

Biogeosciences Discuss., author comment AC3
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Reply on RC2

Yuan Dong et al.

Author 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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Response to Anonymous Referee #2

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.

Response: We thank the reviewer for overall positive comments.

- 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.

Response: We have added more discussion by proving new insights on how size-selective feeding varying temporally during the environmental change. In particular, we have discussed the seasonal change of grazing impacts of

microzooplankton on various size-classes of phytoplankton prey.

- 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.

Response: Thanks for pointing out these. The net growth rate is the same as the apparent growth rate (ϵ). The intrinsic growth rate is the same as the natural growth rate (m_0). In the revised manuscript, we have re-defined the confusing terms specifically and unified them 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.

Response: Thanks for this good suggestion. We have rewritten the discussion section to more focus on the size-selective feeding of microzooplankton in the revised manuscript.

- 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.

Response: We thank the reviewer for these constructive comments. We have taken all of them during our 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.

Response: Done. The sentence has been rewritten as "Microzooplankton are generally the dominant herbivores in the marine ecosystem (Calbet and Landry, 2004), regulating not only primary productivity but also carbon export via

vertical migration/pellet sinking and nutrient recycling by mixotrophy (Steinberg and Landry, 2017)”.

- 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)”.

Response: Thanks for pointing out this. It was less than one hour. The duration has been clarified in the revised manuscript.

- 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.

Response: Sampling collections were made at the offshore station 500 m away from the pier. The incubation experiment was conducted at the pier with the running seawater for temperature control in the incubator directly taking from the nearby surface seawater (There was no difference in temperature detectable between the sampling seawater and the seawater near the pier).

- L128: 5 $\mu\text{mol l}^{-1}$ NaNO_3 , 0.5 $\mu\text{mol l}^{-1}$ KH_2PO_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)?

Response: We did not choose the Redfield N:P of 16 in our nutrient-enriched experiments as the N:P ratio about 10 is sufficient for a large phytoplankton growth due to a persistent high N/P ratio of the local surface seawater driven by river discharge, similar to those used by Chen et al (2009).

- L145: The intrinsic growth rate (μ_0) is calculated as the sum of the net growth rate without nutrient enrichment (ϵ_{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)

Response: Sorry for the confusing terms. We have clearly redefined these rates into three distinct groups (ϵ : apparent growth rate; m_0 : natural growth rate; m_n : nutrient-enriched growth rate) and we have also differentiated them between rate for total community and rate for each size-class. We have unified them throughout the manuscript. The relevant text in the method section is now written as the attached PDF (text1.pdf)

- 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.

Response: Agree. We have deleted this in the revised manuscript.

- L210: 1220 ind L⁻¹

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?

Response: Agree. We have provided the carbon biomass of ciliates in the revised manuscript.

- L218, L238: natural growth rates

What is "natural growth rate"? μ_0 , μ_n or others? Please define and classify them clearly.

Response: The natural growth rate here is μ_0 . We have clarified it in the revised text and in the figure legend.

- 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.

Response: The natural growth rate is μ_0 . We have unified the definition in the Method section and unified them throughout the manuscript.

- L233: intrinsic growth rates

This might be μ_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.

Response: Yes it is μ_0 . We have verified the definition of these terms in the method section and unified them throughout the manuscript.

- L235, L238: the nutrient enriched growth rate

Same to the others (see above).

Response: Done. We have clarified it in the method section.

- L247, L250: constant

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

Response: It was not well written originally. It should be "salinity (and nutrients) was relatively less fluctuated". These factors (salinity and nutrients) were not correlated with growth rate during this period of time.

- 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.

Response: P-limitation of nano-autotrophs growth was only found during April and May 2019, which cannot represent the general relationship between P and nano-autotroph throughout the whole year. That is why we do not see a correlation between P and nanophytoplankton growth during the RDA analyses.

- 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.

Response: Agree. We have applied microzooplankton biomass to the statistical tests.

- 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.

Response: Agree, we have removed the discussion of chemical defense in this paragraph to more focus on size-dependent selectivity in the revised manuscript.

- 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.

Response: Agree, we have rewritten the sentence as "The grazing mortality rate of nano-cells was more correlated to picoplankton biomass as well as all the size-fractionated Chl-a concentrations than the other factors".

- 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.

Response: We thank the reviewer for this suggestion. We have rewritten this part as "A reverse correlation of ciliate with the microphytoplankton grazing rate could likely be explained by selective grazing of microzooplankton on nano- and pico-phytoplankton community (this will be further discussed in next few paragraphs)".

- 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)

Response: Agree. These references have been properly cited in the revised manuscript.

- 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.

Response: Thanks for the great comments. We decide to add a brief introduction on size-selective feeding at the beginning of the Discussion section while keeping the detail discussion of size-selective grazing in the original paragraph. We have also revised the paragraph to focus directly on size-selective grazing of microzooplankton. The new sentences in the beginning of the discussion are written as “The size-fractionated dilution experiment provides us a good opportunity to examine the temporal change of size-selective microzooplankton grazing at the Wanshan station. We focus on grazing rate of microzooplankton on total phytoplankton community (m) as well as on various size-classes (m_{micro} , m_{nano} , and m_{pico}). We present evidences of size-selective grazing of microzooplankton on small phytoplankton, which may have a great impact on the temporal dynamics of plankton community in the coastal ocean..... ”.

- L374: available in the Supplement

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

Response: Data are available at the National Earth System Science Data Center, China (<http://data.scio.ac.cn/metaData-detail/1405396650095489024>). We

have clarified this in the revised manuscript.

- L560: chlorophyll a concentration and the size-fractionated percentages

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

Response: Done. We have revised the text accordingly.

- 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).

Response: Done. We have calcified it in the revised manuscript.

- 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.

Response: The error bars for growth and grazing rates are standard errors not standard deviations. We have corrected this in the revised manuscript. The standard error was calculated from the regression of the 10 data points (5 dilution factors) for each dilution experiment.

- L589: phytoplankton growth rate

Again, which growth rate? If they are μ_0 or μ_n , 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.

Response: Thanks for constructive comments. The phytoplankton growth rate here is μ_0 , which is the sum of the apparent growth rate of raw-seawater (e_{raw}) and the grazing rates (m). Since the apparent growth rate (e_{raw}) was completely independent of the grazing rate (m), we think it is still appropriate to do regression analyses between u_0 and m . The same approach has been used in the paper of Calbet and Landry (2004). On the other hand, we have also added the seasonal change of grazing impacts (m/μ_0) of microzooplankton on various size-classes of phytoplankton prey to the revised manuscript.

- L590: NSCS outside PRE

All abbreviations should be spelled out in figure caption.

Response: Done.

Please also note the supplement to this comment:

<https://bg.copernicus.org/preprints/bg-2021-226/bg-2021-226-AC3-supplement.pdf>