Quantification of dimethyl sulfide (DMS) production in the sea anemone *Aiptasia* sp. to simulate the sea-to-air flux from coral reefs

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Abstract. The production of dimethyl sulfide (DMS) is poorly quantified in tropical reef environments but forms an essential process that couples marine and terrestrial sulfur cycles and affects climate. Here we quantified net aqueous DMS production and the concentration of its cellular precursor dimethylsulfoniopropionate (DMSP) in the sea anemone *Aiptasia* sp., a model organism to study coral-related processes. Bleached anemones did not show net DMS production whereas symbiotic anemones produced DMS concentrations (mean ± standard error) of 160.7 ± 44.22 nmol g^{-1} dry weight (DW) after 48 h incubation. Symbiotic and bleached individuals showed DMSP concentrations of 32.7 ± 6.00 and 0.6 ± 0.19 µmol g^{-1} DW, respectively. We applied these findings to a Monte-Carlo simulation to demonstrate that net aqueous DMS production accounts for only 20% of gross aqueous DMS production. Monte-Carlo based estimations of sea–to–air ~~DMS~~ fluxes of gaseous DMS 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 gaseous 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). The large difference between simulated gross and quantified net aqueous DMS ~~-~~production ~~rates~~ in corals suggests that the current and future potential for ~~DMS~~ its production in tropical reefs is critically governed by DMS consumption processes. Hence, more research is required to assess the sensitivity of DMS-consumption pathways to ongoing environmental change to address the impact of predicted degradation of coral reefs on DMS production in tropical coastal ecosystems and its impact on future atmospheric DMS concentrations and climate.

1 Introduction

The DMSP-catabolite DMS is a biogenic volatile organic compound (BVOC) that provides the dominant natural source of marine sulfur to the atmosphere with a release of 28.1 Tg S per year (Lana et al., 2011). This biogenic sulfur affects cloud formation and climate (Vallina and Simó, 2007), and represents the key link in marine and terrestrial sulfur biogeochemical cycling (Bates et al., 1992). However, atmospheric DMS constitutes only a small fraction of the total DMSP and DMS produced in the sea. Less than 20% of dissolved DMSP is directed towards DMS production in planktonic communities (Kiene et al., 2000), and further chemical and biological loss processes including the conversion to dimethyl sulfoxide (DMSO), methanethiol, and formaldehyde by

DMS-oxidising bacteria (Kiene and Bates, 1990; Lidbury et al., 2016), severely limit its availability for sea–to–air transfer, a limiting step for functioning in climate-cooling.

The cnidarian symbiont *Symbiodinium* sp. is a strong producer of DMSP and DMS (Steinke et al., 2011). Hence, the symbiotic sea anemone *Aiptasia* sp. (Van Alstyne et al., 2009) and corals from the Great Barrier Reef (Broadbent and Jones, 2004; Jones and King, 2015) have been found to produce high quantities of DMSP and DMS that fuel the microbial biogeochemistry in coral reefs (Raina et al., 2009). Coral bleaching from the expulsion of *Symbiodinium* endosymbionts occurs regularly as an acclimatisation strategy to monthly and seasonal changes in environmental parameters such as light and temperature. However, climate anomalies can lead to prolonged loss of symbionts and death of the coral (Suggett and Smith, 2011). The principal cause of bleaching is the overproduction of harmful reactive oxygen species (ROS) mostly originating from the photoinhibition of Photosystem II at increased temperature and irradiance (Tchernov et al., 2011), and *Symbiodinium* can provide clade-specific defences to harmful ROS including enhanced protection against UV radiation (Baker, 2003), higher growth (Little et al., 2004), and increased thermal tolerance (Baker et al., 2004). Since DMSP and DMS readily scavenge ROS (Sunda et al., 2002) and algae are known to use DMS to mitigate ROS-induced metabolic damage under sublethal environmental stresses (Archer et al., 2010; Dani and Loreto, 2017), it is possible that they are part of an antioxidant mechanism that leads to the scavenging of ROS and production of DMSO in symbiotic cnidarians (Gardner et al., 2016; Jones and King, 2015).

Tropical sea anemones belonging to the genus *Aiptasia* provide a powerful model organism to investigate the cnidarian host–symbiont relationship in the context of climate change (Baumgarten et al., 2015; Belda-Baillie et al., 2002). In contrast to corals, these anemones can be grown under the presence and absence of their symbionts. This offers unique opportunity to start dissecting the complex interactions between the main DMSP producer (*Symbiodinium* sp.), its host (*Aiptasia*) and the associated microbial community that, taken together, make up the anemone holobiont that releases DMS into the environment. Since information on the sea–to–air flux of DMS and other BVOCs from tropical reefs is scarce (Exton et al., 2014), this study quantified for the first time net aqueous DMS production (net DMS_{aq} production) in *Aiptasia* sp. and used this data together with information on measured DMSP concentration within anemone holobionts ($DMSP_H$) to simulate anemone gross aqueous DMS production (gross DMS_{aq} production) and coral–derived sea–to–air ~~DMS~~ flux of gaseous DMS (net DMS_g flux).

2 Methods

2.1 Anemone husbandry, bleaching and biomass estimation

The symbiotic tropical sea anemone *Aiptasia* c.f. *pallida* was kept under standard growth conditions in glass aquaria filled with artificial seawater (ASW; 32 g L^{-1} Reef Salt; D-D H_2Ocean) inside an incubator (SANYO Versatile Environmental Test Chamber MLR-351) set to 26°C and 12h:12h light/dark cycle at a light intensity of 80 µmol m^{-2} s^{-1}. No attempts were made to remove bacteria from the anemones since antibiotic treatment is often detrimental to *Symbiodinium* growth (Yost and Mitchelmore 2009) and we expect the microbial community to be representative of laboratory-grown *Aiptasia*. ASW was changed weekly and the anemones were fed with freshly-hatched brine shrimps (*Artemia salina*, reefphyto) every 2 weeks.

