High levels of solar radiation offset impacts of ocean acidification on calcifying and non-calcifying strains of *Emiliania huxleyi*

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ABSTRACT: Coccolithophores, a globally distributed group of marine phytoplankton, showed diverse responses to ocean acidification (OA) and to combinations of OA with other environmental factors. While their growth can be enhanced and calcification be hindered by OA under constant indoor light, fluctuation of solar radiation with ultraviolet irradiances might offset such effects. In this study, when a calcifying and a non-calcifying strain of *Emiliania huxleyi* were grown at 2 CO_2 concentrations (low CO_2 [LC]: 395 µatm; high CO_2 [HC]: 1000 µatm) under different levels of incident solar radiation in the presence of ultraviolet radiation (UVR), HC and increased levels of solar radiation acted synergistically to enhance the growth in the calcifying strain but not in the non-calcifying strain. HC enhanced the particulate organic carbon (POC) and nitrogen (PON) productions in both strains, and this effect was more obvious at high levels of solar radiation. While HC decreased calcification at low solar radiation levels, it did not cause a significant effect at high levels of solar radiation, implying that a sufficient supply of light energy can offset the impact of OA on the calcifying strain. Our data suggest that increased light exposure, which is predicted to happen with shoaling of the upper mixing layer due to progressive warming, could counteract the impact of OA on coccolithophores distributed within this layer.

KEY WORDS: Ocean acidification · Fluctuating light · UV radiation · Coccolithophore · Calcification

INTRODUCTION

Coccolithophores are a key group of marine primary producers, which play an important role in the ocean carbon cycle through photosynthesis as well as calcification (Hutchins 2011). They are also important in the sulphur cycle as one of the dominant producers of dimethylsulphide (DMS), which is known to play an import role in the climate system (Malin & Erst 1997). However, such roles are thought to be affected by ocean acidification (OA) and associated chemical changes, including increases in HCO_3^-, CO_2 and proton concentration ($[H^+]$), and decreases in CO_3^{2-} and pH (Riebesell & Tortell 2011). OA is known to affect the cellular calcification rate of coccolithophores (Riebesell & Tortell 2011) and influence their photosynthesis as well as elemental compositions (Riebesell et al. 2000, Gao et al. 2009, Müller et al. 2010, Jin et al. 2013).

In parallel with OA, daily levels of solar radiation exposure integrated within the upper mixed layer (UML) have been predicted to rise in future oceans with progressive global warming due to intensified ocean stratification and UML shoaling (Sarmiento et al. 2004). Studies of the interactive effect of OA with light on coccolithophores (Zondervan et al. 2002, Feng et al. 2008, Rokitta & Rost 2012, Zhang et al. 2015) have shown that their responses to OA, in

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terms of growth, photosynthesis and calcification, are strongly modulated by light. However, most of the previous studies applied constant light, which is very different from fluctuating solar radiation that phytoplankton cells are naturally exposed to. Fluctuating light treatments have been shown to substantially impact on elemental compositions, photosynthetic efficiencies and growth of phytoplankton, e.g. by van Leeuwe et al. (2005), Kropuenske et al. (2009), Shatwell et al. (2012), Jin et al. (2013), and Hoppe et al. (2015); however, the magnitude of the responses differed greatly among these studies. Such discrepancies could be attributed to different environmental conditions (e.g. temperature, CO_2 levels, nutrient availability), which may alter the responses of phytoplankton to varying irradiance (Jin et al. 2013, Hoppe et al. 2015).

OA and fluctuating irradiance levels have the potential to synergistically affect phytoplankton, e.g. coccolithophores (Jin et al. 2013) and diatoms (Boelen et al. 2011, Hoppe et al. 2015), though we know little about their interactions to date. Fluctuating light reduced growth and strongly altered the effects of OA on photosynthesis of an Antarctic diatom, *Chaetoceros debilis* (Hoppe et al. 2015). Significant interactions between fluctuating light and OA were detected for pigment production rates and particulate organic carbon (POC) cell content, whicht were not affected by OA under constant light regimes (Hoppe et al. 2015). Similarly, a combination of OA and fluctuating light decreased carbon fixation, compared to fixation at an ambient CO_2 level and under constant light, in the coccolithophore *Gephyrocapsa oceanica* (Jin et al. 2013). The growth of another species of coccolithophore, *Emiliania huxleyi*, was higher under indoor constant light, but became lower under outdoor fluctuating photosynthetic active radiation (PAR) (even with equivalent daily dose), and even further declined with addition of solar ultraviolet radiation (UVR, 280–400 nm) (Xing et al. 2015). Therefore, it is likely that changing levels of light and UVR interact with other abiotic factors to influence phytoplankton physiology.

Experiments in laboratory, using, for example, programmed lamps (Hoppe et al. 2015) or a solar simulator (Jin et al. 2013) to mimic natural mixing regimes, may not reflect natural solar irradiance, as they neglect the influence of solar UVR. UVR at 305 and 340 nm can penetrate (at 1% of surface intensity) as deep as 56 and 118 m, respectively, in open oceans (Tedetti et al. 2007). Solar UVR therefore has the potential to impact on natural coccolithophores (e.g. *E. huxleyi*, that is found in the entire photic zone). For example, UVR decreases photosynthetic carbon fixation, calcification and growth of coccolithophores (Gao et al. 2009, Guan & Gao 2010); however, these responses could be modulated by other environmental factors, such as OA (Sobrino et al. 2008, Gao et al. 2009, Li et al. 2012, Jin et al. 2013, Xu & Gao 2015). OA increases the sensitivity of phytoplankton to UVR by increasing photoinhibition (Sobrino et al. 2008). In contrast, the studies of Li et al. (2012) and Jin et al. (2013) showed that OA could act antagonistically to reduce UV-induced photoinhibition of photosynthetic carbon fixation in a diatom and a coccolithophore (Li et al. 2012, Jin et al. 2013). Moderate levels of UVR may stimulate production of particulate inorganic carbon (PIC) in *E. huxleyi* (Xu & Gao 2015). However, we still know little about the interactive effects of OA and solar radiation (including PAR and UVR with natural fluctuating regimes) on coccolithophores and the mechanisms involved.

The decreased calcification rate under OA is partially caused by increasing energy costs of calcification (Irie et al. 2010, Raven 2011, Raven & Crawfurd 2012), which is required to sustain a sufficient supply of CO_3^{2-} in the coccolith-forming vesicle, and net H^+ efflux (Raven 2011). Therefore, based on the documented knowledge on the effects of OA and/or light intensity on *E. huxleyi*, we hypothesize that increased levels of supplied energy with high levels of solar radiation may offset the negative effects of OA on calcification of *E. huxleyi*. To test this hypothesis, we chose *E. huxleyi* PMLB 92/11, a calcifying strain, and *E. huxleyi* CCMP 2090, a non-calcifying strain, and grew them over a range of solar radiation intensities under natural solar radiation at ambient and elevated CO_2 concentrations as projected for the end of this century (IPCC 2013), and then investigated the effects on growth, POC production and calcification.

MATERIALS AND METHODS

Culture conditions

Emiliania huxleyi strains PML B92/11 (calcifying) and CCMP 2090 (non-calcifying) were originally obtained from coastal waters off Bergen, Norway and from the South Pacific, respectively. Monospecific cultures of the 2 strains were grown semi-continuously by partially renewing the medium every 24 h with fresh medium pre-equilibrated at ambient (low CO_2 [LC]: 395 µatm) or elevated (high CO_2 [HC]:1000 µatm) pCO$_2$ levels. To maintain a stable carbonate system in the semi-continuous cultures,

