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Effect of elevated pCO_2 on the production of dimethylsulphoniopropionate (DMSP) and dimethylsulphide (DMS) in two species of *Ulva* (Chlorophyceae)

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Abstract Concentrations of the secondary algal metabolite dimethylsulphoniopropionate (DMSP) and its breakdown product, the climate-active trace gas dimethylsulphide (DMS), are sensitive to changes in pCO_2. Data on the response of marine macroalgae to future pCO_2 levels are lacking. Here we report the first measurements of DMSP and DMS production in two species of chlorophyte macroalgae (*Ulva lactuca* and *U. clathrata*). Laboratory cultures were grown in pH–stated medium that received pulses of CO_2 to create pCO_2 conditions ranging from ambient (432 µatm) to future (1514 µatm). Intracellular concentration of DMSP remained unaffected in both species (101 ± 21 and 69 ± 20 mmol g^{-1} FW in *U. lactuca* and *U. clathrata*, respectively) but significant differences in extracellular production of DMSP and DMS were observed for *U. lactuca*: Whilst production of total DMSP (the sum of external DMSP and DMS) was different between replicated experiments, the percentage of total DMSP produced throughout each experiment increased significantly by up to 65% with increasing pCO_2 to 1514 µatm. In contrast, DMS production decreased from 0.4 to 0.25 nmol g^{-1} FW h^{-1}. This decrease was not a linear function of pCO_2 but an almost 50% step-wise loss of DMS production was indicated between 635 and 884 µatm, a pCO_2 predicted for the next 100 years. Since *Ulva* spp. form massive harmful blooms ('green tides') that can occur free-floating and faraway (>100 km) from the coast, they may provide a locally significant source of DMS to the remote marine boundary layer.

Keywords Ocean acidification · Dimethylsulphide (DMS) · Dimethylsulphoniopropionate (DMSP) · Macroalgae · *Ulva lactuca* · *Ulva clathrata*

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Introduction

The secondary algal metabolite dimethylsulphoniopropionate (DMSP) and its breakdown product dimethylsulphide (DMS) have numerous intracellular functions. The regulation of their production seems linked to cellular fitness and environmental conditions. DMSP can contribute significantly to the cell's osmotic pressure (Kirst 1996; Edwards et al. 1988) and may replace nitrogen-containing osmolytes, for example glycine betaine, under N-limiting conditions (see references in Stefels 2000). It further acts as a compatible solute (Kirst 1996) and as a cryoprotectant at low temperatures (Nishiguchi and Somero 1992; Karsten et al. 1996). DMSP and its breakdown products DMS, acrylate, dimethylsulphoxide and methane sulphinic acid are active scavengers of harmful reactive oxygen species (ROS). Hence, DMSP itself and the enzymatic

breakdown of DMSP may provide an antioxidant system that protects cells during elevated oxidative stress (Sunda et al. 2002; Ross and Van Alstyne 2007). This is consistent with the observation of high DMS production in association with various stressors (Wolfe et al. 2002; Vallina and Simó 2007; Archer et al. 2010). DMSP also acts as a carbon or sulphur store and its synthesis may prevent the depletion of important nitrogen precursors during periods of N-limitation (Stefels 2000; Stefels et al. 2007). In addition, the enzymatic conversion of DMSP to DMS has been suggested to be part of an anti-grazing defence mechanism in phytoplankton (Wolfe et al. 1997) and macroalgae (Van Alstyne and Houser 2003). There is also the possibility that DMS production during grazing can stabilise marine food webs (Lewis et al. 2011) and functions in multitrophic interactions (Pohnert et al. 2007).

Ocean acidification is the inevitable result of the ongoing increase in atmospheric pCO_2 (Raven et al. 2005). In comparison to a pre-industrial mean oceanic pH of 8.2 at a pCO_2 of 280 µatm, current pH is around 8.1 (385 µatm) and is predicted to decrease to pH 7.8 by the year 2100 (ca 750 µatm; Turley 2008). Investigations from laboratory studies, mesocosm experiments and ship-board incubations suggest that DMSP and DMS metabolism is sensitive to changes in pCO_2. These studies have produced mixed results: Kim et al. (2010) show a significant increase in DMS concentration that could be attributed to increased growth and grazing rates of heterotrophic dinoflagellates. Wingenter et al. (2007) suggest an increase in DMS concentrations, whereas Vogt et al. (2008a) show no significant ($p > 0.05$) change in DMS concentration during the same mesocosm experiment. Hopkins et al. (2010) and Avgoustidi (2006) present a decrease of DMS concentrations under elevated pCO_2. As far as we are aware, the effect of future CO_2 increase on DMSP and DMS in macroalgae has not been investigated.

