Author's responses

Dear editor,

Thanks very much for your and the referees' 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.

Best regards,

Linbin Zhou

Point-by-point responses to the comments

Author's responses to comments from anonymous referee #1

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.

Response:

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.).

Response:

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 Sections 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.

Response:

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.

Response:

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.

Response:

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.

Response:

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 Sections 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 (when grazing saturation occur, less data point may result into large standard errors), and the standard errors of each parameters used for the calculation. As a result, it is not easy to precisely calculate the standard errors, so we did not take into account 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/μ_n 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.

Author’s responses to comments from anonymous referee #2

This is the first data set for microzooplankton grazing rate on phytoplankton via the dilution assay in the oligotrophic South China Sea (SCS) during summer and winter monsoon seasons. There are relatively few estimates of protist grazing impact on phytoplankton in low chlorophyll tropical waters, compared to the number of dilution assay experiments that have been done in coastal temperate and high latitude ocean systems. The authors evaluated the idea that microzooplankton preferentially graze on nanophytoplankton rather than smaller picophytoplankton, resulting in the dominance of picophytoplankton in the SCS.

I reviewed an earlier version of this paper. In general, the authors have revised the text in response to my comments on that version and incorporated relevant literature as suggested. The paper provides useful data, however I still think there is too much unfounded speculation in the discussion.

Response:

We would like to delete the speculation about the potential direct effects of salinity on phytoplankton growth in Section 4.3 (the last paragraph in Page 6301, and lines 1-14 in Page 6302).

Specific comments on the present version of the paper:

1) p. 6286 lines 19-21: The low m/u (< 50% on average) indicates low remineralization of organic matter mediated by microzooplankton and the increased importance of the phytoplankton-mesozooplankton grazing pathway.’

This inference is still questionable and should be revised. The bulk of remineralization of organic matter in the sea is by bacteria and bacterivores (e.g. Ducklow 1983), not by microzooplankton, and certainly not by mesozooplankton. However, in some cases, protistan herbivory that includes grazing of photosynthetic bacteria (Synechococcus and Prochlorococcus) may result in a greater carbon and nutrient flows through protist herbivory than through protist bacterivory (Sakka et al. (2000).

Low m/u may also be a consequence of mismatch of phytoplankton growth to protist grazing rates. As explained in Sherr et al. (2009), when conditions of nutrients and light are favorable for growth, phytoplankton blooms can quickly develop, while protist grazers lag the growth. However, when nutrient are used up and the bloom has peaked, protist grazing rate can exceed phytoplankton growth rate.

Ducklow HW (1983) Production and the fate of bacteria in the oceans. Bioscience 33:

494–501.

Sakka A, Legendre, Gosselin M & Delesalle B (2000) Structure of the oligotrophic planktonic food web under low grazing of heterotrophic bacteria: Takapoto Atoll, French Polynesia. Mar. Ecol. Prog. Ser. 197: 1–17.

Sherr, E.B., Sherr, B.F., 2009. Capacity of herbivorous protists to control initiation and development of mass phytoplankton blooms. Aquat. Microb. Ecol. 57, 253-262

Response:

We agree that the bulk of remineralization of organic matter in the sea is ultimately done by bacteria and bacterivores. However, as microzooplankton (< 200 µm) consumes most of the daily primary production in the sea, we would like to say a great carbon and nutrient flows go through and are mediated by microzooplankton, mainly consisting of protist. As the proportion of daily primary production consumed by microzooplankton become low (as indicated by low m/µ), we think that it is reasonable to say that the remineralization of organic matter mediated by microzooplankton will also be low.

When the proportion of daily primary production consumed by microzooplankton was low, i.e. the mismatch between microzooplankton grazing and phytoplankton growth became significant, high proportion of the daily primary production would involve in the formation and gravitational settling of large aggregates by phytoplankton (including picophytoplankton), and/or the consumption of those aggregates by mesozooplankton (Lomas et al, 2011; Richardson and Jackson, 2007), although it was not very likely that mesozooplankton directly consumed the picophytoplankton (Table 2).

We revised the sentence as “The low m/µ (< 50% on average) indicates low remineralization of organic matter mediated by microzooplankton and mismatch between the µ and m”

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.

2) p. 6301 lines 8-9: 'The high μ/μ_n (approximate to or higher than one) indicated that phytoplankton growth was less even not nutrient-limited during the summer cruise (Table 1).' And

p. 6301 Lines 17-18: 'Prochlorococcus has been found seldom occurred in less saline seawaters (Partensky et al., 1999).'

The English is still rough in places, as in these two sentences, and should be corrected throughout the text by a native English speaker.

Response:

We rewrote the sentence in p. 6301 lines 8-9 as 'The high μ/μ_n (approximate to or higher than one) indicated that phytoplankton growth was only slightly or even not nutrient-limited during the summer cruise (Table 1).'

The sentence in p. 6301 Lines 17-18 was deleted.

3) p 6301 lines 3-5: '. . .negative correlation of SSS with silicate and phosphate in the present study may also suggest alternative explanation. Salinity has been found the major environmental determinant of microbial community. . .'

and

p. 6301 lines 6-19: 'Salinity has been found the major environmental determinant of microbial community (including the cyanobacteria) composition in the global level (Lozupone and Knight, 2007). Fu and Bell (2003) demonstrated that low salinity was harmful to the growth, Chl a content, nitrogen fixation and alkaline phosphatase activity of the cyanobacteria Trichodesmium. We speculate that low salinity may also go against the growth of other cyanobacteria such as Prochlorococcus and Synechococcus in the oligotrophic seawater in the SSCS, although there is little (if any) data examining the salinity impact on pico-phytoplankton growth, and thus lead to the lower u of pico-phytoplankton during the winter.'

This speculation about role of salinity in the distribution of coccoid cyanobacteria and Prochlorococcus is an issue I had with the initial version of this paper. I still think this is not a valid conclusion and should be deleted from the paper. It is much more likely that seasonal differences in nutrient availability and light levels had the primary effects on phytoplankton community composition. I don't think the study of Fu and Bell on growth response to salinity for Trichodesmium isolated from a Great Barrier Reef lagoon is relevant to this study. Fu and Bell used salinities as low as 22 psu, and found decreased growth at 29 psu, which are lower salinities than reported here. Also, Trichodesmium is a large filamentous cyanobacterium and is not a good model for pico-cyanobacteria.

First, the data presented in Table 1 shows that the May-June SSS ranged from 32.4 to 30.05 psu; and the data in Table 2 shows that the November SSS ranged from 31.5 to 32.35 psu. These salinities are not very different in the two seasons.

Second, as I explained in my first review, there is really no data suggesting that salinity is an important factor in distribution of picophytoplankton in general, or of Prochlorococcus specifically. The distribution of Prochlorococcus seems to coincide with temperate to tropical low nutrient ocean waters, which are also typically more saline than coastal waters. I could not find any study of relation of Prochlorococcus growth to salinity. The seasonal differences in SSS, from 32-33 ppt in May-June to 28-29 ppt in November, are not very great. Santic et al. (2011, not cited in the paper) found Prochlorococcus in the Adriatic Sea over a similar range of salinities as that reported in this study. Synechoccus is typically more abundant in coastal zones than in the higher salinity open ocean (Zubkov et al. 2000 cited, Sherr et al 2005 not cited) and can also be abundant in lower salinity habitats such as the Chesapeake Bay (Wang et al 2011 not cited).

Santic et al. 2011. Distribution of Synechococcus and Prochlorococcus in the central Adriatic Sea. ACTA ADRIAT. 52(1): 101 - 114 (available on-line)

Sherr, E.B., Sherr, B.F., Wheeler, P.A. 2005. Distribution of coccoid cyanobacteria and small eukaryotic phytoplankton in the upwelling ecosystem off the Oregon coast during 2001 and 2002. Deep-Sea Research II 52:317-330.

Wang et al. 2011. Abundance and Distribution of Synechococcus spp. And Cyanophages in the Chesapeake Bay. Appl. Environ. Microbiol. 77 no. 21 7459-7468

Response:

Following the comments, the speculation about the roles of salinity has been deleted (the last paragraph in Page 6301, and lines 1-14 in Page 6302).

4) p. 6301 lines 15-22: ’The freshwater cap could also impact the microzooplankton grazing indirectly. First,the formation of freshwater cap may inhibit the migration of mesozooplankton (e.g.copepods) into the water with lower salinity (Grindley, 1964) and change the mesozooplankton composition in the water column (Zhou et al., 2015b), which can release the mesozooplankton grazing pressure on ciliates, then through trophic cascades increase the ciliate grazing on nanoflagellates (HNF) (Chen et al., 2012), reducing the abundance of HNF the main grazer on pico-phytoplankton (Safi and Hall, 1999), and releasing the grazing pressure on pico-phytoplankton (Klauschies et al., 2012). Second, as discussed above, the impeding of freshwater cap on phytoplankton accesses to nutrients could lead to poor food quality of phytoplankton as prey, and thus reduce the grazing activity of microzooplankton. Both the arguments suggest that the SSS

decrease could result in low microzooplankton grazing rate on pico-phytoplankton such as that observed in the winter cruise.' I am also not convinced there was much if any freshwater cap. Were depth profiles of salinity and water density done? In Tables 1 and 2 data on salinity is only reported for 0 and 25 m water depths. In November there was at most a little over 1 psu difference between the two depths - hardly a freshwater cap. The effects of such a minor difference would not be likely to affect any biological processes or activity. I would delete this speculation from the paper.

Response:

We would like to retain the speculation. Firstly, the SSS between the two seasons were indeed significantly different statistically ($p < 0.01$, see in Table 5). Secondly, the salinity discrepancy between surface water and that at 25 m in depth was several times higher during the winter cruise (0.99) than the summer cruise (0.17) (Table 1 and Table 2). Thirdly, the low salinity cap may be stronger during or just after the large rainfall when bad sea conditions usually occur and limit work at sea. When the sea become calm suitable for work at sea, the low salinity surface seawater could become more saline, and the absolute salinity discrepancy between surface water and that at deeper layer could be smaller during the winter cruise.

Therefore, we would like to use the "freshwater cap" to describe the contrasted SSS conditions between the two seasons, especially the low SSS during the winter cruise in the southern South China Sea.

5) p. 6304 lines 22-25: 'Secondly, large rainfall and the resulted SSS decrease may decouple the phytoplankton (especially the pico-phytoplankton) growth and microzooplankton grazing through directly or indirectly influencing the phytoplankton growth and microzooplankton grazing as discussed in Sect. 4.3.'

This is also unfounded speculation as explained above, and should be deleted.

Response:

We would like to rewrite the sentence as "Secondly, large rainfall and the resulted SSS decrease may decouple the phytoplankton (especially the pico-phytoplankton) growth and microzooplankton grazing through indirectly influencing the phytoplankton growth and microzooplankton grazing as discussed in Sect. 4.3" .

6) p. 6305 lines 7-10: ' The low m/µ, i.e. the high growth differential over grazing indicates low remineralization of organic matter mediated by microzooplankton and the increased importance of the phytoplankton- mesozooplankton grazing pathway (Landry et al., 1998).'

I am still uncomfortable with this statement for two reasons: 1) the reader might think that overall protist nutrient remineralization rates are lower, when as explained in the first comment, most remineralization is due to bacteria and bacterivorous protists; and 2) there is no data on strength of mesoplankton grazing in this region. To infer that lower protist herbivory implies higher mesoplankton grazing is a stretch, unless there is some data, e.g. on mesozooplankton stocks, in this region. The authors could speculate that mesozooplankton grazing may be enhanced due to differences in phytoplankton community composition, perhaps, and call for further work. But it is not correct to state this as fact.

Response:

We rewrote the sentence as "The low m/µ, i.e. the high growth differential over grazing indicates low remineralization of organic matter mediated by microzooplankton and mismatch between the primary production and microzooplankton grazing".

Correction List

Line 8, P. 6286: replace “but” by “rather than”.

Lines 20-21, P. 6286: replace “and the increased importance of the phytoplankton-mesozooplankton grazing pathway” by “mismatch between the μ and m”.

Lines 2-3, P. 6287: change “Phytoplankton growth provides alomst. . . ” to “Phytoplankton provide almost . . .”.

