

1 **Ocean acidification has different effects on the production of DMS and DMSP measured in cultures of**
2 ***Emiliania huxleyi* and a mesocosm study: a comparison of laboratory monocultures and community**
3 **interactions**

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14 **Environmental Context**

15 About 25% of CO₂ released into the atmosphere by human activities has been absorbed by the oceans, resulting
16 in a process known as ocean acidification. This investigation focuses on the acidification effects on marine
17 phytoplankton and subsequent production of the trace gas dimethylsulphide (DMS), a major route for sulphur
18 transfer from the oceans to the atmosphere and the land. Increasing surface water pCO₂ has differential effects
19 on the growth of different phytoplankton groups, and has resulted in varying responses in net community DMS
20 production and therefore DMS release to the atmosphere.

21 **Abstract**

22 The human-induced rise in atmospheric carbon dioxide since the industrial revolution has led to increasing
23 oceanic carbon uptake and changes in seawater carbonate chemistry, resulting in lowering of surface water pH. In
24 this study we investigated the effect of increasing pCO₂ on concentrations of volatile biogenic dimethylsulphide
25 (DMS) and its precursor dimethylsulphoniopropionate (DMSP), through monoculture studies and community
26 pCO₂ perturbation. DMS is a climatically important gas produced by many marine algae: it transfers sulphur into
27 the atmosphere and is a major influence on biogeochemical climate regulation through breakdown to sulphate
28 and formation of subsequent cloud condensation nuclei (CCN). Overall, production of DMS and DMSP by the
29 coccolithophore *Emiliania huxleyi* strain RCC1229 was unaffected by growth at 900 µatm pCO₂, but DMSP
30 production normalised to cell volume was 12% lower at the higher pCO₂ treatment. These cultures were
31 compared with community DMS and DMSP production during an elevated pCO₂ mesocosm experiment with the
32 aim of studying *E. huxleyi* in the natural environment. Results contrasted with the culture experiments and
33 showed reductions in community DMS and DMSP concentrations of up to 60% and 32% respectively at pCO₂ up to
34 3000 µatm, with changes attributed to poorer growth of DMSP-producing nanophytoplankton species, including

35 *E. huxleyi*, and potentially increased microbial consumption of DMS and dissolved DMSP at higher $p\text{CO}_2$. DMS and
36 DMSP production differences between culture and community likely arise from pH affecting the inter-species
37 responses between microbial producers and consumers.

38 Introduction

39 Since the 1750s, atmospheric carbon dioxide concentrations have increased from 280 to close to 400 μatm today
40 due to anthropogenic inputs from burning fossil fuels, cement production and land use changes.^[1] The
41 atmospheric $p\text{CO}_2$ concentrations projected for 2100 are in the range 350 – 840 μatm ; the majority of climate
42 change scenarios project continuing increases over coming decades, with the possibility of decline through
43 immediate change to low-carbon economies.^[2] Approximately 25% of the total CO_2 emitted into the atmosphere
44 by anthropogenic activities has been absorbed into the oceans to date, making the oceans a crucial sink for CO_2 ,
45 with other sinks including the atmosphere (~45%) and land-based vegetation (~30%).^[3] Dissolution of CO_2 in
46 seawater results in the formation of carbonic acid, which readily dissociates to release H^+ and lower the pH, an
47 effect termed ‘ocean acidification’. Surface ocean pH levels will very likely be up to 0.4 units lower by 2100, a
48 concomitant 150 % increase in H^+ ions, which will decrease the carbonate saturation state and result in increasing
49 dissolution of calcium carbonate in surface waters.^[4,5]

50 *Emiliania huxleyi* is a globally distributed haptophyte which produces calcite plates (coccoliths) covering the cell
51 surface. Large-scale blooms of *E. huxleyi* occur in temperate shelf seas, including the North West European
52 continental shelf in early summer,^[6] and total global production of calcite by *E. huxleyi* makes it the most
53 productive calcifying organism on Earth.^[7] Under conditions of elevated $p\text{CO}_2$ in an ocean acidification scenario,
54 calcite production by *E. huxleyi* has been found to typically decrease.^[8,9] Calcium carbonate formation is a
55 reaction that liberates CO_2 ($\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}$), and any reduction in calcification rate can act as
56 a negative feedback on rising surface water $p\text{CO}_2$.^[10] Over longer timescales, calcite and organic carbon
57 production by calcifying phytoplankton, and subsequent post-bloom settlement of this material through the
58 water column is a major route for carbon transport from the surface oceans to storage in deeper waters.^[11]
59 Decreased surface pH could affect growth and subsequent calcite production and carbon fixation by *E. huxleyi*
60 and have a significant impact on global cycling and removal of carbon in the future ocean.^[8]

61 *E. huxleyi* is also a significant producer of dimethylsulphoniopropionate (DMSP), a compound produced by many
62 phytoplankton species for several suggested purposes: as an osmoregulatory compound,^[12] cryoprotectant,^[13]
63 anti-oxidant,^[14] grazing defence^[15] or chemoattractant.^[16,17] DMSP is recognized as a significant part of the sulphur
64 and carbon fluxes through marine microbial food webs, providing a reported 0.5 to 6 % of total carbon demand
65 and between 3 and 100 % of total sulphur demand by marine bacteria^[18] and major phytoplankton groups.^[19]
66 Breakdown of DMSP is a significant source of dimethylsulphide (DMS), a volatile compound released through the
67 surface microlayer to the atmosphere where it oxidises to form sulphate-containing particles. These particles can

act as cloud condensation nuclei (CCN) in the troposphere, where cloud formation can reflect the Sun's energy back into space, with implications for global climate regulation.^[20,21] The marine DMS-associated global sulphur flux to the atmosphere has been calculated at 28.1 Tg S per year.^[22]

Previous community $p\text{CO}_2$ perturbation experiments in natural waters have identified changes in DMS and DMSP concentrations as $p\text{CO}_2$ increased.^[23–28] Here we investigated the effects of elevated $p\text{CO}_2$ on DMS and DMSP production in a low-bacterial abundance monoculture of *E. huxleyi* (strain RCC1229), and progressed to investigate the effect of $p\text{CO}_2$ on a community known to contain a natural *E. huxleyi* population. The hypotheses of this investigation were that elevated $p\text{CO}_2$ would affect the physiology of the *E. huxleyi* cell and result in lower production of intracellular DMSP, which would result in lower DMS production. On a community level, elevated $p\text{CO}_2$ may stimulate primary productivity, resulting in increased community DMSP synthesis and higher DMSP concentrations.^[29] In contrast, an increase in bacterial productivity at elevated $p\text{CO}_2$ would create a greater demand for sulphur and increase DMS and DMSP consumption.^[30,31] This investigation aimed to determine if changes in DMS and DMSP concentrations under high $p\text{CO}_2$ are a result of physiological changes in the *E. huxleyi* cell, or changes in microbial inter-species responses to elevated $p\text{CO}_2$, nutrient competition and DMSP consumption.

83

84 ***Emiliania huxleyi* Culture Setup**

E. huxleyi strain RCC1229 was chosen for its high level of calcification and origin in the North Sea (as a strain isolated close to the location of the mesocosm experiment) and grown in autoclaved aged natural seawater medium enriched with ESAW (Enriched Seawater Artificial Water) nutrients (starting concentration $186.7 \mu\text{mol L}^{-1}$ NO_3 and $20.1 \mu\text{mol L}^{-1}$ PO_4) and vitamins.^[32] The stock culture was treated for 2 days with a broad-spectrum antibiotic mixture^[33] to significantly reduce bacterial abundance, before regular reinoculation into fresh medium to maintain exponential growth for 10 days prior to $p\text{CO}_2$ perturbation (day T0). All cultures were maintained at 15°C in a 16:8 light/dark cycle with light at $180 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Cells were grown in a semi-continuous culture, with three replicate cultures exposed to $900 \mu\text{atm } p\text{CO}_2$ and three replicate control cultures treated with air at ambient $p\text{CO}_2$ ($395 \mu\text{atm}$) Prior to inoculation, the medium was filter sterilised, decanted into two bespoke vessels and pre-sparged to the $p\text{CO}_2$ treatment concentration using pre-prepared CO_2 gas mixtures (BOC Ltd, UK). Cultures were grown in 1 L Erlenmeyer flasks with 500 mL of pre-prepared sterile medium and sufficient inoculum to provide a starting cell count of $120,000 \text{ cells mL}^{-1}$. Cultures were grown over 4 day periods to cell densities of ca. $1,000,000 \text{ cells mL}^{-1}$ before re-inoculation into fresh medium to keep the culture in exponential growth. Flasks were sealed with ground glass Quikfit stoppers modified to enable inlet and outlet gas lines. Aqueous phase bubbling of the cultures was avoided but the headspaces of each flask were flushed daily with the respective treatment gas for 10 minutes at a rate of 30 mL

101 min⁻¹ through a 0.2 µm Minisart filter (Sartorius Ltd, Epsom, U.K.). Samples were extracted from the flasks
102 through a luer-lock sealed opening in the base of the flask; to prevent contamination of the culture, all sampling
103 from this outlet used sterile luer fittings on 25 mL glass syringes.

104 *Measurement of Biological Parameters*

105 Culture samples for cell volume, cell counts, pH, DMS and total DMSP (DMSP_T) were taken daily 7h after the onset
106 of the light period. Cell volume and counts were measured in triplicate from live culture using a Coulter Multisizer
107 III (Beckman Coulter Ltd, High Wycombe, U.K.). Average growth rates were determined for each inoculation
108 period as $\ln(N_1/N_0)/(t_1 - t_0)$, with cell counts N_0 and N_1 taken at the time points t_0 and t_1 respectively. All six
109 cultures were examined under x100 magnification using an Olympus BX40F-3 fluorescence microscope and no
110 non-calcified cells could be identified from multiple prepared samples. For pH analysis, 20 mL of culture from
111 each flask was analysed daily at 15°C by the standard potentiometric technique^[34,35] using a Seven Easy S20 probe
112 with automatic temperature adjustment (relative accuracy ±0.01 Mettler-Toledo Ltd, Beaumont Leys, U.K.) using
113 NBS buffers.

