Increasing \( pCO_2 \) correlates with low concentrations of intracellular dimethylsulfoniopropionate in the sea anemone *Anemone viridis*

Esther M. Borell\(^1\,*\), Michael Steinke\(^2\,*\), Rael Horwitz\(^1,3\) & Maoz Fine\(^1,3\)

\(^1\)The Interuniversity Institute for Marine Sciences, Eilat 88000, Israel
\(^2\)Coral Reef Research Unit, School of Biological Sciences, University of Essex, Colchester CO4 3SQ, U.K
\(^3\)The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel

**Keywords**
- Chlorophyll, \( CO_2 \) vent, DMSP, primary research article, protein, superoxide dismutase, zooxanthellae.

**Correspondence**
Esther M. Borell, The Interuniversity Institute for Marine Sciences, Eilat 88000, Israel
Tel: +972 (0)8 6360 170; Fax: +972 (0)8 6374 329; E-mail: estherborell@yahoo.co.uk

**Funding Information**
This study was funded by ASSEMBLE Marine, the EU MedSeA project, and an Israel Science Foundation grant to M.F. E.M.B. was funded by the Minerva fellowship program.

Received: 10 June 2013; Revised: 3 December 2013; Accepted: 6 December 2013

Ecology and Evolution 2014; 4(4): 441–449
doi: 10.1002/ece3.946

*These authors contributed equally to the preparation of this manuscript.

**Abstract**

Marine anthozoans maintain a mutualistic symbiosis with dinoflagellates that are prolific producers of the algal secondary metabolite dimethylsulfoniopropionate (DMSP), the precursor of the climate-cooling trace gas dimethyl sulfide (DMS). Surprisingly, little is known about the physiological role of DMSP in anthozoans and the environmental factors that regulate its production. Here, we assessed the potential functional role of DMSP as an antioxidant and determined how future increases in seawater \( pCO_2 \) may affect DMSP concentrations in the anemone *Anemone viridis* along a natural \( pCO_2 \) gradient at the island of Vulcano, Italy. There was no significant difference in zooxanthellae genotype and characteristics (density of zooxanthellae, and chlorophyll a) as well as protein concentrations between anemones from three stations along the gradient, V1 (33.23 \( \mu \text{atm} \) CO\(_2\)), V2 (682 \( \mu \text{atm} \)) and control (463 \( \mu \text{atm} \)), which indicated that *A. viridis* can acclimate to various seawater \( pCO_2 \). In contrast, DMSP concentrations in anemones from stations V1 (33.23 ± 8.30 fmol cell\(^{-1}\)) and V2 (34.78 ± 8.69 fmol cell\(^{-1}\)) were about 35% lower than concentrations in tentacles from the control station (51.85 ± 12.96 fmol cell\(^{-1}\)). Furthermore, low tissue concentrations of DMSP coincided with low activities of the antioxidant enzyme superoxide dismutase (SOD). Superoxide dismutase activity for both host (7.84 ± 1.37 U·mg\(^{-1}\) protein) and zooxanthellae (2.84 ± 0.41 U·mg\(^{-1}\) protein) at V1 was 40% lower than at the control station (host: 13.19 ± 1.42; zooxanthellae: 4.72 ± 0.57 U·mg\(^{-1}\) protein). Our results provide insight into coastal DMSP production under predicted environmental change and support the function of DMSP as an antioxidant in symbiotic anthozoans.

**Introduction**

Dimethylsulfoniopropionate (DMSP) is a secondary metabolite that is produced and accumulated at high intracellular concentrations (typically hundreds of mmol L\(^{-1}\)) by many microalgae (Keller et al. 1989) including members of the dinoflagellates (Caruana et al. 2012). It is the precursor of dimethylsulfide (DMS), the main natural source of reduced sulfur released to the atmosphere (Bates et al. 1987; Kettle and Andreea 2000). Despite controversy about its function in a negative feedback mechanism to global warming (Quinn and Bates 2001), the atmospheric oxidation products of DMS play a major role in the formation of clouds, cloud albedo, and thus in the regulation of global climate (Charlson et al. 1987; Simó 2001). Cellular production of DMSP is complex, and concentrations are affected by a multitude of abiotic variables including salinity, nutrients, light, and temperature (Stefels 2008; Stefels et al. 2007). This is further complicated by a suite of biological processes governing the direct enzymatic cleavage of DMSP (Yost and Mitchelmore 2009), and bacterial (Johnston et al. 2008) and fungal (Kirkwood et al. 2009) degradation. High levels of atmospheric CO\(_2\) continue to increase aqueous pCO\(_2\) and result in the concomitant decrease in seawater pH (Cal-
with microalgae (760 poorly understood. Data from laboratory incubations with microalgae (760 µatm; Avgoustidi et al. 2012) and field mesocosm experiments with a mixed natural phytoplankton population (750 µatm; Hopkins et al. 2010) suggest a decrease in DMSP while several strains of the coccolithophore Emiliania huxleyi respond with an increase in intracellular DMSP under elevated pCO₂ (790 and 1000 µatm) and a 4 to 6°C increase in temperature (Spielmeyer and Pohnert 2012; Arnold et al. 2013). Similarly, varied is the response of DMSP to pCO₂ in seaweeds; Kerrison et al. (2012) found no changes in DMSP in Ulva spp at 550–1250 µatm, while Borell et al. (2013) reported an increase in DMSP in Ulva lactuca following exposure to 4000 µatm.

