Biogeosciences Discuss., 12, C3635–C3644, 2015
www.biogeosciences-discuss.net/12/C3635/2015/

Biogeosciences
Discussions

Interactive comment on "Seasonal and size-dependent variations in the phytoplankton growth and microzooplankton grazing in the southern South China Sea under the influence of the East Asian monsoon" *by* L. Zhou et al.

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Received and published: 16 July 2015

Dear reviewer, Thanks very much for your time on reviewing our MS. Your comments are helpful for us to improve the MS, please see following our point-by-point responses to the comments. The attached "Supplement" is a revised marked-up manuscript version.

Best regards, Linbin Zhou

Point-by-point responses to the comments

"General comments: This paper is an ordinary study that reports the results of dilution experiments and discusses every point relevant with the results. The study area is interesting and was indeed not often investigated before. There are some of the weak points that I need to address before recommending for publication for 'Biogeosciences'. The data themselves are certainly useful (although some of the nutrient and growth rate data seem weird as I discuss below). But, unfortunately, there are no data for microzooplankton biomass. The approach of analysis is crude and breaks down sometimes (certainly needs improvement). Some of the discussions are interesting, but some are unfounded and highly speculative. I would suggest narrowing down to one or two main points."

Responses: We delete the speculation about the potential effects of low salinity on phytoplankton growth in section 4.3 (the last paragraph in Page 6301, and lines 1-14 in Page 6302). Unfortunately, we do not have the microzooplankton biomass data.

"One weird result is the relatively low m/μ values in such an oligotrophic and tropical environment. It is not very likely that mesozooplankton grazing consumed the rest of the primary production left by microzooplankton, given that the majority of the phytoplankton are smaller than 3 μm (The authors' Table 2). Direct sinking is not possible either, also because of the small size of the phytoplankton. Of course, it is possible that μ can highly exceed m in non steady-state conditions. But for the long run, growth must largely balance mortality (including microzooplankton and mesozooplankton grazing, viral lysis, direct sinking, etc.)."

Responses: Yes, the m/μ values were relatively low, but the low values are consistent with the high vertical biogenic particle fluxes in the prevailing periods of the monsoons in the southern SCS (Wan et al., 2010). Although neither direct sinking of picophytoplankton, nor direct grazing of the primary production within picophytoplankton by mesozooplankton can occur, formation and gravitational settling of large aggregates by picophytoplankton, and/or consumption of those aggregates by mesozooplankton could contribute to substantial vertical POC export (Lomas et al, 2011; Richardson and

Jackson, 2007). Therefore, we considered that the mismatch between primary production and microzooplankton grazing could let substantial proportion of the picophytoplankton production be exported to the deep water, and account for the high vertical biogenic particle fluxes observed in the southern South China Sea. We agree that the episodic enhancement and weaking of the monsoons (especially in winter) could lead to non-steady-state conditions favourable to the mismatch (decoupling) between phytoplantkon growth and microzooplankton grazing, and μ highly exceed m may occur. we discussed this in section 4.5 and 4.6

Lomas, M. W. and Moran, S. B.: Evidence for aggregation and export of cyanobacteria and nano-eukaryotes from the Sargasso Sea euphotic zone, Biogeosciences, 8, 203-216, 2011. Richardson, T. L. and Jackson, G. A.: Small phytoplankton and carbon export from the surface ocean, Science, 315, 838-840, 2007. Wan, S., Jian, Z., Cheng, X., Qiao, P., and Wang, R.: Seasonal variations in planktonic foraminiferal flux and the chemical properties of their shells in the southern South China Sea, Sci. China Earth Sci., 53, 1176-1187, 2010.

