

**Author's response for Referee #2:**

The manuscript “Role of *Calanus sinicus* (Copepoda, Calanoida) on dimethylsulfide production in Jiaozhou Bay” by Juan et al attempts to understand the role of copepod *Calanus sinicus* in DMS production in Jiaozhou Bay through insitu observations and lab experiments. The authors followed a yearly cycle of insitu observations on temperature, salinity, Chl *a*, TBC, zooplankton enumeration and speciation, DMS, DMSPp and DMSPd at 10 stations in the Jiaozhou Bay. They also performed lab experiments wherein they conducted zooplankton grazing experiments on select phytoplankton species to see the impact on DMS production. Though the hard work put in by the authors is commendable, there is a major disconnect between observations and lab experiments. Their observation on DMSP transfer from phytoplankton to copepod body, fecal pellet to seawater is not new and has been proposed quite some time back (Tang et al; Belviso et al). The authors mention that data from the field measurements showed that *Calanus sinicus* did not have any apparent effect on DMS/DMSP production and then the authors go ahead to perform a complex grazing experiment to see the impact of grazing on DMS production. If the field data did not show any connect, what was the aim to perform the lab experiments? Perhaps the authors should have checked the gut content to see what species the copepod preferred to feed on? This might have given some clues on how to proceed. Also, the insitu observation part is not clear. Some of the concerns with regards to this work is jotted below.

**Response:** The dilution experiment have been added in the revised submission, and the sentence of “Data from the field experiment showed that *C. sinicus* has no apparent effect on DMS/DMSP production” have been deleted .

The gut contents of *Calanus sinicus* were checked, the results showed that the *C. sinicus* preferred to feed on diatom *Chaetoceros curvisetus* and *Thalassiosira nordenskioldi*, suggesting that diatom (DMSP-poor algae) were the preferable diet for *C. sinicus*, which has been added in 4.1. Dilution

experiments of three stations had been done on the shipboard, and the supplement details has been added in the revised manuscript according to the two referees' suggestions.

1. The authors mention time series sampling at 10 locations, but figure 2 shows data for only one site, which is this site? Or is this averaged data? If averaged then include standard deviation.

**Response:** Figure 2 shows data for the average data of 10 locations, and the standard deviation were added in the Figure 2.

2. What is the reason for the increase in DMSP<sub>p&d</sub> (and marginal increase in DMS) during September 2010? What is the major phytoplankton species during spring? As this might have answered the high DMSP observed during that time.

**Response:** September is in the season of autumn (not spring) in Jiaozhou Bay. Zheng et al (2014) and Luo et al (2016) investigated the species composition and abundance of phytoplankton in 2010 and 2011 in Jiaozhou Bay (Table 4), respectively. From their results, we found that the major phytoplankton species in September 2010 were diatom *Skeletonema costatum* and *Coscinodiscus asteromphalus*, and dinoflagellate *Ceratium fusus*, with the predominancy of 0.17, 0.13 and 0.10, respectively (Table 4). The dinoflagellate/diatom ratio in September is about 0.2 (Fig. 9). On the other hand, the mean bacteria abundance in September is the highest in the year (Fig. 2). Therefore, the dinoflagellate and bacteria might explain the increase in DMSP<sub>p</sub> and DMSP<sub>d</sub> (and marginal increase in DMS) during September 2010. These were added in the part 4.1.

3. In terms of copepod, the authors mention *Calanus sinicus* as the predominant copepod, but that does not seem to be the case as *Eurytemra pacific* was also dominant during three sampling with April 2011 showing maximum abundance.

**Response:** There is a mistake for the use of the word “predominant”, so “predominant” has been

replaced with “dominant”. See Page 7 Line 2.

4. In the feed (diet) experiment it is clear that the copepods prefer *I. galbana* and *C. curvisetus* compared to *E. Huxleyi* and *Gymnodinium* sp. There is not much difference in DMS production in the treatment when compared to the control. On the contrary DMS production dropped in the case of *E. Huxleyi* and *Gymnodinium* sp. in comparison to *I. galbana* and *C. curvisetus* which showed marginal increase in DMS production.

**Response:** Yes, the copepods prefer *I. galbana* and *C. curvisetus* compared to *E. Huxleyi* and *Gymnodinium* sp. Although there is not much difference in DMS production in the treatment when compared to the control, but we have repeated several times and found that DMS production increased in the case of copepod grazing on *I. galbana* and *C. curvisetus*, on the contrary DMS production dropped in the case of *E. Huxleyi* and *Gymnodinium* sp. The cellular DMSP concentration in algae might be the reason.

5. Line 26: ...in May 2011) and had no apparent....

**Response:** “and has no apparent effect on DMS/DMSP production” has been deleted. The sentence were changed into “Data from the field experiment showed that *C. sinicus* was the dominant copepod in Jiaozhou Bay (up to 123 individuals m<sup>-3</sup> in May 2011) and preferred to graze on diatom. DMS and DMSP concentrations not only depend on phytoplankton abundance, but also phytoplankton species and other factors.”, see Page 1 Line 16-18.

6. Introduction: Line 37-38: The authors mention that there was close scrutiny on DMSP, what kind of scrutiny, please elaborate.

**Response:** “recently came under close scrutiny” has been removed according to the comments of referee #1. See Page 1 Line 27.

7. Line 48: what kind of biotic and abiotic factors? Elaborate.

**Response:** “Numerous abiotic and biotic factors” has been changed into “Numerous biotic factors (i.e., phytoplankton, zooplankton, bacteria, and virus) and abiotic factors (i.e., temperature, salinity, light, and nutrient)”. See Page 2 Line 4-5.

