Ocean acidification effects on haploid and diploid *Emiliana huxleyi* strains: why changes in cell size matter

M. Brady Olson^a, Tristen A. Wuori^a, Brooke A. Love^a, Suzanne L. Strom^a

^aShannon Point Marine Center, Western Washington University, 1900 Shannon Point Road, Anacortes, Washington, USA

Corresponding author: M. Brady Olson, brady.olson@wwu.edu

Abstract

The effects of ocean acidification (OA) on *Emiliania huxleyi* non-calcifying haploid strain CCMP 374 (*s374*) and calcifying diploid strain CCMP 2668 (*s2668*) were investigated in this study. Both *E. huxleyi* strains were cultured semi-continuously for ~20 generations under three $p\text{CO}_2$ levels, one representing equilibrium with contemporary atmospheric $p\text{CO}_2$ and two representing different OA scenarios projected for the end the century. During the course of the experiments, measurements were made for cell size in *s374* and coccosphere size in strain *s2668*, cellular intrinsic growth rates, and the per cell (*s374*) and per coccosphere (*s2668*) concentrations of particulate organic carbon (POC) and nitrogen (PON), particulate inorganic carbon (PIC), chlorophyll *a* (chl *a*) and particulate dimethylsulfoniopropionate (DMSP_p). In agreement with other studies, elevated $p\text{CO}_2$ led to a small increase in POC and PON in the diploid strain, but not in the haploid strain. Although the PIC was static across $p\text{CO}_2$ treatments in the diploid calcifier, PIC:POC was reduced under OA due to the disproportionate increase in POC. The concentration of *E. huxleyi* DMSP_p cell⁻¹ increased (*s2668*) or responded non-linearly (*s374*) to OA. Exposure to OA caused cell and coccosphere volumes to increase by 18% and 37% from the ambient to high $p\text{CO}_2$ treatment in the haploid and diploid strain, respectively, despite equivalent cross-treatment growth rates. Therefore, when normalized to cell and coccosphere volume, small to negligible increases in cellular constituents under OA translated into decreased cellular and coccosphere densities (e.g. mass vol⁻¹) of those same constituents. This produces seemingly contradictory cellular responses to OA depending on data normalization, and highlights the need to cautiously interpret OA experimental data when cell and coccosphere size is not reported. A consistent OA-induced increase in cell and coccosphere size has implications for *E. huxleyi* cellular physiology, ecology and trophic interactions, as cell size is a master

characteristic that governs cellular rate processes, predator encounter rates, and predator ingestion efficiencies. Further, because POC and PON did not scale with volume increases, there is a dilution in the carbon and nitrogen concentration in *E. huxleyi* cells under OA. This result could have cascading effects on planktonic food webs as predator ingestion rates and growth efficiencies adjust.

Key words: Ocean acidification, CO₂, *Emiliana huxleyi*, cell volume, food webs

Abbreviations: *s374*, haploid non-calcifying *Emiliana huxleyi*; *s2668*, diploid calcifying *Emiliana huxleyi*; POC, particulate organic carbon; PON, particulate organic nitrogen; PIC, particulate inorganic carbon; DOC, dissolved organic carbon; 1N, haploid *Emiliana huxleyi* cells; 2N, diploid *Emiliana huxleyi* cells; CCM, CO₂ concentrating mechanisms; DMSP_p, particulate dimethylsulfoniopropionate; DMS, dimethyl sulfide; chl *a*, chlorophyll *a*; GF/F, glass fiber filter

1. Introduction

The haptophyte *Emiliania huxleyi* is numerically the ocean's most abundant and cosmopolitan coccolithophore (Winter and Siesser, 1994). Because of its capacity to form dense blooms in mid- to high-latitudes with surface areas exceeding $9 \times 10^5 \text{ km}^2$ (Raitso et al., 2006) and cell concentrations exceeding $10^6 \text{ cells L}^{-1}$ (Berge, 1962), *E. huxleyi* populations contribute significantly to global carbon cycling, most notably through production of particulate organic and inorganic carbon (POC and PIC), and sulfur cycling through production of particulate dimethylsulfoniopropionate (DMSP_p) and its cleavage product, dimethyl sulfide (DMS). Populations of *E. huxleyi* are linked to higher trophic-level production by serving as a resource for micro- and macrozooplankton (Harris, 1994; Nejstgaard et al., 1997). For these reasons, *E. huxleyi* is one of the most intensively studied model phytoplankton species. Given that coccolithophores in general, and *E. huxleyi* in particular, precipitate a significant portion of the global ocean's CaCO_3 (Milliman, 1993), and the recognition of the potential effects that ocean acidification (OA) will have on calcifying organisms, *E. huxleyi* has been intensively thrust into the research spotlight in recent years.

The link between calcification and OA results from the series of surface ocean CO_2 -driven reactions that, under rising atmospheric CO_2 , will increase surface ocean $[\text{H}^+]$, and lower calcite saturation state (Ω_{Ca}) to levels where calcification may become energetically unfavorable ($\sim \Omega_{\text{Ca}} < 1$) (Doney, 2009). Because *E. huxleyi*'s CO_2 -dependent reactions are substrate-limited under contemporary $p\text{CO}_2$ (Paasche, 2002), reliance upon cellular modifications to increase enzymatic efficiency, e.g. carbon concentrating mechanisms (CCMs), has the potential to realign *E. huxleyi* cellular energy budgets in an acidifying ocean, with resultant cascading biological effects.

While there are some consistencies in *E. huxleyi* responses to OA across strains, i.e. a general decrease in PIC:POC with elevated $p\text{CO}_2$ (Findlay et al., 2011; Meyer and Riebesell, 2015), most empirically-derived observations show variable and conflicting OA-induced cellular responses in *E. huxleyi*, including: alterations in intrinsic growth rate (e.g. Bach et al., 2013; Iglesias-Rodriguez et al., 2008; Jones et al., 2013; Langer et al., 2009; McCarthy et al., 2012; Müller et al., 2010), photosynthetic rate (e.g. Borchard and Engel, 2012; Feng et al., 2008; Rokitta and Rost, 2012), production of POC (e.g. Barcelos e Ramos et al., 2010; Hoppe et al., 2011; Shi et al., 2009) and dissolved organic carbon (DOC) (Engel et al., 2004; Riebesell et al., 2007), transparent exopolymeric particle concentration (Engel et al., 2004; Pedrotti et al., 2012), dimethyl sulfide (DMS) and DMSP_p production and storage (Arnold et al., 2013; Spielmeyer and Pohnert, 2012), and elemental stoichiometry (Feng et al., 2008; Iglesias-Rodriguez et al., 2008; Riebesell et al., 2007). The reported changes in *E. huxleyi* cell division rates likely have cascading effects on other cellular parameters, and may be the mechanism explaining the accumulation of POC and PON, DMSP_p, and changes in cell size, the latter being a widely overlooked aspect of *E. huxleyi* studies (e.g. Engel et al., 2014; Langer et al., 2009; Richier et al., 2011).

