Phytoplankton growth and microzooplankton grazing in the central and northern South China Sea in the spring intermonsoon season of 2017

Dawei Chen[1, 2, 3], Congcong Guo[1, 2], Linhui Yu[3], Yuanzhen Lu[3], Jun Sun[1, 2*]

[1] Tianjin Key Laboratory of Marine Resources and Chemistry, Tianjin University of Science and Technology, Tianjin 300457, China

[2] Research Centre for Indian Ocean Ecosystem, Tianjin University of Science and Technology, Tianjin 300457, China

[3] State Key Laboratory of Tropical Oceanography, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

Abstract

Phytoplankton growth rates and mortality rates were experimentally examined at 21 stations during the 2017 spring intermonsoon (April to early May) in the northern and central South China Sea (SCS) using the dilution technique, with emphasis on a comparison between the northern and central SCS areas which had different environmental factors. There had been higher temperature but lower nutrients and chlorophyll *a* concentrations in the central SCS than those in the northern SCS. The mean rates of phytoplankton growth (μ_0) and microzooplankton grazing (m) were (0.88±0.33) d^{-1} and (0.55±0.22) d^{-1} in the central SCS, and both higher than those in the northern SCS with the values of μ_0 ((0.81±0.16) d^{-1}) and m ((0.30±0.09) d^{-1}), respectively. Phytoplankton growth and microzooplankton grazing rates were significantly coupled in both areas. The microzooplankton grazing impact (m/μ_0) on phytoplankton was also higher in the central SCS (0.63±0.12) than that in the northern SCS (0.37±0.06). The microzooplankton abundance was significantly correlated with temperature in the surface. Temperature might more effectively promote the microzooplankton grazing rate than phytoplankton growth rate, which might contribute to higher m and m/μ_0 in the central SCS. Compared with temperature, nutrients mainly affected the growth rate of phytoplankton. In the nutrient enrichment treatment, the phytoplankton growth rate (μ_n) was higher than μ_0 in the central SCS, suggesting phytoplankton growth in the central SCS was nutrient limited. The ratio of μ_0/μ_n was significantly correlated with nutrients concentrations in the both areas, indicating the limitation of nutrients was related to the concentrations of background nutrients in the study stations.

Key words: dilution technique, phytoplankton growth, microzooplankton grazing, South China Sea, spring intermonsoon season

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1 Introduction

Marine phytoplankton is the main primary producer and plays a crucial role in marine ecosystems and the photosynthesis of phytoplankton is the key to the conversion of inorganic carbon to organic carbon in the pelagic food web (Sun, 2013). Microzooplankton are the zooplankton with body lengths of less than 200 μm including flagellates, ciliates, heterotrophic dinoflagellates and small metazoan larvae (Jyothibabu et al., 2008). Microzooplankton are the major grazers of phytoplankton, and control the growth of phytoplankton by grazing which is called "top-down" control (Landry and Calbet, 2004; Lehman, 1991). Notably, they can consume 60%–80% of primary production in the sea and regulate phytoplankton community composition via se-

lective grazing, and affect the ultimate fate of carbon flow from microbial loop to the traditional food web (Banse, 2007; Landry and Calbet, 2004; Schmoker et al., 2013; Strom and Welschmeyer, 1991).

The main way to obtain the knowledge about the importance of microzooplankton in the marine food web is the dilution experiments (Landry and Hassett, 1982). The dilution technique has been extensively used to estimate phytoplankton growth rates and simultaneously estimates mortality rates due to microzooplankton grazing (Schmoker et al., 2013). Since it was proposed, the dilution technique has been widely conducted in different ecosystems and these studies have provided great insight into understanding microzooplankton grazing and phytoplank-

ton growth in marine ecosystems (Landry and Calbet, 2004; Schmoker et al., 2013).

The South China Sea (SCS) is a semi-closed, secondary large marginal sea around the world located in the western Pacific. The SCS is heavily influenced by the southwest summer monsoon during the top of May to September, while is under the influence of the northeast winter monsoon during November to the next April (Su, 2004). Within this monsoon system, the central South China Sea (CSCS) is characterized with annual high temperature in surface water (26–29°C), while the salinity sometimes less than 34 influenced by rainfall and various water masses (Li and Su, 2000). The upper water column is often stratified and remains oligotrophic, therefore, the level of primary productivity is low due to depletion of inorganic nutrients (Wong et al., 2007). The ecological environment in the northern South China Sea (NSCS) is more complicated including estuaries, bays, shelves, slopes and open seas, and is susceptible to various physical processes such as typhoon, coastal upwelling, mesoscale eddy, river plume, etc. (Gong et al., 1992). The more complex physical processes may lead to phytoplankton blooms and the increase in primary production in the NSCS (Ning et al., 2004; Zheng and Tang, 2007; Hu et al., 2014).

There are already some studies on microzooplankton grazing and phytoplankton growth in the SCS, but most of them are concentrated in the region of the NSCS in summer or winter (e.g., Su et al., 2007; Zhou et al., 2011; Chen et al., 2013). So far, there are only few studies (Chen et al., 2009) investigating the basin area of the CSCS and none focus on comparison between the northern

and central SCS with different environmental conditions, especially during the spring intermonsoon period.

2 Materials and methods

2.1 *Study area*

A total of 21 experiments were conducted in the northern and central SCS during the Open Cruise Project in central South China Sea of National Nature Science Foundation of China, aboard the R/V *Shiyan 1* from 30 March to 6 May, 2017 (Fig. 1).

For identifying spatial patterns, we classified the stations into two groups according to geography. Stations C34, C31, C26, C22 and Stations C2, L5, C9, C13 were located along 18°N transect and perpendicular to the shoreline in the NSCS, respectively. The remaining stations were located in the central region of the SCS (Fig. 1). Moreover, according to bathymetry: Station C2 was only station located in the shelf (bottom depth ≤ 100 m); Stations L5, C9, C34 were located in the slope (100 m < bottom depth ≤ 2 000 m); Stations C64, C66, C70 were distributed in waters around the Nansha Islands with bottom depth of about 2 000 m; the bottom depth of remaining stations were more than 3 000 m besides Station C31 (Fig. 1).

