Red coralline algae as a source of marine biogenic dimethylsulphoniopropionate

Nicholas A. Kamenos1,2,*, Sarah C. Strong2, Damodar M. Shenoy3, Samuel T. Wilson3, Angela D. Hatton3, P. Geoffrey Moore4

1Department of Geographical and Earth Sciences, and 2Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK
3Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Oban, Argyll PA37 1QA, UK
4University Marine Biological Station Millport, Millport KA28 0EG, UK

ABSTRACT: The biogenic gas dimethylsulphide (DMS), derived from dimethylsulphoniopropionate (DMSP), plays an important role in the Earth’s albedo, and thus climate regulation, through the formation of aerosols and cloud condensation nuclei. It is estimated that biogenic sources of DMS contribute 42% (mean) of the atmospheric sulphur burden and, significantly, >90% of that contribution is derived from marine sources. Phytoplankton, macroalgae and corals are thought to be the main producers of marine biogenic DMSP. Red coralline algae (known as maerl or rhodoliths) cover extensive areas of seabed, yet despite their widespread global distribution, maerl-forming coralline algae have received little or no attention regarding their DMSP productivity. In the present study we report for the first time the occurrence of DMSP in 2 species of maerl. DMSP concentrations were found to average 1914 nmol g⁻¹ for soft tissue and estimated to be 637 µmol m⁻² for maerl beds. In incubation experiments, maerl led to a dissolved DMSP (DMSPd) increase at a rate of 57.4 to 767.6 nmol m⁻² d⁻¹ in surrounding seawater, indicating that maerl contributes to DMSPd concentrations in the adjacent water column. Results show that maerl beds are a previously undiscovered source of DMSP in the marine environment. Further study is warranted to assess the significance of maerl as a source of DMSP and the role coralline algae may play in the biogenic sulphur cycle.

KEY WORDS: Maerl · Rhodolith · Coralline algae · Dimethylsulphoniopropionate · DMSP · Dimethylsulphide · DMS · Climate regulation

INTRODUCTION

In the marine environment, interest in dimethylsulphonopropionate (DMSP) focuses primarily on the role this compound plays in the production of the climate gas dimethylsulphide (DMS). The ocean–atmosphere flux of DMS accounts for one-quarter of global sulphur emissions, and DMSP is the principal natural source of atmospheric sulphur from the marine environment and a key component of the biogeochemical sulphur cycle (Liss et al. 1997). Furthermore, a proposed phytoplankton–cloud–climate feedback loop, whereby DMS-derived cloud condensation nuclei affect the Earth’s radiation balance through increased albedo (Charlson et al. 1987), has stimulated considerable research into this gas and its precursors.

Due to their extensive geographical distribution, phytoplankton are considered to be the main source of oceanic DMSP. However, a number of benthic marine organisms have also been shown to produce or contain DMSP. Early work identified macroalgae as an important source, with DMSP concentrations between 0 and 85 µmol g⁻¹ in live seaweed (Reed 1983, Karsten et al. 1994). Other benthic sources of DMSP, include tropical corals (Hill et al. 1995, Broadbent et al. 2002), coral mucus and mucus ropes (Broadbent & Jones 2004) and cord-grass (Dacey et al. 1994). Corals are thought to be among the highest benthic producers of DMSP due to
their endosymbiotic zooxanthellae (Broadbent et al. 2002), a finding that has fuelled research into their potential to mitigate climate change. For example, coral reefs have been shown to increase daytime atmospheric DMS concentrations after low tides (Jones & Trevena 2005).

Maerl (Fig. 1) is a non-geniculate, subtidal, red coralline algae. These algae are also known as rhodoliths, but are herein referred to as maerl—a term that encompasses several taxa (Rhodophyta: Corallinales) (Giraud & Cabioch 1976). Maerl is found in areas characterised by strong water movement (tidal and/or wave action) in the photic zone (Grall & Hall-Spencer 2003) and is slow-growing, fragile and easily damaged (Hall-Spencer & Moore 2000). Unlike most biogenic carbonates, which suffer restricted distribution (e.g. corals), maerl is widely distributed from polar (Schwarz et al. 2005) to tropical (Littler et al. 1991) shallow seas in extensive shallow-water beds such as those that extend from 2° N to 25° S on the Brazilian shelf (Milliman 1977). The structural heterogeneity and abundance of maerl lead to a primary production (area-normalised) which exceeds that of frondose macroalgae (Littler et al. 1991). While fleshy red algae are known to contain DMSP (e.g. Polysiphonia sp., 150 nmol g⁻¹ fresh wt; Karsten et al. 1994), in non maerl-forming red coralline algae (e.g. Corallina sp.) only trace DMSP concentrations have been detected (Reed 1983). Despite its global distribution, maerl has received little or no attention regarding its DMSP content and productivity. Here, we present the first measurements of DMSP in maerl and estimations of DMSP concentrations occurring within natural maerl beds.