Three months before the start of our measurements, symbiotic anemones were bleached following a cold-shock protocol described in Muscatine et al. (1991). Briefly, they were starved for three weeks, gently removed from their attachment site and transferred to individual 4.92 mL glass vials containing ASW at 26°C. After attachment of the anemones to the glass surface, the water was replaced with cold (4°C) ASW, the vials were closed, kept in the fridge for at least 4 h before replacing the ASW medium and transferring the vials to 26°C. After 1–2 days, anemones were microscopically checked for symbionts using a dissecting microscope and, in case of visually incomplete bleaching, the protocol was repeated. Bleached anemones were kept in darkness but all other growth conditions remained the same.

For biomass estimation, the anemones were anaesthetised in a 1:1 solution of ASW and 0.37M $MgCl_2$, and placed under a dissecting microscope equipped with an eyepiece graticule that was calibrated to the selected magnification. Two oral disk diameters per individual were measured from photographs. Dry and wet weights (DW and WW, respectively) were estimated using the non-linear model for composite treatment proposed earlier (Clayton Jr and Lasker, 1985), and the assumption that the water content in sea anemones is 85% (Brafield and Chapman, 1983).

2.2 Experimental design

Before the start of the experiment, bleached and symbiotic anemones were acclimated for 2 months at standard growth conditions in darkness or light, respectively. At the beginning of the experiment, anemones were haphazardously selected for four treatments (n=6 each): Symbiotic light, symbiotic darkness, bleached light and bleached darkness. Samples were incubated for 48 h together with six ASW-filled control vials, before quantifying net DMS production and DMSP concentration.

2.3 Quantification of ~~holobiont~~ $DMSP_H$ concentration~~and DMS production~~

$DMSP_H$ (i.e. DMSP in ~~individual~~ anemone~~s~~ holobionts) was indirectly quantified after equimolar hydrolytic conversion to DMS in 3 mL of 0.5M NaOH. DMS was then measured using gas chromatography with flame-photometric detection (GC–FPD) as described earlier (Franchini and Steinke, 2017). Briefly, depending on the amount of DMSP in the specimen, either headspace direct injection of gaseous phase or the more sensitive in vial purging of aqueous phase techniques were used to quantify DMS. For the former technique, 200 µL of headspace were directly injected into the gas chromatograph (GC-2010, Shimadzu, Milton Keynes, UK). For the latter technique, the NaOH in the vials was purged for 6 min with nitrogen (30 mL min^{-1}) and this sample gas dried with a Nafion counter flow drier (Permapure MD-050-72F-2, Fluid Controls Limited, Aldermaston, UK) and cryogenically enriched at -150°C using a purpose-built purge-and-trap apparatus, before heating the enriched sample to 90°C and flushing it into the gas chromatograph for quantification. Both techniques were calibrated using DMSP standard solutions (Franchini and Steinke, 2017).

2.4 Quantification of holobiont net DMS_{aq} production

To quantify the net production of DMS released into the aqueous medium by the holobiont (net DMS_{aq} production), individual anemones were transferred into 3 mL fresh ASW inside 4.92 mL vials and incubated for 48 h. Vials without anemones served as the control. Net DMS_{aq} production was calculated as the difference in

DMS concentration between control vials and vials with anemones after quantification of DMS using the in vial purging of aqueous phase technique (Franchini and Steinke, 2017).

2.5 Simulating the coral-driven sea–to–air DMS_g flux

The coral-driven sea–to–air flux of gaseous DMS (net DMS_g flux) was estimated in four steps: (i) simulating the holobiont gross DMS_{aq} production rate using quantified holobiont DMSP concentration, (ii) calculating the ratio (*R*) between measured net and simulated gross DMS_{aq} production, (iii) simulating coral gross DMS_{aq} production rate, and (iv) converting coral gross DMS_{aq} production to coral net DMS_{aq} production (under the assumption that *R* is similar for anemones and corals) and subsequently to sea–to–air flux using conversion parameters from the literature (Fig. 1; Tables 1 and 2).

Holobiont DMSP concentration from this study was used to simulate the gross DMS_{aq} production rate defined as the total amount of DMS_{aq} produced over time by *Symbiodinium* of clade *i* within the host. Data for cellular DMS production of fee-living *Symbiodinium* from four clades were used as a proxy for gross DMS production (Steinke et al., 2011). The equation describing the holobiont gross DMS_{aq} production rate ($holobiont\ gross_r\ DMS_{aq}$) took the form:

$$holobiont\ gross_r\ DMS_{aq} = \sum_i \left(\frac{DMSP_H \times \frac{N_i}{N_{tot}}}{cDMSP_i} \times cV_i \times cDMS_{aq,i} \right) \quad (1)$$

where $DMSP_H$ is the measured DMSP within the anemone holobiont, N_i is the number of *Symbiodinium* cells of clade *i* (with i= clades A1, A2, A13, B1; see below), and N_{tot} is the total number of cells of different *Symbiodinium* clades (i.e. $\sum_i N_i$). Note that *N* does not reflect the actual number of symbionts within anemones but was arbitrarily set in order to calculate the proportion of clade *i* among all clades within anemones. This made it possible to generate symbiont communities of different relative compositions during our simulations. Values for $cDMSP_i$ (i.e. cellular DMSP concentration for *Symbiodinium* clade *i*), $cDMS_{aq,i}$ (i.e. cellular DMS_{aq} production rate for *Symbiodinium* clade *i*), and cV_i (cell volume for *Symbiodinium* clade *i*) specific to the free-living *Symbiodinium* clades A1, A2, A13 and B1 were obtained from Steinke et al. (2011) (Table 2; Fig. 1).

The term $DMSP_H \times \frac{N_i}{N_{tot}}$ reflected the contribution of clade *i* to the amount of DMSP within the holobiont. This was divided by $cDMSP_i$ to estimate the number of clade *i* cells per anemone biomass, which was then multiplied by cV_i to obtain the volume occupied by clade *i* per anemone biomass. This biomass-normalized volume was subsequently multiplied by $cDMS_{aq,i}$ to estimate the gross DMS_{aq} production rate per anemone biomass for clade *i*. The sum across all 4 clades yielded the gross DMS_{aq} production rate per anemone biomass.