Line 15, P. 6287: change “indicates” to “induces”.

Line 19, P. 6287: change “ Landry et al., 1998, 2011” to “Landry et al., 1995, 1998, 2011”

Line 1, P. 6288: delete “phytoplankton” and change to “pico-sized prey”.

Line 14, P. 6294: replace “exclude” by “excluding”.

Line 16, P. 6294: replace “Taken” by “Taking”.

Line 15, P. 6297: insert “Landry et al. (1995) reported an average μ of 0.83 ± 0.42 d^{-1} and m of 0.72 ± 0.56 d^{-1} in Feburary–March, and an average μ of 0.98 ± 0.31 d^{-1} and m of 0.57 ± 0.17 d^{-1} in August–September in the central equatorial Pacific Ocean. These results are similar to ours observed during the summer cruise.” before “Landry et al. (2011) . . . ”

Lines 26-27, P. 6298: change “ Landry et al., 2011” to “ Landry et al., 1995, 2011”.

Lines 1-3, P. 6300: change the sentence to “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.”.

Line 8, P. 6301: replace “less” by “only slightly or”.

Lines 14-29, P. 6301 and Line 1-14, P. 6302: delete the last paragraph in P. 6301, and lines 1-14 in P. 6302.

Line 24, P. 6304: delete “directly or”.

Lines 9-10, P. 6305: replace “the increased importance of the phytoplankton-mesozooplankton grazing pathway (Landry et al., 1998)” by “mismatch between the primary production and microzooplankton grazing”.

Lines 25-26, P. 6306: replace “the increased importance of the phytoplankton-mesozooplankton grazing pathway” by “mismatch between the μ and m”.

Line 11, P. 6311: insert a reference “Landry, M. R., Constantinou, J., and Kirshtein, J.: Microzooplankton grazing in the central equatorial Pacific during February and August, 1992, Deep-Sea Res. II, 42, 657-671, 1995.”.

A marked-up manuscript version

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

Linbin Zhou[1], Yehui Tan[1*], Liangmin Huang[1], Zifeng Hu[1,2], Zhixin Ke[1]

[1] CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

*Correspondence to: Yehui Tan (tanyh@scsio.ac.cn)

Abstract

To examine seasonal and size-dependent variations in the phytoplankton growth and microzooplankton grazing in oligotrophic tropical waters under the influence of seasonal reversing monsoon, dilution experiments were conducted during the summer 2009 (21 May to 9 June) and winter 2010 (9 to 18 November) in the southern South China Sea (SSCS). The results showed that environmental variables, phytoplankton biomass, phytoplankton growth rate (μ), microzooplankton grazing rate (m), and correlationship (coupling) between the μ and m, ~~but~~rather than the microzooplankton grazing impact on phytoplankton (m/μ) significantly varied between the two seasons. Higher relative preference index (RPI) for and m on the larger-sized (> 3 μm) phytoplankton than pico-phytoplankton (< 3 μm), indicating significant size-selective grazing by microzooplankton on the larger-sized phytoplankton, were also observed. The μ and m were significantly correlated with salinity and dissolved inorganic nutrients, which indicated that salient seasonal variations in the phytoplankton growth and microzooplankton grazing in the SSCS were closely related to the environmental variables under the influence of the East Asian monsoon. We propose that intermittent arrivals of the northeast winter monsoon could lead to the low μ and m, and the decoupling between the μ and m in the SSCS, through influencing nutrient supply to the surface water, and inducing surface seawater salinity decrease. The low m/μ (< 50% on average) indicates low remineralization of organic matter mediated by microzooplankton and ~~the increased importance of the phytoplankton-mesozooplankton grazing pathway~~ mismatch between the μ and m, and thus probably accounts for part of the high vertical biogenic particle fluxes in the prevailing periods of the monsoons in the SSCS. The size-selective grazing suggests that microzooplankton grazing contributes to the pico-phytoplankton dominance in the oligotrophic tropical waters such as that of the SSCS.

Key words: Phytoplankton growth; Microzooplankton grazing; Seasonal variation; Size-selective; Southern South China Sea; East Asian monsoon

1 Introduction

Phytoplankton growth and microzooplankton grazing are crucial processes relating to ocean biogeochemistry and energy flow in the pelagic food web. Phytoplankton ~~growth~~ provide~~s~~ almost all the primary production in the upper ocean. Microzooplankton (< 200 µm) consumes most of the daily primary production in the sea, and regulates phytoplankton community composition, and affects the ultimate fate of the phytoplankton-derived primary production (Banse, 2007, 2013; Landry and Calbet, 2004; Schmoker et al., 2013).

The dilution technique introduced by Landry and Hassett (1982) has extensively been used to estimate phytoplankton growth and synchronous microzooplankton grazing rates in varied marine ecosystems, although later modification and increasing use has also brought scrutiny and critiques (Dolan and Mckeon, 2004; Gallegos, 1989; Gutiérrez-Rodríguez et al., 2009; Teixeira and Figueiras, 2009). Based on global data collection, Schomoker et al. (2013) pointed out that seasonality of environmental variables and phytoplankton community ~~indicates~~induces seasonal variations in the phytoplankton growth and microzooplankton grazing in polar and sub-polar, temperate regions, and tropical waters with salient seasonal reversing monsoon. However, there are few studies investigating both the phytoplankton growth and microzooplankton grazing in tropical oceans (Caron and Dennett, 1999; Chen et al., 2013; Landry et al., 1995, 1998, 2011), and the seasonality of phytoplankton growth and microzooplankton grazing in tropical waters influenced by salient seasonal reversing monsoon is still less known.

Microzooplankton may modify their food preference depending on the morphology, abundance and quality of prey, which would cause phytoplankton community shift in species and/or size composition (Teixeira and Figueiras, 2009). Phytoplankton community in oligotrophic subtropical and tropical waters is usually dominated by small-sized pico-cells (< 3 µm); the pico-phytoplankton consists of three major groups including *Prochlorococcus*, *Synechoccus* and diverse types of pico-eukaryotic phytoplankton (Zubkov et al., 2000). Microzooplankton including both autotrophic and heterotrophic nanoflagellates can actively consume pico~~-~~ ~~phytoplankton~~-sized prey (An-Yi et al., 2009; Christaki et al., 2005;

Frias-Lopez et al., 2009). Landry et al. (1998) proposed that the balance between microzooplankton grazing and phytoplankton growth was consistent with the dominance of pico-plankton in oligotrophic offshore regions in the Arabian Sea. Significantly higher microzooplankton grazing rates on the large-sized phytoplankton (> 5 μm) than the smaller one (< 5 μm) have been recently reported in the oligotrophic subtropical Northeast Atlantic (Cáceres et al., 2013). Our recent results suggest that size-selective grazing by microzooplankton on nano-sized (3–20 μm) phytoplankton contributes to the pico-phytoplankton dominance in the oligotrophic tropical waters of the South China Sea (SCS) in summer (Zhou et al., 2015a). However, until now, the role played by microzooplankton in the pico-phytoplankton dominance in oligotrophic tropical waters is still less examined.

The southern SCS (SSCS) is characterized with permanent water stratification and oligotrophic conditions in the upper layer, and is affected by seasonal reversing monsoon. During the middle of May to September, the SSCS is under the influence of the southwest summer monsoon, while this area is influenced by the stronger northeast winter monsoon during November to the next April (Su, 2004). Mesoscale eddies with obvious seasonal variation (Fang et al., 2002; Fang et al., 1998; Zhang et al., 2014), and seasonal pattern of higher phytoplankton biomass, primary production and vertical biogenic particle fluxes occur during the prevailing periods of the monsoons and wintertime in the SSCS (Liu et al., 2002; Ning et al., 2004; Wan et al., 2010). There are hundreds of coral shoals, reefs and islands located in the SSCS, called the Nansha Islands. Many researchers including Qingchao Chen, Liangmin Huang and their co-workers, from the South China Sea Institute of Oceanology, Chinese Academy of Sciences, have previously investigated the environment variables, marine species diversity and ecological processes in the coral reef lagoons of the Nansha Islands and its adjacent waters during the 1980s to 1990s (e.g. Chen and Mutidisciplinary Expedition to Nansha Islands, 1991, 1989, 1994, 1996, 1998; Huang and Multidisciplinary Expedition to Nansha Islands, 1997). Their works provide valuable contributions to the understanding of the taxonomic composition and distribution of phytoplankton, bacteria, zooplankton and fish, and ecological processes such as primary production in the SSCS.

However, these results are seldom published in international media even scientific journals in Chinese, and thus less known to the scientific community. So far, there is no data reported on the microzooplankton grazing in this region. We hypothesize that seasonal changes in both the phytoplankton growth and microzooplankton grazing is expected in the tropical waters influenced by salient seasonal reversing monsoon, and microzooplankton contributes to the phytoplankton size composition through size-selective grazing in the SSCS.

To test the hypotheses, we conducted a series of dilution experiments in adjacent waters of the Nansha Islands in the SSCS during two cruises in May to June 2009 and November 2010. Significant seasonal variations in the phytoplankton growth and microzooplankton grazing and apparently size-selective grazing were observed.

2 Material and methods

Dilution experiments (Landry and Hassett, 1982) were conducted at 14 stations during 21 May to 9 June 2009 (summer), and at 10 stations during 9 November to 18 November 2010 (winter). Stations KJ28 and KJ32 were located at the basin waters northwest to the Nansha Islands; station YS was located at the lagoon of the Yongshu Reef, the other stations were distributed in waters around the Nansha Islands (Fig. 1).

Surface seawater was collected, and pre-screened with a 200-μm nylon netting for dilution experiments at each station. Particle-free seawater was obtained by filtering the seawater through a filter with a pore size of 0.22 μm. 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. Measured amount of particle-free seawater was firstly added to the 2.4-L polycarbonate bottles, and unfiltered seawater was added and filled the bottles. Four dilution treatments of 25, 50, 75 and 100% unfiltered seawater were prepared for the summer experiments, and another four dilution treatments of 37.5, 58.3, 79.2 and 100% unfiltered seawater were prepared for the winter experiments. All the bottles were enriched with additional nutrients (final concentrations of

0.5 μM NH_4Cl, 0.03 μM KH_2PO_4, 1.0 nM $FeSO_4$ and 0.1 nM $MnCl_2$) to promote constant phytoplankton growth. Two bottles were filled with unfiltered seawater without nutrient enrichment served as no nutrient controls. Another two bottles filled with unfiltered seawater were sacrificed for initial samples of chlorophyll *a* (Chla). 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)

Seawater was filtered through a sequence of 3-μm pore size polycarbonate filter and GF/F filter for size-fractionated Chla of the larger-sized (> 3 μm) and pico- (< 3 μm) phytoplankton. Total Chla was calculated as the sum of the two size fractions or directly sampled by filtering 0.5 to 1 L seawater on the GF/F filter. The filters were extracted in 90% acetone at -20°C for 24 h. The Chla concentration was measured by fluorometry using a Turner Designs Model 10 Fluorometer (Parsons et al., 1984).

Seawater temperature, salinity and dissolved inorganic nutrients were also measured. Temperature and salinity were determined by Conductivity-Temperature-Depth probes. Dissolved inorganic nutrients such as silicate, phosphate and nitrate plus nitrite were analyzed with a flow-injection autoanalyzer (Quickchem 8500, Lachat Instruments) following the standard manuals.

Assuming an exponential growth model, the net growth rate (k_d) of phytoplankton in a dilution treatment was calculated according to the formula $k_d = \ln (P_t/dP_0)$, where d is the dilution factor (the proportion of unfiltered seawater), P_t is the Chla concentration after incubation, P_0 is the initial Chla concentration. Phytoplankton growth rates with nutrient amendment (μ_n) and microzooplankton grazing rates (m) were estimated from Model I linear regressions of net growth rate (k) vs dilution factor (d). In situ phytoplankton instantaneous growth rates (μ) were calculated as the sum of m and net growth rate in control bottles without added nutrients.

When saturated or saturated-increasing grazing was observed as a departure from the assumed linear model (Gallegos, 1989; Teixeira and Figueiras, 2009), m and μ were calculated on the basis of the method of Chen et al.(2009a).

