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Interactive comment on "Effects of the interaction of ocean acidification, solar radiation, and warming on biogenic dimethylated sulfur compounds cycling in the Changjiang River Estuary" *by* Shan Jian et al.

Anonymous Referee #1

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The Ocean Acidification (OA) community has seen a surge in research in the past 10-15 years, with several hundreds of quality papers being published each year. More recently, the OA community has turned its attention to a very important question: the OA problem in the context of multiple stressors (temperature, light, nutrients, etc) facing oceanic ecosystems. This issue is fundamentally important but raises questions that are not simple to answer; effective experimental designs are not easy to recreate, the oceanic carbonate system remains a challenge to tackle, and the complexity of statistical analysis related to multiple stressor experiments heightens as stressors

are added to the mix. The challenge is even greater when attempting to undertake a study focused on the impacts of declining pH on climate-active gases that come with their own complex dynamics. However, such undertakings are necessary if we are to increase our level of confidence on the biogeochemical responses, interactions and potential retroactions of these climate-relevant biogenic compounds under OA in a rapidly changing climate. The paper "Effets of the interaction of ocean acidification, solar radiation, and warming on biogenic dimethylated sulphur compounds cycling in the Changjiang River Estuary" is full of promise towards this goal. However I have many concerns as to the soundness of the scientific approach, experimental design, and statistical scheme used. On several occasions, the lack of clarity in descriptions, methods used, and lack of information altogether impedes the comprehension of the objectives and conclusions of this study. The numerous short falls concern both the core content of the research as well as the form of the paper.

1. On the core.

1.1. Questionable methodological approach.

The methodological approach used by the authors is incomplete and raises several concerns. The most alarming one is trying to report the impacts of OA on the dynamics of biogenic sulphur compounds and having only this to say about carbonate system measurements and monitoring throughout the 23-day experiment: p.3 Lines 29-30 "Seawater pH was constantly measured using a pH meter (. . .) and the precision is +/- 0.002." Have the authors taken into account a correction factor based on fluctuating temperature? pH meters are usually calibrated at 25oC and substantial variation in measurements can ensue from variability in temperature. The authors mention that ". . .temperature was continuously controlled by circulating in situ seawater, hot water, and ice.", suggesting that potentially significant variation in temperature occurred during 23 days. Have they monitored salinity throughout the experiment? The pH scale (National Bureau of Standard scale (pHNBS), free scale (pHF), total scale (pHT), or seawater scale (pHSWS)) is not mentioned in the manuscript.

To ensure reproducibility, it is critical to report and monitor at least two variables of the carbonate system of seawater (as well as salinity and temperature) for the entire period of the experiment. The authors do add a separate paragraph at the end of the Analytical procedures section where they mention the following: p.5 Lines 9-12 “Total dissolved inorganic carbon (DIC) was determined by a DIC analyzer (AS-C2, Apollo SciTech Inc., Georgia, USA). 10 A sample of 0.5 mL was acidified by 0.5 mL 10% phosphoric acid and then the extracted CO2 gas was measured using a nondispersive infrared (NDIR) CO2 detector (LI-6262, Li-COR Inc., USA) with a precision of 0.1%. The total alkalinity (TA) and pCO2 in sea water were calculated from DIC and pH using the CO2SYS (as refitted by Dickson and Millero (1987)).” However these measurements seem to have been made only at the beginning of the experiment for three treatments (as seen in Table 1. Preliminary carbonate parameters of three treatments during the incubation experiment) where no replication is shown and where no actual temporal monitoring is available. What is the use of having a single measurement of DIC at the beginning of the experiment with no follow up throughout the experiment? I also question the methods used to measure DIC, do the authors have a reference to cite here? 0.5ml seems like a very small volume for the measurement of DIC. 125 to 250ml samples are usually taken to accurately measure total inorganic carbon.

