Response of comment on “Bottom-up and top-down controls on picoplankton in the East China Sea” by C. Guo et al.

General response:

We sincerely thank all three reviewers for giving us very detailed and useful suggestions. In the manuscript, we tried to evaluate the controlling factors of seasonal and spatial patterns of picoplankton populations in the ECS. According to the comments, there are 2 major problems in this manuscript.

1. The concept and evaluation of the bottom-up and top down control. Two reviewers questioned about the concept of “bottom up” factors that whether it should include hydrographical factors other than nutrient and light resources. We have checked its definition in papers, some of them refer it mainly as nutrient resources, but some also include other hydrographical factors such as temperature and salinity. In the ECS which is a marginal sea at middle latitude subject to strong seasonal variations and diverse water masses, resources of nutrient and light are not the only factors that affect the dynamics of picoplankton polulations. Therefore, the "bottom-up" effects in our manuscript refer to controls on the abundance of picoplankton that derive from both supply of resources (light or nutrients) and from physical factors (temperature and salinity) of the environment. The reviewers also pointed out that the evaluation of the two controls was not appropriate and adequate. The reviewer#2 suggested us to use $\mu 0/\mu n$ as an indicator of bottom-up control. Though it is a good indicator of nutrient limitation on picoplankton growth, we could not justify the system as a bottom-up controlled system simply by $\mu 0/\mu n<1$. In the nutrient limitation region, the grazing pressure could also play an important role. In the revised manuscript, we will use $\mu 0/\mu n$, as well as correlation analysis among cell abundance, bottom-up factors and grazing mortality (m) to comprehensively analyze the two types of control on picoplankton in ECS. The major objective of our study is to investigate the control mechanism. Since it is difficult to quantify the bottom-up controls and top-down pressure, we changed our topic to growth and grazing rate of picoplankton in ECS.

2. The discussion part lack focus and take home messages. We included too many details in the discussion which are somewhat confusing. We also realize the lack of “the evaluation of covariance of the bottom up drivers”, as mentioned by 2 reviewers, when interpreting the relationship between the bottom up factors and picoplankton abundance. Some of our statements may simply describe the correlations but not having a clear justification on the whole story behind the relationships. In the revised manuscript, we’ve re-organized the structure of discussion and tried to highlight the major findings. Description and discussion about those meaningless correlations have been deleted and more reasonable interpretations have been added by considering the covariance of the bottom-up factors. The typographical and grammatical errors were also corrected.

One-by-one response:

Response to Anonymous Referee #1

General comments: The authors present a very interesting dataset looking at the growth and grazing dynamics of picoplankton in the East China Sea. That being said, the manuscript is substantially weakened, by a discussion that lacks focus and clear ‘take home messages’ and throughout by the abundance of typographical and grammatical errors. I would highly recommend the authors restructure their discussion to make it clear and concise what they think is driving the relationships. Also, the authors should have someone fluent in English proofread the manuscript for

typographical and grammatical errors. My other general concern is that throughout the manuscript there are discussions of bottom up controls and specific controls are given attribution for distribution of picoplankton (e.g., temperature) but I don't see anywhere the evaluation of the covariance of the bottom up drivers to ensure the proper variable is being credited. There will be a strong covariance given the nature of the study (seasonal) and the system (river plume to ocean current) in variables as temperature, salinity nutrients. I think this in part leads to some of the lack of focus in the discussion but also greatly weakens the manuscript and should be addressed. In terms of the manuscript's scientific importance, it is important as it will increase the knowledge base on grazing on and growth of phytoplankton in a region where we don't have a lot of data. It doesn't present any intellectual breakthroughs, but that is OK. In terms of the manuscript's scientific quality, parts of it appear fine, although hard to fully evaluate without the presentation of actual experimental data, and the questions regarding ability to resolve Prochlorococcus. The manuscript references other work as appropriate and there is in general a good balance in the presentation, but as mentioned previously the presentation in general is unfocused. In terms of presentation quality the manuscript needs significant help primarily in typographical and grammatical errors, but also in the presentation quality of the figures which differs greatly between them.

[Response]: Please refer to our general response.

Specific comments by section:
Methods: No mention of how Peuks were defined, are they operationally defined? What size cut-off do they represent, etc. more detail is needed, particularly on how well the FACSCaliber did at observing potentially dim *Prochlorococcus*.

[Response] The peuk were defined according to the high red fluorescence and side scatter (proxy of cell size) signals. The cytometic plot graph and differentiation of three populations is shown in Fig. 1. The size of peuk is approximately 2-5 μm.
Generally, we can clearly identify a whole *Prochlorococcus* cluster on the cytometric plot without overlapping with other noise. But sometimes, the dim Pro may overlap with the noise. In this situation, first we define an estimated range of Pro, and then use histogram to show only the cluster we defined. A normal distribution can be observed, and we can obtain the accurate number of the bright half on the histogram of Pro. So the total number of Pro can be obtained by multiplying the bright half with 2.