Size-fractionated (> 3 μm and < 3 μm) phytoplankton growth and mortality rates were estimated for experiments at YS and KJ35 during the summer cruise, and all the experiments during the winter cruise.

Nutrient limitation index indicating nutrient sufficiency for phytoplankton growth was assessed by the ratio μ/μ_n, which is expected to vary with system trophic state (Landry et al., 1998). Net growth rate was also used as a proxy for the actual trophic state of the system being investigated (Calbet et al., 2011). The microzooplankton grazing impact on phytoplankton was assessed by the ratio of microzooplankton grazing to phytoplankton growth rates (m/μ), and measures the extent to which the daily phytoplankton production is consumed and balanced by microzooplankton grazing (Landry et al., 1998).

Grazing selectivity for size-fractionated phytoplankton was analyzed following the relative preference index (RPI) provided by Obayashi and Tanoue (2002) as $\mathrm{RPI} = \frac{[mchla_i]/\sum[mchla_i]}{[chla_i]/\sum[chla_i]}$, where $[mchla]$ is the amount of daily grazed Chla (μg $L^{-1}d^{-1}$), subscript i refers to each size fraction analyzed and $[chla]$ is the concentration of Chla (μg L^{-1}). RPI > 1 indicates positive selection and vice versa.

All the statistical analyses were conducted by using the SPSS 17.0. The average value of the ratio variables such as m/μ was presented as the geometric mean, while other average values were presented as the arithmetic mean.

3 Results

3.1 Environmental variables and Chla

Environmental variables and Chla in surface seawater were different between the two cruises (Table 1 and Table 2). Surface seawater temperature (SST) and salinity (SSS), and the concentration of nitrate plus nitrite were significantly lower (independent t-test, $p < 0.05$ or

0.01), while the concentrations of silicate and phosphate were significantly higher in the winter cruise (independent t-test, $p < 0.05$ or 0.01) than those in the summer cruise. The concentration of phosphate (mean ± sd: 0.04 ± 0.03 μmol L^{-1}) during the summer cruise and that of nitrate plus nitrite (mean ± sd: 0.04 ± 0.02 μmol L^{-1}) during the winter cruise were almost undetectable. The concentration of Chla in the winter cruise (mean ± sd: 0.104 ± 0.024 μg L^{-1}) was about two times that in the summer cruise (mean ± sd: 0.066 ± 0.022 μg L^{-1}) (independent t-test, $p < 0.01$). Pico-phytoplankton (< 3 μm) accounted for most (> 80%) of the total Chla during both the cruises. The proportion of pico-phytoplankton in the winter cruise (mean ± sd: 81.9% ± 5.0 %) was similar to that in the summer cruise (mean ± sd: 83.0% ± 1.8%) (independent t-test, $p > 0.1$).

3.2 Feeding responses in dilution experiments

The detailed results of each dilution experiment were listed in Table 1 and Table 2. Except for linear feeding responses, both the saturated and saturated-increased feeding responses described by Teixeira and Figueiras (2009) occurred in the dilution experiments during both the cruises (Fig. 2a–c). Non-linear feeding responses occurred at eight of the 14 experiments during the summer cruise, while those occurred at four of the 10 experiments during the winter cruise (Table 1 and Table 2). During the winter cruise, substantially negative phytoplankton growth rates (μ) and high microzooplankton grazing rates (m) were observed at KJ53 (Fig. 2d–f). The negative μ obtained at KJ53 and the derivative parameters (e.g. m/μ) were not included for the comparison between seasons and size fractions.

3.3 Comparison of the phytoplankton growth and microzooplankton grazing between the two seasons

The phytoplankton growth and microzooplankton grazing were significantly different between the two cruises. The μ in the winter cruise (mean ± sd: 0.54 ± 0.22 d^{-1}) were significantly lower than those in the summer cruise (mean ± sd: 0.92 ± 0.32 d^{-1}) (independent t-test, $p < 0.01$). The m in the winter cruise (mean ± sd: 0.27 ± 0.13 d^{-1}) were also significantly lower than those in the summer cruise (mean ± sd: 0.46 ± 0.20 d^{-1}) (independent t-test, $p < 0.01$). However, the m/μ ratio was almost equally low both in the summer (geometric mean ± sd: 49% ± 23%) and winter (geometric mean ± sd: 48% ± 33%) cruises.

3.4 Size-dependent phytoplankton growth and microzooplankton grazing

The μ and m varied between size fractions. Higher m on the larger size fraction were observed at YS (0.65 d^{-1} for the size fraction > 3 μm vs 0.33 d^{-1} for the size fraction < 3 μm) and KJ35 (0.39 d^{-1} for the size fraction > 3 μm vs 0.30 d^{-1} for the size fraction < 3 μm) during the summer cruise. The μ of the larger-sized (> 3 μm) and pico- (< 3 μm) phytoplankton were 1.07 d^{-1} and 0.75 d^{-1} at YS, and 0.69 d^{-1} and 0.90 d^{-1} at KJ35, respectively. During the winter cruise, the m on the larger size fraction (mean ± sd: 0.50 ± 0.41 d^{-1}) were higher than those on pico-phytoplankton (mean ± sd: 0.27 ± 0.27 d^{-1}) at seven of the 10 experiments, and the μ of the larger size fraction (mean ± sd: 0.88 ± 0.38 d^{-1}) were also higher than those of pico-phytoplankton (mean ± sd: 0.42 ± 0.31 d^{-1}) at seven of the nine experiments (exclude data at KJ53) (Table 3). The m/μ was higher for the larger size fraction (57.3% at KJ35 and 61.2% at YS) than pico-phytoplankton (33.3% at KJ35 and 44.0% at YS) during the summer cruise, while the m/μ was not significantly different between the two size fractions (41.1% ± 82.8% for the size fraction > 3 μm, and 39.4% ± 21.1% for the size fraction < 3 μm) during the winter cruise (Table 3).

Higher RPI for the larger-sized phytoplankton than pico-phytoplankton was observed during both the cruises (Fig. 3). The RPI for the larger-sized phytoplankton was higher than one (geomean ± sd: 1.44 ± 0.31), while the RPI for pico-phytoplankton was lower than one (geomean ± sd: 0.90 ± 0.07) during the summer cruise. The RPI was significantly (Mann-Whitney test, $p < 0.05$) higher for the larger-sized phytoplankton (geomean ± sd: 1.44 ± 1.57) than pico-phytoplankton (geomean ± sd: 0.61 ± 0.35) during the winter cruise.

3.5 Nutrient limitation to the phytoplankton growth

Nutrient limitation index (μ/μ_n), as well as the net growth rate (μ-m, data not shown), was significantly higher (Mann-Whitney test, $p < 0.05$) during the summer cruise (geometric mean ± sd: 0.80 ± 0.25) than the winter cruise (geometric mean ± sd: 0.54 ± 0.30). The μ/μ_n was approximate to or larger than one at five of the 14 experiments in the summer. In contrast, the

μ/μ_n was apparently lower than one at eight of the nine experiments (excluding~~e~~ data at KJ53) in the winter (Table 1 and Table 2).

3.6 Correlations between the growth and grazing rates with environmental variables

Taking~~en~~ all the data from the two cruises together, the average μ and m were 0.77 ± 0.34 d^{-1} and 0.39 ± 0.20 d^{-1}. The μ were positively correlated with SST (r = 0.43, $p < 0.05$), SSS (r = 0.55, $p < 0.01$), μ/μ_n (r = 0.50, $p < 0.05$), net phytoplankton growth rate (r = 0.83, $p < 0.01$) and m (r = 0.76, $p < 0.01$), but were negatively correlated with phosphate (r = -0.57, $p < 0.01$) and Chla (r = -0.65, $p < 0.01$) (Table 4). The m were also positively correlated with SST (r = 0.62, $p < 0.01$), SSS (r = 0.48, $p < 0.05$) and nitrate plus nitrite (r = 0.54, $p < 0.01$), and was negatively correlated with Chla (r = -0.66, $p < 0.01$) and phosphate (r = -0.54, $p < 0.01$) (Table 4).

3.7 Correlations between the phytoplankton growth and microzooplankton grazing

The positive correlation ($r^2 = 0.57$, $p < 0.01$) between μ and m was observed during the summer cruise (Fig. 4a). However, there was no significant correlation between the total μ and m during the winter cruise. By grouping μ and m separately for each size fraction, it is found that the correlation was significant for the larger size fraction ($r^2 = 0.84$, $p < 0.05$) but not for the pico-phytoplankton ($r^2 = 0.41$, $p = 0.07$) (Fig. 4b).

4 Discussion

4.1 Comparisons with other studies

All the μ (except those obtained at KJ53 during the winter cruise) and m were within the reviewed ranges based on global data collection (Calbet and Landry, 2004; Schmoker et al., 2013). Substantive m but negative μ were observed at KJ53 (Fig. 2d–f), the only station located on the continental shelf with a bottom depth less than 200 m (Fig. 1).

4.1.1 Negative phytoplankton growth rates

Negative μ have been extensively reported in previous dilution experiments (e.g. Burkill et al., 1987; Loebl and Beusekon, 2008; Suzuki et al., 2002; Zhou et al., 2011). Zhou et al. (2013) reviewed that temperature and light regime during incubation, insufficient sampling, contamination of particle-free water and the added nutrients, and decay of phytoplankton blooms could be the possible reasons for the negative μ in dilution experiments. Our measures to mimic the in situ light and temperature during incubation exclude light and temperature from the factors for the substantially negative μ. Insufficient sampling also unlikely contributed to the negative μ since sufficient mixing was conducted before Chla sampling. The substantive m could exclude contamination as the reason for the negative rates, because contamination could not only cause phytoplankton death, but also less microzooplankton grazing. We conjecture that phytoplankton community decay may occur and lead to the negative μ at KJ53. The lowest silicate concentration at KJ53 may be related to the negative μ. Why the lowest silicate concentration occurred and to what extent the low nutrient condition was related to the negative phytoplankton growth is a topic that needs further assessment.

4.1.2 Non-linear feeding responses

Non-linear feeding responses including both the saturated and saturated-increased types occurred in our dilution experiments. Non-linear responses were usually observed in eutrophic waters with high prey abundance (Elser and Frees, 1995; Gallegos, 1989; Teixeira and Figueiras, 2009). Non-linear feeding responses in dilution experiments conducted in the oligotrophic subtropical Northeast Atlantic in summer have also been reported by Quevedo and Anadón (2001) and Cáceres et al. (2013). However, the authors did not explain the underlying reasons for these phenomena. The oligotrophic conditions and low phytoplankton biomass in their study area were similar to those in the oligotrophic tropical waters of the SSCS. Teixeira and Figueiras (2009) proposed that changes in the specific phytoplankton growth rate due to varied nutrient limitation in experimental bottles, in the morality rate related to microzooplankton abundance and feeding behavior and even virus infection could be related to the non-linear responses. As additional nutrients were added in the experiments bottles, we do not think that nutrient limitation was the factor causing the non-linear responses in our experiments. Rather we think that the sufficient nutrients added in the experimental bottles led to high phytoplankton abundance in the less diluted bottles. Microzooplankton may reach a maximum ingestion rate at high food concentration, and the maximum ingestion rate may remain constant despite further increase in prey abundance, which is often used to explain the occurrence of saturated feeding responses in dilution experiments (Gallegos, 1989; Moigis, 2006; Teixeira and Figueiras, 2009), and could explain those in our experiments. While the occurrence of saturated-increased responses implies that decrease in the ingestion rate should be occurred to the further increase in food availability. There is no concluded explanation for the decrease in the ingestion rate. Teixeira and Figueira (2009) proposed that prey selection by microzooplankton in waters with high and diverse food abundance may account for the decrease. We consider that nutrient amendment in the experimental bottles may give rise to relatively higher phytoplankton (food) abundance, leading to decrease in the ingestion rate and accounting for the occurrence of saturated-increased responses in our experiments.

4.1.3 Phytoplankton growth and microzooplankton grazing rates in low latitude tropical waters

There is no study on microzooplankton grazing in the SSCS, especially in waters around the Nansha Islands. If any, Chen et al. (2009b) reported an average μ of 0.75 ± 0.62 d^{-1} and an average m of 0.65 ± 0.51 d^{-1} in the western SCS northwest to the present study waters in summer. These rates were similar to our results observed during the summer cruise (Table 5).