the cell density was maintained within a range of 1.5 to 3.0×10^4 cells ml^{-1} before and after diluting the cultures (Jin et al. 2013). The culture medium was prepared with artificial seawater enriched with Aquil medium (110 µM nitrate, 10 µM phosphorus; Morel et al. 1979). The cultures were maintained indoor for at least 20 generations under a photon flux density of 100 µmol photons m^{-2} s^{-1} (12 h:12 h light:dark period) in a plant growth chamber (GXZ, Ruihua) at a constant temperature of 20°C under the 2 CO_2 levels before being transferred to the outdoor (rooftop) growth experiments under solar radiation. The outdoor cultures were incubated in 250 ml polyethylene bottles, which allowed 81% PAR, 53% UV-A (315 to 400 nm) and 5% UV-B (280 to 315 nm) transmissions under incident solar radiation (determined by spectrophotometry before use). The temperature was controlled at 20°C using a circulating cooler (CTP-3000, Eyela). In the outdoor incubations, the cultures were diluted every 24 h (at 18:00 h before the onset of the dark phase) with freshly prepared medium equilibrated to the target CO_2 levels (HC and LC), with the cell concentrations of about 3×10^4 cells ml^{-1} after the dilutions and of $<9 \times 10^4$ cells ml^{-1} before the dilutions. As the specific growth rates differed under different conditions, the renewed amount of the medium differed. The fresh medium pre-equilibrated at target $p\mathrm{CO_2}$ levels were achieved by aerating (0.5 l min^{-1}) ambient air (outside, rooftop) or air with elevated (1000 µatm, HC) CO_2 from a plant CO_2 incubator (HP1000G-D, Ruihua). For strain CCMP 2090, the outdoor incubation period was from 17 April 2012 to 11 May 2012 (acclimation for at least 6 d, followed by an experimental phase lasting 18 d), with mean daily incident PAR, UVA and UVB doses of 5.3 MJ m^{-2} d^{-1}, 0.85 MJ m^{-2} d^{-1} and 26 kJ m^{-2} d^{-1}, respectively. For strain PML B92/11, the outdoor incubation phase was from 21 May 2012 to 7 June 2012 (acclimation for at least 6 d, experimental phase 7 d), with mean daily incident PAR, UVA and UVB doses of 8.56 MJ m^{-2} d^{-1}, 1.38 MJ m^{-2} d^{-1} and 41.9 kJ m^{-2} d^{-1}, respectively (daily doses of PAR, UVA and UVB are shown in Figs. S1& S2 in the Supplement at www.int-res.com/articles/suppl/m568p047_supp.pdf). Levels of incident solar PAR were achieved with neutral density screens as follows: 6.5, 10, 17, 31 and 55% (54, 83, 141, 257 and 457 µmol photons m^{-2} s^{-1}, respectively) for strain PML B92/11; 10, 17, 31, 51 and 100% (57, 96, 176, 312, and 567 µmol photons m^{-2} s^{-1}, respectively) for strain CCMP 2090. It should be noted that we only mimicked the light intensities at different depths in the upper mixed layer; we did not take into account changes in light quality occur-

ring as a result of the upwelling and downwelling of phytoplankton cells in the water column. Solar PAR and ultraviolet radiation were measured every 1 s with a broadband solar radiometer (ELDONET, Real Time Computer, Germany) and the averaged values over 1-min intervals were recorded.

Carbonate chemistry measurements

We were not able to measure the carbonate chemistry parameters on a daily basis. To maintain stable carbonate chemistry of the cultures, we measured the carbonate chemistry parameters before the experiments to make sure previously applied semi-continuous culture manipulation methods were reliable (Gao et al. 2012, Jin et al. 2013, Jin & Gao 2016). Then, we ran the outdoor incubations by following the reliable protocols as tested in preliminary experiments. In addition, by keeping the cell concentrations below 9×10^4 cells ml^{-1} for most of the time, we were able to limit the total dissolved inorganic carbon (DIC) drawn down to less than 7% in line with the cultivation recommendations of LaRoche et al. 2010. It should be noted that, very occasionally, the cell concentrations were as high as 1.3×10^5 cells ml^{-1} on some days, due to relatively higher growth rates. Therefore, despite diurnal changes in the carbonate chemistry, a significant difference in carbonate system parameters was maintained between the high CO_2 and low CO_2 treatments.

Determination of growth rates

Cell numbers were determined by a particle counter (Z2, Beckman instruments once every 24 h (at 18:00 h before onset of the dark phase) before and after the partial renewal of the medium (performed in laminar flow hood). Growth rate (µ) was calculated by the following equation: $\mu = (\ln N_1 - \ln N_0) / (t_1 - t_0)$, where N_1 and N_0 are the numbers of cells number at time t_1 and t_0 ($t_1 - t_0 = 1$ d), respectively.

Carbon and nitrogen measurements

Samples taken from each replicate culture (3 replicates for each treatment) under 5 solar radiation levels and 2 CO_2 conditions at the end of the outdoor incubations (on 11 May 2012 for non-calcifying strain CCMP 2090 and on 7 June 2012 for the calcifying strain PML B92/11) were filtered onto pre-combusted

(500°C for 5 h) Whatman GF/F filters (diameter 25 mm) and frozen at −20°C. The sampling time was also at 18:00 h. For POC and particulate organic nitrogen (PON) analysis, filters were fumed over saturated HCl for 24 h to remove all inorganic carbon. The samples were analyzed on a Perkin Elmer Series II CHNS/O Analyzer 2400. PIC was calculated as the difference between total particulate carbon (TPC) and POC.

Rates of PIC, POC and PON production were calculated as: production rate = growth rate (μ, d^{-1}) × cellular PIC, POC or PON content (pg $cell^{-1}$).

Data analysis

One-way ANOVA was used to establish differences among the treatments ($p < 0.05$). Two-way ANOVA was used to determine the individual effects of CO_2 and solar radiation levels and their interactions.

RESULTS

Growth

In general, elevated pCO_2 enhanced the growth of both strains (2-way ANOVA, calcifying strain PML B92/11: df = 1, $F = 81.276$, p < 0.001; non-calcifying strain CCMP 2090: df = 1, $F = 17.809$, p < 0.001) (Tables 1 & 2, Fig. 1). Elevated pCO_2 stimulated growth by 15 to 24% in the calcifying strain and by 22 to 28% in the non-calcifying strain with higher stimulation at higher solar radiation levels (Fig. 1). Specifically, in the calcifying strain, growth rates of the HC-grown cells were 15, 16, 24 and 24% higher than that of the LC-grown cells at 10, 17, 31 and 55 of incident solar radiation, respectively (Fig. 1a). A significant interaction between OA and solar radiation was found for the calcifying strain, suggesting that elevated CO_2 and increasing solar radiation were acting synergistically to enhance the growth in this strain (2-way

Table 1. Responses of calcifying strain PML B92/11 and non-calcifying strain CCMP 2090 of *Emiliania huxleyi* grown under ambient (low CO_2 [LC] 395 µatm) and increased (high CO_2 [HC] 1000 µatm) CO_2 conditions under different solar radiation levels (shown as percent of incident PAR and flux in µmol photons m^{-2} s^{-1}). Responses measured were specific growth rate μ (d^{-1}), content (pg $cell^{-1}$) and production (pg $cell^{-1}$ d^{-1}) of particulate organic carbon (POC), particulate organic nitrogen (PON) and particulate inorganic carbon (PIC), and PIC:POC and C:N ratios (pg pg^{-1}). μ_E and μ_S represent the specific growth rate during the whole experimental phase (7 d for PML B92/11, 18 d for CCMP 2090) and on the PIC, POC and PON sampling day (PML B92/11: 7 June 2012; CCMP 2090: 11 May 2012), respectively. Values are mean ± SD (n = 3). nd: no data