“One potential cause is that the authors had inadvertently overestimated the growth rates of the phytoplankton even in nutrient non-amended bottles. This can occur for two reasons. The first is that the light level was not well controlled. In the “Materials and Methods” section, the authors have indicated that “All of the bottles were incubated for 24 h in a deck incubator cooled by running surface seawater and covered with neutral-density screens to simulate in situ light regime. These measures have been proved effective to avoid phytoplankton photoacclimation during the incubation (Zhou et al., 2015a)”. However, the authors did not report how they estimate the “in situ light regime”, which is not so easy to estimate if one needs to take into account the surface irradiance, the depth of surface mixed layer, the light attenuation coefficient and the mixing turnover time in the water column, etc.. The authors did not provide any of the information in the paper. Because all the factors may vary day-to-day, the carbon-to-chlorophyll ratios of the phytoplankton cells could change even if the simulated light

environment perfectly matches the “in situ” condition and the growth rates could be estimated with biases (if not errors). The experiments in Zhou et al. (2015a) were done in different areas and at different times and could not be used to justify the results in the present study. It is a bit weird why the authors did not do similar checks on the cellular fluorescence in this study. The second possibility for overestimating the growth rates is that inadvertent nutrient contamination in the incubation bottles in oligotrophic waters. This is hard to verify, but this possibility cannot fully ruled out.”

Responses: Although the two possibilities proposed by the reviewer cannot be fully ruled out, we don’t think that the phytoplankton growth rates were significantly overestimated. Firstly, we agree that it is not so easy to simulate the natural light regime in the incubator, as it needs to take into account the surface irradiance, the depth of surface mixed layer, the light attenuation coefficient and the mixing turnover time in the water column, etc.. As the reviewer said, all the factors may vary day-to-day, and the carbon-to-chlorophyll ratios of the phytoplankton cells could change even if the simulated light environment perfectly matches the “in situ” condition. Therefore, it is could be feasible and reliable to deal with “the light problem” by using the proved effective empirical treatment as that in Zhou et al. (2015a). In fact, the experiments in Zhou et al. (2015a) were conducted in the South China Sea, and two experiments were conducted in the southern South China Sea, the same area being investigated in the present study. As the South China Sea is located in tropical area, the seasonal change in light irradiance is likely small. Therefore, we believe that the same measures were effective to avoid phytoplankton photoacclimation during the incubation in the present study. Of course, it cannot deny that checking on the cellular fluorescence at each experiment would get the best insurance. Unfortunately, we did not do this. Secondly, our procedures for bottles washing (All the bottles, containers and filters were soaked in 10% HCl for more than 10 h, and thoroughly washed with deionized water and MiliQ-water before each cruise. The bottles and containers were washed with 10% HCl, deionized water and ambient seawater before each experiment.) could minimize the possibility of nutrient contamination in the incubation bottles, especially in the controlled non-nutrient-amended ones. Thirdly, as we discussed in section 4.1 (Comparisons with other studies), our rates of phytoplankton growth (except the negative values obtained at KJ35) were within the reviewed ranges based on global data collection, and similar to the results in the adjacent western South China Sea reported by Chen et al. (2009), and those in the central equatorial Pacific reported by Landry et al. (1995).

Chen, B., Liu, H., Landry, M. R., Dai, M., Huang, B., and Sun, J.: Close coupling between phytoplankton growth and microzooplankton grazing in the western South China Sea, Limnol. Oceanogr., 54, 1084-1097, 2009.

Landry, M. R., Constantinou, J., and Kirshtein, J.: Microzooplankton grazing in the central equatorial Pacific during February and August, 1992, Deep Sea Research II, 42, 657-671, 1995.

Zhou, L., Tan, Y., Huang, L., and Li, G.: Does microzooplankton grazing contribute to the pico-phytoplankton dominance in subtropical and tropical oligotrophic waters?, Acta Ecol. Sin., 35, 29-38, doi:10.1016/j.chnaes.2014.12.007, 2015a.

"My another concern is on the nutrient data. From Table 1, my first impression is that this area might be a "high-nitrate-low-chlorophyll" (HNLC) region! If it were true, this could be a big issue since, to my limited knowledge, iron limitation has not been reported in this area. My first response for Table 2 is that: could the authors mistakenly swap the N column with the P column since the P concentrations were so high? The authors need to double check these data."

