



Role of *Calanus sinicus* (Copepoda, Calanoida) on dimethylsulfide production in Jiaozhou Bay

Juan Yu^{1,2}, Jiyuan Tian³, Zhengyu Zhang¹, Guipeng Yang^{1,2*}, Hongju Chen⁴

¹Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education; College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao, 266100, People's Republic of China

²Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, People's Republic of China

³College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, 266009, People's Republic of China

⁴College of Environmental Science and Engineering, Ocean University of China, Qingdao, 266100, People's Republic of China

Correspondence to: Guipeng Yang (gpyang@ouc.edu.cn)

Abstract. The role of copepod *Calanus sinicus* on the production of dimethylsulfide (DMS)/dimethylsulfoniopropionate (DMSP) in Jiaozhou Bay was evaluated in field and laboratory experiments. Samples at 10 sites in the bay were collected monthly from June 2010 to May 2011 (except for March 2011), and zooplankton species composition was analyzed. The relationship between copepod abundance and DMS or DMSP concentration was investigated. Effects of *C. sinicus* grazing on DMS/DMSP production at different conditions (i.e., algal diets, food concentrations, and salinities) were assessed in the laboratory. Data from the field experiment showed that *C. sinicus* was the predominant copepod in Jiaozhou Bay (up to 123 individuals m⁻³ in May 2011) and has no apparent effect on DMS/DMSP production. In the laboratory experiment, compared with *Gymnodinium* sp. or *Emiliana huxleyi*, *C. sinicus* feeding on *Isochrysis galbana* and *Chaetoceros curvisetus* exhibited increased DMS concentration, whereas high salinity inhibited DMS production. This study indicated that DMSP was transferred from phytoplankton to copepod body, fecal pellet, and seawater through copepod grazing. Our results provided important information to understand the biogeochemical cycle of DMSP in Jiaozhou Bay.

Key words: Copepod; Diet; Dimethylsulfide; Dimethylsulfoniopropionate; Grazing; Salinity

1 Introduction

Dimethylsulfide (DMS) is the most abundant biogenic sulfur gas that may influence planetary climate by forming cloud condensation nuclei that alters global radiation balance (Charlson et al., 1987). The biogeochemical cycling of dimethylsulfoniopropionate (DMSP), which is the main precursor of DMS, recently came under close scrutiny. The marine environment is the major source of DMSP, and DMSP is synthesized by many marine phytoplankton species as an osmolyte (Kirst, 1996). Therefore, the dynamics of DMSP in the ocean will have an important influence on global DMS production. The conversion of DMSP to DMS is regulated by complex trophic processes in the water column, e.g., algal senescence (Nguyen et al., 1988), phytoplanktonic enzyme catalysis (Niki et al., 2000), bacterial activity (Kiene and Linn, 2000), and



zooplankton grazing (Dacey and Wakeham, 1986; Wolfe et al., 2000; Yu et al., 2015). Currently, most studies have focused on field research concerning the spatial and temporal distributions and fluxes of DMS/DMSP (Turner et al., 1996; Wong et al., 2005). Many field studies indicated a weak correlation between DMS and parameters directly related to primary producers (Kettle et al., 1999). Numerous abiotic and biotic factors, e.g., factors other than phytoplankton, might play an important role in DMS and DMSP dynamics. Zooplankton are known to account for a large fraction (10% to 25%, or higher) of the daily oceanic primary production (Lancelot and Billen, 1985), and knowledge about the role of zooplankton grazing in the DMSP biogeochemical processes is scarce. Copepods, a trophodynamic link between primary and tertiary productions, play a key role in the cycling of materials and energy in marine ecosystems (Kiørboe, 1997). Dacey and Wakeham (1986) were the first to show that copepod grazing stimulated DMS production in a laboratory experiment. Other studies have also documented that zooplankton grazing induced DMS production (Belviso et al., 1990; Christaki et al., 1996; Daly and DiTullio, 1996; Leck et al., 1990) because of sloppy feeding (Dacey and Wakeham, 1986; Tang et al., 2000) or enhanced DMSP lyase activities (Wolfe and Steinke, 1996). The DMSP ingested by zooplankton was accumulated in the body and transferred to the upper food chain, and a portion of the DMSP ingested was transferred to fecal pellets, which were subsequently uncoupled as DMS and DMSP productions.

The calanoid copepod *Calanus sinicus* Brodsky is distributed in the East China Sea, the Yellow Sea, and the coastal waters of Japan (Brodsky, 1965) and is one of the predominant zooplankton in the East China Sea, the Yellow Sea, and the Jiaozhou Bay. In this study, we performed field and laboratory experiments to investigate the effects of copepod on DMS and DMSP productions in Jiaozhou Bay. The field experiments revealed the relationship between DMS/DMSP and biotic or abiotic parameter, and the laboratory experiments indicated the changes of DMS, dissolved and particulate DMSP (DMSP_d and DMSP_p), DMSP in copepod bodies (DMSP_z), and DMSP in fecal pellets (DMSP_f) under different conditions of salinities, food concentrations, and diet species. The data obtained in this study will further recognize the role of copepods in the DMSP biogeochemical cycle.

2 Materials and methods

2.1 In situ experiment

2.1.1 Sampling site

Fieldwork was performed at 10 stations of the Jiaozhou Bay from June 2010 to May 2011 once a month, except of March 2011 due to strong wind (Fig. 1). The Jiaozhou Bay (36°7'24.44" N, 120°14'44.3" E), a semi-enclosed bay in Qingdao City (China), separates Huangdao District from Qingdao City and borders on Jiaozhou City. The bay is 32 km long and 27 km wide, with a surface area of 362 km².



2.1.2 Abundance and taxonomic composition of zooplankton

Zooplankton samples were collected by vertical tows using a conical–cylindrical plankton net with a 50 cm mouth opening and a 160 μm mesh size. A flow meter was used to estimate the amount of filtered water. We retrieved the net at 0.3 m s^{-1} to 0.5 m s^{-1} at each station. Samples were then rinsed into cod-end buckets, concentrated, and preserved in 5% formalin for qualitative and quantitative analyses of in situ zooplankton. Taxonomy was determined by optical microscopy. In situ abundance was calculated using tow volume estimates, which were determined by net dimensions and flow meter values.

2.1.3 Abiotic parameters and analyses of chlorophyll *a* (Chl *a*) and bacteria

The shipboard measurements of temperature and salinity of surface water were obtained with the Sea-Bird 911 plus a conductivity–temperature–depth probe. Water samples were filtered using Waterman GF/F filters (nominal pore size of 0.7 μm) to determine Chl *a*. Chl *a* concentrations were measured using a Hitachi F4500 fluorometer according to the methods of Parsons et al. (1984). Based on the procedures of Porter and Feig (1980), bacteria were counted by epifluorescence microscopy (Hitachi F4500; total magnification of $\times 1,000$).

2.2 Incubation experiment

2.2.1 Phytoplankton and zooplankton cultures

Phytoplankton species (*Isochrysis galbana*, *Chaetoceros curvisetus*, *Emiliania huxleyi*, and *Gymnodinium* sp.) were provided by the Marine Microalgae Research Center, Ocean University of China in different sizes and DMSP contents. They are widespread species in Jiaozhou Bay and Chinese coastal waters (Li et al., 2005; Zhong et al., 2001). These algae are described in Table 1, and the sizes of algal cells are obtained by measuring at least 200 cells using a calibrated ocular micrometer with a light microscope at a magnification of $\times 400$. Cell volume was estimated by approximating the shape of each species to an elliptic sphere, which subsequently determined the biovolume according to the method of Verity et al. (1992). In this study, we determined the cellular carbon contents in phytoplankton according to the method of Strathmann (1967).

All algae were cultured with f/2 medium (Guillard, 1975) at 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under a dark/light cycle of 12 h:12 h at a temperature of 15 ± 1 °C. Algae were fed to copepods during their exponential growth phase. Copepods *C. sinicus* were collected from Jiaozhou Bay, Qingdao, China (120°8' E, 36°8' N) in April and May 2011 using a 0.5 m standard ring net equipped with a 160 μm mesh and a solid cod end. The copepods were grown at 15 ± 1 °C in 30 PSU sterilized seawater. In the incubation experiment, adult copepods were utilized as experimental animals.

2.2.2 Ingestion configuration

Four species of algae (*C. curvisetus*, *I. galbana*, *Gymnodinium* sp., and *E. huxleyi*) were utilized to assess dietary effects on changes in ingestion rate (IR), clearance rate (CR), and DMS/DMSP production in a salinity of 30 PSU. *I. galbana* was used



as a single food source, which served as a good-quality food, and four concentrations of *I. galbana* ($10, 15, 20$, and 25×10^4 cells mL^{-1}) and four levels of salinities ($20, 25, 30$, and 35 PSU) were set as concentration and salinity contrasts to obtain ingestion and DMS/DMSP information from food concentration and salinity. Single-factor experiments were performed, and all parameters were run individually.

