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

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General Comments: 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 will try to do a literature review and obtain the average weight values for the important genera used in our study.

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 stating in the ïĆšIntroductionïĆš 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 try to simplify the sampling way so that some monitoring departments may follow.

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 try to present more about the detail we used, e.g. the replication, trophic enrich factor, replications just like the reviewer suggest.

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.

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 will use "trophic preference" in the revised version.

'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.

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.

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 topdown 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.

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 will revise "in predicting" to "to understand".

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 ïAËŻd'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 δ15N in the cells. We don't have the data on the variations of δ15N of source, while we expect the δ15N of source will vary from 0 to 13‰ based on the variances of POM.

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.

P5-5. "water samples were transported to the laboratory as soon as possible". Please give a time estimate. - Response: 1-2 hours driving.

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 δ13C and δ15N, respectively. Based on the equation of trophic level, the lowest change will be less than 0.02 TL.

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 es-

timating species stable isotope composition. - Response: As mentioned above, we will do a literature search.

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 δ15N value. Or it can also be understood by this way, it is multiplying the difference of two trophic levels (calculated from different δ15N value) with 3‰ and 0.5 ‰ for δ15N and δ13C, respectively.

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.

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.

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

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.

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 will clarify this in the M&M.

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.

P9-15. "Copepod d15N : : : being much more consistent with the pattern of microplankton than that of nanoplankton". This seems true for the summer ïAËŻd'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 will remove this unsuitable sentence.

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 δ15N, the deviance explained was relatively lower suggesting that other factors which were not included would contribute another 23% of the deviance of δ15N. But we don't know what are them. The understanding of the reviewer is right.

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 differ-

ent 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.

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 will revise as suggested.

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 will revise this conclusion.

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.

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

ciency', 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 will carefully check the whole MS and change to "feeding efficiency"

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 15N accumulating. However, we didn't have direct data to support our speculation. Thus we are going to change the sentence (P13 Line 5-6) to "The input of sewage-derived 15N-enriched ammonia 5 (domestic sewage and livestock waste) could contribute substantially and swamp the other subtle processes to increase δ15N values of nanoplankton."

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 15N will tend to be higher (as they choose the lighter 14N), and thus doesn't explain this decreasing trend. - Response: Yes, we agree that nutrient-limiting is not frequently happened in coastal area. Nevertheless, 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 14N in cells thus they will show a reducing ratio of 15N in cells (Cifuentes et al., 1988; Fogel and Cifuentes, 1993; Granger et al., 2004). To remove such confuse, we will revise 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."

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 will show the table in Results. And as suggested by another reviewer, we will move the GAM figures (Fig. 3) to supplementary materials.

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 will remove such speculation in discussion.

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.

I am somewhat confused about the discussion of trophic levels of the copepods Paracalanus and Sinocalanus. The authors state that their ïAËŻd'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.

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

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