

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

**Anonymous Referee #2**

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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.

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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, as explained below:

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

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2) p. 6301 lines 8-9: 'The high  $\mu_n$  (approximateto 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.

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 SCS, although there is little (if any) data examining the salinity impact on pico-phytoplankton growth, and thus lead to the lower  $\mu$  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

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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. *Synechococcus* 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

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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 (Klauschie 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.

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.

6) p. 6305 lines 7-10: 'The low  $m=U$ , 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

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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.

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