Furthermore, the authors do not mention the formulations used to calculate all variables with CO2SYS: concentrations of total boron, CO2 solubility (K0), Dissociation constants of carbonic acid (K1 and K2), boric acid (Kb), water (Kw), phosphoric acid (Kp1, Kp2, Kp3), silicic acid (Ksi), hydrogen fluoride (Kf), and bisulfate (Ks), Solubility products of calcite (Kspc) and aragonite (Kspa). A very useful document prepared in the framework of the data management activity of the Ocean Acidification International Coordination Centre of the International Atomic Energy Agency can be found here (OAICCL: www.iaea.org/ocean-acidification) and shares recommendations proposed in the Guidelines for reporting ocean acidification data in scientific journals.

I also worry about trying to measure concentrations of volatile DMS within non-

collapsible barrels from which volumes of water are extracted daily creating more and more headspace over the course of 23 days (figure 3). The authors do not offer any explanation for this, or any suggestions as to how they correct for this problem. We learn that the authors used filtered (what porosity? Axenic? 0.2um, 0.7um?) water to run side-incubations to measure the impact of light on DMS. A first thought here is that any type of filtration process may introduce biases. DMSP-producing communities are notoriously sensitive to filtration, the most common problem is the pressure-induced heightening of the DMSPd pool caused by the rupture of healthy cells. This can artificially enhance pools of DMS through mixing of DMSPd and DMSP-lyase enzymes. This is never mentioned. Also, DMS is controlled by both biology (bacteria, viruses, phyto, zoo) and physics (light, wind). Thus the response of DMS to various light regimes may occur through various pathways (photochemically but also through its impact on primary producers or bacterioplankton). The methodological scheme suggests that the authors here are only investigating the impact of pH and light on photolysis rates of DMS. Yet it is unclear if bacteria/phyto are still present in these experiments (what is the porosity of the filter used?). On page 8 line 25 the authors state the following: “In order to assess the community-level response to the ocean change in future CO2 and light conditions, photolysis rate constants (K, $d-1$) for DMS were calculated.” How is it possible to assess a “community-level” response when filtered water is used presumably to focus on physical processes only (photochemistry)? The authors state objectives that cannot be answered through the methods they use.

The authors use 20L barrels to run their 23 day incubation experiment. Of those 23 days we learn that 18 are onboard the ship and 5 are conducted in the lab (page 3 line 16)? This is very worrisome. No further information is given here. How were the barrels moved? Presumably a lot of mixing occurred during the transit? How were temperatures kept constant during this time? For an experiment looking to identify the impacts of light and temperature, (and pH) on the dynamics of a DMSP-DMS-producing community, this major shift in environmental settings introduces a lot of potential variability that can obscure the response. Yet this is simply glanced over and never mentioned

again. Also, I am curious to know (this could be calculated from the methodologies) how much water was left in the 20L barrels prior to the last sampling day? At least 10L? Or less?

1.2 The authors treat the response of a complex Estuary to OA the same way they would the response of open waters to OA.

The OA problem is increasingly complex in estuarine and coastal waters where freshwater runoff, tidal mixing and high biological activity contribute to variations in CO2 and pH on different time scales. The surface mixed-layer pCO2 can vary spatially and is strongly modulated by biological productivity during the phytoplankton growth season. Surface pHT in Estuaries can also vary significantly within a single tidal cycle, nearly as much as the world's ocean have experienced in response to anthropogenic CO2 uptake over the last century... Studying the impact of OA in these circumstances (high natural variability in pH and possible resilience of communities) is not devoid of interest, but the authors do not state this, they don't even mention these natural fluctuations and how these could affect the communities, on a basic level, are these communities already tolerant to rapid fluctuations in pH? They treat the impact of OA on this very complex ecosystem as if it was the impact of OA on open oceanic waters. This is troublesome.

1.3 Statistical approach

The description of the statistical strategies used by the authors is confusing and their application to the dataset is questionable. The title of the paper is full of promise "Effects of the interaction of OA, solar radiation and warming on biogenic sulphur compounds cycling..." but fails to deliver on that promise. The methodological and statistical approach proposed by the authors does not allow them to explore potential interactive effects of three stressors on the sources and sinks of DMS and DMSP. Multiple stressors can influence a variable independently (additive), or interact to either reduce (antagonistic) or enhance (synergistic) that variable in a nonlinear, unpre-

dictable fashion. I suggest the authors read Todgham and Stillman (2013), Riebesell and Gattuso (2015), Reum et al. (2015), Gunderson et al (2016). A collapsed factorial approach would have been more informative and simpler to interpret, see Boyd et al (2015). Please also see: "SCOR working group 149 https://scor149-ocean.com Changing Ocean Biological Systems (COBS): How will biota respond to a changing ocean?" It contains pertinent information for moving studies from single to multiple drivers.

