**Biogeosciences
Discussions**

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# *Interactive comment on* "Seasonal and spatial comparisons of phytoplankton growth and mortality rates due to microzooplankton grazing in the northern South China Sea" *by* B. Chen et al.

B. Chen et al.

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Dear editor:

First of all, we sincerely thank the reviewers for their insightful comments which have substantially improved the paper. We have seriously taken all the comments into consideration and revised the manuscript accordingly. Please find the point-to-point responses below:

Anonymous Referee #1

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The MS by Chen et al. investigates de trophic impact of microzooplankton on the phytoplankton communities of South China Sea, comparing the grazing effects on the vertical scale, along a shelf to open ocean gradient, and during two contrasted seasons. I should say the authors presented a comprehensive and thorough study. However, as in any study there are always things to be criticized and others to be improved.

The MS addressed several hypothesis, such as the higher microzooplankton grazing impact in oligotrophic than in eutrophic waters, the enhancing effect of temperature on microzooplankton grazing activity and impacts, and the higher relevance of microzooplankton grazing at depth, compared with surface waters. These hypotheses are based on previous knowledge and ecological theories, as explained in the introduction. However, some of the concepts are in my opinion incorrect, or at least debatable. Hypothesis (1) higher microzooplankton grazing impact in oligotrophic areas: As already stated in the introduction evidences exit to prove this is not the case (see review by Calbet and Landry 2004, among others); therefore, there is no actual argument to write this hypothesis. As a matter of fact, the data of the authors corroborates there are no differences in the grazing impact along a trophic gradient. Hypothesis (2) temperature effect: The authors claim there should be a higher grazing impact ($m/\mu o$) in the warm summer than in winter because the different temperature growth coefficient for phytoplankton and microzooplankton growth (Rose and Caron 2007). Actually, the cited paper embraces a rather large gradient of temperatures, observing the theoretically major differences above and below 15°C, temperature at which maximal growth rates of herbivorous protists equaled or exceeded maximal growth rates of phototrophic protists. The average temperatures for the seasons studied here spanned from 21.3 to 29.7°C, range in my opinion too narrow to observe any effect on well-adapted communities. As expected the authors did not find such effect, even presenting evidences of the opposite. I suspect a methodological artifact here that I will discuss below. Hypothesis (3) grazing impact should be greater at depth than in surface waters: This hypothesis is based on the light dependence for phytoplankton growth. The hypothesis seems to be essentially correctly articulated; however, it ignores that the biomass of

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microzooplankton does not have to be necessarily evenly distributed along the vertical column, and the growth inhibitory effects of light at surface.

Therefore, I recommend the authors to readdress their hypotheses in a more convincing way. Furthermore, these hypotheses should be developed properly, not as questions.

[Response] We have revised the introduction part to make the hypotheses more convincing. The notion that microzooplankton grazing impact should be greater in oligotrophic waters is widely accepted. This concept is consistent with the classic view that the efficiency of carbon export should be higher in eutrophic waters than in oligotrophic waters. We have added the arguments that microzooplankton grazing rates on large phytoplankton especially diatoms are usually lower than on small phytoplankton. Large phytoplankton should be more prone to mesozooplankton grazing and sedimentation, which are the major components of carbon export. And we did find that $m/\mu 0$ can decrease with increasing phytoplankton size in a previous study (Chen and Liu L&O 2010). Therefore, it is a little surprising that we did not find a significant correlation between $m/\mu 0$ and Chl a, which needs an explanation. We have added some discussion on this topic in the 'Discussion' part.

For the temperature issue, we have to point out that the temperature difference of 8 degrees is not trivial. If assuming a Q10 of 2, 8 degrees of temperature difference would lead to 1.7 times of rate difference. In our previous analysis of temperature effects on phytoplankton growth and microzooplankton grazing (Chen et al. L&O 2012), we found that the relationships between temperature and phytoplankton growth and microzooplankton grazing rates are monotonous, implying that phytoplankton thermal adaptation does not alter the overall temperature effects. However, we have limited the discussion on the temperature effect and removed section 4.4 because of the large variability of the data.