5 2.2.3 IR and CR

Batches of 10 individual copepods in each of 3 replicates were sorted out and transferred to 250 mL polycarbonate bottles. Three bottles containing no grazers were used as controls. All bottles were topped off with suspensions of algae, sealed with parafilm, and fastened onto a spinning plankton wheel (2 rpm) at 15 ± 1 °C in darkness for 24 h. After a 24-h incubation, copepods and fecal pellets were harvested according to the methods of Tang (2001). Water samples were used to analyze cell density, IR, CR, DMS, DMSP_p , and DMSP_d . The IRs and CRs were calculated according to the equations of Frost (1972). Duplicates of 10 mL aliquots of each algal suspension were placed in 40 mL serum bottles that contained 2 mL of 10 mol L^{-1} KOH solution for DMSP detection. Ten copepods from each bottle were placed individually in serum bottles for DMSP_z (DMSP in the copepod body) measurement before gut clearance. Fecal pellets were separated from detritus with a mouth micropipette, rinsed with filtered seawater, and concentrated onto a 47 mm Whatman GF/F filter by gravity filtration to determine DMSP_f (DMSP in the fecal pellets). All samples were stored at -70 °C until DMSP measurement.

2.2.4 DMS and DMSP determinations

According to the methods of Andreae and Barnard (1983), DMS and DMSP concentrations were determined using the purge-and-trap technique by a gas chromatograph, which was equipped with a flame photometric detector (Shimadzu GC-14B). For DMS measurement, 10 mL seawater was directly introduced into a glass purge chamber. Gravity filtering of samples for DMSP_d was obtained according to the method of Kiene and Slezak (2006) with minor modifications. The filtrate and unfiltered seawater for DMSP_d and total DMSP (DMSP_i , $\text{DMSP}_d + \text{DMSP}_p$) measurements were transferred to a 40 mL serum bottle containing 2 mL of 10 mol L^{-1} KOH solution and kept at 4 °C for at least 24 h to complete cleavage. DMSP_d and DMSP_p concentrations were determined by the total DMS subtracting the DMS in original seawater. The DMSP_f and DMSP_z concentrations were calculated by subtracting DMS and DMSP_d in filtered water.

25 2.3 Statistical analysis

Data were expressed as mean \pm standard deviation. Student's *t* test and one-way ANOVA were used to determine the differences between control and treatment samples. Pearson correlations were utilized to assess the relationships between DMS/DMSP and IRs.



3 Results

3.1 Field experiment

3.1.1 Abiotic parameter

The temperature and salinity in Jiaozhou Bay are described in Fig. 2A. The salinity changed from 29.7 PSU to 32.3 PSU, and the lowest and highest salinities occurred in August 2010 (summer) and April 2011 (spring), respectively. The changing range of temperature was 1.72 °C to 25.3 °C, and the lowest and highest temperatures were observed in December 2010 (winter) and August 2010 (summer), respectively.

3.1.2 Biotic parameters

Chl *a* concentrations in surface water ranged from 0.808 µg L⁻¹ to 11.0 µg L⁻¹, with an average value of 3.09 µg L⁻¹. Chl *a* concentration peaked on April 2011 (spring) (Fig. 2B). The bacterial abundance in surface water ranged from 5.7 × 10⁷ cells L⁻¹ to 1.79 × 10⁸ cells L⁻¹ (average of 9.68 × 10⁷ cells L⁻¹), with a peak value in September 2010 (autumn) (Fig. 2B).

A total of 74 species of zooplankton were identified and were categorized into 5 phyla, 6 classes, 17 orders, 44 families, and 55 genera. Among these species, 31 are Arthropoda, 22 are Coelenterata, 17 are planktonic larvae, 2 are Chaetognatha, 1 are Protozoa, and 1 are Tunicata (Table 2). The species composition of zooplankton varied with months, and the number of species ranged from 19 to 35. The lowest and highest numbers of species occurred in February 2011 (winter) and June 2010 (summer), respectively. In total, 22 copepods were identified, and *Acartia pacifica*, *Acartia biflosa*, *Centropages abdominalis*, *Eurytemora pacifica*, and *C. sinicus* were the predominant copepods (Table 2 and Fig. 3C).

Temporal variations in the mean zooplankton abundance are shown in Fig. 3A. *Noctiluca scintillans* appeared as the dominant zooplankton, contributing 91% of zooplankton abundance on June 2010. Copepods dominated the zooplankton community during winter and spring and accounted for 39% to 83% of the total zooplankton (Fig. 3A). The changing trend of copepod abundance showed that the minimum (7.5 individuals m⁻³) occurred on July 2010, subsequently increased, and peaked (596 individuals m⁻³) on April 2011 (Fig. 3B). The *C. sinicus* abundance ranged from 0.143 individuals m⁻³ to 123 individuals m⁻³, and *C. sinicus* is the dominant copepod from October 2010 to May 2011. The *C. sinicus* abundance showed a similar trend to that of copepod, and *C. sinicus* exhibited the highest and lowest abundances in spring and summer, respectively (Figs. 3B and 3C).

3.1.3 DMS/DMSP

Changes in DMS/DMSP are presented in Fig. 4. The average concentrations of DMSP_d, DMSP_p, and DMS in surface water were 3.96 (1.14–9.93), 10.6 (3.29–21.2), and 1.91 (0.41–3.19) nmol L⁻¹, respectively. Compared with DMSP_d and DMS, DMSP_p has higher concentrations at the same periods. The highest concentrations of DMSP_p, DMSP_d, and DMS in Jiaozhou Bay were observed on September, September, and June 2010, respectively. In comparison, the lowest contents of DMSP_p, DMSP_d, and DMS were observed on November 2010, April 2011, and April 2011, respectively.



3.1.4 Relationships with biotic and abiotic parameters

In Jiaozhou Bay, many factors (biotic and abiotic parameters) could affect DMS and DMSP concentrations, and five factors (salinity, temperature, Chl *a* concentrations, bacterial abundance, and copepod abundance) were selected to investigate their effects on DMS and DMSP in this study. Salinity was negatively correlated with temperature and DMSP_p ($p < 0.05$). In comparison, Chl *a* concentrations were positively correlated with the copepod abundance ($p < 0.01$), and the copepod abundance was positively correlated with *C. sinicus* abundance ($p < 0.05$). Furthermore, positive correlations were observed among DMS, DMSP_d, and DMSP_p ($p < 0.01$). In addition, no significant correlation was observed between DMS/DMSP and zooplankton, copepod, *C. sinicus* abundance or Chl *a* concentration ($p > 0.05$) (Table 3).

3.2 Incubation experiment

3.2.1 Dietary effects on IR, CR, and DMS/DMSP

After *C. sinicus* was fed on four different diets, IRs changed from 0.13×10^3 cells copepod⁻¹ h⁻¹ to 5.55×10^3 cells copepod⁻¹ h⁻¹ and CRs ranged from 0.26 mL copepod⁻¹ h⁻¹ to 0.80 mL copepod⁻¹ h⁻¹ (Fig. 5A). Four species of algae affected IRs and CRs of *C. sinicus* differently, with *C. curvisetus* being the optimum diet for the grazing of *C. sinicus* (IR = 5.55×10^3 cells copepod⁻¹ h⁻¹; CR = 0.80 mL copepod⁻¹ h⁻¹), *I. galbana* being the second, *E. huxleyi* being the third, and *Gymnodinium* sp. being the fourth.

The ingestion of *C. sinicus* of four species of algae would result in different DMS productions (Fig. 5B). With regard to *I. galbana* and *C. curvisetus*, *C. sinicus* grazing promoted DMS production in the treatments compared with the controls, e.g., DMS in *C. curvisetus* treatment by *C. sinicus* grazing was 1.7-fold of DMS in the controls. In comparison, DMS production has an opposite changing trend for *E. huxleyi* and *Gymnodinium* sp., in which the treatments showed lower DMS production than that in the controls.

For four algae species, DMSP_p concentrations in the controls ranged from 48.78 nmol L⁻¹ to 7,165 nmol L⁻¹ and those in the treatments ranged from 27.25 nmol L⁻¹ to 11,055 nmol L⁻¹. In the control and treatment groups, DMSP_p concentrations of *E. huxleyi* and *Gymnodinium* sp. were two to three orders of magnitude higher than those of *I. galbana* and *C. curvisetus* (Fig. 5C). DMSP_p concentrations in the treatments with copepod exhibited diverse changes for four algae species, DMSP_p concentrations for *I. galbana* and *C. curvisetus* decreased, and DMSP_p concentrations for *E. huxleyi* and *Gymnodinium* sp. increased. DMSP_d in the treatments were higher than those in the controls ($p > 0.05$). DMSP_d concentrations in the controls ranged from 10.60 nmol L⁻¹ to 6,595 nmol L⁻¹. In comparison, DMSP_d concentrations ranged from 11.39 nmol L⁻¹ to 10,848 nmol L⁻¹ in the treatments (Fig. 5D). DMSP_d in the treatments were higher than those in the controls ($p > 0.05$).

Significant differences for the DMSP_z and DMSP_f contents of the four diets were observed (Fig. 5E). DMSP_z contents decreased according the following order: *E. huxleyi* > *Gymnodinium* sp. > *I. galbana* > *C. curvisetus*. DMSP_z contents of *C. sinicus* fed on *E. huxleyi* were 23.51-fold of those fed on *C. curvisetus* (Fig. 5E). When compared with DMSP_z contents, DMSP_f of four diets had different contents with the following order: *Gymnodinium* sp. > *E. huxleyi* > *I. galbana* > *C.*



curvisetus. DMSP_f contents of *C. sinicus* fed on *Gymnodinium* sp. were 126.3-fold of those fed on *C. curvisetus* (Fig. 5E). For *C. curvisetus*, *I. galbana*, *Gymnodinium* sp., and *E. huxleyi*, DMSP_z and DMSP_f accounted for 0.58%, 3.2%, 0.57%, 0.14% and 0.16%, 1.04%, 0.70%, 0.43% of the total amounts of DMS/DMSP (DMSP_z + DMSP_f + DMS + DMSP_{d,p}), respectively.

