

Interactive comment on “Variability in copepod trophic levels and in feeding selectivity based on stable isotope analysis in Gwangyang Bay off the southern coast of Korea” by Mianrun Chen et al.

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General Comments: The authors used stable isotope analysis to solve copepod trophism (i.e. food resources and trophic level), which is important to understand the biogeochemistry in estuarine system. The findings on copepod trophism in the manuscript (MS) will contribute for understanding pelagic food webs in the system. These are valuable and positive points in the MS. Nevertheless, I found many doubtful points throughout the MS. The authors simplified the dynamics of copepod community by considering the most dominant copepod species only, and then applied this simplified copepod assemblage to the stable isotope analysis. As a result, trophism of some

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copepod group, especially carnivorous copepods are still questionable. The current method (e.g. Boyesian mixing model) and the assumption (e.g. the body mass of different genera among calanoids are the same) applied to stable isotope analysis may have some limitation to evaluate the real trophism of the copepods in the field, even though most results of copepod trophism in the MS were similar to previous reports. Therefore, I would like to recommend the authors to include an additional explanation on a potential limitation which may occur when you apply the current method and assumption to copepod community, in the revised MS. - Response: We thank the reviewer for his/her careful checking of the manuscript and we agree his/her general assessment of our work and are happy that he found our valuable and positive points in the MS. We agree that the current method has some limitations to study the entire complex planktonic structure. However, the reviewer may misunderstand our data set as we were not just considering the most dominant species only, whereas we considered all appearing copepod genera in statistics when we interpreted the dynamics of the entire copepod community or specific taxonomic groups like calanoids and cyclopoids. We believed that copepod genus can be grouped based on similar feeding behaviors and thus the food web structure can be simplified.

Specific Comments: P6, line 20-22: The author's assumption is questionable. Calanoids consist of many genera or species with various sizes. Even though some large calanoids are not dominant in the sample in terms of abundance, some large calanoids (e.g. *Calanus*) can have important role in terms of biomass or volume. So, the author's assumption may not apply to a mixed copepod community with existence of both small and large copepods. - Response: We admit that this assumption was a little bit inaccurate. Unfortunately, we didn't record the body length of our taxonomic data. As suggested by another reviewer, we will re-calculate their biomasses based on the empirical formula of biomass and body length of different copepod genus and use the ratio of body length among different genera.

In relation to this issue, how did the authors treat copepodite stages of the copepods

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occurred in this study to calculate their abundance or body mass? There is no explanation in the materials and methods. - Response: Copepodites were counted but grouped together with adults.

P8, line 13-19 and Table 1: There is no criterion for dominant species in Table 1. The authors listed only the most abundant copepod species by station and season. I think more than one copepod species would have contributed to copepod community in the field. Please specify a criterion and also show other copepod species if possible (in fact, the information on copepod species composition is poor and not informative in this study). - Response: Species with abundance consisting more than 10% of total assemblage was considered as dominant species. We agree to show the composition table as supplementary materials in the revised version.

P10 line 31-33: More detailed explanation may be needed, like in the case of $\delta^{15}\text{N}$ in Fig. 6A and 6B. - Response: Trophic enrichment [Trophic enrichment (or fractionation factor) from basal food items based on the difference of each sample's $\delta^{15}\text{N}$ between higher trophic level to lower trophic level] was explained in the figure legend. Maybe our explanation makes the reviewer confused. We will explain more careful in Materials and methods in the revised version.

P10 line 18: There are no results for Centropages in Fig. 4, Fig. 5 and Table 1. However, the authors showed the dietary compositions of Centropages as a major omnivorous copepod genera in Fig. 10D. Why? - Response: As the occurrence frequency of Centropages was low, so that the $\delta^{15}\text{N}$ estimated from linear mixing model was insignificant, thus we cannot obtain the isotope bi-plots, trophic level and trophic enrichment of this species. However, we obtained a significant value of $\delta^{13}\text{C}$, and we use the average trophic enrichment of marine calanoids thus we could obtain the information of diet composition using SIAR package.

P11 line 29-32: The authors did not consider either cyclopoids or brackish water calanoids because those are not co-occurred with Labidocera, a surface water species.

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However, I believe that Labidocera has a chance to contact other preys beside *Acartia* and *Paracalanus*, such as cyclopoids and brackish water calanoids. If the authors check the copepod community in summer, not only dominant species but other sub-dominant species (not shown in Table 1), there are many adult and copepodite copepod species that can be a potential prey for Labidocera. So, please add potential prey in Fig. 11. - Response: Agree to do so.

P11 line 32- P12 line 1-2: For *Sinocalanus*, potential prey including brackish water calanoid such as *Pseudodiaptomus* should be tested in Fig. 11. Also, I failed to understand that why *Acartia* was considered as prey for *Sinocalanus* in Fig. 11, considering *Acartia* was not dominant species in autumn in Table 1. - Response: Agree to add *Pseudodiaptomus* as potential prey for *Sinocalanus*. *Acartia* continuously occurred though they were not dominating in the autumn.