The observed variability in the magnitude and direction of *E. huxleyi* biological responses to OA likely results from genetic diversity within populations and across strains (Langer et al., 2009). Further, in addition to elevated $p\text{CO}_2$ /reduced pH, cell culturing techniques and methods to acidify treatment water vary across experimental designs (Meyer and Riebesell, 2015; Shi et al., 2009), and many studies include arrays of environmental co-stressors (e.g. Borchard et al., 2011; Feng et al., 2008; Müller et al., 2012; Rouco et al., 2013), adding to the degree of variability in the direction and magnitude of *E. huxleyi* responses.

The life cycle of *E. huxleyi* is heteromorphic, haplo-diplontic, whereby cells exist as either motile, non-calcified haploid (1N) cells or calcified, non-motile diploid (2N) cells, both of which mitotically divide. Although *E. huxleyi* 1N non-calcifying morphotypes exist in nature, they are difficult to distinguish from other haptophytes, and it is assumed that the calcifying morphotype dominates blooms (Frada et al., 2012). Significant transcriptome divergence exists between the morphotypes, with 2N calcified cells having a 20% richer transcriptome than the 1N non-calcified cells, and $\leq 50\%$ transcript similarity between the 1N and 2N cells (von Dassow et al., 2009). The smaller transcriptome of the 1N *E. huxleyi* may constrain its ecological niche to a limited subset of environmental conditions that are unsuitable for 2N *E. huxleyi* (von Dassow et al., 2009). The degree to which the two morphotypes interconvert through meiosis and syngamy is unknown, but it is speculated that interconversion between ploidy states broadens the capability for *E. huxleyi* to withstand the ocean's hydrodynamic and chemical inconstancy (Rokitta et al., 2011), and viral- and grazer-induced mortality (Frada et al., 2008; Kolb and Strom, 2013).

Studies exploring the effects of OA on 1N *E. huxleyi* cells (e.g. Fiorini et al., 2011; Kottmeier et al., 2014; Leonardos and Geider, 2005; Pedrotti et al., 2012; Rokitta and Rost, 2012; Rokitta et al., 2012) are rare compared to those examining 2N *E. huxleyi* morphotypes. This disparity in research focus may seem intuitive or justified given the uncalcified state of 1N cells and the seeming dominance of the 2N morphotype in nature (Frada et al., 2008). Yet both morphotypes play roles in biogeochemical cycles and in the survival and propagation of *E. huxleyi* populations in nature. As such, comparisons of how the two *E. huxleyi* morphotypes respond to OA are relevant.

In this study, the effects of OA on a haploid (s374) and a diploid (s2668) strain of *E. huxleyi* were explored. Several physiological and morphological cell metrics were measured on these strains over the course of ~20 generations under three $p\text{CO}_2$ concentrations, one representing equilibrium with contemporary atmospheric $[\text{CO}_2]$, and two to different elevated concentrations within the predicted range of future CO_2 projections (IPPC, 2007). Given the wide body of work that has been done regarding *E. huxleyi*'s responses to OA over the last decade, what can one learn from another study? The results presented here will contribute to the growing and sometimes contradictory body of knowledge regarding *E. huxleyi*'s response to OA. But more importantly, highlighted here are two modifications to *E. huxleyi*'s biology that were observed under the single variable of OA. These are: 1) increased *E. huxleyi* cell and coccosphere volumes under OA that were unrelated to intrinsic growth rates and POC mass, and 2) an increase or non-linear response in cellular DMSP_p under OA. In addition to the cellular physiological implications of increased *E. huxleyi* DMSP_p , a change in cellular sulfur dynamics has wide-ranging climate implications because *E. huxleyi* is so prolific across temperate oceans. The cell and coccosphere volume changes, largely uncharacterized in *E. huxleyi* OA studies, may lead to altered cellular physiology, biochemistry and food web ecology in a future acidified ocean.

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2. Materials and Methods

2.1. Species and culturing technique

Strains of *E. huxleyi* s374 and s2668 were obtained from the National Center for Marine Algae and Microbiota (formerly CCMP). Strain 2668 is a representative of *E. huxleyi*'s 2N, non-motile calcifying morphotype, whereas strain s374 is a 1N, flagellated morphotype, and although it lacks coccoliths, produces organic scales on the cell membrane surface (Paasche, 2002). Stock *E. huxleyi* populations were cultured in autoclaved filtered (0.2 μm) seawater obtained from the northern Puget Sound, Washington State, amended with f/50 nutrients. Stock cultures were maintained at 15°C under $\sim 140 \mu\text{mol photons m}^{-2} \text{sec}^{-1}$ and a 14:10 light:dark cycle.

2.2. CO₂ culturing system

Many marine protists, including *E. huxleyi*, are acutely sensitive to mechanical stress, and react poorly when subjected to it (Gifford, 1993; Löder et al., 2010; Wolfe et al., 2002). Because of this, a CO₂ delivery system was designed to minimize mechanical stress (e.g. from direct bubbling) that would otherwise damage delicate protists. Ambient compressed air (Powerex oilless rotary compressor) is scrubbed of CO₂ (Puregas CAS series CO₂ adsorber) prior to mixing with compressed research grade CO₂ using Sierra SmartTrak Mass flow controllers (MFC) to achieve the desired CO₂ treatment concentrations. Downstream of MFCs, each treatment gas flows into a manifold system that regulates pressure and directs gas to 1) 20L carboys where treatment gasses are bubbled into large volumes of media under controlled temperature for at least 24 h, and 2) to Plexiglas boxes housed in a walk-in environmental chamber held at 15°C. A LiCOR Li-820 CO₂ sensor is used to measure CO₂ in gas entering or exiting these system components. During experiments, *E. huxleyi* cultures are placed in the Plexiglas boxes, where gas exchange between the incoming CO₂ treatment gas and the culture media maintains treatment $p\text{CO}_2$ between semi-continuous water changes described below.