5 3.2.2 Food concentration experiment

When *I. galbana* concentrations increased, IRs gradually increased, peaked at 15×10^4 cells mL⁻¹, and subsequently declined (ranged from 2.45×10^3 cells copepod⁻¹ h⁻¹ to 5.12×10^3 cells copepod⁻¹ h⁻¹). In comparison, increased *I. galbana* concentrations induced the decrease in CRs, which ranged from 0.007 mL copepod⁻¹ h⁻¹ to 0.054 mL copepod⁻¹ h⁻¹ (Fig. 6A). DMS and DMSP_p increased with the increase in *I. galbana* concentrations (Figs. 6B and 6C). DMS in the treatments were higher than those in the controls ($p < 0.05$). Moreover, DMS in the treatments ($20.19\text{--}49.84$ nmol L⁻¹) increased by 1.8% to 11% compared with that in the controls ($18.23\text{--}48.96$ nmol L⁻¹). DMSP_p contents in the treatments ($292.44\text{--}945.76$ nmol L⁻¹) were lower than those in the controls ($385.94\text{--}1,319.83$ nmol L⁻¹) because of grazing activity.

DMSP_d increased initially, peaked at 15×10^4 cells mL⁻¹, and decreased with the increase in *I. galbana* concentrations (Fig. 6D). DMSP_d contents in the treatments ($23.54\text{--}235.94$ nmol L⁻¹) were higher than those in the controls ($22.65\text{--}207.86$ nmol L⁻¹) ($p > 0.05$), and those in the treatments increased by 97.95% compared with that in the controls (20×10^4 cells mL⁻¹). A positive correlation between IR and the increment of DMS + DMSP_d was observed ($r = 0.623$, $p = 0.377$, $n = 4$). In addition, a positive correlation between algal concentration and the decrement of DMSP_p was discovered ($r = 0.767$, $p = 0.233$, $n = 4$).

DMSP_z and DMSP_f increased with the increase in *I. galbana* concentrations, peaked at 15×10^4 cells mL⁻¹, and declined (Fig. 6E). A significantly positive relationship was observed between IR and DMSP_f ($r = 0.99$, $p = 0.01$, $n = 4$), accompanied by irrelevance between IR and DMSP_z ($r = 0.07$, $p = 0.93$, $n = 4$). DMSP_z and DMSP_f of four *I. galbana* concentrations (10×10^4 , 15×10^4 , 20×10^4 , and 25×10^4 cells mL⁻¹) accounted for 1.9%, 4.5%, 3.8%, 2.5% and 3.3%, 2.4%, 1.4%, 1.2% of the sum of DMS/DMSP (DMSP_z + DMSP_f + DMS + DMSP_{d,p}) of *I. galbana*, respectively. We observed significant positive correlations between DMS and DMSP_p ($r = 0.893$, $p = 0.003$, $n = 8$), accompanied by significant negative correlations between DMS and DMSP_d ($r = -0.847$, $p = 0.008$, $n = 8$). In addition, significant negative correlations between DMSP_d and DMSP_p were noted in this study ($r = -0.804$, $p = 0.016$, $n = 8$).

3.2.3 Effects of salinity on IRs and DMS/DMSP production

The salinity experiments showed that IRs and CRs increased with the increase in salinities, peaked at 30 PSU (IR = 3.18×10^3 cells copepod⁻¹ h⁻¹; CR = 0.23 mL copepod⁻¹ h⁻¹), and declined (Fig. 7A).

In this study, Fig. 7B shows that increased salinities restrained the production of DMS. Moreover, DMS contents in the treatment decreased from 84.54 nmol L⁻¹ to 26.54 nmol L⁻¹ when salinities increased, which was consistent with the DMS changes in the control (reduction from 41.11 nmol L⁻¹ to 25.90 nmol L⁻¹). The DMSP_d changes indicated a trend similar to



those of DMS contents, that is, increasing salinities decreased DMSP_d contents. Fig. 7D shows that DMSP_d contents decreased from 481.41 nmol L⁻¹ to 72.35 nmol L⁻¹ in the treatment. In comparison, DMSP_d contents in the control reduced from 423.99 nmol L⁻¹ to 66.80 nmol L⁻¹. DMS contents of the treatments were higher than those of the controls ($p > 0.05$) and increased by 106%, 51%, 4.7%, and 2.4% at the salinities of 20, 25, 30, and 35 PSU, respectively.

- 5 The increasing salinities facilitated the accumulation of DMSP_p in the controls and treatments. DMSP_p contents in the treatments increased from 92.20 nmol L⁻¹ to 371.49 nmol L⁻¹, and those in the controls rose from 121.57 nmol L⁻¹ to 532.16 nmol L⁻¹. DMSP_p contents in the controls and treatments at 30 PSU were 2.9- and 1.7-fold of those at 25 PSU, respectively. DMSP_p contents in the treatments were lower than those in the controls because of grazing activity ($p > 0.05$). Furthermore, data analysis showed a positive correlation between IR and the decrement of DMSP_p ($r = 0.945$, $p = 0.055$, $n = 4$).
- 10 DMSP_d contents in the treatments were higher than those in the controls ($p > 0.05$) (Fig. 7D). The relationship between IR and the increment of DMS + DMSP_d proved to be positive ($r = 0.662$, $p = 0.338$, $n = 4$). In comparison, the correlation between the increments of DMS and DMSP_d was negative ($r = -0.955$, $p = 0.045$, $n = 4$).

- DMSP_z ranged from 0.21 nmol L⁻¹ to 5.38 nmol L⁻¹, reached the maximum content at 35 PSU, and reached the minimum content at 20 PSU. When salinities increased, DMSP_z initially increased, subsequently declined, and finally increased (Fig. 7E). Maximum DMSP_z contents at 35 PSU were 25.6-fold, 4.2-fold, and 24.5-fold of those at 20, 25, and 30 PSU, respectively. DMSP_f had a similar trend to DMSP_z in the salinity experiments, and its changing range was 0.78 nmol L⁻¹ to 3.44 nmol L⁻¹. DMSP_f reached the minimum at 20 PSU and the peak at 25 PSU. Compared with 20, 30, and 35 PSU, DMSP_f at 25 PSU increased by 341%, 98%, and 2%, respectively. At 35 PSU, DMSP_z and DMSP_f attained the maximum sum (8.75 nmol L⁻¹), which was 8.84-fold of the minimum at 20 PSU. With regard to DMS/DMSP (DMS + DMSP_{d,p} + DMSP_z +
- 15 DMSP_f) of *I. galbana*, DMSP_z of the four salinities (20, 25, 30, and 35 PSU) accounted for 0.035%, 0.2%, 0.05%, and 1.1%, and DMSP_f of the four salinities accounted for 0.13%, 0.56%, 0.41%, and 0.70%, respectively. Pearson correlation analysis did not detect a significant correlation between IR and DMSP_z ($r = 0.37$, $p = 0.63$, $n = 4$), which coincided with the relationship between IR and DMSP_f ($r = 0.30$, $p = 0.70$, $n = 4$). In addition, DMSP_p was significantly negatively correlated with DMS ($r = -0.721$, $p = 0.044$, $n = 8$) and DMSP_d ($r = -0.918$, $p = 0.001$, $n = 8$). In comparison, DMSP_p was observed to
- 25 be positively correlated with DMSP_f ($r = 0.491$, $p = 0.509$, $n = 4$) and DMSP_z ($r = 0.705$, $p = 0.295$, $n = 4$).

4 Discussion

4.1 Effects of *C. sinicus* on DMS/DMSP in the field study

- In Jiaozhou Bay, *C. sinicus* was the predominant copepod in 74 species of zooplankton identified from years 2010 to 2011. In this study, no significant correlations were observed between zooplankton, copepod, and/or *C. sinicus* abundance and
- 30 DMS/DMSP concentrations in the field study, illustrating that Jiaozhou Bay was a complex ecosystem with different abundances and types of phytoplankton, zooplankton, and/or copepod in the natural environment. Many kinds of copepods inhabited Jiaozhou Bay, and their IRs depended on copepod species, e.g., IRs of *Harpacticus* sp. (Yu et al., 2015) were 10-



fold of those of *C. sinicus* in this study. DMS/DMSP production was affected by phytoplankton species, which was difficult to investigate in the field study. We evaluated the effects of several agents (i.e., food, diet concentration, and salinity) on DMS and DMSP productions in the laboratory study. Our incubation data showed that copepod grazing increased DMS production, which was consistent with previous investigations on the effects of copepod grazing on DMS production (Dacey and Wakeham, 1986; Yu et al., 2015). Consistent with our field study results, no significant correlations between mesozooplankton abundance and the distribution of DMS or DMSP_d were also observed in the Gulf of Maine and St. Lawrence in previous studies (Cantin et al., 1996; Matrai and Keller, 1993). Cantin et al. (1996) concluded that mesozooplankton grazing played a minor role in DMS and DMSP_d productions in the Gulf of St. Lawrence. The variable conditions in the natural environment can explain the reason for the minor role of zooplankton on DMS/DMSP production and the inconsistent results of field and incubation studies.