P14 Line 23-25: I understand that calanoids (both marine and brackish water types) and cyclopoids had different $\delta^{15}\text{N}$ values according to Fig. 6B. However, the authors mentioned the mean value of the group was the same. Please check again. - Response: We may make confuse to the reviewer. In this sentence, we primarily discussed the similar trophic niche among the three major copepod group so that the referring figures should only contain Fig. 4A and Fig. 5. We will delete to refer Fig. 6 in the parenthesis as Fig. 6 was trophic enrichments on different sizes of plankton, which were estimated and averaged from all seasons and all stations.

P15 line 3: There is no result of the brackish water species, *Pseudodiaptomus* in Fig. 4, but in Fig. 5. Why? - Response: We can add the result of *Pseudodiaptomus* in Fig. 4.

P15 line 20-24: *Corycaeus affinis* was evaluated as omnivorous in this study, but as carnivorous in previous reports. What is a possible explanation for this difference? - Response: We believe that the feeding behavior of *Corycaeus* was not fully examined in literature. We have checked the existing literature more carefully about the feeding behavior of *Corycaeus*. However, we found that some reference suggests *Corycaeus*

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are omnivores based on investigation of the contents of fecal pellets (Turner, 1986). There were some large-sized diatoms (*Thalassiofrix* sp.) found in the pellets. Although *Corycaeus* can locate the prey visually, they can sometimes switch to filter-feeding and act as herbivores based on prey concentrations, according to investigation of the size and shape of the cutting edge of the mandibles (Giesecke & González, 2004)

P15 line 27-31: I believe decapod issue is not necessary for this study. Why did the authors include decapod results? - Response: We agree and will delete it in the revised version.

P15 line 31-33: There is no result of *Euterpina* as a genus of benthic harpacticoids in the results section, but only as harpacticoids. However, the authors mentioned *Euterpina* was detritivores in discussion and conclusion. In case of cyclopoids, the trophic level of cyclopoids and *Corycaeus* was presented separately in Fig. 4. Why? - Response: We directly measured the isotope values of the harpacticoid sample which primarily composed by *Euterpina acifrons*, while we didn't have the detail data of different harpacticoids. For Cyclopoids, we estimated the isotopic ratios of different genera and *Corycaeus* were significantly dominated.

P16 line 13-16: Even though *Acartia* dominated the marine calanoids in winter and summer, it is questionable to say that the bulk copepod assemblage with various species prefers large particles (microplankton; Fig. 7A and 7B). Likewise, *Paracalanus* also dominated the marine calanoid community in the more saline region in winter (Table 1), and *Paracalanus* prefers small particles (nanoplankton; Fig. 10). *Paracalanus* and other marine calanoids other than *Acartia* also may have contributed to the feeding selectivity of the bulk copepod assemblage differently. - Response: Yes, the feeding selectivity of the bulk copepod assemblage was a balance of ingestion among different groups. Our results showed a mean value of diet contribution of the bulk sample from all stations at a given season. When the bulk assemblage was shown preferring to feed on large-sized of POM, those species preferring large particle (e.g. *Acartia*) would play

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a more important role in the assemblage in feeding prey. On the other hand, in the spring and autumn when the assemblage was primarily dominated by *Corycaeus* (our result suggested this genus was an omnivorous species and the size-selectivity was less pronounced), the assemblage overall didn't show an apparent size-selectivity.

P16 line 31: *Corycaeus affinis* dominated copepod community in spring and autumn, except for the river mouth. This result is inconsistent with previous reports in the same region; *Corycaeus affinis* was not a dominant species in spring and autumn (Kwon et al. 2001, Jang et al. 2004). I am very curious about the difference. My speculation is that horizontal net towing (0.5-1m depth) in the deeper region in this study may be responsible for potential bias of copepod composition. (Kwon KY, Lee PG, Park C, Moon CH, Park MO. 2001. Biomass and species composition of phytoplankton and zooplankton along the salinity gradients in the Seomjin River estuary. *The Sea, J Korean Soc Oceanogr*, 6: 9-102 Jang MC, Jang PG, Shin K, Park DW, Chang M. 2004. Seasonal variation of zooplankton community in Gwangyang Bay. *Korean J Environ Biol*, 22: 11-29) - Response: It is hard to give a correct speculation for this difference. However, I think annually variation due to ecosystem change is normal. This species is now quite common around the World Ocean and worth to study more carefully in the future. Nevertheless, we used the same sampling way between taxonomic data and isotopic data, as well as among different seasons. It wouldn't have any uncoupling of community composition and the trophic information of the assemblage.