Members of the Ulvales are very common intertidal and subtidal macroalgae that can produce massive harmful blooms or 'green tides', often as a result of eutrophication and aquaculture practices (Liu et al. 2009). They are characterised by high intracellular concentrations of DMSP that typically range between 20 and 100 mmol g^{-1} fresh weight (Van Alstyne and Puglisi 2007) and some species have been shown to enzymatically produce DMS (de Souza et al. 1996; Steinke and Kirst 1996). Due to the susceptibility of Ulvales to anthropogenic eutrophication, and the likely increase in the abundance and frequency of green tides (Ye et al. 2011), it is important to improve our understanding of how environmental change may affect their DMS production. Here we report a series of incubation studies using various pCO_2 concentrations to quantify the response in DMSP and DMS of two common species (*Ulva lactuca* and *U. clathrata*). Data from such experiments may assist with constraining DMS production in coastal environments but, since *Ulva* green tides can also occur free-floating and away from the coastline (Liu et al. 2009), this data may also be useful for the improved quantification of DMS sources to the marine atmosphere.

Methods

Two separate experiments were conducted with *U. lactuca* L. and *U. clathrata* (Roth) C. Agardh., respectively.

Ulva lactuca

Fronds of the cosmopolitan *U. lactuca* were collected from the low intertidal of St Osyth beach, UK (51.771N, 1.077E) in January 2010. Visibly healthy specimens, 50–60 mm in length, without noticeable epiphytes were collected. Discs (16 mm diameter) were excised at the apical end of each specimen avoiding the marginal 5 mm of tissue. This method does not alter the tissue DMSP concentration and helps to standardise the tissue character investigated (Han et al. 2003; Van Alstyne et al. 2007). The discs were kept in an aerated 2 L conical flask with filtered (0.2 µm) enriched North Sea water (ESNW at a salinity of 34; Berges et al. 2001, 2004) within an environmental growth chamber for 2 days before starting the experiment. Temperature was set to 15°C, and light to 110 µmol m^{-2} s^{-1} with a photoperiod of 12 h light:12 h dark.

Twenty healthy discs were haphazardly assigned to five screw-capped 1 L conical flasks, equipped with Teflon-coated rubber septa, containing 800 mL of ESNW, at a final biomass of ca 0.6 g FW L^{-1}. These cultures were then incubated for 7 days under the same temperature, light and photoperiod conditions as above. Aeration with CO_2-free air, produced from a

soda-lime scrubber, was provided at a flow rate of 60–75 mL min^{-1}. The medium was refreshed on days 3 and 5.

A pH–stating system (Brading et al. 2011) maintained the pH of each culture at preset values (±0.01 units) using pH electrodes (Amphel HI 6291005; Hanna Instruments Ltd, Bedfordshire, UK) and pulses of pure CO_2 when required. The cultures were set to maintain the pCO_2 at target levels of 385, 550, 750, 1000 and 1250 µatm with associated pH values of 8.10, 7.96, 7.83, 7.70 and 7.62. This experiment was repeated four times, each with freshly collected *U. lactuca*.

Ulva clathrata

In a further experiment we used a laboratory culture of the filamentous cold-temperate species *U. clathrata* (previously *Enteromorpha clathrata*; laboratory strain 1086) isolated from Disko Island, Greenland (Bischoff and Wiencke 1993). Before experimentation, biomass was grown in aerated conical flasks with enriched artificial seawater (ESAW at a salinity of 35; Berges et al. 2001, 2004) within an environmental growth chamber for 2 weeks. Temperature was set to 10°C, light to 110 µmol m^{-2} s^{-1} at a photoperiod of 12 h light:12 h dark. The culture medium was refreshed twice a week.

Six 1 L screw-capped conical flasks containing 600 mL of ESAW were inoculated with algae at a final biomass of ca 0.5 g FW L^{-1}. These cultures were then incubated for 5 weeks under the same temperature, light and photoperiods as above, and aerated at 60–75 mL min^{-1} with CO_2-free air. The same pH–stating system was set to maintain the pCO_2 of the cultures at targets of either 385 or 750 µatm.

