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# 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 used gas chromatography to quantify net 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 of DMS flux into the atmosphere and demonstrate that net aqueous DMS production accounts for only 0.5–2.0% of gross aqueous DMS production, and that reefs may release up to 15 µmol DMS $m^{-2}$ coral surface area $d^{-1}$ into the atmosphere with 40% probability for rates between 0.5 and 1.5 µmol $m^{-2}$ $d^{-1}$. Conversion to a flux rate normalised to sea surface area (range 0.3–10 with highest probability for 0.3–1 µmol DMS $m^{-2}$ $d^{-1}$) suggests that coral reefs continuously emit DMS at lower rates than the average global oceanic DMS flux of 6.7 µmol $m^{-2}$ $d^{-1}$. The high gross DMS-production rates in corals suggest that it is important to assess the sensitivity of DMS-consumption pathways to environmental change before addressing 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 that provides the dominant natural source of 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 its 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

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(Broadbent and Jones, 2004) 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 could 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), it is possible that they are part of an antioxidant mechanism that leads to the production of DMSO in symbiotic cnidarians (Gardner et al., 2016).

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). Since information on the sea–to–air flux of DMS and other biogenic volatile organic compounds from tropical reefs is scarce (Exton et al., 2014), this study quantified for the first time net aqueous DMS production in *Aiptasia* sp. and uses this data and information on DMSP concentration to model anemone DMS gross production and coral–derived DMS flux to the atmosphere.

## 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 $H_2$Ocean) 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 $m^{-2}$ $s^{-1}$. ASW was changed weekly and the anemones were fed with freshly hatched brine shrimps (*Artemia salina*, reefphyto) every 2 weeks.

Symbiotic anemones were bleached following a cold-shock protocol (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 0.37 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).

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### 2.2 Quantification of DMSP concentration and DMS production

DMSP in individual anemones 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. 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 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).

To quantify net DMS-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 and net DMS production was calculated as the difference in DMS concentration between control and anemone vials after quantification of DMS using the in vial purging of aqueous phase technique.

### 2.3 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 (n=6) were haphazardously selected for four treatments: 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.

### 2.4 Simulating DMS flux and gross production

We simulated daily coral–driven sea–to–air flux of gaseous DMS (*net $DMS_g$ flux*) normalised by coral surface area (CSA; µmol $m^{-2}$ $d^{-1}$) as described in eq. 1:

$$net\ DMS_g\ flux = \underbrace{\left( \sum_{i=A1,A2,A13,B1} \frac{DMSP \cdot \frac{N_i}{N_{A1}+N_{A13}+N_{B1}+N_{A2}}}{cDMSP_i} \cdot CV_i \cdot cDMS_i \right) \cdot TW}_{\text{Coral gross } DMS_{aq} \text{ production}} \cdot \frac{net\ DMS_{aq}}{gross\ DMS_{aq}} \cdot P \quad (1)$$

where the parameters *DMSP*, $N_{A1}$, $N_{A13}$, $N_{A2}$, $N_{B1}$, *net $DMS_{aq}$*, *TW, and P* were variables determined in this study or taken from the literature (Table 1). The values for *cDMSP* (DMSP amount per *Symbiodinium* cell), *cDMS* (aqueous DMS-production rate per *Symbiodinium* cell volume), and *CV* (cell volume) specific for the free living *Symbiodinium* clades *i* (A1, A13, A2, B1) as in Steinke et al. (2011) were kept constant. The equation for *gross $DMS_{aq}$* (anemone gross DMS production) was the same as the coral gross DMS-production equation, but *DMSP* (biomass-normalised DMSP within corals, see Table 1) was replaced with the biomass-normalised DMSP within anemones ($DMSP_A$).



This modeling approach assumes that: i) endosymbionts are the main DMSP/DMS producers within the anemone holobiont (Van Alstyne et al., 2009), ii) there is no difference in DMSP content (*cDMSP*) and $DMS_{aq}$ production rate (*cDMS*) between free-living *Symbiodinium* cells and those living symbiotically, and iii) that DMSP and DMS characteristics in clades A1, A2, A13, and B1 are representative of other symbiont types. Moreover, 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 DMS production was independent of light intensity (see Sect. 3.2) justifies our approach.

