

1 **Seasonal and size-dependent variations in the phytoplankton**
2 **growth and microzooplankton grazing in the southern South China**
3 **Sea under the influence of the East Asian monsoon**

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11

1 **Abstract**

2 To examine seasonal and size-dependent variations in the phytoplankton growth and
3 microzooplankton grazing in oligotrophic tropical waters under the influence of seasonal
4 reversing monsoon, dilution experiments were conducted during the summer 2009 (21 May to
5 9 June) and winter 2010 (9 to 18 November) in the southern South China Sea (SSCS). The
6 results showed that environmental variables, phytoplankton biomass, phytoplankton growth
7 rate (μ), microzooplankton grazing rate (m), and relationship (coupling) between the μ and
8 m , rather than the microzooplankton grazing impact on phytoplankton (m/μ) significantly
9 varied between the two seasons. Higher relative preference index (RPI) for and m on the
10 larger-sized ($> 3 \mu\text{m}$) phytoplankton than pico-phytoplankton ($< 3 \mu\text{m}$), indicating significant
11 size-selective grazing by microzooplankton on the larger-sized phytoplankton, were also
12 observed. The μ and m were significantly correlated with seawater salinity and temperature,
13 and phytoplankton biomass, which indicated that salient seasonal variations in the
14 phytoplankton growth and microzooplankton grazing in the SSCS were closely related to the
15 environmental variables under the influence of the East Asian monsoon. We propose that
16 intermittent arrivals of the northeast winter monsoon could lead to the low μ and m , and the
17 decoupling between the μ and m in the SSCS, through influencing nutrient supply to the
18 surface water, and inducing surface seawater salinity decrease. The low m/μ ($< 50\%$ on
19 average) indicates low remineralization of organic matter mediated by microzooplankton and
20 mismatch between the μ and m , and thus probably accounts for part of the high vertical
21 biogenic particle fluxes in the prevailing periods of the monsoons in the SSCS. The
22 size-selective grazing suggests that microzooplankton grazing partially contributes to the
23 pico-phytoplankton dominance in the oligotrophic tropical waters such as that of the SSCS.

24

25 **Key words:** Phytoplankton growth; Microzooplankton grazing; Seasonal variation;

26 Size-selective; Southern South China Sea; East Asian monsoon

27

1 **1 Introduction**

2 Phytoplankton growth and microzooplankton grazing are crucial processes relating to ocean
3 biogeochemistry and energy flow in the pelagic food web. Phytoplankton provide almost all
4 the primary production in the upper ocean. Microzooplankton ($< 200 \mu\text{m}$) consumes most of
5 the daily primary production in the sea, and regulates phytoplankton community composition,
6 and affects the ultimate fate of the phytoplankton-derived primary production (Banse, 2007,
7 2013; Landry and Calbet, 2004; Schmoker et al., 2013).

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

21 Microzooplankton may modify their food preference depending on the morphology,
22 abundance and quality of prey, which would cause phytoplankton community shift in species
23 and/or size composition (Teixeira and Figueiras, 2009). Phytoplankton community in
24 oligotrophic subtropical and tropical waters is usually dominated by small-sized pico-cells ($<$
25 $3 \mu\text{m}$); the pico-phytoplankton consists of three major groups including *Prochlorococcus*,
26 *Synechoccus* and diverse types of pico-eukaryotic phytoplankton (Zubkov et al., 2000).
27 Microzooplankton including both autotrophic and heterotrophic nanoflagellates can actively
28 consume pico-sized prey (An-Yi et al., 2009; Christaki et al., 2005; Frias-Lopez et al., 2009).

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

11 The southern SCS (SSCS) is characterized with permanent water stratification and
12 oligotrophic conditions in the upper layer, and is affected by seasonal reversing monsoon.
13 During the middle of May to September, the SSCS is under the influence of the southwest
14 summer monsoon, while this area is influenced by the stronger northeast winter monsoon
15 during November to the next April (Su, 2004). Seasonal variation of seawater salinity and
16 temperature, vertical nutrient flux and mixed-layer depth driven by the East Asian Monsoon
17 have been reported (Fang et al., 2002; Liu et al., 2002; Longhurst 2007; Ning et al., 2004).
18 Mesoscale eddies with obvious seasonal variation (Fang et al., 2002; Fang et al., 1998; Zhang
19 et al., 2014), and seasonal pattern of higher phytoplankton biomass, primary production and
20 vertical biogenic particle fluxes during the prevailing periods of the monsoons and wintertime
21 occur in the SSCS (Liu et al., 2002; Ning et al., 2004; Wan et al., 2010). There are hundreds
22 of coral shoals, reefs and islands called the Nansha Islands located in this area, making the
23 SSCS be worthy of a component of the Archipelagic Deep Basins Province (ARCH) defined
24 by Longhurst (2007). Many researchers including Qingchao Chen, Liangmin Huang and their
25 co-workers, from the South China Sea Institute of Oceanology, Chinese Academy of Sciences,
26 have previously investigated the environment variables, marine species diversity and
27 ecological processes in the coral reef lagoons of the Nansha Islands and its adjacent waters
28 during the 1980s to 1990s (e.g. Chen and Mutidisciplinary Expedition to Nansha Islands,
29 1991, 1989, 1994, 1996, 1998; Huang and Multidisciplinary Expedition to Nansha Islands,

1 1997). Their works provide valuable contributions to the understanding of the taxonomic
2 composition and distribution of phytoplankton, bacteria, zooplankton and fish, and ecological
3 processes such as primary production in the SSCS. However, these results are seldom
4 published in international media even scientific journals in Chinese, and thus less known to
5 the scientific community. So far, there is no data reported on the microzooplankton grazing in
6 this region. We hypothesize that seasonal changes in both the phytoplankton growth and
7 microzooplankton grazing is expected in the tropical waters influenced by salient seasonal
8 reversing monsoon, and microzooplankton contributes to the phytoplankton size composition
9 through size-selective grazing in the SSCS.