2.2 *Dilution experiment*

Seawater for dilution experiment was collected from 0.5 m depth using a clean plastic bucket, then was pre-screened through a 200 μm mesh and gently pooled into polycarbonate carboys, called the initial seawater (ISW). The particle-free water (PFW)
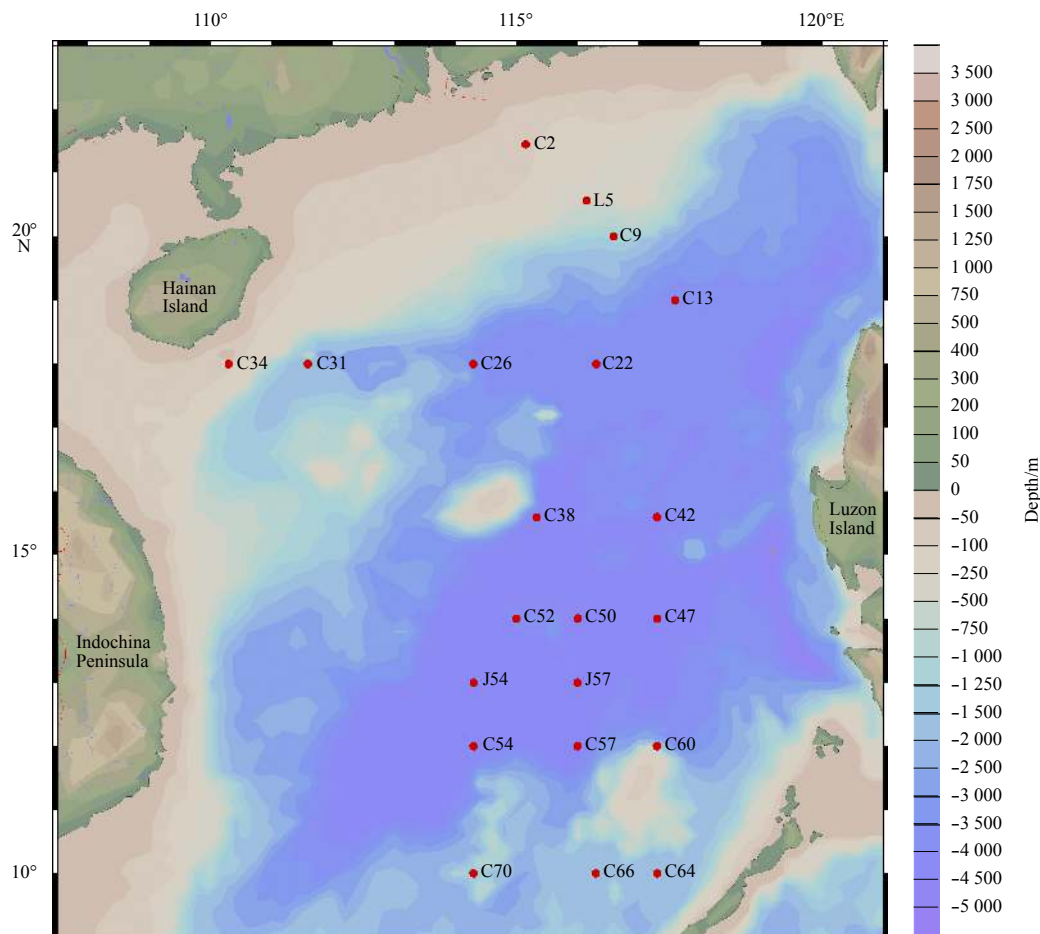

Fig. 1. Map of the experimental stations in the South China Sea.

was prepared by filtering the same seawater through a capsule filter with a pore size of 0.22 μm. For the dilution experiments, the ISW was diluted by PFW with a dilution series of 20%, 40%, 60%, 80% and 100% (ISW: (ISW plus PFW)) into 1.5 L transparent polycarbonate bottles in triplicate. In order to comply with the assumption of dilution technique (Landry and Hassett, 1982), all the bottles were enriched with additional nutrients (final concentrations of 0.5 μmol/L NH_4Cl, 0.03 μmol/L KH_2PO_4, 0.5 μmol/L Na_2SiO_3 and 1.0 nmol/L $FeSO_4$) to promote constant phytoplankton growth rates. Three same bottles were filled with ISW without nutrient addition as control treatment. To simulate *in situ* conditions, all of the incubation bottles were placed in a deck incubator for 24 h with temperature controlling by running surface seawater and covered with neutral-density screens for light regime control. All the filtration apparatus, mesh, carboy containers and incubation polycarbonate bottles were soaked in 10% HCl and thoroughly rinsed with MiliQ-water and filtered seawater prior to each experiment.

Triplicate seawater samples from the ISW were collected at the beginning of each incubation experiment to determine the initial Chlorophyll *a* (Chl *a*) concentrations. The ISW samples of 500 to 1 000 mL were filtered through 25 mm Whatman GF/F glass fiber filters under low vacuum. After filtration, the filters were stored in the dark at –20°C immediately until analysis. For each incubation bottle, the same procedures were used to determine Chl *a* concentrations to calculate phytoplankton growth rates and microzooplankton grazing rates after incubation.

Seawater of 100 mL from ISW was filtered with a 0.45 μm acetate fiber membrane and stored in –20°C for nutrient analysis.

Water samples from ISW for phytoplankton (1 L) and microzooplankton (2 L) taxonomy analyses were fixed with buffered formalin (final concentration 2%) and 1% Lugol's solution, separately. These samples were stored cool in the dark until they were counted using the Utermöhl method (Utermöhl, 1958).

2.3 *Sample analyses*

The filters of Chl *a* samples were extracted with 5 mL 90% acetone and stored in darkness for 24 h at –20°C. Then, the Chl *a* concentrations were measured with a Turner-Designs Trilogy fluorometer (Welschmeyer, 1994). Nutrients (nitrate plus nitrite, phosphate, silicate, ammonium) were measured by a continuous flow AutoAnalyzer (Bran+Luebbe) on the basis of standard procedures (Hansen and Koroleff, 2007).

The data of water temperature and salinity were obtained using a CTD (Sea Bird 911 Plus).

For phytoplankton qualitative and quantitative analyses, the sample of 1 L was concentrated to 10 mL after sedimentation for 24 h. The supernatant was siphoned, then sub-sample were identified and counted to species level using an inverted microscope at magnifications of 200× or 400× according to the method of Utermöhl (1958). The phytoplankton taxonomy identification was carried out as described by Jin et al. (1965) and Yamaji (1966).

For enumeration of the microzooplankton, the samples were concentrated to 100 mL using a silicone tube after 24 h sedimentation. Then sub-samples were chosen and placed in Utermöhl chamber to determine species composition and abundance after settling for several hours with the same way as phytoplankton. The microzooplankton was classified into three main categories: ciliates, copepod nauplii, and heterotrophic dinoflagellates. Ciliates were classified into aloricate ciliates and loricate tintinnids according to their cilia and shapes (Zhang et al., 2012, 2015). Heterotrophic dinoflagellates were identified and counted in this

study from the phytoplankton microscopy (Sun and Guo, 2011). The copepod nauplii were discriminated as one group and one single species.

2.4 *Date analyses*

Followed the standard data analysis procedures for the dilution method presented by Landry and Hassett (1982), the prey apparent growth rate (AGR, d^{-1}) after incubation in each incubation bottle was assumed as the following equation independently:

$$\text{AGR} = \frac{\ln(P_t/P_o)}{t},$$

where *t* is the duration of the incubation in days and P_0 and P_t represent the initial and final concentrations of Chl *a*, respectively. During the nutrient-added treatment, the phytoplankton growth rate (μ_n, d^{-1}) and microzooplankton grazing rate (*m*, d^{-1}) were calculated by least-square regression between AGR with nutrient addition and dilution factors (ISW:ISW plus PFW). The *m* and μ_n values were calculated as the absolute value of the slope and the intercept of the linear regression equation, respectively. *In situ* phytoplankton instantaneous growth rates (μ_0, d^{-1}) were calculated as the sum of *m* and the AGR without nutrient addition in the control group (Landry, 1993).

