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Interactive comment on "Quantification of dimethyl sulfide (DMS) production in the sea anemone *Aiptasia* sp. to simulate the sea-to-air flux from coral reefs" *by* Filippo Franchini and Michael Steinke

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Review of the paper "Quantification of dimethyl sulfide (DMS) production in the sea anemone Aiptasia sp. to simulate the sea-to-air flux from coral reefs" by Filippo Franchini and Micheal Steinke

The paper presented net DMS production and DMSP concentrations in cultures of 48h incubated sea anemones Aiptasia sp. with and without its symbiont Symbiodinium. These data together with literature values were used to estimate the gross DMS pro-

duction within the anemones and the potential amount of anemone derived DMS emitted to the atmosphere.

This study presents an interesting aspect of the role and influence of sea anemones for the biogeochemical pathways of DMS and DMSP in coral reefs. It shows that even when the production of anemones inside of the polyp is relatively high most of the DMS and DMSP is rapidly consumed and degraded due to microbial activities surrounding the anemones showing again the importance of these sulfur species for the microbial world. Additionally, this study showed that the amount of anemone/coral reef derived DMS for atmospheric processes might be less important than it was thought before suggesting coral reefs as less important hot spots compare with phytoplankton spring blooms in boreal regions. However, the method part of the paper is difficult to understand due to very short descriptions that missing some important details resulting in confusion of the reader. Thus, I suggest publishing this paper after major revision.

Author response (AR) 7: We thank referee 2 for the positive comments and very helpful suggestions for improvements to our manuscript. Following the reviewer's comments, we conducted a major revision of our manuscript that resulted in extensively updated Methods sections (sections 2.5 and 2.6). We also re-analysed our simulation and included confidence intervals for cellular DMSaq for the four Symbiodinium clades in Steinke et al. (2011) in our analysis. This changed the magnitude of the net/gross DMSaq production ratio (R) but not the final outcome of the model. The order of Figures and Tables was changed and added a new Table 2 showing parameters extracted from Steinke et al. (2011).

Major comments The reader gets easily confused by the different terms "net DMS production" and "DMS gross production" and which of the terms are measured or calculated/ estimated. Figure 2 was very helpful to understand but it is mentioned only in the last section of the paper. Please define/specify in your method parts the different terms in one to two sentences and make clear how you determined it.

AR 8: We clarified which parameter was measured or modelled at the end of the introduction and at the beginning of Method Section 2.5. Net aqueous DMS production and DMSP concentration within anemones were measured. Gross aqueous DMS production in anemones and coral-driven sea-to-air DMS flux were simulated.

The anemone gross DMS production calculation is confusing and difficult to understand when it is explained together with the DMS flux calculation in one equation. For a better understanding please explain first the gross DMS production separately and give more information about the different parameter you used in the equation.

AR 9: In Section 2.5 we separated the modelling approach into four steps. (i) Simulation of anemone gross DMSaq production rate from measured DMSP and information from the literature (Tables 1 and 2). (ii) Calculation of the ratio (R) between measured net and simulated gross DMSaq production. (iii) Simulation of coral gross DMSaq production rate. (iv) Conversion of coral gross DMSaq production to coral net DMSaq production using R and subsequently calculation of the sea–to–air flux. More information for each parameter in the model and an improved explanation of the model are included in the revised version of our manuscript.

It is not completely clear why you chose certain parameters. For instance, why you used DMSP from Yancey et al. 2010 when you have directly measured DMSP and biomass in your incubations?

AR 10: Table 3 was presented to compare the DMSP concentration measured in our study with those in previous studies. Anemone gross DMS production rate was simulated from anemone holobiont DMSP values (DMSPH) measured in our study and not those in Yancey et al. 2010. However, because we did not work with corals, coral gross DMS production rate was simulated starting from coral DMSP values (DMSPC) found in the literature (i.e. Yancey at al. 2010, Tab. 1).

Why you chose for NÂËĞnA1, NA2 and so on cell number maximum of 100? Is this a reasonable amount for anemone symbionts in your cultures?

AR 11: The number of Symbiodinium clade cells did not represent the real number within the anemones. It was set arbitrarily to 100 and it randomly changed within the simulation framework in order to generate different community compositions (see updated Section 2.5). Setting N = 1000 did not change the outcome of the model (see caption Table 1).

And please give more information about your previous study Steinke et al. 2011 regarding DMS and Symbiodinium you refer to in this study.

AR 12: This information is now included in the new Table 2.

How did you determined TW?

AR 13: Data for tissue weight (TW) were based on various coral species and taken from Thornhill et al. 2013 (see Table 1). We clarified this in the revised text in section 2.5.

And why is P between 0 and 20 % reasonable for your experiment. Why is the equation for gross DMSaq in anemone the same as the coral gross DMS-production equation (p 3, L 35)? Did you replace the TW for corals in this equation with the TW of the anemone?