Quantification of growth

Growth of the algae was measured as the fresh weight increase day^{-1}. All algal thalli within each culture were quickly blotted dry using paper tissues (Kimtech, UK) and weighed to within 0.1 mg, before the start of the light cycle on days 4 and 7 in the *U. lactuca* experiment, and twice a week during the experiment with *U. clathrata*. Immediately thereafter, the thalli were returned to the culture vessel. Growth (g) was calculated as % increase of FW per day according to the following equation:

$$g = \left(\left(\left(T_1/T_0\right)^{1/n}\right) - 1\right) \times 100$$

where T_0 and T_1 are the algal fresh weights at the initial and following time points and n is the number of days between these time points.

Quantification of total inorganic carbon

Water samples were taken from each culture using bubble-free sampling with 10 or 20 mL glass syringes through a Teflon sampling tube integrated in the screw-caps of the conical flasks. Samples for total inorganic carbon (Ci) were taken on day 0, 3, 5 and 7 in the *U. lactuca* experiment and twice a week during the experiment with *U. clathrata*. Samples were filtered through an in-line filtration unit (Swinnex, Millipore, UK) holding a glass fibre filter (25 mm diameter, 0.7 µm pore size; type GF/F, Whatman, UK). Glass vials (24 mL volume) were completely filled from the bottom to prevent atmospheric exchange with the sample, sealed with Parafilm® (Menashu, WI, USA) and analysed immediately for Ci using a carbon analyzer (TOC-V CSH; Shimadzu, UK). Carbonate chemistry was calculated using Seacarb (Gattuso and Lavigne 2009).

Quantification of sulphur fractions

Quantification of the sulphur fractions followed the methods described in detail by Steinke et al. (2011) using a gas chromatograph (GC-2010, Shimadzu, Milton Keynes, UK) equipped with a 30 m × 0.53 mm × 5 µm capillary column (HP-1, Agilent, Wokingham, UK) and a flame photometric detector. Carrier gas (He) was supplied at 10.56 mL min^{-1} (linear velocity 80 cm s^{-1}) and the flame gases of compressed air and H_2 were set to 70 and 60 mL min^{-1}, respectively. A purge-and-trap method was used for the cryogenic enrichment of DMS for quantification of aqueous DMS and DMS production (Vogt et al. 2008b).

Aqueous DMS

Periodically we checked the concentrations of aqueous DMS in 10 mL samples of culture that was passed through an in-line filtration unit at low pressure. Since the cultures were constantly aerated, these measurements produced no detectable amounts of aqueous

DMS and, therefore, concentrations were well below the operational detection limit of 0.3 nM DMS (the lowest concentration used in the calibration).

DMS production

Gas samples were taken between 4 and 6 h into the light cycle, on the final day of the experiment with *U. lactuca*, and after 1, 2, 3 and 5 weeks in the experiment with *U. clathrata*. The outflow was collected for 3 min in Tedlar bags (Sigma-Aldrich) at a known outflow rate. Either four (*U. clathrata*) to five (*U. lactuca*) pseudoreplicates were taken. A gas sample (120 mL) was cryogenically enriched and quantified as described in Steinke et al. (2011).

Total DMSP

In the experiment with *U. lactuca*, triplicate samples from each pCO_2 treatment were taken for total DMSP ($DMSP_t$ = particulate DMSP + dissolved DMSP + DMS) from the media just before the start of the light period and 6 h into the light period on day 7. Samples (9.5 mL) were transferred to 22 mL glass vials. After addition of 0.5 mL of 10 M NaOH (final concentration 0.5 M) the vials were immediately closed using a Teflon-coated rubber septum. After incubation at 30°C for at least 12 h, headspace (200 µL) was injected into the GC calibrated against a series of standards from 0.25 to 300 µM DMSP. These were produced by adding a known quantity of DMSP into 22 mL vials containing, 9.5 mL of ESNW and 0.5 mL of 10 M NaOH. $DMSP_t$ production rate was calculated from the average of each triplicate sample, assuming a linear production rate over the 6 h. Since this provided only one calculated production rate per culture, the variation was conservatively estimated by adding together the standard errors calculated from triplicates at both time points.

Dissolved and particulate DMSP

In the experiment with *U. clathrata*, triplicate samples for particulate DMSP ($DMSP_p$) and dissolved DMSP ($DMSP_d$) were taken from each culture in weeks 1, 2, 3 and 5. This sampling was conducted six hours after the algal thalli had been transferred into fresh medium at the start of the light cycle. Gravity filtering of samples for $DMSP_d$ followed a protocol that was modified after Kiene and Slezak (2006). The first few mL of filtrate were rejected, and a 3 mL sample was transferred to a 4.92 mL glass vial containing 50 µL of concentrated sulphuric acid and sealed. After at least 2 days, the sample was basified with 358 µL of 10 M NaOH and the vial immediately resealed. After incubation at 30°C for at least 12 h, headspace (200 µL) was directly injected into the gas chromatograph for quantification. Since DMS was always below our detection limit (0.3 nM), no corrections for DMS were applied.