Many marine organisms, especially those belonging to the anthozoans such as corals and sea anemones, maintain a mutualistic symbiosis with DMSP-producing dinoflagellates in the genus Symbiodinium, commonly known as “zooxanthellae” (Hill et al. 1995; Van Alstyne et al. 2009). These are thought to make a substantial contribution to the amount of DMS entering the atmosphere (Jones et al. 1994; Broadbent and Jones 2004; Jones and Trevena 2005). While the functional role of DMSP in macroalgae and free living phytoplankton is fairly well understood (e.g., Stefels 2000), significant questions remain regarding the physiological roles of DMSP in symbiotic anthozoans and the environmental factors that regulate its production. While current evidence suggests that DMSP in anthozoans is produced solely by the algal symbionts (Van Alstyne et al. 2009), high concentrations of DMSP are also found in the tissue of the coral host implying translocation of DMSP from symbiont to host, which indicates that DMSP furnishes an important role as secondary metabolite in both symbiotic partners (Broadbent et al. 2002; Yost et al. 2010). However, information on how either of the partners (i.e., animal vs. alga) affect specific DMSP concentrations is not available.

Symbiotic corals and anemones are frequently exposed to hyperoxic conditions within their tissues that lead to the production of harmful reactive oxygen species (ROS) (Dykens and Shick 1984; Lesser 2006) and DMSP, and its enzymatic breakdown products, DMS, acrylate, dimethyl sulfoxide, and methane sulfonic acid are potent scavengers of ROS (Sunda et al. 2002). Because high concentrations of DMSP were observed in stressed corals following thermal bleaching and exposure to ROS-inducing copper (Broadbent et al. 2002; Yost et al. 2010), DMSP has been proposed to function as an antioxidant in these organisms. While OA is expected to have adverse consequences for various biological processes (Kroeker et al. 2010), in anemones it may improve photosynthetic performance (Suggett et al. 2012; Towanda and Thuesen 2012) and result in a lessened production of ROS. In higher plants, for example, elevated concentrations of CO₂ have been shown to decrease concentrations of antioxidant enzymes such as superoxide dismutase (SOD) (Schwanz et al. 1996), an important antioxidant enzyme in both anthozoan hosts and their symbiotic zooxanthellae (Lesser 2006).

Here, we investigated the total tissue concentrations of DMSP (i.e., DMSP in the tissue of both animal and algae) in the Mediterranean sea anemone Anemonea viridis along a natural CO₂ seawater gradient that arises from a cold vent system along the shore of the island Vulcano, Italy. Our aims were to test (1) whether DMSP in A. viridis is sensitive to different CO₂ concentrations along the gradient and (2) whether cellular DMSP concentrations are related to activity of SOD.

**Materials and Methods**

Samples were collected along the sublittoral in Levante Bay, North Vulcano Island (38°25′N, 14°57′E), NE of Sicily, Italy (Fig. 1) in May 2012. The Bay is located on the eastern side of the isthmus of Vulcano island and is characterized by the presence of submarine gas vents that release CO₂ creating an extensive CO₂/pH gradient that runs parallel to the northeastern coast of the island. This site has been used extensively as a natural laboratory for OA studies (Johnson et al. 2011; Arnold et al. 2012;
Suggett et al. 2012; Boatta et al. 2013) as the average pH ranges from 6.05 to 8.29 at ~350 m from the vent site (Johnson et al. 2011; Boatta et al. 2013) providing an environment representing possible future CO2 scenarios.

The sea anemone A. viridis is a dominant benthic organism in Levante Bay and becomes increasingly abundant with increasing pCO2 (Suggett et al. 2012). We selected three sampling stations compatible with previous studies (Johnson et al. 2011; Arnold et al. 2012; Suggett et al. 2012; Boatta et al. 2013) at ~260 m (V1, high pCO2), ~300 m (V2, intermediate pCO2), and ~400 m (control) distance to the vents (Fig. 1). The stations were shallow (1–2 m), and sampling was carried out by snorkeling. Between 5 and 10, tentacles were clipped from each of 16 haphazardly selected anemones (oral disk size 2.5 to 3.5 cm) at every station using a pair of scissors. The samples were stored on ice, transported to the laboratory immediately, and frozen at −20°C in order to minimize the degradation of DMSP and antioxidant enzymes.