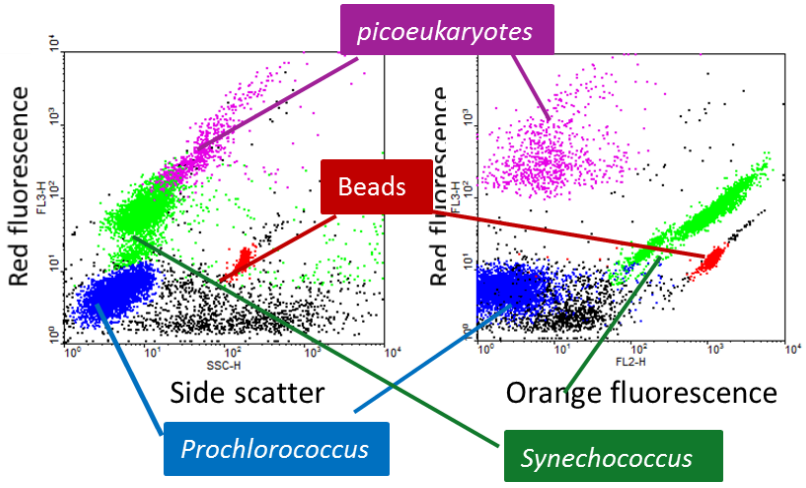

Fig. 1 typical cytometic plot graph of picoplankton populations.

More information on error in the dilution experiment details. Did all the experiments show a significant relationship between change in algal abundance and fraction of seawater? Were they all equally good or were some relationships potentially skewed by weak relationships? It is well known that photoacclimation is a real problem, in this case particularly with the ability to resolve Prochlorococcus. It already appears that there are fewer grazing estimates for Prochlorococcus, not entirely attributed to their lack of presence in the coastal ocean. Results The authors need to show as a figure a set of actual dilution experiment data, not just the reduced coefficients/surface plots. This relates back to the evaluation of error in a prior comment.

[Response]: We have added a supplementary table to show the detailed information of dilution experiments. We agree that photoacclimation is one problem when estimating the rates based on pigment (Chl *a*). However, here we use the abundance of picoplankton to do the calculation, so photoacclimation should not be a problem in this study

In section 3.2, the depth averaged cell abundance values over the upper 150m is presented. First, there needs to be some mention of how this is done, more so than stating the trapezoid rule as there are many stations that don't go to 150m. Second, does presenting the data in this way have any meaning given that 1) surface populations, particularly of Pro are very dim, 2) that there is clear structure in the vertical profile of abundance, and 3) for Euks which are likely to be operationally defined you are not only averaging over depth but very likely over different populations comprised of cells of different sizes, nutrient requirements, etc.

[Response]: In numerical analysis, the trapezoid rule is a method for approximating the definite integral. The trapezoidal rule works by approximating the region under the graph of the function f(x) as a trapezoid and calculating its area. It follows that $\int_a^b f(x)\,dx \approx (b-a)[\frac{f(a)+f(b)}{2}]$.
The a and b in the formula are cell abundances in the nearest two depths. By adding all trapezoid area together followed by being divided by depth, we obtained the depth integrated abundance. However, as the reviewer mentioned, presenting data in this way has some problems. So we have used the surface abundance instead of the depth averaged cell abundance in the revised manuscript.

Table 4: it looks like the ratio for Prochlorococcus in summer, in the Kuroshio Current stations, is inverted. For Syn, winter, plume there is a decimal place error. Please carefully go through the tables and look for other errors. Figure 1: 'labellbed' is a typo Figure 6: this should be split into two figures as there are some many panels that I can't read the axes even when blown up to 400% scale. Figure 7: missing the (C) to reference that panel. Figure 8A: missing 'r=..'

[Response]: Changes have been made according to the reviewer's suggestions. For Table 4, the ratio for Pro in summer in Kuroshio region is correct. The "abnormal value" is because we obtained $m>0$, $\mu<0$ in one experiment in this region in summer. When we calculated the average number, values<0.1 was excluded. For Fig. 8A, we did not show the r value, because the correlation is not significant.

Discussion: P8217 L20- bottom up controls are generally viewed as positive relationships. I don't think that the negative relationship of nutrients and Pro abundance is a direct bottom up control, rather that Pro doesn't grow in the coastal environment and that is where the nutrients are highest. The genome streamlining is a response to chronic nutrient limitation, so would not be an explanation for low abundance in the coastal regime.

[Response]: We agree with the reviewer's point. The absence of Pro in coastal area could also be due to the top-down protist grazing. In the coastal area with high nutrient concentrations, large algae (e.g., diatoms) have advantages and they could over-compete Pro. At the same time, high grazing pressure

resulted from high protist abundance in the coastal area lead to efficient consumption of Pro. Both of the factors resulted in the disappearance of Pro.