The filter was placed in a 4.92 mL vial containing 3 mL of 0.5 M NaOH for the determination of $DMSP_p$. After incubation at 30°C for at least 12 h, headspace (8 µL) was injected directly into the gas chromatograph. It is important to note that $DMSP_p$ represents particulate DMSP released from the macroalgal thalli due to cellular fragmentation and the release of reproductive cells, and so differs from intracellular DMSP as described below.

Intracellular DMSP

Algae were sampled for intracellular DMSP immediately following sampling for the other parameters. Eight individual thalli were haphazardly selected and a 15–20 mg portion was cut from this material. These were immediately placed in individual 4.92 mL vials containing 3 mL of 0.5 M NaOH. After incubation at 30°C for at least 12 h, headspace (8 µL) was injected directly into the gas chromatograph.

Statistical analyses

Microsoft Excel 2007 and Mintab statistical software (V.13.20) were employed for statistical testing. Data was tested for normality using the Anderson–Darling test and homogeneity of variance with an F-test. Where possible, nested-analysis of variance (ANOVA) was used to test differences between conditions and experimental run or week of incubation, followed by pairwise tests to determine significant groupings. If parametric conditions were not satisfied, a Kruskal–Wallis (K–W) test was used. Linear regressions were used to determine significant trends in the data.

Results

Incubation experiments

Carbonate chemistry

In *U. lactuca*, the mean pCO_2 of the cultures was 432, 635, 884, 1182 and 1514 µatm during the incubation, higher than the targeted values of 385, 550, 750, 1000 and 1250 µatm. In *U. clathrata*, the mean pCO_2 of the cultures during the incubation was 461 and 881, higher than the targeted values of 385 and 750 µatm. These increases in pCO_2 were likely due to a net increase in the cultures' carbonate alkalinity during the incubation.

Growth rate

The mean growth rate of *U. lactuca* was 11.4 ± 1.4% FW day^{-1}, and was not significantly different between pCO_2 conditions or the four experimental runs ($p > 0.05$; Table 1). The mean growth rate of *U. clathrata* was 13.7 ± 0.05% FW day^{-1} and was not significantly different between pCO_2 conditions ($p > 0.05$); however, over the 5 week incubation period significant differences were found between the individual incubation weeks (nested ANOVA, $p < 0.001$, $F_{1,8,20} = 5.81$) with growth rates ranging from 9.2 ± 2.2 to 19.2 ± 5.0% FW day^{-1}.

Table 1 Growth rate, intracellular concentration of DMSP and the production rates of total, particulate and dissolved DMSP ($DMSP_t$, $DMSP_p$, $DMSP_d$, respectively) in cultures of *Ulva lactuca* and *U. clathrata* following incubation at different levels of pCO_2 for 1 week

	Ulva lactuca			*Ulva clathrata*		
Parameter	pCO_2	Mean	SD	pCO_2	Mean	SD
Growth rate (% FW day^{-1})	432	11.6	0.4	461	15.3	0.02
	635	12.3	0.7			
	884	10.9	0.6	881	14.0	0.02
	1182	11.0	0.8			
	1514	11.1	0.9			
Intracellular DMSP (mmol g^{-1} FW)	432	99	20	461	69	3
	635	98	4			
	884	100	17	881	76	5
	1182	103	11			
	1514	101	10			
DMS production rate (nmol h^{-1} g^{-1} FW)	432	0.46*a*	0.07	461	6.23	0.73
	635	0.40*a*	0.11			
	884	0.27*b*	0.08	881	5.40	0.63
	1182	0.19*b*	0.06			
	1514	0.21*b*	0.08			
$DMSP_t$ production rate (µmol h^{-1} g^{-1} FW)	432	6.8	5.6	No data		
	635	2.1	5.5			
	884	6.6	6.4			
	1182	10.7	8.6			
	1514	10.9	9.8			
$DMSP_p$ production rate (µmol h^{-1} g^{-1} FW)	No data			461	32.8	19.8
				881	56.3	17.5
$DMSP_d$ production rate (nmol h^{-1} g^{-1} FW)	No data			461	3.4	1.7
				881	5.0	13.4