**MATERIALS AND METHODS**

**Sample collection.** *Lithothamnion glaciale* and *Phymatolithon calcareum* maerl species were collected using SCUBA from Loch Sween (56° 02’ N, 05° 36’ W; depth: 4 m chart datum [CD]) and Brodick Bay, Isle of Arran (55° 32.77’ N, 05° 05.41’ W; depth: 12 m CD), Scotland. Samples were either immediately prepared for analysis as described below or stored in small seawater tanks under ambient conditions for longer-term experiments on dissolved DMSP (DMSPₐ) production.

**Calculation of live maerl mass.** Mass of live maerl m⁻² was calculated by placing 1 (*Lithothamnion glaciale*) or 3 (*Phymatolithon calcareum*) layers (as they occurred in situ) of maerl thalli into plastic aquaria with a bottom area of 1 m² (n = 30 aquaria). Thalli were subsequently weighed (whole mass). Percentage mass of soft tissues (where DMSP is produced) on the maerl high Mg-calcite skeleton was calculated by removal of the soft tissue using 10 M NaOH treatment and re-weighing the damp carbonate skeleton. This was conducted separately for thalli tips and the remaining ‘skeleton’ of each species (n = 17 per species).

**DMSP quantification.** Approximately 2 g of thallus tips (*Lithothamnion glaciale*) or whole thalli (*Phymatolithon calcareum*) were gently cleaned using a soft brush to remove attached sediment and washed in sterile seawater. Samples were placed in 20 ml chromatography vials containing a known volume of Milli-Q water. Four ml of 10 M NaOH were added and the vial topped-up with Milli-Q water and sealed with polytetrafluoroethylene (PTFE) septum crimp lids, leaving no headspace. The vials were then incubated for 24 h in the dark at room temperature before being analysed. The addition of NaOH results in a 1:1 conversion of DMSP to DMS. This DMS was pre-concentrated using a purge and cryogenic trap technique (Turner et al. 1990), and quantified using an analytical system consisting of a Varian 3400 gas chromatograph fitted with a pulse flame photometric detector and a Chromosil 330 column. The gas chromatograph was operated using a temperature program (40 to 85°C), and the DMS retention time on the column was approximately 3.4 min. The detector output was monitored on a Hewlett-Packard 3390A reporting integrator. Concentrations were calculated from a DMSP standard (Research Plus) calibration curve and converted to DMSP g⁻¹ of thalli. Detection limits for DMSP, DMSPₐ (see next section) and DMS were 5 nmol S l⁻¹ seawater/NaOH with an analytical precision of within 3%.

**DMSPₐ quantification.** In addition to analysing DMSP concentrations in maerl, an experiment was performed to assess whether maerl thalli also contributed to the dissolved fraction of DMSP in the water.
column. *Lithothamnion glaciale* thalli (36.5 to 49.7 g) were used from the same source as above. Thalli were placed in 360 to 490 ml of sterile seawater (volume dependent on the mass of maerl used), which had been pre-analysed for DMS and DMSP<sub>d</sub> to ensure any background concentrations could be accounted for. The containers were covered with Parafilm® and incubated at 12°C on a 12 h light:12 h dark regime (irradiance: 5.4 µE m<sup>–2</sup> s<sup>–1</sup>) for 94 d. At 0, 1, 51, 75 and 94 d, seawater in which thalli had been incubated was analysed for dissolved DMS (DMS<sub>d</sub>) and DMSP<sub>d</sub> using the analytical system described above. Samples were collected and filtered gently through AP Millipore depth filters to ensure no particulate material remained. Purged samples were then incubated in the presence of 10 M NaOH for 24 h in PTFE-lined crimp top vials, with no headspace. Again, any DMSP was quantified following its conversion to DMS using the analytical system described above.

**Statistical analyses.** All statistical analyses were performed using R v. 2.2.0 statistical software. Data were transformed to meet parametric test assumptions. One-way ANOVAs were used throughout.

