

Dear Editor,

We finished the revision of the manuscript according to the questions and advices of the four reviewers. The following are the details of our responses (in blue color) to questions and advices of every reviewer.

The work of reviewers help improve the quality of the manuscript. We thank the thoughtful advice of the reviewers and hope the revision successfully answered the questions.

Best wishes

Wuchang Zhang

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Reviewer #4 (CC3): The manuscript "Decoding pelagic ciliate (Protozoa, Ciliophora) community divergences in size spectrum, biodiversity abd driving factors spanning global five temperature zones" by Wang and collaborators uses an impressive dataset on the distribution of pelagic ciliates over different ecological regions, describing important community features such as size and species composition using environmental parameters to contextualize their findings. While the data is fantastic and should be published, the current version of the manuscript still needs further work. I provide some specific comments below:

1) The language should be revised and the text can be streamlined in several parts. A clear example is the title, which is rather long and not really informative.

Response: We accepted suggestions and revised the whole manuscript accordingly. As for title, it conveyed the three main themes of this manuscript, thus we revised into "Decoding pelagic ciliate (Ciliophora) community divergences in size spectrum, biodiversity and driving factors spanning global five temperature zones" in revised manuscript.

2) Considering that you target only one planktonic group, maybe the normalized size spectra approach is not the best to describe the variation in sizes (as also discussed by the authors in the manuscript). Could simple metrics, such as the average size be more informative?

Response: We agreed with your viewpoint that the normalized size spectra approach is not the best to describe the variation in sizes for only one planktonic group. While regarding different temperature zones, there were several variations for pelagic ciliates lived in oceanic habitat. We tried to find out their divergences in size spectra aspect. Actually, we used the average size of each ciliate size-fraction (for instance, we used the 15 μm size-fraction in 10–20 μm size-fraction) in size spectrum analysis in the manuscript. We also revised accordingly in lines 114–115 in revised manuscript.

Lines 114–115: Furthermore, we select the average value (15, 25, 35, 45 μm ,..., etc) of each size-fraction of both loricate ciliate and tintinnid as the counting criterion for

ciliate size spectra (Wang et al. 2024b).

3) The authors should also consider other traits than size to describe the communities, such as the presence/absence of lorica and trophic mode could be more meaningful than the normalized size spectra.

Response: Dear reviewer, thank you very much for proposing these valuable suggestions (the presence/absence of lorica and trophic mode) for pelagic ciliate trait study. To be honest, we counted the presence/absence of tintinnid lorica only in recent two years (starting from 2023 in the Arctic Ocean). Thus relative data was not recorded in the Bering Sea, North Pacific, tropic western Pacific and Indian Ocean. Therefore, we can not conduct this trait structure. Concerning trophic mode, pelagic ciliate belonged to the top grazer of the microbial food web. To date, we already start to study its role in the microbial food web, and relative study in tropic western Pacific have been published in Marine Pollution Bulletin. The other one relate to the Arctic Ocean just submitted in the Global Biogeochemistry Cycles. In the near future, we will put more emphasis on uncovering trophic mode of pelagic ciliate in marine ecosystem.

4) I have reservations about how the statistical methods were used by the authors. A constrained ordination using the entire data set might be more appropriate than the ordination analysis. In addition, the relationship between the community and environmental variables could be done with a more comprehensive model (e.g. GLM that also includes zone and depth as independent variables).

Response: Dear reviewer, we appreciate for your valuable advice that using the entire data set might be more appropriate than the ordination analysis. To be honest, we hold the similar viewpoint with reviewer 2 that the large differences in the relationship between biological and abiotic organisms in different temperature zones may be mainly caused by the difference in the selection of sampling areas, rather than the fundamental differences between temperature zones. Therefore, the stratigists we have adopted were compared the internal correlation among each temperature zone at specific sampling depth (0, 50, 100, and 200 m). In Figure S10, we just want to find out the linear relation between ciliate and each environmental factor at all sampling depth among each temperature zone. Additionally, we are really sorry that we did not conduct a comprehensive GLM model due to our complex data in both latitudinal and vertical directions, thus we have no idea on how we conduct this model.

5) Considering that seasonality is also important to modulate protozoan communities, are all the datasets comparable in this regard?

Response: We acknowledged that seasonality is important to modulate protozoan communities, but this phenomenon was obvious in both temperate and polar seas. Regarding tropic seas in both the Pacific and Indian Ocean, the community structure including vertical distribution pattern, abundance and biomass values, species composition were almost same (e.g., Sohrin et al., 2010; Li et al., 2018; Wang et al., 2019a, 2020, 2022b). In other words, seasonality might not be a driving factor for pelagic ciliate community in tropic seas.

References:

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- Li, H. Zhang, W. Zhao, Y. Zhao, L. Dong, Y. Wang, C. Liang, C. and Xiao, T.: Tintinnid diversity in the tropical West Pacific Ocean, *Acta Oceanol. Sin.* 37, 218–228, doi:10.1007/s13131-018-1148-x, 2018.
- Wang, C. Li, H. Zhao, L. Zhao, Y. Dong, Y. Zhang, W. and Xiao, T.: Vertical distribution of planktonic ciliates in the oceanic and slope areas of the western Pacific Ocean, *Deep-Sea Res. II* 167, 70–78, doi:10.1016/j.dsr2.2018.08.002, 2019a.
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- Wang, C. Li, H. Dong, Y. Zhao, L. Grégori, G. Zhao, Y. Zhang, W. and Xiao, T. Planktonic ciliate trait structure variation over Yap, Mariana and Caroline seamounts in the tropical western Pacific Ocean. *J. Oceanol. Limnol.* 39, 1705–1717. doi:10.1007/s00343-021-0476-4, 2021.
- Wang, C. Zhao, Y. Du, P. Ma, X. Li, S. Li, H. Zhang, W. and Xiao, T.: Planktonic ciliate community structure and its distribution in the oxygen minimum zones in the Bay of Bengal (eastern Indian Ocean), *J. Sea Res.* 190, 102311, doi:10.1016/j.seares.2022.102311, 2022b.

6) Some of the sampling campaigns occurred over relatively large areas, which could be sampling over systems divided by oceanographic features, such as fronts. Was the intra-zone variability taken into account?

Response: We aware that sampling campaigns occurred over relatively large areas and the marine pelagic ciliate community exhibited some differences by oceanographic features. Actually, the intra-zone variability was taken into account during writing process. In a whole, compared to different temperature zones, we found that the intra-zone variability of ciliate community was negligible.