The fraction of DMS_{aq} released into the water by the anemones was calculated as the ratio (*R*) between the measured net DMS_{aq} production and the simulated gross DMS_{aq} production rate multiplied by the incubation period (i.e. 48 h). Thus, *R* accounted for the reaction of DMS with ROS and microbial DMS_{aq} consumption pathways mostly related to anemone or coral membrane-associated microorganisms (Fig. 1). The equation for the simulated daily coral-driven sea–to–air flux of gaseous DMS (*net DMS_g flux*) normalised by coral surface area (CSA; µmol m^{-2} d^{-1}) took the form:

$$net\ DMS_g\ flux = coral\ gross_r DMS_{aq} \times TW \times R \times P \quad (2)$$

where $coral\ gross_r DMS_{aq}$ is the simulated coral gross DMS_{aq} production rate calculated as in eq. 1 but replacing $DMSP_H$ with $DMSP_C$ (i.e. biomass-normalized DMSP within corals), *TW* is the coral tissue weight normalized by coral surface area, and *P* is the percentage of net DMS_{aq} production escaping into the atmosphere (Fig. 1; Table 1). Note that the range of *TW* was based on values for different coral types (branching, plating, and massive corals) but no efforts were made to explicitly explore different coral types at this stage.

2.6 Data analysis

~~Data extrapolation from graphical representations of previously published studies was performed through freelyavailable digitising software (Plot Digitizer,version 2.6.6).~~Graphical representations as well as statistical and sensitivity analyses were performed using the R software (R Project for Statistical Computing, version 3.1.1). Datasets for net DMS_{aq} production from light and dark treatments and for comparison between net and gross DMS_{aq} productions were checked for normality and equal variance using a Shapiro-Wilk normality test and Levene's test for homogeneity of variance, respectively. Since all datasets showed non-normal distributions, mono-factorial analyses were performed using the Kruskal-Wallis rank sum test. Treatment and production type were treated as factors (independent variables) with two levels each (light and darkness, and net and gross, respectively). Simulations and sensitivity analysis were performed through the R software package *pse* (Chalom and Knegt Lopez, 2016), following a similar approach to that described in the tutorial by Chalom *et al*. (2013). Briefly, after specifying Equations 1 and 2 and defining all parameters with respective uncertainty ranges and distributions (Tables 1 and 2), we randomly generated 500 values for holobiont $gross_r$ DMS_{aq} and net DMS_g flux through a Monte-Carlo simulation using the LHS (Latin Hypercube Sampling for uncertainty and sensitivity analyses) function within the *pse* package. This function feeds the simulation framework formed by Equations 1 and 2 with random values for each parameter within the specified ranges. Resulting simulation outcomes were collected and used to generate probability distribution plots. Finally, using the LHS function, partial rank correlation coefficients (prcc) were calculated, which indicate the influence of a parameter on the simulation outcome (with 1 = maximum positive influence and -1 = maximum negative influence; 0 = no influence on simulation outcome). These coefficients were used to assess the response (sensitivity) of our simulation framework to variations in each variable.

3 Results and Discussion

3.1 Symbionts are the main source of DMSP and DMS in *Aiptasia*

Symbionts were the main source of DMSP and our data for symbiotic and bleached anemones are in general agreement with the earlier findings (Table 3) (Van Alstyne et al., 2009; Yancey et al., 2010). However, using the more sensitive in vial purging method compared to the headspace sampling performed by Van Alstyne *et al*. (2009), bleached anemones kept in darkness for 2 months showed an average DMSP concentration of 0.6 ± 0.19 µmol g^{-1} DW (n=6). Additional microscopic observation revealed small clusters of symbiont cells within *Aiptasia* tentacles suggesting that bleaching was incomplete, hence, individuals were not aposymbiotic. Whether aposymbiotic anemones produce DMSP as demonstrated for corals (Raina et al., 2013) requires further investigation.

We quantified for the first time the net DMS_{aq} -production in *Aiptasia* and demonstrated that the symbiont is the main source of DMS (Fig. 2a). Bleached individuals showed net DMS_{aq} production above the limit of detection but below the limit of quantification at 1.2 ± 0.62 nM which is equivalent to a production rate of 3.6 pmol DMS in 3 mL over a 48 h incubation.

3.2 Effect of light on DMS production

Net DMS_{aq} production in dark was the same as in light treatments (Fig. 2a). Although light has been shown to affect the cycling of DMS (Galí et al., 2013; Toole and Siegel, 2004), our results indicate that acclimated symbiotic *Aiptasia* produced 52 to 332 nmol DMS g^{-1} DW (mean = 160.7 ± 44.22 nmol g^{-1} DW; n = 6) over a 48h incubation period with no significant difference between the light and dark treatments (P=0.42; Fig. 2a). Various DMS removal processes affect the amount of DMS that could be detected in the water surrounding the anemones and our measurements represent net DMS_{aq} production rates. Microbial consumption of DMS is concentration dependent and affected by the microbial diversity and presence of DMS-consuming bacteria (Schäfer et al.2010). Although the microbial community associated with the holobiont surface is probably conservative, exchanging the seawater medium (ASW) at the start of our incubations likely resulted in a low abundance of free-living bacteria in comparison to the natural setting. Furthermore, consumption of DMS may be sensitive to light since photosynthetically derived O_2 could stimulate heterotrophic respiration of DMS. In fact, the activity and population size of DMS-oxidising bacteria are higher during oxic/light than during anoxic/dark conditions (Jonkers et al., 2000). Moreover, light is expected to increase ROS that could oxidise DMS and produce DMSO, hence, contributes to DMS consumption (Fig. 1). This scenario suggests that DMS consumption could be higher during the day than at night, and that, as a consequence, net production should show the opposite pattern. However, light can also result in an increase of DMS in phytoplankton suggesting a direct link between acute photo-oxidative stress and DMSP synthesis but the physiological basis for this is unclear (Archer et al., 2010).