114 *DMS and DMSP Analysis*

115 DMS samples were extracted by injection of 2mL of filtered culture into a PTFE purge and cryotrap system and
116 purged with oxygen-free nitrogen (OFN) for 5 minutes at 80 mL min⁻¹. Samples were trapped in a PTFE sample
117 loop suspended above liquid nitrogen and held at -150°C, before immersion in boiling water and injection into a
118 Shimadzu GC2010 gas chromatograph (GC) with a Varian Chrompack CP-Sil-5CB column (30m, 0.53mm ID) and
119 flame photometric detector (FPD). The GC was operated isothermally at 60°C and DMS eluted at 2.1 minutes; the
120 GC was calibrated using liquid DMSP standards treated with 10M NaOH in the concentration range 5.07 – 406.2
121 nmol L⁻¹ (7% analytical error through analysis of 10 samples). Six-point calibrations were performed weekly and
122 checked daily for instrument drift, and the resulting calibrations typically produced linear regression with $r^2 > 0.99$.
123 The same method was used when participating in the AQA 12-23 international DMS analysis proficiency test in
124 February 2013 and achieved close agreement with the concentration of the test material.^[36]

125 Triplicate DMSP_T samples from each flask were prepared in 4 mL headspace vials by the addition of 0.5 mL 1M
126 NaOH to 3 mL of culture and sealed using PTFE screw caps and PTFE/ silicone septa. All DMSP vials were stored
127 for 24 hours at 30°C before an MPS2 Twister multi-purpose autosampler (Gerstel, Mülheim, Germany) equipped
128 with a 250 µL Hamilton syringe sampled 100 µL of headspace from each vial and injected it into the GC-FPD as set
129 up above.

130 **Mesocosm Experiment Setup**

131 The experiment was performed at the Marine Biological Station at Espegrend, University of Bergen, Norway from
 132 6th May to 12th June 2011, with nine cylindrical thermoplastic polyurethane (TPU) mesocosm enclosures (ca 75 m³,
 133 25m water depth) anchored approximately 100 m apart and 1 mile offshore in the Raunefjord (60.265°N, 5.205°E)
 134 at a water depth of 55 to 65m. Each enclosure was supported by an 8m tall floating frame and capped with a
 135 polyvinyl chloride (PVC) hood.^[37] Over 95% of the incoming photosynthetically active radiation (PAR) was
 136 transmitted by the TPU and PVC materials, with near 100% absorbance of incoming UV radiation. The mesocosms
 137 were filled on the 1st May 2011 (day T-7) by lowering the bags through the CO₂ under-saturated post-bloom water
 138 column with the bottom openings covered with 3 mm mesh to exclude larger organisms. Full exclusion of the
 139 mesocosms from the surrounding waters occurred 3 days later: the lower opening was fitted with a sediment trap
 140 and the upper openings were raised above the water surface.^[37]

141 The carbonate chemistry of the water was altered by the addition of CO₂-saturated, filtered fjord water to alter
 142 the dissolved inorganic carbon (DIC) concentrations while keeping alkalinity constant.^[38] This water was added to
 143 7 mesocosms depending on the target *p*CO₂ concentrations over a 5 day period, starting on the 8th May 2011 (day
 144 T0). This was done with a bespoke dispersal apparatus ('Spider') that was lowered through the bags to ensure
 145 even distribution of CO₂-rich waters throughout the water column. Two mesocosms were designated controls and
 146 received no addition of CO₂ enriched water (M2 and M4, 280 µatm). The range of target *p*CO₂ was 390 to 3000
 147 µatm across the seven enriched mesocosms (M6, 390 µatm; M8, 560 µatm; M1, 840 µatm; M3, 1120 µatm; M5,
 148 1400 µatm; M7, 2000 µatm; M9, 3000 µatm) taking into account IPCC projections up to the year 2300 and
 149 beyond,^[2] in order to identify the change in different parameters to increasing *p*CO₂. *p*CO₂ and pH were calculated
 150 from the coulometric measurement of DIC^[39] and spectrophotometric determination of pH^[40] using the
 151 stoichiometric equilibrium constants for carbonic acid^[41,42]. No further perturbation was made to the carbonate
 152 system once the experiment had commenced. Inorganic nutrients were added to each mesocosm on T14 to
 153 stimulate phytoplankton growth. The inside of the mesocosm walls was cleaned regularly with a ring-shaped,
 154 double-bladed wiper to prevent biofilm growth.^[37] After termination of the experiment, one small hole was
 155 detected in the bag of M2 which had led to non-quantifiable water exchange, so the results from this mesocosm
 156 were removed from the analysis.

157 *DMS and DMSP Extraction and Analysis*

158 An integrated water sampler (IWS, Hydrobios GmbH, Kiel, Germany) was used every morning to collect samples
 159 from the full 25m water depth of all nine mesocosms. Samples for DMS and DMSP analysis were collected in an
 160 amber bottle from the laminar flow of the IWS using Tygon tubing and the bottle was allowed to overflow for
 161 twice the volume before the tube was removed and the glass stopper firmly inserted to prevent air bubbles and
 162 atmospheric contact. DMS samples (40 mL) were injected into a purge and cryotrap system^[43] through a 25 mm
 163 Whatman GF/F (GE Healthcare Life Sciences, Little Chalfont, England) and were purged with oxygen-free nitrogen
 164 (OFN) at 80 mL min⁻¹ for 10 minutes. Gas samples passed through a glass wool trap to remove aerosols and water

165 droplets, and a series of two nafion counterflow driers operating at 180 mL min⁻¹, before DMS was trapped in a
166 stainless steel sample loop held above liquid nitrogen at -150°C.

167 DMS samples were injected into an Agilent 6890 gas chromatograph equipped with a 60m DB-VRX capillary
168 column (0.32 mm ID, 1.8 µm film thickness, Agilent J&W Ltd) according to the programme outlined by Hopkins *et*
169 *al.*^[24] Analysis was by an Agilent 5973 quadrupole mass spectrometer operated in electron ionisation (EI), single
170 ion mode (SIM), and was calibrated using a gravimetrically prepared liquid DMS standard diluted in HPLC-grade
171 methanol to the required concentration in the range 0.04 – 7.64 nmol L⁻¹ (10% analytical error for triplicate
172 measurements). GC-MS Instrument drift was corrected using 2 µL of a diluted deuterated DMS (D₆-DMS) as a
173 surrogate analyte.^[44,45] Five-point calibrations were performed weekly, and checked daily, and the linear
174 regression from the calibrations typically produced values $r^2 > 0.98$.

175 DMSP_T samples were prepared for later analysis using the acidification method of Curran *et al.*^[46,47] by storing 7
176 mL of unfiltered aliquots of seawater in 8 mL glass sample vials (Labhut, Churcham, UK) with 0.35 µL of 50%
177 H₂SO₄. All samples were stored in the dark at room temperature for 8 weeks prior to analysis. DMSP_T was
178 extracted by purging of 2 mL unfiltered sample with 1 mL 10M NaOH with OFN for 5 minutes at 80 ml min⁻¹,
179 before analysis by GC-FPD as described above.

180 *Additional Measurements*

181 Water samples were collected from the IWS every first or second day, and phytoplankton abundances were
182 determined with a FACS Calibur flow cytometer (Becton Dickinson) equipped with an air-cooled laser providing 15
183 mW at 488 nm with standard filter set-up. The counts were obtained from fresh samples with the trigger set on
184 red. Discrimination of *Synechococcus* spp., *Emiliania huxleyi*, and autotrophic picoeukaryotes, cryptophytes and
185 other autotrophic nanoeukaryotes was based on dot plots of side-scatter signal (SSC) versus pigment
186 autofluorescence (Chlorophyll-*a* and phycoerythrin).^[48]

187 For determination of chlorophyll-*a* (Chl-*a*) concentrations, aliquots of 250-500 mL of sample from the IWS were
188 also filtered onto GF/F and stored frozen for 24 hours prior to homogenisation in 90% acetone with glass beads.
189 The mixture was centrifuged at 800 x g and the Chl-*a* concentrations were determined on a Turner AU-10
190 fluorometer.^[49] Further samples were extracted in 100 % acetone and analysed by high performance liquid
191 chromatography (WATERS HPLC with a Varian Microsorb-MV 100-3 C8 column),^[50] with phytoplankton
192 community composition calculated using the CHEMTAX algorithm by converting the concentrations of marker
193 pigments to the Chl-*a* equivalents.^[51,52]

194 *Statistical Analysis*

195 Statistical analysis was performed using Minitab v16. All data were checked for normality using an Anderson –
196 Darling test prior to statistical analysis, and were transformed where necessary. Equal variance was confirmed

197 using Levene's Tests. One-way ANOVA combined with Tukey's post analysis tests were used on the DMS and
198 DMSP data to determine differences between the mesocosms at different $p\text{CO}_2$ concentrations. Spearman's Rank
199 Correlation was also used to determine the relationships between $p\text{CO}_2$ and DMS and DMSP concentrations over
200 the course of the experiment, as well as the relationships between different community variables and the trace
201 gas concentrations. Two-tailed t-tests were used to determine differences between the control and CO_2
202 treatments during the laboratory studies.

203

204 ***E. huxleyi* High $p\text{CO}_2$ Culture Experiment Results**

205 *Growth Parameters*

206 pH within the CO_2 treatment cultures started at a mean of 7.43 immediately following inoculation compared to
207 7.90 in the air control (Figure 1a). As the culture grew, the pH gradually increased in all flasks, but in the CO_2
208 treatment cultures pH was significantly lower than for the air control ($T=7.68$, $p<0.01$), and re-inoculation reduced
209 the pH in all cultures. Mean pH for the entire experiment was 7.72 in the CO_2 treatment and 8.13 in the control.
210 Cultures from both treatments grew exponentially for four days after inoculations 1, 2 and 3, and for five days in
211 the fourth and fifth inoculations. Cell counts at the end of each inoculation period ranged from 6.3×10^5 to $1.34 \times$
212 10^6 cells mL^{-1} , and there was no increase in cell count with elevated CO_2 (Figure 1b), with the average specific
213 growth rate 0.47 d^{-1} in both treatments. Cell volume varied in *E. huxleyi* cultures so the data are presented as total
214 cell volume (Figure 1c), and was used to calculate mean individual cell volume, which increased in the 900 μatm
215 CO_2 treatment as the experiment progressed (Figure 1d). Mean cell volume in the control treatment was $46.0 \pm$
216 $12.0 \mu\text{m}^3$ and in the CO_2 treatment was $53.4 \pm 13.8 \mu\text{m}^3$, and cells showed a 20% increase in volume during the
217 fifth inoculation compared to the control treatment ($T=-3.65$, $p<0.01$).