Hourly light recordings were carried out for three consecutive days at each station close to the seabed at a depth of 1–2 m with HOBO Pendant® Temperature/Light data loggers (Onset, Pocasset, MA). The logged light data (Table 1) were converted from lux to µmol·m−2·s−1 PAR (Table 1) (Thimijan and Heins 1983). The pH (NBS scale) together with temperature at each station was measured haphazardly once every day for 4 days (YSI Professional Plus, Handheld Multiparameter Instrument, Yellow Springs, OH), and water samples were taken and frozen at −20°C for measurements of total alkalinity (TA). TA was quantified with a Metrohm 862 compact titrosampler (Cohen 2011). The pCO2 levels were calculated from CO2SYS (Pierrot et al. 2006) and the constants of Mehrbach et al. (1973). Seawater parameters are shown in Table 1.

### Table 1. Measurements of pH and TA taken at four consecutive days in May 2013, calculated pCO2 and mean midday (12:00–13:00 h) light intensities over three consecutive days along a pCO2 gradient at the island of Vulcano, Italy.

| Station | pH (NBS scale) | TA (mmol·kg−1) | pCO2 (µatm) | Light (µmol·m−2·s−1) |
|---------|----------------|----------------|-------------|----------------------|
| V1      | 7.22           | 2.598          | 4808        | 588 ± 85             |
|         | 7.24           | 2.573          | 4506        |                       |
|         | 7.70           | 2.561          | 1468        |                       |
|         | 7.54           | 2.487          | 2144        |                       |
| V2      | 8.04           | 2.488          | 600         | 650 ± 71             |
|         | 7.94           | 2.493          | 778         |                       |
|         | 7.93           | 2.491          | 800         |                       |
|         | 8.07           | 2.472          | 552         |                       |
| Control | 8.16           | 2.464          | 427         | 643 ± 24             |
|         | 8.10           | 2.480          | 506         |                       |
|         | 8.14           | 2.468          | 453         |                       |
|         | 8.13           | 2.469          | 466         |                       |

There were no significant differences in light intensity (n = 21, ±SD) between the stations (F2, 60 = 1.62, P = 0.207). Seawater temperatures ranged from 18.5 to 19.5°C.

### Sample processing

Half of the tentacles were processed for DMSP, total protein, and zooxanthellae characteristics (i.e., chlorophyll a (chl a) and zooxanthellae densities) (n = 16) at the sampling site. Samples were weighed (CT 1202, Citizen, accuracy 0.01 g) and homogenized in sterile filtered seawater (FSW, 0.2 µm) with an electric homogenizer (DIAX 100 homogenizer Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). One milliliter of the homogenate was used directly for quantification of total DMSP (see below). The remaining homogenate was divided into subsamples before freezing these, and the remaining tentacles for SOD analyses and the determination of the zooxanthellae genotype at −20°C. The samples were then transported on dry ice to the Interuniversity Institute for Marine Sciences (IUI), Israel, where they were stored at −80°C pending analyses.

### Total protein

Total protein content was determined after Bradford (1976) using a commercial kit (Bio-Rad, Laboratories, Hercules, CA). In brief, for the protein extraction, 100 µL of the tissue homogenate of each sample was sonicated on ice with a Branson Sonifier B12 (Branson Sonic Power Co., Danbury, Connecticut) for 20 sec and centrifuged at 2900 g for 20 min. Protein concentrations were assessed spectrophotometrically (Multiskan spectrum, Thermo Fisher Scientific Inc., Waltham, MA) at 595 nm using bovine albumin as the standard.

Zooxanthellae characteristics and genotype for measurements of chl a, zooxanthellae densities, and protein, 2 mL of homogenate was centrifuged (1900 g at 4°C) and resuspended four times in FSW. Resuspended zooxanthellae were used for chl a extraction in acetone (100%) at 4°C in the dark for 24 h. Concentrations were determined spectrophotometrically (Jeffrey and Humphrey 1975). Zooxanthellae densities were quantified from 4 replicate counts using a Neubauer haemocytometer. Zooxanthellae densities and chl a were normalized to protein concentration.

To determine the zooxanthellae genotypes, nucleic acid extractions (n = 5) were conducted using a modified Promega Wizard genomic DNA extraction protocol (LaJeunesse et al. 2003). Symbiont identity was characterized by denaturing gradient gel electrophoresis (DGGE) fingerprinting of the partial 5.8S and internal transcribed spacer (ITS) region 2 (LaJeunesse 2002). The region was
amplified using a touch-down thermal cycle profile with the primers "ITS2clamp" and "ITSSintfor2" (LaJeunesse and Trench 2000), and the PCR products resolved on denaturing gels (45–80% of 7 mol·L⁻¹ urea & 40% formamide) using a CBS Scientific system (Del Mar, CA) for 16 h at 115 volts. The dominant band of the symbiont’s DGGE profile was excised, reamplified, and cycle-sequenced to provide the ITS2 sequence that dominates the symbiont’s genome.

