

Ocean Sci. Discuss., author comment AC2
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Reply on RC2

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Author comment on "Carbon and nitrogen dynamics in the coastal Sea of Japan inferred from 15 years of measurements of stable isotope ratios of *Calanus sinicus*" by Ken-ichi Nakamura et al., Ocean Sci. Discuss., <https://doi.org/10.5194/os-2021-74-AC2>, 2021

Response to Reviewer#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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) 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 variables including isotopic signature of POM is still inevitable.

We appreciated 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 using 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).

Please also note the supplement to this comment:

<https://os.copernicus.org/preprints/os-2021-74/os-2021-74-AC2-supplement.pdf>