The grazing impact on phytoplankton by microzooplankton was often expressed by the ratio of microzooplankton grazing rate to phytoplankton growth rate (m/μ_0) and other indices as follows. The percentage of phytoplankton standing stock (P_i, %/d) and potential primary production (P_p, %/d) ingested by the microzooplankton per day were calculated using the following equations (Verity et al., 1993):

$$P_i = (1 - e^{mt}) \times 100\%,$$

$$P_p = (e^{\mu_0 t} - e^{(\mu_0 - m)t})/(e^{\mu_0 t} - 1) \times 100\%,$$

where *t* is the time (d); *m* is microzooplankton grazing rate (d^{-1}); μ_0 is phytoplankton instantaneous growth rate (d^{-1}).

A Tukey test was performed to compare the differences of various environmental and biological parameters between the NSCS and CSCS in present study. Pearson test was operated to test the correlation between variables in present study. $p < 0.05$ or 0.01 was used as the significance level. All tests were performed using SPSS 14.0.

3 Result

3.1 *Environmental variables and Chl a*

The sampling information and geographical, physical, and chemical parameters at the twenty-one stations are presented in Table 1. Surface water temperature was quite high and was always above 25°C. The temperature was lowest at Station L5 with the value of 25.29°C and highest at Station C47 (29.60°C). The average water temperature was (28.17±1.32)°C. Surface salinity ranged from 33.02 to 34.32 with an average value of 33.68±0.31. Temperature increased gradually from north to south in our study area, while salinity was the opposite. The nutrients concentrations of surface water varied among stations (Table 1). The maximum concentration of nitrate plus nitrite was 0.76 μmol/L at Station C22 (besides Station C26). Silicate concentration ranged

Table 1. Environmental factors of experimental sites

Station	Date	Time	Depth/m	T/°C	S	NO_x/μmol·L⁻¹	SiO_3^{2-}/μmol·L⁻¹	PO_4^{3-}/μmol·L⁻¹	NH_4^-/μmol·L⁻¹	Chl a/μg·L⁻¹
CSCS										
C38	Apr. 1	8:40	4 180	29.36	33.41	0.56	1.08	0.06	0.31	0.08
C42	Apr. 26	12:15	4 033	29.46	33.53	0.32	0.91	0.03	0.46	0.08
C47	Apr. 15	12:00	4 143	29.60	33.59	0.54	0.96	0.01	0.12	0.12
C50	Apr. 16	15:30	4 140	29.19	33.57	0.13	1.03	BLQ	0.01	0.07
C52	Apr. 17	3:05	3 752	28.59	33.53	0.14	1.48	BLQ	0.01	0.11
C54	Apr. 2	7:35	3 386	28.45	33.32	0.24	2.12	BLQ	0.34	0.08
C57	Apr. 4	19:15	4 194	28.44	33.64	0.29	1.14	0.06	0.31	0.13
C60	Apr. 5	17:25	3 480	28.83	33.02	0.46	0.82	0.01	0.36	0.07
C64	Apr. 13	21:40	1 736	29.45	33.58	0.23	0.94	BLQ	0.26	0.10
C66	Apr. 13	8:20	1 648	29.47	33.83	0.45	1.08	0.02	0.60	0.09
C70	Apr. 11	21:00	2 042	29.31	33.65	0.44	0.82	0.01	0.36	0.06
J57	Apr. 8	10:50	4 200	28.23	33.35	0.26	0.94	BLQ	0.56	0.09
J54	Apr. 9	2:30	3 386	28.49	33.42	0.45	1.28	0.03	0.60	0.09
NSCS										
C34	Apr. 20	19:50	159.7	27.7	33.62	0.50	1.50	0.02	0.47	0.10
C31	Apr. 21	13:30	2 452	28.04	33.74	0.19	1.04	0.01	0.22	0.11
C26	Apr. 22	17:20	3 573	26.22	34.03	4.49	2.28	0.03	2.99	0.18
C22	Apr. 23	18:30	3 935	28.06	33.97	0.76	2.10	0.07	0.75	0.21
C13	Apr. 30	4:50	3 747	26.82	34.17	0.60	1.87	0.04	0.49	0.15
C9	May 1	2:00	148	27.13	33.94	0.30	1.51	BLQ	1.25	0.12
L5	May 2	8:00	324	25.29	34.32	0.65	1.55	0.03	0.14	0.10
C2	May 4	12:00	96.2	25.34	34.01	0.11	1.65	BLQ	0.51	0.12

Note: NO_x represents nitrate plus nitrite concentration, and BLQ below the limit of determination (0.008 μmol/L for PO_4^{3-}).

from 0.65 to 2.12 μmol/L and was highest at Station C54 in the central basin (besides Station C26). The phosphate concentration was very low (the maximum was only 0.07 μmol/L) and was below the detection limit (0.008 μmol/L) at seven of twenty-one stations. The ammonium concentration ranged from 0.01 to 0.60 μmol/L, except Stations C26 and C9 with the values of 2.99 and 1.25 μmol/L, respectively. The Chl a concentrations of the originally sampled seawater ranged from 0.06 to 0.21 μg/L with an average value of (0.11±0.04) μg/L, and the high values were shown in the NSCS (Stations C22 and C26).

Special emphasis should be placed at Station C26. There were unusually high nutrient concentrations which were far more than other stations and lower temperature than ambient sites due to cold eddy (Tian et al., 2016). The total dissolved nitrogen (nitrate, nitrite and ammonium) concentration was highest at Station C26, with a value of 7.48 μmol/L, about ten times of the average value of remaining twenty stations (0.74 μmol/L). The silicate concentration was also highest at Station C26 (2.28 μmol/L), about 2 fold of the average value of other stations (1.28 μmol/L). However, the highest Chl a concentration was not found at Station C26 but Station C22 with the value of 0.21 μg/L. There was a sub maximum of Chl a concentration at Station C26 (0.18 μg/L).

The Pearson correlation analyze results between different environmental variables were showed in Table 2. The temperature was extremely negative correlated with surface salinity and Chl a concentrations ($p<0.01$ or 0.05) (Table 2). The concentration of nitrate plus nitrite showed extremely positive correlated with ammonium concentration and silicate concentration ($p<0.05$ or 0.01). The Chl a concentration was negative correlated with surface temperature ($p<0.05$), while positive correlated with surface salinity, silicate, ammonium and phosphate concentrations ($p<0.05$ or 0.01), respectively (Table 2).

There were obvious differences in different environmental variables between the NSCS and CSCS (Table 3). Surface water temperature in the CSCS ((28.99±0.49)°C) was higher than that in the NSCS ((26.83±1.12)°C) (t-test, $p<0.05$), but there was no statistically significant difference in surface salinity (t-test, $p=0.865$). The dissolved nitrogen (nitrate plus nitrite, ammonium) concentrations were lower in the CSCS than those in the NSCS (t-test, $p<0.05$). Similarly, the Chl a concentration in the CSCS ((0.09± 0.02) μg/L) was also lower than that in the NSCS ((0.14±0.04) μg/L) (t-test, $p<0.05$).