P8218 L13 – Syn has also been shown to grow in response to nanomolar additions of nitrate (Glover et al. 2007). Thus the separation of the two cyanobacteria lineages is not purely driven by nutrients.

[Response]: We agree with the reviewer that Syn are able to grow at low nutrient levels and the separation of Pro and Syn was not only driven by nutrients. Here, we just want to discuss the effect of nutrient on Syn, but not saying it is the factor that leads to the population succession. We think the population transition is mainly depended on the balance between growth and grazing. Higher growth rate of Syn can be observed in transitional region than in Kuroshio region and growth rate of Pro in this region. The higher growth potential of Syn and diatoms is an important advantage for them to dominant when conditions allow. At the same time, however, the increased biomass also drives the increase of grazing pressure, as indicated by the close couplings between growth rate and grazing mortality of picoplankton populations. Therefore, the mortality effect on slower growing taxa like Pro can advance beyond a growth-rate sustainable level, and Syn can replace Pro as the dominant population with increasing trophic conditions.

P8219-8221 – discussion of top-down control. There are a lot of ideas being mentioned and it seems like the authors are trying to mention everything that might control rates rather than what does appear to control grazing rates. This section is hard to follow and come away with a take home message. For example, at the end of P8221/beginning of 8222, it is suggest, I think, that Pro and Syn are actually controlled by bottom up processes. P8223 L5-10 – "Microzooplankton consumed an average of â´Lij60 %, 69 % and 65 % biomass production of Pro, Syn and peuk, respectively, in ECS, and the proportion of m / was higher in summer than that in winter due to a steeper decrease in grazing than in growth, suggesting a stronger top-down control in summer." I don't understand this sentence, how does a stronger decrease in grazing than growth lead to a higher m/u?

[Response]: We agree that the section lacked focus and included too many details. We have revised it accordingly.
The sentence is not clear, and we have revised it to "…and the proportion of m/μ was lower in winter than that in summer due to a steeper decrease in grazing than in growth in winter, suggesting a stronger top-down control in summer".

Response to Anonymous Referee #2

General comments

This manuscript used two sets of sampling data collected in August 2009 (summer) and January 2010 (winter). These authors discussed the spatial distribution of three picophytoplankton groups (Prochlorococcus, Synechococcus and picoeukaryotes) in both seasons, measured growth rate and grazing morality using dilution experiments among picophtoplankton population, then looked for the controlling mechanism of these variations and evaluated both the bottom-up and top-down controls in microbial food web of different marine environments in both seasons. First, I was so confused with the concept of "bottom-up control and top-down control". When one system was amended with full resources such as organic carbon or nutrients (temperature and salinity generally excluded) and one of the components increased, we can say that the component is mainly under "bottom-up control". And if no other component responded, the component may be more under "top-down control". However, if the gross (intrinsic) growth rate was stimulated by the amendment (0/n), the bottom-up control was effective. Here I would like to emphasize, the top-down and bottom-up controls are a matter of relative importance in pelagic ecosystem. We can evaluate the relative importance just by comparing intrinsic growth rates, grazing rates, and net growth rates within a range of environmental parameters (such as nutrient supplies) varying temporally or

spatially. At this point, I feel that data presentation in the present manuscript is insufficient to discuss this issue and also does not give a proper reason to justify both the bottom-up and top-down controls in regulating the trophic transfer in microbial food web of different marine ecosystem in different seasons.

[Response]: Please refer to our general response.

Next, the data were insufficient for meaningful statistical analysis such as table 6, which included seasonal, spatial, and depth variations, and many results or discussions in this manuscript did not have sound statistic support to, for examples, distribution trend of picophyplankton from inshore to off shore, therefore a clear conclusion about significant seasonal or spatial pattern of picophytoplankton was impossible. I believe that the average abundance of picoplankton (over top 150 m) was inappropriate to discuss "the horizontal distribution of piccoplankton", because diverse environmental condition exists in the upper water column, for example, the depth of plum is about 10 m. Therefore, the average abundance could not represent the true distribution pattern of picophytoplankton in a pelagic ecosystem affected by different water masses.

[Response]: In Table 6, we used all data together to do the analysis, and try to get a general relationship between the parameters. Separating the data according to different season, region and depth may lead to insufficient data to do the statistical analysis. The distribution trend from inshore to offshore was described according to the average regional abundance and the spatial distribution figure. We have added some statistical analysis to show the trend in the revised manuscript. Also, the surface abundance has been used instead of the depth averaged abundance in the revised manuscript.

I believe that the major consumer of picophytoplankton is nanoflagellate, not microzooplankton. The authors cited many Landary's papers to support their viewpoint, but they must realize that Landary's results were acquired with dilution experiment, therefore with the consumers including not only micozooplankton, as believed by these authors, but also nanozooplankton. I suggest the authors must rewrite these parts in the manuscript.