Solar radiation level		μ_E	μ_S	POC		PON		PIC		PIC:POC	C:N
%	Flux			Content	Prod.	Content	Prod.	Content	Prod.		
Calcifying-HC											
6.5	54 ± 17	0.59 ± 0.11	0.59 ± 0.02	16.9 ± 0.8	10.0 ± 0.8	2.1 ± 0.1	1.3 ± 0.1	3.4 ± 0.2	2.0 ± 0.1	0.2 ± 0.02	9.3 ± 0.3
10	83 ± 26	0.62 ± 0.12	0.72 ± 0.05	15.6 ± 1.1	11.3 ± 1.3	2.1 ± 0.2	1.5 ± 0.2	4.2 ± 0.5	3.0 ± 0.2	0.3 ± 0.05	8.6 ± 0.6
17	141 ± 45	0.66 ± 0.08	0.77 ± 0.00	16.1 ± 1.0	12.4 ± 0.8	2.4 ± 0.4	1.8 ± 0.3	5.1 ± 0.8	4.0 ± 0.6	0.3 ± 0.04	8.0 ± 1.0
31	257 ± 82	0.64 ± 0.13	0.80 ± 0.04	17.2 ± 2.7	13.8 ± 2.7	2.3 ± 0.4	1.9 ± 0.4	4.1 ± 0.9	4.1 ± 0.9	0.3 ± 0.07	8.6 ± 1.5
55	456 ± 145	0.62 ± 0.16	0.85 ± 0.07	21.1 ± 2.9	18.0 ± 3.8	2.8 ± 0.5	2.4 ± 0.5	3.8 ± 0.7	3.2 ± 0.7	0.2 ± 0.05	8.9 ± 1.5
Calcifying-LC											
6.5	54 ± 17	0.62 ± 0.10	0.60 ± 0.04	14.0 ± 1.4	8.5 ± 0.4	2.0 ± 0.4	1.2 ± 0.2	8.5 ± 1.0	5.1 ± 0.4	0.6 ± 0.07	8.5 ± 0.9
10	83 ± 26	0.73 ± 0.09	0.65 ± 0.13	16.5 ± 2.6	10.5 ± 0.3	2.1 ± 0.7	1.4 ± 0.3	7.8 ± 1.9	5.0 ± 0.9	0.5 ± 0.09	9.3 ± 1.6
17	141 ± 45	0.78 ± 0.06	0.64 ± 0.12	16.0 ± 0.7	10.2 ± 0.9	2.0 ± 0.2	1.3 ± 0.1	8.5 ± 0.9	5.4 ± 0.9	0.5 ± 0.07	9.4 ± 0.9
31	257 ± 82	0.84 ± 0.08	0.68 ± 0.10	17.9 ± 1.9	12.3 ± 2.6	2.1 ± 0.3	1.5 ± 0.3	8.8 ± 2.6	5.9 ± 1.0	0.5 ± 0.2	9.8 ± 1.5
55	456 ± 145	0.81 ± 0.08	0.57 ± 0.12	19.1 ± 2.8	10.7 ± 0.9	2.1 ± 0.3	1.2 ± 0.0	5.5 ± 1.2	3.1 ± 1.0	0.3 ± 0.1	10.7 ± 0.8
Non-calcifying-HC											
10	57 ± 30	0.50 ± 0.26	0.40 ± 0.03	6.3 ± 0.7	2.6 ± 0.4	1.2 ± 0.2	0.5 ± 0.1	nd	nd	nd	6.1 ± 0.4
17	96 ± 52	0.57 ± 0.25	0.47 ± 0.37	4.9 ± 0.8	2.3 ± 1.2	1.0 ± 0.2	0.5 ± 0.3	nd	nd	nd	5.7 ± 0.2
31	176 ± 95	0.71 ± 0.34	0.51 ± 0.13	5.4 ± 0.8	2.7 ± 1.6	1.2 ± 0.1	0.6 ± 0.1	nd	nd	nd	5.4 ± 0.6
55	312 ± 168	0.77 ± 0.30	0.89 ± 0.10	5.2 ± 0.2	4.6 ± 0.7	1.1 ± 0.1	1.0 ± 0.1	nd	nd	nd	5.4 ± 0.2
100	567 ± 305	0.72 ± 0.29	0.76 ± 0.14	7.4 ± 0.9	5.7 ± 1.8	1.3 ± 0.2	1.0 ± 0.2	nd	nd	nd	6.9 ± 1.4
Non-calcifying-LC											
10	57 ± 30	0.36 ± 0.17	0.14 ± 0.07	6.7 ± 1.4	0.9 ± 0.3	0.8 ± 0.2	0.1 ± 0.0	nd	nd	nd	9.6 ± 1.2
17	96 ± 52	0.44 ± 0.15	0.26 ± 0.06	5.7 ± 1.3	1.5 ± 0.2	0.7 ± 0.1	0.2 ± 0.0	nd	nd	nd	9.6 ± 0.9
31	176 ± 95	0.50 ± 0.16	0.30 ± 0.13	5.5 ± 0.3	1.6 ± 0.5	0.8 ± 0.0	0.2 ± 0.1	nd	nd	nd	7.7 ± 1.1
55	312 ± 168	0.57 ± 0.16	0.44 ± 0.04	5.9 ± 1.0	2.6 ± 0.6	0.8 ± 0.2	0.4 ± 0.1	nd	nd	nd	8.7 ± 0.9
100	567 ± 305	0.52 ± 0.20	0.41 ± 0.02	6.0 ± 0.9	2.5 ± 0.5	0.9 ± 0.3	0.4 ± 0.1	nd	nd	nd	8.5 ± 1.4

Table 2. Significance levels of responses of *Emiliania huxleyi* grown under ambient (LC) and increased (HC) CO_2 levels. The significance of differences in the content and production of POC, PON and PIC, and in PIC:POC and C:N ratios was analysed by 1-way ANOVA for cells of calcifying strain PML B92/11 and non-calcifying strain CCMP 2090 of *E. huxleyi* exposed to different solar radiation levels. df = 1, 4 for all *F*-statistics; nd: no data

| Solar radiation level (%)	POC content F	POC content p	POC production F	POC production p	PON content F	PON content p	PON production F	PON production p	PIC content F	PIC content p	PIC production F	PIC production p	PIC:POC F	PIC:POC p	C:N F	C:N p
Calcifying strain																
6.5	9.27	0.038	8.70	0.042	0.54	0.503	0.51	0.515	70.2	0.001	146.8	<0.001	94.8	<0.001	2.09	0.222
10	0.29	0.616	1.04	0.365	0.00	0.947	0.80	0.422	10.6	0.031	12.8	0.023	13.0	0.022	0.42	0.551
17	0.01	0.914	10.5	0.031	2.08	0.222	11.3	0.028	25.9	0.007	5.87	0.072	21.7	0.010	3.43	0.137
31	0.13	0.741	0.44	0.542	0.49	0.521	11.3	0.028	5.43	0.080	5.37	0.081	2.73	0.174	1.73	0.259
55	0.70	0.448	10.5	0.032	4.95	0.090	16.1	0.016	4.36	0.105	0.01	0.923	2.74	0.173	11.5	0.027
Non-calcifying strain																
10	0.21	0.667	31.4	0.005	5.10	0.087	28.0	0.006	nd	nd	nd	nd	nd	nd	23.6	0.008
17	2.15	0.217	1.31	0.316	8.52	0.043	3.72	0.126	nd	nd	nd	nd	nd	nd	58.4	0.002
31	0.04	0.844	5.59	0.077	43.9	0.003	23.6	0.008	nd	nd	nd	nd	nd	nd	11.2	0.029
55	1.22	0.331	15.3	0.017	6.41	0.065	39.4	0.003	nd	nd	nd	nd	nd	nd	37.4	0.004
100	3.42	0.138	9.18	0.039	4.55	0.010	22.5	0.009	nd	nd	nd	nd	nd	nd	2.21	0.212
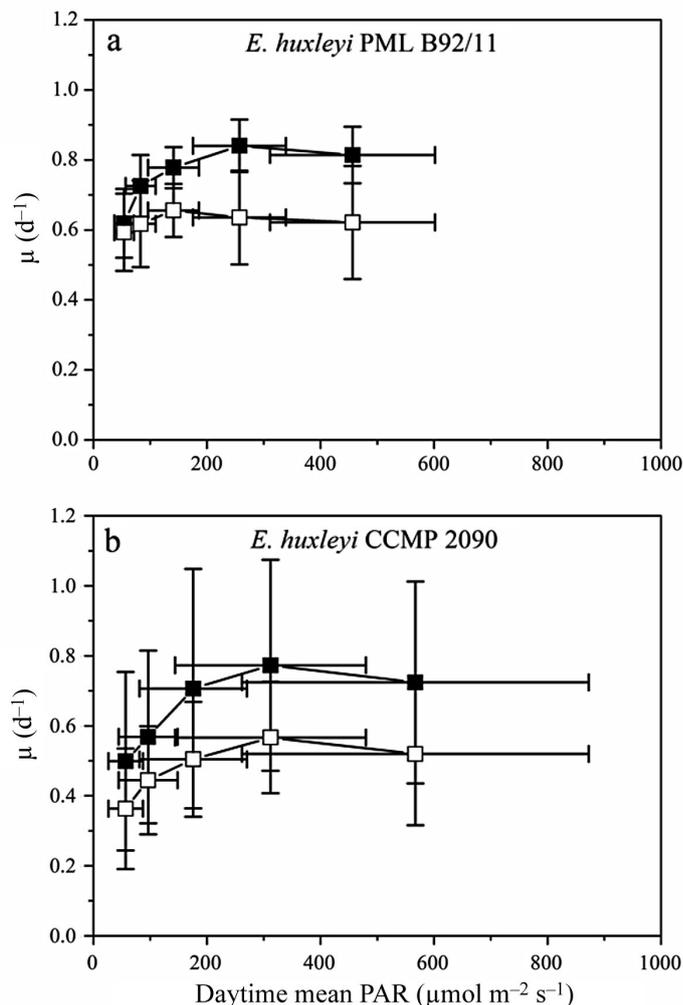
Fig. 1. Growth rates (μ) of *Emiliania huxleyi* grown under (□) ambient (low CO_2 [LC], 395 µatm) and (■) increased (high CO_2 [HC], 1000 µatm) CO_2 conditions: (a) calcifying strain PML B92/11 under 6.5, 10, 17, 31, 55% levels of incident solar radiation; (b) non-calcifying strain CCMP 2090 under 10, 17, 31, 55 and 100% levels of incident solar radiation. Values are mean ± SD for replicate cultures (n = 3) in outdoor experimental phases (7 d for strain PML B92/11 and 18 d for strain CCMP 2090). Horizontal and vertical error bars denote standard deviations for variation of solar radiation and μ, respectively