There are few studies on microzooplankton grazing in low latitude tropical waters such as the SSCS. Landry et al. (1995) reported an average μ of 0.83 ± 0.42 d^{-1} and m of 0.72 ± 0.56 d^{-1} in Feburary–March, and an average μ of 0.98 ± 0.31 d^{-1} and m of 0.57 ± 0.17 d^{-1} in August–September in the central equatorial Pacific Ocean. These results are similar to ours observed during the summer cruise. Landry et al. (2011) reported a low average μ of 0.43 ± 0.14 d^{-1} and m of 0.31 ± 0.11 d^{-1} in the high-nutrient-low-chlorophyll (HNLC) equatorial Pacific Ocean. These rates are similar to the rates observed during the winter cruise in the present study. Yang et al. (2004) investigated the phytoplankton growth and microzooplankton grazing in the western Pacific waters with latitudes similar to those of the SSCS. Their reported μ and m

in summer were 0.35 to 0.75 d^{-1}, and 0.51 to 0.67 d^{-1}, which are similar to our results observed during the summer cruise. Landry et al. (1998) showed an average μ of 0.5 d^{-1} and an average m of 0.6 d^{-1} at the oligotrophic stations in the subtropical and tropical Arabian Sea. Caron and Denett (1999) reported the m of 0.35 ± 0.18 d^{-1} and 0.30 ± 0.17 d^{-1}during the northeast monsoon season and spring intermonsoon season in the Arabian Sea, respectively. The m were similar to the low m (0.27 ± 0.13 d^{-1}) observed during the northeast monsoon season in the present study.

The growth and grazing rates of pico-phytoplankton estimated in the present study fall into the middle range of those rates of the main pico-phytoplankton groups (*Prochlorococcus*, *Synechococcus* and eukaryotic pico-phytoplankton) reported in previous studies (reviewed data in Table II in Hirose et al., 2008). Based on cell cycle analysis, Yang and Jiao (2002) reported the in situ *Prochlorococcus* growth rate of 0.54 d^{-1} at the chlorophyll maximum layer in the SSCS in May. This rate is lower than the pico-phytoplankton growth rate observed (at YS and KJ35) during the summer cruise, but slightly higher than that (0.42 d^{-1} on average) observed during the winter cruise. Lower average growth rate (0.15 d^{-1}) of *Prochlorococcus* in the subtropical and tropical Atlantic has been reported by Zubkov et al. (2000).

4.2 Seasonal variations in phytoplankton growth and microzooplankton grazing

Our results showed pronounced seasonal changes in the phytoplankton growth and microzooplankton grazing, but not the microzooplankton grazing impact on phytoplankton (m/μ), in the SSCS. Many studies have found pronounced seasonality in the phytoplankton growth and microzooplankton grazing in temperate and high latitude waters (e.g. Calbet et al., 2008; Kim et al., 2007; Lawrence and Menden-Deuer, 2012; Loebl and Beusekon, 2008). Nevertheless, there are few studies on the seasonality of the microzooplankton grazing in oligotrophic tropical waters. From the perspective of phytoplankton growth , Landry et al. (2011) reported that μ was slightly higher in December than September in the HNLC waters of the equatorial Pacific, but the rate was not significantly different between periods of the southwest monsoon and the early northeast monsoon in the Arabian Sea (Landry et al., 1998); while Caron and Denett (1999) demonstrated that μ was approximately twice as high during the northeast monsoon season than the spring intermonsoon period in the Arabian Sea. Chen et al. (2013) observed that μ in surface water of the northern SCS was significantly higher in the summer than winter. However, the previous studies showed no seasonal variation in microzooplankton grazing in the HNLC waters of the equatorial Pacific (Landry et al., 1995, 2011), the Arabian Sea (Caron and Dennett, 1999; Landry et al., 1998) and oligotrophic surface waters of the northern SCS (Chen et al., 2013). Significantly lower m in the summer than winter at the chlorophyll maximum layer in the northern SCS has been reported by Chen et al. (2013). From the data published by Quevedo and Anadón (2001), we calculated that the μ and m were higher in the summer (0.61 ± 0.43 d^{-1} and 0.49 ± 0.28 d^{-1}) than in the spring (0.33 ± 0.27 d^{-1} and 0.29 ± 0.18 d^{-1}) in the oligotrophic subtropical Northeast Atlantic. Lower average μ and m for pico-phytoplankton in the winter than summer have been recently reported in the subtropical East China Sea (Guo et al., 2014). These results are consistent with the significant seasonal variations in the phytoplankton growth and microzooplankton grazing in the SSCS in the present study.

4.3 East Asian monsoon influencing seasonal variations in phytoplankton growth and microzooplankton grazing by affecting nutrient supply and salinity decrease in surface water

Seasonal variations in environmental variables, related to the East Asian monsoon, may directly and /or indirectly influence phytoplankton growth and microzooplankton grazing. Significant seasonal changes in a host of environmental variables including salinity, dissolved inorganic nutrients, and phytoplankton biomass were indeed observed (Table 5). The correlationship between μ and m with the environmental variables indicate that seasonal variations in the phytoplankton growth and microzooplankton grazing were related to the contrasting environmental conditions under the influence of the East Asian monsoon (Table 4).

Many studies have showed increased m during the warmest seasons and reduced m and μ in winter months (e.g. Ara and Hiromi, 2009; Lawrence and Menden-Deuer, 2012). Both the μ and m were positively correlated with temperature in the present study. However, the mean seasonal temperature discrepancy was less than 0.4°C and not significant (independent t-test, p = 0.055) (Table 5).We do not think this slightly temperature variation alone could account for the substantial decrease in the μ and m observed in the northeast monsoon season. Substantive m at near freezing temperature in the Arctic Ocean have been reported (Franzè and Lavrentyev, 2014; Sherr et al., 2013; Sherr et al., 2009). This suggested that the physiological effect of temperature was ~~less~~ not important for the m variation in the Arctic Ocean, ~~rather than~~let alone in the tropical waters of the SSCS.

We think 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. The significantly higher concentration of dissolved inorganic nutrients such as silicate and phosphate could support the nearly twice as high Chla concentration during the northeast monsoon season than the summer. The higher concentrations of silicate, phosphate and Chla are consistent with previous results of relatively higher dissolved nutrients, phytoplankton biomass and primary production in surface waters of the SCS in winter (Liu et al., 2002; Ning et al., 2004). It is easy to understand that the stronger northeast monsoon could increase nutrient supply to the surface layer by enhancing vertical mixing and basin-scale uplift of nutricline depth (Liu et al., 2002). However, this kind of nutrient supply in the SSCS could be episodically influenced by intermittent arrivals of the strong northeast monsoon in the form of strong cold air. Therefore, we infer that nutrient supply to the surface water under the influence of the northeast monsoon may stimulate sporadic enhancement in phytoplankton production and biomass.

The arrival of strong northeast monsoon may bring not only nutrient supply to the surface water as a result of enhanced vertical mixing, but also SSS decrease due to heavy frontal rainfall when the cold air meet the warm and wet local air. A strong rainy season is usually developed from October to January in the SSCS (Wyrtki, 1961). The remote sensing data from the Tropical Rainfall Measuring Mission indeed showed that apparently larger rainfall occurred during the winter than summer (Fig. 5). The large rainfall could dilute and freshen the surface seawater (as those observed during the winter cruise). In addition, after the passage of the strong cold air, the winds over the SSCS are always weak. The low-wind condition could facilitate the less saline surface water overlying on the more saline deep water to form the "freshwater cap" (Zeng et al., 2009). The salinity discrepancy between surface water and that at 25 m in depth was several times higher during the winter cruise (0.99) than the summer cruise (0.17) (Table 1 and Table 2), indicating that a freshwater cap formed during the winter cruise in the SSCS.

The freshwater cap could enhance water stratification and block nutrient supply to the surface layer, thus nutrients such as nitrogen in surface water could be depleted by the phytoplankton community, and lead to nutrient limitation to the growth of the phytoplankton community with already increased biomass. The high μ/μ_n (approximate to or higher than one) indicated that phytoplankton growth was only slightly or ~~less~~ even not nutrient-limited during the summer cruise (Table 1). Similar results in the oligotrophic subtropical Northeast Atlantic have also been reported (Cáceres et al., 2013; Quevedo and Anadón, 2001). In contrast, extremely lower concentration of nitrate plus nitrite and severe nutrient limitation indicated by the lower μ/μ_n (0.54 ± 0.30) were observed during the winter, which may account for part of the low μ (Table 5).

~~Except for impeding phytoplankton accesses to nutrients, other factors associated with the SSS decrease may impact phytoplankton growth. Previous studies showed that salinity played an important role in the pico-phytoplankton distribution in estuaries (e.g. Mitbavkar et al., 2012). *Prochlorococcus* has been found seldom occurred in less saline seawaters (Partensky et al., 1999). Guo et al. (2014) reported that salinity was positively correlated with *Prochlorococcus* abundance in summer, and with abundances of all the main pico-phytoplankton groups (*Prochlorococcus*, *Synechococcus* and eukaryotic pico-phytoplankton) in winter, but was negatively correlated with the growth rates of *Synechococcus* and eukaryotic pico-phytoplankton, across the salinity gradient from the inshore to the offshore regions in the subtropical East China Sea. Dissolved nutrients co-varied with salinity were suggested as the reason for the correlation. This could be true in the reported areas with significant spatial salinity gradients caused by river plume, as low salinity means high nutrients supply from the river plume. The correlation between SSS and dissolved inorganic nutrients in the present study may to some degree support this argument~~

~~(Table 4). In addition, trace metals from wet atmospheric deposition (by rainfall) could be associated with the rainfall-induced SSS decrease, and toxic to the pico-phytoplankton growth (Paytan et al., 2009), and lead to the lower μ of pico-phytoplankton than that of the larger-sized phytoplankton during the winter.~~

~~However, the positive correlation between phytoplankton growth rates and SSS, but negative correlation of SSS with silicate and phosphate in the present study may also suggest alternative explanation. Salinity has been found the major environmental determinant of microbial community (including the cyanobacteria) composition in the global level (Lozupone and Knight, 2007). Fu and Bell (2003) demonstrated that low salinity was harmful to the growth, Chla content, nitrogen fixation and alkaline phosphatase activity of the cyanobacteria *Trichodesmium*. We speculate that low salinity may also go against the growth of other cyanobacteria such as *Prochlorococcus* and *Synechococcus* in the oligotrophic seawater in the SSCS, although there is little (if any) data examining the salinity impact on pico-phytoplankton growth, and thus lead to the lower μ of pico-phytoplankton during the winter.~~

The freshwater cap could also impact the microzooplankton grazing indirectly. First, the formation of freshwater cap may inhibit the migration of mesozooplankton (e.g. copepods) into the water with lower salinity (Grindley, 1964) and change the mesozooplankton composition in the water column (Zhou et al., 2015b), which can release the mesozooplankton grazing pressure on ciliates, then through trophic cascades increase the ciliate grazing on nanoflagellates (HNF) (Chen et al., 2012), reducing the abundance of HNF the main grazer on pico-phytoplankton (Safi and Hall, 1999), and releasing the grazing pressure on pico-phytoplankton (Klauschies et al., 2012). Second, as discussed above, the impeding effect of freshwater cap on phytoplankton accesses to nutrients could lead to poor food quality of phytoplankton as prey, and thus reduce the grazing activity of microzooplankton. Both the arguments suggest that the SSS decrease could result in low microzooplankton grazing rate on pico-phytoplankton such as that observed in the winter cruise.