Data show the mean and standard deviation (SD; $n = 4$ for *U. lactuca* and $n = 3$ for *U. clathrata*). *Italicised letters* indicate significant statistical groupings ($p < 0.05$)

Intracellular DMSP

No change in intracellular DMSP was detected for any pCO_2 conditions in either species ($p > 0.05$). The intracellular DMSP content of *U. lactuca* was 101 ± 21 mmol g^{-1} FW, however, there was a significant difference between experimental runs. The freshly collected material showed an increase from 85 ± 6 mmol g^{-1} FW in the first run to 109 ± 6 mmol g^{-1} FW in the fourth consecutive run (nested ANOVA, $p < 0.001$, $F_{4,15,129} = 4.19$). In *U. clathrata,* intracellular DMSP was 68.9 ± 20.0 mmol g^{-1} FW with no variation between the incubation weeks ($p > 0.05$).

DMS production rate

In *U. clathrata* DMS production rate was not statistically different between the two treatments with rates of 6.23 ± 0.73 nmol g^{-1} FW h^{-1} at 461 µatm and 5.40 ± 0.63 nmol g^{-1} FW h^{-1} at 881 µatm. However, each week the mean DMS production at 881 µatm was 10–19% lower in comparison to 461 µatm. In contrast, the DMS production rate in *U. lactuca* was significantly different between pCO_2 conditions (nested ANOVA, $p < 0.0001$, $F_{4,15,72} = 11.87$), declining from 0.46 ± 0.07 nmol h^{-1} g^{-1} FW at 432 µatm, to 0.21 ± 0.08 nmol h^{-1} g^{-1} FW at 1514 µatm (Fig. 1). DMS production rate did not change significantly with different experimental runs ($p > 0.05$). Post hoc nested ANOVAs established two significantly different groupings at 432 and 635 µatm, and 884, 1182 and 1514 µatm ($p < 0.01$). A regression of the data established a significant decline in DMS production rate with increasing pCO_2 ($p < 0.0001$, $df = 1, 90$) with a decrease in DMS production of 27.6% at 884 µatm and 65.6% by 1514 µatm (Fig. 1) in comparison to production at the lowest pCO_2 level of 432 µatm. However, the grouping of the pCO_2 conditions may also suggest a step function with a sudden almost 50% loss of DMS production at a tipping point between 635 and 884 µatm (Fig. 1).

Total DMSP

The $DMSP_t$ production rate from *U. lactuca* initially showed no significant differences between pCO_2 conditions, but there was a significant difference of

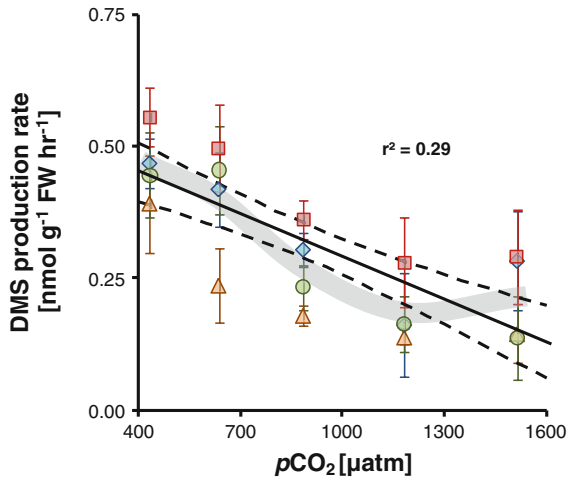

Fig. 1 Production of DMS in *Ulva lactuca* incubated for 1 week over a range of pCO_2 levels. Data shown are from experimental runs 1–4 and are indicated by *triangles*, *diamonds*, *squares* and *circles*, respectively. Each symbol represents the mean ± one standard deviation. A linear regression and 95% confidence intervals are shown. The *grey line* shows the mean of experimental runs and indicates a sharp decrease in DMS production between 635 and 884 µatm

up to ten fold between experimental runs (K–W, $p < 0.005$, $df = 3,17$; Fig. 2a). When the results for each pCO_2 were expressed as a percentage of total $DMSP_t$ released during a specific experimental run, a significant regression was found ($r^2 = 0.28$, $p < 0.05$, $df = 1,18$; Fig. 2b). This indicates that, despite considerable variation between experimental runs, the production of $DMSP_t$ increased by 65% at 1514 µatm in comparison to 432 µatm.

Particulate DMSP and dissolved DMSP

The production of both $DMSP_p$ and $DMSP_d$ were not significantly affected by pCO_2 in the experiment with *U. clathrata* ($p > 0.05$).