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

14

15 **2 Material and methods**

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

21 Surface seawater was collected, and pre-screened with a 200- μm nylon netting for dilution
22 experiments at each station. Particle-free seawater was obtained by filtering the seawater
23 through a filter with a pore size of 0.22 μm . All the bottles, containers and filters were soaked
24 in 10% HCl for more than 10 h, and thoroughly washed with deionized water and
25 MiliQ-water before each cruise. The bottles and containers were washed with 10% HCl,
26 deionized water and ambient seawater before each experiment. Measured amount of
27 particle-free seawater was firstly added to the 2.4-L polycarbonate bottles, and unfiltered
28 seawater was added and filled the bottles. Four dilution treatments of 25, 50, 75 and 100%

1 unfiltered seawater were prepared for the summer experiments, and another four dilution
2 treatments of 37.5, 58.3, 79.2 and 100% unfiltered seawater were prepared for the winter
3 experiments. All the bottles were enriched with additional nutrients (final concentrations of
4 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
5 phytoplankton growth. Two bottles were filled with unfiltered seawater without nutrient
6 enrichment served as no nutrient controls. Another two bottles filled with unfiltered seawater
7 were sacrificed for initial samples of chlorophyll *a* (Chla). All of the bottles were incubated
8 for 24 h in a deck incubator cooled by running surface seawater and covered with
9 neutral-density screens to simulate in situ light regime. These measures have been proved
10 effective to avoid phytoplankton photoacclimation during the incubation (Zhou et al., 2015a)

11 Seawater was filtered through a sequence of 3- μm pore size polycarbonate filter and GF/F
12 filter for size-fractionated Chla of the larger-sized ($> 3 \mu\text{m}$) and pico- ($< 3 \mu\text{m}$) phytoplankton.
13 Total Chla was calculated as the sum of the two size fractions or directly sampled by filtering
14 0.5 to 1 L seawater on the GF/F filter. The filters were extracted in 90% acetone at -20°C for
15 24 h. The Chla concentration was measured by fluorometry using a Turner Designs Model 10
16 Fluorometer (Parsons et al., 1984).

17 Seawater temperature, salinity and silicate concentration were also measured. Temperature
18 and salinity were determined by Conductivity-Temperature-Depth probes. Silicate in seawater
19 was analyzed with a flow-injection autoanalyzer (Quickchem 8500, Lachat Instruments)
20 following the standard manuals.

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

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

4 Size-fractionated ($> 3 \mu\text{m}$ and $< 3 \mu\text{m}$) phytoplankton growth and mortality rates were
5 estimated for experiments at YS and KJ35 during the summer cruise, and all the experiments
6 during the winter cruise.

7 Nutrient limitation index indicating nutrient sufficiency for phytoplankton growth was
8 assessed by the ratio μ/μ_n , which is expected to vary with system trophic state (Landry et al.,
9 1998).. The microzooplankton grazing impact on phytoplankton was assessed by the ratio of
10 microzooplankton grazing to phytoplankton growth rates (m/μ), and measures the extent to
11 which the daily phytoplankton production is consumed and balanced by microzooplankton
12 grazing (Landry et al., 1998).

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

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

21

22 **3 Results**

23 **3.1 Environmental variables and Chla**

24 Environmental variables and Chla in surface seawater were different between the two cruises
25 (Table 1 and Table 2). Surface seawater temperature (SST) and salinity (SSS) were
26 significantly lower (independent t-test, $p < 0.05$ or 0.01), while the concentration of silicate
27 was significantly higher in the winter cruise (independent t-test, $p < 0.05$) than that in the

1 summer cruise. Vertical profiles of these variables also demonstrated the significant seasonal
2 variations (Fig. 2). The concentration of Chla in the winter cruise (mean \pm sd: 0.104 ± 0.024
3 $\mu\text{g L}^{-1}$) was about two times that in the summer cruise (mean \pm sd: $0.066 \pm 0.022 \mu\text{g L}^{-1}$)
4 (independent t-test, $p < 0.01$). Pico-phytoplankton ($< 3 \mu\text{m}$) accounted for most ($> 80\%$) of
5 the total Chla during both the cruises. The proportion of pico-phytoplankton in the winter
6 cruise (mean \pm sd: $81.9\% \pm 5.0\%$) was similar to that in the summer cruise (mean \pm sd: 83.0%
7 $\pm 1.8\%$) (independent t-test, $p > 0.1$).

8 **3.2 Feeding responses in dilution experiments**

9 The detailed results of each dilution experiment were listed in Table 1 and Table 2. Except for
10 linear feeding responses, both the saturated and saturated-increased feeding responses
11 described by Teixeira and Figueiras (2009) occurred in the dilution experiments during both
12 the cruises (Fig. 3a–c). Non-linear feeding responses occurred at eight of the 14 experiments
13 during the summer cruise, while those occurred at four of the 10 experiments during the
14 winter cruise (Table 1 and Table 2). During the winter cruise, substantially negative
15 phytoplankton growth rates (μ) and high microzooplankton grazing rates (m) were observed at
16 KJ53 (Fig. 3d–f). The negative μ obtained at KJ53 and the derivative parameters (e.g. m/μ)
17 were not included for the comparison between seasons and size fractions.

18 **3.3 Comparison of the phytoplankton growth and microzooplankton grazing** 19 **between the two seasons**

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

27 **3.4 Size-dependent phytoplankton growth and microzooplankton grazing**

1 The μ and m varied between size fractions. Higher m on the larger size fraction were observed
2 at YS (0.65 d^{-1} for the size fraction $> 3 \text{ }\mu\text{m}$ vs 0.33 d^{-1} for the size fraction $< 3 \text{ }\mu\text{m}$) and KJ35
3 (0.39 d^{-1} for the size fraction $> 3 \text{ }\mu\text{m}$ vs 0.30 d^{-1} for the size fraction $< 3 \text{ }\mu\text{m}$) during the
4 summer cruise. The μ of the larger-sized ($> 3 \text{ }\mu\text{m}$) and pico- ($< 3 \text{ }\mu\text{m}$) phytoplankton were
5 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
6 cruise, the m on the larger size fraction (mean \pm sd: $0.50 \pm 0.41 \text{ d}^{-1}$) were higher than those on
7 pico-phytoplankton (mean \pm sd: $0.27 \pm 0.27 \text{ d}^{-1}$) at seven of the 10 experiments, and the μ of
8 the larger size fraction (mean \pm sd: $0.88 \pm 0.38 \text{ d}^{-1}$) were also higher than those of
9 pico-phytoplankton (mean \pm sd: $0.42 \pm 0.31 \text{ d}^{-1}$) at seven of the nine experiments (exclude
10 data at KJ53) (Table 3). The m/μ was higher for the larger size fraction (57.3% at KJ35 and
11 61.2% at YS) than pico-phytoplankton (33.3% at KJ35 and 44.0% at YS) during the summer
12 cruise, while the m/μ was not significantly different between the two size fractions ($41.1\% \pm$
13 82.8% for the size fraction $> 3 \text{ }\mu\text{m}$, and $39.4\% \pm 21.1\%$ for the size fraction $< 3 \text{ }\mu\text{m}$) during
14 the winter cruise (Table 3).