Quantification of DMSPₜ and SOD

For the quantification of total DMSP (DMSPₜ = sum of particulate and dissolved DMSP and DMS), 1 mL of homogenate was added to 2 mL 0.5 mol·L⁻¹ NaOH in a gas-tight, screw cap headspace vial. This alkaline hydrolysis rapidly converts DMSP to equimolar concentrations of DMS, which can be quantified using gas chromatographic methods with direct injection of headspace (Steinke et al. 2011). Results were expressed as femtomole DMSPₜ per milligram protein.

Samples for SOD measurements (n = 10) were homogenized in cold HEPES buffer pH 7.3 (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Biological Industries, 03-025-1B). A 2-mL subsample was taken for the analyses of protein concentrations and zooxanthellae densities. To separate the host from the zooxanthellae fraction, a 2-mL aliquot was centrifuged (2900 g for 20 min) and resuspended twice in HEPES buffer. The supernatant was used for analysis of animal SOD. Zooxanthellae contained in the pellet were resuspended in 0.5 mL of buffer and sonicated on ice for 20 sec. The zooxanthellae were centrifuged (2900 g for 20 min), and the supernatant was analyzed for algal SOD. The combined activities of three types of SOD (Cu/Zn-, Mn-, and Fe-SOD) in both animal host and zooxanthellae were determined spectrophotometrically at 450 nm using the Superoxide Dismutase Assay Kit (Cayman Chemical Company, Ann Arbor, MI) following manufacturer’s instructions. In brief, SOD activity was assessed by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was defined as the amount of enzyme required for 50% inhibition of cytochrome c reduction. SOD activity for each sample was expressed as units (U) of enzyme activity per milligram protein.

Data analyses

Data were checked for homogeneity of variances using the Cochran’s C-test and analyzed using one-way ANOVA. The data for zooxanthellae densities were ln(x)-transformed prior to the analysis. Repeated measurements one-way ANOVA was used to determine whether there were significant differences in midday (12:00–13:00 h) light intensities between stations. Student–Newman–Keuls (SNK) tests were used for post hoc multiple comparisons. All data were analyzed using WinGMAV (EICC, University of Sydney, Australia).

Results

There were no significant differences in chl a content (F₂, ₄₅ = 1.12, P = 0.334), zooxanthellae densities (F₂, ₄₅ = 0.32, P = 0.724), and protein concentrations (F₂, ₄₅ = 1.00, P = 0.376) between anemones at the different stations. Chl a content (mg chl a mg⁻¹ protein ± SE) varied between 3.8 ± 1.23 at the control station, 3.43 ± 1.5 and V₂, and 4.41 ± 1.66 at V₁. Zooxanthellae densities (cells mg⁻¹ protein ± SE) averaged to 1.14 ± 0.18 × 10⁶ at the control site and decreased with increasing pCO₂ to 1.1 ± 0.13 × 10⁶ at V₂ and 0.94 ± 0.07 × 10⁶ at V₁. Similarly, protein concentration (mg protein g⁻¹ FW) was highest at the control site (39.87 ± 2.82) and decreased with increasing pCO₂ to 38.09 ± 5.07 at V₂ and 35.24 ± 5.37 at V₁.

There was no variation in zooxanthellae genotype. Anemones of all three stations were associated with symbionts of Symbiodinium type A19. pCO₂ had a significant effect on DMSPₜ concentrations in the tentacles of A. viridis both when normalized to cell (F₂, ₄₅ = 6.47, P = 0.028; Fig. 2A) and to protein (F₂, ₄₅ = 13.96, P = 0.000; Fig. 2B). Irrespective of normalization indices, DMSPₜ concentrations from the control station were about 35% higher (51.85 ± 6.26 fmol cell⁻¹ or 172.12 ± 10.13 nmol·mg⁻¹ protein; equivalent to 6.49 ± 0.19 µmol·g⁻¹ fresh weight (FW)) than concentrations in tentacles from stations V₁ (33.23 ± 8.30 fmol cell⁻¹ or 121.72 ± 7.05 nmol·mg⁻¹ protein; equivalent to 3.95 ± 0.25 µmol·g⁻¹ FW) and V₂ (34.78 ± 8.69 fmol cell⁻¹ or 114.51 ± 7.69 nmol·mg⁻¹ protein; equivalent to 4.54 ± 0.18 µmol·g⁻¹ FW). Increased levels of pCO₂ also had a significant effect on the SOD activity in the anemone tentacles. In both, host (F₂, ₂₇ = 4.07, P = 0.028) and zooxanthellae fractions (F₂, ₂₇ = 3.36, P = 0.049), the SOD activity was significantly lower in tentacles from station V₁ (host: 7.84 ± 1.37; zooxanthellae: 2.84 ± 0.41 U·mg⁻¹ protein) compared with the control station (host: 13.19 ± 1.42; zooxanthellae: 4.72 ± 0.57 U·mg protein; Fig. 3).