4.4 Monsoon related vertical mixing and biological activity affecting the contrasting seasonal shifts of nutrients

Why the seasonal shift of nitrate plus nitrite was different from those of phosphate and silicate is open to discuss. The seasonal varied pattern and concentrations of phosphate and silicate, and the summer concentration of nitrate plus nitrite, in surface seawater of the SSCS, were consistent with the results reported by Ning et al. (2004). They explained that the low phosphate concentration in surface seawater probably resulted from phytoplankton consumption and less supplementation from deep water because of the strong thermocline in summer. Relatively high concentrations of phosphate and silicate (Table 2 and Table 5) could be supplied from deep water, as a result of vertical mixing caused by the strong northeast monsoon. However, the nitrate plus nitrite concentration in the present study was more than one order of magnitude lower in the winter than summer. The nearly undetectable nitrate plus nitrite concentration in the winter is 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, and proposed that nitrogen was the limiting factor for the primary production.

We propose that nutrients in surface waters were influenced by both vertical mixing and phytoplankton consumption and release. In the summer, the low even depleted concentrations of phosphate and silicate could be resulted from phytoplankton consumption and less supplementation from deep water because of the strong thermocline formed in summer. In addition, the probably high nitrogen fixation in the SCS (Wong et al., 2002) may not only provide a large input of nitrogen to the surface water, supporting the growth of nitrogen fixers and other phytoplankton (Foster et al., 2011; Groszkopf et al., 2012; Mulholland and Bronk, 2004) and preventing a depletion of the nitrate plus nitrite in the surface water, but may also lead to a more severe phosphorus-depleted condition, as nitrogen fixation consumes a large amount of phosphorus (Dyhrman et al., 2006; Zehr and Paerl, 2008). In the winter, the strong northeast monsoon could induce vertical mixing and large rainfall, both of them may cause community decay of nitrogen fixers. The harmful effects of low salinity on *Trichodesmium* growth and nitrogen fixation, and the influence of rainfall on the occurrence of *Trichodesmium* blooms have been reported (Fu and Bell, 2003; Lugomela et al., 2002). As discussed in Section 4.3, after the passage of the strong northeast monsoon, nitrogen in the form of nitrate plus nitrite could be firstly depleted by phytoplankton consumption, leading to the extremely low concentration of nitrate plus nitrite observed during the winter cruise.

4.5 Decoupling between phytoplankton growth and microzooplankton grazing influenced by the winter monsoon

The different correlationship between μ and m suggested that coupling between phytoplankton and microzooplankton also varied between the two seasons. Close coupling between phytoplankton and microzooplanktong grazing indicated by the positive correlation between μ and m were broadly reported in previous studies (discussion in Zhou et al., 2011). Our results also showed that the microzooplankton grazing was closely coupled with the phytoplankton growth in the summer cruise, but was not in the winter cruise. We consider that the influence of the northeast monsoon could break the coupling between phytoplankton growth and microzooplankton grazing in winter. Firstly, the comings of strong northeast monsoon supply nutrients from deep water to the surface by enhancing vertical mixing. This episodic input of nutrients could break the coupling between phytoplankton and microzooplankton by stimulating μ overwhelming corresponding m (Irigoien et al., 2005). Secondly, large rainfall and the resulted SSS decrease may decouple the phytoplankton (especially the pico-phytoplankton) growth and microzooplankton grazing through ~~directly or~~ indirectly influencing the phytoplankton growth and microzooplankton grazing as discussed in Section 4.3.

4.6 Implications of the low microzooplankton grazing impact on phytoplankton

As discussed in Section 4.1.3, our data of the μ and m were similar to most of the previous results observed in low latitude tropical waters, but the m/μ in the SSCS was relatively lower (< 50% on average) than those reported in the previous studies except that a similar value (49%) in the Arabian Sea during the northeast monsoon season has been reported by Caron and Denett (1999). Our m/μ is also lower than the mean values in most of the global sea regions (Schmoker et al., 2013). The low m/μ, i.e. the high growth differential over grazing indicates low remineralization of organic matter mediated by microzooplankton and mismatch between the primary production and microzooplankton grazing. ~~the increased importance of the phytoplankton-mesozooplankton grazing pathway (Landry et al., 1998)~~. As a result, potentially high vertical biogenic particle fluxes via the formation of phytoplankton aggregates and consumption of those aggregates by mesozooplankton could occur (Legendre and Rassoulzadegan, 1996; Schmoker et al., 2013). Higher vertical biogenic particle fluxes in the prevailing periods of the monsoons than between the monsoons in the SSCS was indeed observed (Wan et al., 2010). Our results suggest that the high growth differential over microzooplankton grazing may account for part of the high vertical biogenic particle fluxes in the SSCS.

4.7 Size-selective grazing contributes to the pico-phytoplankton dominance in the oligotrophic waters of the SSCS

Size selectivity of microzooplankton grazing have been proposed in previous studies (e.g., Burkill et al., 1987; Froneman and Perissinotto, 1996; Huang et al., 2011; Kuipers and Witte, 1999; Sun et al., 2004), and varied patterns of the size selectivity have been reported. For example, higher grazing rate on smaller phytoplankton has been reported accounting for the larger-sized phytoplankton dominance in eutrophic waters (e.g. Strom et al., 2007), but no such pattern were found in other studies (Lie and Wong, 2010; Safi et al., 2007; Strom and Fredrickson, 2008; Zhou et al., 2011). In contrast, Zhou et al. (2015a) found that microzooplankton selectively grazed on nano-phytoplankton (3–20 μm) in the oligotrophic waters of the SCS in summer, and proposed that the size-selective grazing on nano-phytoplankton contributes to the pico-phytoplankton dominance there. Higher microzooplankton grazing rate on the large-sized phytoplankton (> 5 μm) than the smaller one (< 5 μm) has also been recently reported in the oligotrophic subtropical Northeast Atlantic (Cáceres et al., 2013).

The higher RPI (Fig. 3) for and *m* (Table1 and Table 2) on the larger-sized phytoplankton than pico-phytoplankton suggests that microzooplankton selectively grazed on the larger-sized phytoplankton in the SSCS both in the summer and winter. The size-selective grazing balanced part of the relative higher growth rate of the larger-sized phytoplankton (> 3 μm), and led to the nearly equal grazing impact (m/μ) on both the larger-sized phytoplankton and pico-phytoplankton in the winter (Table 3). As a result, microzooplankton grazing maintained the pico-phytoplankton dominance in the study waters during the winter. Therefore, on the basis of the results observed in the present study and those reported by Zhou et al. (2015a) and Cáceres et al. (2013), we propose that microzooplankton grazing contributes to the pico-phytoplankton dominance in oligotrophic subtropical and tropical waters such as that of the SCS.

5 Conclusions

Significant seasonal variations in μ and m as well as environmental variables under the influence of the East Asian monsoon were observed in the SSCS. Nutrient supply to the surface influenced by vertical mixing and SSS decrease related to large rainfall were considered as the main factors accounting for the significant low μ and m, and the decoupling between the μ and m in the SSCS in the winter.

The m/μ did not significantly vary between the two seasons. The low m/μ (< 50% on average), i.e. the high growth differential over microzooplankton grazing indicates low remineralization of organic matter mediated by microzooplankton and ~~the increased importance of the phytoplankton-mesozooplankton grazing pathway~~mismatch between the μ and m, and may account for part of the high vertical biogenic particle fluxes in the prevailing periods of the monsoons in the SSCS.

Significant size-selective grazing on the larger-sized (> 3 μm) phytoplankton was observed in the SSCS both in the summer and winter, which indicates that microzooplankton grazing contributes to the pico-phytoplankton dominance in the oligotrophic tropical waters of the SCS.

Author contribution

Linbin Zhou designed the experiments and carried them out. Yehui Tan and Liangmin Huang supervised the projects. Zifeng Hu provided the monthly rainfall data and the Figure 5. Zhixin Ke provided part of the nutrient data. Linbin Zhou prepared the manuscript with contributions from all authors.

Acknowledgments

We thank the captain and crew of R/V *Shiyan 3* for their assistance, Shengfu Wang and Shuai Xing for providing part of the nutrient data. This worked was supported by the National Natural Science Foundation of China (Grant no. 41130855; 41276162), the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant no. XDA05030403; XDA11020203).

References

An-Yi, T., Chin, W.-M., and Chiang, K.-P.: Diel patterns of grazing by pigmented nanoflagellates on *Synechococcus* spp. in the coastal ecosystem of subtropical western Pacific, Hydrobiologia, 636, 249-256, 2009.

Ara, K. and Hiromi, J.: Seasonal variability in plankton food web structure and trophodynamics in the neritic area of Sagami Bay, Japan, J. Oceanogr., 65, 757-779, 2009.

Banse, K.: Do we live in a largely top-down regulated world?, J. Biosci., 32, 791-796, 2007.

Banse, K.: Reflections about chance in my career, and on the top-down regulated world, Annu. Rev. Mar. Sci., 5, 1-19, 2013.

Burkill, P. H., Mantoura, R. F. C., Llewellyn, C. A., and Owens, N. J. P.: Microzooplankton grazing and selectivity of phytoplankton in coastal waters, Mar. Biol., 93, 581-590, 1987.

Cáceres, C., Taboada, F. G., Höfer, J., and Anadón, R.: Phytoplankton growth and microzooplankton grazing in the subtropical Northeast Atlantic, PLoS ONE, 8, e69159, doi:10.1371/journal.pone.0069159, 2013.

Calbet, A. and Landry, M. R.: Phytoplankton growth, microzooplantkon grazing, and carbon cycling in marine systems, Limnol. Oceanogr., 49, 51-57, 2004.

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.

Calbet, A., Trepat, I., Almeda, R., Salo, V., Saiz, E., Movilla, J. I., Alcaraz, M., Yebra, L., and Simó, R.: Impact of micro-and nanograzers on phytoplankton assessed by standard and size-fractionated dilution grazing experiments, Aquat. Microb. Ecol., 50, 145-156, 2008.

Caron, D. A. and Dennett, M. R.: Phytoplankton growth and mortality during the 1995 Northeast Monsoon and Spring Intermonsoon in the Arabian Sea, Deep-Sea Res. II:, 46, 1665-1690, 1999.

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, 2009a.

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, 2009b.

Chen, B., Zheng, L., Huang, B., Song, S., and Liu, H.: Seasonal and spatial comparisons of phytoplankton growth and mortality rates due to microzooplankton grazing in the northern South China Sea, Biogeosciences, 10, 2775-2785, 2013.

Chen, J.-Y., Tsai, A.-Y., Gong, G.-C., and Chiang, K.-P.: Grazing pressure by ciliates on the nanoflagellate community in a subtropical pelagic continental shelf ecosystem: small ciliates (of< 45 μm) are major consumers of the nanoflagellate community, Zool. Stud., 51, 1308-1318, 2012.

Chen, Q. and Mutidisciplinary Expedition to Nansha Islands, C. A. o. S. (Eds.): Proceedings of Studies on Marine Organisms in Nansha Islands and Adjacent Waters I, Ocean Press, Beijing, China, 1991.

Chen, Q. and Mutidisciplinary Expedition to Nansha Islands, C. A. o. S.: Research Reports on the Nansha Islands and its Adjacent Waters, Beijing, China, 820 pp., 1989.

Chen, Q. and Mutidisciplinary Expedition to Nansha Islands, C. A. o. S. (Eds.): Studies on Marine Fauna and Flora and Biogeography of the Nansha Islands and Neighboring Waters I, Ocean Press, Beijing, China, 1994.

Chen, Q. and Mutidisciplinary Expedition to Nansha Islands, C. A. o. S. (Eds.): Studies on Marine Fauna and Flora and Biogeography of the Nansha Islands and Neighboring Waters II, Ocean Press, Beijing, China, 1996.

Chen, Q. and Mutidisciplinary Expedition to Nansha Islands, C. A. o. S. (Eds.): Studies on Marine Fauna and Flora and Biogeography of the Nansha Islands and Neighboring Waters III, Ocean Press, Beijing, China, 1998.

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.

Christaki, U., Vázquez-Domínguez, E., Courties, C., and Lebaron, P.: Grazing impact of different heterotrophic nanoflagellates on eukaryotic (Ostreococcus tauri) and prokaryotic picoautotrophs (Prochlorococcus and Synechococcus), Environ. Microbiol., 7, 1200-1210, 2005.

Dolan, J. R. and Mckeon, K.: The reliability of grazing rate estimates from dilution experiments: Have we over-estimated rates of organic carbon consumption?, Ocean Sci., 1, 21-36, 2004.