3.2 *Phytoplankton composition*

A total of 92 species in 46 genera of phytoplankton belonging

Table 2. Pearson correlation analyses result between different environmental variables

	T/°C	S	NO_x/μmol·L⁻¹	SiO_3^{2-}/μmol·L⁻¹	PO_4^{3-}/μmol·L⁻¹	NH_4^-/μmol·L⁻¹	Chl a/μg·L⁻¹
T/°C	1	-0.707**	-0.344	-0.645**	-0.036	-0.392	-0.454*
S		1	0.308	0.514*	0.248	0.322	0.596**
NO_x/μmol·L⁻¹			1	0.503*	0.230	0.898**	0.509*
SiO_3^{2-}/μmol·L⁻¹				1	0.245	0.526*	0.691**
PO_4^{3-}/μmol·L⁻¹					1	0.135	0.490*
NH_4^-/μmol·L⁻¹						1	0.533*
Chl a/μg·L⁻¹							1

Note: * $p<0.05$; ** $p<0.01$.

Table 3. Comparisons of environmental variables between the CSCS and NSCS

Region	T/°C	S	NO_x/μmol·L^{-1}	SiO_3^{2-}/μmol·L^{-1}	PO_4^{3-}/μmol·L^{-1}	NH_4^-/μmol·L^{-1}	Chl a/μg·L^{-1}
CSCS	28.99±0.49	33.50±0.20	0.35±0.15	1.11±0.36	0.02±0.02	0.34±0.19	0.09±0.02
NSCS	26.83±1.12	33.98±0.22	0.95±1.45	1.68±0.39	0.03±0.02	0.85±0.93	0.14±0.04
p	<0.02	0.865	0.02	0.615	0.954	<0.02	<0.05

Table 4. Species composition and abundance of plankton in the experimental waters

Plankton	Classified group	Genera	Taxa	Abundance/cells·L^{-1} or ind.·L^{-1}
Phytoplankton	Bacllariophyta	25	46	587–2 500
	Dinophyta	12	41	121–1 157
	Cyanophyta	2	2	151–11 864
	Chrysophyta	2	3	0–16
Microzooplankton	Tintinnida	18	50	7–106
	Aloricate ciliates	8	12	12–91
	Copepod nauplli	1	1	0–51
	Heterotrophic dinoflagellates	3	12	49–190

to Bacillariophyta, Dinophyta, Cyanophyta and Chrysophyta were identified in the initially sampled waters of twenty-one stations in the surface (Table 4). Bacillariophyta was the most diversified group in which 46 species belonging to 25 genera were recorded. Dinophyta contributed for species composition with 41 species belonging to 12 genera. Although species in Cyanophyta and Chrysophyta were also observed, they were very few and recorded more sporadically.

The phytoplankton abundances ranged from the minimum of 1 546 cells/L to the maximum of 14 016 cells/L, with an average value of 4 843 cells/L. Further, the mean cell abundance of cyanobacteria (*Trichodesmium thiebautii* mainly) was 3 303 cells/L which accounted for 68% of total phytoplankton abundance. *Trichodesmium thiebautii* was the pre-dominanted specie and the cell abundance was 15 cells/L (Station C52) to 11 864 cells/L (Station C22) with an average value of 3 277 cells/L. Abundances of diatom and dinoflagellate at different stations were showed in Fig. 2. The mean cell abundances of diatom and dinoflagellate were 1 247 cells/L and 289 cells/L, separately. *Thalassionema nitzschioides*, *Thalassionema frauenfeldii*, *Synedra* spp., and *Thalassiothrix longissima* were the most dominant diatoms in most experimental stations. Dinoflagellates such as *Prorocentrum lenticulatum*, *Prorocentrum minimm* and *Protoperidinium* sp., etc., also appeared much more in some sites.

There was significant higher phytoplankton abundance in the

NSCS than that in the CSCS, due to the higher abundances of dinoflagellate and cyanobacteria (*t*-test, *p*<0.05 or *p*<0.01), but there was no significant differences in diatom abundance between the NSCS and CSCS.

3.3 *Microzooplankton community structure*

In the initially sampled waters, there were sixty-two ciliate species identified belonging to 26 genera (Table 4). Ciliates were categorized into loricate tintinnids with 50 species and aloricate ciliates with 12 species. Loricate tintinnids mainly consisted of species in the genera of *Tintinnopsis* (13 species), *Eutintinnus* (8 species) and *Rhabdonella* (13 species). Aloricate ciliates mainly consisted of species in the genera of *Strombidium* including *S. sulcatum*, *S. tintinnodes*, *S. paracalkinsi* and *S. conicum*, and *Apostrombidium, Rimostrombidium*. The abundances of ciliates ranged from 34 ind./L to 183 ind./L, and were mainly contributed by *Strombidium* with high abundance. Due to high species diversity of *Tintinnopsis*, the abundances of *Tintinnopsis* was also high in some stations.

The copepod nauplii were identified in 18 experimental stations, and the maximum abundance was 51 ind./L, which accounted for 30% of microzooplankton abundance at Station C13.

Heterotrophic dinoflagellates in genera of *Protoperidinium*, *Gymnodinium* and *Gyrodinium* mainly were identified in all ex-
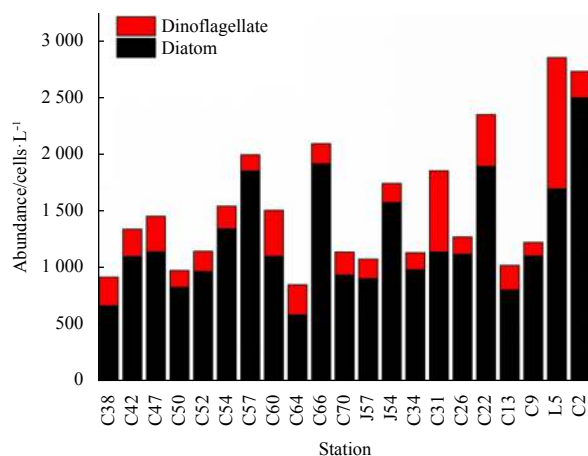
Fig. 2. Abundance composition (cells/L) of diatom and dinoflagellate in the surface layer of experimental sites.
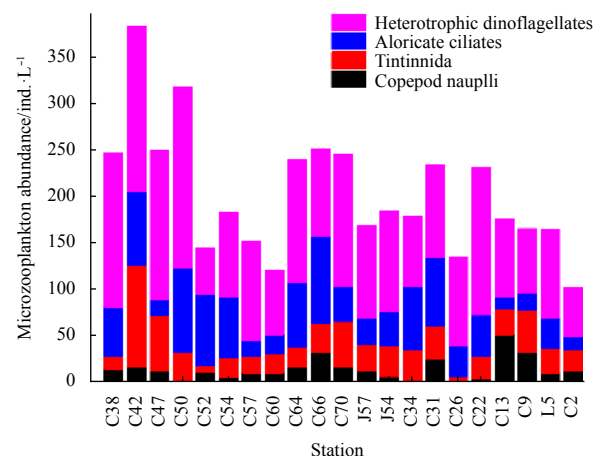
Fig. 3. Microzooplankton abundance (ind./L) of different groups in the surface layer of experimental stations.

perimental stations. The abundance of heterotrophic dinoflagellates was higher (108 ind./L on average) than that of ciliates (77 ind./L on average) at sixteen experimental stations (Fig. 3).

