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

Filippo Franchini and Michael Steinke
Coral Reef Research Unit, School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, UK

Correspondence: Michael Steinke (msteinke@essex.ac.uk)

Received: 28 February 2017 – Discussion started: 3 March 2017
Revised: 29 June 2017 – Accepted: 24 September 2017 – Published: 21 December 2017

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 fluxes of gaseous DMS showed that reefs may release 0.1 to 26.3 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 0.1 to 17.4, with the highest probability for 0.3 to 1.0 µmol m⁻² SSA d⁻¹) suggests that coral reefs emit gaseous DMS at lower rates than the average global oceanic DMS flux of 4.6 µmol m⁻² SSA d⁻¹ (19.6 Tg sulfur per year). The large difference between simulated gross and quantified net aqueous DMS production in corals suggests that the current and future potential for 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 in order 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 (dimethylsulfoniopropionate)-catabolite DMS (dimethyl sulfide) is a biogenic volatile organic compound (BVOC) that provides the dominant natural source of marine sulfur to the atmosphere with a release of 19.6 Tg S per year (Land et al., 2014). 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 cnidian 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 a 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 flux of gaseous DMS (net DMS$_g$ flux).

2 Methods

2.1 Anemone husbandry, bleaching and biomass estimation

The symbiotic tropical sea anemone *Aiptasia* cf. *pallida* was kept under standard growth conditions in glass aquaria filled with artificial seawater (AW; 32 g L$^{-1}$ Reef Salt; D-D H$_2$Ocean) inside an incubator (SANYO Versatile Environmental Test Chamber MLR-351) set to 26$^\circ$C and 12h (12h) light (dark) cycle at a light intensity of 80µmol 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*. AW 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 3 weeks, gently removed from their attachment site and transferred to individual 4.92 mL glass vials containing AW at 26$^\circ$C. After attachment of the anemones to the glass surface, the water was replaced with cold (4$^\circ$C) AW, the vials were closed and kept in the fridge for at least 4 h before replacing the AW medium and transferring the vials to 26$^\circ$C. After 1–2 days, anemones were microscopically checked for symbionts using a dissecting microscope and, in the 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 AW and 0.37 M 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 haphazardly 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 AW-filled control vials, before quantifying net DMS production and DMS concentration.

2.3 Quantification of DMSP$_H$ concentration

DMSP$_H$ (i.e. DMSP in anemone holobionts) was indirectly quantified after equimolar hydrolytic conversion to DMS in 3 mL of 0.5 M 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 a direct headspace injection of the 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 was 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\textsubscript{aq} production

To quantify the net production of DMS by the holobiont (net DMS\textsubscript{aq} production; the release of DMS into the aqueous medium over time), 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\textsubscript{aq} production was calculated as the difference in DMS concentration between control vials and vials with anemones after quantification of DMS the in-vial purging of the aqueous phase technique (Franchini and Steinke, 2017).

2.5 Simulating the coral-driven sea-to-air DMS\textsubscript{g} flux

The coral-driven sea-to-air flux of gaseous DMS (net DMS\textsubscript{g} flux) was estimated in four steps: (i) simulating the holobiont gross DMS\textsubscript{aq} production rate using quantified holobiont DMS production, (ii) calculating the ratio (R) between measured net and simulated gross DMS\textsubscript{aq} production, (iii) simulating coral gross DMS\textsubscript{aq} production rate, and (iv) converting coral gross DMS\textsubscript{aq} production to coral net DMS\textsubscript{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\textsubscript{aq} production rate defined as the total amount of DMS\textsubscript{aq} produced over time by Symbiodinium of clade \(i\) within the host. Data for cellular DMS production of free-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\textsubscript{aq} production rate (holobiont gross\textsubscript{aq} DMS\textsubscript{aq}) took the following form:

\[
\text{holobiont gross}_{aq} \text{DMS}_{aq} = \sum \left( \frac{\text{DMSP}_H \times N_i}{\text{DMSP}_i} \times cV_i \times c\text{DMS}_{aq,i} \right),
\]