P16 line 32: The authors concluded that *Pseudodiaptomus* was a detritivore, feeding on small phytoplankton cells. However, recent paper (Kayfetz and Kimmerer 2017) showed that *P. forbesi* in San Francisco Bay is rather omnivores feeding on various kinds of preys including centric diatom, pennate diatom, diatom (7-15_m), flagellates, flagellate (7-15_m), dinoflagellate and ciliate in the laboratory. (Kayfetz K, Kimmerer W. 2017. Abiotic and biotic controls on the copepod *Pseudodiaptomus forbesi* in the upper San Francisco Estuary. *Mar Ecol Prog Ser*, 581: 85-101) - Response: The reviewer may misunderstand our conclusion or our explanation may cause some confusing. We

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found that *Pseudodiaptomus* were able to feed on plankton based on the mixing models and showed that *Pseudodiaptomus* preferred small-sized particle comparing the two major prey items (Fig.10 C). However, the $\delta^{15}\text{N}$ of *Pseudodiaptomus* estimated from the bulk sample was so low that we speculated that the detritus with low $\delta^{15}\text{N}$ may contribute to the balance the $\delta^{15}\text{N}$ of *Pseudodiaptomus*. Thus, we concluded that *Pseudodiaptomus* were primarily an omnivorous species which preferred on small-sized particle by filter-feeding and was also strongly influenced by detritus.

P17 line 5-6: The authors mentioned that harpacticoids contributed to total copepod diet, preferring microplankton in winter (Fig. 7A), because harpacticoid preferred microplankton (Fig. 9D). However, harpacticoids are not a dominant group in winter (see Table 1). - Response: Although the contribution of harpacticoids to the total assemblage feeding may be weaker than the dominant species such as *Acartia*, the copepod feeding selectivity was a balance from all existing individuals including both dominating species and other species.

P17 line 9-13: The authors used the Bayesian mixing model to estimate the relative contribution of copepods to the carnivore diets, and the prey copepods which were not occurred with predatory copepods according to Table 1 were not considered in the model processing. However, this assumption or process may brings bias when evaluate the prey copepod contribution to predators in reality. The authors did not consider some copepod prey for *Labidocera* and *Sinocalanus*, but not *Tortanus* in Fig. 11. I guess that *Labidocera* who living on surface also may contact copepods other than *Acartia* and *Paracalanus* (for example, according to Table 1, in summer *Labidocera rotunda* co-occurred with *Tortanus* as well as *Acartia* spp.). Therefore, the brackish calanoids and cyclopoid also need to be included in potential prey for *Labidocera*. The same logic can be applied to *Sinocalanus*. Although *Sinocalanus tellenus* dominated in autumn with *Paracalanus* and *Corycaeus*, only *Acartia* was considered as prey for *Sinocalanus*, but not brackish water calanoid such as *Pseudodiaptomus*. Please consider all potential prey for *Labidocera* and *Sinocalanus* like in the case of *Tortanus* in

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Fig. 11A. Also, it is not clear whether the dietary composition of the carnivorous genera in Fig. 10 was for a season or for the four seasons. Please specify appropriate season for each carnivorous copepods (e.g. all season or particular season) so that we can guess the potential prey for the carnivorous copepods. - Response: We will try to do so by considering all potential prey for *Labidocera* and *Sinocalanus* as suggested by the reviewer. By carefully check the taxonomic data set, we agree that the brackish calanoids *Pseudodiaptomus* should be included as a potential food source for *Labidocera*, as they co-occurred. However, when we observed *Labidocera* during the summer, we found that cyclopoid species didn't occur or may be in extremely low abundance. In such case we don't agree to consider cyclopoid species as potential food source for *Labidocera*. The dietary composition of the carnivorous genera in Fig. 10 was for all four seasons, as we used the all samples to estimate a mean isotope ratio for each genus.

P28 Fig.4: Please indicate which genera are the brackish calanoids or marine calanoids in Fig. 5(B) and/or Fig. 5. Also, please specify whether the result of decapods or harpacticoids is for spring and/or winter samples. - Response: I think the reviewer is saying the Fig.5 and Fig.6 (B). We have specified them in figure legend in the revised version.

P33 Fig.9: Please indicate appropriate season for each copepod group and decapods. - Response: Except decapods, all genera are averaged from all seasons, which we will indicate them in the figure legend in the revised version. And we will remove decapods, as suggested by the reviewer in one of the above comments.

Technical Corrections: P15 line 11: 'brackish stations in autumn and saline stations in winter' instead of 'brackish stations in winter and saline stations in autumn' - Response: Agree to revise.

P 15 line 24: 'Turner, 1984' instead of 'Turner, 1986' - Response: Agree to revise.

P16 line 20: 'Fig. 10B' instead of 'Fig. 9B' for *Paracalanus* - Response: Agree to

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revise.

P17 line13: 'Sinocalanus preferred Paracalanus to Acartia and/or cyclopoids.' instead of 'Sinocalanus preferred cyclopoids to Acartia.' - Response: We have revised it according to revised mixing model analysis by including more potential food items, as suggested by the reviewer mentioned above.

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