4.2 Dietary effects on copepod grazing and DMS/DMSP production

Different diets contain various DMSP contents, which affected DMS production induced by copepod grazing. DMSP-rich algae (*E. huxleyi* and *Gymnodinium* sp.) were not the preferable food for copepod *C. sinicus* in the incubation study because the released acrylic acid from DMSP in algae prevents them from copepod grazing. Based on the species of the diets, algae produced the corresponding DMS and DMSP (DMSP_d and DMSP_p) contents, which might be different. For example, when comparing *Gymnodinium* sp. and *E. huxleyi* with *C. curvisetus* and *I. galbana*, we determined that cellular DMSP_p in *Gymnodinium* sp. and *E. huxleyi* were one to two orders of magnitude higher than those in *C. curvisetus* and *I. galbana* (see Table 1). In terms of *I. galbana*, our detection results on cellular DMSP_p production were consistent with those reported by Niki et al. (2000), indicating that cellular DMSP_p production in given algae was approximately invariable.

When *Gymnodinium* sp. and *E. huxleyi* (DMSP-rich phytoplankton) were grazed by *C. sinicus*, acrylic acid or DMSP released from algae protected them from being grazed and stimulated the increase in DMSP_p. In the natural environment, acrylic acid or DMSP depressed copepod appetites and forced them to ingest other DMSP-poor phytoplankton. Thus, DMSP-poor *C. curvisetus* became the favorite diet of copepods among four algae species, elucidating that the *C. sinicus* fed on *C. curvisetus*, which evidently promoted DMS production in this study. Further studies have revealed that DMSP_d was a feeding inhibitor, and DMSP_p, DMSP_d, DMS, and acrylic acid constituted a cellular antioxidant system involved in the scavenging of hydroxyl radicals (Strom et al., 2003; Sunda et al., 2002). Non-DMS-producing phytoplankton species in a mixture of prey were preferentially selected by grazers, and single DMS/DMSP-rich diet decreased the food intake of copepods, e.g., copepod *Harpacticus* sp. had inferior IRs and PPRs when fed on DMS/DMSP-rich alga *Prymnesium parvum* (Wolfe et al., 1997; Wolfe and Steinke, 1996; Yu et al., 2015). In this study, DMSP-rich *Gymnodinium* sp. and *E. huxleyi* repressed copepod grazing, elucidating the antipredator ability of these algae, which in turn reduced DMS/DMSP production.

Our results showed that DMSP in copepod bodies and fecal pellets accounted for 0.035% to 4.5% and 0.13% to 3.3% of DMS and DMSP in this study, illustrating that the ingestion of *C. sinicus* transferred DMSP from phytoplankton to the



copepod bodies and fecal pellets. When compared with *Harpacticus* sp., lower DMSP_f and DMSP_z contributions in *C. sinicus* attributed to the lower IRs of *C. sinicus*.

Based on the *C. sinicus* abundance on May 2011, the maximum DMSP_z (0.02–7 nmol L⁻¹) and DMSP_f (0.1–12 nmol L⁻¹) of *C. sinicus* in the seawater of Jiaozhou Bay were achieved. The results of our study and that of Tang (2001) showed that DMSP_p in copepod bodies and fecal pellets was an essential part of DMSP flux in the ocean. In addition, another sink of DMSP in the ocean was achieved by microbial processes. The results of Tang et al. (2001) and Dong et al. (2013) indicated that copepods and their pellets harbored a dense population of DCB (DMSP-consuming bacteria), which played an important role in DMSP degradation.

DMSP_z and DMSP_f contents were strongly associated with *C. sinicus* grazing. Their changing trends were consistent with that of IR, which depended on the critical concentration of phytoplankton. IRs of *C. sinicus* increased steadily with the increase in algal concentration below the critical concentration and decreased above the critical concentration. Critical concentration differed depending on the copepod and algal species, which were confirmed by this study and other investigations (Yu et al., 2015).

4.3 Effects of salinity on copepod grazing and DMS/DMSP

In this study, low salinity induced high DMS production in seawater, whereas high salinity increased DMSP_p (intracellular DMSP) and decreased DMSP_d, which were consistent with the conclusion of Variamuthy et al. (1985). DMSP, an osmolyte possessing cytomembrane permeability (Kirst, 1996), was excluded from cells when salinity decreased, illustrating the aforementioned DMSP changes. *C. sinicus* grazing promoted DMS production in this study, which was consistent with the results obtained by many investigators (Belviso et al., 1990; Christaki et al., 1996; Daly and DiTullio, 1996; Leck et al., 1990). The decrement of DMSP_p changed into DMS and DMSP_d by copepod grazing, which was consistent with the positive relationships between IR and the increment of DMS + DMSP_d and/or the decrement of DMSP_p. The increment of DMS was significantly negatively correlated with the increment of DMSP_d, indicating that the cleavage of DMSP_d was the source of DMS by DMSP lyase. Many reports on the location of DMSP lyase in the cell have been published. Wolfe and Steinke (1996) indicated that the DMSP lyase location of *E. huxleyi* CCMP 370 was in the membrane bound inside cells. Stefels and Dijkhuizen (1996) reported that DMSP lyase of *Phaeocystis* was membrane-bound and located extracellularly. Cellular locations and functions of DMSP lyase might differ depending on algal species.

This study confirmed that DMS production was affected by primary producers, e.g., algae. DMS/DMSP in algae transferred to the food web by predation, and several researchers investigated the effects of zooplankton grazing on DMS production with copepods and krill, indicating that the breakage of algal cells through sloppy feeding may increase DMSP_d production (Dacey and Wakeham, 1986; Kaamatsu et al., 2004). When DMSP_p concentrations changed, DMSP_f and DMSP_z concentrations were altered correspondingly, which was verified by other results in which the DMSP defecation rate of copepod *Acartia tonsa* feeding on *Tetraselmis impellucida* (prasinophyte) was closely related to food concentration and DMSP_z content (Tang, 2001). Salinity changes altered the osmotic pressure surrounding copepod and algae cells, which in



turn adjusted DMSP (intracellular DMSP_p, DMSP in tissues, and DMSP in gut content). Copepods contained more DMSP at high salinity, indicating the osmoregulatory function of DMSP (Tang et al., 1999).

5 Conclusions

In the present study, field and incubation experiments were performed to investigate the effects of *C. sinicus* grazing on DMS production in Jiaozhou Bay. Copepods (*C. sinicus*) indicated no apparent effect on DMS/DMSP production in the field experiment. Appropriate diets and salinities facilitated DMS/DMSP production, e.g., *C. sinicus* feeding on *I. galbana* and *C. curvisetus* exhibited increased DMS production at 30 PSU in the laboratory experiment. *C. sinicus* grazing promoted the productions of DMS and DMSP_d, and DMS was released mainly from DMSP_d and low salinity increased DMS production. Copepods and fecal pellets supplied substantial DMSP_p into the water column and were important to the biogeochemical cycling of DMS.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. This work was financially supported by the National Key Research and Development Program of China (Grant No. 2016YFA0601302), the National Natural Science Foundation for Creative Research Groups (Grant No. 41521064), the Major International Joint Research Project of NSFC (Grant No. 41320104008), the AoShan Talents Program supported by the Qingdao National Laboratory for Marine Science and Technology (Grant No. 2015ASTP), and the National Natural Science Foundation of China (Grant No. 41776085). This study is MCTL Contribution No. 156.

References

- Andreae, M.O. and Barnard, W.R.: Determination of trace quantities of dimethyl sulfide in aqueous solutions, *Anal. Chem.*, 55, 608–612, <https://doi.org/10.1021/ac00255a006>, 1983.
- 20 Belviso, S., Kim, S.K., Rassoulzadegan, F., Krajka, B., Nguyen, B.C., Mihalopoulos, N., and Buat-Menard, P.: Production of dimethylsulfonium propionate (DMSP) and dimethylsulfide (DMS) by a microbial food web, *Limnol. Oceanogr.*, 35, 1810–1821, <https://doi.org/10.4319/lo.1990.35.8.1810>, 1990.
- Brodsky, K.A.: Variability and systematic of the species of the genus *Calanus* Copepoda. 1. *Calanus pacificus* Brodsky and *Calanus sinicus* Brodsky sp. n, *Exp. Fauna Seas.*, 3, 22–71, 1965.
- 25 Cantin, G., Levasseur, M., Gosselin, M., and Michaud, S.: Role of zooplankton in the mesoscale distribution of surface dimethylsulfide concentrations in the Gulf of St. Lawrence, Canada, *Mar. Ecol. Prog. Ser.*, 141, 103–117, <https://doi.org/10.3354/meps141103>, 1996.
- Charlson, R.J., Lovelock, J.E., Andreae, M.O., and Warren, S.G.: Oceanic phytoplankton, atmospheric sulphur, cloud albedo, and climate, *Nature*, 326, 655–661, <https://doi.org/10.1038/326655a0>, 1987.



