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Title: Variability in copepod trophic levels and in feeding selectivity based on stable isotope analysis in Gwangyang Bay off the southern coast of Korea

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Rebuttals and responses to reviews

We appreciate four anonymous reviewers for the constructive comments and extremely useful reviews on the manuscript. The texts referred to by the reviewer are indicated, in the responses, by page and line numbers of the revised version. We have tried to revise carefully in line with the suggestions made by the reviewers as follows:

Reply to Referee #1

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 copepod group, especially carnivorous copepods are still questionable.

The current method (e.g. Bayesian 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 with 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 o in the revised msr 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. (see Supplementary Table S1 and relating results and discussion in the revised ms)

In relation to this issue, how did the authors treat copepodite stages of the copepods 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. (see P5, Line 9 in the revised ms)

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. (see Supplementary Table S2 and P8, Lines 13–14 in the revised ms)

P10 line 31-33: More detailed explanation may be needed, like in the case of delta 15N in Fig. 6A and 6B.

Response: We have added more explanation on it. (see P11, Lines 14–18 in the revised ms)

P10 line18: 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: Based on new method, we now have obtained the result of *Centropages*. (see Figs. 3–6 and relating result and discussion in the revised ms)

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. 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. (see Fig.8A and relating results and discussion in the revised ms)

P11 line 32- P12 line 1-2: For *Sinicalanus*, 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. (see Fig. 8B and relating results and discussion in the revised ms)

P14 Line23-25: I understand that calanoids (both marine and brackish water types) and cyclopoids had different delta 15N 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 groups 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. Based on revised modeling test, they were indeed different trophic niche. We have revised accordingly. (see P15, Lines 9–14 in the revised ms)

P15 line 3: There is no result of the brackish water species, *Pseudodiaptomus* in Fig.4,

but in Fig. 5. Why?

Response: We have added the result of *Pseudodiaptomus*in Fig.4. (see Fig. 3B and P10, Lines 13–24 in the revised ms in the revised ms)

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: Based on the revised version of model test, *Corycaeus affinis* was indeed a carnivorous species. We have revised accordingly. (see Fig. 3B, Fig. 8C, P16, Lines 2–6)

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 have deleted it in the revised version. (see Figs. 3–7 in the revised ms).

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 detrivores 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. (see Figs. 3 and P16, Lines 5–9 in the revised ms).

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 (Table1),

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 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 carnivorous species (*Corycaeus*, *Tortanus*) and dominated by both *Paracalanus* (preferring small-sized particle) and *Acartia* (preferring large-sized particle), the assemblage overall didn't show an apparent size-selectivity. (see P16 Lines 16–29 and P17, Lines 3–17 in the revised ms)

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. (see P18 Lines 12–17 in the revised ms)

P16 line 32: The authors concluded that Pseudodiaptomus was a detrivore, 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 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 $\delta^{15}N$ of *Pseudodiaptomus* estimated from the bulk sample was so low that we speculated that the detritus with low$\delta^{15}N$ may contribute to the balance the$\delta^{15}N$ 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. (see P 17, Lines 26–33 and P18, Lines 1–8 in the revised ms)

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 *Acartia*, the copepod feeding selectivity was a balance from all existing individuals including both dominating species and other species. (see P18 Lines 15–18 in the revised ms)

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 Fig. 11A.Also, it is not clear whether the dietary composition of the carnivorous genera in Fig. 10was 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 dataset, we agree that the brackish calanoids *Pseudodiaptomus* should be included as a potential food source for *Labiocera*, as they co-occurred. However, in our taxonomic data set, when we observed *Labidocera* during the summer, we found that cyclopoid species and 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. (see Fig. 8A, B, and P17, Lines 11–33, P18, Lines 1–4 and 8–11)

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. (see Fig. 3 and Fig. 4B, and P6, Lines 16–22 in the revised ms)

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 indicate them in the figure legend in the revised version. And we have removed decapods, as suggested by the reviewer in one of the above comments. (see Figs. 3–6 in the revised ms)

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. (see P15, Line 22 in the revised ms)

P 15 line 24: 'Turner, 1984' instead of 'Turner, 1986'

Response: Agree to revise. (see P16, Line 6 in the revised ms)

P16 line 20: 'Fig. 10B' instead of 'Fig. 9B' for Paracalanus

Response: Agree to revise. Now it is Fig. 7E. (see P17, Line 6 in the revised ms)

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. (see P18, Line 5–8 in the revised ms)

Reply to Referee #2

General Comments:

Chen et al report seasonal and spatial variations of copepods on 13C and 15N values in a temperate estuarine system. They present a nice descriptionof these data and use a lot of mathematical analysis models (linear mixing models,Bayesian isotopic mixing models and generalized additive models) to deeply analyzethe trophic structure of plankton. I am in favor of some salient results on averagedtrophic position of different copepods and contribution of two size fractions of diets.These kinds of results are hard to be obtained by direct measure as copepod communityis highly complex so that the individual samples are difficult to separate, which alsoclaimed by the authors. Although the size-selective feeding behaviors of copepods arenot new in literature, the patterns shown in this manuscript are reasonable. More important,it still provides a powerful technique to treat such investigation data that can befollowed by readers and provide insight biogeochemical information about the trophicinteraction between copepods and primary producers. Therefore, this is potentially avery useful paper providing important information and methods for the biogeochemicalstudy (i.e., food resources and trophic levels) in the complex coastal ecosystem, aswell as the influence of the freshwater input in an estuary.The main shortfall of this manuscript is that it can only provide the trophic information ofseveral major genera of copepods. Genera with low biomass or appearance frequencylike Euchaeta, Calanus and Oithona, which are also popular in the world ocean cannotbe treated by the same way. In addition, I would like to suggest more discussion aboutthe uncertainty or disadvantages of these analysis models. And, reasons for someresults on feeding pattern of some species are not discussed enough. For example,what are the mechanism of the feeding selectivity of the three carnivorous genera like Tortanus,Labidocera, and Sinocalanus? Finally, it will be more visual if the authorscan provide a conceptual map about the planktonic food web from their conclusion,showing the relationship and the seasonal differences of the energy flow on this mapas well? Overall, I recommend this manuscript for publication

in Biogeosciences with minor revision. Some specific comments are indicated below.

Response: We appreciate for the reviewer's positive comments. We also admit the criticism of the shortfall of this paper. It is hard to estimate the isotope ratio of those species that contribute a very small fraction in total copepod biomass in GwangyangBay. However, we believe that the same way can be applied to *Euchaeta*, *Calanus* and *Oithona* when they dominate in community and have relatively high biomass. Such cases were commonly found in adjacent waters like East China Sea and South China Sea. As pointed out by other reviewers, we have also tried to increase the discussion of the potential prey of the three carnivorous genera *Tortanus*,*Labidocera*, and *Sinocalanus* in the revised version. (see Fig. 8, P17, Lines 26–33 and P18, Lines 1–11 in the revised ms)

Specific comments:

1. A reason or a reference to calculate trophic enrichment is needed.

Response: We have added it to Page 7 Line 24. To remove confuse, we have also explained it more careful in figure legend. (see Fig. 5 and P7, Lines 21–23 in the revised ms)

2. L21 _m

3. How about the errors or residual (eq. 4) for Linear regression models?

Response: We have addd the errors of the model tests. (see P10, Lines 4–5 and Lines 13–14 in the revised ms)

4. Y-axes in Figure 5 to 11 need plural number.

Response: We have revised accordingly. (see Figs. 4–8 in the revised ms)

5. Increase the resolution of Figure 3.

Response: We have done in the separate pdf version for each figure. (see all figures in the revised ms)

Reply to Referee #3

General Comments:

I find a problem in the way the authors estimated the weight differences between cyclopoids and calanoids randomly, as well as assuming that the weight of all calanoid genera was the same. In particular, because the authors have the taxonomic information already, I suggest they do a literature review and obtain the average weight values for each of the copepod genera/species used in the study, and apply these to the bulk regressions. I believe this is especially important as the authors are trying to extrapolate significantly more results than what they measured (i.e. genera-specific isotope values from a mixed community), that the approach be as precise as possible.

Response: We thank the valuable recommendation of the reviewer. We have tried to do a literature review and obtain the average weight values for the important genera used in our study. (see Supplementary Table S1 in the revised ms)

In general I appreciate the effort to expand upon simple d13C and d15N bulk measurements for more detailed information on a community. However, in the case of copepods, if the authors do/did intend to investigate these relationships in detail, why not simply measure the values of individual genera? They state that too much material is required, but methodological advances these days mean that an individual Calanus female can indeed be analyzed (60ugC, 10ugN), as 5 ugN is typically the lower limit of standard bulk analyses (and low-N methods methods have been developed to go down to 1 ugN). Cyclopoids would require greater number, but following the authors assumptions of $0.1<x<1$, that would be about 10 individuals. When certain problems arise, such as Paracalanus and Sinocalanus having lower d13C values than any measured prey, it would seem the authors acknowledge them, but then continue their analyses, e.g. calculate a TL (presumably based on prey that has been shown to not be consistent with their isotope values) in the same was as for the other genera.

Response: We agree to the reviewer's consideration. However, we missed to do so when the investigation was conducted. As state in the ″Introduction″ part, it is too time-consuming to obtain enough weight for specific genus and isotope analysis for different subgroups requires great expertise in isolating species from highly complex mixtures. Besides, we have tried to simplify the sampling way so that some monitoring departments may follow. (see P6, Lines 13–25 in the revised ms)

One gets the sense that by plugging it into GAMs and regression models, the error sources and magnitudes are lost. I would like to see a quantitative test of the biases inherent in this Bayesian model, and how confident the authors can be that this approach is recovering the actual copepod diets. Given this approach and the number of assumptions that lie within, uncertainty relating to the model (as well as replication, independently) should be presented, discussed, and assessed explicitly with the other sources of uncertainty. This should be done with both the particle feeders and the carnivorous species, and the effects of including or excluding different species types should also be assessed.

Response: The reliability of Bayesian mixing model is fully discussed in literature (Phillips and Koch, 2002; Phillips and Gregg, 2003; Moore and Semmens, 2008; Ward et al., 2010; Parnell et al., 2010, 2013). We are not good at extrapolating this model. However, we have tried to present more about the details we used, e.g. the replication, trophic enrich factor, sources just like the reviewer suggest. (see P7, Lines 11–27 in the revised ms)

Finally, consistent with the point I discuss above, the authors mention a 'simple energy flow' in the abstract and discussion. But I wonder if this methodological approach allows for more complex flows. The actual isotopic values were not measured, but inferred from mass balance of dominant genera, and Bayesian approaches, and the violation of the underlying assumptions was not determined. How would a more complex picture emerge? In fact, the problem of Paracalanus and Sinocalanus having

lower d13C values could hint at more complexity, yet it is assumed perhaps that this is due to unmeasured food sources and then ignored.

I think if the authors address the issues posed above (and specifics below), the MS is suitable for publication.

Response: We admit that a complex picture cannot be fully understood from this paper. The estimated values only can provide a mean value and a standard error, thus they cannot explicit exactly the same situation of different seasons and no dynamics picture can be found. But the mechanism to estimate the isotopic ratio of a mixing sample, which is mixed by different species with different masses, is clear. Our results only suggest the potential trophic position of those examined genera. For *Paracalanus* and *Sinocalanus*, their low 13C was significantly estimated from the samples containing certain amount of them. We believe the data are true and correct. Although we fail to provide information of all potential food items for them, they show the role in interacting with plankton and other copepods. Frankly speaking, we didn't intend to give detail information of the biology of each genus, but aims to investigate their potential roles in regulating the abundance of the two size fractions of plankton. (see P18, Lines 20–424 in the revised ms)

Specific comments:

Abstract.

P1 – 10. The word 'trophism' is introduced yet does not technically mean what the authors define it as (food resources and trophic levels), and is not used within the field's jargon as such either. I would prefer 'trophic structure' or 'trophic interactions', or 'trophic preferences'.

Response: Agree to change. We have changed into ″trophic preference″ in the revised version. (see P1, Line 11 and P4, Line 1 in the revised ms)

'Temperature-related' seasonal variations – The effect of temperature from season was not separated in this study, it should be simply 'seasonal variations'.

Response: Agree to change. (see P1, Lines 14–15 in the revised ms)

Introduction

P2-0. “With broad feeding spectra and flexible feeding strategies, the bulk copepod assemblage is omnivorous depending on dominant species or group”. Omnivo- rous or what? Consider changing to something like ‘displays varying degrees of herbivory/ omnivory/carnivory, depending on dominant: : :’

Response: Agree to change. (see P2, Lines 4–5 in the revised ms)

P2-5. “In turn, TLs of a diverse: : :”. I assume the authors here refer to the average trophic position of the assemblage, and thus should be ‘TL’ (singular). “Because copepods play a fundamental role in feeding on phytoplankton as primary consumers”. Consider re-phrasing as ‘Because copepods rely significantly on phytoplankton as prey’, otherwise the expectation of this phrase is that the second half will refer to the top down effect of copepods on phytoplankton, and not the bottom-up effect of phytoplankton on copepods. ‘feeding on phytoplankton as primary consumers, so the seasonal and spatial’. Delete ‘so’.

Response: Agree to change. (see P2, Line 9 in the revised ms)

P2-15. “Therefore, the assessment of the trophic position (: : :) of copepods within a complex planktonic food web is critical in predicting the ecological relationships between predator and prey”. This phrase seems redundant, isn’t the study about assessing these ecological relationships? I don’t understand the prediction part.

Response: To avoid confuse, we have revised ″in predicting″ to ″to understand″. (see P2, Line 21 in the revised ms)

P3. 0. “In contrast, the d15N values of primary producers increase from being nutrient sufficient (high fractionation) to nutrient-limiting (low fractionation) and are especially high in anthropogenic wastewater nitrogen inputs”. Would the later simple swamp the

fractionation effect? The literature on ïA¸d'15N of different nutrients in the ocean (nitrate, ammonia, urea) shows ranges that are much larger than fractionation factors, e.g. these vary by about 20‰ compared to 3.4‰ of fractionation. Can you comment on how much you expect the source to vary along the river gradient?

Response: The parentheses here indicate the consequences. For example, rich nutrients will cause high fractionation of primary producer. And they would continuously accumulate their $\delta^{15}N$ in the cells. We don't have the data on the variations of $\delta^{15}N$ of source, as we expect theδ15N of source will vary from 0 to 13‰ based on the variances of POM. (see P3, Line 5–7)

Materials and methods

P4-25. Could you mention the average volume filtered per tow, as the net was equipped with a flow meter?

Response: Sure, we can provide this. (see P5, Lines 3–4 in the revised ms)

P5-5. "water samples were transported to the laboratory as soon as possible". Please give a time estimate.

Response: 1–2 hours driving. (see P5, Line 14 in the revised ms)

P6-5. The analytical precision of 0.2‰ and 0.3‰ for d13C and d15N, respectively, seems a bit high. Could you estimate what is the lowest change in TL that you can estimate based on this instrument error?