218 *DMS and DMSP Dynamics*

219 Aqueous DMS was measured daily (Figure 2a) alongside the cell count and volume analyses, and was normalised
220 to cell number (Figure 2b). During the first two culture periods up to T9, DMS was in the range $6.5 - 65.1 \text{ nmol L}^{-1}$,
221 but during the following three culture periods, DMS increased sequentially to higher concentrations up to a mean
222 of $328.8 \pm 56.1 \text{ nmol L}^{-1}$ in the CO_2 treatment and $296.8 \pm 69.2 \text{ nmol L}^{-1}$ in the control at T23. DMS data normalised
223 to cell volume showed no effect of CO_2 treatment on the DMS production ($T=0.77$, $p=0.444$) but was on average
224 80% lower in the first inoculation compared to the final inoculation period with a range of $0.6 - 11.5 \text{ mmol L}^{-1}$ cell
225 volume (CV).

226 DMSP_T concentrations increased exponentially with cell count (Figure 2c) from a mean of $505.3 \pm 118.7 \text{ nmol L}^{-1}$
227 (control) and $504.9 \pm 140.2 \text{ nmol L}^{-1}$ (CO_2) in the initial days of inoculation to $4444.5 \pm 1127.2 \text{ nmol L}^{-1}$ (control)

and $4180.2 \pm 1000.0 \text{ nmol L}^{-1} (\text{CO}_2)$ on the final day of each inoculation. DMSP_T normalised to cell volume varied over the course of the experiment, within the range $16.7 - 202.1 \text{ mmol L}^{-1} \text{ cell volume (CV)}$ and was 12% lower in the CO_2 treatment than the control over the entire experiment (Figure 2d; $T=3.71$, $p<0.01$, $n=138$). The measured DMS: DMSP_T ratio was calculated (Figure 2e) with a mean of 0.04. The ratio had a sharp peak on T19 in both treatments, reaching a maximum of 0.23 in the CO_2 treatment, but over the course of the experiment, increased $p\text{CO}_2$ had no significant effect on the DMS: DMSP_T ratio. A summary of the *E. huxleyi* culture results is given in Table 1.

Mesocosm Experiment Results

Changes in Physical Oceanographic Conditions

Inorganic nitrate and phosphate concentrations in the mesocosms were measured at $1.54 (\pm 0.30) \mu\text{mol L}^{-1}$ and $0.21 (\pm 0.01) \mu\text{mol L}^{-1}$ respectively on T-1 of the experiment, with addition of artificial inorganic nutrients to all mesocosms on T14 to stimulate phytoplankton growth (mean concentrations $5.0 \pm 0.2 \mu\text{mol L}^{-1} \text{NO}_3$ and $0.16 \pm 0.02 \mu\text{mol L}^{-1} \text{PO}_4$ after addition). Maximum nutrient concentrations measured in the fjord were $1.73 \mu\text{mol L}^{-1} \text{NO}_3$ and $0.06 \mu\text{mol L}^{-1} \text{PO}_4$. Outgassing of CO_2 and carbon fixation by phytoplankton caused a gradual $p\text{CO}_2$ decline and pH increase in CO_2 -enriched mesocosms (Figure 3). The average pH before nutrient addition ranged between pH 8.13 ± 0.01 in the control mesocosms and pH 7.31 ± 0.12 in M9 (3000 μatm), the highest $p\text{CO}_2$ mesocosm. After nutrient addition, pH ranged between pH 8.14 ± 0.01 in the control mesocosms and pH 7.49 ± 0.05 in the highest $p\text{CO}_2$ mesocosm. Temperature varied between 6.8°C at the beginning and 10.0°C at the end of the experiment.

Changes in Community Composition

Three phases were identified from the fluorometric Chl-*a* data (Figure 4a): phase 1 as the initial bloom prior to artificial nutrient addition, phase 2 as the artificial nutrient-induced bloom and phase 3 as post-bloom. The initial Chl-*a* concentrations in all mesocosms were $2.2 \pm 0.1 \mu\text{g L}^{-1}$ at T-1 and rapidly increased in a similar manner in all treatments during the phase 1 bloom (Figure 4a), peaking on T3 in all mesocosms except for M9 (3000 μatm) which continued to increase until $4.1 \mu\text{g L}^{-1}$ on T5. A clear differentiation between $p\text{CO}_2$ treatments was seen after T3, with Chl-*a* concentrations higher in the high $p\text{CO}_2$ treatment until the beginning of phase 2 at T9, after which they dropped below the Chl-*a* concentrations of the control and medium $p\text{CO}_2$ mesocosms. During the phase 2 nutrient-induced bloom after T14, Chl-*a* concentrations were lower at high $p\text{CO}_2$, and peaked around T19-T20, before declining through phase 3 until the end of the experiment. Several different phytoplankton species were significant contributors to the total Chl-*a* throughout the experiment as measured by HPLC pigment data, including diatoms (~35%), cryptophytes (~22%), chlorophytes (~20%) and haptophytes (~19%; Figure 4b). Other taxa, including cyanobacteria, dinoflagellates and chrysophytes made a minor (<4%) contribution to the total Chl-

260 *a.* Haptophyte equivalent Chl-*a* showed a peak in all $p\text{CO}_2$ treatments during phase 1, with maximum
 261 concentrations of $0.84 \mu\text{g L}^{-1}$ in the control mesocosms, and there were no significant differences between any
 262 treatments during this phase ($F=0.73$, $p=0.669$, $n=98$). The phase 1 haptophyte equivalent Chl-*a* was coincident
 263 with the peak in DMSP_T concentrations (Figure 5b). The difference between elevated $p\text{CO}_2$ treatments became
 264 more apparent after the initial bloom (T7 to T17) and after the nutrient induced bloom in phase 2 (T22 to T29),
 265 with significantly lower haptophyte equivalent Chl-*a* concentrations in the higher $p\text{CO}_2$ treatments ($F=16.74$,
 266 $p<0.01$, $n=189$) from T9 compared to the low and medium $p\text{CO}_2$ mesocosms. During the period T3 to T10, mean
 267 net growth rates for the haptophytes in the three high $p\text{CO}_2$ mesocosms ($1400\text{-}3000 \mu\text{atm}$) were -0.2 d^{-1} ,
 268 compared to the mean net growth rate in the low $p\text{CO}_2$ mesocosms ($280\text{-}390 \mu\text{atm}$) at -0.06 d^{-1} . Haptophyte
 269 growth rates during the artificial bloom in phase 2 were subsequently higher in the high $p\text{CO}_2$ mesocosms over
 270 the period T10 to T20 at 0.1 d^{-1} compared to 0.02 d^{-1} in the low $p\text{CO}_2$ mesocosms and 0.06 d^{-1} in the medium (540-
 271 $1120 \mu\text{atm}$) mesocosms, but overall haptophyte Chl-*a* remained lower throughout phase 2 into phase 3. The
 272 mean calculated percentage contribution of the haptophyte Chl-*a* to total Chl-*a* was $25 \pm 11 \%$ in the low $p\text{CO}_2$
 273 mesocosms, but $15 \pm 5 \%$ in the highest, and this difference was pronounced in the post-bloom periods (Figure
 274 4c).

275 Calcified (C-form) *E. huxleyi* was the only haptophyte to be identified and enumerated using flow cytometry
 276 (Figure 4d) however this method was not able to identify individual non-calcified haptophyte species; all these
 277 were combined in the small nanophytoplankton ($2\text{-}6 \mu\text{m}$) group with *E. huxleyi* (Figure 4e). Abundance of calcified
 278 *E. huxleyi* cells increased in abundance during phases 2 and 3 when the majority of other groups declined in
 279 abundance. *E. huxleyi* peaked on T29 in the control ($280 \mu\text{atm}$) at $\sim 3000 \text{ cells mL}^{-1}$, and a distinct effect of $p\text{CO}_2$
 280 treatment was observed, with significantly lower abundance in the high $p\text{CO}_2$ mesocosms ($F=13.45$, $p<0.01$,
 281 $n=112$). The nanophytoplankton group ($2\text{-}6 \mu\text{m}$) showed a similar pattern to the haptophyte equivalent Chl-*a* with
 282 a peak during each bloom period, but did not show significantly lower nanophytoplankton abundance at high
 283 $p\text{CO}_2$ during the post-bloom period of Phase 2 (T9-T15) directly following the initial bloom, which was notable in
 284 the haptophyte equivalent Chl-*a*. After T15, significantly lower cell abundance was identified in the in the highest
 285 $p\text{CO}_2$ mesocosms, yet higher abundance was seen in the in the medium $p\text{CO}_2$ mesocosms compared to the
 286 control. Net nanophytoplankton growth rates were comparable between all mesocosms for the period T5 to T15,
 287 in contrast to the haptophyte Chl-*a*, yet were lower in the high $p\text{CO}_2$ mesocosms during the period T15 to T20.
 288 Nanophytoplankton abundance ranged from ~ 3000 to $33500 \text{ cells mL}^{-1}$ in all mesocosms, with maximum
 289 abundance in M8 ($560 \mu\text{atm}$) during Phase 2. Calcified *E. huxleyi* cells contributed less than 5% to the total
 290 nanophytoplankton during Phases 1 and 2 in all $p\text{CO}_2$ treatments, but increased in the low and medium $p\text{CO}_2$
 291 treatments to 27 % at the end of Phase 3 (Figure 4f).

292 *DMS*

DMS concentrations were measured from T12 to T29 for the mesocosms only in phases 2 and 3 (Figure 5a). Until T19, DMS concentrations were below 1 nmol L⁻¹ and from T20 onwards it increased in all pCO₂ treatments. A clear effect of increased pCO₂ is seen from the start of measurements on T12, with DMS concentrations in the highest pCO₂ treatments (2000 and 3000 µatm) significantly lower than the low (280 and 390 µatm) and medium pCO₂ (560, 840 and 1120 µatm) conditions (F=5.52, p<0.01, n=175), and these trends continued until T29. Maximum DMS concentrations were reached in M6 (390 µatm) on T29 at 4.9 nmol L⁻¹, compared to 0.76 nmol L⁻¹ measured in M9 (3000 µatm) on T28. During phases 2 and 3, DMS concentrations in the high pCO₂ treatments were 60% lower than the control and the medium pCO₂ treatments 33% lower. Mean DMS concentrations plotted against the mean pCO₂ for phases 2 and 3 showed a clear decreasing relationship as pCO₂ increased (Figure 6a; $\rho=-0.595$, p<0.01, n=140), however with only three mesocosms at pCO₂ higher than 1000 µatm, it is difficult to determine the exact nature of the DMS/ pCO₂ relationship at these higher pCO₂.