Discussion

Many experimental approaches to quantify the effects of increasing seawater pCO₂ on the physiology and performance of organisms suffer from a lack of adequate
exposure times. In contrast, natural CO₂ vents, such as the one used in this study, provide unique experimental settings to assess the adaptive capacities of organisms to long-term increase in pCO₂/decrease in pH at different biological levels (Hall-Spencer et al. 2008; Fabricius et al. 2011; Suggett et al. 2012).

Elevated pCO₂ had no effect on chl a and zooxanthellae densities in A. viridis. This is in agreement with earlier observations by Towanda and Thuesen (2012) who found no significant changes in zooxanthellae densities or chl a concentrations in Anthopleura elegantissima following exposure to ~2270 µatm pCO₂ under laboratory conditions. By contrast, a recent study on A. viridis along the CO₂ gradient at Vulcano reported significantly higher zooxanthellae densities in animals growing under high pCO₂ conditions compared with the intermediate and control sites (Suggett et al. 2012).

This discrepancy in results is likely the result of methodological differences in the quantification of algal cells.

While Suggett et al. (2012) expressed zooxanthellae densities as cells per surface area of tentacle, cell numbers in our study and that by Towanda and Thuesen (2012) were normalized to mg of protein. This makes the comparison of results difficult. The handling of anemones greatly influences tentacle contraction, which may subsequently have led to a bias in surface area determination and thus an underestimation of tentacle surface area at station V1. This could also explain why the average zooxanthellae densities along the gradient reported by Suggett et al. (2012) were about an order of magnitude lower than those determined in this study.

The lack of differences in protein concentrations in the tentacles of A. viridis between stations indicated that A. viridis was in fact well acclimated to the high seawater pCO₂ at this site (e.g., Langenbuch and Portner 2003). In contrast, DMSPt concentrations were significantly higher under control pCO₂ conditions than at elevated pCO₂ at stations V1 and V2. This is consistent with the results from earlier studies showing increased cellular DMSP and DMS concentrations in response to CO₂ limitation in different phytoplankton species (Sunda et al. 2002) and, at increased CO₂, a decrease in DMS concentration during a mesocosm experiment (Hopkins et al. 2010) and DMS production in E. huxleyi laboratory cultures (Arnold et al. 2013). Increased DMSP concentrations within different algal species were also observed in response to UV radiation, temperature, and nutrient (N, Fe) limitation, factors that can cause oxidative stress (Sunda et al. 2002; Harada et al. 2009; McLennon and DiTullio 2012). DMSP concentrations in anthozoans can vary significantly with Symbiodinium clade (Steinke et al. 2011), which in turn may
change within the host depending on their environmental optima (LaJeunesse et al. 2010). However, anemones in the Mediterranean commonly feature zooxanthellae belonging to clade A only (Savage et al. 2002; Visram et al. 2006). Our results corroborate this and additionally showed that zooxanthellae in anemones of all three stations belonged to the same ITS2 “type”A19, as was previously demonstrated for A. viridis in Levante Bay by Suggett et al. (2012). This supports the notion that the differences in DMSP concentrations between stations were indeed influenced by ambient CO2 conditions and not the result of the genetic makeup of the zooxanthellae.

Previous studies reported enhanced photosynthetic rates of A. viridis at station V1 (Suggett et al. 2012) and of A. elegantissima following exposure to increased pCO2 under laboratory conditions (Towanda and Thuesen 2012), suggesting that elevated pCO2 with proximity to the vent site alleviated CO2 limitation, increased photosynthetic efficiency, and thereby decreased the generation of ROS (Asada 1994; Suggett et al. 2008). The notion that anemones exposed to elevated pCO2 experienced less oxidative stress than under control pCO2 is further supported by the low SOD activity in tentacles from station V1. DMSP concentrations normalized to both cell and protein content appeared to be more sensitive to increased pCO2 than SOD, showing a significant decrease at station V2 relative to the control, whereas the decrease in SOD activity toward higher pCO2 was gradual and significantly different only for station V1 relative to the control. This may be attributed to the specific phenotypic plasticity of DMSP and SOD in response to environmental cues (Ross and Van Alstyne 2007). DMSP concentrations may be thus enhanced only below a certain pCO2 threshold or above a given accumulation of ROS when the capacity of resident ROS scavenging systems is exceeded (Shen et al. 1997). In addition to enhanced photosynthesis, Suggett et al. (2012) observed increased growth of A. viridis in proximity to station V1, suggesting a link between growth and CO2-mediated stimulation of metabolism. Against the background of our results, it is also conceivable that enhanced growth of A. viridis was facilitated by a decreased energy investment in to the synthesis of antioxidant compounds such as SOD or DMSP.