Response: We have re-checked the precision of the instrument during the period we measured the samples. The analytical precision should be 0.1 and 0.05 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Based on the equation of trophic level, the lowest change will be less than 0.02 TL. (see P6, Line 11 in the revised ms)

P6.15. The weight difference between cyclopoids and calanoids was generated randomly. I don't understand why the information from the species identification was

not used for this purpose. What is the error associated with this type of computation? I would really suggest the authors do a literature search of the mean weights of the difference species and genera enumerated in their samples, and use this information to estimate both cyclopoid/calanoid weights, and the weights of the different calanoid genera. If the composition has already been estimated, it makes no sense to make these assumptions that only introduce greater error into an already indirect way of estimating species stable isotope composition.

Response: As mentioned above, we have doe a literature search. (see Supplementary Table S1 and P6, Lines 13–22 in the revised ms)

P7-20. 'fractionation factors used in the model estimation were calculated from TLs'. I don't understand this statement, it sounds like 3‰ and 0.5 ‰ were assumed (logically) and not calculated. Please clarify.

Response: The sentence of "fractionation factors used in the model estimation were calculated from TLs' means that the fractionation factor between two trophic levels, based on the difference of each $\delta^{15}N$ value. Or it can also be understood by this way, it is multiplying the difference of two trophic levels (calculated from different $\delta^{15}N$ value) with 3‰ and 0.5 ‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. (see P7, Lines 22–23 in the revised ms)

Results

The authors discuss their seasonal results in the context of 'temperature'. I would prefer to see this discussed as 'seasonal', since temperature variability within a season was not tested and hence the driver of the observed effects cannot be unequivocally stated to be temperature. Rather, they are probably a combined effect of the changes that co-occur with each season and should be stated as such.

Response: We do test the partial effect of temperature on variation of plankton isotope based on GAM results (Fig.3). However, we agree to avoid too much emphasis on temperature but on season. (see Supplementary Fig. S1 and P9, Linse 13–32 in the

revised ms)

P8-10. 'Despite insignificant spatial variability, higher Chl a concentrations generally occurred in the middle of the bay'. This is not obvious from the values in the table. Please explain in more detail or remove.

Response: Agree to revise. (see P8, Line 8–12 in the revised ms)

P8-25. Please give a mean value for copepod d13C as done for the groups above (nanoplankton and microplankton)

Response: Agree to revise. (see P8, Line 30 in the revised ms)

P9-0. "Overall, seasonal succession of winter-spring, spring-summer, and summer autumn were apparent for all plankton groups". Not clear what this means. There appears to be significant overlap in values for the nanoplankton, and no clear increasing progression from winter to autumn, as increases/decreases seem to interchange.

Response: Yes, seasonal pattern for nanoplankton was not clear. We decide to delete this sentence. (see P9, Line 6 in the revised ms)

P9-5. It isn't clear to me how the coefficients of variation are calculated. The range of d15N values encountered is less than that for d13C, although the spatial progressions are less monotonic. Please clarify in the methods how this is calculated.

Response: We calculated the coefficients of variation by dividing the standard error with mean value. We have tried to clarify this in the M&M. (see P8, Line 2 and P9, Line 7 in the revised ms)

P9-10. The result for the microplankton is inconsistent with the figure. In the figure, the highest value for d15N is 10‰ at the bay in spring. There is no 16.2 value.

Response: We may have a typo. It should be 10.2 at station 9. Thanks for the reviewer's careful check. (see P9, Line 18 in the revised ms)

P9-15. “Copepod d15N : : : being much more consistent with the pattern of microplankton than that of nanoplankton”. This seems true for the summer ïA¸d’15N values, and quite the opposite for the winter values. Regardless, there is such high variability that it is hard to tease out any clear pattern of spatial/seasonal co-variability.

Response: Yes, it is true. We have removed this unsuitable sentence. (see P9, Lines 25–27 in the revised ms)

P9-20. The GAM result is very interesting. Perhaps it reflects the food-web processes that affect d15N disproportionally and were not included in the GAM?

Response: Here the deviances explained by GAM suggest those factors combined to influence the dependent factor. For $\delta^{15}N$, the deviance explained was relatively lower suggesting that other factors which were not included would contribute another 23% of the deviance of $\delta^{15}N$. But we don’t know what are them. The understanding of the reviewer is right. (see Table 2, Table 3, and P9, Lines 25–P10, Line 32 in the revised ms)

P10-20. It is not clear to me how the trophic levels of brackish copepods can be calculated, when their 13C values do not support the sampled nanoplankton and/or microplankton as their food source. I also don’t understand how later in figure 6 they show up enriched, but in figure 4 they are depleted with respect to this food source. The differences between these two figures should be stated clearly as they show different results.

Response: The brackish copepods in this study were defined by empirical taxonomy, including *Pseudodiaptmus* and *Sinocalanus*. The trophic levels were calculated by the formula shown in M&M and figure legend. We admit that there may be some confuse, while we don’t think the two figures show different results. Firstly, the results of brackish copepods were averaged from *Pseudodiaptmus* and *Sinocalanus*, while our result showed more insight information that they were different.

Secondly, as mentioned above, we didn't intend to give detail information of the biology of each genus, but aims to investigate their potential roles in regulating the abundance of the two size fractions of plankton. Thus, we were unable to provide detail information of all potential diet sources of them, whereas they still have enrichment factor on lower trophic levels such as nanoplankton and microplanton. (see P10, Lines 25–32 in the revised ms)

P10-25. "The enrichment values for nanoplankton feeding on marine and brackish water calanoids: : :". This phrase says that nanoplankton are feeding on copepods. That's not right, it should say something like 'enrichment values for marine and brackish water: : : feeding on nanoplankton'.

Response: Sorry for a mistake here. We have revised as suggested. (see P11, Lines 6–12 in the revised ms)

P11-5. I disagree with the statement (based on the figure) that "the proportions of the two size fractions of POM averaged from all four seasons contributing to copepod diets at different stations were also distinctly different except for station 8 (Fig. 8)". It seems that the error bars overlap at station 1 (hence not different), and stations 6 and 7. I might be missing something but then it should be clarified.

Response: Yes, we found that error bars were indeed overlapping. We have revised this conclusion. (see P11, Lines 19–30 in the revised ms)

P11-10. Does 'spring data available' mean 'only spring shown'?

Response: Yes, we obtained enough amounts of decapods for isotopic analysis only at the spring. However, as suggested by another reviewer, we decide to delete the part of decapods as it was not related to the topic of this study. (see P11, Line 27 in the revised ms)

The authors discuss size-selective feeding of calanoids in the context of 'filtering

efficiency', yet they are not true filter feeders, they are suspension feeders that trap and handle particles (Paffenhofer et al, 1982, Mar Bio 67:2), which has different implications for particle handling. This is an important distinction that should be observed throughout the MS.

Response: OK, we have carefully checked the whole MS and change to ″feeding efficiency″ (see P17, Line 7 in the revised ms)

Discussion

P13-0. It seems to me that the sewage explanation deserves a bit more attention. If the authors can't rule it out it means that this could contribute substantially and swamp the other subtle processes discussed in the 15N-enriched ammonia section.

Response: Yes, we also believe that sewage was important for ^{15}N accumulating. However, we didn't have direct data to support our speculation. Thus we have changed the sentence to ″The input of sewage-derived ^{15}N-enriched ammonia 5 (domestic sewage and livestock waste) could contribute substantially and swamp the other subtle processes to increase $\delta^{15}N$ values of nanoplankton″. (see P13, Line 20–22 in the revised ms)

P13-5. "Furthermore, the fractionation effect of phytoplankton will be reduced when phytoplankton became nitrogen-limited and take up nitrogen with little fractionation". I am unsure that this effect could be significant in a coastal areas such as this one. Moreover, if phytoplankton reduce their fractionation, it would mean that their ^{15}N will tend to be higher (as they choose the lighter ^{14}N), and thus doesn't explain this decreasing trend.

Response: Yes, we agree that nutrient-limiting is not frequently happened in coastal area. However, substantial reduction of nutrients from different seasons or from different stations and the mis-match of high phytoplankton and low nutrients were normal. When phytoplankton reduce fractionation, they will select more ligther ^{14}N in cells thus they will show a reducing ratio of ^{15}N in cells (Cifuentes et al., 1988; Fogel

and Cifuentes, 1993; Granger et al., 2004). To remove such confuse, we have revised this sentence to ″Furthermore, the fractionation effect of phytoplankton will be reduced when nutrients substantially decreased and phytoplankton would take up nitrogen with little fractionation and stored relatively light of nitrogen isotope.″ (see P13, Lines 24–25 in the revised ms)

P13-10. I would like to see table with the GAM results. It would be nice to have these presented first in the results, and later discussed. It would also be interesting to see the different variables tested and the ones found to be significant within this table.

Response: Agree to revise. We have tried to show the table in Results. And as suggested by another reviewer, we will move the GAM figures (Fig. 3). (see P9, Lines 13–32, P13, Lines 12–P14, Line 12, and Tables 2–3 in the revised ms)

P13-20. But see Gutierrez-Rodriguez et al (2014, L&O, vol:59, i5) on negligible trophic enrichment of heterotrophic protists.

Response: Thank the reviewer's reference. We agree the negligible trophic enrichment of heterotrophic protists, thus we have removed such speculation in discussion of the revised ms.

P14-0. "Because of different feeding behaviors and fractionation effects of copepods, the variability of trophic positions of copepod assemblage depends on the overall composition of species and is determined by dominant species." Change to ": : :the variability of the average community trophic position depends on the overall composition of species and is determined by the dominant species."

Response: Agree to revise. (see P14, Lines 13–14 in the revised ms)

I am somewhat confused about the discussion of trophic levels of the copepods Paracalanus and Sinocalanus. The authors state that their ïA¸d'13C values are lower than all measured food sources, which would imply that their food source has not been

adequately measured. How then are these organisms included in the trophic level (TL) component of the paper? A bit of clarification on this topic would really help the reader.

Response: Agree to do so. The trophic level in this study was defined as trophic position relative to nanoplankton, which was considered as the trophic baseline. (see P10, Lines 25–26 in the revised ms)

P17-10. This paragraph explaining the Bayesian mixing model methods/results should be moved to the results section.

Response: Agree to revise. Instead, we have added some more discussion on this part. (see P17, Lines 26–P18, Lines 4)

Reply to Referee #4

General Comments

This manuscript provides results from seasonal and spatial variation in the stable isotopes 13C and 15N of POM and copepods along a salinity gradient in Gwangyang Bay, off the southern coast of Korea. The authors combined this information with linear mixing models, Bayesian isotopic mixing models and generalized additive models to derive a statement on food selectivity and trophic level of copepods. In general, this manuscript is very well structured and provides valuable information on the flow of matter through the food web. Still, some concerns have to be clarified before publication.

Response: We appreciated the positive comments of the reviewer and have followed the suggestion to improve the manuscript.

Specific comments.

Introduction

1. Page 3, line 7: Please give more information here on the usage of different N sources and enrichment factors.

Response: Agree. Accordingly, we have explained more information here based on literature. (see P3, Lines 8–11 in the revised ms)

2. Page 3, line 19: “highly mixed species”- Please clarify, mixed with what?

Response: Here the “highly mixed species” means the assemblage contained too many different species and those species had similar size. So such species were hard to be sorted out from the assemblage based on current microscopic technique. To remove confuse, we have revised it to ″high diversity of the assemblage and …″. (see P3, Line 21 in the revised ms)

3. Page 3, line 21: Instrument sensitivity has increased and compound specific analysis (CSI) of stable isotopes in amino acids make it possible to track diets of mesozooplankton and determine their trophic position.

Response: Yes, of course. We admit that highly developed instrument can do so. But for doing so, researchers still need taxonomic expertise to sort out the species from a complex mixture to prepare the sub-sample. It requires a lot of lab processing works. (see P3, Lines 24–28 in the revised ms)

4. Page 3, line 21: Please give some reason why this site was chosen.

Response: The stations were chosen based on salinity regime and different geographic characteristics, e.g. stations 1–3 are river sites with extremely low salinity, stations 4–6 are in the central bay with moderate salinity, while stations 7–9 are in the channel towards to the open ocean with relatively high salinity (see P4, Lines 26–29 in the revised ms).

Material and Methods

5. General: why did the authors not use literature data on average weight values for each of the species investigated instead of assigning the weight to each group?

Response: In the revised version, we have searched for the literature data just like suggestion of this comment and also suggested by other reviewers. (see Supplementary Table S1 and P6, Line 13–22 in the revised ms)

6. General: How where copepodite stages treated regarding abundance and body mass?

Response: They were averaged to adults. (see P5, Line 9 in the revised ms)

7. Page 4, line 15: Change to “increasing”.

Response: Agree and we have revised accordingly. (see P4, Line 16 in the revised ms)

8. Page 4, line 16: Specify “in the middle of Gwangyang Bay.

Response: Agree. We have revised to ″in the middle part of the Gwangyang Bay″. (See P4, Line 18 in the revised ms)

9. Page 4: Please add information on when sampling took place- day or night?

Response: We all sample at the day time. We have add edsuch explanation in M&M. (see P5, Line 1 in the revised ms)

10. Page 5, line 11: “pico- and nano- sized phytoplankton”. Doesn’ t sampling with a mesh also include nanozooplankton like heterotorphic and mixotrophic flagellates- so it does not only comprise phytoplankton?!

Response: Here the plankton less than 20 micron but larger than GF/F (0.78 micron) were defined as nanoplankton. Thus they contains both phytoplankton and heterotrophs. (see P5, Lines 13–17 in the revised ms)

11. Page 6, line 29: something is missing at the end of the sentence- “illustrated in figures?”.

Response: The figures here do not mean citations. We try to explain that the mean and standard deviations were illustrated by forms of figures. To remove confuse, we can delete this sentence in the revised version. (see P6, Line 30 in the revised ms)

Results and Discussion

12. There are too many figures. Some might be moved to the supplemental section, e.g. Fig. 3, 7,8,11

Response: We agree to do so. We have moved Figs. 3, 7, and 8 to supplementary materials, but no Fig.11. We believe that Fig.11 is relatively important for readers and other reviewer want to know more about the information of the feeding of carnivorous species. (See Supplementary Figs. S1–S3 and Fig. 8 in the revised ms)

13. Page 12, line 16: What is a “heavy carbon pool”, give an example?

Response: The phrase is located at ″Page 12, line 24″. ″Heavy carbon pool″ here means the dissolved inorganic carbon pool in which the carbon was primarily composed by heavy carbon (^{13}C). (see P13, Lines 5–7 in the revised ms)

14. Page 12, line 31: Wording! Please revise “much reduced”.

Response: We have changed it to “low”. (see P13, Line 16 in the revised ms)

15. Page 13, line 15: “with low fractionation effects”- give example.

Response: Now we have deleted this kind of discussion about temperature in the revised ms, as suggested by other reviewers.

Conclusion

16. Please provide a simplified figure of the energy flow for the different seasons.

Response: Based on revised estimation, we have tried to provide such simplified figures. (see Fig. 9 and P8, Line 20–24in the revised ms)

I hope that these revisions are satisfactory and that the revised version will be acceptable for publication in Biogeosciences.