304 *Total DMSP*

DMSP_T was measured on alternate days from T-1 and showed different patterns to DMS (Figure 5b). DMSP_T concentrations were similar in all treatments on T-1 (38.5 ± 4.3 nmol L⁻¹ mean), and increased to a peak on T4, after which concentrations decreased. No difference between mesocosms was identified during phase 1 for DMSP_T (F=0.42, p=0.916, n=58). A difference between mesocosms was more apparent for DMSP_T during phases 2 and 3, with concentrations in the high (1400 – 3000 µatm) and medium pCO₂ treatments (560 – 1120 µatm) 32% and 14% lower respectively than the low pCO₂ mesocosms during both phases. This change seems to have been driven by the net DMSP production rate over the period T5 to T12, where the high pCO₂ mesocosms (1400-3000 µatm) showed a loss rate of -0.12 d⁻¹ compared to the low pCO₂ mesocosms (280-390 µatm) at -0.04 d⁻¹. This higher loss rate, similar to that of the haptophyte equivalent Chl-*a*, influences the concentrations in the later part of phase 2 and during phase 3: DMSP_T concentrations increased to a peak at T22 in all treatments, with the highest concentrations of 81.8 nmol L⁻¹ in M1 (840 µatm) but the lowest at 26.3 nmol L⁻¹ in M9 (3000 µatm). DMSP_T concentrations then decreased at the start of phase 3, before increasing again in all treatments on T29, with the lowest concentrations measured in the highest pCO₂ treatments. A summary of the DMS, DMSP and relevant cell abundance is given in Table 1.

319 *Relationships between DMS, DMSP and Biological Parameters*

The community composition proxies (HPLC pigments and flow cytometry data) were analysed alongside the DMS and DMSP data to determine the potential sources of DMS and DMSP within the mesocosm communities. Using Spearman's Rank Correlation analysis, concentrations of DMS and DMSP_T showed significant positive correlation to each other ($\rho=0.339$, p<0.01, n=135), and the ratio between the two compounds (Figure 5c) was relatively stable below 0.02 in all treatments during phase 2, but increased to around 0.06 in phase 3 corresponding to an increase in DMS concentration. The ratio of DMS: DMSP_T was unaffected by CO₂ treatment: mean ratios were

326 plotted against mean $p\text{CO}_2$ in all mesocosms, and showed no change with increasing $p\text{CO}_2$ (Figure 6c; $\rho=0.289$,
327 $p=0.083$, $n=62$).

328 DMSP_T showed positive correlation with Chl-*a* ($\rho=0.400$, $p<0.01$, $n=117$), and an examination of the mean DMSP_T:
329 Chl-*a*_{Hapto} ratio for each mesocosm plotted against mean $p\text{CO}_2$ for the entire experiment showed no effect of
330 increased $p\text{CO}_2$ (Figure 6d; $\rho=-0.01$, $p=0.920$, $n=99$). DMS showed negative correlation with total Chl-*a* ($\rho=-0.406$,
331 $p<0.01$, $n=136$). Correlations between DMS and all phytoplankton abundances and Chl-*a* contributors showed
332 that DMS concentrations correlated only with the haptophyte-equivalent Chl-*a* ($\rho=0.508$, $p<0.01$, $n=126$) and
333 calcified *E. huxleyi* abundance ($\rho=0.615$, $p<0.01$, $n=136$), however the latter only reached 3000 cells mL⁻¹ in M4
334 (290 μatm) on T29 (Figure 4d). DMSP_T correlation with haptophyte equivalent Chl-*a* was also strong ($\rho=0.635$,
335 $p<0.01$, $n=121$), with relatively weak correlation with the nanophytoplankton ($\rho=0.283$, $p<0.01$, $n=117$) and no
336 relationship with calcified *E. huxleyi* abundance. In addition, there was weak correlation between DMSP_T and the
337 diatoms ($\rho=0.301$, $p<0.01$, $n=121$). The ratios of DMS and DMSP_T to nanophytoplankton (2-6 μm) abundance
338 (Figures 7a and b) and haptophyte equivalent Chl-*a* (Figures 7c and d) were calculated on a daily basis, and
339 showed a limited effect of elevated $p\text{CO}_2$. The haptophytes were significant contributors to the DMSP pool given
340 the strong correlations with DMSP_T and relatively high contribution to the total Chl-*a* (Figure 4c) while calcified *E.*
341 *huxleyi* contributed to only a small percentage of the total haptophyte assemblage (Figure 4f) and subsequently
342 the DMSP production. Calcified *E. huxleyi* were of greater importance to DMSP production during phase 3 of the
343 experiment when the abundance was highest. It is highly likely that a large proportion of the nanophytoplankton
344 (2-6 μm ; Figure 4e) were non-calcified DMSP-producing haptophyte cells, although no determination of species
345 composition could be made. Non-calcified *E. huxleyi* cannot be distinguished from other non-calcified
346 haptophytes of the same size by flow cytometry (Aud Larsen, Pers. Comm.).

347 Discussion

348 A number of mesocosm experiments investigating the effect of elevated $p\text{CO}_2$ on the community structure have
349 been performed, and several of these have measured the effects on DMS and DMSP concentrations. These are
350 summarised in Table 2, alongside experiments on clonal *E. huxleyi* cultures which also measured DMS and DMSP
351 versus CO_2 concentrations. The ranges in DMS and DMSP concentrations from the mesocosm experiment in this
352 study are within those seen in previous Bergen mesocosm studies,^[24,25,28,53,54] and the Korean and Svalbard
353 mesocosm experiments, where microbial communities from neither location contained a significant abundance of
354 *E. huxleyi*.^[23,26,27] During this experiment no single group dominated the community at any time; there were high
355 abundances of diatoms, cryptophytes, chlorophytes and haptophytes, but only the haptophytes were significantly
356 correlated with DMSP concentrations. The $p\text{CO}_2$ range used by us was broader than in any previous investigation,
357 with mesocosms at 2000 and 3000 μatm ; the aim being to identify trends of different community parameters
358 beyond the $p\text{CO}_2$ projected for the year 2100. The change in $p\text{CO}_2$ in the system occurred relatively rapidly over 3-
359 5 days (Figure 3), and the community response would have favoured those species with less efficient carbon

concentrating mechanisms (CCMs),^[55–57] as well as those better suited to rapid environmental change. Over the course of the experiment, the $p\text{CO}_2$ decreased in all the treated mesocosms, with the result that the artificial bloom was at a lower mean $p\text{CO}_2$ for each mesocosm than the initial bloom, but the communities would have been exposed to the perturbed conditions for a longer time period. Differences were identified between treatments for a number of community parameters: chlorophytes, picoeukaryotes and cyanobacteria showed a strong positive response in high $p\text{CO}_2$, while haptophyte and diatom growth was negatively affected at the highest $p\text{CO}_2$. These responses were more pronounced during the latter phases of the experiment.

Community Development and *E. huxleyi* Growth

The total Chl-*a* concentrations in the mesocosms showed both positive and negative effects of CO_2 during the three different phases, a scenario which was also identified during a mesocosm experiment in Svalbard^[52], and is a result of different phytoplankton assemblages responding to elevated $p\text{CO}_2$ at different times of the experiment. Of importance to this investigation, haptophyte-equivalent Chl-*a*, nanophytoplankton and calcified *E. huxleyi* cells showed reduced abundance under increased $p\text{CO}_2$ during phases 2 and 3, either as a direct result of CO_2 on the groups, or as a result of differential nutrient-induced competition between groups such as diatoms and picoeukaryotes at the higher availability of DIC,^[52,58,59] as was previously identified during the Svalbard mesocosm experiment in 2010. In contrast, Endres *et al.*^[31] identified significantly higher marine bacterial abundance and activity in the high $p\text{CO}_2$ mesocosms during the same period. Calcified *E. huxleyi* cell counts during the mesocosm experiment were unexpectedly low (up to 3000 cells mL^{-1}) in comparison to some previous experiments (e.g. up to 70,000 cells mL^{-1} in Steinke *et al.*^[53] and up to 50,000 cells mL^{-1} in Delille *et al.*^[60]) and there was no analysis performed on calcification rates in *E. huxleyi* or evaluating coccolith formation. Analysis of the phytoplankton community by flow cytometry was unable to identify other calcified coccolithophore species than *E. huxleyi*, however the mismatch between the pattern of haptophyte equivalent Chl-*a* and the abundance of calcified *E. huxleyi* cells identified by flow cytometry indicate the presence of non-calcified haptophyte cells which were enumerated only as nanophytoplankton (2-6 μm). Previous investigations at Espegrend Marine Biological Station have identified non-calcified *E. huxleyi* cells within the coastal phytoplankton community.^[61] Indeed, in a mesocosm experiment in the Raunefjord in 2008, a significant number (up to 40,000 cells mL^{-1}) of non-calcified haptophyte cells were identified in the natural population through the use of COD-FISH (combined CaCO_3 optical detection with fluorescent in-situ hybridisation) techniques.^[62,63]

Calcification rates were not measured during our mesocosm and laboratory culture experiments, but previous mesocosm studies have identified reductions in calcification under elevated $p\text{CO}_2$,^[60,64] which has been suggested as a negative feedback on surface water $p\text{CO}_2$.^[10] As mentioned above, non-calcified *E. huxleyi* cells do occur in natural and mesocosm assemblages, but their presence is not indicative of lower calcification rates. Overall,

understanding of the non-calcified life-stages of *E. huxleyi* is very scant, and requires further investigation into the physiological changes that occur in the different forms (haploid and diploid, calcified and non-calcified). In addition, other non-calcifying haptophytes were likely present in the community and contributing to the haptophyte Chl-*a* signal. In terms of DMSP production, a single investigation found that DMSP production was increased by up to 0.4 pg cell⁻¹ in a non-calcified *E. huxleyi* strain (N-Form diploid RCC1242) under 790 µatm *p*CO₂ compared to an ambient *p*CO₂ control, while a calcified strain (C-form diploid RCC1731) showed no CO₂ effect.^[65] Further studies of DMSP production from diploid calcified and non-calcified (haploid and diploid) strains in the laboratory and non-calcified cells in the field are certainly warranted, as well as further investigation into the DMSP production of the haploid life-stages, which has never been previously investigated.