Responses: We have checked the nutrient data, and make sure that the presentation of these data is no problem. We do not fully know the reasons for the contrasted distributions of nutrients between the two seasons, and it is still open to discuss. We have discussed in Section 4.4 that the seasonal varied pattern and concentrations of phosphate and silicate, and the summer concentration of nitrate plus nitrite in surface seawater of the southern South China Sea were consistent with the results reported by Ning et al. (2004), The nearly undetectable nitrate plus nitrite concentration in the

winter is significantly lower than and different from the results reported by Ning et al. (2004), but similar to the results reported by Chen et al. (1997). Chen et al. (1997) documented that the concentration of nitrate plus nitrite was undetectable in adjacent waters of the Nansha Islands in winter. We also discussed the possible reasons for the high concentration of nitrate plus nitrite (0.90 μmol L-1 on average) in the summer, and high concentration of phosphate (0.35 μmol L-1 on average) in the winter. Until now, it is still open to discuss.

Ning, X., Chai, F., Xue, H., Cai, Y., Liu, C., and Shi, J.: Physical-biological oceanographic coupling influencing phytoplankton and primary production in the South China Sea, J. Geophys. Res., 109, C10005, doi:10.1029/2004jc002365, 2004. Chen, S., Huang, L., and Han, W.: Studies on limiting factors of primary production of Nansha Islands sea area. In: A Study on Ecological Processes of Nansha Islands Sea Area I, Huang, L. (Ed.), Science Press, Beijing, China, 37-48, 1997.

"I would argue that the RPI index cannot be used to infer whether microzooplankton grazing contributes to the dominance of picophytoplankton in oligotrophic waters, because the RPI index does not include growth rate. The variations of phytoplankton biomass are determined by both growth and loss (including grazing, sinking, etc.) rates. Higher m on larger phytoplankton does not directly lead to the dominance of smaller phytoplankton. In eutrophic waters, we can also observe higher m and RPI index on larger phytoplankton, which does not necessarily indicate the dominance of picophytoplankton in eutrophic waters. It is simply because larger phytoplankton also grow faster."

Responses: We agree that the phytoplankton dynamics are influenced by both the phytoplankton growth and loss (including that caused by zooplankton grazing). However, by using the RPI index provided by Obayashi and Tanoue (2002), we could examine the pure effects of microzooplankton grazing on the size composition of the whole phytoplankton assemblage. If the RPI index include growth rate, we could not directly know the contribution of microzooplankton grazing. Our result does not contradict with the argument that change in phytoplankton biomass and size composition is influenced by phytoplankton growth, but provides evidence that the size-selective grazing by microzooplankton grazing was in favor of the dominance of pico-phytoplankton in the studied waters

Obayashi, Y. and Tanoue, E.: Growth and mortality rates of phytoplankton in the northwestern North Pacific estimated by the dilution method and HPLC pigment analysis, J. Exp. Mar. Biol. Ecol., 280, 33-52, 2002.

"Particularly, one point that needs to be addressed is that, when inferring the mechanisms controlling the growth and grazing on phytoplankton, one must bear in mind that correlation does not lead to causation. There are so many factors that may affect the growth and grazing of phytoplankton. It would be misleading to attribute most of the variations to one or two environmental factors (e.g. rainfall) only based on correlation."

Responses: That is true. We think that the correlationship of μ and m with the environmental variables indicate that seasonal variations in the phytoplankton growth and microzooplankton grazing were related to the varied environmental conditions under the influence of the East Asian monsoons (Table 4). We discussed the possible influences of temperature, dissolved inorganic nutrients and factors associated with large rainfall and the resulted surface salinity decrease, on the growth and grazing. We proposed that changes in dissolved inorganic nutrients and other factors associated with SSS may be the main divers for the variations in the phytoplankton growth and microzooplankton grazing observed in the present study.

"Specific comments: 1. Abstract P. 6286, line 6-9. I am a little confused by this sentence. Does this mean m/μ did not vary significantly between the two seasons?"

