

**Author's response for Referee #1:**

This work looked at the relationship between copepod grazing and DMS(P) production. Laboratory experiments were conducted using the copepod *Calanus sinicus* and four phytoplankton species with varying morphologies and intracellular DMS(P) concentrations. Field measurements of zooplankton species and abundances, and DMS(P) concentrations were also conducted at monthly intervals. For reasons outlined below, I recommend that the authors separate the lab and field measurements and focus on publishing the lab studies in a journal such as Marine Ecology Progress Series.

**Response:** In order to find the effects of copepod *Calanus sinicus* on DMS production in Jiaozhou Bay, we investigate the DMS(P) concentration and the zooplankton including copepod *Calanus sinicus* abundances in situ, according to the comments of the referee, dilution experiment were added in the revised manuscript, which has been done in the experiment from June 2010 to May 2011 to evaluate the role of zooplankton on DMS(P) production, and the laboratory experiment further investigated the effects of the impact factors (such as different food, salinity and food concentrations) on the copepod grazing and therefore DMS production.

**Major comments**

(1) The major issue associated with this manuscript is the design of the field study. It is not clear to me why the authors would measure DMS(P) and zooplankton species composition and abundance, in order to determine the influence of grazing. I would have thought that dilution grazing experiments (see the work of Mike Landry) are an appropriate method to look at the effect of grazing on DMS(P). The identification and abundance of zooplankton are insufficient to determine their relevance to water-column DMS(P) concentrations. The alternative would have been to measure phytoplankton composition/abundance.

**Response:** According to the previous study (Yu et al., 2015), we found that the DMS(P) release varied

depending on the species and the abundance of copepod, so we measured DMS(P) concentrations and zooplankton species composition and abundance to declare the relationships between DMS(P) concentrations and zooplankton species composition or abundance. From the data, we can find that copepod *Calanus sinicus* is the dominant copepod in Jiaozhou Bay. On the other hand, we know that zooplankton grazing can release the DMSP in algae cell. The referee's comment is right, the identification and abundance of zooplankton are insufficient to determine their relevance to water-column DMS(P) concentrations. In fact, the dilution grazing experiments had been done in situ to evaluate the effect of zooplankton grazing on DMS(P) from June 2010 to May 2011, we did not put the data in the previous submission because that copepod numbers were usually low ( $< 1 \text{ L}^{-1}$ ) compared with microzooplankton. According to the referee's comments, we have put these data into the revised manuscript (See 2.1.3, 3.1.3, Table 3, Fig. 4.). We did not measure phytoplankton composition/abundance, while Zheng et al (2014) and Luo et al (2016) investigated the species composition and abundance of phytoplankton in 2010 and 2011 in Jiaozhou Bay, respectively. Therefore, we cited their data in order to understand DMSP variation in Jiaozhou Bay (see 4.1, Table 4, and Fig. 9) .

(2) ①What is the motivation for varying salinity in some lab experiments? ②How will this affect the intracellular DMS(P) concentrations of the phytoplankton if it is an osmolyte? ③Were the DMS(P) concentrations measured at the different salinities?

**Response:** ① Firstly, we found that the salinity in Jiaozhou Bay varied depending on different months; Secondly, multiple laboratory studies have confirmed that salinity can significantly influence the ingestion rates of copepod and the S-compounds production of phytoplankton (Tang et al., 1999; Yu et al., 2015). Therefore, varying salinities were set up in lab experiments to investigate the effects of

salinity on copepod grazing and the variations of DMSP and DMS concentrations. See 2.2.2.

② In our study, DMS and DMSP<sub>d</sub> (extracellular DMSP) concentrations decreased with salinity increase; conversely, the higher salinity stimulated the DMSP<sub>p</sub> (intracellular DMSP) accumulation (Fig. 8), which was in accordance with observation of a benthic diatom documented by van Bergeijk et al. (2003) and *Skeletonema costatum* documented by Yang et al. (2011). When the salinity went up or down, intracellular DMSP, as an osmotically active compound, was accumulated or released in order to help algal cells adjust their osmotic potential (Kirst, 1996). See 4.3.

③ Yes. The algae *Isochrysis galbana* and the copepod *Calanus sinicus* were cultured in different salinities, and the DMS(P) samples were get from the varying salinity environment and then they were measured, see Fig. 8.

(3) In my experience, copepods will pretty much eat anything if they are hungry enough. Of course, this will have a big effect on IR and CR. Did you starve the grazers prior to adding the prey phytoplankton?

**Response:** Yes, we starve the grazers prior to adding the prey phytoplankton, and the sentence “In order to ensure that the copepods were hungry to graze the diets, copepods were starved for 24 h before they were fed with phytoplankton.” was added in the manuscript, see Page 5 Lines 2-3. In spite of starvation, copepods can detect and react to plumes of DMS (Steinke et al., 2006), and the released acrylic acid from DMSP in algae prevents them from copepod grazing. Thus, although they are hungry enough they also can detect and react to the DMSP-rich or DMSP-poor algae, and then they will eat more for DMSP-poor algae and less for DMSP-rich algae.

(4) If you ever repeat the laboratory grazing experiments, you could include a treatment with antibiotics? This will inhibit any bacteria that metabolize DMS and you could see how relevant they

are.