where DMSP\textsubscript{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. \(\sum N_i\)). Note that \(N\) does not reflect the actual number of symbionts within anemones but was arbitrarily set to 100 in order to calculate the proportion of clade \(i\) among all clades within anemones (setting \(N\) to 10\(^3\) or 10\(^6\) did not change the final outcome of the simulation). This made it possible to generate symbiont communities of different relative compositions during our simulations. Values for cDMSP\textsubscript{i} (i.e. cellular DMSP concentration for Symbiodinium clade \(i\)), cDMS\textsubscript{aq,i} (i.e. cellular DMS\textsubscript{aq} production rate for Symbiodinium clade \(i\)), and cV\textsubscript{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\textsubscript{H} \(\times N_i\) reflected the contribution of clade \(i\) to the amount of DMSP within the holobiont. This was divided by cDMSP\textsubscript{i} to estimate the number of clade \(i\) cells per anemone biomass, which was then multiplied by cV\textsubscript{i} to obtain the volume occupied by clade \(i\) per anemone biomass. This biomass-normalised volume was subsequently multiplied by cDMS\textsubscript{aq,i} to estimate the gross DMS\textsubscript{aq} production rate per anemone biomass for clade \(i\). The sum across all four clades yielded the gross DMS\textsubscript{aq} production rate per anemone biomass.

The fraction of DMS\textsubscript{aq} released into the water by the anemones was calculated as the ratio (\(R\)) between the measured net DMS\textsubscript{aq} production and the simulated gross DMS\textsubscript{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\textsubscript{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\textsubscript{g} flux) normalised by coral surface area (CSA; \(\mu\text{mol m}^{-2} \text{d}^{-1}\)) took the following form:

\[
\text{netDMS}_{g} \text{flux} = \text{coral gross}_{aq} \text{DMS}_{aq} \times \text{TW} \times R \times P,
\]

where coral gross\textsubscript{aq} DMS\textsubscript{aq} is the simulated coral gross DMS\textsubscript{aq} production rate calculated as in Eq. (1) but replacing DMSP\textsubscript{H} with DMSP\textsubscript{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\textsubscript{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 exploit different coral types at this stage.

2.6 Data analysis

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\textsubscript{aq} production from light and dark treatments and for comparison between net and gross DMS\textsubscript{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 analyses 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 Eqs. (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, $DMS_{aq}$ 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 Eqs. (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 posi-
Symbiodinium clades of incubation. to a production rate of 3.6 pmol DMS in 3 mL over a 48 h limit of quantification at 1.2 ± DMS source of DMS (Fig. 2a). Bleached individuals showed net inhibition.

Aiptasia cells within microscopic observation revealed small clusters of symbiont generation. Whether aposymbiotic anemones produce DMSP as demonstrated for corals (Raina et al., 2013) requires further investigation. 

Table 2. Parameters for cellular DMSP concentration (cDMSP), cell volume (cV) and cellular net DMSaq production rate (cDMSaq) in four clades of Symbiodinium sp. (data from Steinke et al., 2011).

<table>
<thead>
<tr>
<th>Symbiodinium clade</th>
<th>cDMSP (fmol cell⁻¹)</th>
<th>cV (µm³ cell⁻¹)</th>
<th>cDMSaq (mmol L⁻¹ cV h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>98.0 ± 4.18</td>
<td>415 ± 9.5</td>
<td>0.32 ± 0.112</td>
</tr>
<tr>
<td>A2</td>
<td>126.8 ± 8.59</td>
<td>763 ± 24.2</td>
<td>0.06 ± 0.018</td>
</tr>
<tr>
<td>A13</td>
<td>85.6 ± 22.03</td>
<td>419 ± 34.5</td>
<td>0.10 ± 0.029</td>
</tr>
<tr>
<td>B1</td>
<td>39.3 ± 2.33</td>
<td>237 ± 19.7</td>
<td>0.04 ± 0.025</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Aiptasia species</th>
<th>DMSP (µmol g⁻¹ DW)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. pallida</td>
<td>22.7 ± 8.00 (2)</td>
<td>ND (3) Van Alstyne et al. (2009)</td>
</tr>
<tr>
<td>A. pachella</td>
<td>54.7 ± 15.20 (3)</td>
<td>NI Yancey et al. (2010)</td>
</tr>
<tr>
<td>A. cf. pallida</td>
<td>32.7 ± 6.00 (6)</td>
<td>0.6 ± 0.19 (6) This Study</td>
</tr>
</tbody>
</table>

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

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, and 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 DMSaq production in Aiptasia and demonstrated that the symbiont is the main source of DMS (Fig. 2a). Bleached individuals showed net DMSaq 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 DMSaq 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 48 h 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 DMSaq 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 O2 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 DMSaq 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 DMSaq production rate (cDMSaq) between free-living Symbiodinium cells and those living