There were no significant differences in abundances of ciliates (*t*-test, *p*=0.566) and heterotrophic dinoflagellates (*t*-test, *p*=0.114) between the NSCS and CSCS. But in the whole sites, the abundances of ciliates and heterotrophic dinoflagellates were positively correlated with temperature, respectively (*p*<0.05 or *p*<0.01) (Fig. 4).

3.4 *Phytoplankton growth and microzooplankton grazing rates*

Phytoplankton growth and microzooplankton grazing rates from the dilution experiments are shown in Table 5. Phytoplankton growth rates (μ_0) at different experimental stations ranged

from 0.45 to 1.52 ((0.85±0.28) d^{-1}) and microzooplankton grazing rates (*m*) ranged from 0.20 to 0.87 ((0.45±0.22) d^{-1}). The microzooplankton grazing pressure on the phytoplankton community expressed by the percentages of phytoplankton standing stock (P_i, %/d) and potential primary production (P_p, %/d) that were ingested by microzooplankton, is also shown in Table 5. The microzooplankton consumed 18%–58% ((35.15±12.78)%/d) of standing stocks and 37%–91% ((62.90±16.64)%/d) of potential primary productivity for total phytoplankton among experimental stations. Moreover, the specific value of m/μ_0, ranged from 0.29 to 0.89 with the average value of 0.53±0.17.

The phytoplankton growth and microzooplankton grazing rates were (0.81±0.16) d^{-1} and (0.30±0.09) d^{-1} in the NSCS, and were lower than those in the CSCS with the values of (0.88±0.16) d^{-1}
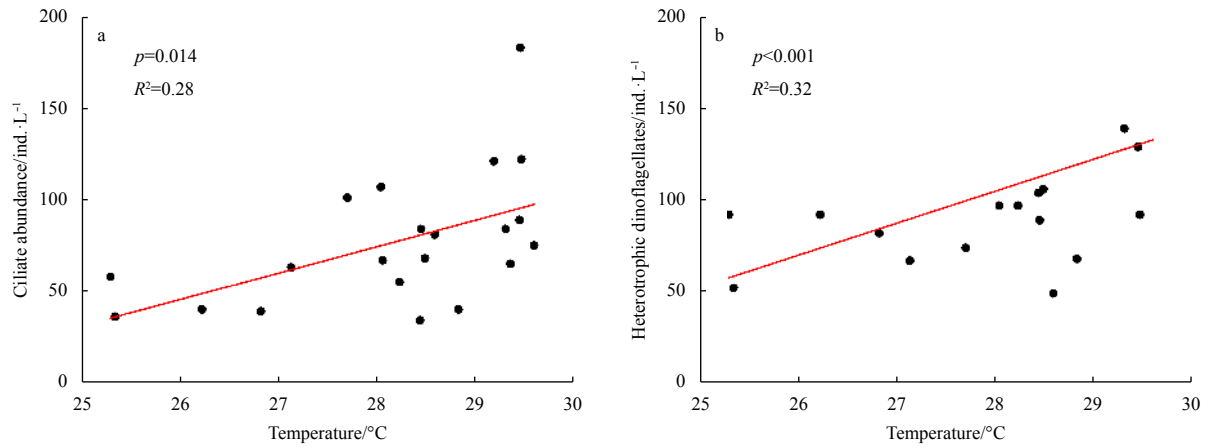

Fig. 4. Relationships between microzooplankton abundance (ind./L) and temperature (°C). a. Ciliate and b. heterotrophic dinoflagellates.

Table 5. Summary of phytoplankton growth (μ, d^{-1}) and microzooplankton grazing (*m*, d^{-1}) rates for all the dilution experiments and relevant experimental parameters

Station	m/d^{-1}	μ_n/d^{-1}	μ_0/d^{-1}	m/μ_0	P_i/d^{-1}	P_p/d^{-1}	μ_0-m/d^{-1}	μ_0/μ_n	R^2
CSCS									
C38	0.39	0.71	0.65	0.60	32.29	67.57	0.26	0.92	0.76
C42	0.78	1.74	1.52	0.51	54.16	69.32	0.74	0.87	0.83
C47	0.38	0.91	0.68	0.56	31.61	64.08	0.30	0.75	0.72
C50	0.87	1.27	1.20	0.73	58.05	83.15	0.33	0.94	0.94
C52	0.43	1.08	0.78	0.55	34.95	64.53	0.35	0.72	0.82
C54	0.51	1.11	0.99	0.52	39.95	63.57	0.48	0.89	0.86
C57	0.67	1.46	0.96	0.70	48.83	79.13	0.29	0.66	0.83
C60	0.47	0.87	0.53	0.89	37.50	91.15	0.06	0.61	0.91
C64	0.81	1.69	1.22	0.66	55.51	88.77	0.41	0.72	0.73
C66	0.86	1.79	1.23	0.70	57.68	81.51	0.37	0.69	0.69
C70	0.33	0.85	0.68	0.49	28.11	59.97	0.35	0.80	0.44
J57	0.35	0.82	0.45	0.78	29.53	81.49	0.10	0.55	0.51
J54	0.27	0.66	0.55	0.49	23.66	55.93	0.28	0.83	0.55
NSCS									
C34	0.40	0.97	1.05	0.38	32.97	50.72	0.65	1.08	0.69
C31	0.46	1.07	1.00	0.46	36.87	58.33	0.54	0.93	0.68
C26	0.28	0.57	0.78	0.36	24.42	45.09	0.50	1.37	0.61
C22	0.20	0.58	0.59	0.34	18.13	40.67	0.39	1.02	0.39
C13	0.32	0.81	0.70	0.46	27.85	54.40	0.38	0.86	0.62
C9	0.30	0.79	0.89	0.34	25.92	43.98	0.59	1.13	0.78
L5	0.25	0.68	0.78	0.32	22.12	40.84	0.53	1.15	0.69
C2	0.20	0.96	0.68	0.29	18.13	36.74	0.48	0.71	0.50

Note: R^2 means regression coefficient.