[Response]: We totally agree that the consumer of picoplankton is mainly nano-sized heterotrophic protists. In the paper, we define microzooplankton here as <200 μm grazers, which therefore include nanozooplankton (2-20μm grazers). We have checked the places citing Landry's paper. They are mostly in the introduction part. We've revised the inappropriate places accordingly.

The three water systems were defined in the manuscript, but the standard of water system in the text (p 8207 L 24-p 8208 L4) was not consistent with that in table 1. Please check and give a reasonable standard. Lastly, many mistakes and erroneous values were found in the manuscript. The authors should carefully check their manuscript before submitting it to journal.

[Response]: We defined the water system based on the hydrographic features combined with location of the stations. In the paper, we state " Characterized by distinct hydrographic features and the locations, stations can be roughly divided into 3 water systems: stations in coastal water affected by Yangtze River input was defined as salinity near or below 31psu (Gong et al., 1996); stations of Kuroshio warm current located in the outer shelf and deep water region with relatively high temperature and high salinity was defined as salinity near or higher than 34 psu (Gong et al., 1996; Jiao et al., 2005); and stations situated between the two were defined as transitional station." The cut-off is not a very strict line. For the values around the cut-off lines, we took the location of the station into consideration when determine which system the stations belong to.
We are very sorry for the mistakes and erroneous values in our manuscript. We've carefully checked the values in our manuscript and corrected those wrong numbers.

Special comments
Title: “ picoplankton” or “picophytoplankton”ïij§ If it is picophytoplankton, please replace picoplankton with picophytoplankton throughout the manuscript.

[Response]: Changes have been made according to the reviewer’s suggestion.

P 8204 L 9-10: Based on Table 5, the average growth rate should be 0.36, 0.81, 0.85. P 8204 L 16-17: Based on Table 5, protest grazing consumed should be 112%, 79%, 74%

[Response]: Changes have been made according to the reviewer’s suggestion.

P8205 L3-8: This paragraph is neither major result nor conclusion, and should be deleted from the Abstract.

[Response]: Changes have been made according to the reviewer’s suggestion.

P8206 L5-18: the major consumer of picoplankton is nanoflagellaye not microzooplankton.

[Response]: Changes have been made according to the reviewer’s suggestion.

P8208 L4: add the model and manufacturer of CTD

[Response]: The CTD used is Sea-Bird's 911. We’ve added the information.

P 8210 L10: the authors did not show the incubation time in method of dilution experiment.

[Response]: The incubation time is 24h. We’ve added the information.

P 8211 L3: The hydrographic patterns in both seasons were similar, increasing from inshoe to offshore, not contrasting.

[Response]: Yes. We’ve deleted the sentence.

P 8211 L15: the nutrient unit should be moleL-1 not g L-1, check the text and table 1

[Response]: The nutrient unit in Table 1 should be μmol L^{-1}. Sorry for the mistake.

P 8211 L26: “the range of Chl a is 0.07-35.3g L-1”, but ranging from 0 to 1g L-1 in fig. 2I.

[Response]: The range of Chl *a* should be 0.07-3.53. Sorry for the typo. Although we set the scale bar as 0-1, the figure still showed the true value of Chl *a*.

P 8212 L15: I believe that the horizontal distribution pattern of picoplankton abundance is better represented by surface data than by the average abundance. The statistical analysis was also done with surface data.

[Response]: We have used the surface data instead of the depth average data.

P 8212 L20-21: “but much higher in Kurshio than in transitional in winter”, this result was not confirmed with proper statistical test. The similar situation occurred throughout the manuscript, please check and correct.

[Response]: Statistical analysis of t-test has been added to verify whether the comparison is significant.

P 8212 L26: Results of Spearman's rank correlation and stepwise regression analyses on Pro were different, how do you explain it?

[Response]: It is possibly because the Pro abundance after log transformation actually did not follow a good normal distribution which is the prerequisite for step-wise correlation. We have revised the Table 3 accordingly.

P 8213 L9: 2.1 should be 13.8
P 8213 L11-12: 6.4 and 29 folds should be 18.2 and 2.8.
P 8213 L20: decreased by 2-3 times (ïij§) please check
P 8213 L21: 5.2 and 9.8 should be 4.4 and 6.1

[Response]: Corrections have been made.

P8213 L28: Is temperature bottom-up factorïij§

[Response]: The "bottom-up" effects in our manuscript refer to controls of picoplankton that comes from the supply of resources (light or nutrients) or from physical factors such as temperature of the environment.

P8214 L15: 84% should be 112%
P8214 L28: 0.82 should be 0.83

[Response]: Corrections have been made.

P8216 L22: "microzooplankton consumed 10.05 in coastal region", this value was not found in Table 9 b.

[Response]: 10.05 should be 8.80. Correction has been made.

P 8217 L8-14: In this paragraph, the hydrographic condition in summer and winter was described. In reality, the hydrographic condition is very different from the description with different water currents intruding into the East China Sea in both seasons, respectively. Not only Kuroshio and fresh water from Yangtze River intruded into ECS.