There have been a number of studies on the effect of elevated *p*CO₂ on different strains of *E. huxleyi*, isolated from different geographical areas,^[9,65–70] but never using the strain RCC1229. This strain was chosen due to its origins in the North Sea close to the Bergen coast (58.42°N, 3.21°E), and likely similar genotype to the natural *E. huxleyi* identified during the Bergen mesocosm experiment. Despite this, calcified cell abundance in the mesocosms showed a significant decrease at 840 µatm *p*CO₂, but no such effect was identified during the culture experiments at the comparable *p*CO₂. While the culture experiments were nutrient replete, *E. huxleyi* within the mesocosms showed significant growth during phase 3 after the artificial bloom, when concentrations of inorganic nitrate and phosphate were low. Although RCC1229 was isolated close to the location of the mesocosm experiment, there is still likely significant genetic difference between the strain and the wild population. The physiological responses between different strains to increased *p*CO₂ have not been uniform: in general carbon fixation has increased,^[65,68,70,71] but three strains investigated by Langer *et al.*^[66] showed the opposite effect. *E. huxleyi* has shown varying sensitivity of growth rate to *p*CO₂ in the laboratory and the field. A previous mesocosm experiment identified decreased net specific growth rate from 0.5 d⁻¹ to 0.43 d⁻¹ in the highest *p*CO₂ mesocosms,^[72] and the reduced haptophyte equivalent Chl-*a* concentrations and calcified *E. huxleyi* abundance values seen in our medium and high *p*CO₂ mesocosms support this. However, in the laboratory, varying responses have been identified for different *E. huxleyi* strains where growth rates either increased,^[9,70,73] remained unchanged as in this study^[65,69,74] or decreased.^[66,75,76] Specific growth rates during the *E. huxleyi* RCC1229 experiment were lower (0.48 d⁻¹ for the 900 µatm *p*CO₂ treatment and 0.47 d⁻¹ for the ambient CO₂ control) than found previously for that strain under near-identical growth conditions at the same temperature (0.67 d⁻¹),^[77] and was likely a result of methodological differences in culturing which can be a significant problem in comparing growth rates between different investigations.^[78] The growth rate of calcified RCC1229 was not affected by 900 µatm *p*CO₂, whereas the abundance of calcified cells decreased in the 840 µatm *p*CO₂ mesocosm. A significant shift to a larger cell size was identified during the RCC1229 culture experiment, which reinforces the findings of Arnold *et al.*^[69] using non-calcifying strain CCMP373, suggesting that the cell size increase is not linked to additional coccolith production. Increased POC production at higher *p*CO₂ has been linked to larger cell size.^[79] The long-term studies of Lohbeck *et al.*^[80] with over 500 generations of single and multi-clonal experiments found

a decrease in cell size as $p\text{CO}_2$ increased. These variations in growth rate and carbon fixation limit the use of a single *E. huxleyi* strain as a representative of all coccolithophores and haptophytes in the natural environment. In contrast, Franklin *et al.*^[81] identified *E. huxleyi* as a good model for the coccolithophores as a whole, particularly in terms of DMSP production, but only examined two strains of *E. huxleyi*. Comparison of the experiments described here and existing studies on *E. huxleyi* suggest sufficient genetic diversity and plasticity in natural populations to at least partially adapt as surface water $p\text{CO}_2$ increases.^[80] *E. huxleyi* has shown significant advancement into polar waters since the first half of the 20th century due to expansion of the thermal window,^[82,83] but the effect of ocean acidification on these blooms is still unclear. Future laboratory high CO_2 experiments should focus on species other than *E. huxleyi*, and on other significant DMSP producers which would allow for better analysis of community development in mesocosm studies such as this.

DMSP and DMS

DMSP concentrations measured in the mesocosms were strongly correlated with haptophyte equivalent Chl-*a* and nanophytoplankton abundance, but not calcified *E. huxleyi* abundance. Although these groups were unlikely to be the sole producers of DMSP, the negative effect of acidification on the bloom dynamics of these groups had significant influence on the lower DMSP concentrations measured in the high $p\text{CO}_2$ mesocosms. DMSP correlated well with haptophyte Chl-*a*, with DMSP: Chl-*a*_{Hapto} ratios of 10-60 nmol μg^{-1} were in strong agreement with those identified in a previous mesocosm experiment.^[28] During the period T9-T14, the increased DMSP: Chl-*a*_{Hapto} ratio in the high $p\text{CO}_2$ mesocosms was a result of the lower haptophyte Chl-*a*, likely due to nutrient competition, particularly with picoeukaryotes at the higher $p\text{CO}_2$ mesocosms during the natural post-bloom phase, and not a direct result of elevated $p\text{CO}_2$. The DMS: DMSP ratio was unaffected by the change in $p\text{CO}_2$ (Figure 6c), and therefore the reduction in DMSP would explain a proportion of the 60% reduction in DMS concentrations measured in the mesocosms. In a number of previous mesocosm experiments, measured DMS and DMSP concentrations were found to be negatively affected by increased $p\text{CO}_2$,^[24,25,27] but in others the effect was either temporally offset,^[28] or showed differential responses in DMS and DMSP.^[23] While the DMSP_T concentrations in the RCC1229 *E. huxleyi* experiment showed no significant difference between treatments, DMSP_T was 12% lower in the 900 μatm $p\text{CO}_2$ treatment when normalised to cell volume (Figure 2d). In contrast, pH-stat laboratory experiments on clonal *E. huxleyi* cultures showed either no effect of elevated $p\text{CO}_2$, or increased DMSP production^[65,69,84] when the $p\text{CO}_2$ was equivalent to that of our mid or high range mesocosm experiments (>800 μatm). DMS concentrations in the laboratory cultures showed no significant difference when normalised to cell volume, with no pronounced differences in *E. huxleyi* biomass, implying that microbial interaction occurs within the mesocosms which is limited in the cultures. Clearly, mesocosm experiments assess the community response to increasing $p\text{CO}_2$ whereas laboratory experiments investigate the physiological changes within a single species and the effect these have on the production of DMSP and DMS; the greater response to acidification in the mesocosms compared to the laboratory experiment implies that there is a strong community interaction in the

net production of DMS and DMSP. The DMSP producers showed no immediate DMSP-response upon addition of the CO₂-enriched waters to the mesocosms (Figure 7b and d) over the T-1 to T3, implying that DMSP production is not a direct response to changing environmental conditions.

The poor relationship of DMS with Chl-*a* has been reported several times, both regionally^[85–87] and in data analysis-global modelling studies^[88], due to the likely differential DMSP synthesis of phytoplankton groups, variability in community DMSP-to-DMS conversion yields, and DMS loss rate constants^[89]. Total DMSP measured in the mesocosms included the intracellular particulate DMSP (DMSP_p) and extracellular dissolved DMSP (DMSP_D). DMS and DMSP_T have often been found decoupled, particularly during the ‘summer paradox’ of delayed DMS maxima compared to DMSP maxima and phytoplankton maximum abundance,^[22,90,91] driven by grazing-induced particulate DMSP transformation. DMSP is degraded through two separate pathways,^[92]: demethylation to methylmercaptopropionate (MMPA)^[93] or cleavage to DMS with production of either acrylate or 3-hydroxypropionate through the ‘DMSP-Lyase’ pathway,^[92,94] and can be intracellular or extracellular by marine bacteria in the surrounding waters.^[95,96] These routes regulate the gross DMS production rates in seawater, and thereby affect the flux of sulphur to the atmosphere. Previous studies on DMSP-lyase activity showed variations in the pH optimum, from pH 5 in a number of haptophyte *Phaeocystis* spp.^[97] and coccolithophore *Gephyrocapsa oceanica*,^[81] to pH 8 in the bacterium *Ruegeria lacuscaerulensis*^[98] and *Pseudomonas doudoroffii*^[99] and up to pH 10.5 in a further *Phaeocystis* strain.^[100] The implication is that community production of DMS from the cleavage of DMSP is unlikely to be immediately affected by lowered pH as a result of ocean acidification, but individual species with optimal pH above 8 will find it increasingly difficult to cleave DMSP at higher atmospheric *p*CO₂.

The DMSP_D pool supports 1-13% of bacterial carbon^[18,101] and 3-100% of bacterial sulphur^[18] demand, by the breakdown pathways diverting sulphur away from DMS production.^[102,103] Increased consumption of the DMSP_D pool by bacteria would affect not only the DMSP_T concentrations but also reduce DMS production from the cleavage pathways. Bacterial transformation of DMS to DMSO has been identified as the removal pathway for the majority of DMS,^[104] further reducing the DMS concentrations during the greater bacterial activity at higher *p*CO₂.

In the laboratory experiments, bacterial abundance was kept low by treatment with antibiotics prior to the initial inoculation, and were checked by DAPI staining at the end of the experiment, when bacterial abundances were found to be low. During the mesocosm experiment, bacterial abundance increased by 28% in the high *p*CO₂ treatments in comparison to the low *p*CO₂ mesocosms, and showed three times higher leucine aminopeptidase activity as a proxy for bacterial enzyme hydrolysis.^[31] This higher bacterial abundance at high *p*CO₂ could result in greater consumption of DMSP from the dissolved phase as a greater bacterial abundance and activity is likely to drive an increased demand for sulphur sources, as well as drive greater conversion of DMS to DMSO. Bacterial loss processes for both DMS and DMSP could account for the lower concentrations of both compounds at elevated *p*CO₂, while not affecting the DMS: DMSP ratio.

During phase 3 of the experiment, there was an increase in DMS concentration which was not explained by corresponding increases in DMSP_T (Figure 5c), haptophyte Chl-*a* (Figure 7c) or nanophytoplankton abundance (Figure 7a), but which was unaffected by elevated *p*CO₂ (Figure 6c) and implied that DMS turnover and loss processes were similar in all mesocosms. A study by Pinhassi *et al.*^[105] in microcosms identified that DMSP was utilised as a sulphur source and removed by bacterioplankton more during the bloom phase (i.e. phase 2) than during senescence (i.e. phase 3), potentially resulting in greater availability of DMSP_D during phase 3 for conversion to DMS. Scarratt *et al.*^[106] identified a direct relationship of DMS concentrations with DMSP_D in short-term incubations, which would imply a greater contribution of dissolved DMSP to the measured DMSP_T in phase 3 of the mesocosm experiment, after the artificial nutrient-induced bloom in phase 2.

Summary

A significant reduction in DMS and DMSP concentrations was identified during a mesocosm experiment designed to study the effects of elevated *p*CO₂ on a coastal phytoplankton community. The major DMSP producers were identified as nanophytoplanktonic haptophytes which showed lower biomass under elevated *p*CO₂. The same effect was not observed during laboratory culture experiments on a calcifying strain of *E. huxleyi* (RCC1229), which indicates that consumption and turnover of DMSP_D and DMS in surface waters at elevated *p*CO₂ by the microbial community is as important as gross DMSP production in determining the concentrations of DMS and DMSP in (future) acidified waters. Elevated *p*CO₂ affected the growth of calcified *E. huxleyi* and nanophytoplankton (2-6 µm) which would have contained non-calcified haptophyte cells, and the reduction in abundance significantly contributed to the lower DMSP concentrations at high *p*CO₂.