In summary, while our data support the notion of an antioxidant functional role for DMSP in symbiotic non-calcifying anthozoans, more work is required to establish an explicit link between oxidative stress and DMSP dynamics in symbiotic cnidarians. Sea anemones are dominant organisms in many tropical and temperate coastal environments (e.g., Manuel 1981; Russo et al. 1994; Venn et al. 2008). The apparent sensitivity of DMSP concentration in A. viridis to pCO2 thus indicated that a doubling in current seawater pCO2 by the end of the century (Caldeira and Wickett 2005) could significantly decrease cellular DMSP concentration in these animals, which may further result in a decreased supply of DMSP and DMS to future coastal ecosystems.

Acknowledgments

Thanks to Marco Milazzo, the staff at Vulcano Blu resort, and Eugenio Viviani for their logistical support and help with the sampling, and to John Green from the University of Essex for assistance with the quantification of DMSP. We are grateful for the help of Mark Warner and D. Tye Pettay for the analyses of the *Symbiodinium* genotypes. We also thank two anonymous reviewers whose critical comments greatly improved the final version of this manuscript. This study was funded by ASSEMBLE Marine, the EU MedSeA project, and an Israel Science Foundation grant to M.F. E.M.B. was funded by the Minerva fellowship program.

Conflict of Interest

None declared.

References

Arnold, T., C. Mealey, H. Leahy, A. W. Miller, J. M. Hall- Spencer, M. Milazzo, et al. 2012. Ocean acidification and the loss of phenolic substances in marine plants. PLoS ONE 7:e35107.

Arnold, H. E., P. Kerrison, and M. Steinke. 2013. Interacting effects of ocean acidification and warming on growth and DMS-production in the haptophyte coccolithophore *Emiliania huxleyi*. Glob. Change Biol. 19:1007–1016.

Asada. 1994. Production and action of active oxygen species in photosynthetic tissues. Pp. 77–105 in C. H. Foyer and P. M. Mullineaux, eds. Causes of photooxidative stress and amelioration of defense systems in plants. CRC Press, London.

Avgoustidi, V., P. D. Nightingale, I. Joint, M. Steinke, S. M. Turner, F. E. Hopkins, et al. 2012. Decreased marine dimethyl sulfide production under elevated CO2 levels in mesocosm and in vitro studies. Environ. Chem. 9:399–404.

Bates, T. S., R. J. Charlson, and R. H. Gammon. 1987. Evidence for the climatic role of marine biogenic sulphur. *Nature* 329:319–321.

Boatta, F., W. D’Alessandro, A. L. Gagliano, M. Liotta, M. Milazzo, R. Rodolfo-Metalpa, et al. 2013. Geochemical survey of Levante Bay, Vulcano Island (Italy), a natural laboratory for the study of ocean acidification. *Mar. Pollut. Bull.* 73:485–494.

Borell, E. M., M. Steinke, and M. Fine. 2013. Direct and indirect effects of high pCO2 on algal grazing by coral reef
herbivores from the Gulf of Aqaba (Red Sea). Coral Reefs 32:937–947.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254.

Broadbent, A. D., and G. B. Jones. 2004. DMS and DMSP in mucus ropes, coral mucus, surface films and sediment pore waters from coral reefs in the Great Barrier Reef. Mar. Freshw. Res. 55:849–855.

Broadbent, A., G. Jones, and R. Jones. 2002. DMSP in corals and benthic algae from the Great Barrier Reef. Estuar. Coast. Shelf Sci. 55:547–555.

Caldeira, K., and M. E. Wickett. 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. J. Geophys. Res. 110:C09S04.

Caruana, A., M. Steinke, S. Turner, and G. Malin. 2012. Concentrations of dimethylsulphoniopropionate and activities of dimethylsulphide-producing enzymes in batch cultures of nine dinoflagellate species. Biogeochemistry 110:87–107.

Charlson, R. J., J. E. Lovelock, M. O. Andreae, and S. G. Warren. 1987. Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate. Nature 326:655–661.

Cohen, S. (2011) Measuring gross and net calcification of a reef coral under ocean acidification conditions: methodological considerations. MSc thesis, Tel Aviv, Bar Ilan University.

Dykens, J. A., and M. Shick. 1984. Photobiology of the symbiotic sea anemone, Anthopleura elegantissima: Defenses against photodynamic effects, and seasonal photoacclimatization. Biol. Bull. 167:683–697.

Fabricius, K. E., C. Langdon, S. Uthicke, C. Humphrey, S. Noonan, and G. De, et al. 2011. Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nat. Clim. Chang. 1:165–169.

Hall-Spencer, J. M., R. Rodolfo-Metalpa, S. Martin, E. Ransome, M. Fine, and S. M. Turner, et al. 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454:96–99.

Harada, H., M. Vila-Costa, J. Cebrian, and R. P. Kiene. 2009. Effects of UV radiation and nitrate limitation on the production of biogenic sulfur compounds by marine phytoplankton. Aquat. Bot. 90:37–42.