- Christaki, U., Belviso, S., Dolan, J.R., and Corn, M.: Assessment of the role of copepods and ciliates in the release to solution of particulate DMSP, *Mar. Ecol. Prog. Ser.*, 141, 119–127, <https://doi.org/10.3354/meps141119>, 1996.
- Dacey, J.W.H. and Wakeham, S.G.: Oceanic dimethylfide: production during zooplankton grazing on phytoplankton, *Science*, 233, 1314–1316, <https://doi.org/10.1126/science.233.4770.1314>, 1986.
- 5 Daly, K.L. and DiTullio, G.R.: Particulate dimethylsulfoniopropionate removal and dimethylsulfide production by zooplankton in the Southern Ocean, in: *Biological and environmental chemistry of DMSP and related sulfonium compounds*, edited by: Kiene, R.P., Visscher, P.T., Keller, M.D., Kirst, G.O., Springer, Boston, MA, 223–238, 1996.
- Dong, Y., Yang, G.-P., and Tang, K.M.: Dietary effects on abundance and carbon utilization ability of DMSP-consuming bacteria associated with the copepod *Acartia tonsa* Dana, *Mar. Biol. Res.*, 9, 809–814, <https://doi.org/10.1080/17451000.2013.765587>, 2013.
- 10 Frost, B.W.: Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus Pacificus*, *Limnol. Oceanogr.*, 17, 805–815, <https://doi.org/10.4319/lo.1972.17.6.0805>, 1972.
- Guillard, R.R.L.: Culture of phytoplankton for feeding marine invertebrates, in: *Culture of Marine Invertebrate Animals*, edited by: Smith, W.L., Chanley, M.H., Springer, Boston, MA, 29–60, 1975.
- 15 Kasamatsu, N., Kawaguchi, S., Watanabe, S., Odate, T., and Fukuchi, M.: Possible impacts of zooplankton grazing on dimethylsulfide production in the Antarctic Ocean, *Can. J. Fish. Aquat. Sci.*, 61, 736–743, <https://doi.org/10.1139/f04-072>, 2004.
- Kettle, A.J., Andreae, M.O., Amouroux, D., Andreae, T.W., Bates, T.S., Berresheim, H., Bingemer, H., Boniforti, R., Curran, M.A.J., DiTullio, G.R., Helas, G., Jones, G.B., Keller, M.D., Kiene, R.P., Leck, C., Levasseur, M., Malin, G., Maspero, M., Matrai, P., McTaggart, A.R., Mihalopoulos, N., Nguyen, B.C., Novo, A., Putaud, J.P., Rapsomanikis, S., Roberts, G., Schebeske, G., Sharma, S., Simó, R., Staubes, R., Turner, S., and Uher, G.: A global database of sea surface dimethylsulfide (DMS) measurements and a procedure to predict sea surface DMS as a function of latitude, longitude, and month, *Global Biogeochem. Cy.*, 13, 399–444, <https://doi.org/10.1029/1999GB900004>, 1999.
- 20 Kiene, R.P. and Linn, L.J.: Distribution and turnover of dissolved DMSP and its relationship with bacterial production and dimethylsulfide in the Gulf of Mexico, *Limnol. Oceanogr.*, 45, 849–861, <https://doi.org/10.4319/lo.2000.45.4.0849>, 2000.
- Kiene, R.P. and Slezak, D.: Low dissolved DMSP concentrations in seawater revealed by small-volume gravity filtration and dialysis sampling, *Limnol. Oceanogr.: Methods*, 4, 80–95, <https://doi.org/10.4319/lom.2006.4.80>, 2006.
- Kjørboe, T.: Population regulation and role of mesozooplankton in shaping marine pelagic food webs, *Hydrobiologia*, 363, 13–27, <https://doi.org/10.1023/A:1003173721751>, 1997.
- 30 Kirst, G.O.: Osmotic adjustment in phytoplankton and macroalgae, in: *Biological and environmental chemistry of DMSP and related sulfonium compounds*, edited by: Kiene, R.P., Visscher, P.T., Keller, M.D., Kirst, G.O., Springer, Boston, MA, 121–129, 1996.
- Lancelot, C. and Billen, G.: Carbon-nitrogen relationships in nutrient metabolism of coastal marine ecosystem, *Adv. Aquat. Microbiol.*, 3, 263–321, 1985.



- Leck, C., Larsson, U., Bågander, L.E., Johansson, S., and Hadju, S.: Dimethyl sulfide in the Baltic Sea: Annual variability in relation to biological activity, *J. Geophys. Res.*, 95, 3353–3363, <https://doi.org/10.1029/JC095iC03p03353>, 1990.
- Li, Y., Li, R., Wang, Z., Zhu, M., Sun, P., and Xia, B.: A preliminary study on phytoplankton community structure and its changes in the Jiaozhou Bay, *Adv. Mar. Sci.*, 23, 328–334, 2005. (in Chinese with English abstract)
- 5 Matrai, P.A. and Keller, M.D.: Dimethylsulfide in a large-scale coccolithophore bloom in the Gulf of Maine, *Contin. Shelf Res.*, 13, 831–843, [https://doi.org/10.1016/0278-4343\(93\)90012-M](https://doi.org/10.1016/0278-4343(93)90012-M), 1993.
- Nguyen, B.C., Belviso, S., Mihalopoulos, N., Gostan, J., and Nival, P.: Dimethyl sulfide production during natural phytoplanktonic blooms, *Mar. Chem.*, 24, 133–141, [https://doi.org/10.1016/0304-4203\(88\)90044-8](https://doi.org/10.1016/0304-4203(88)90044-8), 1988.
- Niki, T., Kunugi, M., and Otsuki, A.: DMSP-lyase activity in five marine phytonplankton species: its potential importance in
10 DMS production, *Mar. Biol.*, 136, 759–764, <https://doi.org/10.1007/s002279900235>, 2000.
- Parsons, T.R., Maita, Y., and Lalli, C.M. (Eds.): A manual of chemical and biological methods for seawater analysis, Pergamon Press, Oxford, 184 pp., 1984.
- Porter, K.G. and Feig, Y.S.: The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, 25, 943–948, <https://doi.org/10.4319/lo.1980.25.5.0943>, 1980.
- 15 Stefels, J. and Dijkhuizen, L.: Characteristics of DMSP-lyase in *Phaeocystis* sp. (Prymnesiophyceae), *Mar. Ecol. Prog. Ser.*, 131, 307–313, <https://doi.org/10.3354/meps131307>, 1996.
- Strathmann, R.R.: Estimating the organic carbon content of phytoplankton from cell volume or plasma volume, *Limnol. Oceanogr.*, 12, 411–418, <https://doi.org/10.4319/lo.1967.12.3.0411>, 1967.
- Strom, S., Wolfe, G., Slajer, A., Lambert, S., and Clough, J.: Chemical defense in the microplankton II: Inhibition of protist
20 feeding by β -dimethylsulfoniopropionate (DMSP), *Limnol. Oceanogr.*, 48, 230–237, <https://doi.org/10.4319/lo.2003.48.1.0230>, 2003.
- Sunda, W., Kieber, D.J., Kiene, R.P., and Huntsman, S.: An antioxidant function for DMSP and DMS in marine algae, *Nature*, 418, 317–320, <https://doi.org/10.1038/nature00851>, 2002.
- Tang, K.W.: Defecation of dimethylsulfoniopropionate (DMSP) by the copepod *Acartia tonsa* as functions of ambient food
25 concentration and body DMSP content, *J. Plankton Res.*, 23, 549–553, <https://doi.org/10.1093/plankt/23.5.549>, 2001.
- Tang, K.W., Dam, H.G., Visscher, P.T., and Fenn, T.D.: Dimethylsulfoniopropionate (DMSP) in marine copepods and its relation with diets and salinity, *Mar. Ecol. Prog. Ser.*, 179, 71–79, <https://doi.org/10.3354/meps179071>, 1999.
- Tang, K.W., Fenn, T.D., Visscher, P.T., and Dam, H.G.: Regulation of body dimethylsulfoniopropionate (DMSP) content by the copepod *Temora longicornis*: a test of four mechanisms, *Mar. Biol.*, 136, 749–757,
30 <https://doi.org/10.1007/s002270000309>, 2000.
- Tang, K.W., Visscher, P.T., and Dam, H.G.: DMSP-consuming bacteria associated with the calanoid copepod *Acartia tonsa* (Dana), *J. Exp. Mar. Biol. Ecol.*, 256, 185–198, [https://doi.org/10.1016/S0022-0981\(00\)00314-2](https://doi.org/10.1016/S0022-0981(00)00314-2), 2001.
- Turner, S.M., Malin, G., Nightingale, P.D., and Liss, P.S.: Seasonal variation of dimethyl sulphide in the North Sea and an assessment of fluxes to the atmosphere, *Mar. Chem.*, 54, 245–262, [https://doi.org/10.1016/0304-4203\(96\)00028-X](https://doi.org/10.1016/0304-4203(96)00028-X), 1996.