3.3 From anemones to corals: Net vs. gross DMS_{aq} production and net DMS flux

Using our measurements of DMSP concentration and net DMS production in anemones to extrapolate to coral reef environments provides an initial route to assess overall DMS production in tropical coastal ecosystems where DMS and DMSP data coverage is relatively poor. To highlight the basis of our approach and discuss possible limitations we will first examine five major assumptions in our approach:

(i) Endosymbionts are the main DMSP and DMS producers within anemones and corals. Juvenile corals of the high DMSP-producing genus *Acropora* that were aposymbiotic (free from *Symbiodinium* symbionts) showed a low level of DMSP production. This suggests that corals and possibly other cnidarians can be a cryptic source of DMSP that is not generated by their endosymbiotic partner. Our bleached anemones were not aposymbiotic in our experiment and showed low DMSP concentrations with DMS production below the level of quantification. This confirms a previous study that could not detect DMSP in aposymbiotic anemones (Table 3; Van Alstyne et al., 2009) and supports our assumption that the endosymbionts are the main producers of DMSP and DMS.

(ii) There is no difference in cellular DMSP content (*cDMSP*) and DMS_{aq} production rate ($cDMS_{aq}$) between free-living *Symbiodinium* cells and those living symbiotically. Symbionts in corals contained about 10 to 300

fmol DMSP cell^{-1} (Yost and Mitchelmore 2010), while Borell et al. (2014; 2016) reported concentrations ranging from about 20 to 50 fmol DMSP cell^{-1}. These values for both corals and anemones were similar to the 4 free-living *Symbiodinium* clades investigated by Steinke et al. (2011) (39.3 to 126.8 fmol DMSP cell^{-1}; Table 2) and suggest that free-living and endosymbiotic *Symbiodinium* likely produce similar amounts of cellular DMSP. As far as we are aware, Steinke et al. (2011) present the only data for $cDMS_{aq}$ in *Symbiodinium* so that this assumption cannot be tested against other published information.

(iii) DMSP and DMS characteristics in *Symbiodinium* clades A1, A2, A13, and B1 are representative of other symbiont types. Symbiont community composition varies depending on the geographic region. In the Red Sea and in the Sea of Oman clade A1 was found to be one of the most abundant (Ziegler et al. 2017), while clade B1 was found to be abundant in Caribbean reef-building coral *Orbicella faveolata* (Kemp et al. 2015). Both clades seemed to play a minor role in the Indo-Pacific (Yang et al. 2012). Although having information about DMS and DMSP characteristics for more clades might improve simulation accuracy, such values seem to play a minor role in shaping the DMS sea–to–air flux (see below and Figure 3).

(iv) The ratio between net and gross DMS_{aq} production calculated for anemones also applies to corals, an assumption that is currently impossible to test due to the lack of published information.

(v) Finally, light intensity does not significantly affect $cDMS_{aq}$. Although light conditions in the experiment conducted by Steinke *et al.* (2011) (350 µmol m^{-2} s^{-1}, 14h:10h light/dark cycle) were different from those adopted here, the evidence that net DMS_{aq} production was independent of light intensity (see Sect. 3.2) is in support of our assumption.

Aiptasia is an accepted model to investigate the cnidarian host–symbiont relationship (Baumgarten et al., 2015; Belda-Baillie et al., 2002). However, the microbial communities on the surface of corals and anemones may differ, leading to potential differences in DMS biogeochemistry. Corals produce vortical ciliary flows that may not only limit the attachment of pathogens to the host surface, but also prevent accumulation of oxygen that could inhibit the photosynthetic activity of their endosymbionts (Shapiro et al. 2014). Whether those ciliary flows are also present in anemones has to be investigated. Last but not least corals calcify and this might change the allocation of resources within the host with consequences on the type of relationship with their symbionts under stress conditions.

The adopted simulation framework suggests that gross DMS production of ~1 µmol g^{-1} over 48 h is up to 7 times higher than the net production of ~0.15 µmol g^{-1} ($P < 0.001$) (Fig. 2b). Additionally, the percentage of the gross production escaping into the water surrounding the anemones ranged from 1 to 120% with 60% probability for 5 to 30% (Fig. 2c). It is proposed that the remaining 70 to 95% reacts with ROS or is consumed in other ways by the host and surface-associated microorganisms (Fig. 1). It has been demonstrated that the coral host not only controls the population size of various *Symbiodinium* clades inside the symbiosomes (Kemp et al., 2014), but it also actively modifies the microenvironment on their surface (Barott et al., 2015), both with consequences for DMSP concentration and DMS production. Furthermore, DMS production is significantly different between the *Symbiodinium* clades (Table 2) so that the relative abundance of clade A1 affected coral–driven sea–to–air DMS fluxes (see N_{A1} in Figure 3), which ranged from 0 to 25 µmol m^{-2} d^{-1} with 40% probability for values between 0.5 and 1.5 µmol m^{-2} CSA d^{-1} (Fig. 2d). The other clades had minor influence on sea–to–air DMS fluxes, because even if corals accommodate high DMS-producing endosymbionts leading to high gross DMS_{aq} production rates, the amount of DMS emitted into the atmosphere is more strongly affected

by physico–chemical variables including temperature (affects DMS solubility) and wind speed (drives sea–to–air transfer), and depends more critically on net DMS_{aq} production that is the result of several DMS-consumption processes (Fig.1; Fig. 3). Simulation parameters that highly influenced the DMS flux were the percentage of aqueous DMS escaping into the atmosphere (P), the coral tissue weight normalised by coral surface area (TW), coral DMSP ($DMSP_C$), holobiont DMSP ($DMSP_H$), and anemone net DMS_{aq} production (Fig. 3). This is not surprising since P shapes the amount of dissolved DMS escaping into the atmosphere, TW is a proxy of reef structural complexity and the higher it is the larger is the surface area per unit of biomass available to accommodate DMS-producing symbionts, and $DMSP_C$ and $DMSP_H$ are proportional to the total number of symbionts. Since $DMSP_H$ is used to simulate anemone gross DMS_{aq} production and subsequently to estimate R, higher $DMSP_H$ will decrease R, resulting in decreased DMS flux (Fig. 3). Finally, the higher the pool of DMS dissolved in the water (net DMS_{aq} production) the higher the chance that DMS will escape into the atmosphere.