For the comparisons of grazing rate between surface and DCM layers, it is of course

true that microzooplankton biomass is another factor that affects grazing rates. But as long as the two depths are within the mixed layer, microzooplankton biomass and grazing rates should be similar, but phytoplankton growth rates decrease with increasing depth. We have added the statement 'other things being equal...' in the argument of the introduction section. And the result is true that when surface and DCM waters were within the mixed layer in winter, $m/\mu 0$ was indeed greater at DCM waters than at surface. It is also possible that photo-inhibition can cause a reduction of phytoplankton growth rate at surface waters, but this effect seemed not pronounced in our dataset.

Specific comments Methods I see in the methods the authors used 5 non-replicated dilution levels in 1.2 L bottles, being the most diluted level 15% of natural seawater. For oligotrophic waters, such small volume may not capture correctly the variability of grazers, especially in the diluted treatments. This could be at least corrected for by using replicates, which seems was not the case. It is true, most of the rates obtained in the study are based on high regression coefficients, but we do not have information of the significance of any of these regression lines. I ask the authors to comment on this and to include significance levels.

[Response] The dilution technique is labor-intensive and there is a tradeoff between the number of experiments conducted and the data accuracy of each experiment. Landry et al. (2008, Deep-Sea Res. II 55: 1348-1359; 2009, Prog. Oceanogr. 83: 208-216; 2010, Deep-Sea Res. II 58: 524-535; 2011, MEPS 421:13-24) have developed a 'mini-dilution' technique with only two dilution bottles. Although they sacrificed the data accuracy in each experiment, the benefit of a large data coverage overweighs the weakness of less accurate data. We kind of followed Landry et al.'s approach by using only five dilution bottles but tried to do as many experiments as possible. The volume of 1.2 L is typical in dilution experiments. Variability of grazers is always a problem in dilution experiments, but so far we do not have a better technique to estimate the in situ microzooplankton grazing effect on the community level. The work by Landry et al. (1995, MEPS 120: 53-63) has largely proved the validity of the dilution technique with

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the presence of a number of potential problems. We admit that the linear regressions of some of our experiments are insignificant (marked by * in the data appendix). But they represent a small portion of our experiments.

Units: in many occasions L and m3 are mixed in the same line. I would recommend choosing one.

[Response] We have unified the unit to L.

The incubations were conducted at surface temperature, irrespectively of the depth the water was collected from. This should not represent a problem in winter, when the water column was mixed, but could represent a severe thermic shock for the communities inhabiting in these waters in summer. Perhaps this can explain the reduced m/Bz at DCM in summer. Please, comment on that.

[Response] We fully agree that this is a problem for measuring phytoplankton growth and microzooplankton grazing rates at DCM layers. Actually, it is a common problem for all rate measurements at deeper waters (see also Landry et al. MEPS 2011). We have no idea of how this thermal shock affects the rate measurements. We are currently doing experiments investigating this problem. It may be the reason for the reduced m/Bz at DCM in summer. We have added this point in the discussion.

Include details on how the microzooplankton samples were collected.

[Response] Details added.

Regarding the latter, the authors write" dinoflagellates known to have phagotrophic ability (such as Gyrodinium, Protoperidinium) were included in the biomass of microzooplankton". I understand there is a need for tracing a line to distinguish phytoplankton than microzooplankton. However, the phagotrophic capacity may not be the right one, given most (if not all) of the dinoflagellates may have phagotrophic abilities. If the authors consider only the two previously indicated genus of dinoflagellates, I suggest changing the term phagotroph to heterotroph.

[Response] We have changed the term to 'heterotrophic'.

Please, indicate the cases with positive slopes.

[Response] We have indicated the cases with positive slopes in the supplemental data.

I am happy to see the authors made an effort to correct the phytoplankton growth rates for photoacclimation, even if simulating actual light conditions. However, I do not really see if these corrections were applied to $\mu$o, and what was their magnitude. This information is required. As a matter of fact in the results there are extremely high phytoplankton growth rates (e.g., S9, 10 summer, surface, among others), considering the chlorophyll biomass, the nutrient (only nitrate is reported) availability, and the biomass of grazers that could be actively recycling inorganic nutrients. Basic mass balance calculations show that is rather unrealistic to expect such growth rates. Please, comment on this. Please, explain the reasons for choosing the mixed layer definition.