2.3. *Emiliania huxleyi* characterization experiments

Autoclaved filtered seawater from the nutrient-rich northern Puget Sound, Washington State, was used in experiments. Prior to experiments this seawater, containing ambient concentrations of 27.9 and 29.1 μM nitrate+nitrite, and 2.4 and 2.5 μM phosphate for *s2668* and *s374* experiments, respectively, was amended with f/50 nutrient concentration to ensure that cells were not nutrient limited during experiments. Media was pre-equilibrated as described above to three $p\text{CO}_2$ treatments, ambient (A; ~ 400 ppmv), moderate (M; ~ 750 ppmv), and high (H; ~ 1000 ppmv). Both strains were inoculated at very low cell densities (~ 2000 cells ml^{-1}) into pre-equilibrated media, with five replicate cultures in each $p\text{CO}_2$ treatment. After inoculation, cultures were placed in Plexiglas boxes supplied with corresponding CO_2 gas, and housed in an environmental chamber maintained at 15°C . Experimental cultures were supplied with $134\text{--}156$ $\mu\text{mol photons sec}^{-1} \text{ m}^{-2}$, near fully-saturated light intensity for *E. huxleyi* growth at 15°C (Nielsen, 1997), on a 14:10 light:dark cycle. Experimental cultures in each $p\text{CO}_2$ treatment grew in batch mode until reaching 1×10^5 cells ml^{-1} , after which time they were diluted daily for 14 days (18 and 20 generations for *E. huxleyi s374* and *s2668*, respectively). Dilutions were carried out 2 h after initiation of the light cycle with media pre-equilibrated to respective $p\text{CO}_2$ treatment concentrations, restoring $p\text{CO}_2$ of cultures which had been drawn down slightly by the physiological activity of the cells. Prior to daily dilutions, cell densities were determined using a Bd FACSCalibur flow cytometer. Cell densities were used to calculate the volume of culture to remove in order to return experimental flasks to 1×10^5 cells ml^{-1} after 24 h of growth. Dilution volumes averaged $\sim 60\%$ of the initial culture volume. Throughout the time course of the experiment, culture removed from each treatment replicate during daily dilutions was used to characterize carbonate chemistry (Table 1), and the following *E. huxleyi* cellular metrics: cellular

volume, intrinsic growth rates, chlorophyll *a* (chl *a*), PIC and POC, particulate organic nitrogen (PON), and DMSP_{*p*}.

2.4. Carbonate chemistry

Seawater pH was measured daily using a Metrohm Ecotrode combined electrode, calibrated with TRIS and AMP buffers on the total scale (Dickson et al., 2007). 20 ml subsamples were carefully transferred into glass scintillation vials via syringe. Vials were filled to overflowing with no headspace and kept in a 20°C water bath for 1 h prior to pH measurement. 100 ml subsamples were taken every other day for measurement of total alkalinity (TA). Samples were gravity-filtered through GF/F filters, stored at 6°C and titrated using open-cell potentiometric gran titration (Metrohm 888 Titrando titrator) for total alkalinity within two weeks of sampling (Dickson et al, 2007). Remaining carbonate parameters were calculated with CO2SYS (Lewis and Wallace, 1998) using the constants of Mehrbach et al. (1973) refit by Dickson and Millero (1987), and Dickson (1990). Estimated precision of 10 µeqkg⁻¹ in TA was determined against an artificial seawater standard. Combined with a precision of 0.02 in pH, uncertainty in calculated *p*CO₂ is approximately 20 µatm in the ambient treatment and 40 µatm in the high treatment.

2.5. Cell and coccosphere volume

A Photometrics CoolSNAP camera attached to an Olympus CH30 microscope was used to image live *E. huxleyi* cells from each of the five *p*CO₂ treatment replicates for each strain over the duration of the experiments. Cell diameter (*s*374) and coccosphere diameter (*s*2668) of the first twenty cells in each treatment replicate were measured using Image-Pro Plus 5.0 software. Cell and coccosphere volumes were calculated assuming spherical cellular geometry.

2.6. Cellular intrinsic growth rates

Intrinsic growth rate measurements were made daily ($n = 10$) in each *E. huxleyi* treatment replicate during the course of the experiments. Flow cytometer counts of *E. huxleyi* cell numbers were used to calculate growth rates according to $\mu = \ln(N_2) - \ln(N_1)/t_2 - t_1$, where N_2 is the cell concentration before dilution on day t_2 , and N_1 is the cell concentration after dilution on the previous day (t_1).

2.7. Particulate carbon and nitrogen

Duplicate 60 ml subsamples from each treatment replicate were filtered through 13 mm GF/F filters that were previously muffled at 450°C for 4 h. One of the two paired filters was placed in a tin and one in a silver capsule; both were dried at 60°C for 24 h. Filters in silver capsules were acid-fumed for 24 h to drive off PIC. POC and PON were quantified using a CE Elantech Flash EA 112 elemental analyzer. PIC was calculated by subtracting POC from total particulate carbon.

2.8. Coccolith number and area

On the final day of sampling (day 14), small aliquot samples of *s2668* were filtered onto 0.2 μm black polycarbonate filters, and allowed to air dry for 24 hours. Filters were subsequently mounted onto scanning electron microscopy sample stubs, and sputter coated. A Vega TS 5136 MM SEM was used to image between 220 to 340 cells from each treatment. From these images, Image J software was used to calculate the area of the coccosphere, and to count the number of coccoliths visible on each coccosphere. For enumerating the number of coccoliths, only coccoliths with the entire central area visible were counted. Individual coccoliths oriented exclusively in the x-y plane were measured for area.

2.9. Cellular chlorophyll *a*

10 ml subsamples from each treatment replicate were taken for measurement of chl *a*. Samples were gravity-filtered through GF/F filters and stored at -70°C until analysis (< 1 month). Upon analysis, filters were extracted in 90% v/v acetone for 24 h in darkness at -16°C. After extraction, chl *a* was quantified using a Turner Designs 10-AU fluorometer using the acidification method of Parsons (1984).

2.10. DMSP_p

Subsamples of 25 ml from each treatment replicate were gravity-filtered onto GF/F filters for analysis of DMSP_p. Filters were muffled at 500 °C for 24 h prior to use. Immediately after filtration, the GF/F filters were placed into muffled glass vials containing 3 ml of 10 N NaOH. Vials were sealed with butyl septa and vortexed. Standards were prepared at the same time as sample collection. Samples and standards were stored in the dark until analysis according to Wolfe et al. (2002). Cellular DMSP_p was measured using a Shimadzu GC-14A gas chromatograph equipped with a flame photometric detector.

2.11. Data treatment

The biological metrics analyzed for both *E. huxleyi* strains were averaged over the time course of the experiment for each of the 5 replicates in each *p*CO₂ treatment. These values for each replicate were then averaged to give a treatment mean. This approach minimizes any sampling variability at the different time points associated with details of culturing methods, but maintains independent replicates and treatment differences if they exist. Further, with the exception of intrinsic growth rates (10 time points), all other metrics were sampled at only 3 to 4 time points, thus prohibiting any rigorous time-inclusive statistical tests. Due to the considerable

time required for SEM analysis, coccolith metrics were analyzed from only the last day of the s2668 experiment. All data are presented with error estimates of one standard deviation, with the exception of cell volume presented in Fig. 1. Time-averaged treatment values for each biological metric displayed normal distributions and variances were homogenous, thus treatment differences were assessed using ANOVA with Tukey post-hoc comparisons (SPSS 17).