Table 6. Comparisons of dilution experimental parameters between the CSCS and NSCS

Region	m/d^{-1}	μ_n/d^{-1}	μ_0/d^{-1}	m/μ_0	P_i/d^{-1}	P_p/d^{-1}
CSCS	0.55±0.22	1.15±0.40	0.88±0.33	0.63±0.12	40.91±12.33	73.09±11.57
NSCS	0.30±0.09	0.80±0.19	0.81±0.16	0.37±0.06	25.80±6.67	46.35±7.46
p	<0.01	=0.02	<0.02	<0.05	<0.02	<0.05
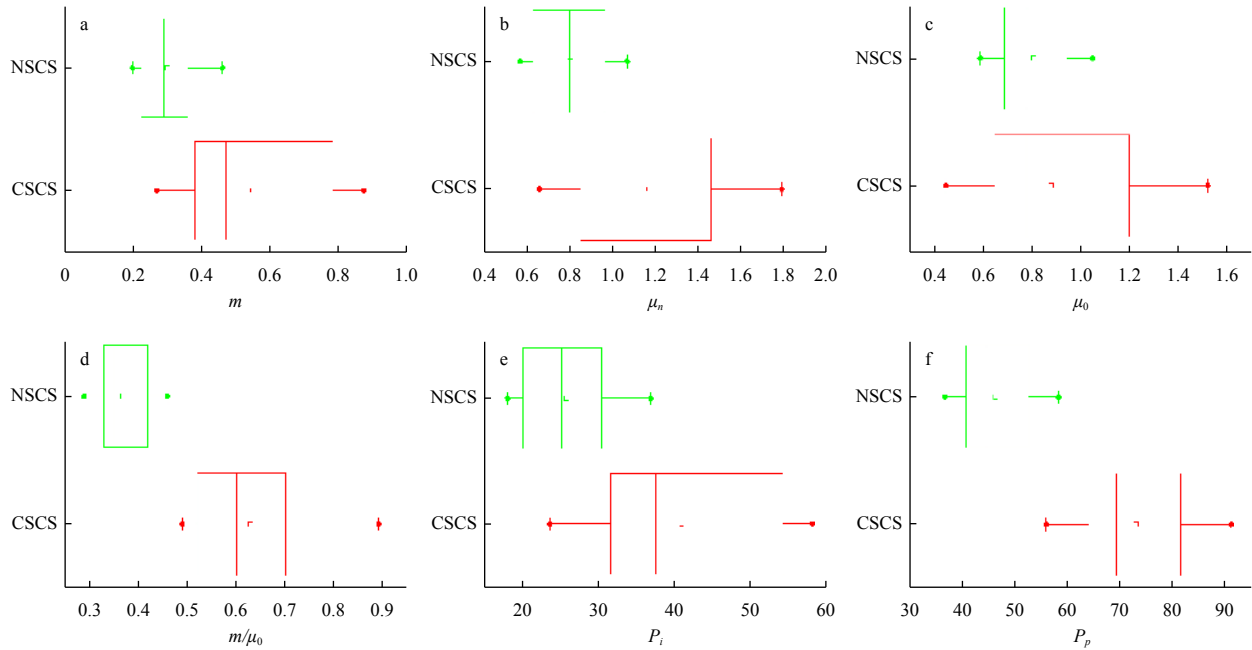
Fig. 5. Box plots of the microzooplankton grazing rate (m), phytoplankton growth rate (μ_0 and μ_n) and microzooplankton grazing pressure expressed by the rate of m/μ_0, P_i and P_p.

and (0.55±0.22) d^{-1}, separately (*t*-test, *p*<0.05 or *p*<0.01) (Table 6, Fig. 5). The microzooplankton grazing pressure on phytoplankton (m/μ_0, P_i, P_p) in the CSCS were (0.63±0.12), (40.91± 12.33), and (73.09±11.57) d^{-1}, respectively, which were also higher than those in the NSCS with the values of (0.37±0.06), (25.80±6.67), and (46.35±7.46) d^{-1}, respectively (*t*-test, *p*<0.05) (Table 6, Fig. 5).

Correlation analysis showed that the microzooplankton grazing rates were positively correlated to phytoplankton growth rates in both the NSCS (*n*=13, *p*<0.01) and CSCS (*n*=8, *p*<0.01) (Fig. 6).

Nutrient limitation indices (μ_0/μ_n) were scattered over a wide area, from 0.55 to 1.37 with the average value of 0.87±0.20, suggesting that nutrients had different effects among stations in this study area. In the NSCS with the higher nutrient concentrations, the ratio was 1.03 on average, indicating phytoplankton growth rates estimates in the nutrient addition treatments (μ_n) were almost the same as the estimates in control group (μ_0) and no nutrient limitation in the NSCS. But in the CSCS, the μ_0/μ_n ratio was just 0.77 on average and the ratios were below 1 at all the thirteen sites, suggesting that nutrients became the limiting factors for phytoplankton growth in the CSCS. The difference of μ_0/μ_n
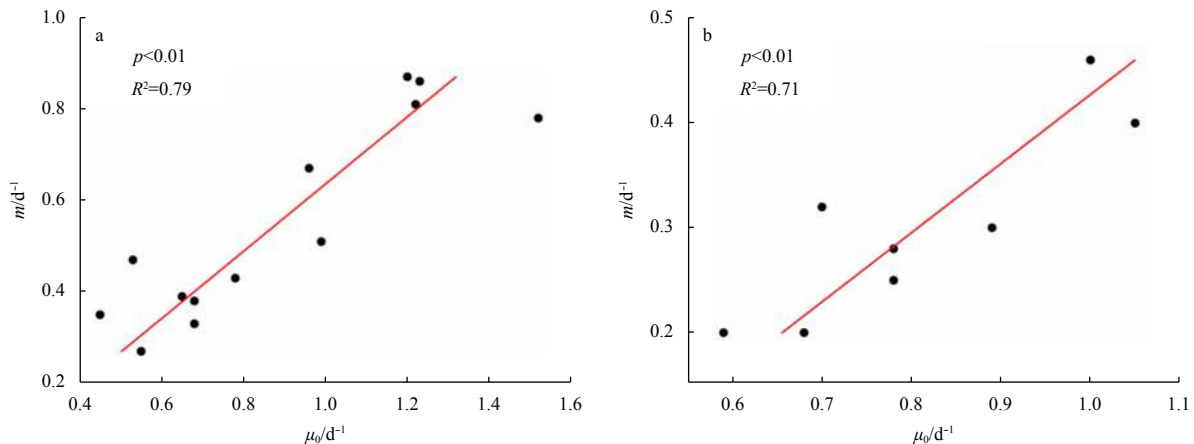

Fig. 6. Microzooplankton grazing rate (m, d^{-1}) as a function of phytoplankton growth rate (μ_0, d^{-1}). a. NSCS and b. CSCS.