The range of sea–to–air DMS fluxes obtained from our simulation is in good agreement with earlier measurements on *Acropora intermedia*, a dominant staghorn coral in the Indo-Pacific region, which generated a sea–to–air flux of 0.55 to 1.13 µmol m^{-2} CSA d^{-1} (Fischer and Jones, 2012). Converting CSA-normalised fluxes into fluxes normalised to sea surface area (SSA) requires information on coral cover and reef rugosity, i.e. the unit-less ratio between the reef real surface area and its projected area with a ratio of 1 indicating a flat reef while rugosity values >1 indicate increasing structural complexity. Assuming a coral cover of 22% in the Indo-Pacific (Bruno and Selig, 2007) and an average rugosity of 3 (Storlazzi et al., 2016), we can calculate a maximum flux of about 17 µmol DMS m^{-2} SSA d^{-1} with highest probabilities for fluxes ranging from 0.3 to 1 µmol DMS m^{-2} SSA d^{-1}. This flux is in good agreement with modelled fluxes based on continuous DMS measurements during the wet and dry seasons at Heron Island in the southern Great Barrier Reef that show coral-derived DMS fluxes of 0.2 µmol DMS m^{-2} SSA d^{-1} (Swan et al., 2017). Taken together, this suggests that coral reefs likely continuously emit DMS at lower rates than the short-lived DMS 'hotspots' of phytoplankton blooms in the North Atlantic (20.69 to 26.93 µmol m^{-2} SSA d^{-1}; Holligan et al., 1993) or at high latitudes (21.87 µmol m^{-2} SSA d^{-1}; Levasseur et al., 1994). Furthermore, our estimated sea-to-air flux from coral reefs is also lower than the global oceanic flux that is calculated at 6.7 µmol m^{-2} SSA d^{-1} (equivalent to 28.1 Tg S y^{-1} in Lana et al., 2011) and are in agreement with earlier findings that suggest coral environments enhance the dominant oceanic DMS flux by just 4% during the wet season and 14% during the dry season (Swan et al., 2017). While our calculated fluxes refer to fully submersed reefs, it is important to note that tidally-exposed corals such as the strongly DMS producing *Acropora* spp. (3,000–11,000 µmol DMS m^{-2} SSA d^{-1}) may provide substantial short 'bursts' of DMS to the atmosphere that last for several minutes during and after periods of aerial exposure (Hopkins et al., 2016). These bursts can be further enhanced when corals experience hypoosmotic stress from rain (Swan et al., 2017).

Our study suggests that net DMS_{aq} production and the resulting sea–to–air flux from coral reefs are under strong control of DMS-consumption pathways. Furthermore, DMS and its massively abundant precursor DMSP (Broadbent and Jones, 2004) are likely key metabolites that significantly fuel microbial activity in tropical coastal ecosystems (Raina et al., 2009). Hence, predicting future DMS concentration in the tropical atmosphere and its effect on climate requires an assessment of the sensitivity of DMS-consumption processes in reefs under environmental change.

4 Data availability

The datasets supporting this article will be made publicly available upon manuscript acceptance.

5 Author contribution

F. Franchini and M. Steinke conceived and designed the study, interpreted the data and wrote the manuscript. F. Franchini performed the experiments, and collected and analysed the data. Both authors gave final approval for publication.

6 Competing interests

The authors declare that they have no conflict of interest.

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Table 1: Parameters used for the simulation. DMS, dimethylsulfide; DMSP, dimethylsulfoniopropionate; DW, dry weight; N/A, not applicable. Note that the simulation was unaffected when setting N (the arbitrary number of clade-specific *Symbiodinium* cells) to a maximum value of 1000.

Parameter	Description	Unit	Range		Source
			min	max	
$DMSP_H$	Biomass-normalised DMSP within the anemone holobiont	µmol g^{-1} DW	15.09	51.82	This study
net DMS_{aq}	Biomass-normalised net aqueous DMS production	nmol g^{-1} DW in 48h	52.15	332.25	This study
TW	Coral tissue weight normalised by coral surface area (CSA)	mg DW cm^{-2}	2.58	11.51	Thornhill et al. (2013)
$DMSP_C$	Biomass-normalised DMSP within corals	µmol g^{-1} DW	52.36	84.00	Yancey et al. (2010)
$N_{A1, A2, A13, B1}$	Arbitrary number of clade-specific *Symbiodinium* cells	N/A	0	100	–
P	Percentage of aqueous DMS escaping into the atmosphere	%	1	20	Bates et al. (1994)

Table 2: Parameters for cellular DMSP concentration (cDMSP), cell volume (cV) and cellular net DMS_{aq} production rate ($cDMS_{aq}$) in four clades of *Symbiodinium* sp. (data from Steinke et al.2011).

Symbiodinium clade	cDMSP (fmol $cell^{-1}$)	cV (μm^3 $cell^{-1}$)	$cDMS_{aq}$ (mmol L^{-1} cV h^{-1})
A1	98.0 ± 4.18	415 ± 9.5	0.32 ± 0.112
A2	126.8 ± 8.59	763 ± 24.2	0.06 ± 0.018
A13	85.6 ± 22.03	419 ± 34.5	0.10 ± 0.029
B1	39.3 ± 2.33	237 ± 19.7	0.04 ± 0.025

Table 3: Biomass-normalised DMSP within symbiotic or bleached anemones (mean ± se) in this and two previous studies. Sample size (n) in parentheses. DMSP, dimethylsulfoniopropionate; DW, dry weight; ND, not detectable; NI, not investigated.