15 Higher RPI for the larger-sized phytoplankton than pico-phytoplankton was observed during
16 both the cruises (Fig. 4). The RPI for the larger-sized phytoplankton was higher than one
17 (geomean \pm sd: 1.44 ± 0.31), while the RPI for pico-phytoplankton was lower than one
18 (geomean \pm sd: 0.90 ± 0.07) during the summer cruise. The RPI was significantly
19 (Mann-Whitney test, $p < 0.05$) higher for the larger-sized phytoplankton (geomean \pm sd: 1.44
20 ± 1.57) than pico-phytoplankton (geomean \pm sd: 0.61 ± 0.35) during the winter cruise.

21 **3.5 Nutrient limitation to the phytoplankton growth**

22 Nutrient limitation index (μ/μ_n) was significantly higher (Mann-Whitney test, $p < 0.05$)
23 during the summer cruise (geometric mean \pm sd: 0.80 ± 0.25) than the winter cruise
24 (geometric mean \pm sd: 0.54 ± 0.30). The μ/μ_n was approximate to or larger than one at five of
25 the 14 experiments in the summer. In contrast, the μ/μ_n was apparently lower than one at eight
26 of the nine experiments (excluding data at KJ53) in the winter (Table 1 and Table 2).

27 **3.6 Correlations between the growth and grazing rates with environmental** 28 **variables**

1 Taking all the data from the two cruises together, the average μ and m were $0.77 \pm 0.34 \text{ d}^{-1}$
2 and $0.39 \pm 0.20 \text{ d}^{-1}$. The μ were positively correlated with SST ($r = 0.43, p < 0.05$), SSS ($r =$
3 $0.55, p < 0.01$), μ/μ_n ($r = 0.50, p < 0.05$), net phytoplankton growth rate ($r = 0.83, p < 0.01$)
4 and m ($r = 0.76, p < 0.01$), but were negatively correlated with Chla ($r = -0.65, p < 0.01$)
5 (Table 4). The m were also positively correlated with SST ($r = 0.62, p < 0.01$) and SSS ($r =$
6 $0.48, p < 0.05$), but was negatively correlated with Chla ($r = -0.66, p < 0.01$) (Table 4).

7 **3.7 Correlations between the phytoplankton growth and microzooplankton** 8 **grazing**

9 The positive correlation ($r^2 = 0.57, p < 0.01$) between μ and m was observed during the
10 summer cruise (Fig. 5a). However, there was no significant correlation between the total μ
11 and m during the winter cruise ($r^2 = 0.12, p > 0.1$). By grouping μ and m separately for each
12 size fraction, it is found that the correlation was significant for the larger size fraction ($r^2 =$
13 $0.84, p < 0.05$) but not for the pico-phytoplankton ($r^2 = 0.41, p = 0.07$) (Fig. 5b).

14

15 **4 Discussion**

16 **4.1 Comparisons with other studies**

17 All the μ (except those obtained at KJ53 during the winter cruise) and m were within the
18 reviewed ranges based on global data collection (Calbet and Landry, 2004; Schmoker et al.,
19 2013). Substantive m but negative μ were observed at KJ53 (Fig. 3d–f), the only station
20 located on the continental shelf with a bottom depth less than 200 m (Fig. 1).

21 **4.1.1 Negative phytoplankton growth rates**

22 Negative μ have been extensively reported in previous dilution experiments (e.g. Burkill et al.,
23 1987; Loebel and Beusekon, 2008; Suzuki et al., 2002; Zhou et al., 2011). Zhou et al. (2013)
24 reviewed that temperature and light regime during incubation, insufficient sampling,
25 contamination of particle-free water and the added nutrients, and decay of phytoplankton
26 blooms could be the possible reasons for the negative μ in dilution experiments. Our measures
27 to mimic the in situ light and temperature during incubation exclude light and temperature

1 from the factors for the substantially negative μ . Insufficient sampling also unlikely
2 contributed to the negative μ since sufficient mixing was conducted before Chla sampling.
3 The substantive m could exclude contamination as the reason for the negative rates, because
4 contamination could not only cause phytoplankton death, but also less microzooplankton
5 grazing. We conjecture that phytoplankton community decay may occur and lead to the
6 negative μ at KJ53. The lowest silicate concentration at KJ53 may be related to the
7 phytoplankton community decay and the negative μ . Why the lowest silicate concentration
8 occurred and to what extent the low nutrient condition was related to the negative
9 phytoplankton growth is a topic that needs further assessment.

10 **4.1.2 Non-linear feeding responses**

11 Non-linear feeding responses including both the saturated and saturated-increased types
12 occurred in our dilution experiments. Non-linear responses were usually observed in
13 eutrophic waters with high prey abundance (Elser and Frees, 1995; Gallegos, 1989; Teixeira
14 and Figueiras, 2009). Non-linear feeding responses in dilution experiments conducted in the
15 oligotrophic subtropical Northeast Atlantic in summer have also been reported by Quevedo
16 and Anadón (2001) and Cáceres et al. (2013). However, the authors did not explain the
17 underlying reasons for these phenomena. The oligotrophic conditions and low phytoplankton
18 biomass in their study area were similar to those in the oligotrophic tropical waters of the
19 SSCS. Teixeira and Figueiras (2009) proposed that changes in the specific phytoplankton
20 growth rate due to varied nutrient limitation in experimental bottles, in the mortality rate
21 related to microzooplankton abundance and feeding behavior and even virus infection could
22 be related to the non-linear responses. As additional nutrients were added in the experiments
23 bottles, we do not think that nutrient limitation was the factor causing the non-linear
24 responses in our experiments. Rather we think that the sufficient nutrients added in the
25 experimental bottles led to high phytoplankton abundance in the less diluted bottles.
26 Microzooplankton may reach a maximum ingestion rate at high food concentration, and the
27 maximum ingestion rate may remain constant despite further increase in prey abundance,
28 which is often used to explain the occurrence of saturated feeding responses in dilution
29 experiments for eutrophic ecosystems (Gallegos, 1989; Moigis, 2006; Teixeira and Figueiras,

1 2009), and could explain those in our experiments. While the occurrence of
2 saturated-increased responses implies that decrease in the ingestion rate should be occurred to
3 the further increase in food availability. There is no concluded explanation for the decrease in
4 the ingestion rate. Teixeira and Figueira (2009) proposed that prey selection by
5 microzooplankton in waters with high and diverse food abundance may account for the
6 decrease. We consider that nutrient amendment in the experimental bottles may give rise to
7 relatively higher phytoplankton (food) abundance, leading to decrease in the ingestion rate
8 and accounting for the occurrence of saturated-increased responses in our experiments.