Hill, R. W., J. W. H. Dacey, and D. A. Krupp. 1995. Dimethylsulfinioproionate in reef corals. Bull. Mar. Sci. 57:489–494.

Hopkins, F. E., S. M. Turner, P. D. Nightingale, M. Steinke, D. Bakker, and P. S. Liss. 2010. Ocean acidification and marine trace gas emissions. Proc. Natl Acad. Sci. 107:760–765.

Jeffrey, S. W., and G. F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanz. 167: S191–S194.

Johnston, V. R. 2012. A study of marine benthic algae along a natural carbon dioxide gradient. Ph.D thesis. University of Plymouth, Plymouth.

Johnson, V. R., C. Brownlee, R. E. M. Rickaby, M. Graziano, M. Milazzo, and J. M. Hall-Spencer. 2011. Responses of marine benthic microalgae to elevated CO2. Mar. Biol., 160:1813–1824.

Johnston, A. W. B., J. D. Todd, L. Sun, M. N. Nikolaidou-Katsaridou, A. R. J. Curson, and R. Rogers. 2008. Molecular diversity of bacterial production of the climate-changing gas, dimethyl sulphide, a molecule that impinges on local and global symbioses. J. Exp. Bot. 59:1059–1067.

Jones, G. B., and A. J. Trevena. 2005. The influence of coral reefs on atmospheric dimethylsulphide over the Gerat Barrier Reef, Coral Sea, Gulf of Papua and Solomon and Bismarck Seas. Mar. Freshw. Res. 56:85–93.

Jones, G. B., M. A. J. Curran, and A. D. Broadbent (1994) Dimethylsulphide in the South Pacific. Pp. 183–190 in O. Bellwood, H. Choaot, N. Saxena, eds. Recent advances in marine science and technology 1994. James Cook University Press, Townsville.

Keller, M. D., W. K. Bellows, and R. R. L. Guillard. 1989. Dimethyl sulfide production in marine phytoplankton. Pp. 167–182 in E. S. Saltzman and W. J. Cooper, eds. Biogenic sulfur in the environment. American Chemical Society, Washington DC.

Kerrison, P., D. J. Suggett, L. J. Hepburn, and M. Steinke. 2012. Effect of elevated pCO2 on the production of dimethylsulphoniopropionate (DMSP) and dimethyl sulphide (DMS) in two species of Ulva (Chlorophyceae). Biogeochemistry, 110:5–16.

Kettle, A. J., and M. O. Andreae. 2000. Flux of dimethylsulfide from the oceans: a comparison of updated data sets and flux models. J. Geophys. Res. 105:26793–26808.

Kirkwood, M., J. D. Todd, K. L. Rypien, and A. W. B. Johnston. 2009. The opportunistic coral pathogen Aspergillus sydowii contains dddP and makes dimethyl sulfide from dimethylsulfiniopropionate. ISME J. 4:147–150.

Kroeker, K. J., R. L. Kords, R. N. Crim, and G. G. Singh. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol. Lett. 13:1419–1434.

LaJeunesse, T. C. 2002. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Mar. Biol. 141:387–400.

LaJeunesse, T. C., and R. K. Trench. 2000. Biogeography of two species of Symbiodinium (Freudenthal) inhabiting the intertidal sea anemone Anthopleura elegantissima (Brandt). Biol. Bull. 199:126–134.

LaJeunesse, T. C., W. K. W. Loh, R. van Woesik, O. Hoegh-Guldberg, G. W. Schmidt, and W. K. Fitt. 2003. Low symbiont diversity in southern Great Barrier Reef corals,
relative to those of the Caribbean. Limnol. Oceanogr. 48:2046–2054.

Lajeunesse, T., D. T. Pettay, E. M. Sampaio, N. Phongsuwan, B. Brown, and D. O. Obura, et al. 2010. Long-standing environmental conditions, geographic isolation and host–symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus Symbiodinium. J. Biogeogr. 37:785–800.

Langenbuch, M., and H. O. Pörtner. 2003. Energy budget of hepatocytes from Antarctic fish (Pachycara brachycephalum and Lepidonotothen kempii) as a function of ambient CO2; pH-dependent limitations of cellular protein biosynthesis? J. Exp. Biol. 206:3895–3903.

Lesser, M. P. 2006. Oxidative stress in marine environments: Biochemistry and physiological ecology. Annu. Rev. Physiol. 68:253–278.

Manuel, R. L. 1981. British anthozoa, Linnean society of London, synopses of the British fauna, London, Academic Press.

McLenon, A. L., and G. R. DiTulio. 2012. Effects of increased temperature on dimethylsulfoniopropionate (DMSP) concentration and methionine synthase activity in Symbiodinium microadriaticum. Biogeochemistry 110:17–29.

Mehrbach, C., C. H. Culberson, H. JE, and P. RM. 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18:897–907.

Pierrot, D. E., E. Lewis, and D. W. R. Wallace. 2006. MS Excel program developed for CO2 system calculations. ORNL/CDIA-105a, Carbon dioxide information analysis center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, TN.