Aiptasia Species	DMSP (μmol g^{-1} DW)		Source
	Symbiotic	Bleached	
A. pallida	22.7 ± 8.00 (2)	ND (3)	Van Alstyne et al. (2009)
A. puchella	54.7 ± 15.20 (3)	NI	Yancey et al. (2010)
A. cf. *pallida*	32.7 ± 6.00 (6)	0.6 ± 0.19 (6)	This Study

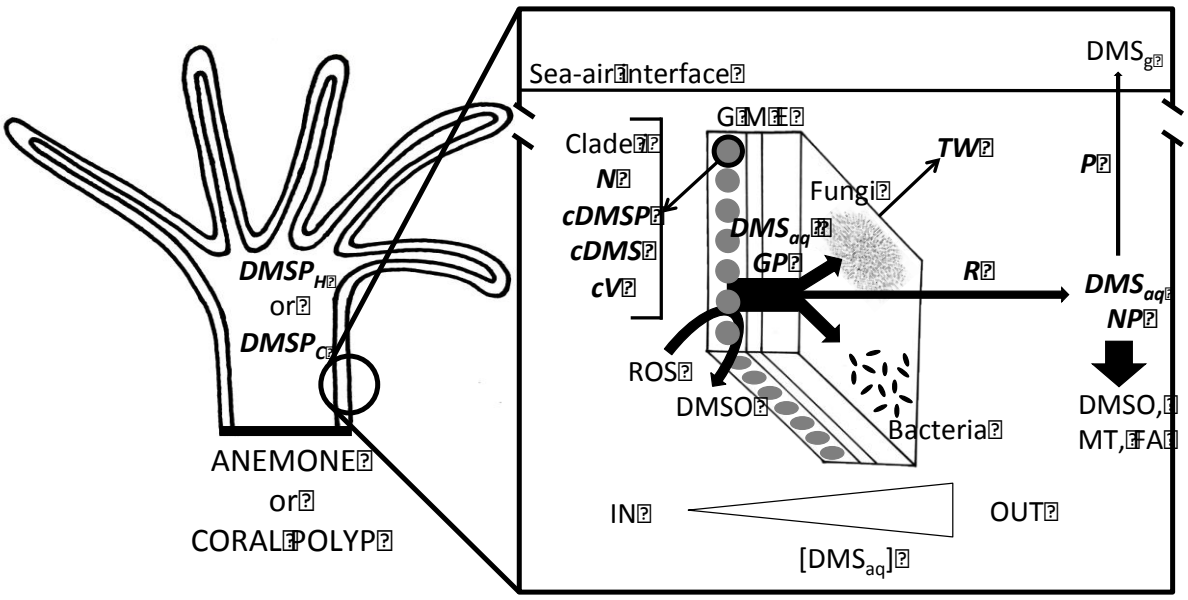

Figure 1: Magnification of a coral polyp and its cell layers with particular emphasis on the pathway of DMS from its production by endosymbionts (grey circles) to its release to the atmosphere. Note that symbols in bold italics describe the parameters fed into the simulation framework. The host with a particular tissue weight (TW) accommodates a number *N* of cells for *Symbiodinium* clade *i* containing DMSP (cDMSP, cellular DMSP), producing and releasing DMS (cDMS, cellular DMS production rate), and having a particular volume (cV, cellular volume). All cells of all clades *i* form the total DMSP concentration within the anemone holobiont or corals ($DMSP_H$ and $DMSP_C$, respectively). Measured net DMS_{aq} production (DMS_{aq} NP) is a fraction R of the gross DMS_{aq} production (DMS_{aq} GP). The remaining DMS (i.e. 1-R) is available to scavenge reactive oxygen species (ROS) and/or is consumed by surface-associated microbes. Once dissolved, a fraction P of the net DMS_{aq} production escapes into the atmosphere, while most of it is biologically transformed by free-living bacteria in the water column to, for example, DMSO, methanethiol (MT) and formaldehyde (FA). DMS, dimethylsulfide; DMS_g, gaseous DMS; DMS_{aq}, aqueous DMS; DMSO, dimethyl sulfoxide; G, gastrodermis; M, mesoglea; E, epidermis.