Response: Yes. According to this comments, we rewrote the sentence as "The results showed that environmental variables, … and correlationship (coupling) between the μ and m, rather than the microzooplankton grazing impact on phytoplankton (m/μ) significantly varied between the two seasons."

2. Introduction P. 6287, line 2-3, change to: "Phytoplankton provide almost . . ." Line 15, change "indicates" to "induces"

Response: Corrected.

"P. 6287, Line 17-19, in fact, the microzooplankton studies in tropical oceans are not so few. Please also see Landry et al. DSR II 1995 and Quevedo & Anadón MEPS 2001."

Response: Following the comments, we cited the study by Landry et al. DSR II 1995, and compared their results with ours in Section 4.13. The study area in Quevedo & Anadón MEPS 2001 was located in oligotrophic subtropical Northeast Atlantic, and we compared their results with ours in Section 4.1.2 and 4.2.

"P. 6288, line 1, delete "phytoplankton" and change to "pico-sized prey"."

Response: Corrected.

3. Materials and methods P. 6290, line 1-2, the authors did not set up a dilution level below 20%. It is recommended by some authors (e.g. Gallegos 1989; Strom and Fredrickson 2008) to use a highly diluted bottle to deal with the possible grazing saturation.

Response: No, we did not. It was better to set up the highly diluted treatment, unfortunately, we did not do it.

4. Results P. 6293, the last paragraph. Please take into account the standard errors of each μ and m measurements when comparing the large size and small size fractions. I would guess many of the differences were insignificant.

Response: Ideally, it is better to take into account the standard errors. However, as we could see in the methods for estimating the μ and m in Chen et al. (2009), all the μ and m were not directly measured. The standard errors were influenced by the number of data point used for the regression, and the standard errors of each parameters used for the calculation. As a result, it is not easy to precisely calculate the standard errors, and we are afraid that it would make little sense to use the standard errors for comparison.

Chen, B., Liu, H., Landry, M. R., Chen, M., Sun, J., Shek, L., Chen, X., and Harrison, P. J.: Estuarine nutrient loading affects phytoplankton growth and microzooplankton grazing at two contrasting sites in Hong Kong coastal waters, Mar. Ecol.-Prog. Ser., 379, 77-90, 2009.

"P. 6294, line 14, change 'exclude' to 'excluding'. Line 16, "Taking all the data. . ."."

Response: Corrected.

5. Discussion P. 6300, line 1-3. What does this mean? Does it mean that the physiological effect of temperature is strong in the SSCS? But in the text above, you already wrote that the temperature variation was small.

Response: We rewrote the sentences as "This suggested that the physiological effect of temperature was not important for the m variation in the Arctic Ocean, let alone in the tropical waters of the SSCS."

Table 4: The correlations between μ and m/μ (and μ/μn, μ–m) make little sense since these variables are not independent with each other.

Response: We agree that these variables are not independent with each other, but as the nutrient limitation index μ/μn and net growth rate (μ–m) are proxy for the actual system trophic state (Landry et al., 1998; Calbet et al., 2011), and the m/μ measures the extent to which the daily phytoplankton production is consumed and balanced by microzooplankton grazing (i.e. the amount of potential recycled nutrients), the correlation between μ with these variables may tell us information that the phytoplankton growth rates were related to the actual trophic state and the recycled nutrients mediated by microzooplankton.

Landry, M. R., Brown, S. L., Campbell, L., Constantinou, J., and Liu, H.: Spatial patterns in phytoplankton growth and microzooplankton grazing in the Arabian Sea during monsoon forcing, Deep-Sea Res. II, 45, 2353-2368, 1998. Calbet, A., Saiz, E.,

Almeda, R., Movilla, J. I., and Alcaraz, M.: Low microzooplankton grazing rates in the Arctic Ocean during a Phaeocystis pouchetii bloom (Summer 2007): fact or artifact of the dilution technique?, J. Plankton Res., 33, 687-701, 2011.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C3635/2015/bgd-12-C3635-2015-supplement.pdf

Interactive comment on Biogeosciences Discuss., 12, 6285, 2015.