**Response:** According to referee's comments and the methods of Agostini et al. (2016), we have repeated the laboratory grazing experiments and the antibiotics (0.025 g L<sup>-1</sup> penicillin G potassium + 0.08 g L<sup>-1</sup> streptomycin sulphate + 0.04 g L<sup>-1</sup> neomycin sulphate) were used to inhibit the bacteria in the copepod culture, and the relevant were analyzed in the paper. The following was added in the revised manuscript:

1) 'According to the methods of Wolfe and Steinke (1996), the algal culture was detected by epifluorescence microscopy following staining with acridine orange and by plating on 1% peptone agar plates to check for bacterial growth. No bacterial contamination was found in any of the experimental cultures.' See Page 4 Lines 14-16. 2) 'Copepods were rinsed with sterilized seawater before the beginning of the grazing experiment.' See Page 5 Line 3. 3) 'We ran preliminary experiment to check the effects of the bacteria on DMS concentration. The treatment with antibiotics (0.025 g L<sup>-1</sup> penicillin G potassium + 0.08 g L<sup>-1</sup> streptomycin sulphate + 0.04 g L<sup>-1</sup> neomycin sulphate) were used to inhibit the bacteria in the algal culture. When *C. sinicus* were fed on the four diets (*I. galbana*, *C. curvisetus*, *E. huxleyi*, *Gymnodinium* sp.), no significant differences were found between DMS concentrations in the control (without antibiotics) and those in the treatment (with antibiotics) (data no shown). Therefore, the copepod cultures were not treated with antibiotics in our laboratory experiment to obtain axenicity. Yost and Mitchelmore (2009) reported that antibiotic treatment negatively affected algal growth, which was the other reason for not using antibiotics.' See Page 5 Lines 22-29.

**Smaller comments:**

(5) Page 1, Line 13 The field work should be referred to as measurements and not experiments.

**Response:** The sentence has been replaced with "in field measurements and laboratory experiments",

see Page 1 Line 13.

(6) Page 1 Line 27 Remove this ‘recently came under close scrutiny’.

**Response:** ‘recently came under close scrutiny’ has been removed, see Page 1 Line 27.

(7) Page 3 Line 8 remove ‘a conductivity–temperature–depth probe’

**Response:** ‘a conductivity–temperature–depth probe’ has been removed, see Page 3 Line 29.

(8) Page 3 Line 9 Waterman or Whatman?

**Response:** ‘Whatman’ is correct, and ‘Waterman’ has been replaced with ‘Whatman’, see Page 3 Line

30.

(9) Page 4 Line 1 what is meant by ‘which served as a good-quality food,’

**Response:** ‘which served as a good-quality food,’ means ‘which was a DMSP-poor and favorite food for *C. sinicus*,’ and the sentence has been revised, see Page 4 Line 24.

(10) Page 4 Line 10 I don’t know the equations of Frost (1972) so some description is needed. To measure IR you presumably measure algal abundance before and after grazing?

**Response:** Yes, the initial and final algal concentrations should be measured to obtain IR and CR. The description of the equations of Frost (1972) has been added in the revised manuscript as follows. ‘The IRs and CRs were calculated according to the equations of Frost (1972). Because no significant differences were found in algal concentrations between the initial and final control bottles, the growth constant ( $k$ ) for algal growth was eliminated from the equations, thus yielding:

$$CR = \frac{V \times \ln(C_1 / C_2^*)}{Nt} \quad (1)$$

$$IR = CR \times C_1 \quad (2)$$

Where  $CR$  is the clearance rate ( $\text{mL ind}^{-1} \text{ h}^{-1}$ ),  $IR$  is the ingestion rate ( $\text{cells ind}^{-1} \text{ h}^{-1}$ ),  $C_1$  is the initial algal concentration in control bottles ( $\text{cells mL}^{-1}$ ),  $C_2^*$  is the final algal concentration in the

experimental bottles (cells  $\text{mL}^{-1}$ ),  $t$  is the duration of the experiment (h),  $V$  and  $N$  are the volume (mL)

and number of copepods in the experimental bottles (ind), respectively.' See 2.2.3.

(11) Page 4 Line 15 why were samples stored at  $-70^{\circ}\text{C}$ ? I wasn't aware that this is part of the typical DMS(P) protocol

**Response:** The referee's comment is right, and this is not part of the typical DMS(P) protocol. The sentence 'All samples were stored at  $-70^{\circ}\text{C}$  until DMSP measurement.' has been deleted, see Page 5 Line 21.

(12) Page 5 Line 9 Report chlorophyll concentrations to 1 decimal place Page 5 Line 28 I suspect DMS(P) concentrations should also be reported to 1 decimal place

**Response:** chlorophyll concentrations and DMS(P) concentrations were all changed into 1 decimal place , see Page 6 Line 22 and Page 7 Line 23.

(13) Page 5 Line 30 Replace contents with concentrations

**Response:** 'contents' has been replaced with 'concentrations', see Page 7 Line 25.

(14) Page 6 Line 16 Change 'would result' to 'resulted in'

**Response:** 'would result' has been changed to 'resulted in', see Page 8 Line 11.

(15) Table 3 Why show correlations which are not significant?

**Response:** According to the comments of the referee, Table 3 has been deleted, and the correlations which are significant were described in the text.