- Vairavamurthy, A., Andreae, M.O., and Iverson, R.L.: Biosynthesis of dimethylsulfide and dimethylpropiothetin by *Hymenomonas carterae* in relation to sulfur source and salinity variations, *Limnol. Oceanogr.*, 30, 59–70, <https://doi.org/10.4319/lo.1985.30.1.0059>, 1985.
- Verity, P.G., Robertson, C.Y., Tronzo, C.R., Andrews, M.G., Nelson, J.R., and Sieracki, M.E.: Relationships between cell
5 volume and the carbon and nitrogen content of marine photosynthetic nanoplankton, *Limnol. Oceanogr.*, 37, 1434–1446, <https://doi.org/10.4319/lo.1992.37.7.1434>, 1992.
- Wolfe, G.V., Levasseur, M., Cantin, G., and Michaud, S.: DMSP and DMS dynamics and microzooplankton grazing in the Labrador Sea: application of the dilution technique, *Deep Sea Res. I*, 47, 2243–2264, [https://doi.org/10.1016/S0967-0637\(00\)00028-5](https://doi.org/10.1016/S0967-0637(00)00028-5), 2000.
- 10 Wolfe, G.V. and Steinke, M.: Grazing-activated production of dimethyl sulfide (DMS) by two clones of *Emiliania huxleyi*. *Limnol. Oceanogr.* 41, 1151–1160, <https://doi.org/10.4319/lo.1996.41.6.1151>, 1996.
- Wolfe, G.V., Steinke, M., and Kirst, G.O.: Grazing-activated chemical defence in a unicellular marine alga, *Nature*, 387, 894–897, <https://doi.org/10.1038/43168>, 1997.
- Wong, C.S., Wong, S.E., Richardson, W.A., Smith, G.E., Arychuk, M.D., and Page, J.S.: Temporal and spatial distribution
15 of dimethylsulfide in the subarctic northeast Pacific Ocean: a high-nutrient-low-chlorophyll region, *Tellus B*, 57, 317–331, <https://doi.org/10.1111/j.1600-0889.2005.00156.x>, 2005.
- Yu, J., Tian, J.-Y., and Yang, G.-P.: Effects of *Harpacticus* sp. (Harpacticoida, copepod) grazing on dimethylsulfoniopropionate and dimethylsulfide concentrations in seawater, *J. Sea Res.*, 99, 17–25, <https://doi.org/10.1016/j.seares.2015.01.004>, 2015.
- 20 Zhong, S.-L., Wang, Y.-P., Gao, S., Cao, Q.-Y., and Zhu, Y.-H.: Distribution patterns of nannofossil *gephyrocapsa oceanica* in surficial sediments of Jiaozhou Bay, southern Shandong Peninsula, China, *Acta Palaeontol. Sin.*, 40, 505–513, 2001.



Table 1. Sizes and DMSP of phytoplankton cells.

Algae	Dimension (μm)	Biovolume (μm^3)	Carbon (pg cell^{-1})	DMSP (fmol cell^{-1})
<i>C. curvisetus</i>	7.8×4.6	9.04×10^1	1.15×10^1	1.0×10^{-1}
<i>I. galbana</i>	6.0×5.8	1.11×10^2	2.05×10^1	4.0×10^0
<i>Gymnodinium</i> sp.	14.0×12.0	1.13×10^3	1.53×10^2	3.6×10^1
<i>E. huxleyi</i>	2.7×3.2	1.25×10^1	2.94×10^0	3.0×10^1

**Table 2.** Species of zooplankton in Jiaozhou Bay.

Phylum	Species	2010.06	2010.07	2010.08	2010.09	2010.10	2010.11	2010.12	2011.01	2011.02	2011.04	2011.05
Protozoa	<i>Noctiluca scientillans</i> Kofoid et Swezy	1	1	1	1	1	1	1	1	1	1	1
Coelenterata	<i>Ectopleura dumontieri</i> (Van Beneden)					1			1	1		
	<i>Bougainvillia muscus</i> (Allman)	1										
	<i>Sarsia japonica</i> (Nagao)	1	1	1			1		1		1	1
	<i>Rathkea octopunctata</i> (M.Sars)								1	1		
	<i>Turritopsis nutricula</i> (McCrary)									1		1
	<i>Euphysora bigelowi</i> Maas					1						1
	<i>Euphysora knides</i> Huang	1			1			1				
	<i>Zanclea costata</i> (Linne)			1			1					
	<i>Obelia</i> spp.	1		1	1	1	1				1	1
	<i>Clytia hemisphaerica</i> (Linnaeus)	1	1		1	1	1		1	1	1	1
	<i>Eucheilota menoni</i> Kramp					1	1	1				
	<i>Eirene cyclonensis</i> Browne					1	1					1
	<i>Liriope petrophylla</i> (Chamisso et Eysenhardt)						1					
	<i>Sugiura chengshanense</i> (Ling)	1										
	<i>Lovenella</i> sp.				1	1					1	
	<i>Malagazzia carolinae</i> (Mayer)	1	1									
	<i>Proboscicladia flavicirrata</i> Brandt	1							1	1	1	1
	<i>Aequorea conica</i> Browne								1			
	<i>Eutima levuka</i> (Agassiz et Mayer)			1	1							
	<i>Muggiaea atlantica</i> Cunningham			1	1							
	<i>Pleurobrachia globosa</i> Moser				1	1						
Arthropoda	<i>Berbe cucumis</i> Fabricius						1					
	<i>Penilia avirostris</i> Dana		1									
	<i>Evadne nordmanni</i> Loven		1									
	<i>Calanus sinicus</i> Brodsky	1	1	1	1	1	1	1	1	1	1	1
	<i>Paracalanus parvus</i> (Claus)	1			1	1	1		1			1
	<i>Eurytemora pacific</i> Sato							1	1		1	1
	<i>Pseudodiaptomus poplesia</i> Shen							1	1			
	<i>Sinocalanus tenellus</i> (Kikuchi)							1	1			
	<i>Centropages abdominalis</i> Sato	1	1				1	1	1	1	1	1
	<i>Centropages dorsispinatus</i> Thompson et Scott			1	1	1	1					
	<i>Centropages tenuiremis</i> Thompson et Scott	1	1	1	1	1						1
	<i>Calanopia thompsoni</i> A.Scott	1	1	1	1	1	1					
	<i>Labidocera pavo</i> (Dana)			1								
	<i>Labidocera euchaeta</i> Giesbrecht				1		1	1	1	1		
	<i>Labidocera bipinnata</i> Tanaka	1	1	1	1	1	1					1
	<i>Pontellopsis tenuicauda</i> (Giesbrecht)	1										
	<i>Acartia biflosa</i> Giesbrecht	1				1	1	1	1	1	1	1
	<i>Acartia pacifica</i> Steuer	1	1	1	1	1	1					1
	<i>Tortanus dextrilobatus</i> Chen and Zhang	1	1	1	1	1					1	1
	<i>Tortanus spinicaudatus</i> Shen et Bai			1	1							
	<i>Tortanus forcipatus</i> (Giesbrecht)					1	1					
	<i>Oithona similis</i> Claus								1		1	1
	<i>Corycaeus affinis</i> Mcmurrichi		1	1	1		1		1	1		
	Harpacticoida						1	1	1	1		
	<i>Monstrilla</i> sp.				1		1					
	<i>Themisto gracilipes</i> (Norman)										1	1
	<i>Acanthomysis longirostris</i> Li					1	1	1	1	1	1	1
	<i>Acetes japonicus</i> Kishinouye	1										
	Gammaridea	1					1	1			1	1
	<i>Caprella</i> sp.			1	1							1
	<i>Microniscus</i> sp.				1	1						
	<i>Leueon</i> sp.							1	1	1		
Chaetognatha	<i>Sagitta nagae</i> Alvarino			1		1	1	1				1
	<i>Sagitta crassa</i> Tokioka	1	1	1	1	1	1	1	1	1	1	1
Tunicata	<i>Oikopleura dioica</i> Fol					1	1					1
Planktonic larvae	Trochophore larva	1										
	Polychaeta larva	1		1	1	1		1	1	1		1
	Gastropod post larva	1	1	1	1	1					1	1
	Bivalve larva	1		1		1	1		1	1		1
	Nauplius larva (Copepoda)					1					1	
	Nauplius larva (Cirripedia)	1									1	
	Cypris larva	1										
	Macrura larva	1	1	1	1	1					1	1
	Brachyura zoea larva	1	1	1	1	1	1				1	1
	Megalopa larva	1	1	1	1							
	Porcellana zoea larva	1	1	1	1							
	Alima larva	1	1	1								
	Ophiopluteus larva				1							
	Echinopluteus larva				1	1	1	1	1	1		
	Echinodermata larva				1		1					
	Fish eggs	1	1								1	1
	Fish larva	1	1	1					1	1	1	1

“1” means appearance.



Table 3. Pearson correlation analyses between DMS/DMSP and abiotic and biotic parameters.

		Temperature	Salinity	Chl a	Bacteria	Zooplankton	Copepod	<i>C. sinicus</i>	DMS	DMSP _d	DMSP _p
Temperature	r	1									
	p value	0									
Salinity	r	-0.678*	1								
	p value	0.022	0								
Chl a	r	-0.082	0.423	1							
	p value	0.811	0.195	0							
Bacteria	r	0.437	-0.577	-0.202	1						
	p value	0.179	0.063	0.552	0						
Zooplankton	r	0.243	0.379	0.441	-0.116	1					
	p value	0.472	0.25	0.174	0.733	0					
Copepod	r	-0.166	0.448	0.877**	-0.114	0.125	1				
	p value	0.626	0.167	0	0.738	0.714	0				
<i>C. sinicus</i>	r	-0.225	0.382	0.315	-0.387	-0.027	0.698*	1			
	p value	0.507	0.247	0.345	0.239	0.938	0.017	0			
DMS	r	0.302	-0.353	-0.264	0.104	0.385	-0.47	-0.329	1		
	p value	0.367	0.287	0.434	0.762	0.242	0.144	0.323	0		
DMSP _d	r	0.454	-0.588	-0.227	0.56	0.117	-0.241	-0.219	0.812**	1	
	p value	0.161	0.057	0.503	0.073	0.732	0.475	0.518	0.002	0	
DMSP _p	r	0.477	-0.603*	-0.103	0.538	0.218	-0.205	-0.229	0.787**	0.900**	1
	p value	0.138	0.049	0.762	0.088	0.521	0.546	0.499	0.004	0	0

*, Correlation is significant at the 0.05 level (2-tailed).