[Response]: We agree that other water masses/currents, such as Yellow Sea cold water mass, Taiwan warm current, also intrude ECS. But the Kuroshio current and Yangtze River freshwater input are the two major water masses that affect seasonal and spatial patterns of hydrographical conditions in ECS.

P 8217 L23-26: If the ability of Pro to utilize nutrient is significantly weaker than other picophytoplankton, why they are abundant in very oligotrophic environment.

[Response]: Pro have strong ability to utilize nutrient under nutrient depleted condition because of their larger surface area to volume ratio. Here, we are saying that Pro lack genes responsible for nutrient-uptake regulation, which may affect their ability of nutrient-uptake regulation.

P 8218 L17-20: In the East China Sea, Syn distributes in intermediate nutrient environment. Please give a statistical support to this conclusion.

[Response]: This description was given according to the distribution pattern of nutrient and Syn abundance in Fig.2 and Fig. 4. Statistical analysis has been added.

P 8219 L4-5: 0.89, 0.9 should be 0.81. 0.85

[Response]: Correction has been made.

P 8219 L10-11: “growth rates were higher in summer than in winter”. This phenomenon does not occur in Pro and the result could not be supported by statistical analysis.

[Response]: We have revised the sentence as ‘‘the average growth rates of Syn and peuk were higher in summer than in winter”.

P 8219 L15-19: There was no statistical support to the decrease trend of growth rate from inshore to offshore What relationship between growth rate and nutrient or salinity should be shown.

[Response]: We described the trend according to the regional average abundance and the spatial distribution pattern figure. Statistical analysis has been added according to the reviewer’s suggestion.

P8219 L26-28: and P8220 L1-4: “the negative correlation between grazing morality and salinity”. This result is not only to show a spatial variation but also to include the seasonal and depth variations. This result was not ideal to explain the spatial distribution.
P8220 L14-18: “Spearman’s correlation in table 6”. The analysis included the seasonal, spatial, and depth variations, therefore I would not accept the explanation about seasonal variation of grazing rate based on the analysis result in table 6.

[Response]: In Table 6, we used all data together to do the analysis, and try to get a general relationship between the parameters, because separating the data according to different season, region and depth may lead to datasets that are too small to do the statistical analysis. We have revised the text and changed the inappropriate discussion.

P8220 L6-14: I believe that the major consumer of picophytoplankton is nanoflagellate not microzooplankton.

[Response]: In the paper, we define microzooplankton as <200 µm grazers, which therefore include nanozooplankton (2-20 µm grazers). We have added this sentence in the introduction part.

P8220 L22: 84%, 78%, 73% should be 115%, 79%, 74%.
P8221 L2-3: I do not know how these authors calculate these ratios

[Response]: Correction has been made. We are so sorry for the wrong calculations.

P8221-P222: “The top-down control on picoplankton by microzooplankton grazing in ECS”. I could not accept this conclusion, because the slopes of Syn and Peuk between growth and grazing rates were 0.87 and 0.56, respectively, and sometime u0/un <1 these data confirmed the bottom-up control of picoplankton abundance in the East China Sea.

[Response]: We think both bottom up and top down control are important here. Revisions were made according to the suggestions.

P8221 L17-28 and P8222 L1-6: the seasonal variation of morality and m/u in the three picophytoplankton communities showed a similar pattern (summer>winter), but the relationship between abundance and morality was significantly different (different correlation coefficient in Fig. 8). These authors explained this phenomenon to be resulted from “different potential mechanism of top-down control”, but the different grazing pressure among the three picophtoplnkton was not observed in the change of grazing morality of

nanoflagellate on picoplankton.

[Response]: We agree with the reviewer's comment that it is not appropriate to say "different potential mechanism of top-down control". The different correlation coefficients between grazing mortality and abundance of three populations suggest different control mechanism of abundance of picoplankton populations. For peuk, the seasonal change of abundance was very small, which was very different from Pro and Syn. Besides the high species diversity which may keep peuk grow well in different seasons, high grazing pressure in summer which can keep the abundance of peuk in check may be also one of the possible reason for the constant abundance of peuk, evidenced by higher m/μ in summer and the negative correlation between grazing mortality and peuk abundance. However, although the seasonal pattern of m and m/ μ of Syn were similar to that of peuk, its grazing mortality did not demonstrate a significant negative relationship with its abundance. There was also no apparent correlation between Pro abundance and its mortality rate. Considering the clear seasonal change in the abundance of Pro and Syn and their narrower spatial distribution especially in winter compared with peuk, we believe that the bottom-up factors might be more important in determining the seasonal pattern of the populations.

P8222 L6-25 : growth rate and grazing rate were higher at surface than at the DCM, Please provide statistical analysis to support the conclusion.

[Response]: Because the data have large variations in different regions, we revised the sentence as "the average growth rate and grazing rate were higher …".

P8223 L2-3: Do salinity and temperature belong to the bottom-up factorsïij§

[Response]: please refer to our general response.