8. Line 49: replace ‘account’ with ‘consume’.

**Response:** ‘account’ has been replaced with ‘consume’. See Page 2 Line 6.

9. Line 51: the authors mentioned that role of zooplankton grazing on DMSP biogeochemical processes are scarce. And later in the same paragraph include detailed studies on the impact of zooplankton grazing on DMS production. The aim for carrying out the grazing work needs to clearly spelt out.

**Response:** There were inconsistency about evaluating zooplankton grazing in the paragraph, therefore, the sentence “and knowledge about the role of zooplankton grazing in the DMSP biogeochemical processes is scarce” has been deleted. See Page 2 Line 7.

10. Line 62: delete ‘Brodsky’.

**Response:** ‘Brodsky’ has been deleted. See Page 2 Line 15.

11. Line 65: what field experiments were performed? Are the authors referring to field measurements?

**Response:** Yes, field experiments were referred to field measurements, and the sentence has been replaced with “field measurements and laboratory experiments”. See Page 2 Line 17.

#### **Materials and methods:**

12. The authors mention collection of samples from 10 stations. ① I assume these are surface samples? How were the samples collected? Niskin sampler or any other sampler? ② Zooplankton samples were collected by vertical tows, mention depth or range of depth from where the vertical tows were done.

**Response:** ① Yes, the water samples were the surface samples, and the samples were collected by a Niskin sampler, see Page 3 Line 30. ② Zooplankton samples were collected by vertical tows from the bottom to the surface, the depth varied from 3 m to 28 m according to different station. See Page 3 Line 2.

13. Line 91: spelling correction 'Whatman' GF/F filters. There are many grammatical errors in the manuscript. Only a few are pointed out. The authors need to correct these.

**Response:** 'Waterman GF/F filters' has been replaced with 'Whatman GF/F filters'. See Page 3 Line 30.

We have checked the grammars in the manuscript.

14. Line 334: Did the authors measure acrylic acid as a deterrent against grazing?

**Response:** No, we did not measure acrylic acid, so 'acrylic acid or' had been deleted. See Page 11 Line 30.

15. Line 345-346: 'In this study, .....which in turn reduced DMS/DMSP production'. This dose not seem to be the case as DMS production was high in *Gymnodinium* sp. and *E. Huxleyi* as seen from control and did not depend on grazing.

**Response:** The comments of the referee is right, and the sentence 'In this study, .....which in turn reduced DMS/DMSP production' has been deleted. See Page 12 Line 6.

16. Figure 1: Include latitude and longitude or specify 'N' and 'E'.

**Response:** 'N' and 'E' were added in the Figure 1.

17. Figure 3: (B) may be deleted as species wise in shown in (C).

**Response:** Figure 3 (B) has been deleted.

18. One of the important parameters that this work lacks is phytoplankton speciation of the natural samples. In the absence of that data, understanding DMSP variation becomes difficult.

**Response:** Zheng et al (2014) and Luo et al (2016) have investigated the species composition and abundance of phytoplankton in 2010 and 2011 Jiaozhou Bay, respectively. According to their data, the changing trend of species composition, abundance of phytoplankton and dinoflagellate/diatom ratio from June 2010 to May 2011 were presented in Table 4 and Fig. 9. The predominance of dinoflagellate *Ceratium fusus* was 0.10 in September 2010 (Table 4). The dinoflagellate/diatom ratios in the three months (July, August and September) were high among the whole year, and the abundances of dinoflagellate and diatom in September were the highest among the three months (Fig. 9), what is more, the bacterial abundance in September was the highest among the year (Fig. 2). Therefore, the occurrence of high abundances of dinoflagellate and bacteria might were the reason of high DMS and DMSP in September 2010. In February, April and May 2011, the dominant phytoplankton were diatom *Rhizosolenia delicatula*, *Skeletonema costatum* and *Skeletonema costatum*, and predominancies were 0.7, 0.99 and 0.68, respectively (Table 4). Although the phytoplankton abundances and Chl *a* contents were high during January 2011 to May 2011, the DMSP<sub>p</sub> and DMSP<sub>d</sub> concentrations were lower than those in September 2010, suggesting that DMSP concentration not only depend on phytoplankton abundance, but also phytoplankton species and other factors. See 4.1, Table 4, and Fig. 9.

19. Grazing by zooplankton on phytoplankton is an important part which results in DMSP going from particulate (within cell) to dissolved (outside) and further the action by DMSP lyase (both by phytoplankton as well bacterial lysis) results in high DMS production. Grazing is studied by looking at the gut content or by isotopic work, neither being done in the present study, it's difficult to address DMS production to grazing.

**Response:** Gut content in the copepod were checked in the field study, and found that *C. sinicus* preferred to graze on diatom *Chaetoceros curvisetus* and *Thalassiosira nordenskioldi* (data not shown),

suggesting that diatom (DMSP-poor algae) were the preferable diet for the copepod *C. sinicus*. See Page 10 Lines 30-32. Isotopic work will be done in the future study to address DMS production to grazing.

20. And finally, there is a complete disconnect between field results and the basis for performing grazing experiments in the laboratory.

**Response:** Dilution experiments of three stations had been done on the shipboard from June 2010 to May 2011, and the data have been added in the revised manuscript according to the two referees' suggestion. We did not put these data in the previous submission because that copepod numbers were usually low ( $< 1 \text{ L}^{-1}$ ) compared with microzooplankton. See 2.1.3, 3.1.3, Table 3, Fig. 4. Then, the grazing experiments were investigated both in field and in laboratory.