Diurnally fluctuating pCO₂ enhances growth of a coastal strain of Emiliania huxleyi under future-projected ocean acidification conditions

Futian Li, Jiekai Xu, John Beardall, and Kunshan Gao

State Key Laboratory of Marine Environmental Science/College of Ocean and Earth Sciences, Department of Marine Biological Science and Technology, Xiamen University, Xiamen 361102, China
Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Jiangsu Ocean University, Lianyungang 222005, China
Jiangsu Institute of Marine Resources Development, Lianyungang 222005, China
School of Biological Sciences, Monash University, Clayton, VIC 3800, Australia

*Corresponding author: tel: 86-592-2187982; fax: 86-592-2187963; e-mail: ksgao@xmu.edu.cn.
†These two authors contributed equally to this work.


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The carbonate chemistry in coastal waters is more variable compared with that of open oceans, both in magnitude and time scale of its fluctuations. However, knowledge of the responses of coastal phytoplankton to dynamic changes in pH/pCO₂ has been scarcely documented. Hence, we investigated the physiological performance of a coastal isolate of the coccolithophore Emiliania huxleyi (PML B92/11) under fluctuating and stable pCO₂ regimes (steady ambient pCO₂, 400 μatm; steady elevated pCO₂, 1200 μatm; diurnally fluctuating elevated pCO₂, 600–1800 μatm). Elevated pCO₂ inhibited the calcification rate in both the steady and fluctuating regimes. However, higher specific growth rates and lower ratios of calcification to photosynthesis were detected in the cells grown under diurnally fluctuating elevated pCO₂ conditions. The fluctuating pCO₂ regime alleviated the negative effects of elevated pCO₂ on effective photochemical quantum yield and relative photosynthetic electron transport rate compared with the steady elevated pCO₂ treatment. Our results suggest that growth of E. huxleyi could benefit from diel fluctuations of pH/pCO₂ under future-projected ocean acidification, but its calcification was reduced by the fluctuation and the increased concentration of CO₂, reflecting a necessity to consider the influences of dynamic pH fluctuations on coastal carbon cycles associated with ocean global changes.

Keywords: calcification, CO₂, coccolithophore, Emiliania huxleyi, fluctuation, ocean acidification, photosynthesis

Introduction

The oceans have absorbed about one-third of the anthropogenically released CO₂ (Sabine et al., 2004), leading to a decrease in seawater pH (0.1 pH units since the preindustrial era) and carbonate ions, along with increases in aqueous CO₂ and bicarbonate, a process known as ocean acidification (OA). The effects of OA on coccolithophores, one of the most important calcifying microalgal groups in the oceans, have been investigated intensively by experiments under elevated CO₂ [see the review by Meyer and Riebesell (2015) and references therein]. Generally, it is suggested, based on both laboratory studies [see the review by Schlüter et al. (2016) and literature therein] and mesocosm investigations (Engel et al., 2005), that OA would inhibit coccolithophore calcification even under varied levels of light, UV, temperature, and nutrients [see the review by Gao et al. (2019)], with the exception of a few studies that indicate enhanced calcification and increased coccolith numbers per cell (Iglesias-Rodriguez et al., 2008; White et al., 2018). In terms of production
of particulate organic carbon (POC) and growth, a range of effects of OA has been documented, with POC quota and growth reported as being unaffected or enhanced (Rokitta and Rost, 2012; Mackey et al., 2015; Zhang et al. 2020). OA treatment might reduce the particulate inorganic carbon quota in Emiliania huxleyi when grown under different levels of light and nutrients (Zhang et al. 2020). On the other hand, elevated CO₂ tends to enhance the production of transparent exopolymer particles and dissolved organic carbon, which has strong implications for carbon export efficiency (Engel et al., 2004). It should be noticed that most of these results were obtained from short-term experiments simulating OA conditions, which cannot be simply extrapolated to the oceans given the gradual decrease in pH over an extended period of time and interactions of multiple environmental factors.

In recent years, more and more studies have investigated the combined effects of seawater acidification and other environmental factors on E. huxleyi. Among these factors, temperature and nutrients seem to play major roles in regulating the physiology of this global important coccolithophore species (Müller et al., 2017; Feng et al., 2018), whereas the lower pH in OA treatments also showed significant impacts on the calcification process (Bodt et al., 2010; Bach et al., 2012). The concurrent changes in CO₂, temperature, and nutrients under future ocean scenarios are supposed to impact the performance of coccolithophores. Decreased growth and primary production with increased exudation of dissolved organic carbon were observed in E. huxleyi cells acclimated to elevated CO₂, elevated temperature, and decreased nutrient conditions, leading to the remarkable change in organic carbon partitioning (Borchard and Engel, 2012; Zhang et al., 2020).