P 8223-224: In summary, many of the authors' conclusions were neither in Discussion nor Result section, such as " microzooplankton consumed an average : : :: : :" or " relative strength: : :.(L3-9)" . I suggest the authors to rewrite the summary, based on the conclusion of manuscript.

[Response]: We have rewritten the summary part.

Response to Anonymous Referee #3

General Comments: This paper concerns the patterns of picoplankton abundance and growth rates and microzooplankton grazing in the East China Sea (ECS) during 2 cruises: one in summer 2009 and one in winter 2010. The authors also measured various physical and chemical parameters to correlate with the abundance and growth/grazing estimates (temperature, salinity, nutrients, chl a). They found that different regions of the ECS were dominated by different components of the microbial community during the 2 cruises. They also found that growth and grazing rates varied seasonally, regionally, and between groups. Using correlation analyses, they tried to estimate the relative impacts of top-down and bottom-up factors on phytoplankton growth rates in this region. Much of the discussion/conclusions/interpretation of the data hinges on the correlation analyses and their interpretations. Of course, correlation is not causation, so discussing these data as if they imply causation without clear justification is a mistake that should be avoided. For instance, for young school children, shoe size strongly correlates with the ability to read, but there is no causal connection here. The missing causal variable is the age of the child. When reading the first section of the discussion, and the abstract, I get the impression that the authors think that there is a causal relationship

between the variables they tested: I doubt that this is the case. If it is the case, they need to make it very clear in their discussion of the data their reasoning. For instance, the strong positive correlation between salinity and all picophytoplankton abundances is not because these phytoplankton need more salt to accumulate. Salinity is a proxy for something: make it clear what that "something" is. The strong negative correlations between the picophytoplankton populations and nutrients in winter also does not mean that high nutrients kill off the phytoplankton: it is more likely this is a proxy for mixing and hence light levels experienced by the phytoplankton. And so on.

Another clarification to make in the text is that the "microzooplankton" grazers include "nanozooplankton" – since PRO and SYN are probably being consumed mainly by these smaller grazers (as is mentioned twice in the discussion). Seawater dilution experiments would of course include nanozooplankton, too. Perhaps the introduction, p. 8206, could state, "As the major consumer of phytoplankton, microzooplankton undertake the most important role in trophic carbon transfer between picoplankton and higher trophic levels. We define microzooplankton here as <200 um grazers, which therefore include nanozooplankton (2-20 um grazers)."

In summary, this paper needs significant editing of its content to streamline it and make its arguments crystal clear. It will be a valuable addition to the literature once these edits are made. As it is currently written, the data suggesting that bottom-up and topdown factors are important controllers on phytoplankton populations is not convincing, even if such statements are intuitively obvious.

[Response]: The reviewer commented on the interpretation of correlation analysis in the manuscript. We totally agree that the correlation does not imply causation. As the reviewer pointed out, some of our statements may simply describe the correlations but not having a clear justification on the whole story behind the relationships. In the revised manuscript, we have deleted some description and discussion about those meaningless correlations and added more reasonable explanation by considering the covariance of the bottom-up factors.

We have added the sentence "We define microzooplankton here as <200 um grazers" to clarify that the consumer of picophytoplankton we talked about included nanozooplankton.

We admit that the paper included too many details without highlighting the important findings and take home messages. We have significantly revised the manuscript to make our argument more clear.

Specific Comments:

Figure 2k: is the blob of red indicating high chlorophyll just south of 32 N and east of 124 E an artifact of the ODV contour plotting routine used? It doesn't look like there are any stations at that location to account for the high values. If VG gridding was used in ODV, it is better to use a low number for X & Y scale-length and have a white area on the plot where no data is, than pick a high number to fill in the x-y space where no data occurs. Please check and modify as appropriate.

[Response]: Yes, the bolb of red indicates the high chlorophyll concentration at the stations at that location. We used value of 40 for x and y scale when doing the DIVA gridding in ODV. Maybe it's too high to reflect the real condition. We have lower the value and revised the figure according to the reviewer's suggestion.

Figure 2L and 3L: is the high chlorophyll at the second to last station on the PN transect (to the east) real? Is this the transition zone or the Kuroshio region? The PN transect data for chlorophyll is hard to see in the figures provided: the description in the text (p. 8212) doesn't really match what we can see in Figure 3.

[Response]: There is a high chlorophyll value (0.97 μg/L) at surface layer at station PN03 which is in the Kuroshio region. We missed this station when calculating the average, max, and min values in Kuroshio region. Sorry for the mistake. Corrections have been made in the context and Table 1.

There is no description in the methods for how microzooplankton abundance was obtained: please add (first

mention is on page 8215 in the results section).

[Response]: The microzooplankton abundance was obtained by counting under microscope after fixation by Lugol's solution. We have added the information in the method part.

