November 1st, 2021

Dear Editor,

We have attached a revised version of our manuscript "Biophysical controls on seasonal changes in the structure, growth, and grazing of the size-fractioned phytoplankton community in the northern South China Sea" by Yuan Dong, Qian Li and other coauthors.

We greatly appreciate the constructive comments provided by the editor and three reviewers. They have been very constructive, contributing significantly to improve the overall quality of our paper. We have carefully addressed all their points in the revised manuscript, and have detailed our changes in the response to reviewers (below). We hope that you will now find our manuscript suitable for publication.

Sincerely yours,

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Response to Anonymous Referee #1

General comments

1. The manuscript reports a study of size-fractioned phytoplankton growth and grazing rates in the northern South China Sea. The authors used the well established dilution method to measure community grazing rates and growth rates of the different size fractions --- micro-, pico- and nano- components of the phytoplankton. The results were then discussed in the context of the different environmental parameters.

The experiments appear to be done and the data analyzed carefully. The amount of work involved is quite impressive, and it generates quite interesting insights into the dynamics of the different phytoplankton size fractions in the region. It is a valuable contribution to the basic biological oceanography of the northern South China Sea.

Response: Thanks to the reviewer for positive comments on this work.

Specific comments

2. Introduction: The hydrographical conditions described are not unique to NSCS. To give the paper a broader appeal, perhaps the authors can explain better the ecological or biogeochemical significance of the studied area?

Response: We thank the reviewer for the good suggestion. We have revised the last part of the introduction section to better present the ecological and biogeochemical significance of our study area. The new text is written as " ... Seasonal changes in growth and grazing rates, as well as size-selective prey preference at a coastal site such as Wanshan could be crucial for understanding the temporal dynamics of food-web structure, carbon export, and nutrient recycling in the coastal ocean (e.g. Steinberg and Landry, 2017). Moreover, our results here may be of great value for modeling the size-structured planktonic ecosystem and associated element cycles as temporal variabilities of these processes are often not well represented in current biogeochemical models (e.g. Li et al., 2011; Sailley et al., 2015)."

3. Likewise, the background biological information seems lacking. The authors only briefly cited a few papers on diatom blooms, nutrient limitation and microzooplankton grazing, but no details are provided. It would be helpful to say more about the plankton community in the area (if known), and give a stronger justification (than just “it remains largely unknown…”) how this study can improve our understanding of the area in a meaningful way.

Response: We thank the reviewer for this suggestion. We have provided the detail biological background of the Wanshan station to the revised manuscript. The relevant sentences are

rewritten as "Previous studies have suggested that phytoplankton community in the coastal waters near Wanshan was dominated by diatoms with intense blooms occurring in response to strong eutrophication (e.g. Li et al., 2013). The dominant diatom species here were *Skeletonema costatum* in the summer and *Eucampia zoodiacus* in the winter. There were also intense grazing of phytoplankton by microzooplankton (mainly ciliates and dinoflagellates) and mesozooplankton (mainly copepods) (Chen et al., 2015)".

4. Materials and Methods: Initial Chl-a was estimated based on the dilution factor, instead of direct measurements (line 135). This seems rather dubious for a study that so critically depends on accurate Chl-a measurements for calculating growth rates and grazing rates. Can the authors provide any ancillary data to confirm the reliability of their estimation?

Response: Actually, we have conducted experiments to verify that the calculated initial Chl-a concentration was not significantly different from that of the direct measurement ($t=0.5$, $n=22$, $p=0.31$). We have clarified this in the revised manuscript.

5. While it is commendable that the authors used 5 dilution levels (line 127), can the authors confirm how well the data points fit on a linear regression for calculating grazing rates (line 145)? Perhaps the authors can display the actual "apparent growth rate vs. fraction seawater" and the corresponding statistics in supplementary?

Response: The statistic details (including R-value and p-value) of the linear regression between apparent growth rate and dilution factors for each experiment had been shown in the supplementary Table S2.

6. Results: Please include and explain the "nutrient limitation index" (line 252) in the Method section.

Response: Done. We have added the detail description of "nutrient limitation index" to the revised manuscript.

7. Line 322: "The negative effect of… salinity and nutrients." This part is a bit confusing; please revise.

Response: Done. The sentence has been rewritten as "A negative correlation of salinity with the abundance of smaller zooplankton in the Jiaozhou Bay of the South Yellow Sea has been attributed to the discharge of eutrophic freshwater (Wang et al., 2020). This may likely be also true at the Wanshan station given the negative correlation between salinity and nutrients. The input of low-salinity/high-nutrient water stimulates phytoplankton growth and thus the growth of small zooplankton grazing on them".