3. Results

3.1. $p\text{CO}_2$.

The CO_2 culturing system proved effective at maintaining steady carbonate chemistry in treatment replicates over the course of the two 14 d experiments (Table 1). Although average $p\text{CO}_2$ was slightly below pre-equilibrated media concentrations due to the physiological activity of the cells, drawdown of carbon was less than 2% relative to the media in all cases. For s374, time-averaged $p\text{CO}_2$ was 400 ± 69 ppmv, 665 ± 12 ppmv, and 871 ± 70 ppmv, and for s2668, time-averaged $p\text{CO}_2$ was 323 ± 14 ppmv, 664 ± 31 ppmv, and 800 ± 23 ppmv, for the ambient, moderate and high treatments, respectively.

3.2. Cell and coccosphere size

Both *E. huxleyi* strains showed a significant increase in volume with increasing $p\text{CO}_2$ (Fig. 1; Tables 2 and 3). Strain 374 increased in cell volume (μm^3) from 71.52 ± 4.50 and 73.20 ± 5.34 in the ambient and moderate treatments, respectively, to 82.43 ± 1.81 in the high treatment. These differences were statistically significant ($p < 0.005$), with the high treatment yielding cell volumes larger by ~18% than the ambient and moderate treatments.

The *E. huxleyi* s2668 showed a significant step-wise increase ($p < 0.001$) in coccosphere volume across all $p\text{CO}_2$ treatments (Fig. 1B; Table 3). Coccosphere volumes in the ambient, moderate and high treatments were 192.51 ± 7.35 , 229.00 ± 5.76 , and $264.37 \pm 13.06 \mu\text{m}^3$, respectively. This corresponds to an 18 and 37% increase in coccosphere volume under moderate and high $p\text{CO}_2$ treatments, respectively, compared to volume in the ambient treatment.

Two considerations are needed when interpreting the cell size results. First, can images of live cells resolve relatively small differences in cell volume and diameter? The cell and coccosphere volumes are calculated assuming cells are spherical. Apparent settling and motility of cells during imaging indicate that cells were not under pressure from the cover slip to a degree which would distort their spherical shape. While observed differences in diameter are close to the potential resolution of the imaging, the large number of cells analyzed ($n=700$), and the consistency of this response through repeated trials add to the reliability of this statistical result. Secondly, a direct comparison between the size measurements in the 2 strains is inappropriate given that for s2668, coccosphere volume was assessed rather than cell volume. Because coccoliths obscured visualization of s2668's cell membrane, it was impossible to determine visually whether the stronger volumetric response in s2668 was attributable to the cell, the coccolith morphology and arrangement, or both. Due to this constraint, volumetric comparisons of cellular constituents between strains (described below) should be avoided, and the observed concentration changes across treatments in s2668 should be interpreted cautiously.

3.3. *Emiliana huxleyi* growth rate

Both strains of *E. huxleyi* exhibited similar growth rates (μ , d^{-1}) across $p\text{CO}_2$ treatments (Tables 2 and 3). Time-averaged growth rates in s374 were 0.89 ± 0.09 , 0.91 ± 0.10 , and 0.94 ± 0.07 in the ambient, moderate and high treatments, respectively. Strain 2668 growth rates were

slightly higher than those observed for *s374*, averaging 1.00 ± 0.15 , 0.99 ± 0.16 , and 1.02 ± 0.08 , for the ambient, moderate and high treatments, respectively.

3.4. Particulate organic and inorganic carbon

Elevated $p\text{CO}_2$ had no significant effect on POC cell^{-1} in *s374* (Fig. 2A; Table 2). POC across the three treatments was approximately 12 pg cell^{-1} . When normalized to cell volume, POC ($\text{fg } \mu\text{m}^{-3}$) in *s374* was, on average, lower in the high $p\text{CO}_2$ treatment (148.60 ± 5.70) compared to the ambient (171.50 ± 014.00) and moderate treatments (174.00 ± 20.80). Strain 2668 had nearly 2x more POC cell^{-1} than *s374* in all $p\text{CO}_2$ treatments ($p < 0.001$). On average *s2668* cells cultured under high $p\text{CO}_2$ contained more pg POC cell^{-1} (23.61 ± 1.05) than cells cultured under ambient (21.71 ± 1.77) and moderate (21.90 ± 1.55) $p\text{CO}_2$ (Fig. 2B; Table 3). These differences correspond to high $p\text{CO}_2$ cultured *s2668* cells having 8.8% and 7.4% more POC than ambient and moderate $p\text{CO}_2$ cultured cells, respectively. This trend, however, was reversed on a volumetric basis. Here, moderate and high $p\text{CO}_2$ -cultured *s2668* cells had significantly less POC μm^{-3} of coccosphere volume ($p < 0.01$ and $p < 0.001$, respectively) than cells cultured under ambient $p\text{CO}_2$ (Fig. 2B; Table 3). This is directionally similar to the non-significant trend seen in *s374* when POC was normalized to cell volume.

Strain 2668 did not demonstrate a significant calcification response to elevated $p\text{CO}_2$ on a cell^{-1} basis (Fig. 3; Table 3). The cellular mass of PIC (pg PIC cell^{-1}) was 16.86 ± 3.64 , 15.01 ± 1.63 , and 17.11 ± 1.83 for the ambient, moderate and high $p\text{CO}_2$ treatments, respectively. On a volumetric basis, however, there was a significantly greater ($p < 0.03$) concentration of PIC ($\text{fg } \mu\text{m}^{-3}$ per coccosphere volume) in the ambient $p\text{CO}_2$ cultured cells (87.80 ± 19.80) than in cells cultured under moderate (65.60 ± 7.20) and high (64.80 ± 6.80) $p\text{CO}_2$ (Fig. 3; Table 3).

A significant decrease in the ratio of PIC to POC (PIC:POC) with elevated $p\text{CO}_2$ for *s2668* was observed (Table 3). The PIC:POC was highest in the ambient treatment (0.83 ± 0.10), and was significantly greater ($p < 0.04$) than that of the moderate (0.69 ± 0.09) and high (0.70 ± 0.05) $p\text{CO}_2$ treatments.

3.5. *Coccolith number and area*

Coccoliths surrounding *s2668* cells from the moderate and high $p\text{CO}_2$ treatments were significantly larger ($p < 0.001$) than coccoliths surrounding cells cultured under ambient $p\text{CO}_2$ (Fig. 4; Table 4). As expected with larger coccoliths, when the number of visible coccoliths is normalized to area, significantly fewer coccoliths on the moderate and high $p\text{CO}_2$ cultured cells were visible per μm^2 ($p < 0.001$).