ANOVA, df = 4, F = 4.996, p < 0.001) (Fig. 1a, Tables 1 & 2). For the non-calcifying strain, growth rates were 27.2, 21.8, 28.6, 26.7 and 28.2 higher in the HC-grown cells at 10, 17, 31, 55 and 100% of incident solar radiation, respectively (Fig. 1b). No significant interaction between the solar radiation and CO_2 levels was found in this strain (2-way ANOVA, df = 4, F = 0.802, p = 0.524; Fig. 1b, Tables 1 & 2).

Cellular POC content and POC production rate

Elevated CO_2 did not affect the cellular POC in the calcifying strain (2-way ANOVA, df = 1, F = 0.838, p= 0.371; Fig. 2a) or the non-calcifying strain (2-way ANOVA, df = 1, F = 0.143, p = 0.709; Fig. 2c, Tables 1 & 2). Moreover, there was no consistent difference in cellular POC content across the different solar radiation levels (Fig. 2a,c). Due to the CO_2-stimulated growth rate, calculated POC production was significantly higher in HC-grown compared to LC-grown cells (Fig. 2b,d, Table 3), and the increment increased within the solar radiation levels (e.g. for the calcifying strain there was an increment of 25% at the 6.5% light level, and an increment of 44% at the 55% light level) (Table 3). In both strains, POC production increased with increasing solar radiation, with a slope of 0.016 (n = 5, R^2 = 0.98) and 0.004 (n =

5, R^2 = 0.26), respectively, in the HC- and LC-grown calcifying strain (Fig. 2b), and a slope of 0.007 (n = 5, R^2 = 0.92) and 0.003 (n = 5, R^2 = 0.73), respectively, in the HC- and LC-grown non-calcifying strain (Fig. 2d). There was a significant interaction between CO_2 and solar radiation on POC production in the calcifying strain, suggesting that elevated CO_2 and increasing solar radiation acted synergistically to enhance the POC production (2-way ANOVA, df = 4, F = 3.212, p = 0.034; Fig. 2d, Tables 1 & 2).

Cellular PON content and PON production rate

Similar to the cellular POC content, no consistent change with solar radiation intensity was observed in cellular PON content (Fig. 3). However, in both strains the cellular PON content was higher in HC-
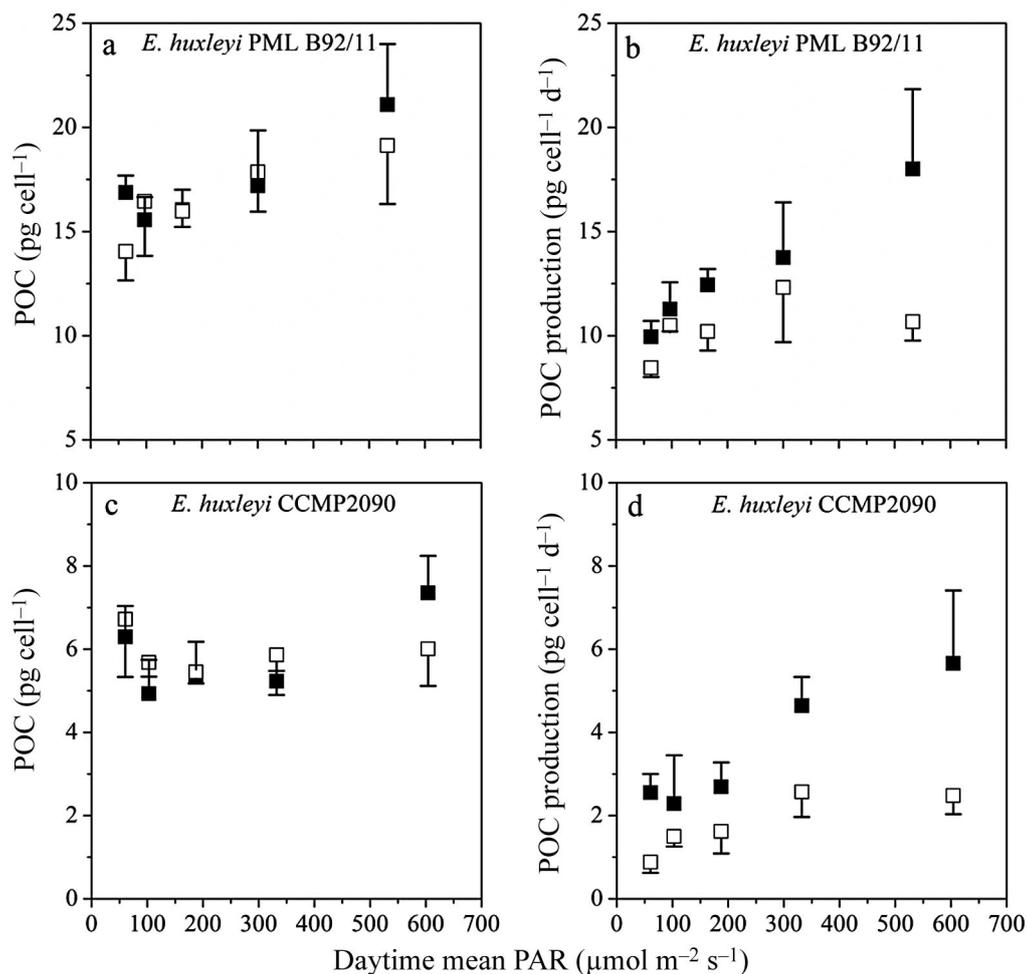

Fig. 2. Particulate organic carbon (POC, pg cell^{-1}) and POC production rates (pg cell^{-1} d^{-1}) of *Emiliania huxleyi* grown under (□) ambient (LC) and (■) increased (HC) CO_2 conditions and different solar radiation levels: (a) POC cell content and (b) production rates for calcifying strain PML B92/11; (c) cell content and (d) production rates for non-calcifying strain CCMP 2090. Values are mean and SD (n = 3)

Table 3. Two-way ANOVA analysis of individual and inter-active effects of pCO_2 and solar radiation (SR) on growth, cell content and production of POC, PON and PIC, and PIC:POC and C:N ratios in calcifying strain PML B92/11 and non-calcifying strain CCMP 2090 of *Emiliania huxleyi*