### RESULTS

The area-normalised mass of *Lithothamnion glaciale* and *Phymatolithon calcareum* was determined to be (mean ± SD) 12.20 ± 0.56 and 3.95 ± 0.29 kg m<sup>–2</sup>, respectively. Only 5.37 ± 2.79% (*L. glaciale*) and 4.53 ± 4.45% (*P. calcareum*) of the maerl mass was composed of live, DMSP-producing cells, with the remaining ~95% of the mass attributable to the calcitic maerl skeleton. The concentration of DMSP in both whole mass (nmol g<sup>–1</sup>) \(F_{2,29} = 20.10, p = 0.0001\), Table 1) and in soft tissues (nmol g<sup>–1</sup>) \(F_{2,29} = 25.87, p = 0.0001\), Table 1) was significantly higher in *P. calcareum* than in *L. glaciale*.

Mean DMSP g<sup>–1</sup> concentrations for whole maerl thalli of *Lithothamnion glaciale* and *Phymatolithon calcareum* (Table 1) were converted to mean DMSP m<sup>–2</sup>, using the mass of maerl in 1 m<sup>2</sup> of seabed (Table 2). The area-normalised DMSP content (standing stock) was estimated to be 617.1 and 678.3 µmol m<sup>–2</sup> for *L. glaciale* and *P. calcareum*, respectively, with no significant difference observed between the species \(F_{2,29} = 0.93, p = 0.406, \) Table 2).

In incubation experiments, DMSP<sub>d</sub> was detected once epithelial sloughing (natural loss of the outer epithelium) started to occur (Fig. 2, between Days 1 and 51). As sloughing continued, DMSP<sub>d</sub> concentrations increased (Fig. 2). DMSP<sub>d</sub> concentrations were determined for individual thalli and converted to DMSP<sub>d</sub> nmol m<sup>–2</sup> using the mass of maerl m<sup>–2</sup>. We observed DMSP<sub>d</sub> to increase at a rate of between 57.4 and 767.6 nmol m<sup>–2</sup> d<sup>–1</sup> (Fig. 2). It should be noted that DMS was not detected at any point during the incubation experiments.

**DISCUSSION**

Maerl may be an important source of DMS, with concentrations of between 0.28 and 6.25 µmol DMSP g<sup>–1</sup>
soft tissue. Our data show that DMSP concentrations observed in *Phymatolithon calcareum* were greater than those in *Lithothamnion glaciale*. Both maerl species had substantially greater DMSP concentrations than those expected for other calcified Rhodophyta and non-calcified Phaeophyta, and within the range observed for both un-calcified Rhodophyta (excluding the genus *Polysiphonia*) and Chlorophyta (Table 1). It is of value to note that salt marsh cord-grass *Spartina alterniflora* has also been observed to contain high DMSP concentrations (80 to 280 µmol g–1 dry wt [fresh wt not available]) (Dacey et al. 1994).

*Phymatolithon calcareum* and *Lithothamnion glaciale* have similar mean growth rates (Blake & Maggs 2003, Kamenos et al. 2008); thus, it is unlikely that growth rates are responsible for observed differences in algal DMSP content (Table 1). Similarly, despite faster growth in the geniculated red coralline algae *Corallina officinalis* (~17 mm yr–1; Blake & Maggs 2003), only trace concentrations of DMSP have been detected (Reed 1983).

Whilst benthic sources of DMSP are easier to identify due to their static nature, phytoplanktonic organisms, including coccolithophores and dinoflagellates (e.g. *Prorocentrum* sp.; Keller et al. 1989), and the blooms they produce, are generally considered to be among the highest biogenic DMSP producers (Andreae 1990). Direct comparisons between phytoplanktonic and maerl DMSP contributions are difficult to make. However, in comparison with other benthic DMSP sources, we observed maerl beds to contain similar or slightly lower concentrations of DMSP m–2 than spatially averaged coral reefs (adjusted for ~50% typical coral coverage; Broadbent et al. 2002), similar DMSP content to intertidal and subtidal marine sediments, and around 4000% more DMSP m–2 than tropical benthic algal stands (Table 2). The high DMSP standing stocks we observed indicate that maerl may be amongst the largest macroalgal DMSP producers (Table 1).