Dyhrman, S. T., Chappell, P. D., Haley, S. T., Moffett, J. W., Orchard, E. D., Waterbury, J. B., and Webb, E. A.: Phosphonate utilization by the globally important marine diazotroph Trichodesmium, Nature, 439, 68-71, 2006.

Elser, J. J. and Frees, D. L.: Microconsumer Grazing and Sources of Limiting Nutrients for Phytoplankton Growth - Application and Complications of a Nutrient-Deletion/Dilution-Gradient Technique, Limnol. Oceanogr., 40, 1-16, 1995.

Fang, W. D., Fang, G., Shi, P., Huang, Q., and Xie, Q.: Seasonal structures of upper layer circulation in the southern South China Sea from in situ observations, J. Geophys. Res., 107, 3202, doi:10.1029/2002JC001343, 2002.

Fang, W. D., Guo, Z. X., and Huang, Y. T.: Observational study of the circulation in the southern South China Sea, Chinese Sci. Bull., 43, 898-905, 1998.

Foster, R. A., Kuypers, M. M. M., Vagner, T., Paerl, R. W., Musat, N., and Zehr, J. P.: Nitrogen fixation and transfer in open ocean diatom-cyanobacterial symbioses, The ISME J. 5, 1484-1493, 2011.

Franzè, G. and Lavrentyev, P. J.: Microzooplankton growth rates examined across a temperature gradient in the Barents Sea, PLoS ONE, 9, e86429, doi: 10.1371/journal.pone.0086429, 2014.

Frias-Lopez, J., Thompson, A., Waldbauer, J., and Chisholm, S. W.: Use of stable isotope-labelled cells to identify active grazers of picocyanobacteria in ocean surface waters, Environ. Microbiol., 11, 512-525, 2009.

Froneman, P. W. and Perissinotto, R.: Structure and grazing of the microzooplankton communities of the Subtropical Covergence and a warm-core edyy in the Altantic sector of the Southern Ocean, Mar. Ecol.-Prog. Ser., 135, 237-245, 1996.

Fu, F. X. and Bell, P. R. F.: Effect of salinity on growth, pigmentation, N-2 fixation and alkaline phosphatase activity of cultured Trichodesmium sp, Mar. Ecol.-Prog. Ser., 257, 69-76, 2003.

Gallegos, C. L.: Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics, Mar. Ecol.-Prog. Ser., 57, 23-33, 1989.

Grindley, J. R.: Effect of low-salinity water on the vertical migration of estuarine plankton, Nature, 203, 781-782, 1964.

Groszkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M. M. M., Lavik, G., Schmitz, R. A., Wallace, D. W. R., and LaRoche, J.: Doubling of marine dinitrogen-fixation rates based on direct measurements, Nature, 488, 361-364, 2012.

Guo, C., Liu, H., Zheng, L., Song, S., Chen, B., and Huang, B.: Seasonal and spatial patterns of picophytoplankton growth, grazing and distribution in the East China Sea, Biogeosciences, 11, 1847-1862, 2014.

Gutiérrez-Rodríguez, A., Latasa, M., Mourre, B., and Laws, E. A.: Coupling between phytoplankton growth and microzooplankton grazing in dilution experiments: potential artefacts, Mar. Ecol.-Prog. Ser., 383, 1-9, 2009.

Hirose, M., Katano, T., and Nakano, S.-I.: Growth and grazing mortality rates of Prochlorococcus, Synechococcus and eukaryotic picophytoplankton in a bay of the Uwa Sea, Japan, J. Plankton Res., 30, 241-250, 2008.

Huang, B., Xiang, W., Zeng, X., Chiang, K.-P., Tian, H., Hu, J., Lan, W., and Hong, H.: Phytoplankton growth and microzooplankton grazing in a subtropical coastal upwelling system in the Taiwan Strait, Cont. Shelf Res., 31, S48-S56, 2011.

Huang, L. and Multidisciplinary Expedition to Nansha Islands, C. A. o. S. (Eds.): A Study on Ecological Processes of Nansha Islands Sea Area I, Science Press, Beijing, China, 1997.

Irigoien, X., Flynn, K. J., and Harris, R. P.: Phytoplankton blooms: a 'loophole' in microzooplankton grazing impact?, J.Plankton Res., 27, 313-321, 2005.

Kim, S., Park, M. G., Moon, C., Shin, K., and Chang, M.: Seasonal variations in phytoplankton grwoth and microzooplankton grazing in a temperate coastal embayment, Korea, Estuar. Coast.Shelf S., 71, 159-169, 2007.

Klauschies, T., Bauer, B., Aberle-Malzahn, N., Sommer, U., and Gaedke, U.: Climate change effects on phytoplankton depend on cell size and food web structure, Mar. Biol., 159, 2455-2478, 2012.

Kuipers, B. R. and Witte, H. J.: Grazing impact of microzooplankton on different size classes of algae in the North Sea in early spring and mid-summer, Mar. Ecol.-Prog. Ser., 180, 93-104, 1999.

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.

Landry, M. R. and Calbet, A.: Microzooplankton production in the oceans, ICES J Mar. Sci., 61, 501-507, 2004.

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

Landry, M. R. and Hassett, R. P.: Estimating the grazing impact of marine micro-zooplankton, Mar. Biol., 67, 283-288, 1982.

Landry, M. R., Selph, K. E., Taylor, A. G., Décima, M., Balch, W. M., and Bidigare, R. R.: Phytoplankton growth, grazing and production balances in the HNLC equatorial Pacific, Deep-Sea Res. II, 58, 524-535, 2011.

Lawrence, C. and Menden-Deuer, S.: Drivers of protistan grazing pressure: seasonal signals of plankton community composition and environmental conditions, Mar. Ecol.-Prog. Ser., 459, 39-52, 2012.

Legendre, L. and Rassoulzadegan, F.: Food-web mediated export of biogenic carbon in oceans: hydrodynamic control, Mar. Ecol.-Prog. Ser., 145, 179-193, 1996.

Lie, A. A. Y. and Wong, C. K.: Selectivity and grazing impact of microzooplankton on phytoplankton in two subtropical semi-enclosed bays with different chlorophyll concentrations, J. Exp. Mar. Biol. Ecol., 390, 149-159, 2010.

Liu, K. K., Chao, S. Y., Shaw, P. T., Gong, G. C., Chen, C. C., and Tang, T. Y.: Monsoon-forced chlorophyll distribution and primary production in the South China Sea: observations and a numerical study, Deep-Sea Res. I, 49, 1387-1412, 2002.

Loebl, M. and Beusekon, J. E. E. V.: Seasonality of microzooplankton grazing in the northern Wadden Sea, J. Sea Res., 59, 203-216, 2008.

Lozupone, C. A. and Knight, R.: Global patterns in bacterial diversity, P. Natl Acad. Sci. USA, 104, 11436-11440, 2007.

Lugomela, C., Lyimo, T. J., Bryceson, I., Semesi, A. K., and Bergman, B.: Trichodesmium in coastal waters of Tanzania: diversity, seasonality, nitrogen and carbon fixation, Hydrobiologia, 477, 1-13, 2002.

Mitbavkar, S., Rajaneesh, K., Anil, A., and Sundar, D.: Picophytoplankton community in a tropical estuary: Detection of *Prochlorococcus* like populations, Estuar. Coast.Shelf S, 107, 159-164, 2012.

Moigis, A. G.: The clearance rate of microzooplankton as the key element for describing estimated non-linear dilution plots demonstrated by a model, Mar. Biol., 149, 743-762, 2006.

Mulholland, M. R. and Bronk, D. A.: Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by Trichodesmium IMS101, Aquat. Microb. Ecol., 37, 85-94, 2004.

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.

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.

Parsons, T. R., Maita, Y., and Lalli, C. M.: A Manual of Chemical and Biological Methods for Seawater Analysis, Pergamon Press, Oxford, 1984.

Partensky, F., Blanchot, J., and Vaulot, D.: Differential distribution and ecology of *Prochlorococcus* and *Synechococcus* in oceanic waters: a review, Bulletin-Institut Oceanographique Monaco-Numero Special, 19, 457-476, 1999.

Paytan, A., Mackey, K. R. M., Chen, Y., Lima, I. D., Doney, S. C., Mahowald, N., Labiosa, R., and Post, A. F.: Toxicity of atmospheric aerosols on marine phytoplankton, P. Natl Acad. Sci. USA, 106, 4601-4605, 2009.

Quevedo, M. and Anadón, R.: Protist control of phytoplankton growth in the subtropical north-east Atlantic, Mar. Ecol.-Prog. Ser., 221, 29-38, 2001.

Safi, K. A., Brian Griffiths, F., and Hall, J. A.: Microzooplankton composition, biomass and grazing rates along the WOCE SR3 line between Tasmania and Antarctica, Deep-Sea Res. I, 54, 1025-1041, 2007.

Safi, K. A. and Hall, J. A.: Mixotrophic and heterotrophic nanoflagellate grazing in the convergence zone east of New Zealand, Aquat. Microb. Ecol., 20, 83-93, 1999.

Schmoker, C., Hernández-León, S., and Calbet, A.: Microzooplankton grazing in the oceans: impacts, data variability, knowledge gaps and future directions, J Plankton Res., 35, 691-706, 2013.

Sherr, E. B., Sherr, B. F., and Ross, C.: Microzooplankton grazing impact in the Bering Sea during spring sea ice conditions, Deep-Sea Res. Part II: Topical Studies in Oceanography, 94, 57-67, 2013.

Sherr, E. B., Sherrr, B. F., and Hartz, A. J.: Microzooplankton grazing impact in the Western Arctic Ocean, Deep-Sea Res. II, 56, 1264-1273, 2009.

Strom, S. L. and Fredrickson, K. A.: Intense stratification leads to phytoplankton nutrient limitation and reduced microzooplankton grazing in the southeastern Bering Sea, Deep-Sea Res. II, 55, 1761-1774, 2008.

Strom, S. L., Macri, E. L., and Olson, M. B.: Microzooplankton grazing in the coastal Gulf of Alaska: Variations in top-down control of phytoplankton, Limnol. Oceanogr., 52, 1480-1494, 2007.

Su, J.: Overview of the South China Sea circulation and its influence on the coastal physical oceanography outside the Pearl River Estuary, Cont. Shelf Res., 24, 1745-1760, 2004.

Sun, J., Dawson, J., and Liu, D.: Microzooplankton grazing on phytoplankton in summer in the Jiaozhou Bay, China, Chinese J. Applied Ecol., 15, 1245-1252, 2004.

Suzuki, K., Tsuda, A., Kiyosawa, H., Takeda, S., Nishioka, J., Saino, T., Takahashi, M., and Wong, C. S.: Grazing impact of microzooplankton on a diatom bloom in a mesocosm as etimated by pigment-specifc dilution technique, J. Exp. Mar. Biol. Ecol., 271, 99-120, 2002.

Teixeira, I. and Figueiras, F.: Feeding behaviour and non-linear responses in dilution experiments in a coastal upwelling system, Aquat. Microb. Ecol., 55, 53-63, 2009.

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.

Wong, G. T., Chung, S.-W., Shiah, F.-K., Chen, C.-C., Wen, L.-S., and Liu, K.-K.: Nitrate anomaly in the upper nutricline in the northern South China Sea-Evidence for nitrogen fixation, Geophys. Res. Lett., 29, 2097, doi:10.1029/2002GL015796, 2002.

Wyrtki, K.: Physical oceanography of the southeast Asian waters, Scripps Institution of Oceanography, La Jolla, California, 1961.

Yang, E., Choi, J., and Hyun, J.-H.: Distribution and structure of heterotrophic protist communities in the northeast equatorial Pacific Ocean, Mar. Biol., 146, 1-15, 2004.

Yang, Y.-h. and Jiao, N.-z.: In situ daily growth rate of *Prochlorococcus* at the chlorophyll maximum layer in the southern South China Sea: an estimation from cell cycle analysis, Chin. J. Oceanol. and Limn., 20, 8-14, 2002.

Zehr, J. P. and Paerl, H. W.: Molecular ecological aspects of nitrogen fixation in the marine environment. In: Microbial Ecology of the Oceans, Kirchman, D. L. (Ed.), John Wiley&Sons, Inc., Hoboken, New Jersey, 2008.