Discussion

During the incubations there was a net increase in the carbonate alkalinity in the cultures. This is in contrast to the usual trend during many incubation studies where alkalinity is often depleted due to photosynthetic activity and calcification of the experimental organism. Macroalgae have been shown to excrete hydroxide (OH^-) in greater amounts than is produced

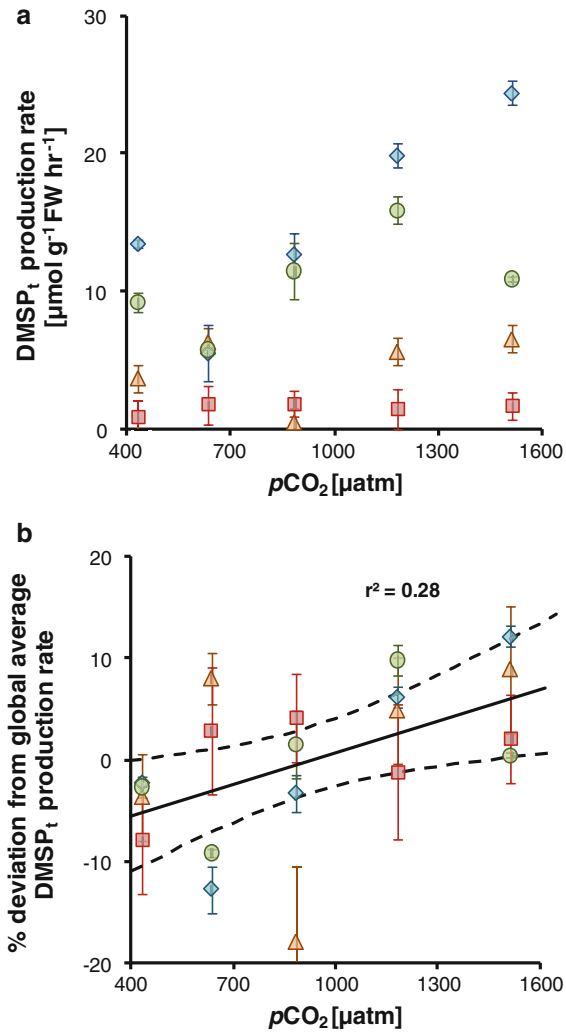

Fig. 2 Production of total DMSP ($DMSP_t$) in *Ulva lactuca* following incubation for 1 week over a range of pCO_2 levels. Data shown are from experimental runs 1–4 and are indicated by *triangles*, *diamonds*, *squares* and *circles*, respectively. Each *symbol* represents the measured value ± estimated error (see "Methods"). **a** Raw data of $DMSP_t$ production indicate high diversity of rates between individual experimental runs. **b** Percentage variation in the global average total $DMSP_t$ released from each run. A linear regression and 95% confidence intervals are shown. A regression line parallel to the *x*-axis at 0% would indicate that there was no change in production under various pCO_2

by the enzymatic conversion of bicarbonate (HCO_3^-) to CO_2 and OH^-, and this may assist with maintaining electroneutrality and pH balance during active HCO_3^- uptake (Cook et al. 1988). These OH^- then react with external CO_2 to form more HCO_3^-. Such a loss of CO_2 is automatically replenished by the pH–stating system. Internal HCO_3^- often exceeds external concentration by an order of magnitude. If some of the imported HCO_3^- were then exported, or leaked, into the external medium, this could lead to the increase in carbonate alkalinity detected by this study. Such fluxes of carbonate system components could be used to dissipate excess light energy as suggested by Tchernov et al. (2003). This phenomenon has not been observed in studies of natural macroalgal ecosystems (Middelboe and Hansen 2007) and is probably unique to the artificial environment of our pH–stating system.

The ramification of this increase, at constant pH, was that the actual mean pCO_2 was greater than initially targeted. This meant that the pCO_2 of 432 and 461 µatm was at the high-end of the range designated as 'control' values in a recent meta-analysis (Hendriks et al. 2010).

Growth and intracellular DMSP

Here we show the first results from a pH–stated ocean acidification experiment with two species of *Ulva*. Similar to the results presented by Israel and Hophy (2002), an increase in pCO_2 did not affect somatic growth in *Ulva*. A CO_2-dependent stimulation of growth was demonstrated in other studies involving *Ulva* spp. (Björk et al. 1993; Gordillo et al. 2003), however, these experiments are not suitable to predict the effects of ocean acidification, as the carbonate chemistry was poorly controlled and unrealistic pCO_2 conditions were chosen (Barry et al. 2010).