9 **4.1.3 Phytoplankton growth and microzooplankton grazing rates in low latitude** 10 **tropical waters**

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

15 There are few studies on microzooplankton grazing in low latitude tropical waters such as the
16 SCS. Landry et al. (1995) reported an average μ of $0.83 \pm 0.42 \text{ d}^{-1}$ and m of $0.72 \pm 0.56 \text{ d}^{-1}$
17 in February–March, and an average μ of $0.98 \pm 0.31 \text{ d}^{-1}$ and m of $0.57 \pm 0.17 \text{ d}^{-1}$ in August–
18 September in the central equatorial Pacific Ocean. These results are similar to ours observed
19 during the summer cruise. Landry et al. (2011) reported a low average μ of $0.43 \pm 0.14 \text{ d}^{-1}$ and
20 m of $0.31 \pm 0.11 \text{ d}^{-1}$ in the high-nutrient-low-chlorophyll (HNLC) equatorial Pacific Ocean.
21 These rates are similar to the rates observed during the winter cruise in the present study.
22 Yang et al. (2004) investigated the phytoplankton growth and microzooplankton grazing in
23 the western Pacific waters with latitudes similar to those of the SCS. Their reported μ and m
24 in summer were 0.35 to 0.75 d^{-1} , and 0.51 to 0.67 d^{-1} , which are similar to our results
25 observed during the summer cruise. Landry et al. (1998) showed an average μ of 0.5 d^{-1} and
26 an average m of 0.6 d^{-1} at the oligotrophic stations in the subtropical and tropical Arabian Sea.
27 Caron and Denett (1999) reported the m of $0.35 \pm 0.18 \text{ d}^{-1}$ and $0.30 \pm 0.17 \text{ d}^{-1}$ during the
28 northeast monsoon season and spring intermonsoon season in the Arabian Sea, respectively.

1 The m were similar to the low m ($0.27 \pm 0.13 \text{ d}^{-1}$) observed during the northeast monsoon
2 season in the present study.

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

12 **4.2 Seasonal variations in phytoplankton growth and microzooplankton grazing**

13 Our results showed pronounced seasonal changes in the phytoplankton growth and
14 microzooplankton grazing, but not the microzooplankton grazing impact on phytoplankton
15 (m/μ), in the SSCS. Many studies have found pronounced seasonality in the phytoplankton
16 growth and microzooplankton grazing in temperate and high latitude waters (e.g. Calbet et al.,
17 2008; Kim et al., 2007; Lawrence and Menden-Deuer, 2012; Loebl and Beusekon, 2008).
18 Nevertheless, there are few studies on the seasonality of the microzooplankton grazing in
19 oligotrophic tropical waters. From the perspective of phytoplankton growth, Landry et al.
20 (2011) reported that μ was slightly higher in December than September in the HNLC waters
21 of the equatorial Pacific, but the rate was not significantly different between periods of the
22 southwest monsoon and the early northeast monsoon in the Arabian Sea (Landry et al., 1998);
23 while Caron and Denett (1999) demonstrated that μ was approximately twice as high during
24 the northeast monsoon season than the spring intermonsoon period in the Arabian Sea. Chen
25 et al. (2013) observed that μ in surface water of the northern SCS was significantly higher in
26 the summer than winter. However, the previous studies showed no seasonal variation in
27 microzooplankton grazing in the HNLC waters of the equatorial Pacific (Landry et al., 1995,
28 2011), the Arabian Sea (Caron and Dennett, 1999; Landry et al., 1998) and oligotrophic

1 surface waters of the northern SCS (Chen et al., 2013). Significantly lower m in the summer
2 than winter at the chlorophyll maximum layer in the northern SCS has been reported by Chen
3 et al. (2013). From the data published by Quevedo and Anadón (2001), we calculated that the
4 μ and m were higher in the summer ($0.61 \pm 0.43 \text{ d}^{-1}$ and $0.49 \pm 0.28 \text{ d}^{-1}$) than in the spring
5 ($0.33 \pm 0.27 \text{ d}^{-1}$ and $0.29 \pm 0.18 \text{ d}^{-1}$) in the oligotrophic subtropical Northeast Atlantic. Lower
6 average μ and m for pico-phytoplankton in the winter than summer have been recently
7 reported in the subtropical East China Sea (Guo et al., 2014). These results are consistent with
8 the significant seasonal variations in the phytoplankton growth and microzooplankton grazing
9 in the SCS in the present study.

10 **4.3 East Asian monsoon influencing seasonal variations in phytoplankton** 11 **growth and microzooplankton grazing by affecting nutrient supply and salinity** 12 **decrease in surface water**

13 Seasonal variations in environmental variables, related to the East Asian monsoon, may
14 directly and /or indirectly influence phytoplankton growth and microzooplankton grazing.
15 Significant seasonal changes in a host of environmental variables including salinity, dissolved
16 inorganic nutrients, and phytoplankton biomass were indeed observed (Table 5). The
17 relationship between μ and m with the environmental variables indicate that seasonal
18 variations in the phytoplankton growth and microzooplankton grazing were related to the
19 contrasting environmental conditions under the influence of the East Asian monsoon (Table
20 4).

21 Many studies have showed increased m during the warmest seasons and reduced m and μ in
22 winter months (e.g. Ara and Hiromi, 2009; Lawrence and Menden-Deuer, 2012). Both the μ
23 and m were positively correlated with temperature in the present study. However, the mean
24 seasonal temperature discrepancy was less than 0.4°C and not significant (independent t-test,
25 $p = 0.055$) (Table 5). We do not think this slightly temperature variation alone could account
26 for the substantial decrease in the μ and m observed in the northeast monsoon season.

27 We think that changes in dissolved inorganic nutrients and other factors associated with SSS
28 may be the main drivers for the variations in the phytoplankton growth and microzooplankton

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

13 The arrival of strong northeast monsoon may bring not only nutrient supply to the surface
14 water as a result of enhanced vertical mixing, but also SSS decrease due to heavy frontal
15 rainfall when the cold air meet the warm and wet local air. A strong rainy season is usually
16 developed from October to January in the SCS (Wyrski, 1961). The remote sensing data
17 from the Tropical Rainfall Measuring Mission indeed showed that apparently larger rainfall
18 occurred during the winter than summer (Fig. 6). The large rainfall could not only bring
19 nutrients such as nitrate in rainwater to the surface water (Kodama et al., 2011), but also
20 dilute and freshen the surface seawater (as those observed during the winter cruise). In
21 addition, after the passage of the strong cold air, the winds over the SCS are always weak.
22 The low-wind condition could facilitate the less saline surface water overlying on the more
23 saline deep water to form the “freshwater cap” (Zeng et al., 2009). The salinity discrepancy
24 between surface water and that at 25 m in depth was several times higher during the winter
25 cruise (0.99) than the summer cruise (0.17) (Table 1 and Table 2), indicating that a freshwater
26 cap formed during the winter cruise in the SCS (Fig. 2).