Parameter	Treatment	df	F	p
Calcifying strain				
Growth	pCO_2	1	81.276	<0.001
	SR	4	10.675	<0.001
	$pCO_2 \times$ SR	4	4.996	<0.001
POC content	pCO_2	1	0.838	0.371
	SR	4	5.462	0.004
	$pCO_2 \times$ SR	4	1.042	0.411
POC production	pCO_2	1	15.891	<0.001
	SR	4	7.113	<0.001
	$pCO_2 \times$ SR	4	3.212	0.034
PON content	pCO_2	1	4.437	0.048
	SR	4	1.019	0.421
	$pCO_2 \times$ SR	4	0.864	0.503
PON production	pCO_2	1	24.399	<0.001
	SR	4	3.831	0.018
	$pCO_2 \times$ SR	4	4.168	0.013
C:N	pCO_2	1	6.214	0.022
	SR	4	1.280	0.311
	$pCO_2 \times$ SR	4	1.780	0.172
PIC content	pCO_2	1	58.205	<0.001
	SR	4	3.325	0.030
	$pCO_2 \times$ SR	4	1.343	0.289
PIC production	pCO_2	1	36.994	<0.001
	SR	4	6.367	0.002
	$pCO_2 \times$ SR	4	3.475	0.026
PIC:POC	pCO_2	1	47.613	<0.001
	SR	4	4.177	0.013
	$pCO_2 \times$ SR	4	2.015	0.131
Non-calcifying strain				
Growth	pCO_2	1	17.809	<0.001
	SR	4	72.758	<0.001
	$pCO_2 \times$ SR	4	0.802	0.524
POC content	pCO_2	1	0.143	0.709
	SR	4	3.875	0.017
	$pCO_2 \times$ SR	4	1.647	0.202
POC production	pCO_2	1	35.928	<0.001
	SR	4	10.753	<0.001
	$pCO_2 \times$ SR	4	2.051	0.126
PON content	pCO_2	1	34.034	<0.001
	SR	4	1.233	0.329
	$pCO_2 \times$ SR	4	0.091	0.984
PON production	pCO_2	1	87.031	<0.001
	SR	4	10.781	<0.001
	$pCO_2 \times$ SR	4	2.174	0.109
C:N	pCO_2	1	76.981	<0.001
	SR	4	2.017	0.131
	$pCO_2 \times$ SR	4	1.488	0.243

compared to LC-grown cells, although differences were not statistically significant for most solar radiation intensities in the calcifying strain (Fig. 3a,c, Table 3). In combination with the higher growth rates, this increase in PON content equates to much higher PON production rates in HC-grown cells, with a maximum increment at the highest solar radiation level (100%) in the calcifying strain and a maximum increment at the solar radiation level of 31% in the non-calcifying strain (Fig. 3b,d, Table 3). PON production increased with increasing solar radiation intensity in HC- and LC-grown cells of both strains. Elevated CO_2 and increasing solar radiation apparently acted synergistically to enhance the PON production in the calcifying strain (2-way ANOVA, df = 4, $F = 4.168$, $p = 0.013$; Fig. 3b, Tables 1 & 2).

C:N ratio

Elevated CO_2 significantly decreased the C:N ratio in both strains. C:N ratios decreased by 7 to 17% (2-way ANOVA, df = 1, $F = 6.214$, p = 0.022) in the calcifying strain and by 20 to 41% (2-way ANOVA, df = 1, $F = 76.981$, p < 0.001) in the non-calcifying strain at different solar radiation levels; however, there was no significant effect of solar radiation on C:N ratio in either strain (2-way ANOVA, all df = 1, both p > 0.05; for detailed results see Table 3) (Fig. 4). In addition, no significant interaction between CO_2 and solar radiation on C:N ratio was observed in either strain (2-way ANOVA, all df = 4, all p > 0.05; for detailed results see Table 3) (Fig. 4).

Cellular PIC content, PIC production and PIC:POC

Elevated CO_2 significantly decreased the cellular PIC content of the calcifying strain by 42 to 63% at low solar radiation levels of 6.5 to 17% (Fig. 5a, Table 3), while no significant differences were found at the high solar radiation levels of 31 and 55% (1-way ANOVA, 31%: $F_{1,4} = 5.43$, p = 0.080; 55%: $F_{1,4} = 4.36$, p = 0.105). PIC production rates were significantly lower in HC- compared to LC-grown cells, falling by 40 and 60% at solar radiation levels of 17 and 6.5%, respectively (Fig. 5b, Table 3). Similar to cellular PIC content, the PIC:POC ratio decreased significantly, by 43 to 72%, at low solar radiation levels of 6.5 to 17% in HC-grown cells (Fig. 5c, Table 3), while PIC:POC in HC-grown cells did not differ from that in LC-grown cells at the high solar radiation levels of 31 and 55% (Fig. 5c, Table 3). Elevated CO_2, however, had significant negative effects on cellular PIC content, PIC production and PIC:POC, and a significant interaction between CO_2 and solar radiation was found in PIC production, suggesting an antagonistic effect of OA and increasing solar radiation (2-way ANOVA, df = 4, $F = 3.475$, p = 0.026).
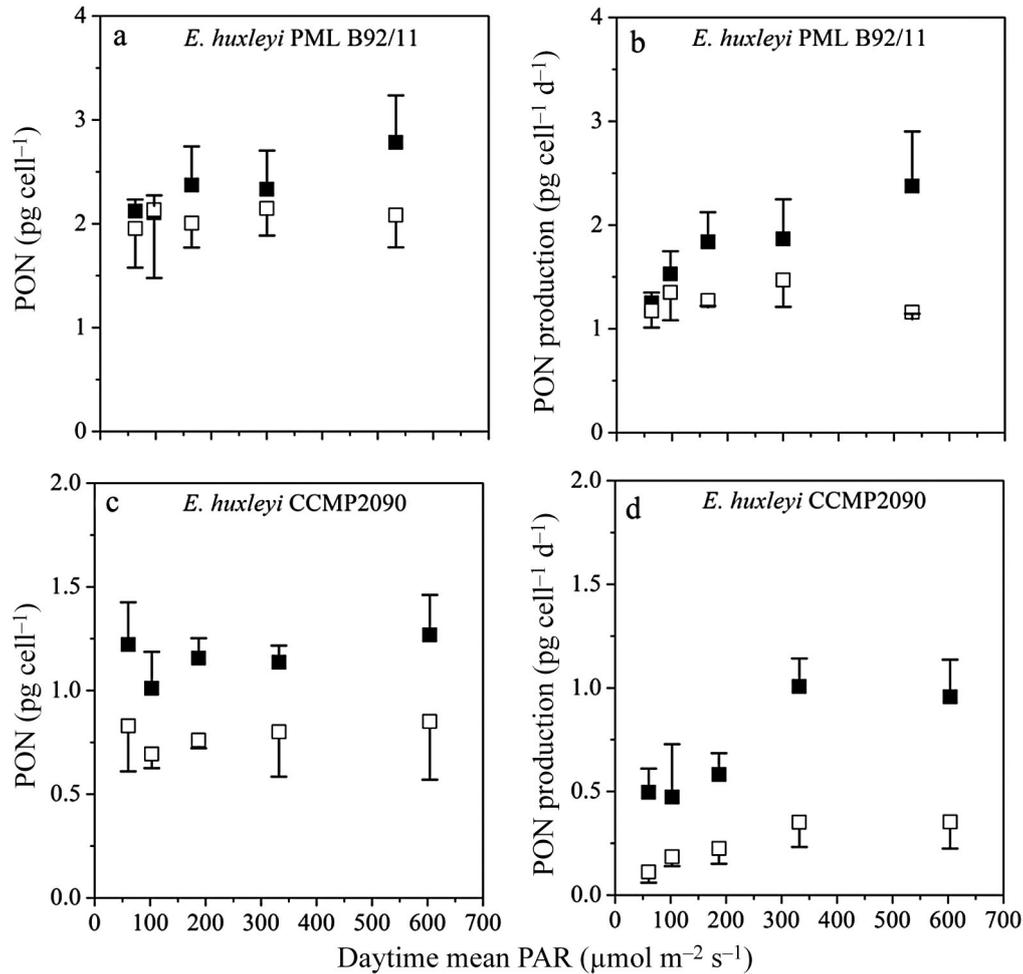
Fig. 3. Particulate organic nitrogen (PON, pg cell^{-1}) and PON production rates (pg cell^{-1} d^{-1}) of *Emiliania huxleyi* grown under (□) ambient (LC) and (■) increased (HC) CO_2 conditions and different solar radiation levels: (a) PON cell content and (b) production rates for calcifying strain PML B92/11; (c) cell content and (d) production rates for non-calcifying strain CCMP 2090. Values are mean and SD (n = 3)

DISCUSSION

Our study investigated the interactive effects of OA and increasing solar radiation on the physiology of a calcifying strain (PML B92/11) and a non-calcifying strain (CCMP 2090) of the coccolithophore *Emiliania huxleyi*. We found that, firstly, OA and increased solar radiation density acted synergistically to enhance growth in the calcifying but not in the non-calcifying strain; secondly, the enhancement of POC and PON production under OA increased with increased solar radiation levels; and thirdly, OA had a negative effect on calcification at low solar radiation levels, which was absent at high solar radiation levels. These results support our hypothesis that high levels of solar radiation can offset the impacts of ocean acidification on *E. huxleyi*.