The intracellular DMSP was also not affected by the differences in pCO_2. As far as we are aware, data on the response of intracellular DMSP to higher CO_2 concentrations are lacking for macroalgae. However, a decrease in CO_2 results in the increase of intracellular DMSP in the diatom *Thalassiosira pseudonana* and the coccolithophore *Emiliania huxleyi,* which may be in response to increased oxidative stress during carbon limitation (Sunda et al. 2002; Bucciarelli and Sunda 2003). This suggests that DMSP is sensitive to a decrease in CO_2 and more information is needed to constrain the effect of increasing CO_2 on intracellular DMSP.

A study by Lee et al. (2009) investigated changes in DMSP during on-deck incubations of a natural phytoplankton community at: ambient conditions, elevated pCO_2 (690 µatm), elevated temperature (ambient + 4°C), and 'greenhouse' conditions (elevated pCO_2 and

elevated temperature) in the North Atlantic spring bloom. While their findings do not suggest a change in intracellular DMSP content under the different treatments, they do indicate a competitive benefit to coccolithophores and pelagophytes from elevated temperatures and greenhouse conditions. This effectively increased the community's DMSP cell^{-1} due to the typically high intracellular concentration that occurs in coccolithophores (Franklin et al. 2010). Nonetheless, the complex interactions in natural communities make direct comparison with laboratory cultures of individual species challenging.

DMS production

We found a highly significant decrease in DMS production over the range of pCO_2 levels examined in *U. lactuca*. A similar significant decrease was not observed in *U. clathrata*. Although a linear relationship between pCO_2 and DMS production was found in *U. lactuca*, the results also suggest a sudden decrease in production of close to 50% between 635 and 884 µatm. Such increases in pCO_2 are likely to be achieved within the next 100 years and may result in a step-wise reduction in DMS production. This is of key concern, since DMS is an important link in the global sulphur cycle, and this change may rapidly alter the global biogeochemical cycling of sulphur via a decrease in DMS emissions to the atmosphere. The oxidation products of volatilised DMS in the marine boundary layer act as a significant source of cloud condensation nuclei (CCN; Shaw 1983). These can increase the backscattering of sunlight and the albedo of clouds above the remote oceans, and so reduce the energy that reaches the Earth's surface (Twomey et al. 1984; Andreae 1990). It was hypothesised by Charlson et al. (1987) that DMS production from phytoplankton may act as a negative feedback to increased global temperatures by increasing planetary albedo in response to climate change. Our study demonstrates that increased pCO_2, the major driver of anthropogenic global warming, may decrease DMS production in chlorophyte macroalgae. Whilst these organisms typically occur in the coastal zone, DMS emissions here are not implicated to affect planetary albedo due to the abundance of land-derived particles which can act as CCN (Simó 2001). However, if a common response is found in other algal taxa throughout the pelagic realm, as demonstrated by some mesocosm experiments (see above), it would suggest a positive feedback on the future increase in atmospheric pCO_2.

Green tides of *Ulva* can occur as substantial free-floating masses in the open sea. The largest recorded bloom was in the Yellow Sea and East China Sea (He et al. 2011), covering an area of between 13,000–30,000 km^2 (Leliaert et al. 2008; Liu et al. 2009) with some masses detected up to 350 km from the nearest coast (He et al. 2011). The DMS produced by these macroalgae likely represents a significant regional source of DMS in affected areas. However, due to the abundance of anthropogenic aerosol in this region (Kim et al. 2009), DMS from these blooms will contribute little to CCN formation and will likely not affect local climate. Nevertheless, it is possible that drifting macroalgal blooms may act as a significant source of CCN to the marine atmosphere along and beyond the continental shelf.

$DMSP_t$, $DMSP_p$ and $DMSP_d$

The production of $DMSP_d$ by algae is thought to be due to either passive leakage of cell contents (Fogg 1983), cell lysis (Nguyen et al. 1988) or overflow production (Stefels et al. 2007). $DMSP_d$ production was not affected by pCO_2 in *U. clathrata* and occurred at a rate similar to DMS production. Although $DMSP_d$ was not measured during the *U. lactuca* experiment, its release is predicted to occur at a similar magnitude to its close relative *U. clathrata*. This indicates that the main contributor to $DMSP_t$ production in *U. lactuca* was $DMSP_p$ production, which in *U. clathrata* was 1000-times greater than the release of $DMSP_d$.