AR 14: P is most sensitive to changes in temperature and wind speed and we selected a range of 1 to 20% based on the data presented in Bates et al. (1994). We have re-written the methods section 2.5 including a clearer description of the simulation with two new equations. The new equation 1 describes the calculation of gross DMS production rate in the anemone holobiont, whereas equation 2 describes the calculation of net DMS flux. TW was used to simulate the sea-to-air DMS flux from coral reefs (not included in Eq. 1). Briefly, Eq. 1 was used to simulate the gross DMSaq production rate in anemones using the DMSPH measured in this study and the data in Steinke et al. (2011). The same equation but with DMSPC instead of DMSPH was used to simulate the gross DMS production rate in corals. This was multiplied by TW to con-

vert biomass-normalized coral gross DMS production rates into CSA-normalized coral gross DMS production rates. The resulting values were finally multiplied by R and P to calculate the sea-to-air DMS flux.

Why are the assumptions on p4 L1-7 are reasonable. Please justify. Have you tested it?

AR 15: In the revised version of the manuscript, we explicitly discuss our five assumptions to provide support for our approach (section 3.3). Some of our assumptions are based on few data available in the literature. For example, it is currently impossible to assess whether the ratio between net and gross DMSg production calculated for anemones also applies to corals.

In your experiment, anemones were the organisms of interest, but you talked a lot about corals and coral surface area, so the reader gets confused if you want to show the impact of anemones or corals. You also said "Using our measurements of DMSP concentration and DMS production in anemones to extrapolate to coral reef environments has its limitations. . ." (p5, L16). Furthermore, on P5 L28: You said that you "normalized to CSA". How did you normalized? Did you assume that anemone coverage in coral reefs was 100% or you assumed that corals and anemones produce similar amounts of DMS so that the composition of the coral reefs (corals or anemones) didn't matter?

AR 16: We are using the anemones (phylum Cnidaria, order Actinaria) as a model system to explore DMS cycling in the globally important coral reefs that are dominated by stony corals (phylum Cnidaria, order Scleractinia). Stony corals are difficult to grow and experiment on. Hence, Aiptasia is often the preferred model to study bleaching and other processes in cnidarians. For example, we would not have been able to conduct a comparison between zooxanthellate and bleached individuals of stony coral species (Fig. 1a), since they have an obligate mutualistic relationship with their endosymbionts. Very little is known about the details of DMS cycling in tropical environments and we

explored the flux of DMS from tropical reefs using the limited published information available in the literature as best as currently possible.

We normalised to CSA by converting biomass-normalised DMS production to surface-normalised DMS production using TW [mg DW cm-2; eq. 2]. We assumed that the DMS production by the endosymbiont Symbiodinium is similar in anemones and corals and that the ratio (R) between net and gross DMS production calculated for anemones (see AR 15) also applies to corals (section 3.3).

Please justify why you can compare anemones and corals and why you can use anemone driven DMS to interpret the amount of DMS produced/released from coral reefs in general. Please say also something about the limitation of this comparison.

AR 17: As suggested by the reviewer, we added this information to the Results and Discussion section 3.3.

In your equation and your Fig 1c, please explain shortly the meaning of the term net DMSaq/gross DMSaq. Does the term say something about the amount of consumed DMS?

AR 18: We added an explanation of this term to the Method section 2.5. Our simulation suggests a ratio of 0.2 suggesting that about 80% of the gross DMSaq is being consumed (likely from reaction with ROS and microbial consumption/catabolism).

The section 2.5 "Data analysis" is very difficult to understand. It needs more details about why and what you were doing with your data. What do you want to say in the first sentence (p4 L10)? Please reword it. Is the mono-factorial analysis well known? Can you shortly say what that mean? What is the R package pse doing, why you used it? The references you gave are very complicated and detailed. It would be great when you give a more general information in your paper. Please, give also a short and general explanation about Monte-Carlo and why you applied it. In the last sentence of section 2.5 (p4 L20-22) is not clear what you have done. Please give more information

how you determined the sensitivity of the variables.

AR 19: We substantially revised the Data Analysis section (section 2.6). The first sentence described how data presented as a column figure in Steinke et al. (2011) were converted into numerical values. In the revised version of our manuscript, we applied original data including error terms in our re-analysis (see AR 7) so that this sentence became obsolete and was removed. Mono-factorial analysis means that the response variable (net DMS production in Fig. 2a) was compared between the two levels ('light' and 'darkness') of the factor 'treatment'. This is principally the same for Fig. 2b but here the factor was the 'production type', i.e. 'net' or 'gross' (2 levels). More information on the pse package and the Monte-Carlo simulation was added to Method Section 2.6 as requested.