Increased pCO_2 and associated seawater chemical changes were reported to have diverse effects on the growth of coccolithophores (Meyer & Riebesell 2015 and references therein), due to species- and strain-specific differences, different methodologies applied as well as different experimental conditions. Our data suggest that increased CO_2 could enhance growth in both a calcifying and non-calcifying strain of *E. huxleyi* under naturally fluctuating solar irradiance (Fig. 1, Table 2), in agreement with previous work on this species (e.g. Langer et al. 2009, Shi et al. 2009, Fiorini et al. 2011), although it should be borne in mind that only constant light was employed in previous studies. Furthermore, the enhancements of elevated CO_2 on POC and PON production were higher under high solar radiation compared with low solar radiation conditions (Figs. 2 & 3). This may be
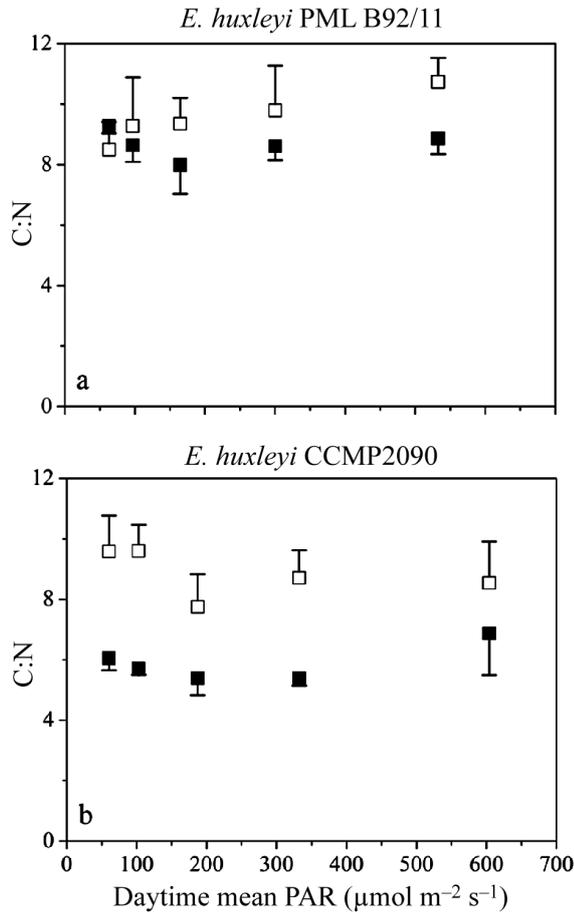
Fig. 4. C:N ratios of particulate organic carbon (POC) to particulate organic nitrogen (PON) in *Emiliania huxleyi* grown under (□) ambient (LC) and (■) increased (HC) CO_2 conditions and different solar radiation levels: (a) calcifying strain PML B92/11; (b) non-calcifying strain CCMP 2090. Values are mean and SD (n = 3)

Fig. 5. (a) Particulate inorganic carbon (PIC, pg cell^{-1}) (b), PIC production rates (pg cell^{-1} d^{-1}) and (c) PIC:POC ratios in the calcifying strain PML B92/11 of *Emiliania huxleyi*, grown under (□) ambient (LC) and (■) increased (LC) CO_2 conditions and different solar radiation levels. Values are mean and SD (n = 3)

due to the additional energetic cost of calcification in the calcifying species under low solar radiation conditions counteracting the positive effect of elevated CO_2 on POC and PON production, leading to the lower POC and PON production in the HC-grown compared to LC-grown cells. Furthermore, [H$^+$] in the cytosol of the coccolithophore *E. huxleyi* has been found to increase instantly (within <10 s) with increasing [H$^+$] in seawater (Suffrian et al. 2011), which could affect POC production (Bach et al. 2011). Consequently, extra energy would be required to maintain the cell's homeostasis or net H$^+$ efflux (Jin et al. 2015), thus less energy would be available for for POC and PON production. However, when the energy availability increases under relatively high solar radiation conditions, increased availability of light could meet the demand of increased energy requirement for calcification and for H$^+$ homeostasis
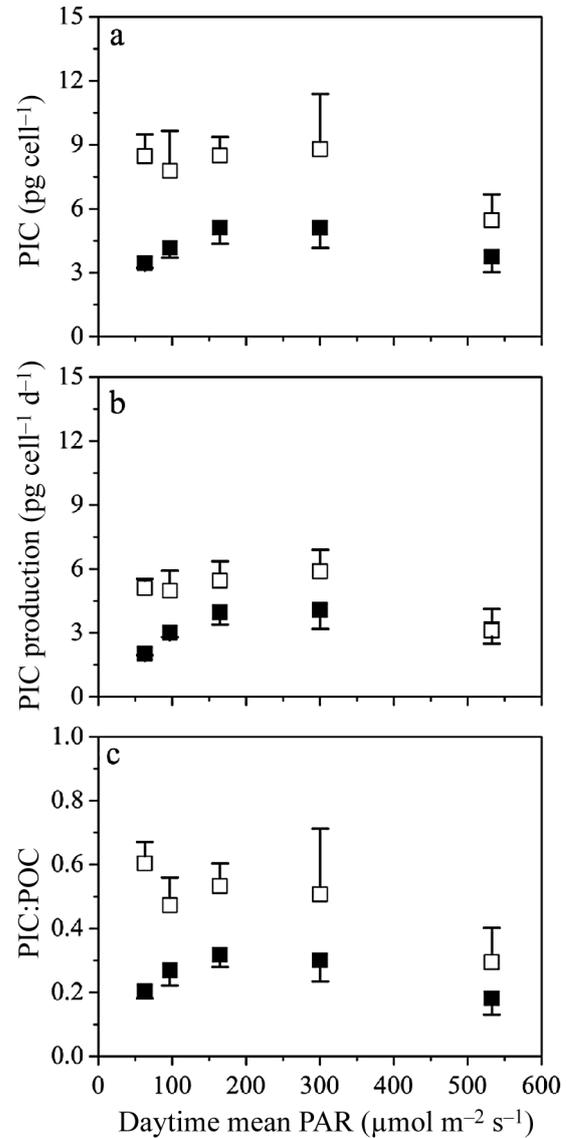
(Rokitta & Rost 2012). Thus, POC and PON production benefited from the CO_2 increase under high solar radiation conditions, and these effects were more pronounced at increased solar radiation levels (Figs. 2 & 3). OA and increasing solar radiation levels acted synergistically to increase POC and PON production in the calcifying but not in the non-calcifying strain (Fig. 2 & 3, Table 3), suggesting that the calcifying strain will benefit more in terms of increased capacity for carbon and nitrogen assimilation under future high CO_2 and high light conditions; however,

the corresponding mechanisms have not yet been determined. While PON production was more up-regulated than POC production under OA conditions, a decrease of C:N was observed in the HC-grown cells (Fig. 4). This is in good agreement with results obtained for another coccolithophore, *Gephyrocapsa oceania*, in which C:N decreased by 13% after the cells had been grown under OA conditions for 670 generations (Jin et al. 2013). The gene for nitrite reductase in phytoplankton can be up-regulated under OA conditions (Li et al. 2017); this would enhance inorganic nitrogen uptake, leading to an increased PON quota and a decreased C:N ratio, as observed in this study (Fig. 4).