5 **, Correlation is significant at the 0.01 level (2-tailed).

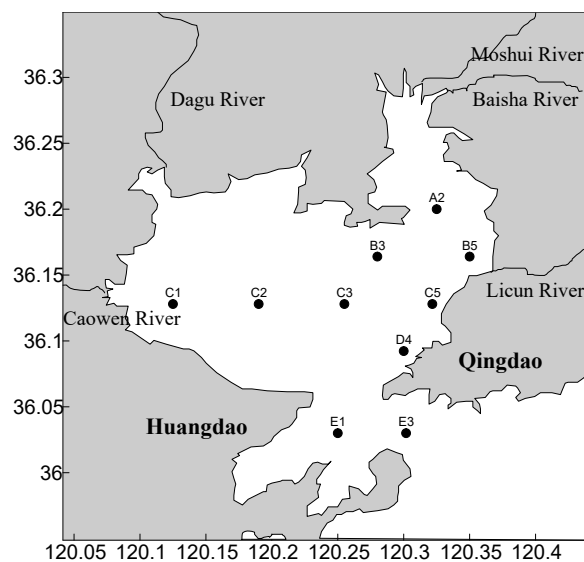


Figure 1. Location of fieldwork sampling stations in Jiaozhou Bay.

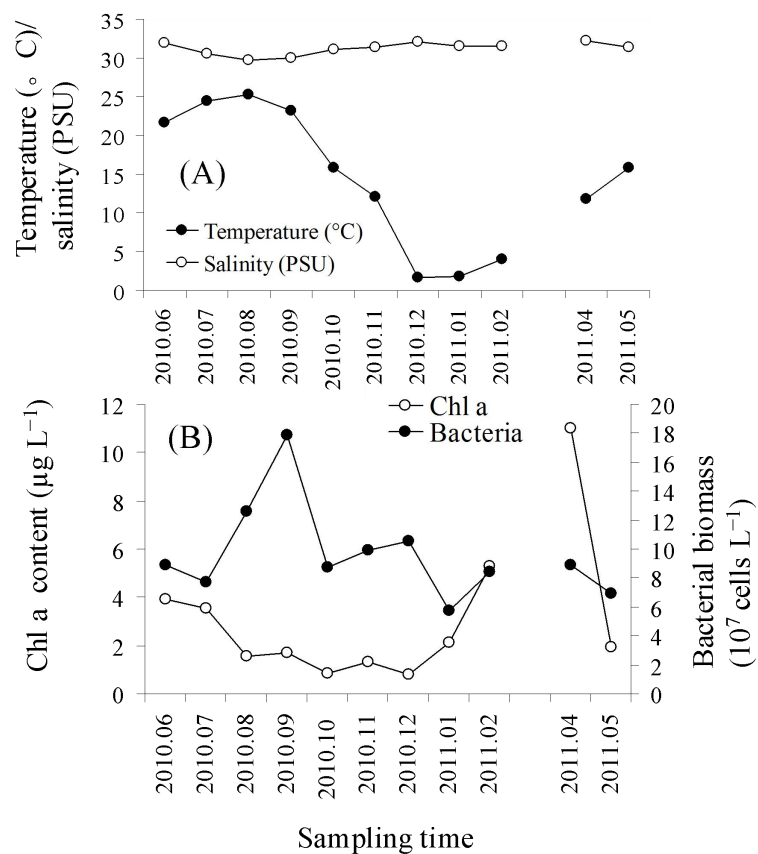


Figure 2. Monthly changes in temperature and salinity (A) and Chl *a* content and bacterial abundance (B) in Jiaozhou Bay.

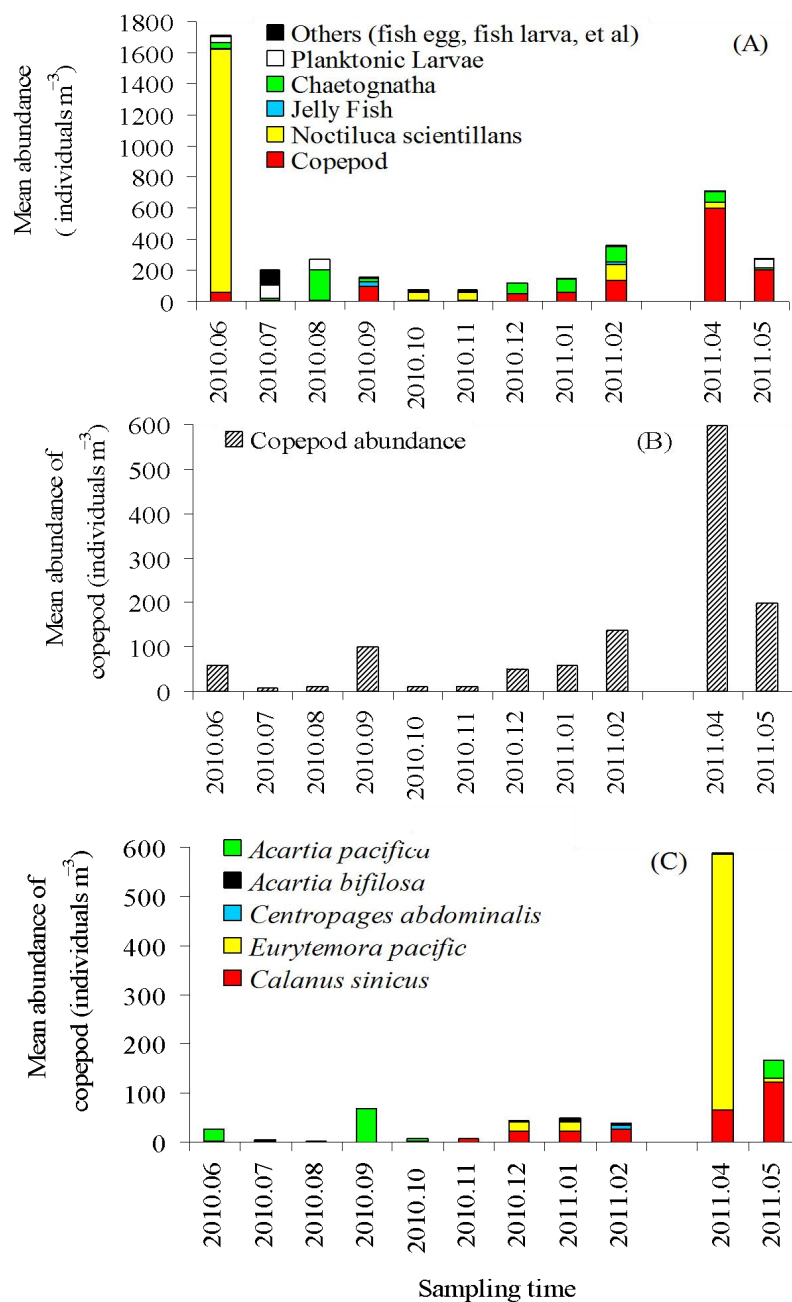


Figure 3. Monthly changes in the mean abundances of zooplankton (A), copepod (B), and predominant copepod (C) in Jiaozhou Bay.

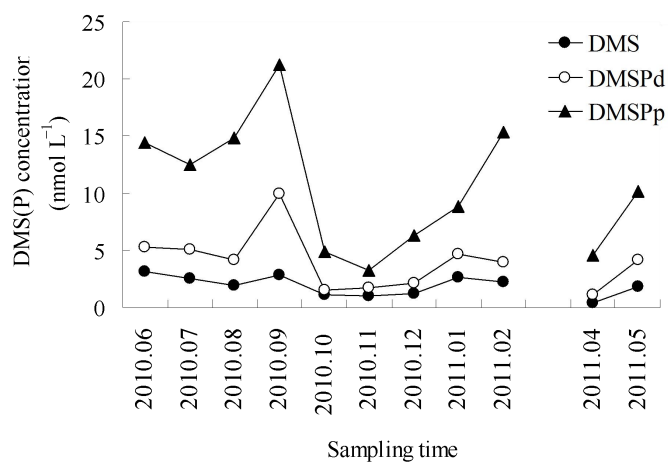


Figure 4. Mean DMS and DMSP concentrations in surface seawater of 10 stations in Jiaozhou Bay.