Sincerely yours,

Chang-Keun Kang

Variability in copepod trophic levels and feeding selectivity based on stable isotope analysis in Gwangyang Bay off the southern coast of Korea

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Abstract. Trophic preference (i.e., food resources and trophic levels) of different copepod groups was assessed along a salinity gradient in the temperate estuarine Gwangyang Bay of Korea, based on seasonal investigation of taxonomic results in 2015 and stable isotope analysis incorporating multiple linear regression models. The $\delta^{13}C$ and $\delta^{15}N$ values of copepods in the bay displayed significant spatial heterogeneity as well as seasonal variations, which were indicated by their significant relationships with salinity and temperature, respectively. Both spatial and temporal variations reflected those in isotopic values of food sources. The major calanoid groups (marine calanoids and brackish water calanoids) had a mean trophic level of 2.2 relative to nanoplankton as the basal food source, similar to the bulk copepod assemblage; however, they had dissimilar food sources based on the different $\delta^{13}C$ values. Calanoid isotopic values indicated a mixture of different genera including species with high $\delta^{15}N$ values (e.g., *Labidocera*, *Sinocalanus*, and *Tortanus*), moderate values (*Calanus sinicus*, *Centropages*, *Paracalanus*, and *Acartia*), and relatively low $\delta^{15}N$ values (*Eurytemora pacifica* and *Pseudodiaptomus*). Feeding preferences of different copepods probably explain these seasonal and spatial patterns of the community trophic niche. Bayesian mixing model calculations based on source materials of two size fractions of particulate organic matter (nanoplankton at < 20 μm vs microplankton at 20–200 μm) indicated that *Acartia* and *Centropages* preferred large particles, *Paracalanus*, *Calanus*, *Eurytemora*, and *Pseudodiaptomus* apparently preferred small particles. *Tortanus* was typically carnivorous with low selectivity on different copepods. *Labidocera* preferred marine calanoids *Acartia*, *Centropages*, and harpacticoids, and on the other hand, *Sinocalanus* and *Corycaeus* preferred brackish calanoids *Paracalanus* and *Pseudodiaptomus*. Overall, our results depict a simple energy flow of the planktonic food web of Gwangyang Bay: from primary producers (nanoplankton) and a mixture of primary producers and herbivores (microplankton), through omnivores (*Acartia*, *Calanus*, *Centropages*, and *Paracalanus*) and detrivores (*Pseudodiaptomus*, *Eurytemora*, and *harpacticoids*) to carnivores (*Corycaeus*, *Tortanus*, *Labidocera*, and *Sinocalanus*).

1 Introduction

Mesozooplankton constitute essential trophic mediators of marine food webs in transferring energy and materials by linking the microbial food web to higher trophic levels. Copepods are a diverse assemblage dominating mesozooplankton communities. With broad feeding spectra and flexible feeding strategies, the bulk copepod assemblage displays varying degree of herbivory/omnivory/carnivory, depending on dominant species or groups (Graeve et al., 1994; Sell et al., 2001; Turner, 2004; Vadstein et al., 2004; Gifford et al., 2007; Chen et al., 2017). The role of copepods in planktonic food webs can be determined by their overall trophic levels (TLs) relative to primary producers. In turn, the TL of a diverse copepod assemblage is balanced from different groups with different feeding preferences and are ultimately determined by species composition. Because copepods rely significantly on phytoplankton as prey, the seasonal and spatial changes in the composition and availability of phytoplankton determine the abundance and feeding behavior of the copepod assemblages.

The most dominant copepod species, such as *Neocalanus*, *Calanus*, *Temora*, and *Paracalanus*, are filter-feeders that perform a size-selective feeding behavior depending on particles effectively retained by feeding appendages of copepods. Large phytoplankton (> 20 μm; mainly diatoms and dinoflagellates) are generally grazed at high rates by copepods, as shown by many field studies in coastal and estuarine waters (e.g., Liu et al., 2005a, b; Chen et al., 2017). Many other field studies have reported that omnivorous species dominate copepod assemblages because of high feeding selectivity on larger microzooplankton that are considered to have higher nutritional quality (e.g., Berk et al., 1977; Fessenden and Cowles, 1994; Calbet and Saiz, 2005; Gifford et al., 2007; Chen et al., 2013). These omnivorous copepods might induce increases in phytoplankton levels indirectly through trophic cascades as they graze intensely on microzooplankton (e.g., ciliates and heterotrophic dinoflagellates) (Nejstgaard et al., 2001; Stibor et al., 2004; Sommer and Sommer, 2006; Zöllner et al., 2009; Chen et al., 2011, 2013). Therefore, the assessment of the trophic position (herbivores, omnivores, or carnivores) of copepods within a complex planktonic food web is critical to understand the ecological relationships between predators and prey.

Stable isotope analysis (SIA) is a reliable technique providing insight into the trophic positions of copepods relative to basal food sources (Grey et al., 2001; Sommer et al., 2005; Hannides et al., 2009; Kürten et al., 2011). Isotopic comparisons with food sources enable us to analyze prey selectivity during predators' feeding history as well as within food web structures (Fry, 2006; Layman et al., 2012). In general, the carbon stable isotope ratio ($\delta^{13}C$) can be useful for tracing food sources because of small fractionation (0.5–1‰ per TL) during trophic transfer, particularly when different food sources at a given period in a specific system have distinct $\delta^{13}C$ values. By contrast, the nitrogen stable isotope ratio ($\delta^{15}N$) can be useful for estimating relative TLs because $\delta^{15}N$ values of consumers generally increase with TL (an average 3.2‰ of enrichment per TL; Post, 2002; Michener and Kaufman, 2007). The development of linear mixing models and the Bayesian mixing model has allowed researchers to predict the proportions of different food sources in the diets assimilated by grazers (Phillips and Koch, 2002; Phillips and Gregg, 2003; Moore and Semmens, 2008; Ward et al., 2010; Parnell et al., 2010, 2013).

Coastal and estuarine environments often experience rapid fluctuations of inorganic carbon and nitrogen inputs in response to diverse oceanographic processes (e.g., coastal currents, upwelling, tidal mixing, and river discharges), which drive spatial and

seasonal heterogeneities in biogeochemical dynamics and isotopic signatures (Rolff, 2000). Indeed, the $\delta^{13}C$ values of suspended particulate organic matter (POM) in estuarine systems increase progressively from the head to the mouth of each estuary because of the lower $\delta^{13}C$ values in terrestrial carbon or sewage materials through river discharge (Cifuentes et al., 1988). In contrast, the $\delta^{15}N$ values of primary producers increase from being nutrient-sufficient (high fractionation) to nutrient-limiting (low fractionation) and are especially high in anthropogenic wastewater nitrogen inputs (McClelland et al., 1997). In addition, different phytoplankton groups utilize different nitrogen sources with different enrichment factors, possibly offering different isotopic pools to grazers (Gearing et al., 1984; Rolff, 2000; Montoya et al., 2002). For example, diatoms primarily utilize nitrate with varying fractionation factor on ^{15}N (0.7–6.2‰) depending on species (Waser, et al, 1998; Needoba et al., 2003), while flagellates primarily utilize ammonia with enrichment factor of 6.5–8‰ (Montoya et al., 1991).

Given that isotopic values of copepods vary in association with copepods' food source by one or two increases in TL values, seasonal and spatial patterns generally follow the trends of their food sources or dominant prey (Grey et al., 2001; Montoya et al., 2002; Kürten et al., 2011). Higher $\delta^{15}N$ values of copepods caused by fractionation rather than food source or by averaging from mixed food sources are evident considering the lowered isotopic values of fecal pellets (Checkley and Entzeroth, 1985; Checkley and Miller, 1989; Tamelander et al., 2006). Furthermore, the effect of the microbial food web on the elevated $\delta^{15}N$ values of copepods cannot be ignored (Rolff, 2000; Kürten et al., 2011). Therefore, variations in isotope signatures of both copepods and POM (including phytoplankton, bacteria, ciliates, and detritus) help to depict the biogeochemical cycles of specific systems (Grey et al., 2001; Montoya et al., 2002; Francis et al., 2011). Nevertheless, because copepods graze preferentially on larger phytoplankton (diatoms and dinoflagellates) and microzooplankton (ciliates and heterotrophic dinoflagellates), we hypothesize that isotopic values of the copepod assemblage will be much closer to those of larger rather than smaller food source plankton.

However, high diversity of the assemblage and size overlap among different species make it hard to determine the relative trophic positions of different subgroups or species. Isotope analysis for different subgroups requires great expertise in isolating species from highly complex mixtures. Moreover, the number of individuals of a specific genus is often insufficient for analysis because of limited instrument sensitivity. Thus, to our knowledge, direct comparisons of different mesozooplankton groups or copepod species are seldom found in the literature (Schmidt et al., 2003; Sommer et al., 2005; Hannides et al., 2009). Here, we estimated isotope values of different copepods by mass balancing linear mixing models from values of bulk samples and taxonomic data of copepods. The allocated masses of calanoids and cyclopoids were achieved from literature and empirical formulas.

Overall, we aimed (1) to understand the seasonal variations and spatial heterogeneity of copepod $\delta^{13}C$ and $\delta^{15}N$ values in a temperate estuarine system (Gwangyang Bay, Korea); (2) to compare the trophic positions of different copepods; and finally (3) to elucidate the compositions of two major size classes (<20 μm and >20 μm) of POM in grazer diets. The dietary composition (nano-vs microplankton) of copepods was estimated using Bayesian isotopic mixing models (Parnell et al., 2010,

2013). The results of this study will provide insights into trophic preference information (i.e., food resources and trophic levels) for different copepod groups and help in understanding the biogeochemistry of this estuarine system.

2 Materials and methods

2.1 Study area

Gwangyang Bay is a semi-enclosed bay system, located on the southern coast of the Korean Peninsula, and is one of the most industrialized coastal areas exposed to anthropogenic pressure. It starts from Seomjin River through Yeosu Channel (between Yeosu Peninsula and Namhae Island) to open ocean (the East China Sea). The bay area covers approximately 145 km^2 and water depth is generally shallow at 2.4–8.0 m in the northern upper-middle Seomjin River estuarine channel compared with 10–30 m in the deep bay channel (Kim et al., 2014). The annual freshwater discharges of Seomjin River are 10.7–39.3 $\times 10^8$ tons. The seasonality of nutrient input from the catchment area (ca. 5×10^3 km^2), including agricultural and forested land, is profound (Kwon et al., 2002). The wet season starts from late spring and the discharge peaks during the summer monsoon period.

Accordingly, the maximum median river discharge varies from 30–95 $m^3 s^{-1}$ in the dry season to 300–400 $m^3 s^{-1}$ in the summer monsoon, with an annual mean of c.120 $m^3 s^{-1}$ (Kim et al., 2014). The tidal cycle of the bay is semidiurnal with maximum ranges of 3.40 m during spring tides and 1.10 m during neap tides. Tidal currents from the Yeosu Channel also strongly influence the system and approximately 82% of the Seomjin River flux is discharged toward this channel. Overall, increasing industrial pollution facilitates eutrophic conditions in the estuarine and related bay waters. Diatoms dominate in the phytoplankton community and density is high in the middle part of the Gwangyang Bay (Kim et al., 2009; data from our parallel study not shown). The distribution patterns of copepods in the Seomjin River during summer were represented by three main salinity zones: an oligohaline zone (predominated by *Pseudodiaptomas koreanus*, *Sinocalanus tenellus*, and *Tortanus dextrilobatus*), a mesohaline zone (predominated by *Acartia ohtsukai* and *Acartia forticrusa*), and a polyhaline zone (predominated by *Acartia erythraea*, *Calanus sinicus*, *Centropages dorsispinatus*, *Labidocera rotunda*, and *Paracalanus parvus*) (Park et al., 2015).

2.2 Sampling and processing

Surface water and net-tow samples were collected seasonally (February, May, August, and November) at nine stations from the head to the mouth of Gwangyang Bay in 2015 (Fig. 1). The stations were chosen based on salinity regime and different geographic characteristics. Stations 1–3 were located in the Seomjin River, stations 4 and 5 were in Gwangyang Bay (the middle part of the estuary), and stations 6–9 were located from the offshore deep-bay channel to the southern mouth of the estuary. On each sampling occasion, water temperature and salinity were determined in situ using an YSI Model 85 probe (YSI Inc., Yellow Springs, OH, USA).

Zooplankton taxonomic samples were collected during daytime by net towing using a plankton net (45 cm diameter, 200 μm mesh size) equipped with a flowmeter (Model 2030R Mechanical Flowmeter, General Oceanics Inc., Miami, FL) and gently hauled horizontally at a subsurface depth of 0.5–1 m with the ship speed at about 1 knot (0.5 m s^{-1}). The average volume filtered per tow was 16.7 ± 5.1 m^3 (mean ± se). Samples were fixed in formalin solution with a final concentration of 5% and then identified and enumerated under a stereomicroscope (SMZ 645; Nikon, Tokyo, Japan) in the laboratory. At each station, one additional net tow was collected for isotope analysis. After collection, specimens were transferred immediately into plastic bottles and preserved in a refrigerator (4 °C) until analysis. In the laboratory, subsamples were picked out from the mixed zooplankton samples under a dissecting microscope. Easily distinguishable zooplankton groups such as harpacticoids were separated from a mixture of calanoids and cyclopoids. Copepodites were counted but grouped together with adults. All subsamples were lyophilized and then homogenized by pulverizing them with a mortar and pestle before isotope analysis.

POM in surface water (0.5–1 m depth) was collected using a 5-l Niskin bottle at a midday high tide at the same time as zooplankton collection. Approximately 20 l seawater collected was first screened through a 200 μm Nitex mesh to remove zooplankton and large-sized particles. The pre-screened water samples were transported to the laboratory as soon as possible within 1–2 h. In the laboratory, water samples were filtered again through a 20 μm Nitex mesh and then filtered onto pre-combusted (450 °C for 4 h) Whatman GF/F glass fiber filters to determine isotope ratios of fine POM (< 20 μm) representing pico- and nano-sized plankton. To obtain enough plankton cells for isotope analysis of coarse POM (≥ 20μm), we collected POM samples by net towing with a plankton net of 50 cm diameter and 20 μm mesh size. After collection, each sample was pre-filtered through a 200 μm Nitex mesh to remove large particles and zooplankton. Both size fractions of samples were prepared in duplicate. Samples for $\delta^{13}C$ measurements were acidified by fuming for about 5 h over concentrated HCl in a vacuum desiccator to remove carbonates, while the samples for $\delta^{15}N$ measurements were not acidified. All the samples were lyophilized and pulverized with a mortar and pestle before isotope analysis.