3.6. *Particulate organic nitrogen*

On average, *s374* showed a slight increase in pg PON cell^{-1} with increasing $p\text{CO}_2$, averaging 0.82 ± 0.10 , 0.92 ± 0.24 , and 1.04 ± 0.11 , for the ambient, moderate and high $p\text{CO}_2$ treatments, respectively (Fig. 5A; Table 2). These differences, however, were not significant. The cell PON concentration was also unchanged on a volumetric basis in *s374*, averaging $\sim 12 \text{ fg } \mu\text{m}^{-3}$ of cell volume in each $p\text{CO}_2$ treatment. PON of *s2668* was higher overall by $\sim 3\text{x}$ than that of *s374* (Figure 5A and B). The pg PON cell^{-1} was significantly greater ($p < 0.009$) in *s2668* under the high $p\text{CO}_2$ treatment (3.90 ± 0.24) compared to ambient (3.35 ± 0.39) and moderate (3.25 ± 0.16) treatments (Fig. 5B; Table 3). As was observed for cell POC concentration, PON decreased on a volumetric basis. Here, $\text{fg PON } \mu\text{m}^{-3}$ of coccosphere volume was greatest in ambient $p\text{CO}_2$ cultured cells, and was significantly greater ($p < 0.02$) than cells cultured under moderate $p\text{CO}_2$ (Fig. 5B; Table 3).

In *s374* POC:PON ratios were, in general, high across all $p\text{CO}_2$ treatments. Ratios decreased with increasing $p\text{CO}_2$ by 9.9% and 17.3% in the moderate and high treatments, respectively, but the decreases were only significant at $p < 0.06$ (Table 2). Strain 2668 had considerably lower POC:PON ratios than *s374*, with ratios adhering closely to Redfield values. No treatment effect on mean *s2668* POC:PON was found (Table 3).

3.7. Cellular chlorophyll *a*

While *s2668* had nearly 2x the chl *a* content of *s374*, no significant difference was observed in chl *a* cell⁻¹ across $p\text{CO}_2$ treatments in either strain (Fig. 6A and B; Tables 2 and 3). When normalized to cell volume, the concentration of chl *a* in *s374* was significantly greater in the ambient compared to high $p\text{CO}_2$ treatment ($p < 0.03$). This same trend was observed in *s2668*, where chl *a* was greater per coccosphere volume in the ambient treatment compared to both the moderate ($p < 0.007$) and high ($p < 0.004$) $p\text{CO}_2$ treatments.

3.8. Particulate dimethylsulfoniopropionate

The cellular concentration of DMSP_{*p*} in *s374* showed a significant non-linear response (Fig. 7A; Table 2). In this case DMSP_{*p*} cell⁻¹ in the high $p\text{CO}_2$ treatment (1.80 ± 0.03) was significantly greater ($p < 0.008$) than in the moderate treatment (1.41 ± 0.29), but similar to DMSP_{*p*} concentrations in the ambient $p\text{CO}_2$ treatment. Despite this, cell volume specific DMSP_{*p*} in *s374* showed no response to treatment. As seen for other cellular constituents, DMSP_{*p*} cell⁻¹ was greater in *s2668* than in *s374* (Fig. 7; Tables 2 and 3). For *s2668*, cells cultured under high $p\text{CO}_2$ had significantly more ($p < 0.001$) DMSP_{*p*} cell⁻¹ by 22% and 19% than cells cultured under ambient and moderate $p\text{CO}_2$, respectively (Fig. 7B; Table 3). This trend was reversed, however, when normalizing DMSP_{*p*} to coccosphere volume. Here, cells from the ambient treatment had

17% more $\text{DMSP}_p \mu\text{m}^{-3}$ of coccosphere volume ($p < 0.04$) than cells cultured under the moderate treatment, and while non-significant, on average 9% more DMSP_p than cells in the high $p\text{CO}_2$ treatment.

4. Discussion

Two findings from this study are emphasized that will add new information and bring fresh perspective to the otherwise wide body of knowledge regarding the effects of OA on *E. huxleyi* biology and ecology. Observed here were increased cellular DMSP_p , and larger cell and coccosphere volumes under OA despite equivalent cross-treatment growth rates, and without inclusion of a co-stressor in the experimental design. The former adds new information about *E. huxleyi*'s cellular DMSP_p response to OA, a comparatively understudied phenomenon. The latter is a novel finding because size differences cannot be attributed to differences in growth rates and were observed with OA as the sole stressor. This result prompts wider discussion about the physiological and ecological tradeoffs of increasing cell size caused by OA.

The generalized OA response of *E. huxleyi* DMSP_p is, like other *E. huxleyi* OA responses, difficult to discern due to complications imposed by differing experimental designs. From the few studies exploring *E. huxleyi*'s DMSP_p response to OA without concurrent temperature stress, results range from a strong decrease in DMSP_p (Avgoustidi et al. 2012), to no change (Arnold et al. 2012; Spielmeyer and Pohnert, 2012 [strain RCC 1731]; Webb et al., 2016), to a modest increase (Spielmeyer and Pohnert, 2012 [strain RCC 1242]). In this study OA increased $\text{DMSP}_p \text{ cell}^{-1}$ in *E. huxleyi* s2668, and although the increase in s374 was non-linear, highest on-average DMSP_p was observed in the high $p\text{CO}_2$ treatment (Table 5). Results for s374 are in agreement with Spielmeyer and Pohnert (2012), who also found an increase in *E.*

huxleyi DMSP_p per cell under elevated $p\text{CO}_2$ in a non-calcifying strain (RCC 1242). Their results showing no increase in DMSP_p in calcifying strain RCC 1731 under elevated CO_2 alone (i.e. without increased temperature), however, are contradictory to what was observed for calcifying *s2668*.

The per cell increase in DMSP_p observed here has the potential for climate feedback, in a manner dependent on the degree to which the DMSP_p is cleaved into its breakdown product, DMS. DMS is a volatile compound that, when outgassed into the atmosphere, can act as cloud condensation nuclei, thus contributing to the atmosphere's albedo. If OA-induced increases in DMSP_p are widespread across bloom-forming *E. huxleyi* strains, increased DMSP production under OA and climate co-stressors may constitute a mechanism that counteracts the warming effect associated with increasing atmospheric CO_2 . Others, however, have shown either static (Webb et al., 2016) or reduced production and concentration of DMSP and DMS (Arnold et al., 2013; Avgoustidi et al. 2012) under OA. As such, the direction and magnitude of *E. huxleyi*-driven sulfur dynamics under OA, and its influence on climate, remain uncertain in an acidifying ocean.