8. Conclusion: Line 359: Perhaps change "in the ocean" to "in the studied area". After all, the measurements are limited to a rather small area.

Response: Done.

9. Presently, the data are discussed rather narrowly within the confine of data patterns and trends, but it is missing what the data tell us about the bigger picture. In the conclusion, the authors state “our findings… ocean biogeochemical modeling… carbon fluxes… microbial food web… future environmental and climate change” (line 329). Related to my comments about the Introduction section, it would strengthen the manuscript if the authors introduce some of these issues in the Introduction, then discuss the results in these context in the Discussion. I believe, doing so will elevate the overall quality and significance of the paper.

Response: We thank the reviewer for the constructive comments. We have rewritten the relevant parts of the introduction and the discussion sections according to his/her suggestions to provide a broader discussion on the impacts of our findings on the general carbon cycle and ecosystem dynamics of the shelf-sea.

In the last paragraph of the introduction, we have added several sentences as " ... Seasonal changes in growth and grazing rates, as well as size-selective prey preference at a coastal site such as Wanshan could be crucial for understanding the temporal dynamics of food-web structure, carbon export, and nutrient recycling in the coastal ocean (e.g. Steinberg and Landry, 2017). Moreover, our results here may be of great value for modeling the size-structured planktonic ecosystem and associated element cycles as temporal variabilities of these processes are often not well represented in current biogeochemical models (e.g. Li et al., 2011; Sailley et al., 2015)".

We have also added the following text to the last paragraph of the discussion as "A seasonal change in size-selective feeding of microzooplankton may be crucial for understanding food web dynamics and the carbon cycle of the coastal ocean. It has been well recognized that temporal change of phytoplankton community structure can be regulated by size-selective herbivory of microzooplankton (Strom et al., 2007; Haraguchi et al., 2018). In addition, an enhanced export production may occur when large phytoplankton such as diatoms can escape from grazing by micrograzers due to size-selective prey preference on small cells (Froneman and Perissinotto 1996). Furthermore, nutrient recycling within the microbial food web can be significantly influenced by selective grazing of microplankton on heterotrophic bacterioplankton (Christaki et al., 2001; Unrein et al., 2007). On the other hand, our results may be also important for ecosystem and biogeochemical modeling. Inaccurate representative of microzooplankton grazing and their prey selection can cause deficiencies of these models and cast doubts on the results of their predictions (e.g. Li et al., 2011; Sailley et al., 2015). In this sense, our finding of seasonal variability of size-selective grazing and their controlling factors should be crucial for not only the parameterization of grazing models but also the prediction of the shifts in the plankton community structure in response to future climate change ".

10. Technical corrections: Overall clearly written, notwithstanding a few minor typos or grammatical errors.

Response: The manuscript has been proofread by a native English speaker and the typos and errors have been corrected in the revised manuscript.

Response to Anonymous Referee #2

General comments

1. 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.

2. 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.

The new paragraphs in the discussion section are written as
" Monthly grazing impact ((m_i/μ_i)) at the Wanshan station reveals a seasonal change of size-selective prey preference of microzooplankton with increased grazing on nanophytoplankton during the winter-spring period (Fig. S3). Large aloricate ciliates (30-50 µm), the dominant micrograzer in our system, are the major consumers of nanoplankton (Bernard & Rassoulzadegan 1990). Increased ingestion of aloricate ciliates on phototrophic nanoflagellate during the winter with lower nanoplankton biomass and productivity has been reported in the coastal waters off Chile (Vargas and Martinez, 2009). Our results are also consistent with the previous finding in the Southern Ocean with microzooplankton preferentially grazing on the nano- and pico-phytoplankton during the winter when the community was dominated by small cells (Froneman and Perissinotto, 1996). Alternatively, it has also been suggested that an increase of grazing on small autotrophs may be caused by microzooplankton growth due to the artifact of removing mesozooplankton from the incubations (Schmoker et al., 2013; Calbet and Saiz, 2013). Meanwhile, this effect should be negligible here since mesozooplankton was barely present in our 200-µm screen during the one-year field study. ".

3. 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 (μ_0). In the revised manuscript, we have re-defined the confusing terms specifically and unified them throughout the manuscript.

4. 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.