Zeng, L., Du, Y., Xie, S.-P., and Wang, D.: Barrier layer in the South China Sea during summer 2000, Dynam. Atmos. Oceans, 47, 38-54, 2009.

Zhang, Z., Qiao, F., and Guo, J.: Subsurface eddies in the southern South China Sea detected from in-situ observation in October 2011, Deep-Sea Res. I, 87, 30-34, 2014.

Zhou, L., Tan, Y., and Huang, L.: Negative phytoplankton growth rates in dilution experiments and the possible causes, J. Trop. Oceanogr., 32, 48-54, 2013.

Zhou, L., Tan, Y., Huang, L., Huang, J., Liu, H., and Lian, X.: Phytoplankton growth and microzooplankton grazing in the continental shelf area of northeastern South China Sea after Typhoon Fengshen, Cont. Shelf Res., 31, 1663-1671, 2011.

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.

Zhou, L., Huang, L., Tan, Y., Lian, X., and Li, K.: Size-based analysis of a zooplankton community under the influence of the Pearl River plume and coastal upwelling in the northeastern South China Sea, Mar. Biol. Res., 11, 168-179, 2015b.

Zubkov, M. V., Sleigh, M. A., and Burkill, P. H.: Assaying picoplankton distribution by flow cytometry of underway samples collected along a meridional transect across the Atlantic Ocean, Aquat. Microb. Ecol., 21, 13-20, 2000.

Table 1 Summary of environmental variables, phytoplankton growth rates with (μ_n, d^{-1}) and (μ, d^{-1}) without nutrient amendment, and microzooplankton grazing rates (m, d^{-1}) in the southern South China Sea in May–June 2009. SST, surface seawater temperature (^oC); SSS, surface seawater salinity; 25-T (S) seawater temperature (salinity) at 25 m in depth; Si, silicate (µmol L^{-1}); P, phosphate (µmol L^{-1}); N, nitrate plus nitrite (µmol L^{-1}); Chla, chlorophyll *a* (µg L^{-1}); n, the number of data points in the linear part; R^2, the determination coefficient of the regression of the linear part; the superscript 'a' denotes a saturated feeding response; the superscript 'b' denotes a saturated-increased feeding response. The significant level (*p)* of regressions when saturated or saturated-increased feeding responses occurred was in some occasions > 0.05 owing to the few points included in the regression.

Station	Date	Depth	SST	SSS	25-T	25-S	Si	P	N	Chla	μ_n	m	μ	R^2	n	p	μ/μ_n	m/μ
KJ28	21-May	2899	29.90	32.90	28.02	33.07	2.0	0.04	0.67	0.087	1.01	0.34	1.09	0.88	6^a	0.006	1.08	0.31
KJ35	22-May	3243	29.60	32.41	29.05	32.91	1.6	0.02	0.71	0.086	1.11	0.30	0.85	0.83	4^a	0.086	0.77	0.35
YS	23-May	28	29.10	32.77	-	-	0.3	0.07	0.53	0.082	0.74	0.40	0.82	0.50	8	0.052	1.12	0.49
KJ41	25-May	1724	29.93	32.83	28.60	33.25	1.7	0.06	2.26	0.079	1.04	0.57	0.84	0.76	8	0.005	0.80	0.68
KJ47	26-May	468	31.03	32.60	29.63	32.90	1.8	0.04	0.74	0.049	1.10	0.61	0.90	0.87	6^b	0.007	0.82	0.67
NS25	28-May	2025	30.12	32.72	29.65	32.74	6.8	0.06	1.46	0.036	1.16	0.71	1.30	0.66	8	0.015	1.12	0.55
NS19	30-May	2057	30.28	32.94	29.18	33.20	1.0	0.04	1.02	0.050	1.51	0.79	1.20	0.61	4^b	0.216	0.79	0.66
KJ39	31-May	2001	29.60	32.89	29.11	33.03	9.0	0.01	0.61	0.050	1.50	0.49	0.83	0.85	8	0.001	0.55	0.59
NS16	31-May	1589	29.88	32.94	29.77	33.01	5.2	0.00	0.82	0.044	1.00	0.46	1.04	0.56	8	0.033	1.05	0.44
KJ65	02-Jun	2999	28.95	32.82	28.78	32.90	3.4	0.05	1.46	0.096	0.64	0.21	0.19	0.90	6^b	0.004	0.29	1.13
KJ69	05-Jun	1522	29.02	32.86	28.93	32.93	6.5	0.03	0.49	0.057	2.30	0.66	1.50	0.99	8	< 0.001	0.65	0.44
KJ73	06-Jun	1785	29.50	32.84	29.24	32.90	6.4	0.05	0.80	0.045	0.78	0.48	0.87	0.30	4^b	0.450	1.11	0.55
NS12	07-Jun	920	29.26	33.05	28.90	33.12	4.6	0.01	0.86	0.062	1.19	0.39	0.88	0.82	6^b	0.013	0.73	0.45
KJ32	09-Jun	4229	28.84	32.97	28.85	33.00	3.2	0.11	0.20	0.105	0.54	0.09	0.54	0.99	4^a	0.006	0.99	0.16

Table 2 Summary of environmental variables, phytoplankton growth rates with (μ_n, d^{-1}) and without (μ, d^{-1}) nutrient amendment, and microzooplankton grazing rates (m, d^{-1}) in the southern South China Sea in November 2010. SST, surface seawater temperature (^{o}C); SSS, surface seawater salinity; 25-T (S) seawater temperature (salinity) at 25 m in depth; Si, silicate (µmol L^{-1}); P, phosphate (µmol L^{-1}); N, nitrate plus nitrite (µmol L^{-1}); Chla, chlorophyll *a* (µg L^{-1}); n, the number of data points in the linear part; R^2, the determination coefficient of the regression of the linear part; the superscript 'a' denotes a saturated feeding response; the superscript 'b' denotes a saturated-increased feeding response. The significant level (p) of regressions when saturated-increased feeding responses occurred was in some occasions > 0.05 owing to the few points included in the regression.

Station	Date	Depth	SST	SSS	25-T	25-S	Si	P	N	> 3 µm Chla	< 3 µm Chla	μ_n	m	μ	R^2	n	p	μ/μ_n	m/μ
KJ28	09-Nov	2538	28.89	32.35	28.74	33.33	8.4	0.37	0.03	0.013	0.120	0.66	0.17	0.45	0.77	8	0.009	0.69	0.38
KJ32	09-Nov	4229	29.07	32.20	28.93	33.50	6.5	0.36	0.02	0.024	0.085	1.30	0.16	0.74	0.37	8	0.108	0.57	0.22
KJ35	10-Nov	2903	28.97	31.86	28.05	32.15	5.9	0.41	0.02	0.013	0.084	1.28	0.43	0.96	0.67	6	0.047	0.75	0.45
KJ39	11-Nov	1996	29.22	31.70	29.41	33.11	20.2	0.34	0.04	0.015	0.091	0.77	0.26	0.28	0.89	4^b	0.059	0.37	0.92
KJ42	12-Nov	1460	29.62	31.46	29.32	32.66	7.3	0.36	0.02	0.017	0.088	0.39	0.13	0.50	0.38	4^b	0.383	1.27	0.25
KJ47	13-Nov	511	29.45	32.31	29.33	32.36	9.7	0.28	0.07	0.037	0.121	1.84	0.46	0.60	0.12	6	0.497	0.33	0.77
KJ50	14-Nov	1259	29.22	31.69	29.18	32.98	6.0	0.36	0.02	0.020	0.069	0.90	0.13	0.43	0.94	4^b	0.030	0.48	0.30
KJ53	14-Nov	145	29.62	32.06	28.92	33.20	3.8	0.36	0.07	0.017	0.050	-2.03	1.01	-2.06	0.65	8	0.015	-	-
KJ65	16-Nov	2100	29.26	31.83	29.27	32.93	6.6	0.36	0.02	0.014	0.077	0.97	0.33	0.28	0.52	6	0.106	0.29	1.15
KJ73	18-Nov	1672	29.42	31.72	29.50	32.84	4.4	0.34	0.07	0.017	0.077	0.87	0.34	0.57	0.94	4^a	0.030	0.66	0.59

Table 3 Comparison of phytoplankton growth and mortality rates between two size fractions in the southern South China Sea in November 2010. μ_n, phytoplankton growth rates with nutrient amendment (d^{-1}); μ, phytoplankton growth rates without nutrient amendment (d^{-1}); m, microzooplankton grazing rates or phytoplankton mortality rates caused by microzooplankton grazing (d^{-1}); the superscript 'a' denotes a saturated feeding response; the superscript 'b' denotes a saturated-increased feeding response. The significant level (p) of regressions when saturated or saturated-increased feeding responses occurred was in some occasions > 0.05 owing to the few points included in the regression.

Size fraction	Station	μ_n	m	μ	R^2	n	p	μ/μ_n	m/μ
< 3 µm	KJ28	0.57	0.15	0.39	0.57	8	0.050	0.69	0.37
	KJ32	1.29	0.16	0.78	0.23	8	0.231	0.60	0.21
	KJ35	1.30	0.50	1.01	0.65	6	0.052	0.78	0.50
	KJ39	0.50	0.12	0.05	0.89	4^a	0.058	0.10	2.42
	KJ42	0.26	0.05	0.33	0.04	4^b	0.791	1.24	0.16
	KJ47	1.27	0.10	0.18	0.00	4^a	0.958	0.14	0.55
	KJ50	0.73	0.01	0.32	0.96	4^b	0.020	0.43	0.03
	KJ53	-2.64	0.89	-2.55	0.59	8	0.027	0.96	-0.35
	KJ65	0.85	0.29	0.15	0.42	6	0.167	0.18	1.88
	KJ73	0.80	0.39	0.58	0.94	6^a	0.001	0.73	0.67
> 3 µm	KJ28	1.20	0.22	0.77	0.68	8	0.012	0.64	0.29
	KJ32	1.34	0.17	0.62	0.65	8	0.015	0.46	0.28
	KJ35	1.14	0.09	0.62	0.26	8	0.192	0.54	0.14
	KJ39	1.71	1.31	1.76	0.86	4^b	0.075	1.03	0.75
	KJ42	0.89	0.38	1.14	0.23	4^b	0.520	1.28	0.34
	KJ47	2.32	0.45	0.69	0.14	8	0.365	0.30	0.65
	KJ50	1.34	0.68	0.97	0.91	4^a	0.046	0.72	0.70
	KJ53	-1.17	1.10	-1.39	0.65	8	0.016	1.18	-0.79
	KJ65	1.23	0.22	0.56	0.16	8	0.328	0.46	0.40
	KJ73	1.03	0.35	0.77	0.92	4^b	0.041	0.75	0.46

Table 4 Pearson correlation analyses between dilution experimental parameters and environmental variables. μ, phytoplankton growth rates without nutrient amendment; μ_n, phytoplankton growth rates with nutrient amendment; *m*, microzooplankton grazing rates; SST, surface seawater temperature; SSS, surface seawater salinity; Si, silicate; P, phosphate; N, nitrate plus nitrite; Chla, chlorophyll *a*

	μ	m	SST	SSS	Si	P	N	Chla	m/μ	μ/μ_n	μ-m
μ	1	$.762^{**}$	$.425^{*}$	$.547^{**}$	-.348	$-.570^{**}$	.369	$-.646^{**}$	-.356	$.496^{*}$	$.827^{**}$
m		1	$.617^{**}$	$.477^{*}$	-.206	$-.536^{**}$	$.539^{**}$	$-.660^{**}$	.252	.163	.266
SST			1	.225	-.289	$-.426^{*}$	$.450^{*}$	$-.542^{**}$	.093	.332	.098
SSS				1	$-.465^{*}$	$-.893^{**}$	$.649^{**}$	$-.519^{*}$	-.061	.293	.396
Si					1	$.484^{*}$	$-.433^{*}$	.304	.239	-.406	-.336
P						1	$-.721^{**}$	$.690^{**}$	.002	-.357	-.383
N							1	$-.541^{**}$	.246	.221	.079
Chla								1	-.007	-.404	-.389
m/μ									1	$-.597^{**}$	$-.748^{**}$
μ/μ_n										1	$.596^{**}$
μ-m											1

**, Significant correlation at the level of 0.01; *, Significant correlation at the level of 0.05.

Table 5 Comparisons of environmental variables, and phytoplankton growth and microzooplankton grazing between May–June 2009 and November 2010 in the southern South China Sea. SST, surface seawater temperature (^{o}C); SSS, surface seawater salinity; Si, silicate (μmol L^{-1}); P, phosphate (μmol L^{-1}); N, nitrate plus nitrite (μmol L^{-1}); Chla, chlorophyll *a* (μg L^{-1}); μ_n, phytoplankton growth rates with nutrient amendment (d^{-1}); μ, phytoplankton growth rates without nutrient amendment (, d^{-1}); m, microzooplankton grazing rates (d^{-1}); p, the significant level. The mean ratio values of μ/μ_n and m/μ were compared based on the Mann-Whitney test, while the other mean values were compared based on the independent t-test.

