same idea of a structure in the way to present the results.

We appreciate the comments. We revised the descriptions of results (L203–230).

L 128. Interannual variations of stable isotope at every station

We revised as suggested (L130).

L 141. Note that we considered ...

We revised as suggested (L143).

Results
L 146. 3.1. Environmental variables, not factors

We revised as suggested (L148).

L 147. Please write fully the acronyms SST SSS SSC at the beginning of each section (here results section).

We revised as suggested (L149, 151 and 153).

L 149. Why giving the range of SST and SSC and not SSS ? Or at least an average and sd ?

We added the range (L152).

L 157. It is unclear of you describes the ANOVA in the methods.

We added the descriptions (L112–116).

L 173. This section could have its own title.

We entitled as “Relationships between environmental parameters” (L193).

L 182. 3.3. title of this section should reflect the findings or objectives and not mention the type of analysis. From line 173 to the end of the results, it would be very nice to link it with objectives of the study (e.g. spatial patterns, temporal patterns, link with environmental data). For instance, l 196, this is the beginning of the temporal aspect it seems.

We revised and entitled as “Temporal variations of residuals” (L219).

Discussion

L 227. This statement needs more support or explanations.

We added the results of POM, and also added discussion. The discussion was completely revised (L232266).

L 229. Carbon and nitrogen in copepod tissues

We revised as suggested (L235).

L 240-244. This is typically an example of redundant sentences that makes the manuscript hard to read. Please condense, rephrase or reorganize.

We reorganized the discussion and sentence (L255–258).

L 251-252. This statement can be tested with POM isotope values (for one given year for instance depending on available data).

We revised based on this comment (L243–278).

L 260-261. This statement needs more support.

We revised, and we reached a conclusion as neither $\delta^{13}C_{bulk}$ nor $\delta^{13}C_{ex}$ of *C. sinicus* is not reflected the $\delta^{13}C$ of POM at the same station (L243–278).

L 277-278. I do not agree with this statement. It doesn't seem that your data support this local hypothesis or need to be explained in a clearer way (that you have local differences in isotope signal

of copepods, then have a spatial hypothesis to be tested). It needs stronger evidences.
L 280-284. This needs to be brought up in the introduction.

We deleted these discussion to clarify our message.

Response to Revewer#2

Recent studies certainly revealed material cycles are changing rapidly on the global scale, while connectivity between coastal (local) and large-scale ecosystem is rather unknown. Based on this background, this study aimed to clarify the anthropogenic effect on coastal ecosystem in terms of carbon and nitrogen cycle through the stable isotopic signature of planktonic copepods. To achieve the goal, the authors examined sample collected four different sites where have specific local oceanographic condition for 15 years. The obtained results were analyzed with basic environmental variables such as temperature, salinity, and chlorophyll-a concentration with the generalized liner model (GLM). However established model outputs were somehow not surprising as the all of the parameters used in the model are covariable affected by progress of season. Consequently, the derived conclusion, "local conditions rather than global-scale trends were the primary determinants of elemental cycles in this coastal ecosystem" was quite vague in the light of the research objectives as they were not successful to determine the anthropogenic effect in the coastal ecosystem. This is largely attributable to their insufficient design of the research plan from sampling, analysis and discussion as shown below.

We appreciate the reviewer #2's comments very much. The comments indicated our plans are insufficient, and we must agree with this comment. We made an effort as much as possible in this revision. We re-checked the raw data, and found the C:N ratio and sampling records of *Calanus* stages. Of course, we considered that our data sets are not perfect after this revision. It was because this study was the result of the long-term monitoring, and the primary aims of the observations were not to identify the changes of oceanic environments. The long-term monitoring is conducted with very limited efforts, and scientists were not same. We believe that it is publish the dataset as papers for the future sciences.