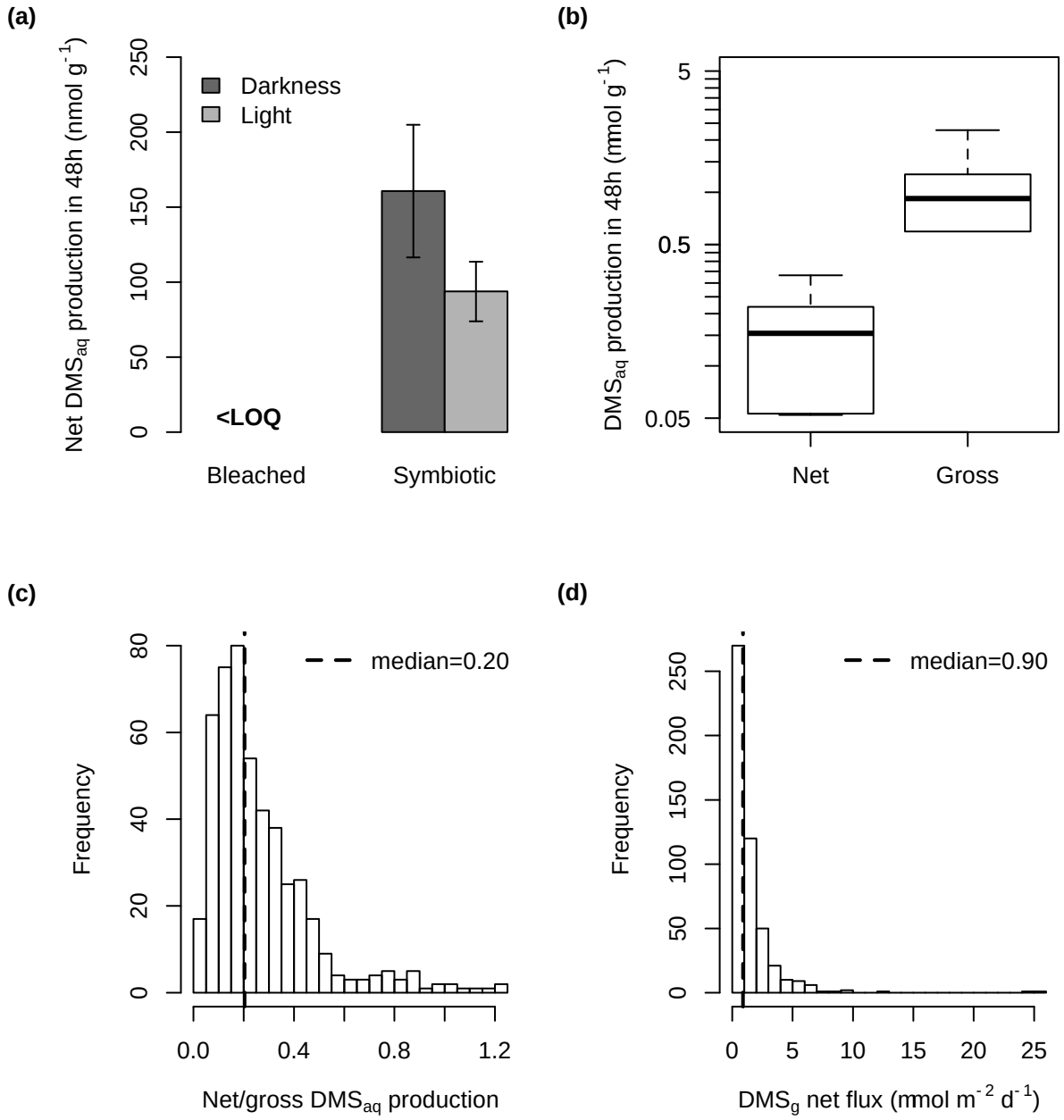

Figure 2: (a) Biomass-normalised (dry weight) net DMS production (mean ± se) for symbiotic and bleached anemones during light and dark treatments (n=6). **(b)** Boxplot showing the difference ($P < 0.001$) between the biomass-normalised (dry weight) observed net DMS_{aq} production (n=6) and the simulated gross DMS_{aq} production after 500 simulations for symbiotic anemones. Boxes show first and third quartile ranges, thick lines indicate median values, and error bars the range of data. Please note the logarithmic scale along the y-axis. **(c)** Distribution for net/gross production ratio after 500 simulations. **(d)** Distribution for coral-driven daily net DMS_g flux into the atmosphere normalised by coral surface area after 500 simulations. LOQ, limit of quantification; DMS_{aq}, aqueous dimethyl sulfide; DMS_g, gaseous dimethyl sulfide.

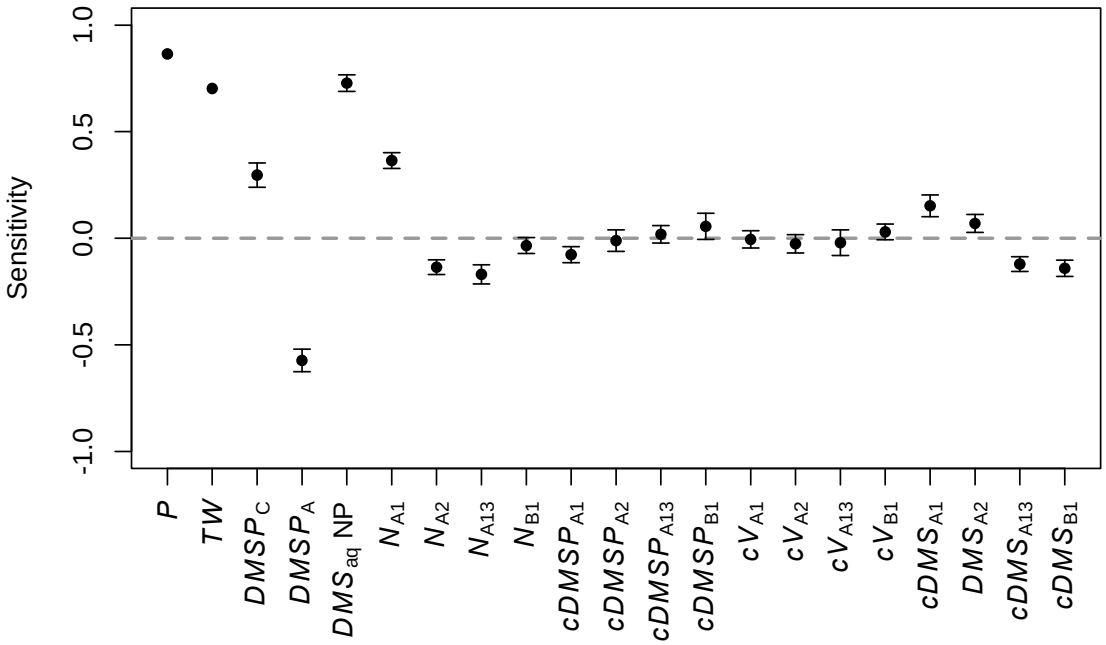

Figure 3: Sensitivity of the variables fed into the simulation framework. Values close to 0 have less influence on the simulation than those departing from 0. Error bars show standard error. Where error bars are invisible they are smaller than the symbol size. DMS_{aq} and DMS_g, aqueous and gaseous dimethyl sulfide; P, percentage of DMS_{aq} escaping into the atmosphere; TW, coral tissue weight normalised by coral surface area; $DMSP_C$, dimethylsulfoniopropionate within corals; $DMSP_H$, dimethylsulfoniopropionate in ~~anemones~~holobionts; N, number of cells for *Symbiodinium* clades A1, A2, A13, and B1; cDMSP, cellular DMSP for *Symbiodinium* clades A1, A2, A13, and B1; cV; cell volume for *Symbiodinium* clades A1, A2, A13, and B1; cDMS, cellular DMS_g production rate for *Symbiodinium* clades A1, A2, A13, and B1.

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

Anonymous Referee #1

Received and published: 15 March 2017

Review of: "Quantification of DMS production in the sea anemone Aiptasia sp. to simulate the sea-to-air flux from coral reefs" by F. Franchini and Michael Steinke.