Several strains of *E. huxleyi* probably operate CO_2-concentrating mechanisms (CCMs) (Sikes & Wheeler 1982, Nielsen 1995, Stojkovic et al. 2013), which are likely to be down-regulated at elevated CO_2 conditions (Stojkovic et al. 2013). Since the operation of CCMs is energetically costly (Raven et al. 2014), the energy saved from CCM down-regulation at elevated CO_2 could be reallocated to other physiological processes, thereby resulting in increased POC and PON production (Figs. 2b,d & 3b,d), which is in good agreement with some previous studies (Riebesell et al. 2000, Iglesias-Rodriguez et al. 2008, Shi et al. 2009, Rokitta & Rost 2012). POC production of marine phytoplankton usually increases with increasing light intensity but decreases at extremely high light levels. However, POC production did not show any decline in either strain, even at the highest light density applied in the present study (Fig. 2b,d). This results provides a physiological basis for the *E. huxleyi* bloom that usually occurs in stratified water under high light intensity (Tyrrell & Taylor 1996).

Calcification is an energy dependent process and normally decreases in coccolithophores under elevated CO_2 conditions (Riebesell et al. 2000, Feng et al. 2008, Gao et al. 2009, Rokitta & Rost 2012, present study). However, we found that OA decreased calcification at low solar radiation levels while no significant differences were detected at high solar radiation levels (Fig. 5a), suggesting the high levels of solar radiation can offset the impacts of ocean acidification on calcification in *E. huxleyi*. This is in agreement with some previous studies that report a decreased PIC quota with elevated CO_2 under low light (e.g. 50 µmol photons m^{-2} s^{-1}), but not under high light (e.g. 300 µmol photons m^{-2} s^{-1}) (Zondervan et al. 2002, Rokitta & Rost 2012). Elevated CO_2 increases the costs of calcification (Irie et al. 2010, Raven 2011, Raven & Crawfurd 2012), thereby lowering available energy and reducing calcification at low light levels.

However, this elevated CO_2 impact may be minimized by increased energy availability under high light (Rokitta & Rost 2012). In this study, *E. huxleyi* cells showed a similar response of calcification to OA as those in the previous works (e.g. Rokitta & Rost 2012), despite being exposed to higher light levels and diel fluctuations of solar radiation in the presence of UVR. The possible reasons for this are as follows: Firstly, fluctuating light can affect energetics in phytoplankton by increasing the costs for maintenance and repair of photoinhibition, thereby potentially altering the allocation of energy to other processes (e.g. calcification) (Dimier et al. 2009). Secondly, cells in the present study were exposed to solar radiation with UVR (280 to 400 nm), which has been shown to enhance PIC production (Guan & Gao 2010, Xu & Gao 2015). Consequently, fluctuations of solar radiation and presence of UVR may act individually or interactively to affect the calcification of coccolithophores. Therefore, the similar responses of calcification to OA in this study and previous studies (e.g. Rokitta & Rost 2012) might be the result of a trade-off between fluctuating light and UVR. In addition, these 2 possible mechanisms would also account for the different growth responses of *E. huxleyi* to OA treatment compared with that of diatoms, whose growth was enhanced under low light conditions but reduced under high sunlight levels (Gao et al. 2012). This conjecture should be investigated.

While higher light intensity appeared to offset OA impact on the calcification of *E. huxleyi* by increasing energy availability, further increasing light levels (to >500 µmol photons m^{-2} s^{-1}) decreased the calcification under both ambient or elevated CO_2 conditions (Fig. 5). This is consistent with previous findings that cellular PIC and PIC:POC in *E. huxleyi* both decreased when the light level was raised from 300 to 500 µmol photons m^{-2} s^{-1} (Xu & Gao 2015), and were lower when *E. huxleyi* was grown at 400 µmol photons m^{-2} s^{-1} (Feng et al. 2008), compared to lower light levels. Since the saturating light level for photosynthesis is much higher than that for calcification in *E. huxleyi* (Balch et al. 1992, Bleijswijk et al. 1994), in our experiments, calcification could have been photoinhibited at relatively high light levels while photosynthesis remained unaffected, leading to markedly decreased cellular PIC quotas and PIC:POC ratios at the high light levels.

Our data showed that the calcifying and non-calcifying *E. huxleyi* strains responded differentially to increasing light levels under OA treatment. As discussed above, calcification should account for this discrepancy. On the other hand, these 2 strains were

isolated from different regions, therefore their temperature reaction norms should be different (Zhang et al. 2014). Although we grew these 2 species at the same temperature level for the purpose of comparison, our results can hardly reflect their responses to OA in their natural habitat (Sett et al. 2014).

Since both pCO_2 (IPCC 2013) and integrated light density in the UML (Sarmiento et al. 2004) are projected to continue to rise in the future ocean, our findings imply that POC production of coccolithophores (both calcifying and non-calcifying) will increase. Our work also showed that, while calcification and ratio of calcification to photosynthesis in *E. huxleyi* may decrease with progressive OA at deeper layers under reduced levels of solar radiation, they might not be affected in the UML, as a result of the shoaling of the UML with ocean warming.

Our study showed that OA and solar radiation can individually and interactively affect the physiology of coccolithophores. However, nutrient availability, ocean warming and deoxygenation (Doney et al. 2012, Gattuso et al. 2015) are also likely to alter effects of OA, so how these factors will interactively impact on phytoplankton under future ocean scenarios is still an open question (Riebesell & Gattuso 2015). Biochemical composition of phytoplankton (e.g. fatty acid composition, phenolic compounds) has been shown to be significantly altered under OA conditions, and these changes could transfer up to secondary producers via trophic energy and nutrient transfer (Rossoll et al. 2012, Schoo et al. 2013, Jin et al. 2015). Furthermore, decreased C:N ratios (shown in this study) can improve the nutritional value of primary producers. Therefore, further knowledge is needed to understand the consequences of OA on food webs and marine biological CO_2 pumps.

LITERATURE CITED

Bach LT, Riebesell U, Schulz KG (2011) Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi.* Limnol Oceanogr 56:2040−2050. doi:10.4319/lo.2011.56.6.2040

Balch WM, Holligan PM, Kilpatrick KA (1992) Calcification, photosynthesis and growth of the bloom-forming coccolithophore, *Emiliania huxleyi.* Cont Shelf Res 12:1353−1374

Bleijswijk JD, Kempers RS, Veldhuis MJ, Westbroek P (1994) Cell and growth characteristics of type A and B of *Emiliania huxleyi* (Prymnesiophyceae) as determined by flow cytometry and chemical analyses. J Phycol 30:230−241

Boelen P, van de Poll WH, van der Strate HJ, Neven IA, Beardall J, Buma AGJ (2011) Neither elevated nor reduced CO_2 affects the photophysiological performance of the marine Antarctic diatom *Chaetoceros brevis.* J Exp Mar Biol Ecol 406:38−45

Braarud T, Gaarder KR, Markali J, Nordli E (1952) Coccolithophorids studied in the electron microscope. Observations on *Coccolithus huxleyi* and *Syracosphaera carterae.* Nytt Mag Bot 1:129−134

Dimier C, Brunet C, Geider R, Raven J (2009) Growth and photoregulation dynamics of the picoeukaryote *Pelagomonas calceolata* in fluctuating light. Limnol Oceanogr 54:823−836

Doney SC, Ruckelshaus M, Duffy JE, Barry JP and others (2012) Climate change impacts on marine ecosystems. Annu Rev Mar Sci 4:11−37

Feng Y, Warner ME, Zhang Y, Sun J, Fu FX, Rose JM, Hutchins DA (2008) Interactive effects of increased pCO_2, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae). Eur J Phycol 43:87−98

Fiorini S, Middelburg JJ, Gattuso JP (2011) Effects of elevated CO_2 partial pressure and temperature on the coccolithophore *Syracosphaera pulchra.* Aquat Microb Ecol 64:221−232

Gao K, Ruan Z, Villafañe VE, Gattuso JP, Helbling EW (2009) Ocean acidification exacerbates the effect of UV radiation on the calcifying phytoplankter *Emiliania huxleyi.* Limnol Oceanogr 54:1855−1862

Gao KS, Xu JT, Gao G, Li YH and others (2012) Rising CO_2 and increased light exposure synergistically reduce marine primary productivity. Nat Clim Change 2:519−523

Gattuso JP, Magnan A, Bille R, Cheung W and others (2015) Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios. Science 349:aac4722

Guan W, Gao K (2010) Impacts of UV radiation on photosynthesis and growth of the coccolithophore *Emiliania huxleyi* (Haptophyceae). Environ Exp Bot 67:502−508

Hoppe CJ, Holtz LM, Trimborn S, Rost B (2015) Ocean acidification decreases the light use efficiency in an Antarctic diatom under dynamic but not constant light. New Phytol 207:159−171