The new paragraphs on size-selective grazing of microzooplankton are written as " Monthly grazing impact (m_i/μ_i) at the Wanshan station reveals a seasonal change of size-selective prey preference of microzooplankton with increased grazing on nanophytoplankton during the winter-spring period (Fig. S3). Large aloricate ciliates (30-50 µm), the dominant micrograzer in our system, are the major consumers of nanoplankton (Bernard & Rassoulzadegan 1990). Increased ingestion of aloricate ciliates on phototrophic nanoflagellate during the winter with lower nanoplankton biomass and productivity has been reported in the coastal waters off Chile (Vargas and Martinez, 2009). Our results are also consistent with the previous finding in the Southern Ocean with microzooplankton preferentially grazing on the nano- and pico-phytoplankton during the winter when the community was dominated by small cells (Froneman and Perissinotto, 1996). Alternatively, it has also been suggested that an increase of grazing on small autotrophs may be caused by microzooplankton growth due to the artifact of removing mesozooplankton from the incubations (Schmoker et al., 2013; Calbet and Saiz, 2013). Meanwhile, this effect should be negligible here since mesozooplankton was barely present in our 200-µm screen during the one-year field study.

A seasonal change in size-selective feeding of microzooplankton may be crucial for understanding food web dynamics and the carbon cycle of the coastal ocean. It has been well recognized that temporal change of phytoplankton community structure can be regulated by size-selective herbivory of microzooplankton (Strom et al., 2007; Haraguchi et al., 2018). In addition, an enhanced export production may occur when large phytoplankton such as diatoms can escape

from grazing by micrograzers due to size-selective prey preference on small cells (Froneman and Perissinotto 1996). Furthermore, nutrient recycling within the microbial food web can be significantly influenced by selective grazing of microplankton on heterotrophic bacterioplankton (Christaki et al., 2001; Unrein et al., 2007). On the other hand, our results may be also important for ecosystem/biogeochemical modeling. Inaccurate representative of microzooplankton grazing and their prey selection can cause deficiencies of these models and cast doubts on the results of their predictions (e.g. Li et al., 2011; Sailley et al., 2015). In this sense, our finding of seasonal variability of size-selective grazing and their controlling factors should be crucial for not only the parameterization of grazing models but also the prediction of the shifts in the plankton community structure in response to future climate change. "

5. 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.

<u>Specific comments</u>

6. 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 <u>carbon export via vertical migration/pellet sinking</u> and <u>nutrient recycling by mixotrophy</u> (Steinberg and Landry, 2017)".

7. 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.

8. 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).

9. L128: 5 μmol l-1 NaNO3, 0.5 μmol l-1 KH2PO4
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 Red-field 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).

10. L145: The intrinsic growth rate (μ0) 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.
1. apparent growth rate at each dilution factor
2. growth rate at non-dilution without nutrients enrichment
3. apparent growth rate at non-dilution with nutrients enrichment
4. 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; μ_0: natural growth rate; μ_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.

11. 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.

12. 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.

13. L218, L238: natural growth rates
What is "natural growth rate"? μ0, μn or others? Please define and classify them clearly.

Response: The natural growth rate here is μ0. We have clarified it in the revised text and in the figure legend.

14. 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.

15. L233: intrinsic growth rates
This might be μ0? 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.

16. L235, L238: the nutrient enriched growth rate
Same to the others (see above).

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

17. 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.

18. 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.

19. 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.

20. 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.

21. 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".

22. 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)".

23. 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.

24. 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.

The new paragraph in the beginning of this discussion section are written as "We address the temporal change in the feeding strategy of microzooplankton by focusing on their grazing on total phytoplankton community (m) as well as on various phytoplankton size-classes (m_{micro}, m_{nano}, and m_{pico}). We present evidence for the size-selective preference of microzooplankton on small autotrophs, which may have a great impact on the temporal dynamics of the plankton community in the coastal ocean ".

We have also substantially revised the paragraph to focus directly on size-selective grazing of microzooplankton. Please refer to our detail response to the point No.4 of the this reviewer.

25. 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.scsio.ac.cn/metaData-detail/1405396650095489024). We have clarified this in the

revised manuscript.

26. 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.

27. 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.

28. 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.

29. L589: phytoplankton growth rate
Again, which growth rate? If they are μ0 or μn, 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 (ε_{raw}) and the grazing rates (m). Since the apparent growth rate (ε_{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.

30. L590: NSCS outside PRE
All abbreviations should be spelled out in figure caption.

Response: Done.

Response to Anonymous Referee #3

General comments

1. The authors showed the size-fractioned phytoplankton community growth and grazing based on the result from dilution experiments. The authors also explained the biophysical factors which controlled the growth and grazing rates of micro-, nano- and pico-phytoplankton. In general, this manuscript is novelty and a board international interest. The experiment was well designed and conducted, the data interpretation was sufficient and accurate. However, the statistical analysis and some data interpretation should be revised and improved.