A number of mesocosm studies, including this one, have shown that the phytoplankton community response to an increase in *p*CO₂ has resulted in lower DMS concentrations than seen in the ambient *p*CO₂ concentrations of today.^[1] This response is representative for the exposure of the current phytoplankton community assemblage to a comparatively rapid increase in *p*CO₂, and does not reflect the adaptation likely to occur in phytoplankton communities with the gradual increase in *p*CO₂ over the next 100 years. A reduction in DMS concentration will affect the atmospheric flux of sulphur from the marine environment. As many of these mesocosm experiments have been performed in a single location in Norway, further large-scale mesocosm experiments should be performed in different oceanic regions, to assess the changes in the parameters measured here for different microbial communities. Further investigations should concentrate on rates of DMSP production and the bacterial consumption of DMS and DMSP to develop a better understanding of the interactions with the microbial community that affect the concentrations of these compounds. DMS and DMSP analyses should also be included in long term (500+ generations) algal culture experiments, to establish if the short-term changes identified here are retained over a longer study period.

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Table 1. Comparison of *E. huxleyi* cell counts and DMS and DMSP_T concentration ranges and means for the mesocosm and the *E. huxleyi* culture experiments. All *E. huxleyi* counts show calcified cells only. The % changes in total measured DMS and DMSP_T concentrations are also shown. NS: Not significant

Experiment	<i>E. huxleyi</i> RCC1229 Culture Experiment		Mesocosm Experiment		
pCO ₂ treatment	390 µatm	900 µatm	390 µatm	840 µatm	3000 µatm
<i>E. huxleyi</i> range (cells mL ⁻¹)	87439 – 1355000	60598 – 1254000	81 – 2004	58 – 1393	15 – 135
Nanophytoplankton (2-6µm) range (cells mL ⁻¹)			2341 – 28628	2373 – 29412	2453 – 20649
DMS range (nmol L ⁻¹)	6.5 – 345.8	11.5 – 366.6	0.4 – 4.9	0.1 – 2.4	0.1 – 0.8
DMS Mean (±SD) (nmol L ⁻¹)	74.5 ± 73.7	77.8 ± 83.4	1.5 ± 1.2	1.0 ± 0.6	0.4 ± 0.2
DMS % Change		NS		-17	-60
DMSP _T range (nmol L ⁻¹)	109.8 – 6233.6	144.1 – 6062.3	21.1 – 67.4	20.3 – 81.9	14.6 – 58.2
DMSP _T Mean (±SD) (nmol L ⁻¹)	1840.2 ± 1621.1	1769.0 ± 1546.5	46.0 ± 12.0	44.5 ± 15.6	28.8 ± 15.2
DMSP _T % Change		NS		-13	-32

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799 **Table 2. Comparison of DMS and DMSP concentrations from this study and previous $p\text{CO}_2$ perturbation experiments. ND –**
800 **not detected, NC – No change. * indicates concentrations were given in mmol DMSP L⁻¹.**

	Location or Culture Strain	$p\text{CO}_2$ Range (μatm)	Range DMS (nmol L ⁻¹)	% change DMS	Range DMSP (nmol L ⁻¹)	% change DMSP	Author
Bergen Mesocosm Experiment, 2011	Raunesfjorden, Norway	280 – 3000	0.09 – 4.92	-60	14.3 – 88.2	-32	This Study
Korean Mesocosm Experiment 2, 2012	Jangmok, Korea	160 - 830	1.0 - 100	-82	10-350	-71	Park <i>et al.</i> 2014 ^[27]
EPOCA Svalbard, 2010	Kongsfjorden, Svalbard	180 - 1420	ND - 14	-60	ND-80	+50	Archer <i>et al.</i> 2013 ^[23]
Korean Mesocosm Experiment 1, 2008	Jangmok, Korea	400 - 900	1 - 12	+80	No Data	No Data	Kim <i>et al.</i> 2010 ^[26]
NERC Microbial Metagenomics Experiment, 2006	Raunesfjorden, Norway	300-750	ND - 50	-57	30 - 500	-24	Hopkins <i>et al.</i> 2010 ^[24]
PeECE III, 2005	Raunesfjorden, Norway	300 - 750	ND - 35	NC	10 - 500	NC	Vogt <i>et al.</i> 2008 ^[28]
PeECE II, 2003	Raunesfjorden, Norway	300 - 750	3 - 30	-40	ND - 300	-40	Avgoustidi <i>et al.</i> 2012 ^[25]
UKOA European Shelf Cruise, 2011	NW European Shelf	340 - 1000	0.5 - 12	+225	5 - 80	-52	Hopkins and Archer 2014 ^[71]
<i>E. huxleyi</i> Batch Experiments	CCMP1516	370 - 760	0.1 – 2.5	-90	500 - 4000	-60	Avgoustidi <i>et al.</i> 2012 ^[25]
<i>E. huxleyi</i> pH stat experiment	CCMP 373	385 - 1000	2.5 – 5.0	NC	84.0 – 200	NC	Arnold <i>et al.</i> 2013 ^[69]
<i>E. huxleyi</i> Semi-continuous Experiment	RCC1242	390 - 790			100 – 270*	+30	Spielmeyer and Pohnert 2012 ^[65]
<i>E. huxleyi</i> Semi-continuous Experiment	RCC1731	390 – 790			50-60*	NC	

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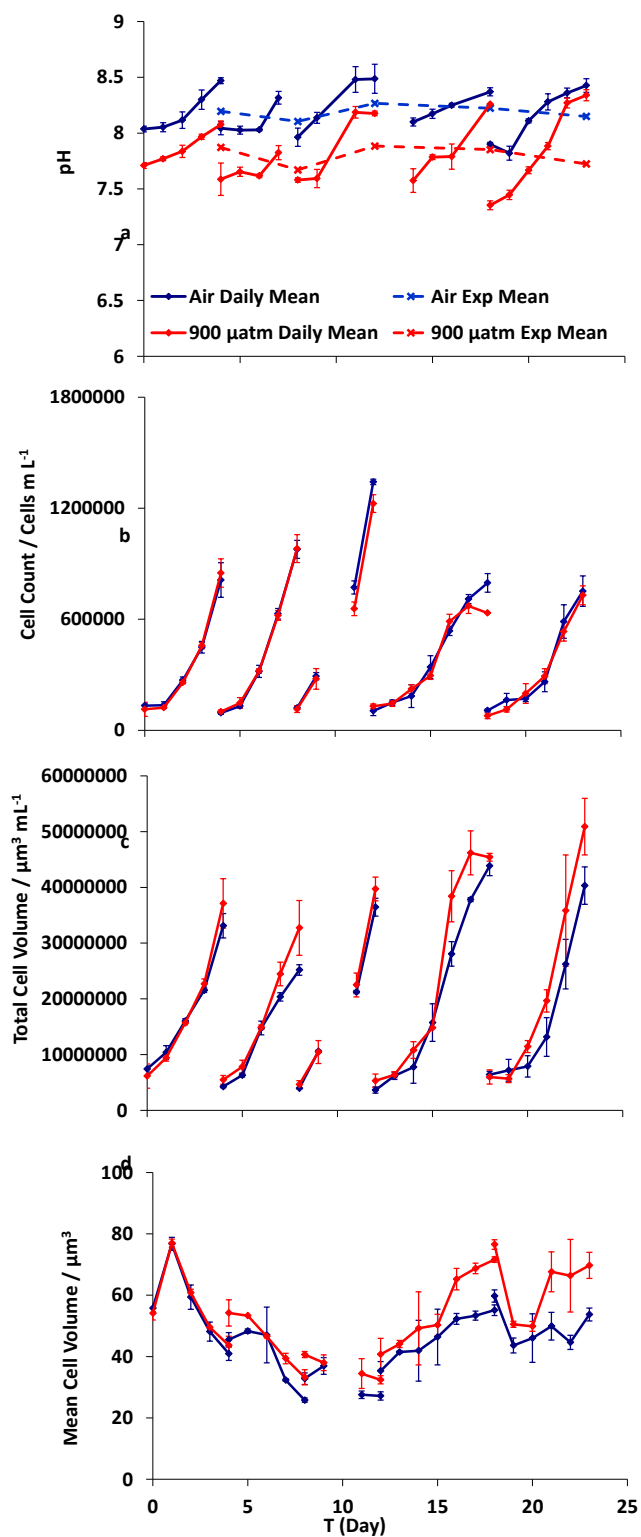


Figure 1. Growth dynamics of the 900 μatm $p\text{CO}_2$ (red) and control (blue) cultures showing the mean and standard deviation as error bars for three replicate flasks for (a) pH, (b) cell count (cells mL^{-1}), (c) total cell volume ($\mu\text{m}^3 \text{mL}^{-1}$) and (d) individual cell volume (μm^3). Dashed lines for pH show the mean pH for each inoculation period across the duration of the experiment.