27 The freshwater cap could enhance water stratification and block nutrient supply to the surface
28 layer, thus nutrients in surface water could be depleted by the phytoplankton community, and
29 lead to nutrient limitation to the growth of the phytoplankton community with already

1 increased biomass. The high μ/μ_n (approximate to or higher than one) indicated that
2 phytoplankton growth was only slightly or even not nutrient-limited during the summer cruise
3 (Table 1). Similar results in the oligotrophic subtropical Northeast Atlantic have also been
4 reported (Cáceres et al., 2013; Quevedo and Anadón, 2001). In contrast, severe nutrient
5 limitation indicated by the lower μ/μ_n (0.54 ± 0.30) were observed during the winter, which
6 may account for part of the low μ (Table 5).

7 The freshwater cap could also impact the microzooplankton grazing indirectly. First, the
8 formation of freshwater cap might inhibit the migration of mesozooplankton (e.g. copepods)
9 into the water with lower salinity and change the mesozooplankton composition in the water
10 column in the studied waters, as similar salinity effects on mesozooplankton have been
11 observed in estuarine and inshore waters (Grindley, 1964; Zhou et al., 2015b), which can
12 release the mesozooplankton grazing pressure on ciliates, then through trophic cascades
13 increase the ciliate grazing on nanoflagellates (HNF) (Chen et al., 2012), reducing the
14 abundance of HNF the main grazer on pico-phytoplankton (Safi and Hall, 1999), and
15 releasing the grazing pressure on pico-phytoplankton (Klauschies et al., 2012). Second, as
16 discussed above, the impeding effect of freshwater cap on phytoplankton accesses to nutrients
17 could lead to poor food quality of phytoplankton as prey, and thus reduce the grazing activity
18 of microzooplankton. Both the arguments suggest that the SSS decrease could result in low
19 microzooplankton grazing rate on pico-phytoplankton such as that observed in the winter
20 cruise.

21 **4.4 Decoupling between phytoplankton growth and microzooplankton grazing** 22 **influenced by the winter monsoon**

23 The different relationship between μ and m suggested that coupling between
24 phytoplankton and microzooplankton also varied between the two seasons. Close coupling
25 between phytoplankton growth and microzooplankton grazing indicated by the positive
26 correlation between μ and m were broadly reported in previous studies (discussion in Zhou et
27 al., 2011). Our results also showed that the microzooplankton grazing was closely coupled
28 with the phytoplankton growth in the summer cruise, but was not in the winter cruise.

1 Previous studies suggested that the lower nutrient concentration and phytoplankton biomass
2 may facilitate the higher coupling between phytoplankton growth and microzooplankton
3 grazing in summer than in winter (Cáceres et al., 2013; Schmoker et al., 2013). We consider
4 that the influence of the northeast monsoon could break the coupling between phytoplankton
5 growth and microzooplankton grazing in winter. Firstly, the comings of strong northeast
6 monsoon supply nutrients from deep water to the surface by enhancing vertical mixing. This
7 episodic input of nutrients could break the coupling between phytoplankton and
8 microzooplankton by stimulating μ overwhelming corresponding m and/or changing the
9 phytoplankton community composition (Irigoien et al., 2005). Secondly, large rainfall and the
10 resulted SSS decrease may decouple the phytoplankton (especially the pico-phytoplankton)
11 growth and microzooplankton grazing through indirectly influencing the phytoplankton
12 growth and microzooplankton grazing as discussed in Section 4.3.

13 Why the coupling between phytoplankton growth and microzooplankton grazing was still
14 significant for the larger size fraction but not for the pico-phytoplankton in winter is still open
15 to discuss. On the basis of the size-fractionated data from two sites (YS and KJ35) in summer,
16 it is found that both the growth and grazing rates for the larger size fraction were comparable
17 between the two seasons, while the growth rates of pico-phytoplankton was apparently lower
18 in winter than in summer. We conjecture that the significant environmental change (such as
19 salinity decrease) in winter may do harm to the growth of pico-phytoplankton dominated by
20 *Prochlorococcus* in the southern SCS (Wang et al., 2016), and as a result, the inhibited
21 pico-phytoplankton growth could contribute to the decoupling between the
22 pico-phytoplankton growth and microzooplankton grazing. To confirm this, further studies on
23 the effects of environmental variables on pico-phytoplankton growth are apparently
24 necessary.

25 **4.5 Implications of the low microzooplankton grazing impact on phytoplankton**

26 As discussed in Section 4.1.3, our data of the μ and m were similar to most of the previous
27 results observed in low latitude tropical waters, but the m/μ in the SCS was relatively lower
28 (< 50% on average) than those reported in the previous studies except that a similar value

1 (49%) in the Arabian Sea during the northeast monsoon season has been reported by Caron
2 and Denett (1999). Our m/μ is also lower than the mean values in most of the global sea
3 regions (Schmoker et al., 2013). The low m/μ , i.e. the high growth differential over grazing
4 indicates low remineralization of organic matter mediated by microzooplankton and mismatch
5 between the primary production and microzooplankton grazing. As a result, potentially high
6 vertical biogenic particle fluxes via the formation of phytoplankton aggregates and
7 consumption of those aggregates by mesozooplankton could occur (Legendre and
8 Rassoulzadegan, 1996; Schmoker et al., 2013). Higher vertical biogenic particle fluxes in the
9 prevailing periods of the monsoons than between the monsoons in the SCS was indeed
10 observed (Wan et al., 2010). Our results suggest that the high growth differential over
11 microzooplankton grazing may account for part of the high vertical biogenic particle fluxes in
12 the SCS.

13 **4.6 Size-selective grazing contributes to the pico-phytoplankton dominance in** 14 **the oligotrophic waters of the SCS**

15 Size selectivity of microzooplankton grazing have been proposed in previous studies (e.g.,
16 Burkill et al., 1987; Froneman and Perissinotto, 1996; Huang et al., 2011; Kuipers and Witte,
17 1999; Sun et al., 2004), and varied patterns of the size selectivity have been reported. For
18 example, higher grazing rate on smaller phytoplankton has been reported accounting for the
19 larger-sized phytoplankton dominance in eutrophic waters (e.g. Strom et al., 2007), but no
20 such pattern were found in other studies (Lie and Wong, 2010; Safi et al., 2007; Strom and
21 Fredrickson, 2008; Zhou et al., 2011). In contrast, Zhou et al. (2015a) found that
22 microzooplankton selectively grazed on nano-phytoplankton (3–20 μm) in the oligotrophic
23 waters of the SCS in summer, and proposed that the size-selective grazing on
24 nano-phytoplankton contributes to the pico-phytoplankton dominance there. Higher
25 microzooplankton grazing rate on the large-sized phytoplankton ($> 5 \mu\text{m}$) than the smaller
26 one ($< 5 \mu\text{m}$) has also been recently reported in the oligotrophic subtropical Northeast
27 Atlantic (Cáceres et al., 2013).