Results from this study demonstrated that 1N and 2N *E. huxleyi* morphotypes increased their volume under elevated $p\text{CO}_2$. This volume increase occurred in the absence of differences in cell growth rates, and for *s2668*, without an increase in PIC mass. Cell volumes of the 1N and coccosphere volumes of the 2N strains increased by 14% and 37%, respectively, from the ambient to high $p\text{CO}_2$ treatment. Because the two *E. huxleyi* ploidy states show genetic disparity (e.g. Rokitta et al., 2012; von Dassow et al., 2009) and occupy different ecological niches (Rokitta et al., 2011; von Dassow et al., 2009; von Dassow et al., 2015), volume increases in response to OA may be a common response among genetically disparate *E. huxleyi* strains.

Despite this, very few OA studies report on cell size. For most that do, their reported changes in *E. huxleyi* volume cannot be uncoupled from concomitant OA-induced changes in population growth rate (e.g. Avgoustidi et al., 2012; Bach et al., 2011; Barcelos e Ramos et al., 2010; De Bodt et al., 2010; Fiorini et al., 2011; Gao et al., 2009; Iglesias-Rodriguez et al., 2008; Jones et al., 2013; McCarthy et al., 2012; Müller et al., 2015; Pedrotti et al., 2012), increased organic C, N and P (Rouco et al., 2013), or decoupled from the effect of additional stressors, e.g. phosphorous limitation (Borchard et al., 2011) and temperature (e.g. Arnold et al., 2013; Borchard et al., 2011; De Bodt et al., 2010; Feng et al., 2008).

Cell size and other phytoplankton morphological characteristics are generally assumed to have evolved in response to selective pressures involving diffusion limitation of resource supply rates (e.g. Chisolm, 1992; Irwin et al., 2006), and sinking from regions with adequate light for photosynthesis. These constraints explain the preponderance of very small cells in oligotrophic environments, while larger cell size can be favored when inorganic nutrients are abundant and turbulence is high. Within the genetically prescribed size range of a given species or strain, however, plasticity exists, and size can respond to environmental cues (Akçaalan et al., 2007; Morabito et al., 2007). It is possible that the low present-day $p\text{CO}_2$ selects for small cells in a manner analogous to reduced levels of nitrogen, phosphorus, and other nutrients. Given that the production, maintenance and operation of CCMs have an energetic cost (Raven, 1991), it is generally accepted that marine algae are sometimes CO_2 limited. The variable expression and activity of CCMs may be driven by the range of DIC concentrations and speciation found in nature today (Raven et al., 2011). Evidence for CO_2 limitation in *E. huxleyi* is found in Richier et al. (2011), who showed down-regulation of carbonic anhydrase (CA) transcripts under OA. Additionally, Bach et al. (2013) showed that *E. huxleyi* genes encoding for CA were upregulated

under low DIC. Similarly, Kottmeier et al. (2014) showed a strong shift in uptake to the energetically less costly CO_2 from HCO_3^- under reduced pH. The relief of chronic CO_2 limitation that comes with OA, in the absence of co-limiting resources and size-selective grazing, may allow *E. huxleyi* to exist closer to the upper edge of its genetically prescribed size range. That the observed size changes reported here were a response to OA and possibly the elevated $p\text{CO}_2$ is supported by the fact that light, nitrogen and phosphorus were at growth-saturating levels during experiments.

A consistent cell size effect under OA has implications for interpretation of existing experimental OA data. A number of the response variables measured here were unchanged on a cell⁻¹ basis, but were reduced under OA when normalized to cell or coccosphere volume, leading to a reduction in intracellular concentration of measured cellular matter and highlighting the disconnect between accumulation of cellular constituents and OA-induced size increases. For example, no change in pg chl *a* cell⁻¹ was found in either strain under OA, but when scaled to cell volume, a reduction in concentration of chl *a* was observed (Table 5). In *s2668* PIC mass was the same across treatments on a cell⁻¹ basis, but when scaled to coccosphere volume, fg PIC μm^{-3} decreased from the ambient to the moderate and high $p\text{CO}_2$ treatments. Strain 2668 did show a small increase in cellular POC, and a larger increase in cellular PON and DMSP_p under elevated $p\text{CO}_2$, but because of the comparatively larger increases in coccosphere volume with elevated $p\text{CO}_2$, this trend was reversed when normalized to volume, such that these same parameters decreased with the increased $p\text{CO}_2$ on a per coccosphere volume basis (Table 5). The degree to which cellular constituents in *s2668* were diluted per cell volume rather than per coccosphere volume cannot be determined in this study, and therefore conclusions about this aspect of the study should be interpreted cautiously. Despite this caveat, the finding that PIC

mass did not increase in *s2668* under OA, and was accordingly reduced per unit coccosphere volume, suggests that the increase in *s2668* coccosphere volume was accompanied by, and perhaps largely a result of, a simultaneous increase in cell volume. If one calculates PIC mass per surface area in *s2668*, PIC mass decreased from ~ 0.11 pg PIC μm^2 to 0.08 pg PIC μm^2 from the ambient to high $p\text{CO}_2$ treatments, respectively. This finding supports the assumption that the coccosphere is thinning, and that the increase in coccosphere volume in *s2668* includes an increase in cell volume. Further, Müller et al. (2012) observed a step-wise increase in *E. huxleyi* coccosphere volumes from low to moderate to high $p\text{CO}_2$. When volumes were re-measured after removal of coccoliths, a step-wise increase in cell volume was also observed. The differences between these two volume measurements within treatments were proportional across $p\text{CO}_2$ treatments. That is, the ratios of coccosphere to cell volume were 1.22, 1.21 and 1.16 for the low, moderate and high $p\text{CO}_2$ treatments, respectively. Therefore, although it remains unknown what proportion of the volume increase in *s2668* is due to changes in the coccosphere versus cell size, if it can be assumed that the changes are proportional as seen in Müller et al. (2012), then the observed trends of reduced concentrations of cellular parameters with OA relative to coccospheres volume would be true on a per cell volume basis as well.

The mechanism that caused both strains of *E. huxleyi* to increase in volume under OA remains elusive. In addition to equivalent cellular growth rates across $p\text{CO}_2$ treatments, POC cell^{-1} did not significantly increase in either strain, and thus did not scale with cell or coccosphere volume. In *s2668* no addition of PIC cell^{-1} across $p\text{CO}_2$ treatments was observed; therefore, addition of PIC mass is unlikely the mechanism by which the coccosphere volume increased in *s2668*. It was found, however, that individual coccoliths were larger and fewer per surface area on *s2668 E. huxleyi* cells cultured under high $p\text{CO}_2$. Plasticity in coccolith

morphology in response to environmental variability, including fluctuation in $p\text{CO}_2$, is well known (Paasche, 2002). Iglesias-Rodriguez et al. (2008), Müller et al. (2012) and Rouco et al. (2013) showed that in addition to increased *E. huxleyi* cell volume under elevated $p\text{CO}_2$, cells were also encased by larger coccoliths. In these instances though, cellular growth rates were either lower under elevated $p\text{CO}_2$ (Iglesias-Rodriguez et al., 2008; Müller et al., 2012), or cells showed elevated PIC under OA conditions (Iglesias-Rodriguez et al., 2008; Müller et al., 2012; Rouco et al., 2013). In this study, the degree to which the larger coccoliths, and by extension, the arrangement of the coccoliths surrounding the cell, contributed to the increase in cell volume is unknown.