Table 1: Please add more explanatory details to the table. I have to assume that the values reported here are averages, with the range of the values in parentheses. Also, it should be mentioned in the legend that total C refers to total phytoplankton carbon. Please confirm and add to table legend.
What jumps out at me in this table is that in the summer the plume area is highly variable for all parameters, suggesting that more than one region is encompassed here. This makes me wonder if using a single value (50) for converting Chl to C is really valid for this area. In general, there should be a discussion of the use of this single value and how it might over or under-estimate the total carbon, and therefore the percent of the total community represented by picoplankton. Also, is there ancillary data suggesting that a diatom bloom, for instance, was going on in the coastal area? With such high chlorophyll values that is what I would expect. Perhaps the light scatter data of the PEUKs indicate that they were larger there? If so, please mention in the text.

[Response]: We have added more details to the legend of Table one according to the reviewer's suggestions.
About the C/Chl ratio, Chang et al. (2003) estimated that the value was 13.0 and 92.8 in coastal and midshelf&Kuroshio zones respectively using POC regression method, and 18.0, 67.4 and 94.4 in coastal, midshelf and Kuroshio zone respectively using cell volume method in ECS. In the revised manuscript, we have used different C/Chl ratio for different regions according to the Chang et al (2003)'s paper.
According to the microscopic data, diatom was dominant in the coastal area in summer.
According to the side scatter signal (indicator of cell size) and red fluorescence signal (indicator of cellular Chl a) of peuk, the high values did not totally coincide with high Chl a region, suggesting peuk was not a major contributor of Chl a in coastal region in summer.

Table 2: The lack of correlation between SYN and the nutrients in the summer is potentially a very interesting result. I don't see it specifically mentioned in the discussion section. It suggests that there is another control on this population; perhaps grazing.

[Response]: According to the below figure, µ0/µn of Syn in Kuroshio stations were all below 1, suggesting nutrient limitation of Syn growth. Therefore, we think Syn was controlled by both nutrients resources and grazing pressure in summer. We have added discussion on this point.

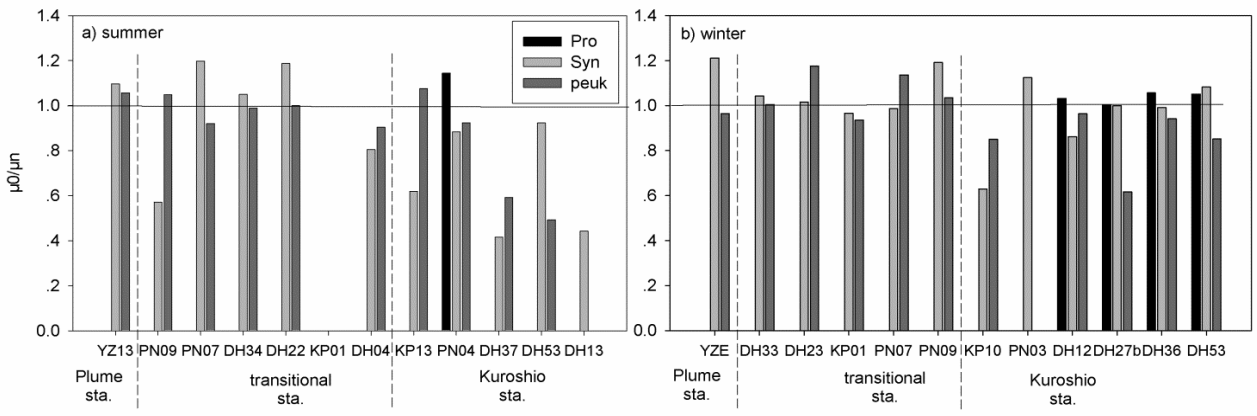

Fig. 2 µ0/µn in plume, transitional and Kuroshio regions

Figure 4: these surface plots are averages over the top 150 m: it would be better to use integrals over that depth interval. Similarly, Figure 2 is just surface distributions: this makes sense for temperature/salinity, but integrals over the upper 150 m (assuming the data are available) of nutrients would be better to compare to integrals of phytoplankton concentration over the same depth range.

[Response]: the surface plot in our manuscript is depth average abundance calculated by trapezoid rule. The other 2 reviewers also questioned about the appropriateness of calculating the depth average abundance, because diverse environmental condition exists in the upper water column and the average abundance could not represent the true distribution pattern of picophytoplankton in a pelagic ecosystem affected by different water masses. Therefore, we have used surface abundance of picoplankton populations to represent the horizontal distribution pattern.

Figure 6: Are the panels labeled with their letters A-L? I can't read which are growth and which are mortality (quality of figure too bad). They are too small to see. This figure should be made bigger, perhaps making a Figure 6A for summer and Figure 6B for winter. It is too hard to see if any real trends are present. It is an interesting way to present the data, however, so it would be worth making it legible!

[Response]: In the revised figure, we have separated the figures of winter and summer, added labels of A-L, and enlarge the font size, according the reviewer's suggestions.