Maerl thalli regularly slough-off epithelial material to prevent fouling (Giraudo & Cabioch 1976), which is likely to be the mechanism for the release of intracellular DMSP (in the sloughed epithelial cells) into the surrounding seawater (DMSPd). Consistent with evaluations of DMSP in macroalgae (White 1982, Dacey et al. 1994, Broadbent et al. 2002), we observed DMSPd concentrations released by *Lithothamnion glaciale* to also be highly variable between individual maerl thalli (Fig. 2). However, the release rates observed in the present study may indicate a continuous flux of DMSP to the water column from maerl beds in the field, as individual thalli within a maerl bed will slough at different times, providing a constant source of DMSPd to the water column. With the latter in mind, it has been estimated that maerl thalli may provide DMSPd at a mean rate of between 57.4 and 767.6 nmol m–2 d–1, although actual DMSPd concentrations around maerl beds have yet to be determined. These values are likely to be the minimal DMSP release estimates, as DMSPd will have been broken down by microbial activity before it could be measured at our 19 to 51 d sampling intervals. This could be particularly relevant between Days 1 and 51 when the initial disturbance of maerl and bacteria on placement into experimental chambers may have caused bacterial-induced DMSP–DMS dynamics. If 57.4 nmol DMSPd m–2 d–1 is taken as a conservative estimate of DMSPd generated by maerl, once combined with the widespread global distribution of maerl beds, it is possible that maerl contributes substantial DMSPd loads to the adjacent water column. Comparison of DMSPd produced by maerl (Fig. 2) with global average (16.9 nmol dm–3) and recently revised maximum bulk water (2.8 nmol dm–3) DMSPd concentrations (Kiene & Slezak 2006) supports our suggestion.

Coral mucus ropes produced during the release of zooxanthellae have been observed to contain DMSP concentrations of up to 54.38 µM (Broadbent & Jones 2004); however, comparisons with maerl are difficult to draw as it appears that a component of DMSP in the coral mucus is due to the post-production adherence of bacteria and plankton to the mucus (Broadbent & Jones 2004). It is possible that sloughed-off maerl epithelial material may perform a similar concentration function to the coral mucus.

It has been suggested that DMSP from coral mucus can be degraded to DMS by bacteria (Broadbent & Jones 2004). Although the seawater used for incubation in the present study was sterile, the maerl thalli...
were not, and so it is likely that the natural bacterial population associated with maerl would have been present in the incubation experiments. However, no DMS was detected; it is possible that DMSP$_d$ was converted to DMS (by either DMSP lyase activity within the maerl or microbial activity) and was then broken down by microbial activity before it could be measured on Day 51. Although cord-grass has also been observed to contain high DMSP concentrations, there is only a 0.4% turnover of that DMSP to DMS (Dacey et al. 1987)—this too, may occur in maerl. Additionally, it is possible that the DMS concentrations present were below analytical detection limits and thus a combination of the above factors may contribute to the apparent absence of any DMS in the incubation seawater.

Previous algal studies suggest that increased light, changes in salinity and temperature, and nitrate limitation may affect intracellular levels of DMSP (Stefels 2000). In other algal species, DMSP is produced as a compatible solute to maintain osmotic balance and stabilize biochemical pathways within the seawater environment (Vairavamurthy et al. 1985, Dacey & Wakeham 1986), in response to oxidative stress such as bleached zooxanthellae in corals (Sunda et al. 2002), or as a cryoprotectant (Malin & Kirst 1997). This may also be the case in maerl, although further investigation is required for confirmation. Thus, it is possible that representatives of tropical or polar maerl genera may produce higher concentrations of DMSP.

Anthropogenic impacts on maerl are detrimental (Wilson et al. 2004). It is important that efforts are made to determine any impacts of ocean acidification (Kuffner et al. 2007) and global change on DMSP production by maerl and the role such production may play in the biogenic sulphur cycle and climate feedback hypotheses.

Empirical (Sciare et al. 2000) and modelling (Gunson et al. 2006) studies provide evidence that biogenic release of DMS may participate in processes of climate regulation, and coral reefs have been shown to influence atmospheric DMS concentrations (Jones & Trevena 2005). Maerl can be a major component of coral reefs (Broadbent et al. 2002), and so may also contribute to the area-normalised DMSP content of tropical reefs. While we have observed maerl beds to contain high area-normalised DMSP concentrations, our finding that little DMS accumulated during incubations with isolated thalli may suggest that maerl is not a major DMS source. Further in situ investigations are required to determine this. Maerl beds do appear to be an important DMSP$_d$ source, especially when considering the extensive maerl beds that occur globally. Clearly, their potential contribution to marine DMSP biogeochemical cycles warrants further investigation.

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