Why you didn't determine the net DMSP production? Is this term not interesting?

AR 20: DMSP is a zwitterion and, in contrast to the freely diffusible DMS, does not easily cross cellular membranes. It is likely that observed concentrations of dissolved DMSP (DMSPd) in previous publications are overestimates stemming from the release of DMSP from expelled/non-symbiotic Symbiodinium in the medium (see discussion in Kiene, R. P., and D. Slezak. 2006. Low dissolved DMSP concentrations in seawater revealed by small-volume gravity filtration and dialysis sampling. Limnology and Oceanography-Methods 4: 80-95). Hence, it is difficult to quantify net DMSP production from an accumulation of dissolved DMSP in medium after 48h incubation. Other studies used isotopic labelling coupled with mass-spectrometric detection of DMSP (e.g. Stefels, J., J. W. H. Dacey, and J. T. M. Elzenga. 2009. In vivo DMSP-biosynthesis measurements using stable-isotope incorporation and proton-transfer-reaction mass spectrometry (PTR-MS). Limnol. Oceanogr. Methods 7: 595-611.), techniques that are not available to us. Assuming that anemone were fully acclimated to our experimental set up and growth is negligible during our 48 incubation period, net DMSP production is likely going to be close to zero since the concentration of DMSP per biomass is typically stable at constant environmental conditions.

Minor comments Abstract P1 L10: Please delete the part with the gas chromatograph. I suggest "Here we quantified the net DMS production and the concentration of its cellular precursor dimethylsulfoniopropionate (DMSP) in the cultured sea anemone Aiptasia sp., : : :"

AR 21: Changed as suggested.

Please show only one number after the decimal place in the abstract, e.g. 44.2 instead of 44.22 (p1 L13) and 6 instead of 6.00.

AR 22: Changed as suggested.

P1 L15: This sentence is very confusing. You say that you simulated the DMS flux and than you present the results of the gross DMS production. I suggest "We applied these findings to a Monte-Carlo simulation to demonstrate that net aqueous DMS production accounts for only 0.5 – 2% of gross aqueous DMS production. Monte Carlo based estimations of DMS fluxes into the atmosphere showed that reefs may release up to : : :"

AR 23: Changed as suggested.

Maybe you can write also a discussion sentence about the DMS flux results in the abstract as you have done for DMS gross production.

AR 24: In the abstract we state: '. . .Monte-Carlo based estimations of sea–to–air DMS fluxes showed that reefs may release up to 25 μmol DMS m-2 coral surface area (CSA) d-1 into the atmosphere with 40% probability for rates between 0.5 and 1.5 μmol m-2 CSA d-1. These predictions were in agreement with directly quantified fluxes in previous studies. Conversion to a flux normalised to sea surface area (SSA) (range 0.3 to 17.0 with highest probability for 0.3 to 1.0 μmol DMS m-2 SSA d-1), suggests that coral reefs emit DMS at lower rates than the average global oceanic DMS flux of 6.7 μmol m-2 SSA d-1 (28.1 Tg sulfur per year). . .'

Section 3.2 You discussed in this section that DMS removal processes under light con-

ditions are faster compared to dark conditions mainly due to microbial consumption. However, in your incubation experiment you didn't see lower DMS concentration in the light treatments compare to the dark treatments. Maybe you should consider and discuss that your incubation experiments didn't contain the microbial diversity as natural environments have. You used artificial seawater (axenic?) for the incubation, thus you might miss important DMS consuming microbes in your experiments resulting in similar DMS concentrations in dark and light treatments.

AR 25: No attempts were made to sterilise the seawater medium. We added information on microbial diversity to our Methods (section 2.1) and a short discussion on the effect of microbial diversity on DMS consumption under Results and Discussion (section 3.2).

P6 L 1: "an average rugosity of 3". Can you say what that means? Is 3 much rugosity or only a little bit? Has rugosity a unit?

AR 26: We added a definition of rugosity to the Results and Discussion (section 3.3).

Fig.1 d: please add the different variables in the figure or color code the dots. It is not clear which point presents which variable in the sensitivity plot. Maybe you can say a little bit about what the different sensitivity numbers mean in the plot, such as "variables close to 0 have less influence on the simulation than variables lower/higher than 0" or something similar.

AR 27: There was an error within the R script in the line coding for the x-axis ticks. We apologise for not having noticed it in the version submitted earlier. Note that we now show the original Figure 1d as a separate Figure 3 in the revised version of our manuscript. We also included the parameters cDMS, CV, and cDMSP for the four Symbiodinium clades that were allowed to vary within the confidence intervals given in Steinke et al. (2011) in our re-analysis. The Figure caption now includes a short description of the sensitivity values: '...Values close to 0 have less influence on the simulation than those departing from 0...'

END OF RESPONSE TO REFEREE 2

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