On page 12 lines 24-26 the authors mention the following: "The effect of the interaction between OA and environmental conditions complicates the overall ecosystem response. Hence, comprehensive consideration of OA and solar radiation can better interpret and understand feedbacks between OA and global climatic change." A very small exploration of interactive effects is shown in Figure 6 for pH and temperature, and there appears to be a subset of information on pH and certain measurements of light in Table 3, but the interactive impacts of solar radiation, pH and temperature together are not explored. I do not see any "comprehensive consideration". Table 3 seems to relate one type of pH with one type of light treatment, but no combinations... pH 8.1 + light control (quartz), pH 8.1 + Mylar D (UVA), pH8.1 + Plexiglass (PAR), pH8.1 + Dark. pH + light control (quartz), pH 8.1 + Mylar D (UVA), pH8.1 + Plexiglass (PAR), pH8.1 + Dark. Same with pH 7.9 or 7.7 with various light treatments. It is not clear what the rational is behind choosing a specific pH with a specific light treatment (?).

The authors mention the following on page 3 lines 11-13: "Moreover, we examined the photolysis rate and concentration changes of DMS under the dual stressors of changing pH and solar radiation/temperature and assessed the coupling effects of OA, solar radiation and warming on biogenic dimethylated sulfur compounds cycling." Where is the basic information on light? How was light measured? How did it vary naturally over the 23 day experiment? Where is the basic information on temperature? How was it measured? How did it vary over time? I do not see this anywhere. This is very concerning. Furthermore, the authors seem to treat light and temperature as a single

fixed factor (light/temperature) (? I may be wrong because the text is convoluted and unclear) and proceed with a two-way ANOVA to disentangle the impacts of pH, light, and temperature on concentrations of sulphur compounds? Ecologically and statistically, this is difficult to justify. pH + Light experiments were carried out under natural ambient solar radiation (lines 139-140) with 3 treatments and 1 control (full light) and then information is inferred for a 5th variable by subtracting the effects of one treatment from another treatment. The authors state that they also ran pH + Temperature experiments (with 1 treatment (+4oC) and control but it sounds like these are separate experiments from the light + pH experiments, however it is rather unclear): Lines 140-142: "Temperature experiments were also carried out with unfiltered water in quartz bottle under in situ temperature at 12 ïĆřC and high temperature at 18 ïĆřC for 8 h. The temperature was continuously controlled by circulating in situ seawater, hot water, and ice." (This alone begs the question of the uniformity of the temperature treatment itself if ice and hot water are necessary to stabilize the temperature (there were likely a lot of fluctuations, yet these are not reported)). In any case, I have serious doubts as to the validity of lumping together Light and Temperature variables into a single fixed factor as it seems to be proposed in the statistical paragraph of the methodological section. Also are the underlying assumptions of normal distribution for the response variables (S compounds) respected? There is no mention of this. The authors state the following at lines 25-28 p.5: "Seawater pH was adjusted with CO2-saturated seawater during the experiment to maintain a stable pH environment. In the first week, pH level was comparatively stable with a better control, but pH fluctuated obviously and was difficult to control in the stable and decline phases of algal growth. As shown in Fig. 1, the pH showed relatively apparent fluctuations on days 9-12 and days 18-20." Again, it is difficult here to conceive that pH was a fixed explanatory variable. Judging by Figure 1, there are several instances when there doesn't appear to be a statistical difference between the pH treatments with very large error bars.