Figure 7, 8,9: Needs better quality graphics, so it is easy to read axes and notes on plots.

[Response]: Revisions have been made according to reviewer's suggestions.

Lines 13-15: Diverse hydrographical conditions as the "driving force" for structuring phytoplankton distributions: I am uncomfortable with this phrasing. If the statement was that the communities were different spatially (in the different water masses) and seasonally within the same areas, then that would be fine, but the "cause" of those changes is likely due to the light, nutrient (hydrographic) and grazing (ecological) dynamics as they change over time and space. So, perhaps the sentence could be re-phrased.

[Response]: The sentence has been re-phrased according to the reviewer's suggestion.

How does temperature directly affect phytoplankton species distributions? Is it temperature directly, or is temperature a proxy for light or nutrients?

[Response]: We believe temperature is the key factor in controlling seasonal distribution patterns of Pro and Syn (much higher abundance in summer) and the spatial patterns in winter (much higher abundance in Kuroshio region). It is not a proxy of light or nutrients.

How is grazing affected by salinity? I see no reason why salinity should directly affect it, so what is the indirect cause of grazing changes? To say that the Yangtze River affects microzooplankton grazing needs more justification than this.

[Response]: We do not think salinity can directly affect grazing activity. Affected by Yangtze River input, increasing trend of salinity and decreasing trend of nutrient concentrations were obtained from inshore to offshore region. Salinity here can be seen as the proxy of nutrient because these two factors co-varied. We have added more discussion on how Yangtze River affecting microzooplankton grazing.

Technical Corrections:
Page 8205: line 13: insert the words "to have" between "them" and "a"
Line 15: change "are" to "have been" Line 26: change "them" to "their carbon" and change "packed" to

“consumed” Line 27: insert “the” between “via” and “microbial”
Page 8206: Line 2: change “previous” to “previously” Line 4: change “feeders” to grazers”; change “rate” to “rates”, change “of” to “and” Line 9: insert “of their” between “rest” and “mortality” Line 10: change “of” to “by” Line 14: change “change” to “changes”
Line 16 & 17: omit the word “the” in both lines Line 19: change “situates” to “is situated” Line 21: insert “the” before “ECS” Line 23: insert “The” before “physical”
Line 24: change “mid-latitude” to “mid-latitudes” and “landmass” to “landmasses” Line 26: change “amount” to “amounts” Line 29: change “Besides” to “Additionally”
Page 8207 Line 23: change “setting on” to “along”
Figure 1 legend: change “line” to “lines”; Change last sentence to: “The PN transect is labeled by a black line.”
Page 8208 Line 3: insert “the” before “transitional” and change “station” to “zone”
Line 4: put in actual model and manufacturer. Line 5: change “membrane” to “membranes”
Line 19: change “according to” to “delineated by”
Page 8209 Line 2: insert “the” before “depth” and before “chlorophyll”; insert “note that” before “at” Line 3: insert “the” before “experiment”and before “surface”
Line 9-11: re-write as “Duplicate sets of 1.2 L bottles were used to establish a nutrient-enriched dilution series, consisting of 15%, 27%, 50%, 73% and 100% natural seawater (10 bottles total).”
Line 14: add “pre-screened through a 200 m nitex mesh” after “seawater”
Line 15: Change “All five bottles: : :” to “Inorganic nutrients were added to all ten bottles (final concentrations of : : :.): : :”
Page 8210 Line 5: insert “the” before “formulas”
Page 8211 Line 10: insert “E” after “125”
Page 8212 Line 12: omit “up-“ and insert “the” after “located in”
Line 16: insert “order of” before “magnitude” Line 17: insert “cells ml-1” after 10ˆ3. Line 22: change “were” to “was”
Line 24: insert “A” before “Negative”
Line 26: omit “existence”
Line 27: change “controlled” to “correlated with” (just because it is correlated, doesn’t mean it directly controls abundance – it is probably a proxy for nutrients or another parameter)
Page 8213 Line 2: add “the” before “PN” Line 4: add in “were” before “distributed”
Line 8: omit “that of” and insert “the trend found for”
Line 11: replace “for” with “by about”
Line 12: change “folds” to “fold”
Page 8214 Line 6: change “Flow cytometry-based” to “Seawater dilution experimentbased”
Line 8: add in “d-1” after “0.1” Line 14: add in “the” after “at” (both times)
Table 4: need the units for microzooplankton abundance on the table (assume cells per mL).
Page 8216 Line 11: omit “sometimes” and put in “always” before “significant” (also there is an extra period on this line that needs to be removed)
Line 26: omit “rest” and replace with “remaining”, replace “contribution” with “consumption”
Page 8217 Line 4-5: omit the end of the sentence, i.e.,“the composition”.
These are not the only grammatical errors, but should give an idea of what needs to improve in the manuscript. I do not list the changes necessary in the discussion/conclusion.

[Response]: We have revised the manuscript according to the reviewer’s suggestions.