Hutchins DA (2011) Oceanography: forecasting the rain ratio. Nature 476:41−42

Iglesias-Rodriguez MD, Halloran PR, Rickaby RE, Hall IR and others (2008) Phytoplankton calcification in a high-CO_2 world. Science 320:336−340

IPCC (Intergovernmental Panel on Climate Change) (2013) Summary for Policymakers. In: Stocker TF, Qin D, Plattner GK, Tignor M and others (eds) Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, p 3−33

Irie T, Bessho K, Findlay HS, Calosi P (2010) Increasing costs due to ocean acidification drives phytoplankton to be more heavily calcified: optimal growth strategy of coccolithophores. PLOS ONE 5:e13436

Jin P, Gao K (2016) Reduced resilience of a globally distrib-

uted coccolithophore to ocean acidification: confirmed up to 2000 generations. Mar Pollut Bull 103:101–108

Jin P, Gao K, Villafañe VE, Campbell DA, Helbling EW (2013) Ocean acidification alters the photosynthetic responses of a coccolithophorid to fluctuating ultraviolet and visible radiation. Plant Physiol 162:2084–2094

Jin P, Wang T, Liu N, Dupont S and others (2015) Ocean acidification increases the accumulation of toxic phenolic compounds across trophic levels. Nat Commun 6:8714

Kropuenske LR, Mills MM, van Dijken GL, Bailey S, Robinson DH, Welschmeyer NA, Arrigoa KR (2009) Photophysiology in two major Southern Ocean phytoplankton taxa: photoprotection in *Phaeocystis antarctica* and *Fragilariopsis cylindrus.* Limnol Oceanogr 54:1176–1196

Langer G, Nehrke G, Probert I, Ly J, Ziveri P (2009) Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry. Biogeosciences 6:4361–4383

LaRoche J, Rost B, Engel A (2010) Bioassays, batch culture and chemostat experimentation. In: Riebesell U, Fabry VJ, Hansson L, Gattuso JP (eds) Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union, Luxembourg, p 81–94

Li Y, Gao K, Villafañe V, Helbling E (2012) Ocean acidification mediates photosynthetic response to UV radiation and temperature increase in the diatom *Phaeodactylum tricornutum.* Biogeosciences 9:3931–3942

Li Y, Zhuang S, Wu Y, Ren H, Chen F, Lin X, et al. (2017) Ocean acidification modulates expression of genes and physiological performance of a marine diatom. PLOS ONE 12:e0170970

Malin G, Erst GO (1997) Algal production of dimethylsulfide and its atmospheric role. J Phycol 33:889–896

Meyer J, Riebesell U (2015) Reviews and syntheses: responses of coccolithophores to ocean acidification: a meta-analysis. Biogeosciences 12:1671–1682

Morel FMM, Rueter J, Anderson DM, Guillard R (1979) Aquil: a chemically definded phytoplankton cullture medium for trace metal studies. J Phycol 15:135–141

Müller M, Schulz K, Riebesell U (2010) Effects of long-term high CO_2 exposure on two species of coccolithophores. Biogeosciences 7:1109–1116

Nielsen MV (1995) Photosynthetic characteristics of the coccolithophorid *Emiliania huxleyi* (prymnesiophyceae) exposed to elevated concentrations of dissolved inorganic carbon. J Phycol 31:715–719

Raven J (2011) Effects on marine algae of changed seawater chemistry with increasing atmospheric CO_2. Biology & Environment: Proc R Irish Academy 111B:1–17

Raven JA, Crawfurd K (2012) Environmental controls on coccolithophore calcification. Mar Ecol Prog Ser 470: 137–166

Raven JA, Beardall J, Giordano M (2014) Energy costs of carbon dioxide concentrating mechanisms in aquatic organisms. Photosynth Res 121:111–124

Riebesell U, Gattuso JP (2015) Lessons learned from ocean acidification research. Nat Clim Change 5:12–14

Riebesell U, Tortell PD (2011) Effects of ocean acidification on pelagic organisms and ecosystems. In: Gattuso JP, Hanson L (eds) Ocean acidification. Oxford University Press, Oxford, p 99–121

Riebesell U, Zondervan I, Rost B, Tortell PD, Richard EZ, Morel FMM (2000) Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature 407:364–367

Rokitta SD, Rost B (2012) Effects of CO_2 and their modulation by light in the life-cycle stages of the coccolithophore *Emiliania huxleyi.* Limnol Oceanogr 57:607–618

Rossoll D, Bermúdez R, Hauss H, Schulz KG, Riebesell U, Sommer U, Winder M (2012) Ocean acidification-induced food quality deterioration constrains trophic transfer. PLOS ONE 7:e34737

Sarmiento J, Slater R, Barber R, Bopp L and others (2004) Response of ocean ecosystems to climate warming. Global Biogeochem Cycles 18:GB3003

Schoo KL, Malzahn AM, Krause E, Boersma M (2013) Increased carbon dioxide availability alters phytoplankton stoichiometry and affects carbon cycling and growth of a marine planktonic herbivore. Mar Biol 160: 2145–2155

Sett S, Bach LT, Schulz KG, Koch-Klavsen S, Lebrato M, Riebesell U (2014) Temperature modulates coccolithophorid sensitivity of growth, photosynthesis and calcification to increasing seawater pCO_2. PLOS ONE 9:e88308

Shatwell T, Nicklisch A, Köhler J (2012) Temperature and photoperiod effects on phytoplankton growing under simulated mixed layer light fluctuations. Limnol Oceanogr 57:541–553

Shi D, Xu Y, Morel F (2009) Effects of the pH/pCO_2 control method on medium chemistry and phytoplankton growth. Biogeosciences 6:1199–1207

Sikes CS, Wheeler A (1982) Carbonic anhydrase and carbon fixation in coccolithophorids. J Phycol 18:423–426

Sobrino C, Ward ML, Neale PJ (2008) Acclimation to elevated carbon dioxide and ultraviolet radiation in the diatom *Thalassiosira pseudonana*: effects on growth, photosynthesis, and spectral sensitivity of photoinhibition. Limnol Oceanogr 53:494–505

Stojkovic S, Beardall J, Matear R (2013) CO_2 concentrating mechanisms in three southern hemisphere strains of *Emiliania huxleyi.* J Phycol 49:670–679

Suffrian K, Schulz KG, Gutowska MA, Riebesell U, Bleich M (2011) Cellular pH measurements in *Emiliania huxleyi* reveal pronounced membrane proton permeability. New Phytol 190:595–608

Tedetti M, Sempéré R, Vasilkov A, Charrière B and others (2007) High penetration of ultraviolet radiation in the south east Pacific waters. Geophys Res Lett 34:L12610

Tyrrell T, Taylor A (1996) A modelling study of *Emiliania huxleyi* in the NE Atlantic. J Mar Syst 9:83–112

van Leeuwe M, van Sikkelerus B, Gieskes WWC, Stefels J (2005) Taxon-specific differences in photoacclimation to fluctuating irradiance in an Antarctic diatom and a green flagellate. Mar Ecol Prog Ser 288:9–19

Xing T, Gao K, Beardall J (2015) Response of growth and photosynthesis of *Emiliania huxleyi* to visible and UV irradiances under different light regimes. Photochem Photobiol 91:343–349

Xu K, Gao K (2015) Solar UV irradiances modulate effects of ocean acidification on the coccolithophorid *Emiliania huxleyi.* Photochem Photobiol 91:92–101

Zhang Y, Klapper R, Lohbeck KT, Bach LT, Schulz KG, Reusch TB, Riebesell U (2014) Between- and within-population variations in thermal reaction norms of the coccolithophore *Emiliania huxleyi.* Limnol Oceanogr 59: 1570–1580

Zhang Y, Bach LT, Schulz KG, Riebesell U (2015) The modulating effect of light intensity on the response of the coccolithophore *Gephyrocapsa oceanica* to ocean acidification. Limnol Oceanogr 60:2145–2157

Zondervan I, Rost B, Riebesell U (2002) Effect of CO_2 concentration on the PIC/POC ratio in the coccolithophore *Emiliania huxleyi* grown under light-limiting conditions and different daylengths. J Exp Mar Biol Ecol 272:55–70