Above all, it appears that the authors did not have clear hypothesis in this study. Although they stated in the abstract as "We hypothesized that the carbon and nitrogen stable isotope ratios (δ13C and δ15N) of the copepod Calanus sinicus, one of the dominant secondary producers of North Pacific coastal waters, would record anthropogenic impacts on the coastal environment of the Japan Sea.", they did not specify what kind of anthropogenic impacts they assumed. For example, in the introduction they mentioned that "long-term trends in the amounts of anthropogenic inputs are not spatially uniform: since 1997 total nitrogen inputs from rivers to Toyama Bay have been decreasing (Terauchi et al., 2014b) and those to Wakasa Bay have been increasing (Sugimoto and Tsuboi, 2017)." However, such topics were not discussed elsewhere in the interpretation of results, which is very *disappointing. Many processes such as input of fertilizer through river, deposition of nitrogen oxides by precipitation, eutrophication, phosphate depletion, hypoxia, and denitrification could be involved with nitrogen isotopic signature. These parameters should have been taken in to account for data interpretation and/or modeling.*

We are sorry for disappointment. We mostly removed the descriptions on the human activities. In our observation area (the Japan Sea), *denitrification with hypoxia* were not observed. Therefore, we can ignore the DO concentration. We revised the aims of this study.

It is also questionable why C. sinicus was selected as proxy to detect the anthropogenic effect in the coastal ecosystem. Certainly, C. sinicus is key species as secondary producer, its isotopic signature is involved with very complex process of phenology which affect the metabolism, lipid storage, and behaviour including vertical distribution. The study period is focused on the timing that C. sinicus commence the maturation to reproduce and perhaps summer dormancy, indicating that isotopic signature was affected not only by environmental variables but also these processes related with phenology. The planktonic copepod population would be affected by water movement as well. These facts imply that C. sinicus was not best proxy to detect the anthropogenic effect in the coastal ecosystem. In my opinion, phytoplankton (POM) would be more appropriate to detect the anthropogenic effect in the coastal ecosystem as it would directly respond above-mentioned environmental parameters. Alternatively, organisms at higher trophic level like fish would be appropriate because of its longer life span which effectively average and accumulate the anthropogenic effect for certain period.

We don't agree with this comment. Zooplankton may not be best for identification the long-term variation of carbon and nitrogen dynamics, but better for monitoring. Of course, the POM directly recorded the changes with the anthropogenic effect. However, our sampling sites are under the influence of rivers, therefore, the spatiotemporal variations of POM values were expected to vary largely based on the mixing ratio of river water and seawaters. These suggested that we need more frequent sampling to detect the trends based on the POM. The fish is used as the detecting trends, and we also conducted. However, to identify the trend based on the fish muscle, the auto-correlation must be considered. In addition, as same as the zooplankton, the fish muscles had seasonality. To minimum the sampling efforts (that is very important for the long-term monitoring), the zooplankton may be best.

I was also disappointed that the authors disregard of the ecology of C. sinicus during the study. It is well known that this species shows ontogenetic change in physiology and behavior during the maturation from CV to adult. As CN ratio between CV and adult is greatly different in C. sinicus (e.g. Pu et al. 2004, JPR 26: 1059-1068), it is clearly inadequate to analyze these two stages altogether with random ratio. I suspect that CN ratio of adults is also different depending on the sex and egg production stage because of eggs contain lots of lipids. Although the authors briefly discussed about the effect of lipid storage on d13C in the discussion, such indefinite argument could have been avoided if they care about the ecology of the target species. If they have the data of population structure of C. sinicus in the sampling station, I recommend to include them in the analysis. Although the authors did not mention at all in the manuscript, it is also well known that population structure, physiological status, vertical distribution of C. sinicus are variable depending on the environment even in the same period (e.g. Pu et al. 2004, JPR 26: 1049-1057 Pu et al. 2004, JPR 26: 1059-1068, Zhou et al. 2016, JPR 38 etc.). These features might be advantageous to achieve the initial goal of this study, yet appropriate data set of environmental varables including isotopic signature of POM is still inevitable.

We apricated to this comment very much. We agree that SI ratio of *C. sinicus* varies with the life stages. This study is results of our long-term monitoring, so the sampling quality is different among the samples; when an expert of zooplankton was in our team, we can divide the samples into copepodite V, adult female, and adult male, however, when an expert was not in, we can only pick up as *C. sinicus.* The recorded data was not all, and thus we made the category “mixed” to treat them in the GLM. The “mixed” was considered as several life stage were mixed, but we did not know. However, significant difference of d13C was not observed among the life stage after correction using C:N ratio. Therefore, in the case of d13C, we can ignore the impact of the life stage by usinf C:N ratio. On the other hand, we cannot ignore the difference of d15N. The difference was small (0.5‰), but we clearly described on this (L293–296). The references which introduced in this comment were referred in the revised MS (L96).