For chlorophyll *a* (Chl *a*) determination, 1-l subsamples of surface water were filtered through Waterman GF/F glass fiber filters. The filters for Chl *a* (including other photosynthetic pigments) were extracted with 95% methanol (5 ml) for 12 h in the dark at −20 °C and sonicated for 5 min to foster cell disruption. Aliquots of 1 ml of the supernatants were mixed with 300 μl of water; 100 μl of this solution was analyzed by reverse-phase high performance liquid chromatography (HPLC, LC-20A HPLC system, Shimadzu Co., Kyoto, Japan) using a Water Symmetry C_8 (4.6 × 150 mm, particle size: 3.5 μm, 100 Å pore size) column (Waters, Milford, MA, USA) and a method derived from Zapata et al. (2000). Quantification of standard pigments was calculated by spectrophotometer with the known specific extinction coefficients after Jeffrey at al. (1997). Sample peaks were identified based on their retention time compared with those of pure standards. Further details on analysis, calibration, and quantification have been given elsewhere (Lee et al., 2011; Kwak et al., 2017).

2.3 Isotope analysis

For measurements of carbon and nitrogen stable isotope ratios, all pre-treated samples were analyzed using a continuous-flow

isotope ratio mass spectrometer (CF-IRMS; Isoprime100, Cheadle, UK) connected to an elemental analyzer (vario Micro cube, Hanau, Germany) following the procedure described by Park et al. (2016). Briefly, powdered samples were sealed in tin combustion cups and filter samples were wrapped with a tin plate. All prepared samples were put into the elemental analyzer to oxidize at high temperature (1030 °C). CO_2 and N_2gaseswere introduced into the CF-IRMS with the carrier being helium gas. Data of isotope values are shown in terms of δX, indicating the relative differences between isotope ratios of the sample and conventional standard reference materials (Vienna Pee Dee Belemnite for carbon, and atmospheric N_2 for nitrogen), which were calculated by the following equations:$\delta X = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 10^3$, where X is ^{13}C or ^{15}N and $R_{sample}$and $R_{standard}$ are the ratios of heavy to light isotope for samples and standards, respectively. International standards of sucrose (ANU $C_{12}H_{22}O_{11}$; National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) for carbon, and ammonium sulfate ($[NH_4]_2SO_4$; NIST) for nitrogen, were used for calibration after analyzing every 5–10 samples. The analytical precision for 20 replicates of urea were approximately ≤ 0.1‰ and ≤ 0.05‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.4 Data analysis

We used multiple linear regression to assign the isotopic value to each species from a mixture sample. As we did not measure the dry weights each species directly, we calculated them using published empirical equation of the relationship between body length and dry weight of each species and we searched literature for the range of body length and their living environment of each species in the 'World of Copepods Database' and related references (Table S1). Those calanoid species primarily living marine environment based on the definition of 'World of Copepods Database' and related references were grouped as marine calanoids in this paper including *Acartia hudsonica*, *Acartia o*morii, *Bestiolina coreana*, *Calanus sinicus*, *Clausocalanus furcatus*, *Centropages abdominalis*, *Centropages dorsispinatus*, *Paracalanus aculeatus*, *Paraeuchaeta plana*, *Labidocera rotunda*, *Labidocera euchaeta*, *Tortanus dextrilobatus*, and *Tortanus forcipatus*. The group of brackish calanoids included *Acartia ohtsukai*, *Acartia erythraea*, *Eurytemora Pacifica*, *Paracalanus parvus*, *Pseudodiaptomus koreanus*, *Pseudodiaptomus marinus* and *Sinocalanus tenellus*.

Assuming that the proportion we picked to do isotope analysis was the same as the proportion in samples for composition analysis, once the weights of different groups or different genera/species were assigned, we computed the isotope ratio for each group by multiple linear regression models as in the following equations:

$$\mathrm{m} \times \delta^{13}C = m_1 \times \delta^{13}C_1 + m_2 \times \delta^{13}C_2 + m_3 \times \delta^{13}C_3 + m_X \times \delta^{13}C_X + error, \quad (1)$$

$$\mathrm{m} \times \delta^{15}N = m_1 \times \delta^{15}N_1 + m_2 \times \delta^{15}N_2 + m_3 \times \delta^{15}N_3 + m_X \times \delta^{15}N_X + error, \quad (2)$$

where m is the weight of the total community and $m_1 - m_X$ are the weight of different groups or genera of each group. $\delta^{13}C_1 - \delta^{13}C_X$and $\delta^{15}N_1 - \delta^{15}N_X$ are the $\delta^{13}C$ and $\delta^{15}N$ values of each group or genus, respectively. We used R software to do the estimation using the whole sampling data set. Insignificant results for sparse species, such as *Bestiolina coreana*,

Clausocalanus furcatus, *Paraeuchaeta plana*, *Oithona davisae*, and *Oncaea venella*, found in Gwangyang Bay are not tested separately, while they were incorporated to respective groups based on their living environment.

Given that the isotopic values of consumers come from their diets and thereby from mixed proportions of different sources, the proportions of each source could be simulated by linear mixing models with a fractionation factor (also called a trophic enrichment factor). For instance, a mass balance mixing model is given by:

$$\delta^{13}C_{consumer} = f_1\delta^{13}C_{source1} + f_2\delta^{13}C_{source2} + \cdots + f_n\delta^{13}C_{source\,n} + \alpha_{Carbon}, \quad (3)$$

$$\delta^{15}N_{consumer} = f_1\delta^{15}N_{source1} + f_2\delta^{15}N_{source2} + \cdots + f_n\delta^{15}N_{source\,n} + \alpha_{Nitrogen}, \quad (4)$$

$$f_1 + f_2 + \cdots + f_n = 1, \quad (5)$$

where f_1–f_n are the proportion of different sources, and α_{Carbon} and $\alpha_{Nitrogen}$ are trophic enrichment factors for $\delta^{13}C$ and $\delta^{15}N$ values, respectively.

Here, a Bayesian isotopic mixing model (available as an open source Stable Isotope Analysis package in R: SIAR) was performed to estimate the relative contribution of nanoplankton (defined by fine POM in the present study) and microplankton (coarse POM) to the copepod diets, as well as copepods to the carnivore diets (Parnell et al., 2010, 2013). The model assumes that each isotopic ratio of consumers follows the pattern of a Gaussian distribution with an unknown mean and standard deviation. The structure of mean values of consumers is a weighted combination of the food sources' isotopic values. The weights make up dietary proportions (given by a Dirichlet prior distribution). The standard deviation is divided up between the uncertainty around the fractionation corrections and the natural variability between all individuals within a defined group (Parnell et al., 2010, 2013). Because the values of consumers calculated from bulk copepod samples using the previous multiple linear regression models were only means and standard errors, we generated a vector consisting of 250 numbers for each group by a random normal distribution function. We then used the default iteration numbers (iterations = 500,000, burin = 50,000) provided by the SIAR package to perform our analysis. Fractionation factors used in the model estimation were estimated by difference of TLs multiplying with 3‰ per TL for $\delta^{15}N$ and with 0.5‰ per TL for $\delta^{13}C$, respectively. TLs were calculated from the $\delta^{15}N$ difference between consumer and source as follows (Post, 2002): $(TL = 1 + (\delta^{15}N_{consumer} - \delta^{15}N_{source})/3)$. Concentrations of isotope per mass among different diets (nanoplankton, microplankton, and major copepod genera) were not considered in this study. Model fitting was done via a Markov Chain Monte Carlo (MCMC) protocol that produces simulations of plausible values of the dietary proportions of each source. More details on model simulation can be found elsewhere (Parnell et al., 2010, 2013).

All statistical analyses were performed using R 3.4.0 software (https://cran.r-project.org/bin/windows/base/). Regression analyses of copepod isotopic values were performed by generalized additive models (GAMs) using the *mgcv* library (Wood and Wood, 2015). Data were smoothed by cubic regression splines and fitted by the family of Gaussian. One-way analysis of variance (ANOVA) was adopted to test seasonal differences in environmental factors and copepod abundances, and Student's *t*-tests were used to test for significant differences in mean $\delta^{13}C$ and $\delta^{15}N$ values between nano- and microplankton. Before

applying ANOVA and *t*-tests, the data were tested for normality of distribution and equal variance; significance was assumed at $P = 0.05$. For the coefficients of variation, we calculated them by dividing the standard error with mean value.

3 Results

3.1 Environmental variability and zooplankton abundances

Environmental factors including temperature, salinity, Chl *a* levels, copepod abundance, dominant species, and percentages of total copepods are shown in Table 1. Water temperature was significantly higher in summer and lower in winter (ANOVA, $P < 0.001$). Spatial variability of salinity was significant, with extremely low values at stations 1 and 2 (the river mouth) and then the values gradually increased to station 5 (the middle of the bay). Chl *a* concentrations ranged from 0.1 to 6.8μg l^{-1} and they were significantly higher in spring and summer than in winter and autumn (ANOVA, $P < 0.01$). The highest Chl *a* concentration occurred in the middle of the bay during the spring, while the lowest concentration was found at the river station during the autumn. Seasonal variability of copepod abundance was significant (ANOVA, $P < 0.01$), with higher abundances in winter when temperatures and Chl *a* concentrations were low.

Detailed abundance composition of copepods is shown in Table S2. Seasonal and spatial variations of dominant species (>10% of total abundance) of copepods were apparent (Table 1). The marine calanoid *Acartia* dominated at the river mouth to the middle part of the bay, while *Paracalanus* dominated at the mouth of the bay during winter. *Acartia* also dominated at the most highly saline stations in summer, except for station 7, where the community was dominated by *Labidocera rotunda*. A brackish water-preferring calanoid species, *Pseudodiaptomus*, dominated stations 1 and 2 at the river mouth in spring and another brackish calanoid species, *Sinocalanus*, dominated station 1 in autumn. At the river-mouth stations in summer, copepods were unexpectedly dominated by the marine calanoid species *Tortanus dextrilobatus*. The cyclopoid species *Corycaeus affinis* mainly dominated the most highly saline stations in spring and autumn.

3.2 Variability of plankton $\delta^{13}C$ and $\delta^{15}N$ values

The $\delta^{13}C$ values of size-fractionated plankton (< 20 and 20–200 μm) and mixed copepod samples showed distinct spatial variations in each season (Fig. 2A–C). The $\delta^{13}C$ values of nanoplankton (< 20 μm POM) ranged from −27.6 to −19.4‰ with a mean of −22.7‰ (Fig. 2A). The lowest $\delta^{13}C$ value of nanoplankton was found at station 1 (the upper stream station of Seomjin River) in spring and the highest at station 9 (the mouth of the estuary) in summer. The $\delta^{13}C$ values of microplankton (20–200 μm POM) ranged from −26.3 to −17.8‰ with a mean of −20.8‰ (Fig. 2B), being significantly higher than those of nanoplankton (paired *t*-test, $t = 7.6$, $P < 0.001$). Its lowest $\delta^{13}C$ value was found at station 2 in spring and the highest at station 8 in winter. Overall, similar to nanoplankton, the microplankton $\delta^{13}C$ values were more negative at the river portion (stations 1–3) and less negative at the mouth of the estuary (stations 7–9). The $\delta^{13}C$ values of mixed copepods ranged from −25.9 to −16.4‰, with a mean of −20.1‰ (Fig. 2C). The lowest at station 1 in autumn and the highest at station 8 in summer. The

spatial variability of copepod $\delta^{13}C$ values followed the pattern of POM $\delta^{13}C$ values. However, the copepod $\delta^{13}C$ values were significantly higher than those of nanoplankton (paired *t*-test, $t = 8.6$, $P < 0.001$) and microplankton ($t = 3.1$, $P = 0.004$). Their $\delta^{13}C$ values were higher in summer and winter than in spring and autumn. At stations 1–3, river input lowered the $\delta^{13}C$ values of nanoplankton during the wet season (spring to summer). At stations 4–9, significantly lower $\delta^{13}C$ values were observed in autumn than in other seasons (ANOVA, $F = 13.4$, $P < 0.001$). For copepods, the autumn values were significantly lower than those in other seasons (ANOVA, $F = 5.9$, $P = 0.004$).

The $\delta^{15}N$ values exhibited wider fluctuations than $\delta^{13}C$ values (coefficients of variation = 29.3% vs. 9.0%, 21.5% vs. 11.8%, and 18.8% vs. 13.1% for nanoplankton, microplankton, and copepods, respectively). The $\delta^{15}N$ values of nanoplankton ranged from 3.2‰ (station 4 in summer) to 8.8‰ (station 1 in winter) with a mean of 5.6‰ (Fig. 2D). There were distinct patterns in the three locations of the bay. The $\delta^{15}N$ values tended to decline with distance from the river mouth, then increased in the middle of the bay, and decreased again toward the mouth of the estuary. The nanoplankton $\delta^{15}N$ values were higher in winter than in other seasons (paired *t*-test, $t = 5.4$, $P = 0.001$ for spring; $t = 3.0$, $P = 0.017$ for summer; $t = 4.1$, $P = 0.004$ for autumn). As indicated by regression analyses between the distribution of nanoplankton $\delta^{15}N$ and environmental factors (Table 2), significant increases in the nanoplankton $\delta^{15}N$ values depend on ammonia (GAM, $F = 4.1$, $P = 0.029$) and Chl *a* (GAM, $F = 3.8$, $P = 0.044$). In addition, the seasonal distribution of nanoplankton $\delta^{15}N$ values was well indicated by their relationship with temperature among different seasons (GAM, $F = -5.5$, $P = 0.013$), decreasing the values in summer to autumn.

Mean $\delta^{15}N$ value of microplankton (7.6‰), ranging from 4.8‰ (station 2 in spring) to 10.2‰ (station 6 in spring), was significantly higher than that of nanoplankton (paired *t*-test, $t = 4.9$, $P < 0.001$). The microplankton $\delta^{15}N$ values were higher in summer than in other seasons (ANOVA, $F = 4.6$, $P = 0.009$), with the spatial trend vanishing in summer. Indeed, spatial trends differed between seasons, increasing progressively from the river mouth to the bay mouth in spring and autumn, and decreasing in winter. As tested by GAM analysis, the microplankton $\delta^{15}N$ values were stepwise elevated by environmental factors including temperature (GAM, $F = 5.0$, $P = 0.015$), salinity (GAM, $F = 5.0$, $P = 0.031$), ammonia (GAM, $F = 4.5$, $P = 0.031$), and nitrate (GAM, $F = 7.8$, $P = 0.010$). Similar to nanoplankton, regression analysis also showed that the microplankton $\delta^{15}N$ values increased significantly with increasing Chl *a* concentrations (GAM, $F = 4.2$, $P = 0.043$).

Copepod $\delta^{15}N$ values ranged from 6.6 to 12.3‰ and were higher in summer than in other seasons (ANOVA, $F = 15.6$, $P < 0.001$). Generalized additive model analysis showed that the deviances of copepod $\delta^{13}C$ and $\delta^{15}N$ values explained by the GAMs were 92.7% and 76.9%, respectively (Table 3). Copepod $\delta^{13}C$ values changed significantly toward increasing salinity (GAM, $F = 7.9$, $P = 0.005$), for both the nanoplankton (GAM, $F = 6.2$, $P = 0.008$) and the microplankton (GAM, $F = 16.4$, $P < 0.001$; Fig. S1A–C). In contrast, temperature was the most important factor to explain the variability of copepod $\delta^{15}N$ values (GAM, $F = 13.6$, $P < 0.001$; Fig. S1D). The microplankton $\delta^{15}N$ value was another important contributor to the variability of copepod $\delta^{15}N$ values (GAM, $F = 3.5$, $P = 0.034$; Fig. S1E), while nanoplankton $\delta^{15}N$ was not (GAM, $P > 0.05$). The Chl *a* concentration influenced the variability of copepod $\delta^{15}N$ values significantly (GAM, $F = 3.3$, $P = 0.047$; Fig. S1F).

