

Biogeosciences Discuss., referee comment RC2  
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## Comment on bg-2021-226

Anonymous Referee #2

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Referee comment on "Biophysical controls on seasonal changes in the structure, growth, and grazing of the size-fractionated phytoplankton community in the northern South China Sea" by Yuan Dong et al., Biogeosciences Discuss.,  
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### General comments

The authors show phytoplankton growth and grazing mortality by microzooplankton based on the result from dilution experiments. In my knowledge, dilution techniques are somewhat difficult for researchers and thus large numbers of data sets have been unavailable. Even under these difficulties, the authors demonstrate excellent data sets not only from the dilution experiments but also detail measurements on environmental variables. I believe that this study provides a good example for phytoplankton dynamics in the fluctuated environments. On the other hand, some disadvantages are found in the present study as follows.

- Local dynamics

Data demonstrations and discussions in the present study are focused on local phytoplankton dynamics. For more broader readers, the authors should provide new insights from these findings. I would like to see how size-selective feeding of microzooplankton on prey is variable under such fluctuating environments.

- Confused terminology

The authors described and discussed some different growth rates of phytoplankton in this manuscript. While these rates are crucial for this manuscript, most of the readers, particular for who are not familiar with dilution experiments, cannot understand the present results due the confused terminology (see specific comments). I recommend that the authors determine these terms specifically and then unify their writings throughout the manuscript.

- Size-selective prey preference

I believe that one of the advantages in this study is size-fractionated dilution experiments providing size- preference of microzooplankton on prey. While considerably excellent results are demonstrated, the authors provided opportunistic discussions (see specific comments) unfortunately. More logical (or comprehensive) discussion would be appreciated for size-selective feeding.

I am afraid to say that current conditions of this manuscript need moderate revisions. I would be very happy if the authors provide more suitable descriptions and discussions on the above issues and conduct major revisions.

#### Specific comments

L35: the cycling of carbon and nutrients in the ocean

Please add brief description why they regulate carbon and nutrients cycle, here.

L99: After returned to the laboratory

Could you tell the readers how many minutes do you take from the study station to land laboratory? I am just wondering whether microzooplankton grazing and excretion affect samples for chlorophyll and nutrients measurements. For our information, you can add the durations here, such as "after return to the laboratory (<1 hour)".

L122: carried out directly at a coastal pier near the sampling site

This description was unclear. We cannot understand where you take water samples for the experiments and incubate these waters in the bottles. All procedures including water sample collections for experiments were conducted at the coastal pier? If so, you need to discuss the regional difference between the station and the coastal pier. Please mention them clearly.

L128:  $5 \mu\text{mol l}^{-1} \text{NaNO}_3$ ,  $0.5 \mu\text{mol l}^{-1} \text{KH}_2\text{PO}_4$

I understand you determine these concentrations based on the previous experiments. In my knowledge, the N:P ratio is also important for regulating phytoplankton growth. Could you provide some explanations why you determine this N:P ratio (ca. 10) far from Redfield ratio (16) and observed ratio (>20)?

L145: The intrinsic growth rate ( $\mu_0$ ) is calculated as the sum of the net growth rate without nutrient enrichment ( $\epsilon_{\text{raw}}$ ) and the grazing rate

The authors should add another equation or alternative description on phytoplankton growth rates. As mentioned later, most of the readers who are not familiar with dilution experiments are confused for several phytoplankton growth rates that the authors mentioned. Currently, at least, the authors used the following growth rates and these terms should be defined clearly in Method section.

- apparent growth rate at each dilution factor
- growth rate at non-dilution without nutrients enrichment
- apparent growth rate at non-dilution with nutrients enrichment
- intrinsic growth rates (growth rate 3 minus microzooplankton grazing)

L192: which may indicate an extra utilization of P compared to other nutrients. Likely, an increased P consumption could occur here given the phosphorus deficiency driven by very high N/P ratios.

This phrase involves some assumptions and discussions. I think this should be deleted or moved to discussion.

L210: 1220 ind L<sup>-1</sup>

Why don't you estimate carbon-based biomass like pico-sized autotrophs? Ciliate/TChl is semi-quantitative values due to the different cell size between aloricates and tintinnids. Numerical abundance of microzooplankton is comparable to the other quantitative numbers like nutrients, growth rates and grazing mortality rates?