1 The higher RPI (Fig. 4) for and m (Table 1 and Table 2) on the larger-sized phytoplankton
2 than pico-phytoplankton suggests that microzooplankton selectively grazed on the
3 larger-sized phytoplankton in the SCS both in the summer and winter. The size-selective
4 grazing balanced part of the relative higher growth rate of the larger-sized phytoplankton (> 3
5 μm), and led to the nearly equal grazing impact (m/μ) on both the larger-sized phytoplankton
6 and pico-phytoplankton in the winter (Table 3). As a result, microzooplankton grazing
7 maintained the pico-phytoplankton dominance in the study waters during the winter.
8 Therefore, on the basis of the results observed in the present study and those reported by Zhou
9 et al. (2015a) and Cáceres et al. (2013), we propose that microzooplankton grazing
10 contributes to the pico-phytoplankton dominance in oligotrophic subtropical and tropical
11 waters such as that of the SCS.

12

13 **5 Conclusions**

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

19 The m/μ did not significantly vary between the two seasons. The low m/μ ($< 50\%$ on average),
20 i.e. the high growth differential over microzooplankton grazing indicates low remineralization
21 of organic matter mediated by microzooplankton and mismatch between the μ and m , and
22 may account for part of the high vertical biogenic particle fluxes in the prevailing periods of
23 the monsoons in the SCS.

24 Significant size-selective grazing on the larger-sized ($> 3 \mu\text{m}$) phytoplankton was observed in
25 the SCS both in the summer and winter, which indicates that microzooplankton grazing
26 contributes to the pico-phytoplankton dominance in the oligotrophic tropical waters of the
27 SCS.

28

1 **Author contribution**

2 Linbin Zhou designed the experiments and carried them out. Yehui Tan and Liangmin Huang
3 supervised the projects. Zifeng Hu provided the monthly rainfall data and the Figure 6. Zhixin
4 Ke provided part of the nutrient data. Linbin Zhou prepared the manuscript with contributions
5 from all authors.

6

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1 **Table 1** Summary of environmental variables, phytoplankton growth rates with (μ_n , d⁻¹) and (μ , d⁻¹) without nutrient amendment, and
 2 microzooplankton grazing rates (m , d⁻¹) in the southern South China Sea in May–June 2009. SST, surface seawater temperature (°C);
 3 SSS, surface seawater salinity; 25-T (S) seawater temperature (salinity) at 25 m in depth; Si, silicate ($\mu\text{mol L}^{-1}$); P, phosphate (μmol
 4 L^{-1}); N, nitrate plus nitrite ($\mu\text{mol L}^{-1}$); Chla, chlorophyll *a* ($\mu\text{g L}^{-1}$); n, the number of data points in the linear part; R², the determination
 5 coefficient of the regression of the linear part; the superscript ‘a’ denotes a saturated feeding response; the superscript ‘b’ denotes a
 6 saturated-increased feeding response. The significant level (*p*) of regressions when saturated or saturated-increased feeding responses
 7 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	Chla	μ_n	m	μ	R ²	n	<i>p</i>	μ/μ_n	m/μ
KJ28	21-May	2899	29.90	32.90	28.02	33.07	2.0	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	0.086	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	0.082	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	0.079	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	0.049	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	0.036	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	0.050	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	0.050	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	0.044	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	0.096	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	0.057	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	0.045	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	0.062	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	0.105	0.54	0.09	0.54	0.99	4 ^a	0.006	0.99	0.16

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

8

Station	Date	Depth	SST	SSS	25-T	25-S	Si	> 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	0.013	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	0.024	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	0.013	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	0.015	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	0.017	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	0.037	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	0.020	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	0.017	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	0.014	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	0.017	0.077	0.87	0.34	0.57	0.94	4 ^a	0.030	0.66	0.59

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

9

Size fraction	Station	μ_n	$m \pm se$	$\mu \pm se$	R^2	n	p	μ/μ_n	m/μ
< 3 μm	KJ28	0.57	0.15 ± 0.06	0.39 ± 0.04	0.57	8	0.050	0.69	0.37
	KJ32	1.29	0.16 ± 0.12	0.78 ± 0.09	0.23	8	0.231	0.60	0.21
	KJ35	1.30	0.50 ± 0.18	1.01 ± 0.15	0.65	6	0.052	0.78	0.50
	KJ39	0.50	0.12 ± 0.15	0.05 ± 0.07	0.89	4 ^a	0.058	0.10	2.42
	KJ42	0.26	0.05 ± 0.18	0.33 ± 0.09	0.04	4 ^b	0.791	1.24	0.16
	KJ47	1.27	0.10 ± 1.67	0.18 ± 1.16	0.00	4 ^a	0.958	0.14	0.55
	KJ50	0.73	0.01 ± 0.05	0.32 ± 0.02	0.96	4 ^b	0.020	0.43	0.03
	KJ53	-2.64	0.89 ± 0.34	-2.55 ± 0.25	0.59	8	0.027	0.96	-0.35
	KJ65	0.85	0.29 ± 0.17	0.15 ± 0.14	0.42	6	0.167	0.18	1.88
	KJ73	0.80	0.39 ± 0.05	0.58 ± 0.03	0.94	6 ^a	0.001	0.73	0.67
> 3 μm	KJ28	1.20	0.22 ± 0.07	0.77 ± 0.05	0.68	8	0.012	0.64	0.29
	KJ32	1.34	0.17 ± 0.05	0.62 ± 0.04	0.65	8	0.015	0.46	0.28
	KJ35	1.14	0.09 ± 0.06	0.62 ± 0.04	0.26	8	0.192	0.54	0.14
	KJ39	1.71	1.31 ± 0.38	1.76 ± 0.19	0.86	4 ^b	0.075	1.03	0.75
	KJ42	0.89	0.38 ± 0.49	1.14 ± 0.24	0.23	4 ^b	0.520	1.28	0.34
	KJ47	2.32	0.45 ± 0.46	0.69 ± 0.33	0.14	8	0.365	0.30	0.65
	KJ50	1.34	0.68 ± 0.15	0.97 ± 0.07	0.91	4 ^a	0.046	0.72	0.70
	KJ53	-1.17	1.10 ± 0.35	-1.39 ± 0.26	0.65	8	0.016	1.18	-0.79
	KJ65	1.23	0.22 ± 0.21	0.56 ± 0.15	0.16	8	0.328	0.46	0.40
	KJ73	1.03	0.35 ± 0.18	0.77 ± 0.09	0.92	4 ^b	0.041	0.75	0.46