3.3 Trophic positions of major groups

Multiple linear regression analyses to estimate mean isotopic values of different copepod groups (i.e., brackish calanoids, marine calanoids, and cyclopoids) from mixed copepod values (excluding harpacticoids) were all significant (R^2= 0.94, P < 0.001 for $\delta^{13}C$; R^2 = 0.78, P < 0.001 for $\delta^{15}N$). The intercepts of the model indicating the errors of Eq. (1) and Eq. (2) were1.1 ± 0.8‰ and 0.3 ± 0.8‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. The two calanoid groups displayed a close mean $\delta^{15}N$ value (around 9‰) but significantly different $\delta^{13}C$ values (−26.3 ± 1.5‰, −20.6 ± 1.6‰, and −20.1 ± 1.2‰ for brackish water calanoids and marine calanoids, respectively; Fig. 3A). Cyclopoids occupied a relative broad trophic niche based on a big coefficient of variation (41.6%), and the mean $\delta^{13}C$ value of cyclopoids (−31.0 ± 6.0‰) was even lower than those of brackish calanoids. The $\delta^{15}N$ values of the three major groups were all higher than the basal food resource (nanoplankton), and relatively higher than microplankton with some overlap of error bar. The values of harpacticoids isolated from the winter (stations 2–9) and spring samples (stations 1) were measured directly. The mean $\delta^{15}N$ values of harpacticoids (6.9 ± 0.6‰) were lower than those of other copepods and microplankton, but relatively higher $\delta^{13}C$ value (−16.7 ± 1.4‰).

The multiple linear regression analysis performed for major copepod genera/species was also significant (R^2 = 0.99, P < 0.001 for $\delta^{13}C$; R^2 = 0.88, P < 0.001 for $\delta^{15}N$). The intercepts were 0.4 ± 0.7‰ and 0.8 ± 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. There were two to three different trophic positions in the mixed copepod assemblages based on different $\delta^{15}N$ values (see patterns in Fig. 3B). The mean $\delta^{15}N$values of *Corycaeus affinis* (13.7 ± 5.6‰), were the highest among taxa, followed by those of *Labidocera* (12.3 ± 3.9‰), *Tortanus* (12.3 ± 1.9‰), *Sinocalanus* (11.3 ± 1.7‰) and *Paracalanus* (11.0 ± 3.6‰). The mean values of *Acartia* (9.4 ± 2.6‰), *Calanus Sinicus* (9.0 ± 1.1‰), *Centropages* (8.1 ± 1.7‰) and *Eurytemora pacifica* (7.4 ± 2.5‰) indicated their trophic positions were relatively lower than those carnivorous species while they were higher than nanoplankton. *Pseudodiaptomus* had a lowest value of $\delta^{15}N$ (5.3 ± 2.5‰) and it was even lower than microplankton and not much different to nanoplankton. Compared to of the two putative food resources (nanoplankton and microplankton), those brackish calanoid genera/species (*Pseudodiaptomus*, *E. pacifica*, *Paracalanus and Sinocalanus*) and *Corycaeus* had lower $\delta^{13}C$ than nanoplankton, while marine calanoid genera/species (*Tortanus*, *Labidocera*, *Calanus*, *Acartia* and *Centropages*) had higher values (Fig. 3B).

The trophic level in this study was defined as trophic position relative to nanoplankton, which was considered as the trophic baseline. Considering the TL of nanoplankton is 1, the TL value of microplankton was calculated to be 0.7 times higher than that of nanoplankton (Fig. 4). As a whole assemblage balanced from different feeding behaviors, as indicated by the standard errors, copepods occupied a 1.2 level higher TL than that of nanoplankton, indicating herbivory (here, herbivory means a trophic level of 2) on nanoplankton with slight omnivory (TL = 2–3) on other dietary sources. The TLs of three major calanoid groups (marine calanoids and brackish calanoids) were similar to the bulk copepod assemblage with mean levels slightly higher than 2. The mean TL value of cyclopoids had apparently higher trophic level than calanoids. In contrast, the mean TL of harpacticoids was very low, reflected by their low $\delta^{15}N$ values.

Among calanoids, the mean TLs of *Eurytemora pacifica* (1 ± 0.5) and *Pseudodiaptomus* (1 ± 0.5) is indicative of their herbivorous and/or detritivorous characteristics. The mean TLs of *Calanus sinicus* (2.0 ± 0.6) and *Centropages* (1.6 ± 0.6) indicated they were primarily herbivorous; and those of *Acartia* and *Paracalanus* were slightly higher than 2 indicating they were primarily omnivorous feeding on both nanoplankton and microplankton. The levels of *Sinocalanus* (3.0 ± 0.7), *Tortanus* and *Labidocera* were higher than 3.

Based on TLs, the mean ^{15}N enrichments of the copepod assemblage were estimated to be 3.4‰ and 1.7‰ for nanoplankton and microplankton, respectively (Fig. 5A, B). The enrichment values on nanoplankton for both marine and brackish calanoids, as well as for the genera of *Acartia* were close to the average value of the copepod assemblage. The trophic enrichment of *Calanus sinicus* on nanoplankton (3.0 ± 1.7‰) was slightly lower than averaged marine calanoids and total copepods, while the enrichment on microplankton (1.7 ± 1.5‰) was similar. *Centropages* (1.9 ± 1.8‰) had much lower enrichment on nanoplankton compared to total copepods and marine calanoids. Four high TL genera *Tortanus*, *Labidocera*, *Sinocalanus* and *Corycaeus* had high enrichments > 6 on nanoplankton and > 3 on microplankton.

The ^{13}C enrichments for total copepods were on average 0.6‰ and 0.3‰ when feeding on nanoplankton and microplankton, respectively (Fig. 5C, D). Patterns were same with enrichments on ^{15}N.The four high TL genera *Tortanus*, *Labidocera*, *Sinocalanus* and *Corycaeus* had high enrichments > 1.2 on nanoplankton and > 0.6 on microplankton. The enrichments of four herbivorous/omnivorous species increased from *Centropages, Calanus, and Acartia to Paracalanus.* In contrast, the brackish calanoid genera *Eurytemora* and *Pseudodiaptomus* had extremely low enrichments of both ^{15}N and ^{13}C.

3.4 Contribution of size-fractionated POM to copepod diets

The Bayesian mixing model calculations showed that the contributions of different sizes of POM to copepod diets varied significantly with season (Student's *t*-test, $P < 0.001$ for all cases; Fig. 7). Size-selective feeding phenomena were particularly apparent in winter (Fig. S2A) and summer (Fig. S2C). Mean contributions of microplankton accounted for about two-thirds of their assimilated diets at all stations in winter and summer, and were almost equal to that of nanoplankton in spring and autumn (Fig. S2B, D). The proportions of the two size fractions of POM averaged from all four seasons contributing to copepod diets were also distinctly different at stations 2–5 and station 9 (Fig. S2). The mean contributions of microplankton to the copepod diets increased gradually from the river mouth up to a peak (0.81 ± 0.11) at the middle part of the bay. Then, the proportion declined gradually to a trough (0.31 ± 0.18) at the deep-bay channel. The proportion then rebounded to a high level again at the bay mouth station.

Three major groups of copepods showed contrasting size-selective feeding behaviours (Fig. 6). Marine calanoids typically preferred feeding on larger particles, with a contributing proportion of 0.63 ± 0.03 (range: 0.50–0.71) for microplankton (Fig. 6A). Harpacticoids had a more apparent size-selective feeding behaviour preferring on microplankton (0.89 ± 0.10) and merely fed on nanoplankton (extremely low reliance of < 0.11; Fig. 6C). In contrast, brackish calanoids preferred feeding on nanoplankton (0.68 ± 0.05) to on microplankton (0.32 ± 0.05). For herbivorous/omnivorous copepod genera/species (e.g., *Acartia*, *C. sinicus*, *Centropages*, *E. pacifica*, *Paracalanus*, and *Pseudodiaptomus*), we tested their feeding preference on the

two size fractions of POM using the Bayesian mixing model (Fig. 7). *Acartia* and *Centropages* significantly preferred large to small particles (Student's *t*-test, $P < 0.001$)with a reliance of > 0.9 on microplankton (Fig. 7A, C),on the other hand, *C. sinicus* and *Paracalanus* apparently preferred small particles (Student's *t*-test, $P < 0.001$) (Fig. 7B, E).The two 'low trophic level' species/genus *Eurytemora pacifica* and *Pseudodiaptomus* had higher selecting on nanoplankton while there were high ranges of proportion of both potential food sources in diets(Fig. 7D, F). Based on TL showed above, we considered the four genera (*Labidocera*, *Sinocalanus*, *Tortanus* and *Corycaeus*) are carnivorous and able to predate other copepods. Thus, we tested the two fractions of POM and some herbivorous/omnivorous species/genera as potential food sources of them. The dietary compositions estimated by Bayesian mixing models showed slightly different among them (Fig. 8). Except *Sinocalnaus*, the other three carnivorous species all showed only negligible reliance on the two size fractions of POM. The marine calanoid genus *Labidocera* preferred predating on *Centropages* (0.35 ± 0.08), followed by harpacticoids (0.15 ± 0.10), *Acartia* (0.13 ± 0.09) and *Calanus* (0.10 ± 0.07) (Fig. 8A). On the contrary, besides of feeding on nanoplankton (0.23 ± 0.03), the brackish calanoid genus *Sinocalanus* also preferred predating on other brackish calanoids *Paracalanus* (0.37 ± 0.02), *Pseudodiaptomus* (0.15 ± 0.03) and followed by cyclopoids (0.09 ± 0.02) (Fig. 8B). *Tortanus* frequently co-occurred with many other copepods and its diet was composed of many different copepod species/genera without apparent selectivity (Fig.8C). The cyclopoid species *Corycaeus affinis* primarily predating on *Paracalanus* (0.67 ± 0.05) and *Pseudodiaptomus* (0.22 ± 0.06).

4 Discussion

4.1 Variability of $\delta^{13}C$ and $\delta^{15}N$ values of plankton with time and space

We found that seasonal variations and the spatial heterogeneity of copepod $\delta^{13}C$ and $\delta^{15}N$ values in Gwangyang Bay followed those of nanoplankton (POM < 20 μm) and microplankton (POM > 20 μm) (Figs 2 and 3). Based on the results of regression analyses, we found that the variability of copepod isotopic values was influenced by salinity (spatial variations), temperature (temporal variations), and isotopic values of food sources (both spatial and temporal variations; Fig. 3). In general, spatial variations were much more pronounced because of the effect of river input and thereby riverine carbon in different salinity regimes. More negative values of three plankton groups (nanoplankton, microplankton, and copepods) were measured near the river mouth, and then the values increased progressively to the mouth of the estuary, indicating an apparently decreasing effect of river runoff and thus the uptake of carbon derived from river-borne terrestrial organic matter. These results are consistent with other studies in estuarine environments (Cifuentes et al., 1988; Matson and Brinson, 1990; Thornton and McManus, 1994; Deegan and Garritt,1997; Fry, 2002). Such spatial distribution patterns have also been found for other primary producers such as seagrasses (reviewed by Hemminga and Mateo, 1996), macroalgae (Lee, 2000), as well as benthic microalgae (Kang et al., 2003), and the pattern will further propagate to consumers such as fish (Melville and Connolly, 2003; Herzka, 2005), oysters (Fry, 2002), mollusks (Antonio et al., 2010), and other benthic macro-invertebrates (Choy et al., 2008).

Seasonal successions of $\delta^{13}C$ values were also apparent, probably because of high river input in spring, elevated productivity in summer, and species successions in autumn. When the wet season started in spring and phytoplankton started to bloom, river input lowered the $\delta^{13}C$ values. The values increased again in summer because of the persistence of phytoplankton bloom (a low fractionation effect because of source limitation) and elevated productivity in summer. Although both river discharge and input of light carbon were low in autumn, the observed $\delta^{13}C$ values were low. This was probably because of the lack of a heavy carbon pool (a dissolved inorganic carbon pool in which the carbon was primarily composed of heavy ^{13}C) due to microbial respiration and species succession (Rau et al., 1990). During the post-bloom period in autumn, phytoplankton show low productivity and Chl *a* concentrations, with low abundance of diatoms but a dominance of flagellates (Baek et al., 2015). Flagellates are known to have more negative $\delta^{13}C$ values than those of diatoms arising from different fractionation effects (Gearing et al., 1984; Cifuentes et al., 1988; Rolff, 2000).

The $\delta^{15}N$ variability of three major plankton groups was relatively complex spatially. Seasonal pattern of nanoplankton $\delta^{15}N$ values, decreasing the values in summer to autumn, can well explained by the relationship between the $\delta^{15}N$ values and temperature, indicated by significant GAM analysis. Spatial trends in the nanoplankton $\delta^{15}N$ values can be explained by three distinct distribution patterns. The first pattern found in the river mouth area exemplifies a declining trend expected by mixing of freshwater planktonic materials, which grew up in water with high levels of dissolved inorganic nitrogen. The second pattern found in the middle part of the bay, in which nitrate inputs were low while the concentrations of ammonia increased (Kwon et al., 2004; own data not shown), characterizes an increase in $\delta^{15}N$ values in association with high Chl *a* concentrations. Fractionations by autotrophic assimilation and bacterial utilization were the most likely source of the ^{15}N-enriched ammonia in nutrient pools of the middle of the bay (Cifuentes et al., 1988). The elevated POM $\delta^{15}N$ values in the middle of the bay may be explained by ^{15}N-enriched ammonia remaining after algal uptake in the river mouth channel (Sato et al., 2006). The input of sewage-derived ^{15}N-enriched ammonia (domestic sewage and livestock waste) could contribute substantially and swamp the other subtle processes to increase $\delta^{15}N$ values of nanoplankton. The third distribution pattern represents declining $\delta^{15}N$ values toward the offshore bay mouth in association with a reduction in the supply of ^{15}N-enriched nutrients from terrestrial sewage. Furthermore, the fractionation effect of phytoplankton will be reduced when nutrients substantially decreased and phytoplankton would take up nitrogen with little fractionation and stored relatively light nitrogen isotope (Cifuentes et al., 1988; Fogel and Cifuentes, 1993; Granger et al., 2004). As indicated by GAM regression analyses between the distribution of nanoplankton $\delta^{15}N$ and environmental factors, significant increases in the nanoplankton $\delta^{15}N$ values depend on ammonia and Chl *a*, further supporting our explanation. The microplankton $\delta^{15}N$ values were stepwise elevated by environmental factors including temperature, salinity, ammonia and nitrate, indicated by significant results of GAM analysis (Table 2). Similar to nanoplankton, regression analysis also showed that the microplankton $\delta^{15}N$ values increased significantly with increasing Chl *a* concentrations (Table 2). One possible mechanism for this pattern is that higher phytoplankton abundance will result in a ^{15}N-enriched nutrient pool because of fractionation during nutrient assimilation (Kang et al., 2009). Nitrate was important for

microplankton, indicative of the role of diatoms (preferring nitrate) in controlling the variation in microplankton $\delta^{15}N$ values, whereas nanoflagellates (preferring ammonia) probably controlled the variation in nanoplankton $\delta^{15}N$ values.