If cells consistently increase in volume under OA, there are potential physiological and ecological consequences of this morphological change. Litchman and Klausmeier (2008) argue that cell size is a ‘master’ trait that governs the occupation of specific ecological niches by phytoplankton. Indeed, cell size influences metabolic rate processes, nutrient uptake rates and half saturation constants, nutrient diffusion, light absorption, sinking velocity, population abundance, and trophic interactions (reviewed in Finkel et al., 2009). While the increases in volume observed for both strains under OA are modest, the power-law functions that govern phytoplankton physiological and ecological processes are a function of volume (Finkel et al. 2010). The cell volume increase of 18% and coccosphere volume increase of 37% from ambient to high $p\text{CO}_2$ for *s374* and *s2668*, respectively, may be substantial enough to drive, at the very least, subtle physiological and ecological changes in *E. huxleyi* populations. For the latter, the selection of prey by planktonic grazers is primarily governed by size (Kjørboe, 2008). That is, prey characteristics such as behavior (e.g. motility) and chemical signature matter less than cell size when grazers select prey, in part because as prey size increases so too does encounter rate

with predators. Additionally, the changes in *E. huxleyi* cell size under OA may bring the population into or out of the optimal prey size spectra for particular, and potentially dominant, grazers (Hansen et al., 1994). While the volume changes observed here may not be large enough to affect the grazing behavior of larger zooplankton (e.g. copepods), they are large enough to affect microzooplankton grazers (Kendall, 2015; Still, 2016). These factors collectively may cause *E. huxleyi* populations to be grazed at higher rates (i.e. increased *E. huxleyi* size increases predator-prey encounter rates and moves cells into optimal prey size window for dominant grazers) or lower rates (i.e. in the absence of a diverse predator community, the size increase under OA moves *E. huxleyi* outside of the prey size spectra for the available grazers). If changes in cell size are widespread, they will influence grazing mortality for *E. huxleyi* populations, and will be a governing mechanism for community composition and the size structure of planktonic communities.

Increased *E. huxleyi* cell volume and decreased concentrations of cellular constituents may affect elemental trophic transfer in planktonic food webs. To illustrate this, consider that a food vacuole or gut cavity will hold fewer large cells than small cells. If, as in the case of s374, the POC quota per cell is the same for the two prey cell sizes, less carbon is consumed per unit grazer vacuole or gut cavity volume when grazers consume larger cells. Under these conditions grazers will be forced, if possible, to increase their ingestion rates in order to meet their own carbon demand and nutrient quotas. Increased grazer ingestion rates can increase production of DOC through sloppy feeding (Møller et al., 2003; Strom et al., 1997), decrease assimilation efficiencies (Landry et al., 1984; Urabe and Watanabe, 1991), increase fecal pellet production (Butler and Dam, 1994), and increase nutrient remineralization (Takahashi and Ikeda, 1975;

Urabe, 1993). These processes have ramifications for the structure, function, and efficiency of the microbial loop and plankton food web.

The results of this study add to the growing body of work elucidating *E. huxleyi*'s responses to OA. As in many other studies, the strains investigated here have been in culture for many years, providing a rich context for interpretation of responses (e.g. Bidle et al., 2007; Nuester et al., 2014; Strom et al., 2003; Strom and Bright, 2009; Wolfe et al., 1997). Although there is no direct evidence that long-term culture of *E. huxleyi* strains influences their OA response relative to naturally occurring populations, it should be borne in mind that adaptation to culture may lead to genetic change that influences physiological and biochemical responses. The data reported here are in agreement with one of the most reported *E. huxleyi* responses to OA, a reduction in PIC:POC (Meyer and Riebesell, 2015). The reduction in this ecologically important ratio resulted from a small increase in POC rather than reduced mass of PIC. Elevated DMSP_p cell⁻¹ was observed in the high *p*CO₂ treatments for both ploidy states. This finding, in the absence of co-environmental stressors and changes in growth rates across treatments, makes this aspect of the study novel. The primary emphasis of this study, however, is the either overlooked or underappreciated aspect of a cell size response to OA. This effect should be considered when interpreting experimental results, as it has implications for understanding cell physiological adjustments to, and ecological consequences of, OA. The increase in cell volume under OA without concomitant changes in growth rate, and either zero (*s*374), or only small increase in POC (*s*2668) suggests a dilution of cellular constituents in OA-exposed *E. huxleyi* cells. As discussed above, this may have cascading effects on the physiology of *E. huxleyi* cells, and on the ecology of *E. huxleyi*-dominated planktonic systems given that cell size is a primary governing trait in the biology and ecology of phytoplankton.

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Table 1

Table 1. Carbonate chemistry parameters within each treatment during both *E. huxleyi* strain characterization experiments. pH and total alkalinity (TA) were measured. All others calculated using CO2sys. Drawdown of carbon relative to the media was less than 2% of total dissolved inorganic carbon (DIC) in all cases. *s374*: *E. huxleyi* non-calcifying haploid strain 374; *s2668*: *E. huxleyi* calcifying diploid strain 2668. Units: $p\text{CO}_2$ (μatm); CO_2 , HCO_3^- , CO_3^{2-} , DIC ($\mu\text{mol kg}^{-1}$); TA ($\mu\text{Eq kg}^{-1}$).

Treatment	Ambient		Moderate		High	
	<i>s374</i>	<i>s2668</i>	<i>s374</i>	<i>s2668</i>	<i>s374</i>	<i>s2668</i>
pH	8.04 ± 0.06	8.04 ± 0.02	7.85 ± < 0.00	7.78 ± 0.01	7.74 ± 0.03	7.69 ± <0.00
$p\text{CO}_2$	400.6 ± 69.3	323.2 ± 13.6	665.1 ± 12.4	664.5 ± 31.7	871.9 ± 70.7	800.4 ± 23.4
CO_2	15.1 ± 2.6	12.2 ± 0.5	24.1 ± 0.5	25.1 ± 1.2	32.9 ± 2.7	30.2 ± 0.9
HCO_3^-	1876.0 ± 50.7	1522.8 ± 29.3	2008.6 ± 25.7	1694.7 ± 23.3	2051.3 ± 38.5	1680.1 ± 30.1
CO_3^{2-}	149.5 ± 15.9	120.5 ± 6.1	101.7 ± 2.3	72.2 ± 2.1	81.0 ± 5.2	58.9 ± 0.9
DIC	2040.2 ± 38.4	1655.5 ± 33.6	2135.3 ± 27.5	1792.0 ± 23.2	2165.2 ± 39.2	1769.2 ± 31.4
TA	2258.1 ± 20.2	1847.0 ± 40.1	2262.9 ± 29.0	1891.4 ± 20.1	2262.9 ± 37.0	1843.3 ± 30.7
Ω calcite	3.6 ± 0.4	2.9 ± 0.2	2.5 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	1.4 ± < 0.0