Quinn, P. K., and T. S. Bates. 2011. The case against climate regulation via oceanic phytoplankton sulphur emissions. Nature 480:51–56.

Ross, C., and K. L. Van Alstyne. 2007. Interspecific variation in stress-induced hydrogen peroxide scavenging by the Ulvoid macroalga Ulva lactuca. J. Phycol. 43:466–474.

Russo, C. A. M., A. M. Solé-Cava, and J. P. Thorpe. 1994. Population structure and genetic variation in two tropical sea anemones (Cnidaria, Actiniidae) with different reproductive strategies. Mar. Biol. 119:267–276.

Savage, A. M., M. S. Goodson, S. Visram, H. G. Trapido-Rosenthal, J. Wiedemann, and A. E. Douglas. 2002. Molecular diversity of symbiotic algae at the latitudinal margins of their distribution: dinoflagellates of the genus Symbiodinium in corals and sea anemones. Mar. Ecol. Progr. Ser. 244:17–26.

Schwanz, P., B. A. Kimball, S. B. Idso, D. L. Hendrix, and A. Polle. 1996. Antioxidants in sun and shade leaves of sour orange trees (Citrus aurantium) after long-term acclimation to elevated CO2. J. Exp. Bot. 47:1941–1950.

Shen, B., R. G. Jensen, and H. J. Bohnert. 1997. Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. Plant Physiol. 113:1177–1183.

Simó, R. 2001. Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. Trends Ecol. Evol. 16:287–294.

Spielmeyer, A., and G. Pohnert. 2012. Influence of temperature and elevated carbon dioxide on the production of dimethylsulfoniopropionate and glycine betaine by marine phytoplankton. Mar. Environ. Res. 73:62–69.

Stefels, J. 2000. Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. J. Sea Res. 43:183–197.

Stefels, J., M. Steinke, S. Turner, G. Malin, and S. Belviso. 2007. Environmental constraints on the production and removal of the climatically active gas dimethylsulphide (DMS) and implications for ecosystem modelling. Biogeochemistry 83:245–275.

Steinke, M., P. Brading, P. Kerrison, M. E. Warner, and D. J. Suggett. 2011. Concentrations of dimethylsulfoniopropionate (DMSP) and dimethylsulphide (DMS) are strain-specific in symbiotic dinoflagellates (Symbiodinium sp., Dinophyceae). J. Phycol. 47:775–783.

Suggett, D. J., M. E. Warner, D. J. Smith, P. Davey, S. Hennige, and N. R. Baker. 2008. Photosynthesis and production of hydrogen peroxide by Symbiodinium (Pyrrophyta) phytootypes with different thermal tolerances. J. Phycol. 44:948–956.

Suggett, D. J., J. M. Hall-Spencer, R. Rodolfo-Metalpa, T. G. Boatman, D. T. Pettay, and V. R. Johnson, et al. 2012. Sea anemones may thrive in a high CO2 world. Glob. Change Biol. 18:3015–3025.

Sunda, W., D. J. Kieber, R. P. Kiene, and S. Huntsman. 2002. An antioxidant function for DMSP and DMS in marine algae. Nature 418:317–320.

Thimijan, R. W., and R. D. Heins. 1983. Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. HortScience 18:818–822.

Towanda, T., and E. V. Thuesen. 2012. Prolonged exposure to elevated CO2 promotes growth of the algal symbiont Symbiodinium muscatinei in the intertidal sea anemone Anthopleura elegansissima. Biol. Open 1:615–621.

Van Alstyne, K. L., V. J. III Dominique, and G. Muller-Parker. 2009. Is dimethylsulfoniopropionate (DMSP) produced by the symbionts or the host in an anemone-zooxanthella symbiosis? Coral Reefs 28:167–176.

Venn, A. A., J. E. Loram, H. G. Trapido-Rosenthal, D. A. Joyce, and A. E. Douglas. 2008. Importance of time and place: Patterns in abundance of symbiodinium clades A and B in the tropical sea anemone Condylactis gigantea. Biol. Bull. 215:243–252.

Visram, S., J. Wiedemann, and A. E. Douglas. 2006. Molecular diversity of symbiotic algae of the genus
Symbiodinium (Zooxanthellae) in cnidarians of the Mediterranean Sea. J. Mar. Biol. Assoc. U.K. 86:1281–1283.
Yost, D. M., and C. L. Mitchelmore. 2009.
Dimethylsulfoniopropionate (DMSP) lyase activity in different strains of the symbiotic alga Symbiodinium microadriaticum. Mar. Ecol. Prog. Ser. 386:61–70.

Yost, D. M., R. J. Jones, and C. L. Mitchelmore. 2010.
Alterations in dimethylsulfoniopropionate (DMSP) levels in the coral Montastrea franksi in response to copper exposure. Aquat. Toxicol. 98:367–373.