$DMSP_p$ production was most likely linked to the release of reproductive cells (haploid gametes and/or diploid spores) during the day, which sometimes resulted in a noticeable increase in the turbidity of the media. These releases occurred, to differing degrees, throughout both experiments. Typically, the increased turbidity would disappear overnight and green deposits would appear on the bottom of the flasks due to the settlement of reproductive cells. Although this cannot be directly linked to an increase in reproductive output, the results of the present study suggest that increased pCO_2 stimulated the release of $DMSP_p$ by *U. lactuca*, a response not observed in *U. clathrata*. Since the cultures were not axenic, the detachment of microbial epiphytes from the thalli may have contributed to $DMSP_p$. However, this was

considered insignificant relative to the visibly noticeable release of reproductive cells.

Inter-week and inter-run variation in parameters

Natural weekly rhythms in growth and reproduction occur in macroalgae, often coordinated through changing environmental conditions (Lüning et al. 2008). This explains the large variation in growth rates in *U. clathrata* over the 5 week experiment and the substantial inter-run differences in $DMSP_t$ production by *U. lactuca*. The inter-run difference in intracellular DMSP in this species may result from the seasonal variation of DMSP content known in naturally occurring macroalgae (Lyons et al. 2007).

Role of bacteria

Bacteria were present in our incubations and it is well established that they affect the cycling of dissolved DMSP and DMS, since multiple metabolic pathways exist in prokaryotes that catabolise DMSP, not all of which result in DMS (Johnston et al. 2008). Furthermore, DMS consumption has also been documented in various bacteria (Schäfer et al. 2010). It may appear desirable to conduct experiments in the absence of bacteria, however, bacteria affect *Ulva* physiology and specific bacteria are essential to produce the typically presented morphological growth (e.g. Marshall et al. 2006). This makes it difficult to separate the effect of bacterial and algal processes on DMS and DMSP dynamics where the results are of interest for the prediction of future DMS production in marine environments.

Advantages and disadvantages of pH–stat methodology

The experimental set-up and the measurements of DMS production relied on a constant supply of air and pulses of CO_2 into the cultures to maintained the pH. As our measurements for aqueous DMS revealed, this efficiently purges DMS from the medium to the stream of waste air. In steady state, the concentration of DMS in the waste air is equivalent to DMS production. This can be quantified via discrete measurements of DMS (this study) or when connected to an on-line Fast DMS Sensor that allows near real-time measurements of DMS production (Green et al. 2011). In contrast to some microalgae, Ulvales and other macroalgae are particularly suitable for such DMS production measurements since the amount of shear stress experienced by individual cells is low under these conditions.

A drawback of using macroalgae for this type of research is that the DMSP content is highly variable even within the same type of tissue (Van Alstyne et al. 2007). This variability hinders the measurement of significant change during acclimation to various environmental conditions without an appropriately large response or large sample size. Another possible complication is that in almost all ocean acidification experiments such as here, pCO_2 was increased within a few minutes in comparison to the relatively slow increase of mean pCO_2 occurring currently in nature. Although macroalgae acclimate to various stressors within 1 week, it cannot be ruled out that many measurements conducted in ocean acidification experiments represent a stress response, and adaptation and selection could operate in the long term.

The pH–stating system tightly controls pH, while the constant bubbling will maintain the pO_2 at around 0.2 atm. This is an artificial environment as macroalgae in natural settings may undergo significant diel variations in pH and O_2 (Middelboe and Hansen 2007). While these natural variations in pH may have a significant impact on algal physiology and, hence, DMS and DMSP production, the standardised conditions of the presented experiments allow greater comparability between conditions and avoid problems associated with variation in complex natural systems.

Conclusion

Increasing pCO_2 from ambient (432 µatm) to future (1514 µatm) resulted in a significant decrease in DMS production in *U. lactuca* that was most pronounced around a 'tipping point' between 635 and 884 µatm. The mean production of DMS in *U. clathrata* was also lower when comparing ambient with future pCO_2 (881 µatm) but this decrease was statistically not significant with the relatively low sample size in our experiment. It is also possible that the response of *Ulva* to an increase in pCO_2 is species-specific so that the tipping points may occur at different pCO_2 with different species. This diversity and the fact that the mechanisms behind such physiological changes are

unknown, make general predictions on the future production of DMS from *Ulva*-dominated environments difficult. *Ulva* is a major contributor to harmful green tides which are predicted to become more dominant in the future (Ye et al. 2011). Importantly, since these can form free-floating blooms in the open sea, its DMS production is not limited to coastal ecosystems but may add significantly to DMS in and around the shelf regions that are traditionally associated with DMS from phytoplankton.

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