### 2.5 Data analysis

Data extrapolation from graphical representations of previously published studies was performed through freely available digitising software (Plot Digitizer, version 2.6.6). Graphical representations as well as statistical and sensitivity analyses were performed using the free R software environment for statistical computing and graphics (R Project for Statistical Computing, version 3.1.1). All data 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. Modelling 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 developing the model function and defining all constants and variables (Table 1) within the R programming environment, we randomly generated 500 values through a Monte-Carlo simulation. Subsequently, these values were used to generate probability distribution plots. Finally, partial rank correlation coefficients were extrapolated in order to assess the response (sensitivity) of our model 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 or bleached anemones are in general agreement with the earlier findings (Table 2) (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-production in *Aiptasia* and demonstrate that the symbiont is the main source of DMS (Fig. 1a). Bleached individuals showed DMS-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.

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### 3.2 Effect of light on DMS production

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. 1a). 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-production rates. 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. 2). 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, based on our results, net production in dark was the same as in light treatments (Fig. 1a).

### 3.3 From anemones to corals: Net vs. gross DMS production and net DMS flux

Using our measurements of DMSP concentration and DMS production in anemones to extrapolate to coral reef environments has its limitations but it provides an initial route to assess overall DMS production in tropical coastal ecosystems where DMS and DMSP data coverage is relatively poor. The adopted model suggests that gross DMS production of ~15 µmol $g^{-1}$ over 48 h is up to 100 times higher than the net production of ~0.15 µmol $g^{-1}$ ($P < 0.001$) (Fig. 1b). Additionally, the percentage of the gross production escaping into the water surrounding the anemones ranged from 0 to 10% with 70% probability for 0.5 to 2% (Fig. 1c). It is proposed that the remaining ≥98% reacts with ROS or is consumed in other ways by the host and surface-associated microorganisms (Fig. 2). 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, although symbiont community composition plays a role in shaping gross DMS production, it does not have a major influence on coral–driven sea–to–air DMS fluxes (Fig. 1d), which ranged from 0 to 15 µmol $m^{-2}$ $d^{-1}$ with 40% probability between 0.5 and 1.5 µmol $m^{-2}$ $d^{-1}$ when normalised to CSA (Fig. 1c). This is because even if corals accommodate high DMS producing endosymbionts leading to high gross DMS-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 production that is the result of several DMS-consumption processes (Fig. 1d; Fig. 2).

The range of sea–to–air DMS fluxes obtained from our model 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 fluxes normalised to coral surface area (CSA) into fluxes normalised to sea surface area (SSA) requires information on coral cover and reef rugosity.

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 10 µmol DMS $m^{-2}$ SSA $d^{-1}$ with highest probabilities for fluxes ranging from 0.3 to 1 µmol DMS $m^{-2}$ SSA $d^{-1}$. 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 $m^{-2}$ $d^{-1}$; (Holligan et al., 1993)) or at high latitudes (21.87 µmol $m^{-2}$ $d^{-1}$; 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 $m^{-2}$ $d^{-1}$ (equivalent to 28.1 Tg S $y^{-1}$ in Lana et al. (2011)). While these fluxes refer to fully submersed reefs, it is important to note that tidally-exposed corals such as the strongly DMS producing *Acropora* spp. may provide significant 'bursts' of DMS to the atmosphere during and after periods of aerial exposure (Hopkins et al., 2016).

Our study suggests that net DMS-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.

## 7 Acknowledgments

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**Table 1:** Parameters used for the modeling approach. DMS, dimethylsulfide; DMSP, dimethylsulfoniopropionate; DW, dry weight; N/A, not applicable.

| Parameter | Description | Unit | Range | | Source |
|---|---|---|---|---|---|
| | | | min | max | |
| $DMSP_A$ | Biomass-normalised DMSP within anemones | 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 | Biomass-normalised DMSP within corals | µmol $g^{-1}$ DW | 52.36 | 84.00 | Yancey et al. (2010) |
| $N_{A1, A13, B1, A2}$ | 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) |

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**Table 2:** 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.

| | DMSP (μmol $g^{-1}$ DW) | | |
|---|---|---|---|
| *Aiptasia* Species | Symbiotic | Bleached | Source |
| *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 |