Season	SST	SSS	Si	P	N	Chla	μ/μ_n	m	μ	m/μ
Summer	29.64 ± 0.60	32.82 ± 0.16	3.8 ± 2.6	0.04 ± 0.03	0.90 ± 0.52	0.066 ± 0.022	0.80 ± 0.25	0.46 ± 0.20	0.92 ± 0.32	0.49 ± 0.23
Winter	29.27 ± 0.25	31.92 ± 0.30	7.9 ± 4.7	0.35 ± 0.03	0.04 ± 0.02	0.104 ± 0.024	0.54 ± 0.30	0.27 ± 0.13	0.54 ± 0.22	0.48 ± 0.33
p	0.055	< 0.01	< 0.05	< 0.01	< 0.01	< 0.01	< 0.05	< 0.01	< 0.01	> 0.1

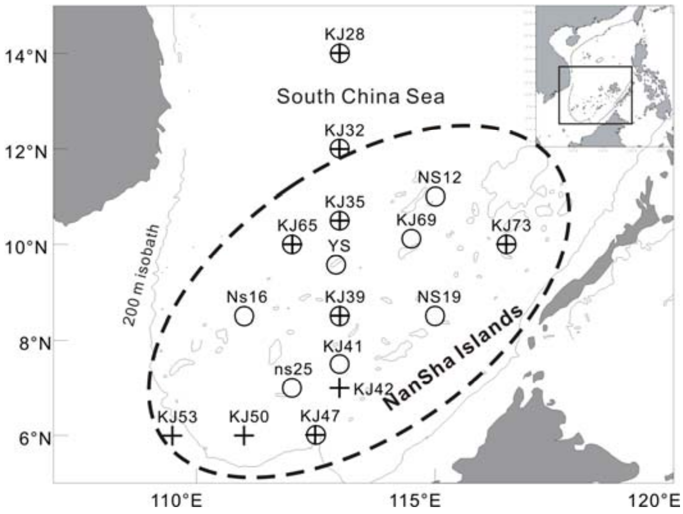

Figure 1. Stations for dilution experiments in the southern South China Sea during May to June 2009 and November 2010. The open circle indicates experimental stations during May to June 2009; the cross indicates experimental stations during November 2010.

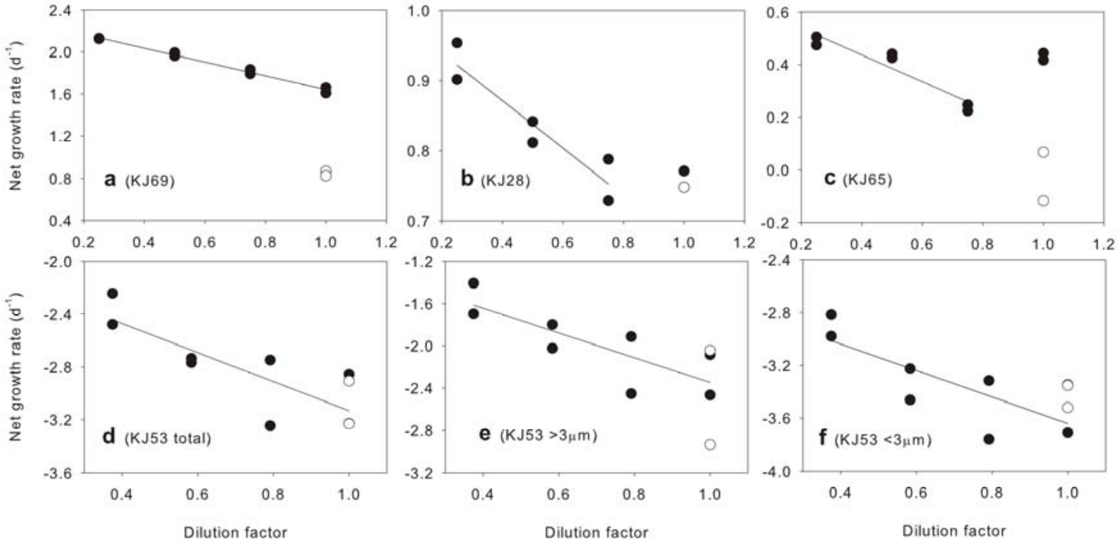

Figure 2. Examples of dilution experiment plots of net phytoplankton growth rate as a function of dilution factor. (**a**) Linear feeding responses in the dilution experiment at KJ69; (**b**) saturated feeding responses in the dilution experiment at KJ28; (**c**) saturated-increasing feeding responses in the dilution experiment at KJ65; (**d–f**) negative phytoplankton growth rates at KJ53, (**d**) for total phytoplankton, (**e**) for the size fraction > 3 µm and (**f**) for the size fraction < 3 µm; the black circle indicates experimental bottles enriched with addition nutrients; the open circle indicates experimental bottles without nutrient amendment.

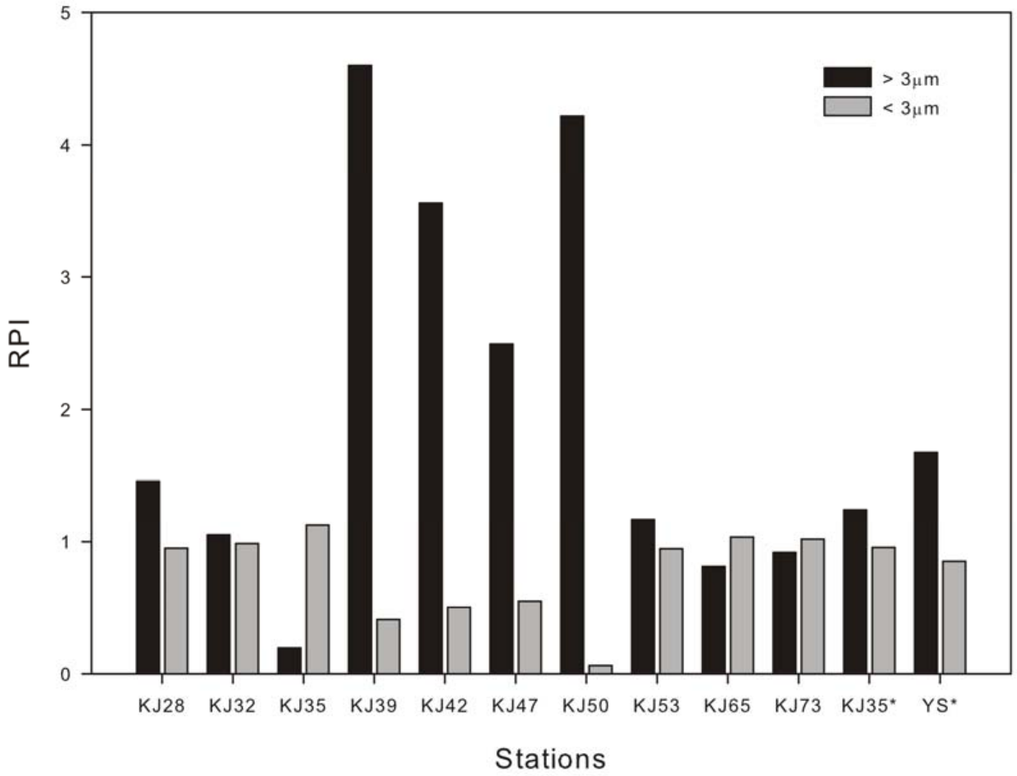

Figure 3. Relative preference index (RPI) for size-fractionated phytoplankton. > 3 μm, size fraction larger than 3 μm; < 3 μm, size fraction smaller than 3 μm; the asterisk indicates experiments conducted in May 2009, others were conducted in November 2010. RPI > 1 indicates positive selection and vice versa.

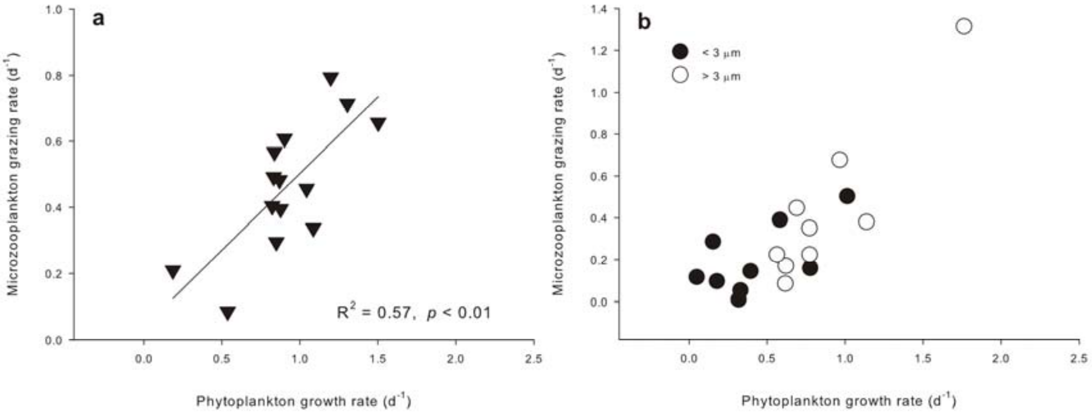

Figure 4. Correlationship between phytoplankton growth and microzooplankton grazing rates. (**a**) May to June 2009; (**b**) November 2010; > 3 μm, size fraction larger than 3 μm; < 3 μm, size fraction smaller than 3 μm.

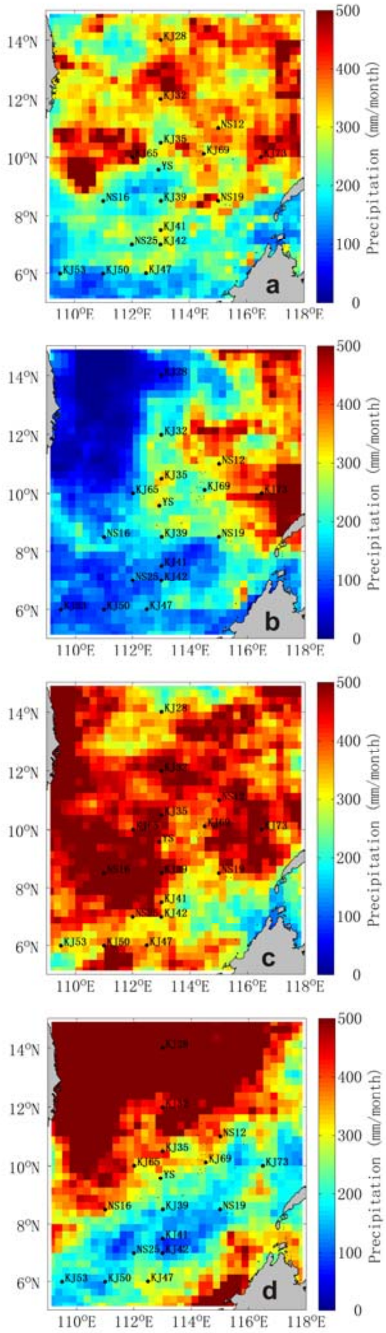

Figure 5. Monthly rainfall in the southern South China Sea estimated on the basis of data from the Tropical Rainfall Measuring Mission. (**a**) May 2009; (**b**) June 2009; (**c**) October 2010; (**d**) November 2010.