Response: We thank the reviewer for overall positive comments.

Specific comments

1. Introduction:
Please add information about phytoplankton community in the study area.

Response: Done. The background information of phytoplankton community has been added to the revised manuscript as "Previous studies have suggested that phytoplankton community in the coastal waters near Wanshan was dominated by diatoms with intense blooms occurring in response to strong eutrophication (e.g. Li et al., 2013). <u>The dominant diatom species here were *Skeletonema costatum* in the summer and *Eucampia zoodiacus* in the winter. There were also intense grazing of phytoplankton by microzooplankton (mainly ciliates and dinoflagellates) and mesozooplankton (mainly copepods) (Chen et al., 2015)</u>".

2. Materials and Methods:
in the estuary system, the ammonium is important nutrient for phytoplankton. So please explain why not analyses ammonium as the control factor?

Response: The reviewer is right about the importance of ammonium in coastal waters. Unfortunately, we did not have ammonium measurements. Nitrate concentration is generally higher than ammonium throughout the year in the PRE (Chen et al., 2009). Thus, we only examine nitrate as a control factor in this study. Further study may need to consider ammonium as well.

3. line128-130,'' Ten incubation bottles were enriched with dissolved inorganic nutrients of 5 µmol l-1, NaNO3, 0.5 µmol l-1 KH2PO4, and 5 µmol l-1, Na2SiO3 to ensure the constant growth of phytoplankton (particularly to avoid nutrient limitation during winter).'' As we know, the N/P Redfield

ratio is 16, Could you explain why you determine this N:P ratio (10) in your manuscript?

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).

4. Results:

Line 238-239, “Generally, the annual average of the nutrient-enriched growth rate (1.68 d-1) was higher than that of the natural growth rate (1.22 d-1), indicating a nutrient limitation of phytoplankton even in this highly eutrophic system”, i think the conclusion needs to be taken with caution, especially in the estuary system.

Response: Agree. We have replaced "limitation" with "deficiency".

5. Discussion:

Line 261, “It is surprising to find negative intrinsic growth rates of nanophytoplankton during April and May 2019”. The authors explained that “nanophytoplankton by itself tends to be limited by phosphorus”. However, there were some similar situations in the Dec. and Feb., and the intrinsic rates of nanophytoplankton was higher. Could you give more information to explain the different results?

Response: We should not expect to get a negative specific growth rate (u0 >=0). A negative specific growth rate of nano-autotrophs during April and May should imply that the dilution technique may not work for nano-cells in these two months So, it is inappropriate to directly compare growth rates of nano-cells between Dec (or Feb) and April (or May).

6. Line 283-284, “Interestingly, we found nanophytoplankton was more controlled by light than the other factors.”, this experiment was conducted in the surface (2m), light should not limit phytoplankton growth. So please explain the reasons why nanophytoplankton was more controlled by light than the other factors.

Response: The reviewer is right about that there should not be light limitation in the surface seawater. Previous study suggested that phytoplankton growth on the west coast of Spitsbergen could be predominantly controlled by solar irradiance on seasonal and inter-annual timescales (van de Poll et al., 2021). The cycle of warming and freshwater discharge in the coastal regions could be driven by solar radiation (van de Poll et al., 2021). This may be a possible explanation why nanophytoplankton in our system was more controlled by light than the other factors.

van de Poll, W.H., Maat, D.S., Fischer, P., et al. (2021), Solar radiation and solar radiation driven cycles in warming and freshwater discharge control seasonal and inter-annual phytoplankton chlorophyll a and taxonomic composition in a high Arctic fjord (Kongsfjorden, Spitsbergen). Limnol Oceanogr, 66: 1221-1236.

7. in the 4.1, there was a strong negative correlation between salinity and phytoplankton growth, but the authors did not discuss the salinity how to influence the phytoplankton. A reasonable explanation may be obtained in terms of salinity.

Response: Coastal phytoplankton species can tolerate a much larger range of salinity than estuarine and oceanic species (e.g. Brand 1984). The negative relationship between salinity and phytoplankton growth should be attributed to nutrients given the tight negative relationship between salinity and nutrients. The correlation between nutrient and salinity at this station was due to the seasonal input of eutrophic freshwater (higher nutrient but lower salinity compared to the offshore oligotrophic seawater).

8. Technical corrections

in the Fig.2, the legend of NO3 and PO4 is not the standard format, please revise it.

Response: Done. We have revised the legend as suggested.