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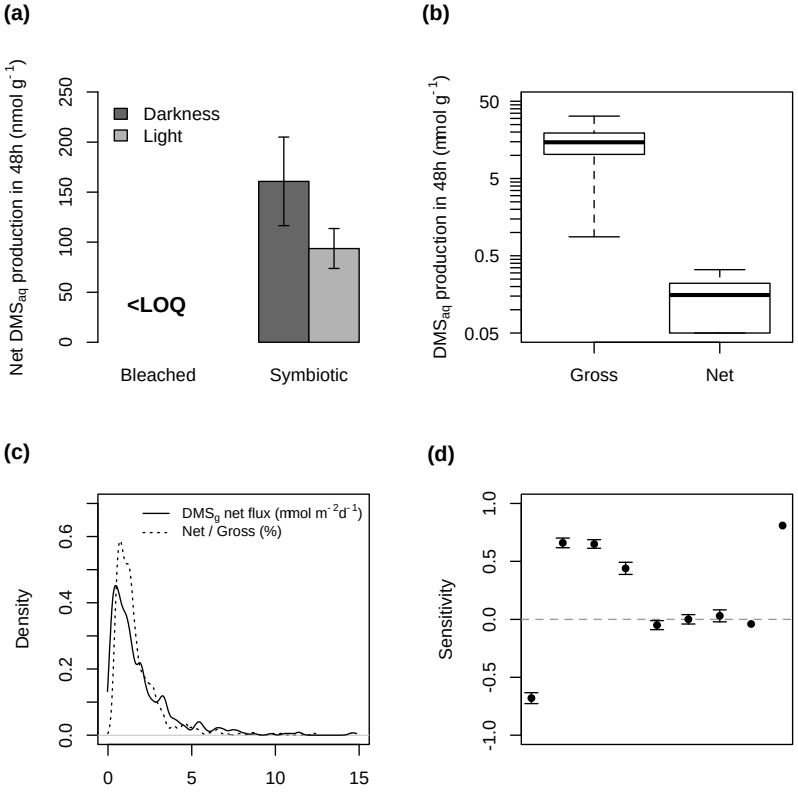


**Figure 1: (a)** Biomass-normalised (dry weight) net DMS production (mean ± se) for symbiotic and bleached anemones during light and dark treatments (n=6). **(b)** Boxplot showing the significant difference ($P < 0.001$) between the biomass-normalised (dry weight) observed $DMS_{aq}$ net production (n=6) and the modelled $DMS_{aq}$ gross production (n=500) 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)** Probability distribution of net / gross production ratio and modelled coral-driven daily $DMS_g$ net flux into the atmosphere normalised by coral surface area (n=500). **(d)** Sensitivity of the variables used in the modelling approach. Error bars show standard error. Where error bars are invisible they are smaller than the symbol size. LOQ, limit of quantification; $DMS_{aq}$ and $DMS_g$, aqueous and gaseous dimethyl sulfide; net $DMS_{aq}$, $DMS_{aq}$ net production; $DMSP_A$, dimethylsulfoniopropionate in anemones; TW, coral tissue weight normalised by coral surface area; DMSP, dimethylsulfoniopropionate within corals; N, number of *Symbiodinium* cells for clades A1, A13, B1, and A2; P, percentage of aqueous DMS escaping into the atmosphere.

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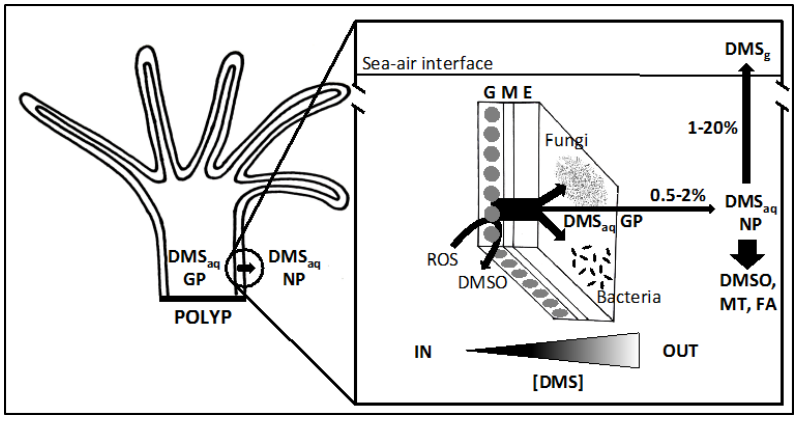


**Figure 2:** Magnification of a coral polyp and its cell layers with particular emphasis on the pathway of DMS from its production by endosymbionts (grey circles) to its release to the atmosphere. Net production (NP) ranges from only 0.5–2% of gross DMS production (GP). The remainder is available to scavenge reactive oxygen species (ROS) and/or is consumed by surface-associated microbes. Once dissolved, 1–20% of the DMS net production escapes to 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.