Table 7. Pearson correlation analyses between dilution experimental parameters and environmental and biological parameters variables

Parameter	m/d^{-1}	μ_n/d^{-1}	μ_0/d^{-1}	m/μ_0	P_i/d^{-1}	P_p/d^{-1}	μ_0/μ_n
T/°C	0.621**	0.496*	0.290	0.671**	0.645**	0.739**	−0.483*
S	−0.330	−0.201	0.038	−0.722**	−0.376	−0.696**	0.547*
NO_x/μmol·L^{-1}	−0.258	−0.359	−0.129	−0.274	−0.268	−0.299	0.619**
SiO_3^{2-}/μmol·L^{-1}	−0.505*	−0.465*	−0.190	−0.654**	−0.520*	−0.695**	0.608**
PO_4^{3-}/μmol·L^{-1}	−0.165	−0.202	−0.143	−0.160	−0.174	−0.206	0.235
NH_4^-/μmol·L^{-1}	−0.286	−0.320	−0.232	−0.343	−0.304	−0.361	0.592**
Chl a/μg·L^{-1}	−0.424*	−0.353	−0.232	−0.502*	−0.449*	−0.539*	0.415
Ciliates abundance/ind.·L^{-1}	0.636**	0.630**	0.785**	0.088	0.626**	0.258	0.019
HD abundance/ind.·L^{-1}	0.376	0.184	0.277	0.198	0.354	0.266	0.053
CN abundance/ind.·L^{-1}	0.142	0.161	0.239	−0.088	0.143	−0.038	0.125
MZP abundance/ind.·L^{-1}	0.590**	0.493*	0.617**	0.167	0.573**	0.308	0.007

Note: MZP represents microzooplankton abundance, CN copepod nauplii, and HD Heterotrophic dinoflagellates. * $p < 0.05$; ** $p < 0.01$.

between the NSCS and CSCS was not statistically significant (t-test, p=0.219), but as a whole it was obviously related to nutrient concentrations (Table 7).

The grazing rate (m) in surface waters was positively correlated with temperature and ciliate and microzooplankton abundances, separately (p<0.01). The temperature was positively correlated with μ_n, (p<0.05), but was not correlated significantly with μ_0 (p=0.203) (Table 7).

4 Discussion

4.1 *Effects of environmental factors on microzooplankton distribution*

Few studies on detailed microzooplankton species composition and abundance in the region of the SCS have been carried out previously. Microzooplankton are important consumers of phytoplankton production in the open ocean (Stelfox-Widdicombe et al., 2000) and play a critical role in the microbial loop (Schmoker et al., 2013). Microzooplankton abundance and metabolism are affected by many factors, such as temperature, oxygen and nutrients (Caron and Hutchins, 2013). Generally, it is believed that temperature and feed concentration are the main factors affecting ciliates (Nielsen and Kicrboe, 1994; Strom et al., 2007; Zhang et al., 2011). Archer et al. (1996) found that the growth rate of dinoflagellates was lower in the low temperature environment, and the temperature restriction of the growth rate was also applicable to ciliates (Rose and Caron, 2007). In addition, Setälä and Kivi (2003) showed the abundance of ciliates was significant correlation with the Chl a concentrations, and Gómez (2007) pointed out that the numbers of loricate tintinnids were mainly limited by diet, so they were distributed in the aquifer with the highest Chl a concentration. In the SCS, Zhou et al. (2015a) also pointed the significantly positive correlation between ciliates abundance and Chl a concentration.

In our experiments, the same results are also found, which the abundances of heterotrophic dinoflagellates and the ciliates are positively correlated with temperature, respectively (Fig. 4). However, there is no significant correlation between microzooplankton abundance and Chl a concentration. This may be due to the complex distribution pattern of microzooplankton, and other factors can also influence the distribution of microzooplankton (Nielsen and Kicrboe, 1994; Quevedo et al., 2003). High productivity waters often correspond to high biomass of microzooplankton (First et al., 2007). However, a different species composition, abundance and prey quality of microzooplankton will lead to different food preference and the changes of phytoplankton communities in the species and size composition (Teixeira and Figueiras, 2009). Most ciliates were resistant to diatom, while some larger heterotrophic dinoflagellates (e.g., *Gyrodinium*) preferentially feed on diatom (Archer et al., 1996; Leising et al., 2005). The *Tintinnopsis* sp. belonging to loricate tintinnids could only incept pico- or nano-phytoplankton because of their small oral diameter (median 38.5 μm), while aloricate ciliates could graze particles as large as themselves (Burkill et al., 1987; Paranjape, 1987; Gifford, 1988). The food preference in species and particle size of phytoplankton may result in no significant correlation between microzooplankton abundance and total Chl a concentration. In nature, the growth of microzooplankton is dependent on the suitable prey, but the abundances and species composition of microzooplankton are controlled by the higher consumers (meso- and macrozooplankton) directly (Porter et al., 1985; Gifford et al., 1995; Lonsdale et al., 2000). In addition, environmental factors may influence the abundance of microzooplankton. Increased irradiance and exposure to ultraviolet radiation may have significant direct effects on the growth and behavior of some heterotrophic protists (Macaluso et al., 2009), which may affect the structure of marine food webs as a result of species-specific differences in ultraviolet sensitivity (Belzile et al., 2006). Oxygen is also necessary for the respiration of microzooplankton (Verity et al., 2002). There is no enough data to determine the specific causes of decoupling between microzooplankton abundance and Chl a concentration in the present study and further study is necessary.

4.2 *Comparisons of rate estimates with previous studies*

It is cautious to compare our data with other studies in the same area or similar environment before discussing environmental effects on microzooplankton grazing rates and phytoplankton growth rates. All the μ and m are within the reviewed ranges based on global data collection (Calbet and Landry, 2004; Schmoker et al., 2013). There are few studies on microzooplankton grazing in the CSCS and NSCS, especially during spring inter-monsoon season. Previous studies in the waters adjacent to our study area are happened in winter and summer. For example, Chen et al. (2009) 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 near our study area in summer, which was similar to our results observed in the CSCS. Zhou et al. (2015b) estimated an average μ of (0.92±0.32) d^{-1} and an average m of (0.46±0.20) d^{-1} in the southern SCS during

summertime, while in winter, the rates were lower with the values of (0.54±0.22) d^{-1} and (0.27±0.13) d^{-1} for μ and m, respectively. Chen et al. (2013) reported that the average values of m were (0.49±0.47) d^{-1} and (0.35±0.21) d^{-1}, and the μ were (0.89±0.45) d^{-1} and (0.61±0.32) d^{-1} for summer and winter in the NSCS, respectively. These rates are similar to our results (Tables 5 and 6).

Globally, there are relatively few studies at similar latitudes in the open ocean. Yang et al. (2004) reported μ and m in summer were 0.35–0.75 d^{-1} and 0.51–0.67 d^{-1}, respectively, in the western Pacific with latitudes similar to those stations of the CSCS in the present study. The μ_0 and m estimated by Landry et al. (1998) showed similar results in the subtropical and tropical Arabian Sea with the mean growth rates of 0.85 and 0.62 d^{-1} in summer and winter, while the mean grazing rates were 0.68 and 0.65 d^{-1}, respectively. Caron and Dennett (1999) showed the μ_0 of (0.84±0.29) d^{-1} and the m of (0.35±0.18) d^{-1} during the northeast monsoon season, while the μ_0 of (0.44±0.19) d^{-1} and the m of (0.30±0.17) d^{-1} during spring intermonsoon season in the Arabian Sea, respectively. Also in the Arabian Sea, Edwards et al. (1999) compared the results of dilution method experiments between during and after the southwest monsoon, which indicated the μ_0 were (0.81±0.47) and (0.68±0.15) d^{-1} during and after the southwest monsoon, meanwhile the m were (0.33±0.19) d^{-1} and (0.41±0.19) d^{-1}, respectively. Although the results of these studies are slightly different, they are all within reasonable range.