[Response]The detailed data are shown in the supplemental Table S1 and S2. The 'Rf/Ri' indicates the extent of photo-acclimation. The reported $\mu 0$ and $\mu$n are phytoplankton growth rates after correction of photoacclimation. We do not think that the phytoplankton growth rates are unrealistically high because of the reasons listed below. First, Droop model tells us that phytoplankton growth rate is usually a function of intracellular nutrient quota (in our cases nitrogen or phosphorus). Although the ambient nutrient concentrations are low, we have no idea about the phytoplankton intracellular nutrient quotas. In fact, Laws (2013, Annual Review of Marine Science 5: 247-268) recently proved that, when nutrient uptake and cell growth reach a balance, ambient nutrient concentration is usually much smaller than the nutrient uptake half-saturation constant because maximal nutrient uptake rate is one-order of magnitude higher than the maximal growth rate. Second, our estimates of phytoplankton growth rates are normal among the values reported in the literature. For example, Landry et al. (1998, Deep-Sea Res. II 45: 2353-2368) reported quite a few estimates of $\mu 0$ exceeding 1 or even 2 d-1 in the Arabian Sea, which is quite similar to South China Sea. As discussed

in the 'Discussion' part, our estimates are quite comparable with other studies at similar latitudes. Third, zooplankton can excrete a substantial amount of nutrients supporting phytoplankton growth. If we assume that the microzooplankton grazing rate is 70% of phytoplankton growth and the growth efficiency of microzooplankton is 30%, then half of the nutrients required for phytoplankton growth can be met by microzooplankton excretion and the rest can be provided by other sources such as mesozooplankton excretion, diffusion, and vertical advection. Considering that there may be multiple trophic levels within the food web, the amount of nutrients excreted could be much higher. In a steady state, phytoplankton uptake of nutrients equals zooplankton excretion plus external input, which does NOT require high ambient nutrient concentration. To summary, low ambient nutrient concentration does not imply low phytoplankton growth rate because of the intense recycling of nutrients within the food web. This definition of mixed layer based on temperature differences is common in oceanography. We have added two references to support this.

Results Include DCM data on table 1 as well.

[Response] Included.

When presenting table 1 refer to the appendix to show where the actual data are.

[Response] We have added the words to refer to the appendix.

Define stratification index.

[Response] This sentence is deleted because discussion of stratification index is not quite relevant here.

I suspect a mistake in the p value at page 16012, line 9 (p should not be < 0.05 if insignificantly different).

[Response] Yes, it has been corrected.

I do not understand the last sentence on page 16012

[Response] We have revised the sentence to 'In surface waters, the ratio of Bz:Chl a was also significantly higher in the summer than in the winter (Wilcoxon test, $p < 0.001$), which might be caused by higher carbon-to-chlorophyll ratios of phytoplankton and/or higher microzooplankton-to-phytoplankton biomass ratios in summer'. Hopefully it is clearer now.

Discussion

Please, rephrase first 5 lines of the discussion to improve for clarity and style.

[Response] Revised.

It makes sense light limits phytoplankton growth. However, I also wonder what would be the consequences of the thermal shock indicated above.

[Response] We agree that the problem of thermal shock might lead to the lower m/Bz at DCM waters in summer. We have added this point in the discussion.

Chapter 4.3. I do not really see the point when referring to phytoplankton size-structure. Were are the data? Besides, I urge the authors to carefully read this section, and others, to revise the differences between m and $m/\mu o$. It seems both concepts are mix-up and they are, obviously, very different.

[Response] We agree that we should not discuss much on phytoplankton size-structure here and we have deleted the unnecessary text. We have carefully revised this section and made clear distinctions between m and $m/\mu o$.

In the same chapter. Introduce better the study by Liu et al (2002).

[Response] During revision, we have removed the discussion on Liu et al. (2002) because it is not quite relevant with the main focus here.

Page 16018 line 20. Please, make some basic calculations to back up microzooplankton recycling is enough to sustain the observed phytoplankton growth rates.

[Response] As noted above, we have deleted this section. We do not claim that micro-zooplankton excretion ALONE can be enough to sustain the observed phytoplankton growth rate, but can meet more than half of the phytoplankton nutrient requirement. Other sources can supply the rest of the nutrients that are needed.

Anonymous Referee #2

Received and published: 25 February 2013

Seasonal and spatial comparisons of phytoplankton growth and mortality rates due to microzooplankton grazing in the northern South China Sea Chen et al.