Table 2

Table 2. Haploid *Emiliania huxleyi* s374 biometrics measured during incubation under ambient (A), moderate (M) and high (H) $p\text{CO}_2$. For POC, PON, chl *a* and DMSP_p, * denotes that cell volume-based values are expressed as fg μm^{-3} of cell volume. Bold values indicate significance at $p < 0.05$. Different lower case letters next to values denote treatments that were significantly different from

s374						
	cell ⁻¹			μm^{-3}		
	A	M	H	A	M	H
Vol. (μm^3)	71.52 ± 4.50^a	73.20 ± 5.34^a	82.43 ± 1.81^b	–	–	–
μ (d ⁻¹)	0.89 ± 0.09	0.91 ± 0.10	0.94 ± 0.07	–	–	–
POC (pg)*	12.23 ± 0.61	12.7 ± 1.41	12.25 ± 0.51	171.50 ± 14.00	174.00 ± 20.80	148.60 ± 5.70
PON (pg)*	0.82 ± 0.10	0.92 ± 0.24	1.04 ± 0.11	11.60 ± 1.80	12.50 ± 3.20	12.60 ± 1.20
POC:PON	12.15 ± 0.37	11.00 ± 1.70	10.21 ± 0.81	–	–	–
Chl <i>a</i> (pg)*	0.10 ± 0.01	0.10 ± <0.00	0.10 ± <0.00	1.45 ± 0.14^a	1.40 ± 0.07^{a,b}	1.25 ± 0.06^b
DMSP _p (pg)*	1.61 ± 0.08^{a,b}	1.41 ± 0.29^a	1.80 ± 0.03^b	22.60 ± 2.00	19.30 ± 4.40	21.80 ± 0.98

each other.

Table 3

Table 3. Diploid *Emiliana huxleyi* s2668 average biometrics measured during incubation under ambient (A), moderate (M) and high (H) $p\text{CO}_2$. For POC, PIC, PON, Chl *a* and DMSP_p, * denotes that volume-based values are expressed as fg μm^{-3} of coccosphere volume. Bold values indicate significance at $p < 0.05$. Different lower case letters next to values denote treatments that were

s2668						
	cell ⁻¹			μm^{-3}		
	A	M	H	A	M	H
Vol. (μm^3)	192.51 ± 7.35^a	229.00 ± 5.76^b	264.37 ± 13.06^c	—	—	—
μ (d ⁻¹)	1.00 ± 0.15	0.99 ± 0.16	1.02 ± 0.08	—	—	—
POC (pg)*	21.71 ± 1.77	21.90 ± 1.55	23.61 ± 1.05	110.20 ± 13.80^a	95.60 ± 4.80^b	92.30 ± 6.40^b
PIC (pg)*	16.86 ± 3.64	15.01 ± 1.63	17.11 ± 1.83	87.80 ± 19.80^a	65.60 ± 7.20^b	64.80 ± 6.80^b
PIC:POC	0.83 ± 0.10^a	0.69 ± 0.09^b	0.70 ± 0.05^b	—	—	—
PON (pg)*	3.35 ± 0.39^a	3.25 ± 0.16^a	3.90 ± 0.24^b	17.50 ± 2.60^a	14.20 ± 0.80^b	14.80 ± 1.00^{a,b}
POC:PON	6.40 ± 0.41	6.84 ± 0.40	6.32 ± 0.37	—	—	—
Chl <i>a</i> (pg)*	0.18 ± 0.02	0.17 ± 0.01	0.20 ± 0.01	0.95 ± 0.12 ^a	0.77 ± 0.05 ^b	0.75 ± 0.02 ^b
DMSP _p (pg)*	2.30 ± 0.16^a	2.37 ± 0.09^a	2.88 ± 0.15^b	11.98 ± 1.22^a	10.35 ± 0.33^b	10.93 ± 0.82^{a,b}

significantly different from each other.

Table 4

Table 4. The area (μm^2) of individual coccoliths that were visible exclusively in the x-y dimension from each cell, and the number of visible coccoliths per unit cell area (μm^{-2}) across each $p\text{CO}_2$ treatment. Differences in both coccolith area and coccoliths μm^{-2} were significant at $p < 0.001$. Letters represent treatments that were significantly different. N represents the number of coccoliths counted and measured for each $p\text{CO}_2$ treatment.

	s2668		
	Ambient ($n = 221$)	Moderate ($n = 275$)	High ($n = 348$)
Coccolith area (μm^2)	5.87 ± 1.14 ^a	6.30 ± 1.26 ^b	6.24 ± 1.28 ^b
Coccoliths (μm^{-2})	0.23 ± 0.04 ^a	0.21 ± 0.04 ^b	0.21 ± 0.03 ^b

Table 5

Table 5. The directional trends in response to elevated $p\text{CO}_2$ for *Emiliana huxleyi* s374 and s2668 *Emiliana huxleyi*. Unless otherwise specified, units are mass cell^{-1} or per unit volume (see tables 2 and 3 for details). Vol: volume. Directional trends with an * represent significant $p\text{CO}_2$ treatment differences. $\uparrow$: increased response; $\downarrow$: decreased response; $\approx$: neutral response; U: non-linear response; NA: non-applicable.

	s374		s2668	
	cell ⁻¹	μm ⁻³	cell ⁻¹	μm ⁻³
Vol. (μm ³)	$\uparrow^*$	NA	$\uparrow^*$	NA
μ (d ⁻¹)	$\approx$	NA	$\approx$	NA
POC	$\approx$	$\downarrow$	$\uparrow$	$\downarrow^*$
PIC	NA	NA	$\approx$	$\downarrow^*$
PIC:POC	NA	NA	$\downarrow^*$	NA
Coccolith area (μm ²)	NA	NA	$\uparrow^*$	NA
Coccoliths (μm ⁻²)	NA	NA	$\downarrow^*$	NA
PON	$\uparrow$	$\approx$	$\uparrow^*$	$\downarrow^*$
POC:PON	$\downarrow$	NA	$\approx$	NA
Chl <i>a</i>				

DMSP_p

$\approx$
U*

$\downarrow^*$
 $\approx$

$\approx$
 $\uparrow^*$

$\downarrow^*$
 $\downarrow^*$