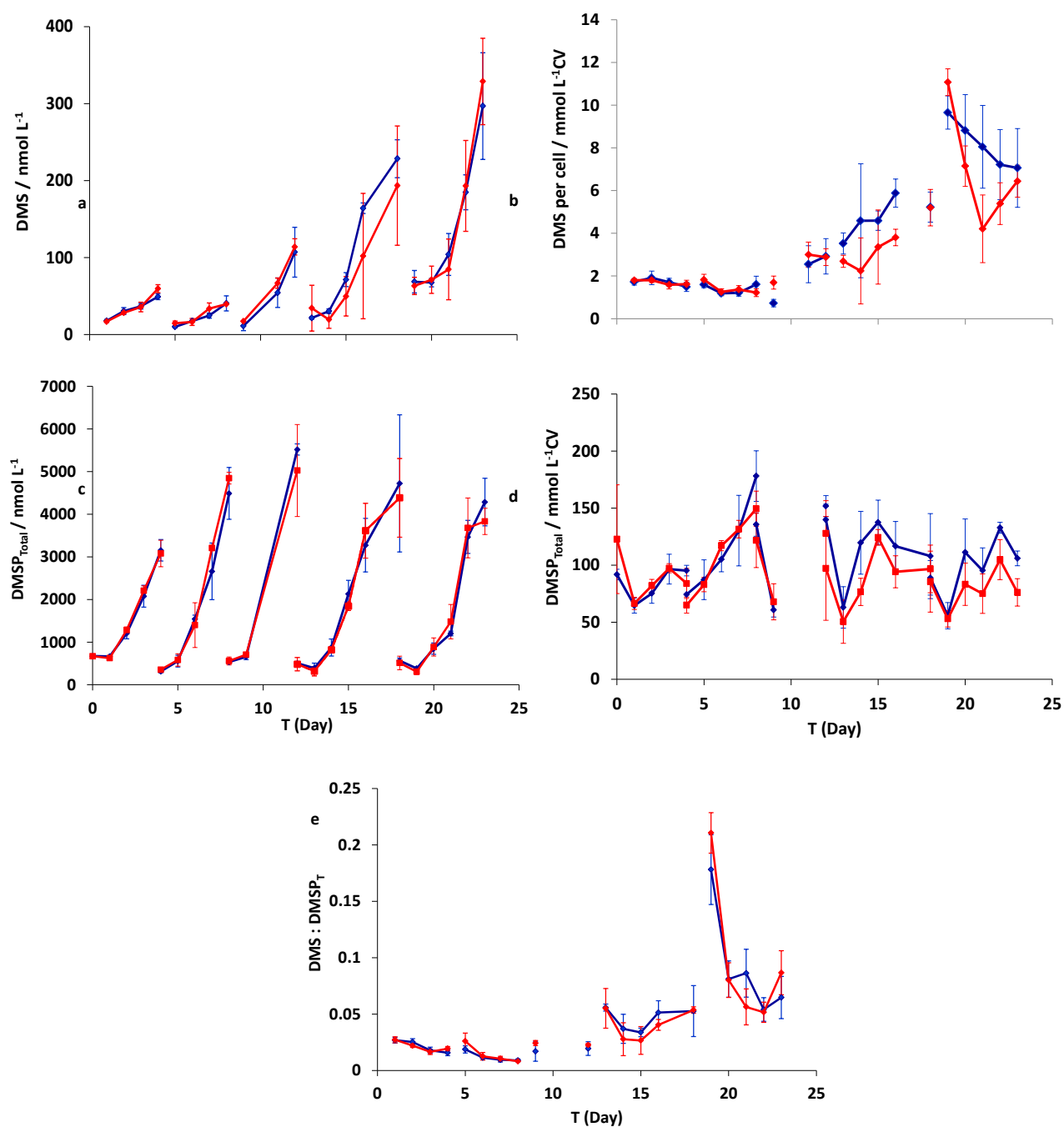


Figure 2. DMS and DMSP dynamics of the 900 μatm $p\text{CO}_2$ (red) and control (blue) treatments, showing the mean and standard deviation as error bars of three replicate flasks for each treatment. (a) DMS concentration (nmol L^{-1}), (b) DMS normalised to cell volume ($\text{mmol L}^{-1}\text{CV}$), (c) DMSP_T concentration (nmol L^{-1}), (d) DMSP_T normalised to cell volume ($\text{mmol L}^{-1}\text{CV}$) and (e) DMS: DMSP_T ratio.

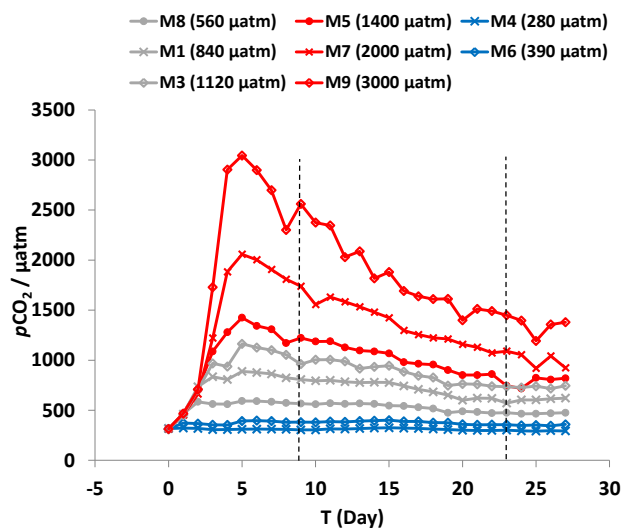


Figure 3. Daily measurements of $p\text{CO}_2$ during the mesocosm experiment. Dashed lines indicate the three phases of the experiment: the initial bloom, the second bloom and the post-bloom phase. Blue lines indicate the low $p\text{CO}_2$ (280 – 390 μatm), grey lines the mid-range $p\text{CO}_2$ (560 – 1120 μatm) and red lines the high $p\text{CO}_2$ (1400 – 3000 μatm).

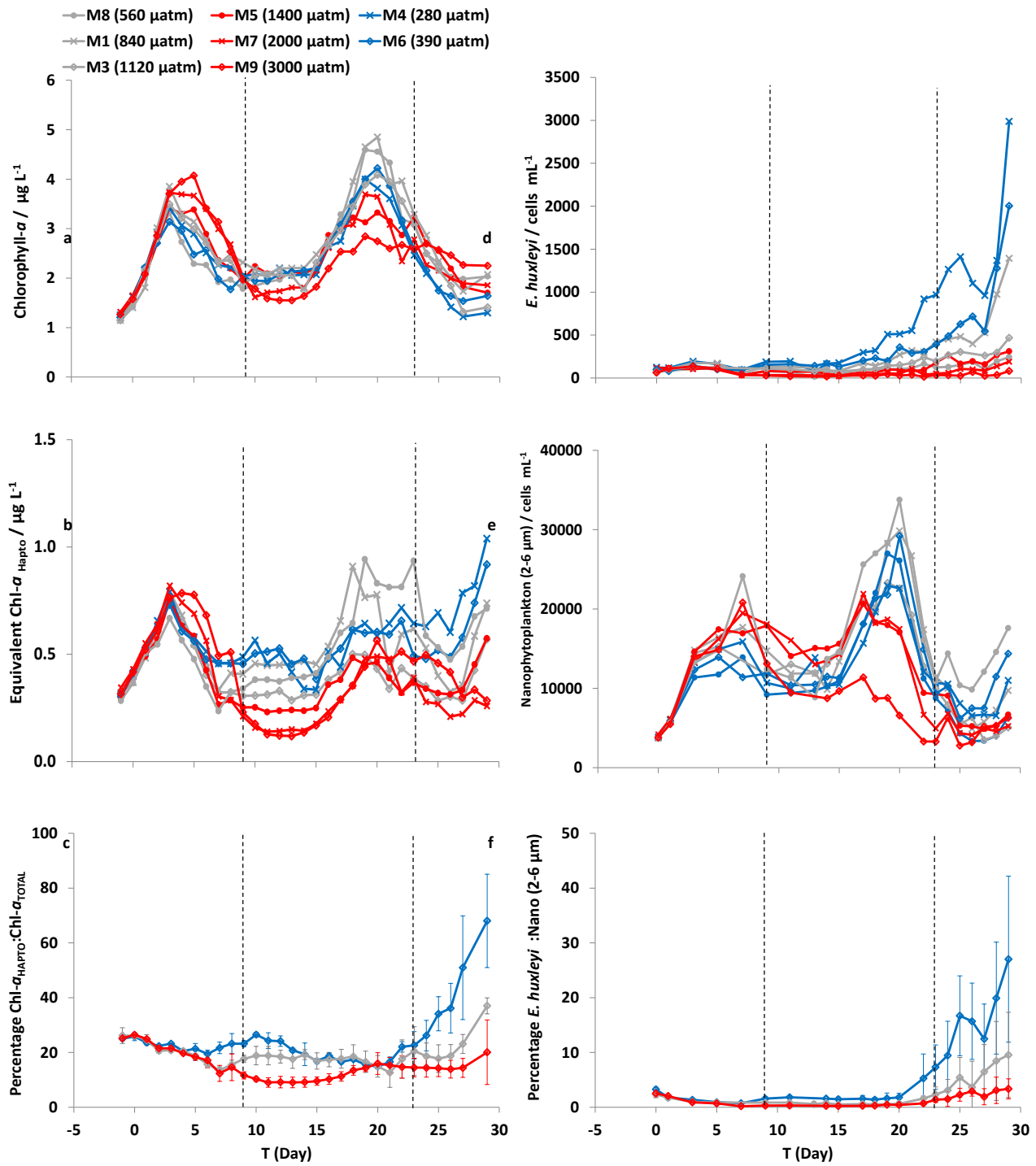


Figure 4. Temporal changes in (a) Chl-*a* ($\mu\text{g L}^{-1}$), (b) haptophyte equivalent Chl-*a* ($\mu\text{g L}^{-1}$), (c) percentage haptophyte Chl-*a*: total Chl-*a*, (d) calcified *E. huxleyi* cell abundance (cells mL^{-1}), (e) small nanophytoplankton including *E. huxleyi* (2-6 μm ; cells mL^{-1}) and (f) percentage *E. huxleyi*: small nanophytoplankton during the mesocosm experiment. Dashed lines indicate the three phases of the experiment: the initial bloom, the second bloom and the post-bloom phase. Blue lines indicate the low (280 – 390 μatm), grey lines the mid-range $p\text{CO}_2$ (560 – 1120 μatm) and red lines the high $p\text{CO}_2$ (1400 – 3000 μatm). Error bars show the standard deviation between all mesocosms of low, medium and high $p\text{CO}_2$.

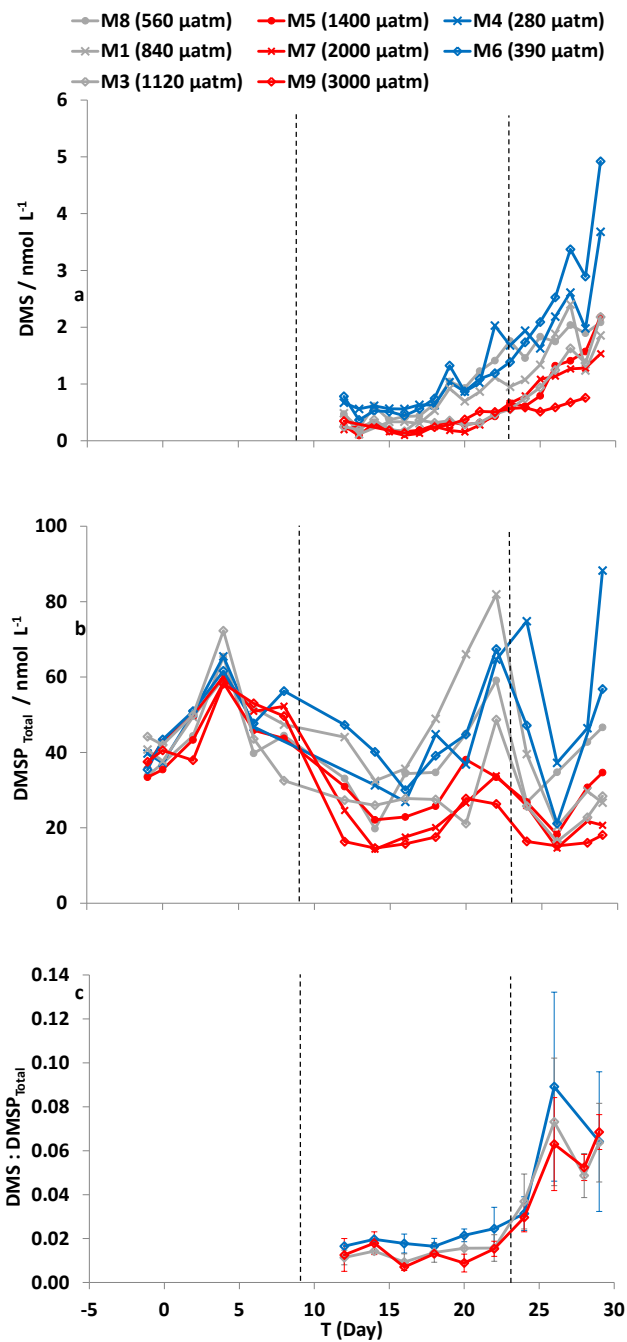


Figure 5. Temporal changes in (a) DMS (nmol L⁻¹) and (b) DMSP_T (nmol L⁻¹) with a single analysis per treatment. Blue lines indicate the low $p\text{CO}_2$ treatments (280 – 390 μatm), grey lines the mid-range $p\text{CO}_2$ treatments (560 – 1120 μatm) and red lines the high $p\text{CO}_2$ treatments (1400 – 3000 μatm). The DMS : DMSP_T ratio was calculated during Phases 2 and 3 of the experiment (c) with error bars showing the standard deviation between all mesocosms of low, medium and high $p\text{CO}_2$. Dashed lines indicate the three phases of the experiment.