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) Box plot showing the difference (P < 0.001) between the biomass-normalised (dry weight) observed net DMSaq production (n = 6) and the simulated gross DMSaq production after 500 simulations for symbiotic anemones. Boxes show first and third quartile ranges, thick lines indicate median values, and error bars show the range of data. Please note the logarithmic scale along the y axis. (c) Distribution of the net / gross production ratio after 500 simulations. (d) Distribution for coral-driven daily net DMSg flux into the atmosphere normalised by coral surface area after 500 simulations. LOQ, limit of quantification; DMSaq, aqueous dimethyl sulfide; DMSg, gaseous dimethyl sulfide.
iii. DMSP and DMS characteristics in Symboidinium 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 Fig. 3).

iv. The ratio between net and gross DMS\textsubscript{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\textsubscript{aq}. Although light conditions in the experiment conducted by Steinke et al. (2011) (350 µmol m\textsuperscript{-2} s\textsuperscript{-1}, 14 h (10 h) light (dark) cycle) were different from those adopted here, the evidence that net DMS\textsubscript{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.
The adopted simulation framework suggests that gross DMS production of \( \sim 1 \mu\text{mol g}^{-1} \) over 48 h is up to 7 times higher than the net production of \( \sim 0.15 \mu\text{mol g}^{-1} \) (\( P \leq 0.001 \)) (Fig. 2b). Additionally, the percentage of the gross production escaping into the water surrounding the anemones ranged from 3 to 120 %. A percentage of gross DMS production escaping into the atmosphere greater than 100 % occurs when the simulated net production exceeds the gross production. This is due to the random sampling of high net-production values (Table 1), and to the calculation of low gross production within the simulation framework. Low gross production arises when a particular combination of parameter values are inserted into Eq. (1). For example, a low symbiont population resulting in low DMSP\(_H\), combined with a population of low DMS-producing Symbiodinium clades with small cell volume (e.g., Symbiodinium clade B1) could result in this output from the simulation framework. The highest probabilities of 65 % were found for net / gross production ratios of 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 Fig. 3), which ranged from 0.1 to 26.3 \( \mu\text{mol m}^{-2} \text{d}^{-1} \) with 40 % probability for values between 0.5 and 1.5 \( \mu\text{mol m}^{-2} \text{CSA d}^{-1} \) (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 (Figs. 1, 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 this 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 larger 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 \( \mu\text{mol m}^{-2} \text{CSA d}^{-1} \) (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 unitless 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 17.4 \( \mu\text{mol m}^{-2} \text{SSA d}^{-1} \), with the highest probabilities for fluxes ranging from 0.3 to 1.0 \( \mu\text{mol m}^{-2} \text{SSA d}^{-1} \). 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 \( \mu\text{mol m}^{-2} \text{SSA d}^{-1} \) (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 \( \mu\text{mol m}^{-2} \text{SSA d}^{-1} \); Holligan et al., 1993) or at high latitudes (21.87 \( \mu\text{mol m}^{-2} \text{SSA d}^{-1} \); Levasseur et al., 1994). Furthermore, our estimated sea-to-air flux from coral reefs is also often lower than the global oceanic flux that is calculated at 4.6 \( \mu\text{mol m}^{-2} \text{SSA d}^{-1} \) (equivalent to 19.6 Tg \( \text{S yr}^{-1} \) in Land et al., 2014) and is 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. (3000–11 000 \( \mu\text{mol m}^{-2} \text{SSA d}^{-1} \)) 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.
F. Franchini and M. Steinke: Quantification of dimethyl sulfide (DMS) production

Data availability. The datasets supporting this article are publicly available at https://doi.pangaea.de/10.1594/PANGAEA.883652.

Author contributions. FF and MS conceived and designed the study, interpreted the data and wrote the paper. FF performed the experiments and collected and analysed the data. Both authors gave final approval for publication.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. The authors thank Sue Corbett, Tania Cresswell-Maynard and John Green for technical assistance. We are grateful to two anonymous reviewers for very constructive comments which greatly helped us to improve our paper. The article processing charges for this open-access publication were covered by the School of Biological Sciences, University of Essex.

References


Chalom, A. and Knecht Lopez, P. I.: PSE: Parameter space extrapolation with latin hypercubes, R package version 0.3.2, available at: https://cran.r-project.org/web/packages/pse (last access: June 2017), 2016.


Hopkins, F. E., Bell, T. G., Yang, M., Suggett, D. J., and Steinke, M.: Air exposure of coral is a significant source of

www.biogeosciences.net/14/5765/2017/
dimethylsulfide (DMS) to the atmosphere, Sci. Rep., 6, 36031, https://doi.org/10.1038/srep36031, 2016

Biogeosciences, 14, 5765–5774, 2017