Dear Colleagues: Dilution technique is the most accepted method to measure phyto-plankton growth and mortality simultaneously. While Calbet and Landry (2004) pointed out that microzooplankton grazing loss accounted for more than half of the daily primary production globally, any systematic patterns have not been evident for the microzoo-plankton grazing on phytoplankton. The authors demonstrated the seasonal (winter vs. summer) and spatial (coastal to oceanic) changes in phytoplankton growth and mortal-ity using the dilution technique in the northern South China Sea where the chlorophyll gradients was shown. Since microzooplankton grazing loss of primary production is still unpredictable process of energy flow in pelagic food web. I believe that the manuscript contributes this field and such kind of topic would be major interest for the readers of Biogeoscience. The manuscript would be recommended for Biogeoscience if the authors could revise the following issues:

General comments 1. The authors should mention more clearly the answers to the three hypotheses in Results and Discussion sections. It's bit difficult to figure out the answers throughout the current manuscript.

[Response] We have made clear answers to the hypotheses in the Discussion sections 4.2 and 4.3.

2. As mentioned in Introduction, the authors want to find a systematic pattern of mi-

crozooplankton grazing on primary production. Although they demonstrated the comparison of many variables among the three domains, between the two seasons and between the two depths, it might be difficult for the readers to understand what is the systematic pattern. It would be nice to show phytoplankton growth, microzooplankton grazing and their ratios along the environmental gradients using ANOVA and/or correlation, even though the systematic pattern was not found in the present study.

[Response] We added a figure (Fig. 7) to show the patterns of $\mu 0$, m, and m/$\mu 0$ along bathymetry gradients and checked whether these trends are significant using spearman correlations.

Specific comments Introduction P16008, L15 More description might be needed for the three hypotheses on the scientific background. The readers might be confused why primary production is variable among the sites and seasons but microzooplankton grazing is steady-state in the hypotheses.

[Response] We have added descriptions on the scientific background in the introduction. For the sentence 'The readers might be confused why primary production is variable among the sites and seasons but microzooplankton grazing is steady-state in the hypotheses', we do not fully understand what the reviewer meant. We do not assume any steady state in the hypotheses, but just hypothesize that, statistically, microzooplankton grazing rates are higher under some conditions than under other conditions.

Materials and methods

P16008, L24 Normal Niskin or X-Niskin?

[Response] Normal Niskin bottles.

P16009, L4 Mesozooplankton are excluded from the seawaters? If not, the authors should mention the potential effects of mesozooplankton grazing on phytoplankton community because small copepods would appear abundantly in the subtropical sites.

[Response] No, we did not use 200 $\mu$m meshes to exclude mesozooplankton to min-

imize damage to delicate protozoan cells. The grazing effect of mesozooplankton on phytoplankton is much lower than that of microzooplankton. A parallel study (M. Chen et al. in revision) showed that mesozooplankton consumed less than 10% of the phytoplankton standing stock in both summer and winter.

Results P16012, L13 Between summer "and" winter

[Response] Corrected. Thanks for pointing it out.

P16013, L3-L5 and L10-L13 It is not clear which domain the authors mentioned here.

[Response] These are comparisons between the two depths for all stations.

P160013, L15 A decreasing trend of "surface" or "DCM" m from shelf to basin waters in summer?

[Response] It is "surface".

P16013, L22 The percentages of daily primary production consumed by microzooplankton (m/$\mu$o) are different from those shown in Figure 6. They are the values in summer? [Response] These are for the pooled dataset. It has been made clear in the text.

P16014, L3-L7 Please indicate the results using table.

[Response] Because this part of results is scattered, we prefer not to use a table to show the results.

Discussion P16014, L18 to P16015, L18 It would be very kind for the readers if the previous and present estimates using dilution technique (i.e. phytoplankton growth and mortality) are listed in table.

[Response] Table added (the new Table 2).

P16017, L9 It is not clear what the authors want to mention. Could they revise this phrase?

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[Response] This sentence has been deleted during revision.

P16017, L26 to P16018, L13 The authors describe the grazing control of phytoplankton biomass instead of bottom-up forces, using the hypotheses suggested by Marra and Barber (2005) and Behrenfeld (2010). It is one of possible explanations but it is better to show more direct evidence from the present results. [Response] We are not able to show more direct evidence using the present results because we do not have time-series data. If we can really prove this hypothesis, it will be a great contribution to this field.

Figures and Tables Table 1 The numbers without parentheses for SST, SSNO3, MLD and DCM are mean, median or the others? [Response] They are the median values.

Bingzhang Chen, On behalf of all coauthors.

Interactive comment on Biogeosciences Discuss., 9, 16005, 2012.