On page 5 Lines 14-20, the authors describe very briefly the statistical approaches used, but these spark more questions than answer anything. "Statistical analysis was

performed using SPSS version 18.0 (SPSS Inc., IBM, USA). Pearson's correlation coefficient and probability (p) were calculated to evaluate the quality of the fit when variables were normally distributed. (How was normality assessed?) T-test was used to determine whether a significant difference existed between two treatments. (WHICH treatment? More information is needed.) "Variability in the concentrations of biogenic dimethylated sulfur compounds was analyzed using two-way ANOVA with pH and temperature/light as fixed factors and concentration as a random factor to understand whether an interaction existed between pH and temperature/light on concentration. (Are the authors suggesting that temperature/light are one fixed factor?) Value at $p \leq 0.05$ was considered statistically significant. Linear correlation analyses were used to determine the response of DMS, DMSO concentrations, and bacterial abundance to OA using Origin 9.1." The authors proceed to establish Pearson's correlations between two variables within a same pH condition. I am not convinced that this is useful. What is the purpose of this exactly? How does it inform us of the complex response of these variables to fluctuations of pH itself? Overall, the statistical schemes proposed by the authors do not seem appropriate, and they do not facilitate the interpretation of possibly complex responses. Table 4 presents these correlation coefficients. What are the degrees of freedom (DF) for these analyses? Why is a coefficient of 0.791 (between DMSOd and chla) significant at the $p < 0.05$ level while a coefficient of 0.778 (between DMSOd and DMS) is significant at the $p < 0.01$ level ? More information is needed about the n and the DF. On pages 10 and 11, the authors discuss these correlations between sulphur compounds and other variables (within a same pH treatment) at length but I am not convinced that these are very informative on the impact of pH itself on the sulphur compounds. When looking at figure 2-3-4-5, the error bars are so wide for the pH treatments (substantial overlapping most of the time, ex: DMSOp), that it is hard to understand what the purpose of these inner-treatment correlations is. A very convoluted text does not help in the matter. An example of this here: "A significant correlation between DMS and DMSOd was observed in LC treatments (Table 4). The result was in accordance with the findings of Hatton et al. (2004) and Zindler-Schlundt

et al. (2015) who showed that DMSO and DMS are closely related because of the direct formation of DMSO via bacterial DMS oxidation. The close link between DMS and DMSOd was not observed in MC and HC treatments. The changes in the production, consumption, and degradation processes which caused by the decreasing pH might mask the relationship between DMS and DMSOd. (Production/consumption and degradation of what exactly?) In addition, DMSPd concentration showed an obvious correlation with DMSOd only in LC treatments for the entire duration of the experiment (Table 4). DMSPd may be cleaved to DMS by specific bacteria that contain DMSP lyase (Curson et al., 2008), followed by the bacterial oxidation of DMS to DMSOd. This relationship was not found in MC and HC treatments. The phenomenon might be caused by the change in phytoplankton community and bacterial oxidation of DMS to DMSO." It is very difficult to follow the logic here. What change in phyto community? What are the authors talking about? They do not show any information on community composition for this study. And this is another important shortcoming of this paper: no phytoplankton identification. DMSP/DMS cycling is intimately linked with species composition, yet we have no idea WHO is there and HOW pH may affect the primary producers responsible for a substantial part of the DMS/DMSP cycling. In the introduction the authors mention this: "Shaw hypothesized that DMS and sulfate aerosols are linked to global climate. This link was further elaborated by Charlson et al. (1987). Consequently, ocean acidification (OA)-induced changes in the primary productivity might impact on the production rate and sea-to-air emission of DMS and these impacts might further affect cloud formation and climate". The authors do not explore the primary production side of things at all. There are no PP rates, no phyto identification, only chla which is a proxy of biomass that does not inform us on whether pH impacts chla levels through physiological processes or through variability in the species composition.

1.4 Out-dated information and statements that are too general.

Page 1 line 28. The information given here is not up to date. The authors reference a paper written in 2000. Progress has been made in the last ca. 15 years. Anthropogenic

carbon dioxide (CO2) emissions have increased atmospheric CO2 concentrations from their pre-industrial value of 280 to ca. 400 μatm in 2016 (NOAA-ESRL).

Page 1 lines 29-30. Under which scenario exactly? Please be precise and use the latest information available. Concentrations of 850 to 1370 μatm are expected by the end of the century under the business-as-usual scenario RCP 8.5 (IPCC, 2013).