As indicated by GAM analysis, the seasonality of copepod$\delta^{15}N$ values was primarily enhanced by temperature, which probably caused an elevated fractionation effect during the rapid assimilation of copepods. The lower explained deviance of $\delta^{15}N$ indicated reflects the food-web processes that affect $\delta^{15}N$ disproportionally and were not included in the GAM analysis. The regression relationship between larger plankton and copepods was significant, whereas the patterns were somewhat decoupled, as they were primarily observed in spring and autumn. This kind of decoupling has also been reported in the open ocean (Montoya et al., 2002), where the transfer of nitrogen from primary producers to zooplankton is weak. A time lag in zooplankton development might cause the mismatch of zooplankton to ^{15}N-enriched POM at the initial stage of nutrient supplies. Indeed, here we found that the high $\delta^{15}N$ values of copepods were primarily observed in summer, while the corresponding $\delta^{15}N$ values of POM started to increase from the winter, and phytoplankton blooming occurred in the spring.

4.2 Trophodynamics and trophic enrichments of copepods

The variability of copepod isotopic values in Gwangyang Bay suggests that the TLs of the copepod assemblage were highly dynamic. Because of different feeding behaviors and fractionation effects of copepods, the variability of the average community trophic position depends on the overall composition of species and is determined by the dominant species. Direct measurements of copepod isotopic values for species levels have been poorly conducted in the literature, although there are still clear patterns in existing reports. In the Southern Ocean copepods, the known carnivores *Euchaeta* and *Heterorhabdus* had high $\delta^{15}N$ values, while the acknowledged omnivores *Calanoides* and *Metridia* were intermediate in position, and *Rhincalanus* had the lowest values (Schmidt et al., 2003). A mesocosm study found that the $\delta^{15}N$ values were increasingly higher in the order *Temora* < *Pseudocalanus* < *Centropages*, suggesting an increase of carnivory in the same manner (Sommer et al., 2005). The trophic positions of primary consumers (*Oithona* and *Neocalanus*) and secondary consumers (*Pleuromamma* and *Euchaeta*) in the North Pacific Subtropical Gyre are estimated to be 2.1 and 2.9, respectively (Hannides et al., 2009). Furthermore, Kürten et al. (2011) reported that the relative trophic positions of zooplankton in the North Sea were high when the assemblage was mainly composed of *Sagitta* and *Calanus*, but low when the assemblage was dominated by *Pseudocalanus* and zoea larvae.

Our study has demonstrated the trophodynamics of estuarine copepods using multiple linear mixing model analysis based on the values of bulk samples and percentages in total biomass, by which the results of estimated $\delta^{13}C$ and $\delta^{15}N$ values were both significant ($P < 0.01$). The estimated $\delta^{13}C$ values varied greatly up to 10.9‰ among groups (from the lowest for cyclopoids to the highest for harpacticoids) and 14.2‰ among genera (from the lowest for *Paracalanus* to the highest for *Centropages*), respectively (Fig. 4). The estimated $\delta^{15}N$ values also varied somewhat up to 4.9‰ among groups, indicating one TL difference among groups if we consider 3–4‰ trophic enrichment of $\delta^{15}N$ between two adjacent TLs (Post, 2002; Michener and Kaufman, 2007). The trophic enrichments calculated by the differences from the basal food sources indicate that the overall enrichments

of copepods were around 3.4‰ and 1.7‰ from nanoplankton and microplankton, respectively (Fig. 6). Our estimation shows that both marine calanoids and brackish water calanoids overall occupy a similar trophic niche (i.e., similar mean $\delta^{15}N$ values), but have contrasting food sources (i.e., different $\delta^{13}C$ values; Fig. 4A). The isotopic values of calanoid copepods indicate a mixture of different genera including both high and low $\delta^{15}N$ values. For example, marine calanoids were mixed by high $\delta^{15}N$ genera (*Tortanus* and *Labidocera*) and low $\delta^{15}N$ genera (*Acartia, Calanus, Centropages*) and moderate $\delta^{15}N$ genera (*Paracalanus* and *Acartia*). Brackish types were mixed by high $\delta^{15}N$ genera (*Sinocalanus*) and low $\delta^{15}N$ genera (*Eurytemora* and *Pseudodiaptomus*). Consequently, the two major calanoids groups (marine and brackish water types) as well as the mixture of all copepod groups were estimated to be on average one TL higher than the nanoplankton base (Fig. 4). However, we do not necessarily conclude that they are herbivores because the nanoplankton studied here represent POM with a size range of 2–20 μm, which may include ciliates, heterotrophic nanoflagellates, and heterotrophic dinoflagellates. Instead, all assemblages mentioned above might be omnivorous with varying levels of relative trophic positions depending on dominant species.

Among calanoids, brackish water species had significantly lower $\delta^{13}C$ values than marine species, indicative of an apparent effect of riverine carbon sources on brackish species through the food web (Fig. 3). These results were consistent with the distribution pattern that most brackish species occurred or dominant in the upper part of Gwangyang Bay. *Sinocalanus tenellus* had contrasting TL value to those of *Pseudodiaptomus* and *Eurytemora* (Fig. 4B), indicating a mixture of brackish water calanoids being close to omnivory with a broad feeding size spectrum. However, as *Sinocalanus* and *Pseudodiaptomus* s were dominant in different seasons (Table 1), the TLs of the copepod assemblage at a specific condition will become relatively carnivorous (*Sinocalanus* dominating) or omnivorous (*Pseudodiaptomus* and *Paracalanus parvus dominating*). *P. parvus* is an important small species (body length ≤1mm) that is widely distributed in coastal and estuarine waters worldwide (Turner, 2004). Our results showed that, similar to other brackish water calanoids, this species was greatly influenced by ^{13}C-depleted dietary sources, dominating both brackish stations in autumn and saline stations in winter (Table 1). This result indicates that *Paracalanus* was well adapted to fluctuating estuarine environments by feeding on prey originating from freshwater or prey that depends on riverine carbon sources. *Acartia,* one of the commonest genus in Gwangyang Bay throughout the year (Table 1), included both marine types (*A. hudsonica and A. Omorii*) and brackish types *(A. ohtsukai*, and *A. erythraea*) based on literature (Table S1). Marine types of *Acartia* primarily occurred or dominated during the winter and spring, while brackish type dominated during the summer. Overall the genus of *Acartia* had higher $\delta^{13}C$ values than those of *Paracalanus*, suggesting their different food sources. *Acartia* is also a widely distributed genus, with a switching feeding behaviour in response to the status of food composition (Kiøboe et al., 1996; Rollwagen-Bollens and Penry, 2003; Chen et al., 2013). The isotopic values of *Acartia* were similar to those of the assemblage of marine calanoids, indicating that this genus is omnivorous, as typical of marine calanoids. The marine calanoids species *C. sinicus* had a close trophic niche to total marine calanoids based on similar $\delta^{15}N$ value and trophic level (Fig. 4), 1 TL higher than nanoplankton suggesting that this species is a typical marine herbivore relative to nanoplankton. Conversely, two other marine calanoid genera, *Tortanus* (*T. dextrilobatus* and *T. forcipatus*) and *Labidocera* (*L. euchaeta* and *L. rotunda*), were primarily carnivorous as indicated by their $\delta^{15}N$ values (Figs. 3B and 4). These

estimated results are consistent with the former experimental tests and field investigations (Ambler and Frost, 1974; Landry, 1978; Conley and Turner, 1985; Hooff and Bollens, 2004). Cyclopoids (primarily *Corycaeus affinis*) dominated copepod assemblages in spring and autumn at the middle part and deep-bay channel of the bay (Table 1). Our data reveal that *Corycaeus* was primarily carnivorous, being 2 TLs higher than nanoplankton (Fig. 4) and prefers feeding on ^{13}C-depleted dietary sources (Fig. 3). This result is consistent with previous reports that the *Corycaeus* genus is carnivorous (Gophen and Harris, 1981; Landry et al., 1985; Turner, 1984).

Isotopic values of microplankton indicate that they are roughly a half TL value higher than nanoplankton. Considering that the sizes of most ciliates and heterotrophic dinoflagellates primarily fall within this size spectrum (20–200 μm), this result suggests an omnivorous trend among the mixed microplankton groups. Similarly, although measured only in winter samples, the benthic copepod group harpacticoids, represented by the species *Euterpina* sp., also differed from calanoids and cyclopoids with low $\delta^{15}N$ values. The TL of harpacticoids estimated from this approach was somewhat misleading because of their unexpectedly low $\delta^{15}N$ values, which probably reflect feeding on detritus or dead organisms that are depleted in ^{15}N (Sautour and Castel, 1993).

4.3 Selective feeding of copepods

Feeding preferences of different groups or genera on two size fractions of POM are of particular importance to explain seasonal and spatial patterns of community trophic niches, and in turn will predict the impacts of the grazer community on lower TLs including phytoplankton and microzooplankton. Because not all possible food sources, such as bacteria, picoplankton, fecal pellets, and dead detritus, were investigated, our Bayesian mixing model calculations might have led to some biased results. Nevertheless, the model results might provide an estimation on what size fractions of dietary sources the grazer community ingest and assimilate. In general, our results highlight that the copepod assemblages have size-selective feeding behaviors, and that these vary with season (Fig. S2) and space (Fig. S3). The feeding selectivity of the bulk copepod assemblage was a balance of ingestion among different groups. The whole copepod assemblage assimilated two-thirds of its food requirement from microplankton in winter and summer, but they fed nearly equally on both size fractions of POM in spring and autumn (Fig. S2). Our results suggest that groups that preferred large-sized prey played a more important role in total assemblage of copepods in winter and summer, during which the assemblage were primarily dominated by marine calanoids (Table 1). On the other hand, in the spring and autumn when the assemblage was primarily dominated by carnivorous species (*Corycaeus*, *Tortanus*) and dominated by both *Paracalanus* (our result suggested this genus was an omnivorous species and the size-selectivity was less pronounced preferring small-sized particle) and *Acartia* (preferring large-sized particle), the assemblage overall didn't show an apparent size-selectivity.

Based on the model results for major copepod groups and genera, marine calanoids preferred feeding on large particles (Figs. 6A and 7A) Such a size-selective feeding preference has been widely reported in many field investigations (Liu et al., 2005a; Jang et al., 2010; Chen et al., 2017). On the contrary, brackish calanoids as a group preferred feeding on small-sized plankton. These results were not well reported in literature. Among calanoids, *Acartia and Paracalanus* both contain marine species and

brackish species. Our model results showed that *Acartia* preferred feeding on large-sized plankton, while *Paracalanus* was contrastingly different. *Acartia*, well studied in literature, are reported to prefer feeding on phytoplankton larger than 20 μm in the coastal water adjacent to the present study area (Jang et al., 2010). Besides, due to nutritional requirements, *Acartia* were reported that they preferred predating microzooplankton such as ciliates and heterotrophic dinoflagellates (Wiadnyana and Rassoulzadegan, 1989; Chen et al., 2013). On the other hand, another dominant calanoid genus, *Paracalanus*, preferred feeding on nanoplankton (Fig. 7E), suggesting that the feeding efficiency, with which the grazers' feeding appendage to retain particles, differs between these two genera. Because of such different feeding preferences, they dominated in different stations or seasons with differing food conditions (Table 1) and frequently co-occurred with little overlap of preferred food particle sizes. Besides, low $\delta^{13}C$ value of *P. parvus* indicated that this wildly distributed species preferred feeding on particles flourished from terrestrial carbon.

Although *C. sinicus* was not dominant in abundance, this species contributes a great amount in biomass due to large body size (Table S1). Similar to *Paracalanus*, *C. Sinicus* preferred feeding on small-sized particles, while the difference is that the relatively high $\delta^{13}C$ value of *C. Sinicus* indicated that this species preferred prey originated from offshore areas. The result is consistent with reports that *C. Sinicus* is a dominant species in Yellow Sea and East China Sea (the offshore shelf waters of this study estuary) (Uye, 2000; Liu et al., 2003). Another marine calanoid genus similar to *Acartia*, *Centropages* (*C. abdominalis* and *dorsispinatus*) apparently preferred feeding on large-sized plankton (this study and Wiadnyana and Rassoulzadegan, 1989). For other two marine calanoid genera —*Labidocera* (*L. rotunda and L. euchaeta*) and *Tortanus* (*T. dextrilobatus and T. forcipatus*) — feeding on two size fractions of POM did not occur, based on no contribution of the two size fractions of POM to their diets (Fig. 8A, C). Model results indicated that *Labidocera and Tortanus* were carnivorous genera, consistent with many reports (Landry, 1978; Mullin, 1979; Turner, 1984; Uye, 1994; Hooff and Bollens, 2004). Our result also showed that *Labidocera* had an apparent selectivity in predating their prey, preferring marine calanoids (*Acartia*, *Calanus* and *Centropages*) and harpacticoids. This feeding selectivity was consistent with the distribution of this species. It never distributed in river stations (stations 1–3) (Table S2) and even dominated at station 7 during the summer (Table 1). Differently, *Tortanus* didn't show selectivity among different prey (Fig. 8C) and thus this genus occurred frequently in different stations (Table S2).

The model results indicated that the size selectivity of brackish water calanoids such as *Pseudodiaptomus* and *Eurytemora* was also apparently for nanoplankton similar to *Paracalanus* (Fig.7 D, F). To our knowledge, the feeding habit of *Pseudodiaptomus* is unknown in the current literature, whereas some field studies suggest that estuarine *Pseudodiaptomus* flourishes by feeding on small phytoplankton cells (< 20 μm) (Froneman, 2004), consistent with the present results. Incorporating the results of low $\delta^{13}C$ values of *Pseudodiaptomus* and *Eurytemora*, we believe that these two genera were able to feed on those prey with small sizes (2–20 μm) and low $\delta^{13}C$ from originated from terrestrial sources, whereas low trophic position and trophic enrichments indicated that these two genera may ingest prey with extremely low $\delta^{15}N$ compared to nanoplankton (e.g., detritus). The detritus with low $\delta^{15}N$ may contribute to the balance the $\delta^{15}N$ of *Pseudodiaptomus* and *Eurytemora*, and thus, they may act as

detritivores besides of herbivores. Another brackish calanoid species —*Sinocalanus tenellus* were more diverse in feeding selectivity (Fig. 8B). It can act as a suspension feeder preferring small-sized plankton as well as a raptorial feeder preferring predating other brackish calanoids and cyclopoids. This species was even reported as a cannibalistic feeder in estuary (Hada and Uye, 1991).