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11

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

5

	μ	m	SST	SSS	Si	Chla	m/μ	μ/μ_n	$\mu-m$
μ	1	.762**	.425*	.547**	-.348	-.646**	-.356	.496*	.827**
m		1	.617**	.477*	-.206	-.660**	.252	.163	.266
SST			1	.225	-.289	-.542**	.093	.332	.098
SSS				1	-.465*	-.519*	-.061	.293	.396
Si					1	.304	.239	-.406	-.336
Chla						1	-.007	-.404	-.389
m/μ							1	-.597**	-.748**
μ/μ_n								1	.596**
$\mu-m$									1

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

7

1 **Table 5** Comparisons of environmental variables, and phytoplankton growth and microzooplankton grazing between May–June 2009
 2 and November 2010 in the southern South China Sea. SST, surface seawater temperature ($^{\circ}\text{C}$); SSS, surface seawater salinity; Si,
 3 silicate ($\mu\text{mol L}^{-1}$); Chla, chlorophyll *a* ($\mu\text{g L}^{-1}$); μ_n , phytoplankton growth rates with nutrient amendment (d^{-1}); μ , phytoplankton
 4 growth rates without nutrient amendment (d^{-1}); *m*, microzooplankton grazing rates (d^{-1}); *p*, the significant level. The mean ratio values
 5 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
 6 t-test. The ratio values of μ/μ_n and m/μ were shown as geometric mean \pm standard deviation, while other parameters were displayed as
 7 arithmetic mean \pm standard deviation.

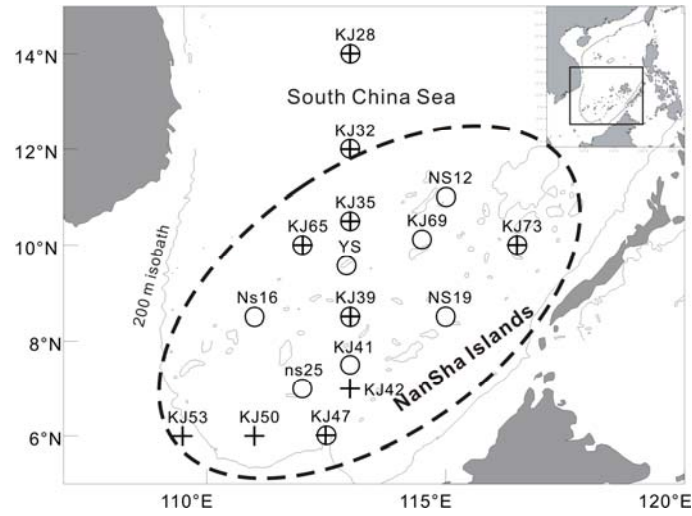
Season	SST	SSS	Si	Chla	μ/μ_n	<i>m</i>	μ	m/μ
Summer	29.64 \pm 0.60	32.82 \pm 0.16	3.8 \pm 2.6	0.066 \pm 0.022	0.80 \pm 0.25	0.46 \pm 0.20	0.92 \pm 0.32	0.49 \pm 0.23
Winter	29.27 \pm 0.25	31.92 \pm 0.30	7.9 \pm 4.7	0.104 \pm 0.024	0.54 \pm 0.30	0.27 \pm 0.13	0.54 \pm 0.22	0.48 \pm 0.33
<i>p</i>	0.055	< 0.01	< 0.05	< 0.01	< 0.05	< 0.01	< 0.01	> 0.1

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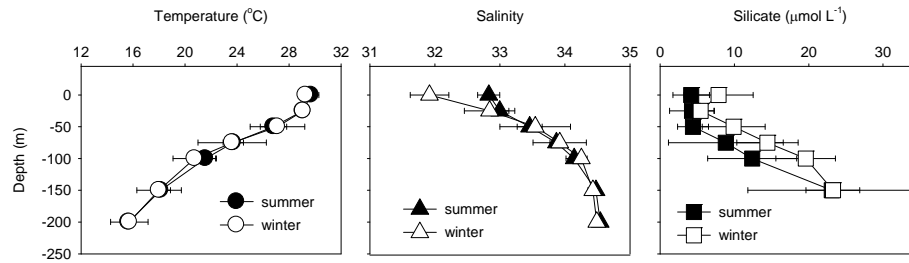


2

3 **Figure 1.** Stations for dilution experiments in the southern South China Sea during May to June
4 2009 and November 2010. The open circle indicates experimental stations during May to June
5 2009; the cross indicates experimental stations during November 2010. The dashed circle shows
6 the schematic area of the Nansha Islands.

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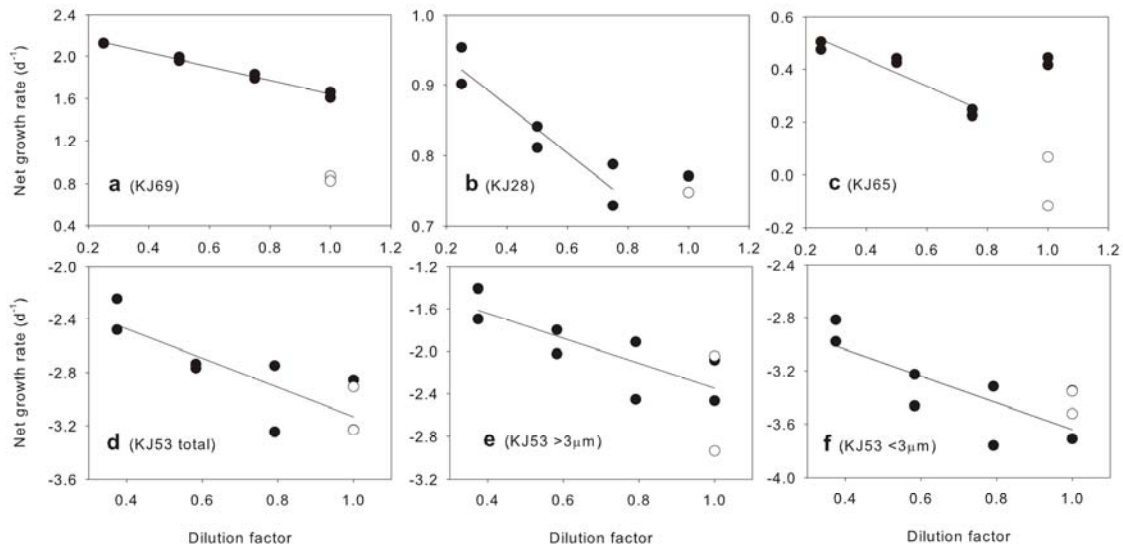
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Figure 2. Vertical profiles of seawater temperature, salinity and silicate concentration in the southern South China Sea during May to June 2009 (summer) and November 2010 (winter). The error bar indicates standard deviation.