Page 1 line 30 and beyond. The authors don't offer any explanation, even if short, as to the underlying processes involved in pH modulation in oceans as a result of CO2 increases in the atmosphere. They should spend a little more time explaining this. That is one the focuses of their paper after all. Also there is a more recent paper by Caldeira and Wickett (2005) suggesting that the surface ocean pH is expected to decrease by an additional 0.3-0.4 units under the RCP 8.5 scenario by 2100.

Page 2 lines 1-2. What is the reference here? There are several consequences of increasing OA. Modifications in DIC is the primary, from which modifications in carbonate system ensue (which includes modifications in saturation states of calcite and aragonite, which by the way the authors don't explain the importance of: why talk about calcite and aragonite?). Modifications in the amount of protons (H+) is another. Etc. Etc.

Page 2 lines 2-4. This is quite a general statement. What changes exactly (less calcite, more H+, more CO2?) will affect what physiological processes exactly, and what type of marine "organism" exactly? There is so much literature on the many aspects of OA and the consequences for marine organisms (Calcifiers? Phyto? Zoo? Fish? Etc), and the potential impacts may be positive, negative, resilience etc... The phrase written is simply too generalist, it should offer at least a glimpse into the multi-tiered discoveries made by the OA research community, or at least focus on the aspects that are relevant to the author's research: DMS-producing microbial communities...

Page 2 lines 19-20. The authors make very general statements without offering much information: "The conversion of DMSP to DMS is controlled by a number of chemical

and biological processes." Yes. But which ones?

These are only some examples of the generalities, more can be found in the manuscript.

2. On the form

As it currently stands, the overall level of language does not meet the high standards of BG. There are countless examples of awkward formulations and missing words throughout the paper, including: "...more CO2 (low pH) will affect physiological process of marine organism." Page 2 line 3 Page 2 lines 5 and beyond : The transition between the impact of OA on they physiology of marine organisms and the oceanic production of climate-relevant gases is not well established.

"Once emitted to atmosphere, DMS...". Page 2 line 10 "DMSO was initially conceived as a sink for DMS. Nevertheless, DMSO was later found a potential source of DMS." Page 2 lines 26-27 "The ocean undergoes multiple environmental changes. Other climatic ecological stressor or factors would probably alter the effects of ocean acidification on the production and consumption process of DMS in both direct and indirect ways." Page 3 lines 2-4 "The photochemical process of DMS in the surface water would change due to the changing light level and seawater pH level." Page 3 line 5

There are so many more examples. I will stop here because I believe a profound language editing needs to be conducted and this goes beyond the scientific mandate of a reviewer.

The introduction offers many general statements that only skim a fraction of the inherent complexity and wealth of information related to OA research. The paper lacks coherence and clarity and there is redundancy in the writing. Formulation of phrases is awkward, making the text very difficult to follow.

Page 2 lines 6-8: Redundancy in the phrase: "It is produced by enzymatic cleavage of dimethylsulfoniopropionate (DMSP) (Gabric et al., 2010), which is synthesized by

marine phytoplankton as a phytoplankton-derived precursor of DMS."

Page 2 lines 16-17: Redundancy in the phrase as well as unclear: "DMSP, as the main precursor of DMS, ..., presents an important effect on the biogeochemical cycle of climatically active trace gas DMS." This is rather vague and redundant. Needs rephrasing.

Page 2 line 21 The word "preferred" is not appropriate.

Page 2 line 26: "DMSO was originally conceived as a sink for DMS." This phrase does not make sense. Needs rewording.

Again, these are only examples, more can be found in the manuscript.

In short, at this point, there is much work to be done before this paper can be considered for publication in BG. First and foremost, there are several uncertainties and questions related to the methodologies used and the experimental design itself. Without a sound experimental plan and measurements, the rest of it (interpretations and conclusions) is useless. Overall, the entire methodological aspects related to the OA part of the experiments are unclear and lacking (carbonate system measurements absent, salinity?, temperature records?). It is unclear whether the temperature and light treatments were separate experiments. And where are those measurements of temperature and light? What about primary production, phytoplankton species composition?The statistical schemes do not seem appropriate. The objectives of the study are not well stated (I am referring here to the Estuarine context in which the experiment takes place). The environmental setting is not clearly established; the authors don't even situate the study area on a map. The level of language is not up to par.

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