L218, L238: natural growth rates

What is "natural growth rate"?  $\mu_0$ ,  $\mu_n$  or others? Please define and classify them clearly.

L230: There was no general difference found among the natural growth rates of three phytoplankton size classes ( $p > 0.05$ ) except April and May 2019

Most of the readers cannot find these results from figures and tables. Which one is for "natural growth rate" in Fig. 5? I believe this "natural growth rate" is not defined in Method section. Once you define these terms, please unify them in texts, figures and tables.

L233: intrinsic growth rates

This might be  $\mu_0$ ? As mentioned above, the authors should indicate the defined terms in Method section since most of the readers are confused for these different growth rates.

L235, L238: the nutrient enriched growth rate

Same to the others (see above).

L247, L250: constant

What do the authors mean? Even when these factors are not fluctuated largely, significant correlations can be found.

L277: Microphytoplankton growth seemed more influenced by phosphate than by other factors.

These results are likely inconsistent with the results and discussions for nano-sized autotrophs. If nano-autotroph growths are associated with P deplete conditions as mentioned above, they would demonstrate similar results of micro-autotrophs. The authors need further discussions or some revisions.

L306: This was likely the case at the Wanshan station when the community grazing rate was poorly explained by the ciliate abundance.

Even though they reveal size-dependent preference on prey, the authors should conduct statistical tests using microzooplankton biomass due to their different cell size.

L309: chemical defense of diatoms to microzooplankton grazing

Just after mentioned "size-dependent selectivity", why do the authors mention chemical defense? This is one of probable mechanisms, but they should discuss size-dependent selectivity first.

L312: size-fractionated

Which size? I could not find larger correlation of all size-fractionated chlorophyll to grazing mortality on nano-autotrophs than those of pico-autotroph biomass in Fig. 6B.

L315: A reverse correlation of ciliate with the grazing rate could likely be explained by trophic cascade with the feeding of omnivorous ciliates on other microzooplankton reducing the overall grazing pressure on phytoplankton (Zollner et al., 2009).

As pointed out above, why don't the authors discuss this issue by size-dependent feeding? All ciliates can graze micro-autotrophs? If trophic cascading effects are likely, this interpretation is very poor due to no evidence from this study.

L334: contribution of mesozooplankton grazing

The authors should add information from the following papers.

Calbet & Landry (1999): LO (10.4319/lo.1999.44.6.1370)

Calbet (2001): LO (10.4319/lo.2001.46.7.1824.)

Liu et al. (2010): MEPS (10.3354/meps0 8550)

Karu et al. (2020): FO (10.1111/fog.12488)

L336: size-selective grazing of microzooplankton

This issue should be more discussed at the beginning of Discussion section due to the central issue derived from size-fractionated dilution experiments. Also, size-selective feeding is associated with many discussions as pointed above. However, even if the authors move this paragraph at the beginning of Discussion section, the readers cannot

catch the authors conclusion for size-selective feeding from the current interpretations. They need major revision on this paragraph.

L374: available in the Supplement

In my understanding, this journal recommends uploading data sets used in this study at accessible website or others.

L560: chlorophyll a concentration and the size-fractionated percentages

chlorophyll a concentration "(red circles and lines)" and the size-fractionated percentages "(columns)"

L574: nutrient enriched phytoplankton growth

For the readers who are not familiar with dilution experiments, they might be confused for these growth rates. The authors should define these terms clearly in Method section and classified thereafter (see above).

L575: standard deviation

How do the authors compute standard deviations? When standard deviations are estimated, at least, they need triplicates for dilution experiment sets (i.e., 10 bottles multiplying 3 experiments). In the methods, you mentioned 10 bottles for each dilution



experiments. I understand the authors can take aliquots from each bottle. However, I believe that they cannot create triplicates of dilution experiments from these aliquots due to same bottles.

L589: phytoplankton growth rate

Again, which growth rate? If they are  $\mu_0$  or  $\mu_u$ , they involve grazing mortality. In the authors' computations, grazing rates at Y-axis are already dependent on growth rates at X-axis before this analysis. Is this okay? On the other hand, correlation or regression is necessary for this analysis? Other researchers demonstrate the ratio of "intrinsic growth rate" (i.e., intercept of dilution equation) to grazing mortality (i.e., slope of dilution equation). This procedure would exclude problematic logics in statistics.

L590: NSCS outside PRE

All abbreviations should be spelled out in figure caption.