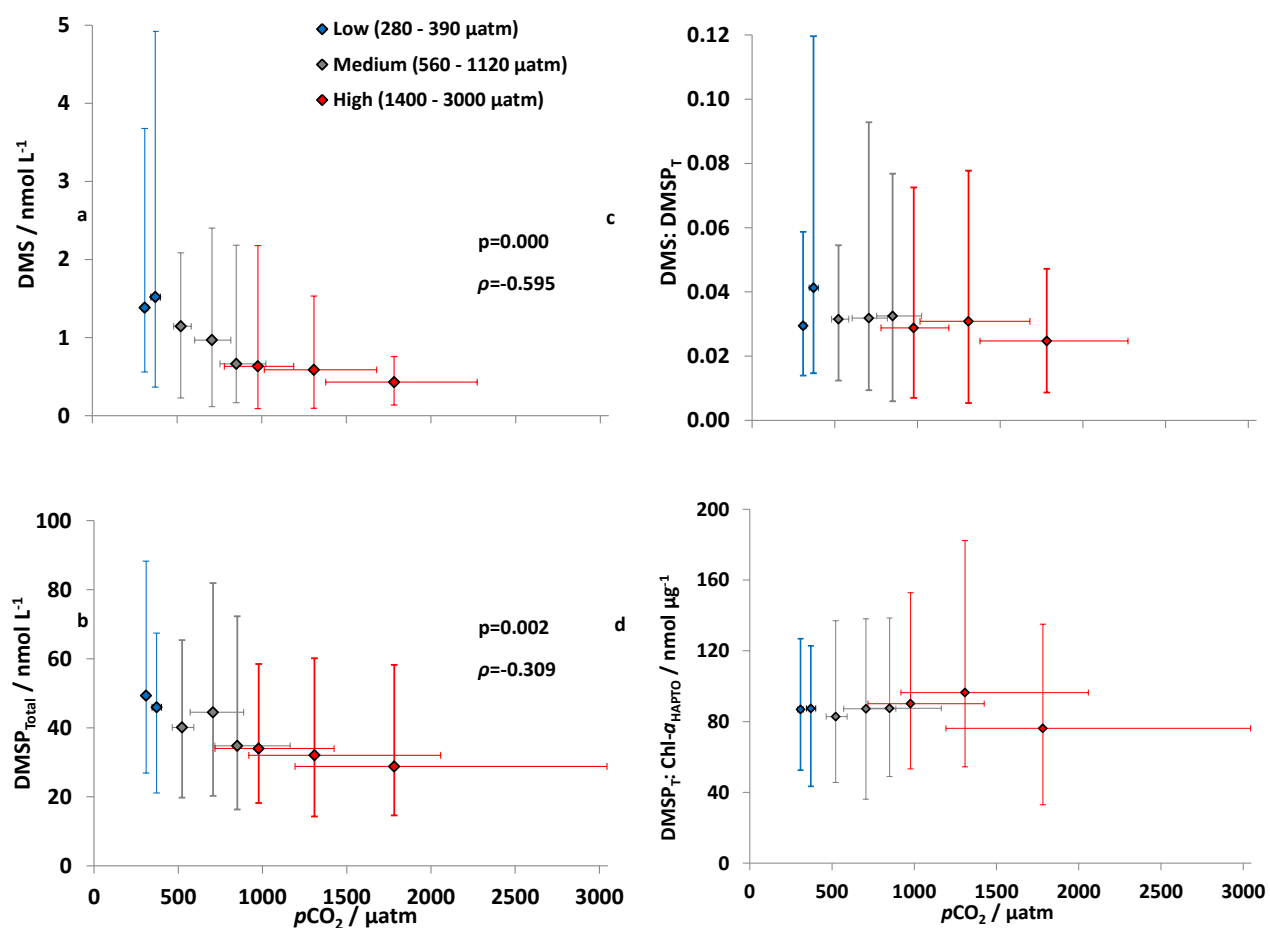


Figure 6. Relationships between $p\text{CO}_2$ and (a) mean DMS concentration (nmol L^{-1}) (b) mean DMSP_T concentration (nmol L^{-1}) (c) mean $\text{DMS} : \text{DMSP}_T$ and (d) mean $\text{DMSP}_T : \text{Chl-}a$ ($\text{nmol } \mu\text{g}^{-1}$) for the low (blue; 280 – 390 μatm), medium (grey; 540 – 1120 μatm) and high (red; 1400 – 3000 μatm) $p\text{CO}_2$ treatments, plotted against the mean $p\text{CO}_2$ in each mesocosm. Error bars show the range of the data on the horizontal and vertical axes. Where significant, the Spearman's Rank Correlation Coefficients (ρ) for the relationships between the variables are shown, with the corresponding p-value.

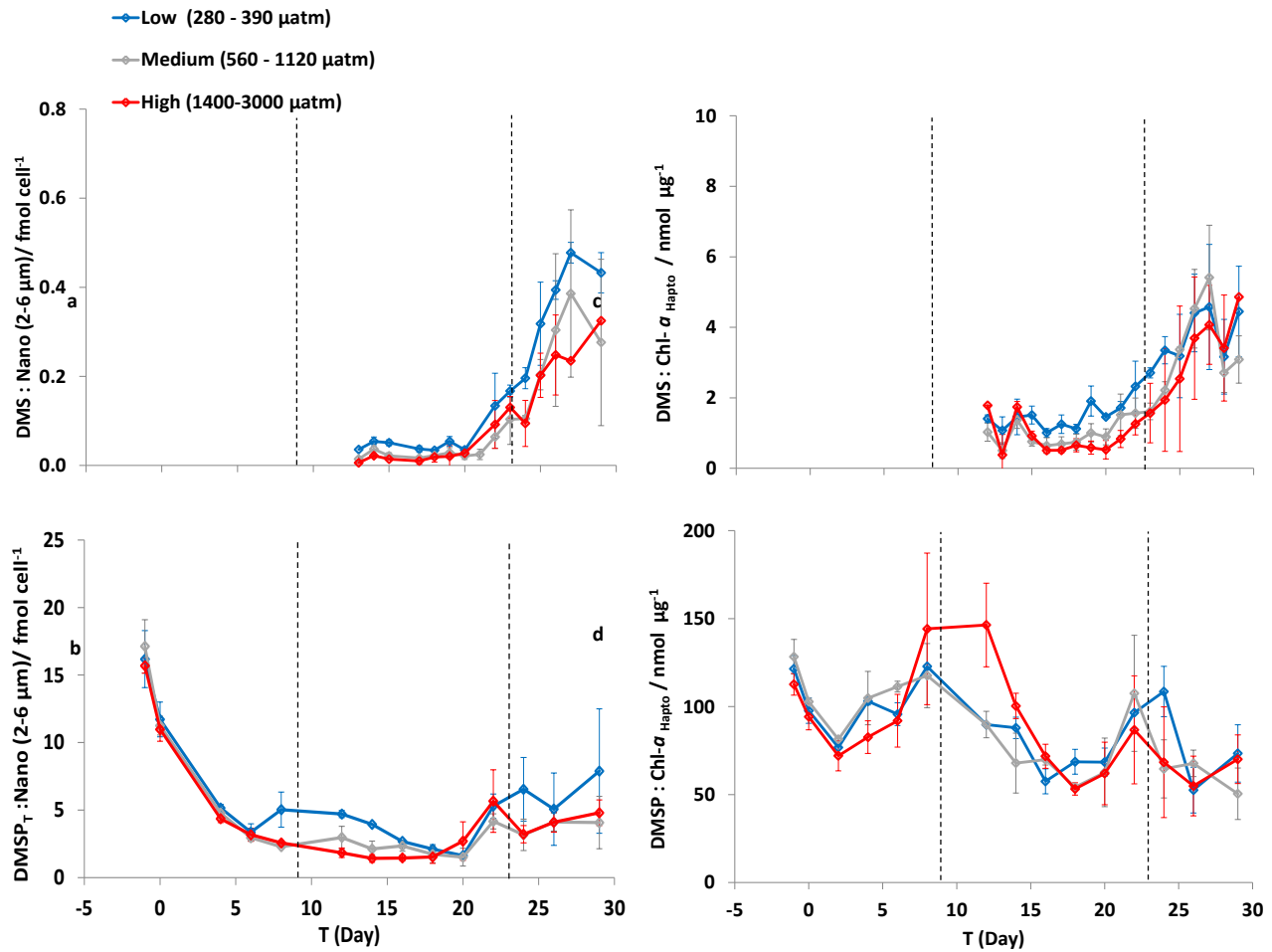


Figure 7. Mean ratios of (a) DMS to nanophytoplankton (2-6μm) (fmol cell⁻¹), (b) DMSP_T to nanophytoplankton (2-6μm) including *E. huxleyi* (fmol cell⁻¹) (c) DMS to haptophyte equivalent Chl-*a* (nmol ug⁻¹), and (d) DMSP_T to haptophyte equivalent Chl-*a* (nmol ug⁻¹) for three different pCO₂ conditions: low (blue; 280 μatm), medium (grey; 390 – 1120 μatm) and high (red; 1400 – 3000 μatm). Error bars show the standard deviation between all mesocosms of low, medium and high pCO₂.