4.3 *Environmental effects in the variations of phytoplankton growth and microzooplankton grazing rates*

Calbet and Landry (2004) pointed that there was a significant positive correlation ($p<0.001$, $R^2=0.37$) between phytoplankton growth and microzooplankton grazing rates based on the dataset collected on open oceans and offshore marine systems. The latest review confirmed this point (Schmoker et al., 2013). In fact, microzooplankton feed passively and always preferentially feed on fast-growing species to gain a stable food source and maintain its own growth. The high coupling of grazing rate and phytoplankton growth rate also promotes ecosystem stability (Sun et al., 2007; Strom, 2002). Similar result was observed in present study, the microzooplankton grazing rate was positively correlated with the phytoplankton growth rate in both study areas ($p<0.01$) (Fig. 6), similar to previous studies in the SCS (Chen et al., 2009; Zhou et al., 2015a).

Previous studies indicated that the phytoplankton mortality by microzooplankton grazing was higher in oligotrophic waters and open ocean, and lower in eutrophic and coastal environments (e. g., Landry et al., 1997; Calbet and Landry, 2004). Similar results have emerged in our experiments. There are obviously higher grazing pressure on phytoplankton (m/μ_0, P_i and P_p) in the CSCS than those in the NSCS with higher nutritional level and biomass. In our study, the temperature in the NSCS was significantly lower than that in the CSCS, while microzooplankton grazing rate was more sensitive to temperature and it meant the growth rate of phytoplankton might increase more slowly with temperature than microzooplankton growth and grazing rates (e.g., López-Urrutia et al., 2006; Rose and Caron, 2007; López-Urrutia, 2008; Chen et al., 2012). The high grazing impact might have been caused by a larger increase of microzooplankton grazing than of phytoplankton growth rates in the CSCS.

Compared with temperature, nutrients played a more significant role in phytoplankton growth in the spring of the SCS (Hu et al., 2014). The open sea of the SCS has very low phytoplankton biomass and primary production due to lack of nutrients (Ning et

al., 2004; Wong et al., 2007; Tan and Shi, 2009) and is generally considered to be nitrogen limited in the open waters (Chen, 2005; Wu et al., 2003). In the spring intermonsoon period, the increase of surface heat and the decay of turbulent mixing as a result of switching monsoon, leaded to the strongest stratification and minimum mixed layer depth in the euphotic layer (Shi et al., 2001), which restrain the vertical nutrient supply and phytoplankton photosynthesis in the upper layer (Hu et al., 2014). The results in the present study confirm that the nutrient limitation index (μ_0/μ_n) is related to nutrients concentrations (nitrate plus nitrite, ammonium and silicate) (Table 7). Caron and Dennett (1999) pointed the ratio of μ_0/μ_n was averaged 0.71 during the spring intermonsoon season in the Arabian Sea, corresponded to samples in which total dissolved inorganic nitrogen concentrations were low. In contrast to spring intermonsoon season, the ratio of μ_0/μ_n was 0.94 on average during the winter northeast monsoon season with higher nutrient concentrations.

However, there was no significant correlation between phytoplankton growth rate and nutrients concentrations, even that the phytoplankton growth rate was higher in the CSCS with lower nutrients concentrations than those in the NSCS (Fig. 5). One possible explanation is that different phytoplankton may have differently responded to nutrients. Phytoplankton community in oligotrophic subtropical and tropical waters is usually dominated by small sized pico-cells (<3 μm), including *Prochlorococcus*, *Synechoccus* and pico-eukaryotic phytoplankton (Zubkov et al., 2000; Liu et al., 2007). Small phytoplankton are better adapted oligotrophic environment because of their larger surface-to-volume ratio, which gives them an advantage in acquiring nutrients and absorbing light energy competing with larger cells (Raven, 1998). Liu et al. (2009) reported that both the abundance and growth rate of *Synechococcus* increased with the water temperature increasing, similar to the results of previous studies (Tsai et al., 2005; Chang et al., 1996). Zhou et al. (2015a) also noted there were higher growth rates of pico-phytoplankton than micro-phytoplankton in the SCS. Furthermore, although the nutrient concentrations are low in the CSCS, phytoplankton may obtain nutrients support from cellular internal recycled nutrients and nutrients remineralization by grazers (Sun et al., 2013; Zhou et al., 2015a). Zhou et al. (2015a) speculated the coupling between microzooplankton grazing and phytoplankton growth rates could helpful sustain the high rate of phytoplankton growth by using the recycled nutrient supply through microzooplankton grazing in the SCS only when the phytoplankton standing stock was in a relatively low level like the CSCS in present study ($p<0.01$, $R^2=0.79$) (Fig. 6a). With the increase of phytoplankton standing stock, there might be a looser coupling between phytoplankton and microzooplankton (Strom, 2002; Irigoien et al., 2005) like the NSCS in present study ($p<0.01$, $R^2=0.71$) (Fig. 6a). High grazing impact on phytoplankton by microzooplankton could be the result of the close coupling between phytoplankton growth and microzooplankton grazing rates (Zhou et al., 2015a). As a result, the higher growth rate and grazing rate are emerged in the CSCS. Meanwhile, the higher grazing impacts in the CSCS than those in the NSCS contribute to maintain higher biomass and primary production in the NSCS.

5 Conclusions

Generally, it is believed that temperature and feed concentration are the major factors affecting microzooplankton abundance. Temperature plays an obvious role in promoting the abundance and distribution of microzooplankton in the present study. Strangely, there is no correlation between microzooplank-

ton abundance and Chl *a* concentration. Some other factors are also playing an unknown role, maybe the nanozooplankton or cascading trophic relationships. Composition and size of phytoplankton, ingestion by the higher consumers (meso- and macrozooplankton) and environmental factors may be the culprit and need further study to determine.

Significant spatial variations in phytoplankton growth rate and microzooplankton grazing rate as well as environmental variables between the NSCS and CSCS, are observed in the present study. There are obviously higher m, μ_0 and grazing impact (m/μ_0) though the nutrients and Chl *a* concentrations are lower in the CSCS than those in the NSCS, separately. Temperature can be one important factor affecting both rates, but more sensitive for grazing rate, which may contribute to higher m and m/μ_0 in the CSCS. Due to the lack of nutrients in the surface layer during the spring intermonsoon season, the growth of phytoplankton is affected and the nutrient limitation index (μ_0/μ_n) is obviously related to nutrient concentrations. Nevertheless, the dominant pico-phytoplankton can effectively utilize nutrients because of their larger surface-to-volume ratio even in oligotrophic waters. Meanwhile, phytoplankton could obtain nutrients support to maintain growth from cellular internal recycled nutrients and nutrients remineralized by grazers. The coupling between microzooplankton grazing and phytoplankton growth may promote nutrient recycling and maintain high grazing impact on phytoplankton.

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