General: The authors use a sea anemone as a model organism to study DMS flux from coral reefs. There are major deficiencies in this approach and I cannot recommend this manuscript for publication. If anything the results are very preliminary and a gross approximation of DMS flux from coral reefs. This is only superficially acknowledged.

Author response (AR) 1: *We thank referee 1 for their comments and can provide reassurance that our results are based on a suitable experimental design where none of the measured data are of preliminary nature. The simulation is based on a series of assumptions that we have clarified in the Results and Discussion section of the revised version. Since information on DMS cycling is severely limited for tropical reefs, we used our model simulation to estimate the flux of DMS from corals and the outcome of our simulation is in excellent agreement with the very few data from previous studies that quantified DMS flux from coral directly (e.g. Fischer and Jones 2012) and with calculated fluxes based on continuous atmospheric DMS measurements at Heron Island (Swan et al., 2017). We added information on the study by Swan et al. (2017) and highlighted the limitations of our study in the revised section 3.3.*

Using artificial seawater and cold shock to 4oC to compare bleached and unbleached samples is not realistic. Generally only a 2oC shock above or below ambient seawater temperatures should be used to stress a coral and would be comparable to studies by Fischer and Jones (2012).

AR2: *We did not use acute cold shock in any of our experiments. We merely used a widely accepted cold-chock protocol for anemones to bleach* Aiptasia *and reduce the number of endosymbionts to compare the production of DMS between symbiotic and bleached individuals. After the cold shock, there was a period of 3 months where the bleached and non-bleached* Aiptasia *were acclimated to our experimental conditions.*

No measurements seem to be made on the actual Symbiodinium concentrations in samples and results are expressed per gram.

AR3: *Our model simulation does not require data on* Symbiodinium *concentrations but uses measurements of the holobiont DMSP concentration ($DMSP_H$; a proxy for* Symbiodinium *concentration in the anemone holobiont) and net DMS production rate (net DMS_{aq}). Other required information was taken from the literature (see Table 1). Following the conventions in*

previously published studies (Van Alstyne et al*. 2009; Yancey* et al*. 2010), we expressed DMSP data in units of µmol g^{-1} DW (Table 3).*

Conversion to surface areas should be shown in a table and compared with other available data so that good comparisons can be made.

***AR4:** All key data on fluxes normalised to coral surface area and sea surface area in our dataset are clearly presented in the text and compared with data in the literature (Fisher and Jones, 2012; Swan et al., 2017). We also discuss information on the global DMS flux estimates (Lana et al., 2012) and from measurements in the North Atlantic and high latitudes (Holligan et al., 1993; Levasseur et al., 1994). We do not feel that the manuscript would benefit from including a table with this information.*

The authors should discuss in length two other important papers that have made good measurements and assessments of DMS flux from coral reefs. These are:

Hopkins, F.E., Bell, T.G., Yang, M., Suggett, D.J. and Steinke, M. (2016) Air exposure of coral is a significant source of dimethylsulphide (DMS) to the atmosphere. Scientific Reports, 6:36031,doi:1038/srep36031.

Swan, H.B., Jones, G.B., Deschaseaux, E.S.M and Eyre, B.D. (2017) Coral reef origins of atmospheric dimethylsulfide at Heron Island, southern Great Barrier Reef, Australia. Biogeosciences, 14, 1-11. Doi 10.5194/bg-14-1-2017

DMS flux can be estimated by both atmospheric and seawater measurements of DMS and the two papers above have shown that corals emit DMS directly to the atmosphere. The submitted paper makes no mention of this in their article.

***AR5:** Information from the publication by Hopkins et al. (2016) was included in our initial submission of the manuscript. We provide more information on their findings in the revised Results and Discussion section to highlight the importance of short 'bursts' of DMS during periods of aerial exposure. The paper by Swan et al. (2017) was not included in our initial submission (it was published one month before our initial submission). We apologise for this oversight and have included a discussion of their relevant key findings in the revised version.*

Their measurements from a sea anemone are therefore a gross underestimate. This is not helped by arbitrarily estimating the number of clade types in the anemone and not measuring them in the anemone. Different clades of zooxanthellae contain different levels of DMSP and produce variable levels of DMS. What data is available and published on DMS and DMSP production from coral reefs and discrete corals (e.g. Acropora-the most abundant coral in the Indo-Pacific) is not used or quoted (see Jones et al. (2007); Jones and King (2015).

***AR6:** Results from our flux simulation are in excellent agreement with the very few published datasets that empirically quantified fluxes from coral based on water and air measurements (see Results and Discussion). Our calculations are based on few parameters including the net DMS production rate that is also used to infer gross DMS production rate. This approach suggests that the potential for DMS production in coral reefs is very high but much of the climatically*

important flux of DMS to the atmosphere, where it exerts its cooling activity, is driven by the consumption of DMS through microbial processes. Hence, we use our research to stress the requirement for a better understanding of these consumption processes if we were to improve our forecasting ability of DMS fluxes under ongoing/future environmental change.

We now include reference to the more recent publication by Jones and King (2015). Data from the chamber experiments presented in the paper by Jones et al. (2007) would have been very useful for inclusion in our manuscript. However, as far as we are aware, these experiments were conducted without biological replication, hence lack statistical analysis of the results (e.g. no error presented in their Figure 7) and are presented with confusing (erroneous?) units. Taken together, this precluded us using data from their study as an authoritative reference to enhance our discussion.

END OF RESPONSE TO REFEREE 1

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

Anonymous Referee #2

Received and published: 30 March 2017

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 production 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 DMS_{aq} 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 DMS_{aq} production. (iii) Simulation of coral gross DMS_{aq} 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 ($DMSP_H$) 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 ($DMSP_C$) found in the literature (i.e. Yancey at al. 2010, Tab. 1).*

Why you chose for NÂˇnA1, 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 $DMSP_H$ measured in this study and the data in Steinke et al. (2011). The same equation but with $DMSP_C$ instead of $DMSP_{HA}$ was used to simulate the gross DMS production rate in corals. This was multiplied by TW to convert 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 DMS_g 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 DMS_{aq} 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 conditions 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