In contrast to both marine calanoids and brackish water calanoids, the cyclopoid species *Corycaeus affinis* was a carnivorous species preferring predating on two brackish species —*Paracalanus and Pseudodiptomus* (Fig. 8D). This species is a widely distributed in all the world's oceans (Turner, 2004) and also frequently dominate in copepod assemblages in spring and autumn in Gwangyang Bay (Table 1). Although the isotopic data for harpacticoid copepods were limited to only one season, we still obtained a clear feeding selectivity pattern based on the Bayesian mixing model (Fig. 7C). Harpacticoids in winter preferred microplankton to nanoplankton if benthic food sources were not considered. Their feeding selectivity contributed to the overall feeding preference of total copepods in this season.

5 Conclusions

Here we have demonstrated the temporal and spatial variability of stable isotope ratios of copepods, which was determined by the isotopic values of two size fractions of POM, and strongly influenced by salinity (spatiality) and temperature (temporality). Such characteristic is a key in understanding the biogeochemical cycles of carbon and nitrogen in Gwangyang Bay. We further used a simple linear mixing model and a Bayesian mixing model to extrapolate from the information derived from the isotopic analysis of bulk copepod samples. The model results were robust and allowed the estimation of the relative TLs and trophic enrichment (fractionation effect) of different groups and dominant genera of copepods, as well as their diet compositions. Temporal and spatial patterns of copepod isotopic traits were further explained by size selectivity on plankton size fractions, as well as the feeding preference of dominant species. Based on such relative trophic positions and feeding preference, we can depict a simple energy flow of the Gwangyang Bay planktonic food web: from primary producers (nanoplankton) and a mixture of primary producers and herbivores (microplankton) through omnivores (represented by *Calanus*, *Centropages*, *Acartia*, and *Paracalanus*) and detrivores (represented by *Pseudodiaptomus* and *Eurytemora*) to carnivores (dominated by *Corycaeu*, *Tortanus*, *Labidocera*, and *Sinocalanus*) (see a simplified energy flow, Fig. 9).

Author contribution: M. Chen and C. K. Kang designed the experiments. D. Kim and C. K. Kang conducted field work, sample collection, and laboratory analyses. M. Chen, H. Liu and C. K. Kang developed the growth model and performed the simulations. M. Chen, H. Liu and C. K. Kang prepared the manuscript with contributions from all co-authors.

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Table 1: Seasonal variations in basic environmental factors, including temperature (T), salinity (S), and chlorophyll *a* (Chl *a*), copepod abundance, and dominant species and the percentage (%) of dominant species in total copepods at the 9 stations in the Gwangyang Bay system.

Seasons	Stations	T (°C)	S (psu)	Chl *a* (μg l^{-1})	Copepod Abundance (ind. m^{-3})	Dominant species	% of dominant species
Winter February 2015	1	7.4	1.4	1.2	65	*Acartia hudsonica*	45
	2	9.1	8.0	2.2	551	*Acartia hudsonica*	89
	3	9.3	23.7	2.3	944	*Acartia omorii*	33
	4	8.5	28.2	1.6	1614	*Acartia omorii*	46
	5	8.4	29.9	1.0	2888	*Acartia omorii*	49
	6	9.1	27.4	0.8	2123	*Acartia omorii*	32
	7	8.7	26.9	1.3	1673	*Paraclanus parvus*	41
	8	9.0	26.9	1.1	2159	*Paraclanus parvus*	30
	9	9.0	26.1	1.0	2690	*Paraclanus parvus*	35
Spring May 2015	1	19.8	0.0	5.0	2265	*Pseudodiaptomus koreanus*	88
	2	19.8	4.7	2.0	175	*Pseudodiaptomus koreanus*; *Tortanus dextrilobatus*	46
	3	19.0	11.2	0.9	324	*Acartia omorii*	53
	4	17.4	27.2	6.2	326	*Corycaeus affinis*	38
	5	17.0	30.1	2.2	266	*Corycaeus affinis*	52
	6	17.0	32.2	6.8	358	*Corycaeus affinis*; *Calanus sinicus*	41
	7	18.0	32.7	5.3	148	*Corycaeus affinis*	73
	8	16.5	32.9	3.8	139	*Corycaeus affinis*	41
	9	16.5	32.8	2.7	150	*Acartia omorii*	81
Summer August 2015	1	26.8	0.4	1.0	53	*Tortanus dextrilobatus*	79
	2	27.4	10.6	4.3	3220	*Tortanus dextrilobatus*	58
	3	27.1	20.5	4.5	784	*Acartia ohtuskai*	71
	4	25.8	28.8	1.6	1401	*Acartia ohtuskai*	62
	5	23.7	32.2	2.8	366	*Acartia ohtuskai*	37
	6	23.9	32.2	2.9	129	*Acartia erythraea*	67
	7	24.1	32.3	2.3	79	*Labidocera rotunda*	60
	8	24.5	32.4	1.6	124	*Acartia erythraea*	93
	9	24.2	32.5	2.4	81	*Acartia erythraea*	55
Autumn November 2015	1	8.8	0.0	0.2	17	*Sinocalanus tellenus*	78
	2	9.9	4.7	0.1	22	*Paracalanus parvus*	32
	3	11.3	15.0	0.5	33	*Paracalanus parvus*	32
	4	12.1	20.9	0.4	32	*Corycaeus affinis*	65
	5	15.4	31.3	0.4	18	*Corycaeus affinis*	62
	6	14.6	31.3	0.4	41	*Corycaeus affinis*	55
	7	14.7	31.8	0.9	113	*Corycaeus affinis*	71
	8	14.8	32.3	1.1	118	*Corycaeus affinis*	30
	9	14.2	32.1	0.4	23	*Corycaeus affinis*	56

Table 2: Coefficients (*F*) and significance levels (*p*) of the effects of variable predictors on $\delta^{15}N$ of nanoplankton (plankton < 20 µm) and microplankton (plankton > 20 µm) using a Generalized Additive Model test. *P*-value < 0.05 indicates significance. The symbol 'n.s.' represents no significance.

Predictors	Nanoplankton		Microplankton	
	F	*p*	*F*	*p*
Temperature	5.493	0.013	5.008	0.015
Salinity	2.790	n.s.	5.001	0.011
Ammonia	4.116	0.029	4.521	0.031
Nitrite	1.436	n.s.	2.128	n.s.
Nitrate	3.292	n.s.	7.795	0.010
Chlorophyll *a*	3.786	0.044	4.159	0.043
Deviance explained	66.3%		73.1%	
R^2	0.526		0.638	

Table 3: Coefficients (*F*) and significance levels (*p*) of the effects of variable predictors on $\delta^{13}C$ and $\delta^{15}N$ copepods using a Generalized Additive Model test. *P*-value < 0.05 indicates significance. The symbol 'n.s.' represents no significance.

Predictors	Copepod $\delta^{13}C$		Copepod $\delta^{15}N$	
	F	*p*	*F*	*p*
Temperature	7.887	0.005	1.03	n.s.
Salinity	0.075	n.s.	13.641	<0.001
Chlorophyll *a*	6.193	0.008	3.272	0.047
Nanoplankton $\delta^{13}C$	16.411	<0.001		
Nanoplankton $\delta^{15}N$			1.086	n.s.
Microplankton $\delta^{13}C$	1.465	n.s.		
Microplankton $\delta^{15}N$			3.456	0.034
Deviance explained	92.7%		76.9%	
AIC test	0.894		0.686	

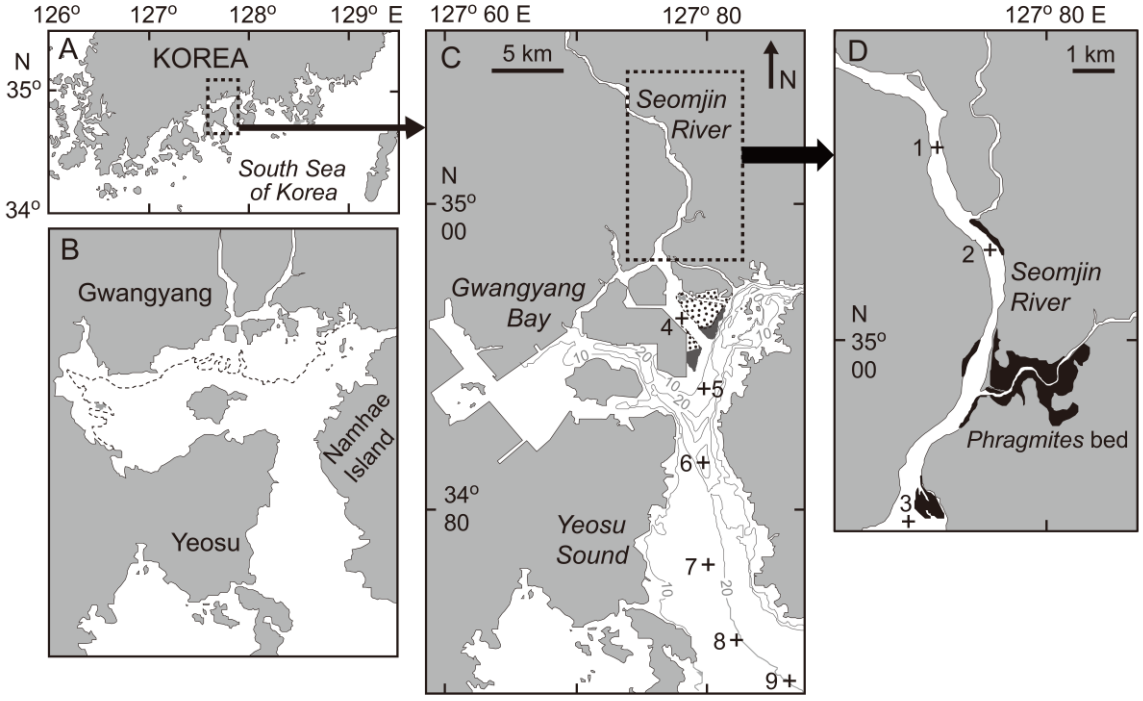

Figure 1: Map showing the location of Gwangyang Bay (A), the appearance of the bay before the reclamation of tidal flats in 1982 (B), the sampling stations in the bay (C), and in the estuarine channel (D). The broken line represents the lowest water line (B); the dotted areas show intertidal beds, the dark gray areas *Zostera* beds (C); and the darker areas *Phragmites* beds (D).

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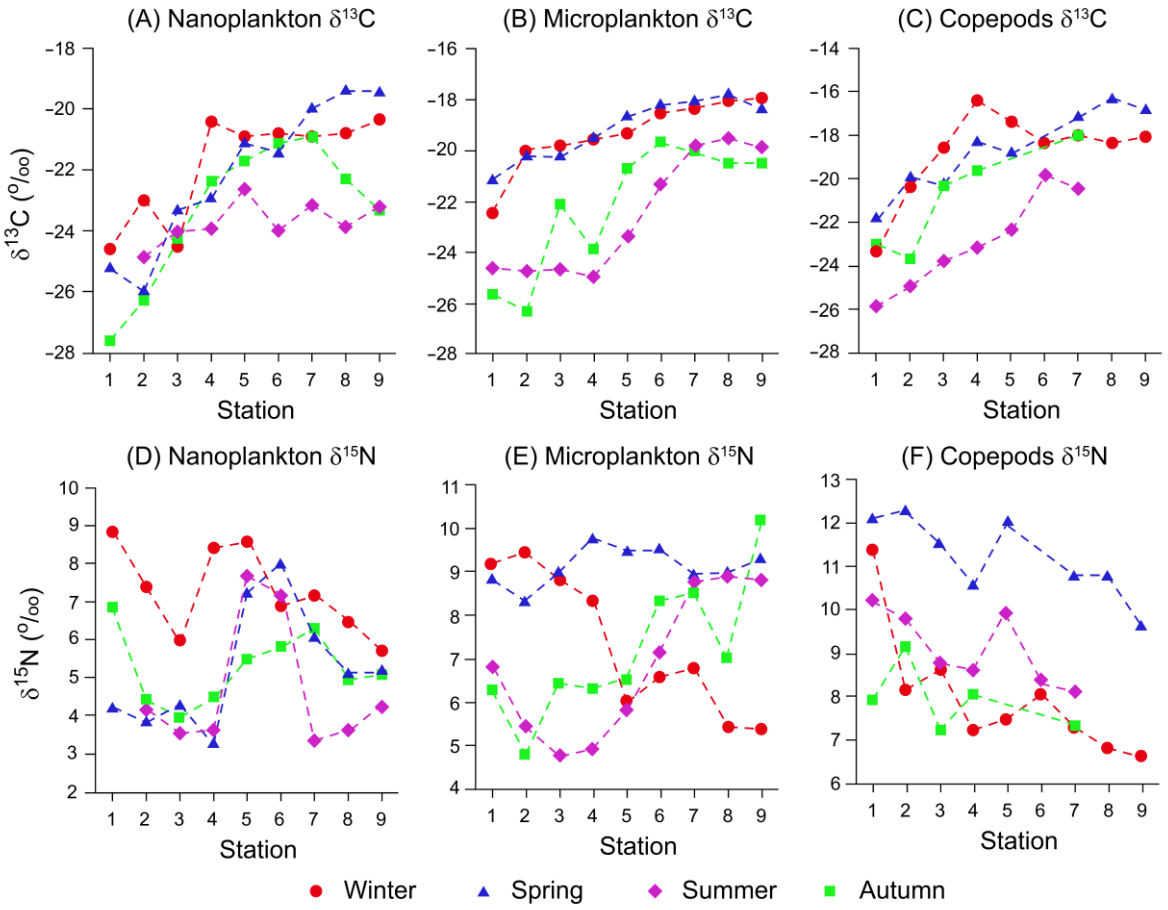

Figure 2: Temporal and spatial variations in plankton $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰).

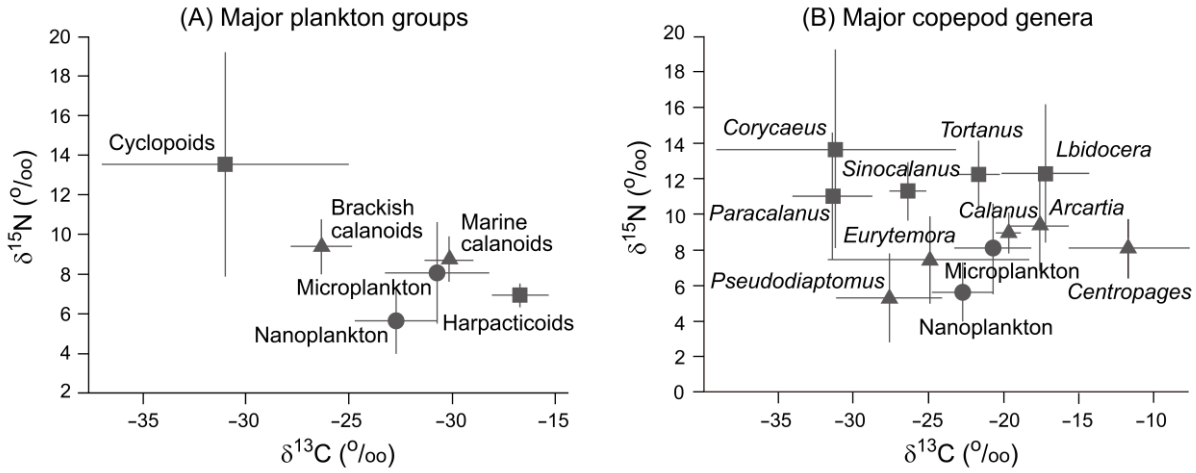

Figure 3: Bi-plots of major plankton group and genus isotopes in Gwangyang Bay.