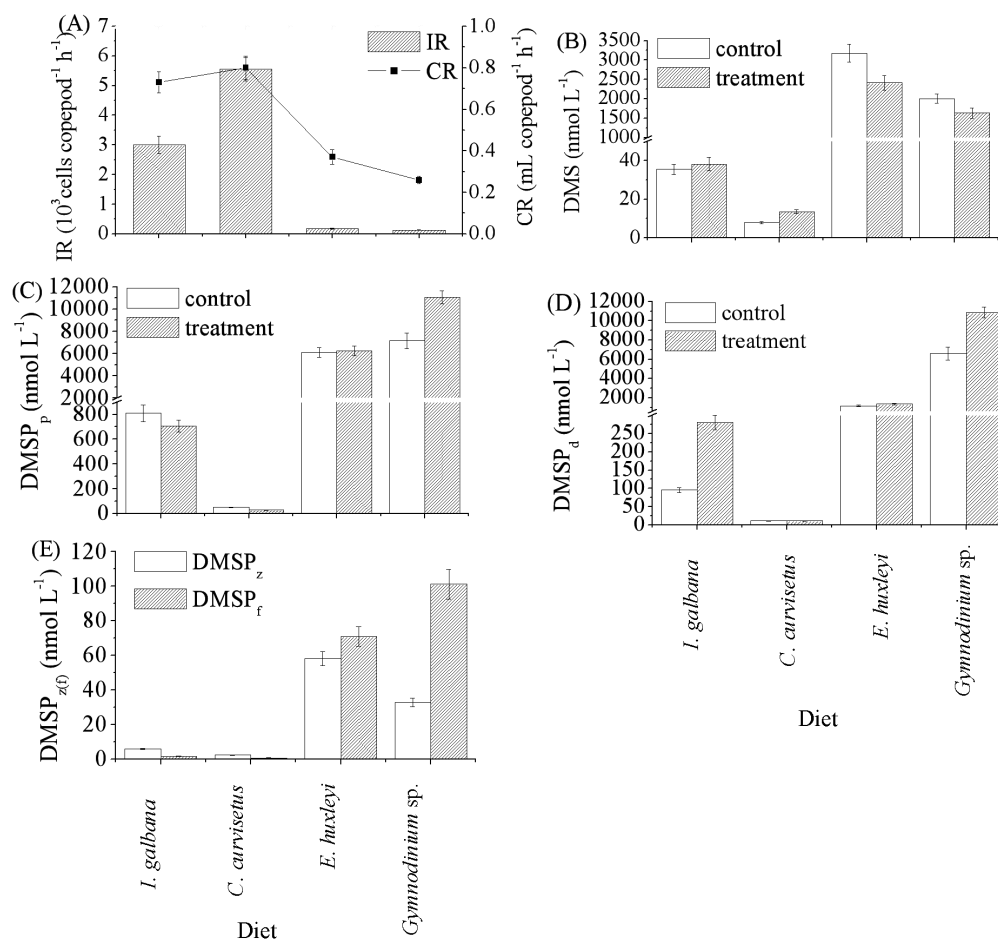


Figure 5. Effects of *Calanus sinicus* grazing on IR and CR (A), DMS (B), DMSP_p (C), DMSP_d (D), and DMSP_{zf} (E) when they preyed on different diets. Error bars represent the standard deviation ($n = 3$).

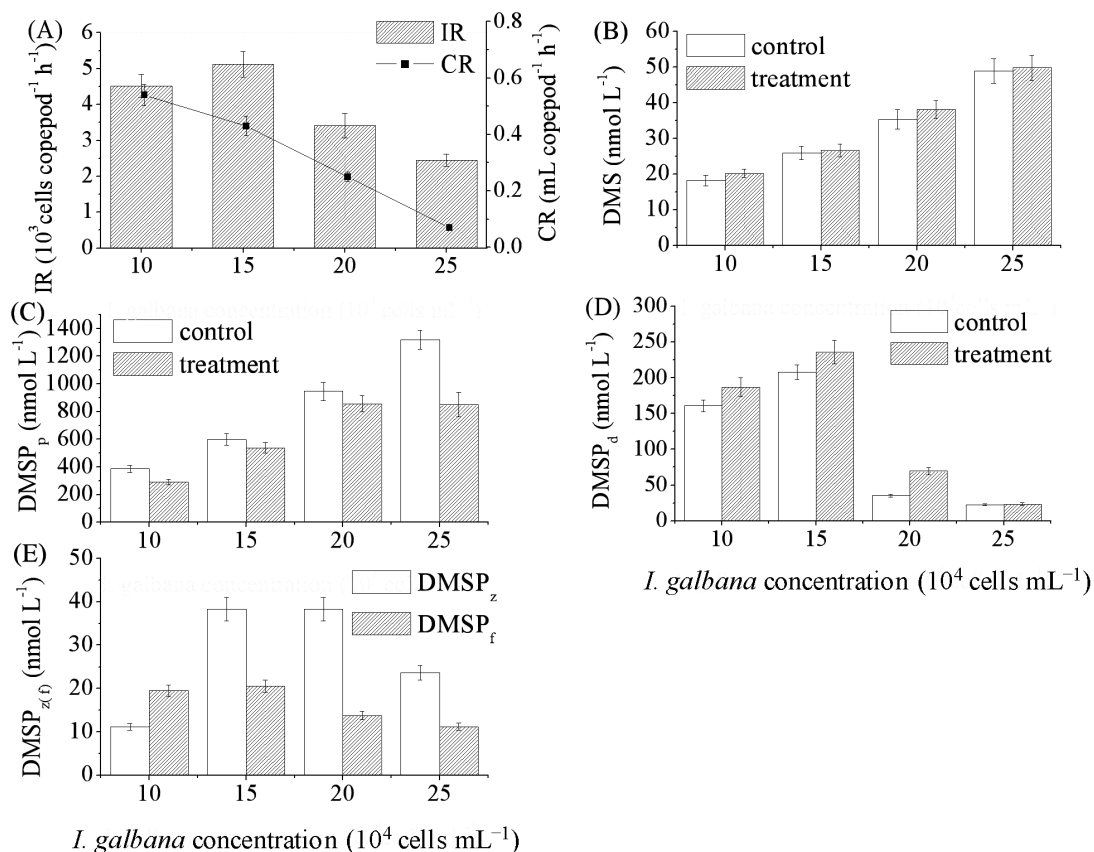


Figure 6. Effects of *C. sinicus* grazing on IR and CR (A), DMS (B), DMSP_p (C), DMSP_d (D), and DMSP_{z,f} (E) when they preyed on different concentrations of *I. galbana*. Error bars represent the standard deviation ($n = 3$).

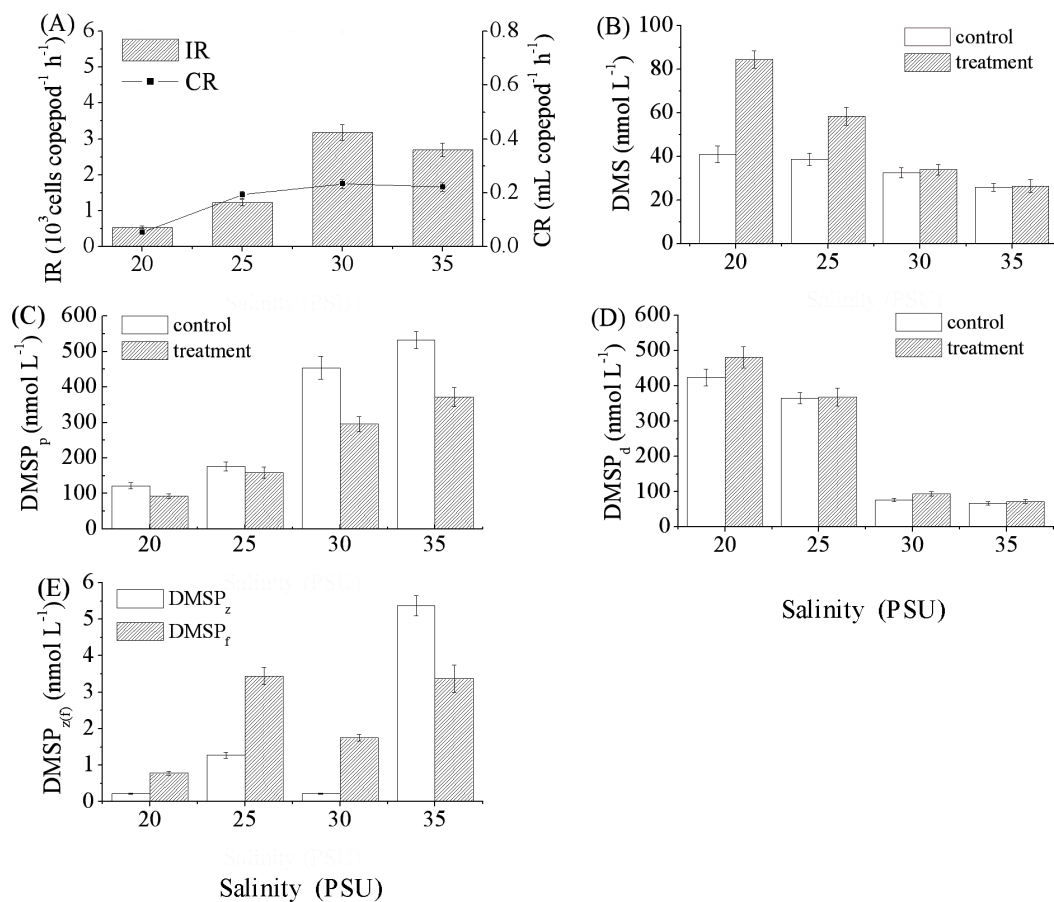


Figure 7. Effects of *C. sinicus* grazing on IR and CR (A), DMS (B), DMSP_p (C), DMSP_d (D), and DMSP_{z,f} (E) when they preyed at different salinities. Error bars represent the standard deviation ($n = 3$).