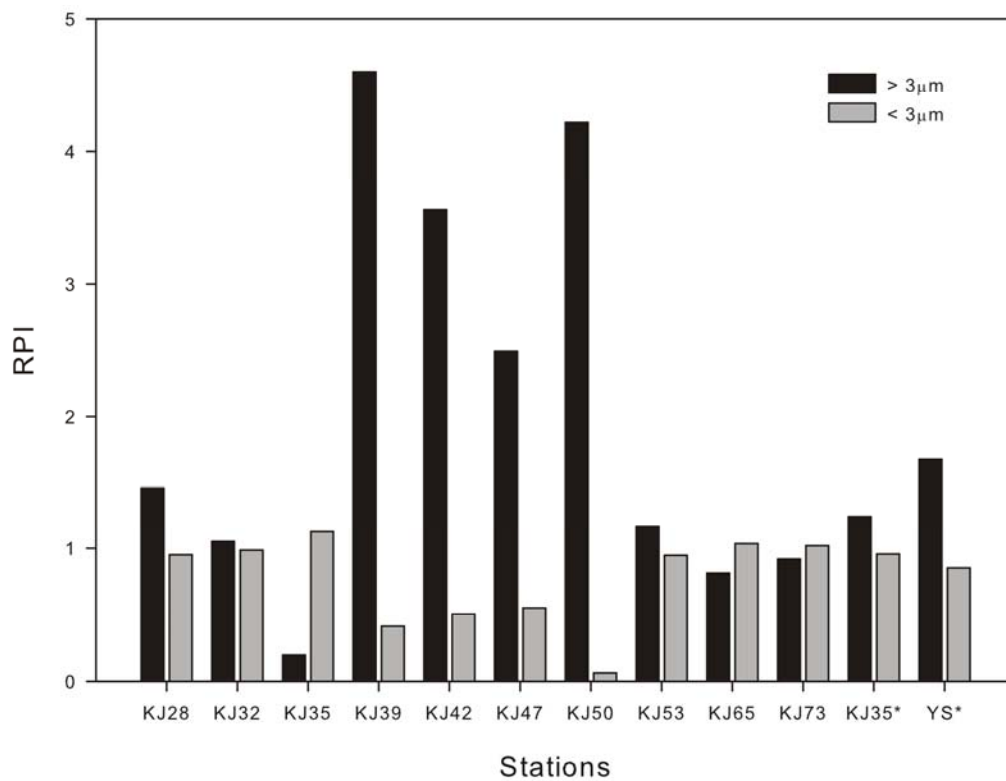
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2

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

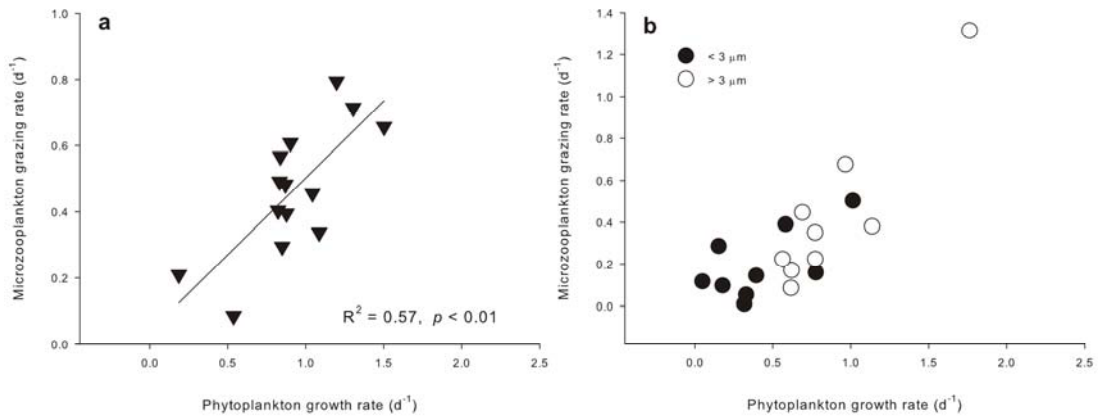
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2 **Figure 4.** Relative preference index (RPI) for size-fractionated phytoplankton. > 3 µm, size
 3 fraction larger than 3 µm; < 3 µm, size fraction smaller than 3 µm; the asterisk indicates
 4 experiments conducted in May 2009, others were conducted in November 2010. RPI > 1
 5 indicates positive selection and vice versa.

6



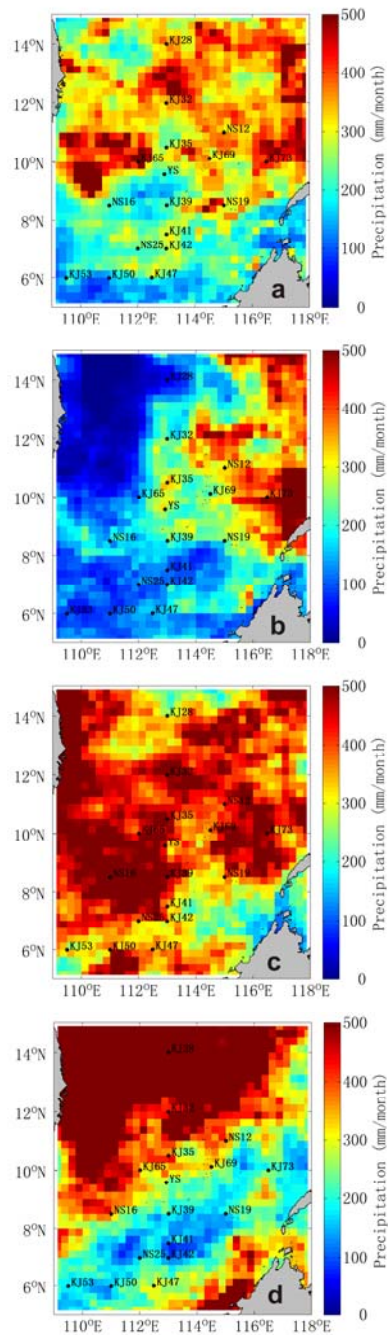
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2 **Figure 5.** Correlation between phytoplankton growth and microzooplankton grazing rates. **(a)**

3 May to June 2009; **(b)** November 2010; > 3 μm, size fraction larger than 3 μm; < 3 μm, size

4 fraction smaller than 3 μm.

5



1

2 **Figure 6.** Monthly rainfall in the southern South China Sea estimated on the basis of data from
 3 the Tropical Rainfall Measuring Mission. (a) May 2009; (b) June 2009; (c) October 2010; (d)
 4 November 2010.

5