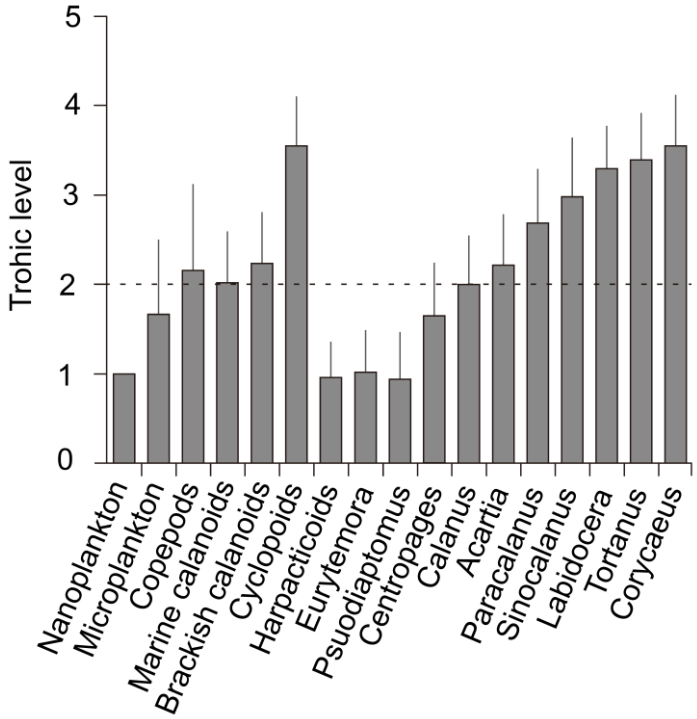

Figure 4: Trophic levels (TLs) of different groups. Nanoplankton were set as TL = 1, while consumers' trophic levels were calculated as: $TL = 1 + (\delta^{15}N_{consumer} - \delta^{15}N_{Nanoplakton})/3$. The reference line indicates the herbivores relative to nanoplankton. However, nanoplankton here might not be truly primary producers as the bulk samples might include heterotrophic flagellates, dinoflagellates, and ciliates, which we could not separate from the collected samples.

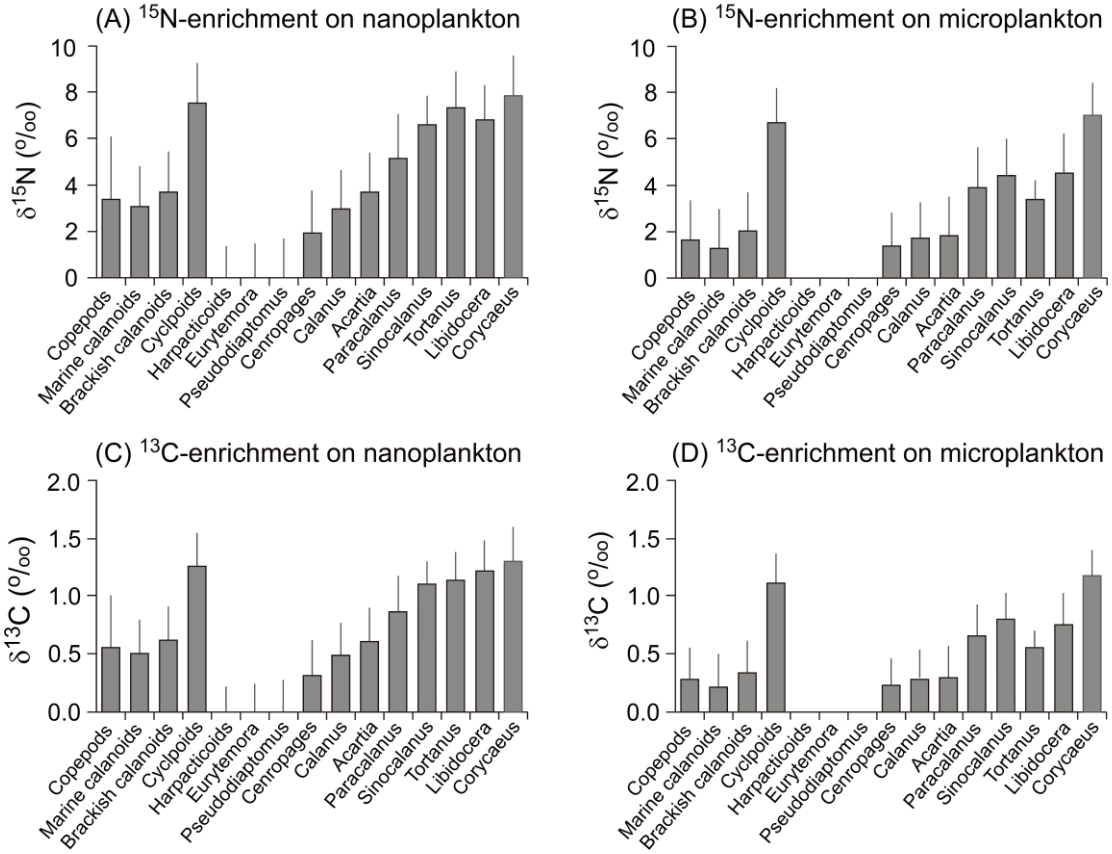

Figure 5: Trophic enrichment (or fractionation factor) from basal food items (nanoplankton: A and C; microplankton: B and D), based on the difference of each sample's $\delta^{15}N$ between higher trophic level to lower trophic level; a 0.5‰ per one trophic level was used to calculate the $\delta^{13}C$ enrichment for each group. Reference lines indicate mean values from all groups.

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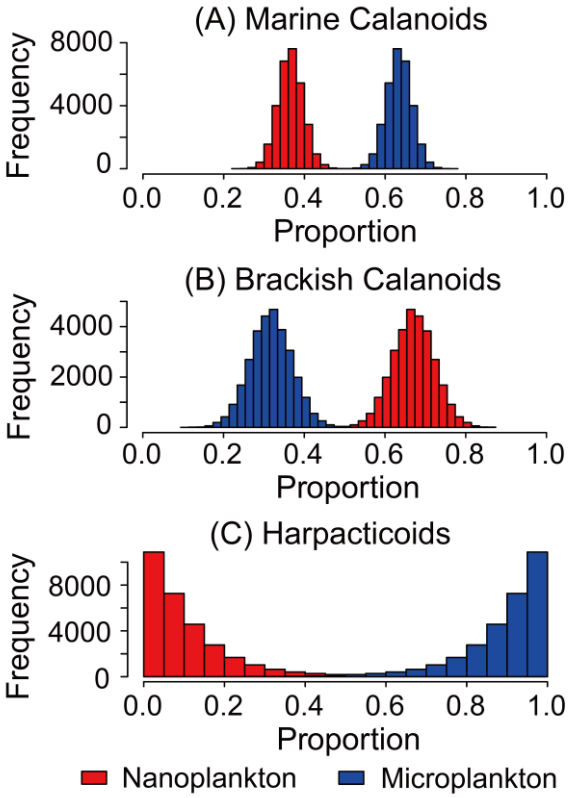

Figure 6: Comparison of the dietary compositions of major copepod groups (A) Marine calanoids, including *Acartia hudsonica*, *Acartia o*morii, *Bestiolina coreana*, *Calanus sinicus*, *Clausocalanus furcatus*, *Centropages abdominalis*, *Centropages dorsispinatus*, *Paracalanus aculeatus*, *Paraeuchaeta plana*, *Labidocera rotunda*, *Labidocera euchaeta*, *Tortanus dextrilobatus* and *Tortanus forcipatus*; (B) Brackish calanoids, including *Acartia ohtsukai*, *Acartia erythraea*, *Eurytemora Pacifica*, *Paracalanus parvus*, *Pseudodiaptomus koreanus*, *Pseudodiaptomus marinus* and *Sinocalanus tenellus*; and (C) Harpacticoids.

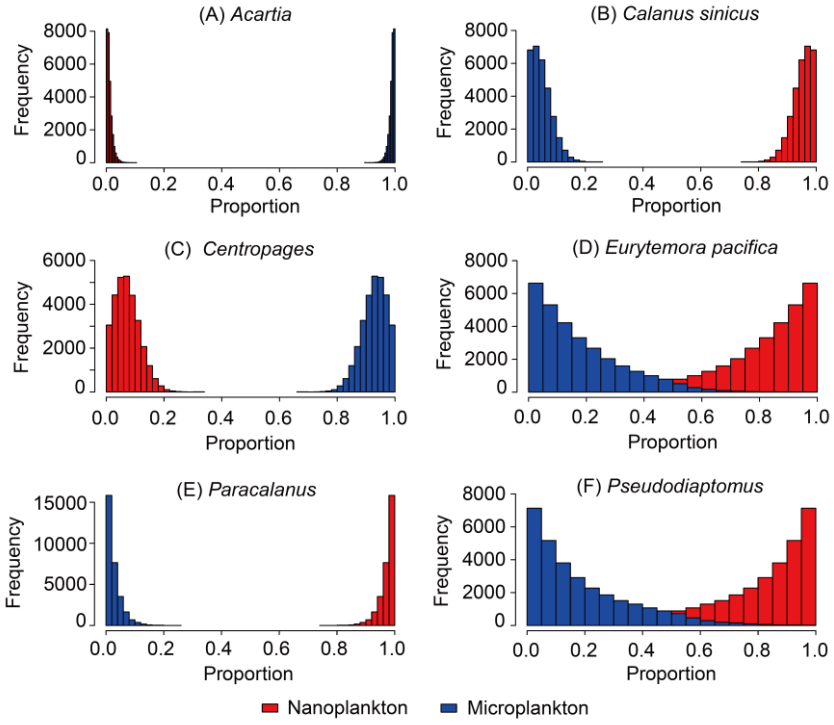

Figure 7: Comparison of the dietary compositions of major omnivorous copepod genera.

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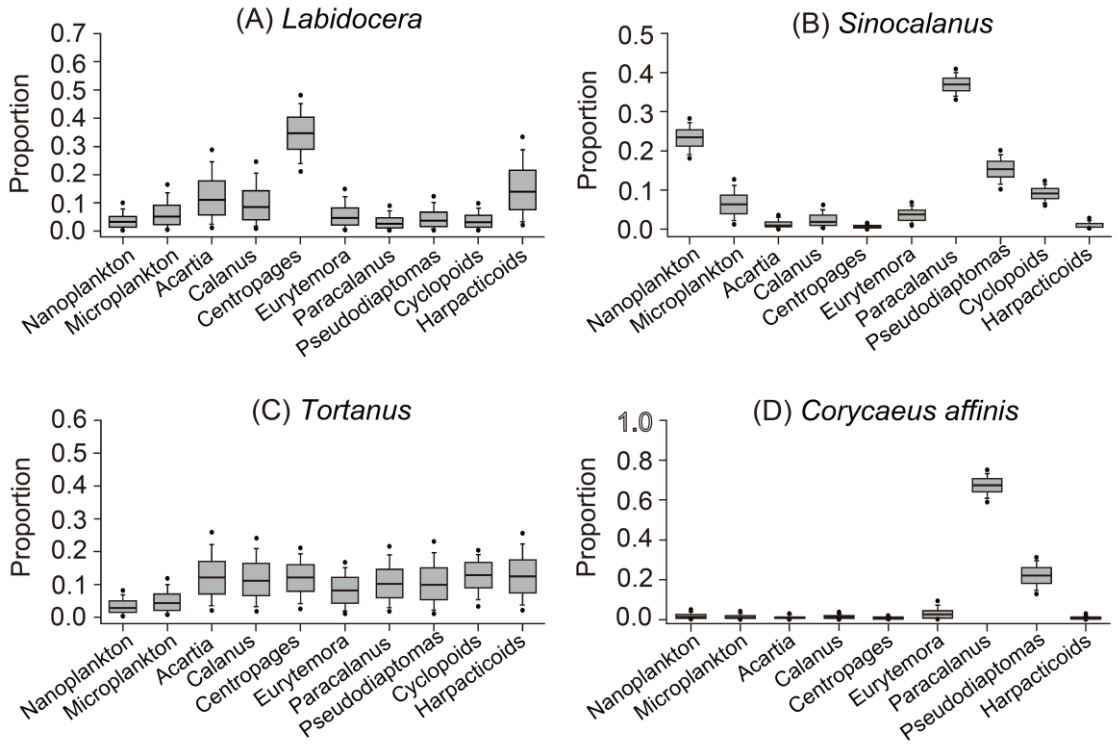

Figure 8: Comparison of the dietary composition of major carnivorous genera. Credibility intervals of 95% (dots), 75% (whiskers), and 25% (boxes) and mean values (lines in the boxes) are shown in boxplots for each source.

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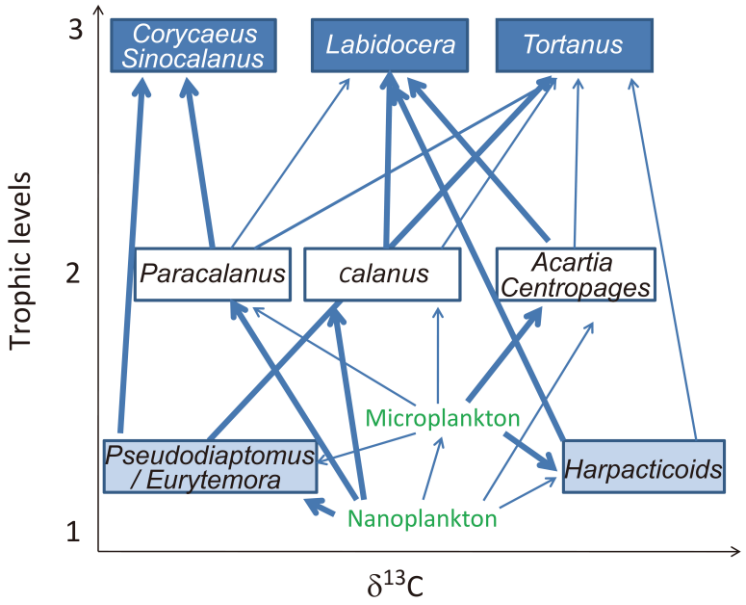

Figure 9: A simplified energy flow figures of Gwangyang Bay plankton and copepods. Arrows indicate feeding relationships. Strong arrows indicate feeding preference.