For coastal regions, the changes in seawater carbonate chemistry, especially pH and pCO₂, are more complex. Compared with the open ocean, pH fluctuations occur more commonly in coastal waters over short time scales from hours to days (Duarte et al., 2013). Such fluctuations in carbonate chemistry of coastal zones are associated with complex interactions between physical and chemical processes as well as biological activities. These processes include tidal cycles, upwellings and nutrient inputs from anthropogenic activities as well as biological removal of CO₂ (Dai et al., 2009; Capone and Hutchins, 2013). Primary producers, both phytoplankton and benthic algae, are photosynthetically active during daytime, resulting in a pH rise, and their respiration leads to a drop in pH during night (Hurd et al., 2011). Such diel variation in some areas can be more than 1 pH unit (Duarte et al., 2013), which well exceeds the value of the pH decrease (∼0.4 units) predicted for future OA by the end of this century (Gattuso et al., 2015).

As a consequence of global climate change and intense anthropogenic disturbance, chemical and biological environments in coastal waters are likely to be more variable in the future. On the one hand, the frequency of periodic phytoplankton bloom episodes is likely to increase, during which phytoplankton photosynthesis consumes dissolved inorganic carbon and raises seawater pH (Gilbert, 2020). On the other hand, with a declining buffering capacity under elevated pCO₂ condition and increasing aqueous CO₂ released by eutrophication-driven respiration associated with heterotrophic dissimilation of organic matter, coastal waters are suggested to be more susceptible to OA than pelagic waters, with coastal pH dropping faster by about 12% compared to pelagic areas (Cai et al., 2011).

Previous studies usually set two or more steady pCO₂ levels to assess the physiological responses of coccolithophores to future OA scenarios (Hurd et al., 2009). Considering the features of the open ocean as described above, where CO₂ conditions are relative stable, such simulations are reasonable. However, this approach may be inappropriate to mimic the highly variable chemical environment of coastal or upwelling waters (Raven et al., 2020). In addition, it usually overlooks the surrounding micro-layers (i.e. diffusion boundary layer, DBL), a “micro-environment”, which can have major implications for cells, since the DBL could buffer organisms from the chemical changes in milieu (Flynn et al., 2012). Although most coccolithophores are oceanic species, some are also distributed in coastal areas (Godrijan et al., 2018; Matson et al., 2019). Therefore, investigations into coastal species’ responses to OA and exploring the mechanisms involved in coping with fluctuating pH and carbonate chemistry are important to our understanding of effects of global change in inshore waters.

There have been limited studies on the responses of marine phytoplankton to fluctuating pH/pCO₂. Our previous work has shown that the coastal (Thalassiosira weissflogii) and oceanic (Thalassiosira oceanica) diatoms differed in their capacity to respond to fluctuating carbonate chemistry (Li et al., 2016). After 400 generations’ adaptation under fluctuating high pCO₂, the green alga Ostreococcus grew smaller and responded more strongly to further pCO₂ increase than cells under the steady regime (Schaum et al., 2016). For coccolithophores, it has been shown that the calcification rate of an estuarine species, Pleurochrysis carterae, increased at elevated pCO₂ when the cells were subjected to diurnally fluctuating carbonate chemistry (White et al., 2018). However, it is still unknown how CO₂ variability affects other coastal coccolithophores. We hypothesized that a coastal strain of E. huxleyi could tolerate fluctuating pH/pCO₂, and show different physiological performance compared with cells grown under steady pCO₂ regimes. Hence, in the present study, we manipulated pCO₂ to mimic the diurnally fluctuating carbonate chemistry of the inshore environment in order to investigate the responses of a coastal E. huxleyi isolate.

**Material and methods**

**Cultures and experimental setup**

*Emiliania huxleyi* (PML B92/11, originally isolated from coastal waters off Bergen, Norway) was cultured in 500 ml polycarbonate bottles with Aquil medium. The medium was prepared from autoclaved artificial seawater with a salinity of 35, enriched with nitrate, phosphate, trace metal, and vitamin according to Aquil recipes (Sunda et al., 2005). Triplicate monospecific cultures were used for each treatment and were illuminated by cool white fluorescent light at an intensity of 150 μmol photons m⁻² s⁻¹. Cultures were maintained at 15°C, under a 12:12 h light:dark cycle. Semi-continuous methods were used and cells were maintained in exponential growth phase by dilution every 3 d. The maximal concentration during culturing was <50 000 cells ml⁻¹ (Supplementary Figure S1), within which the carbonate chemistry was insignificantly affected by biological activities (Jin et al., 2013).

*Emiliania huxleyi* was grown under three treatments: (i) steady ambient pCO₂ level (LCs); (ii) steady elevated pCO₂ level (HCs); and (iii) diurnally fluctuating elevated pCO₂ level (HFC) for at least 14 generations (∼14 d) before sampling commenced. For
the steady pCO₂ regimes, cultures were bubbled (180 ml min⁻¹) with ambient (~400 µatm, LCs) or high pCO₂ (~1200 µatm, HCs) air respectively, though the exact dissolved CO₂ partial pressures were slightly different (384 ± 7 µatm for LCs and 1198 ± 32 µatm for HCs), which was automatically achieved by using a CO₂ enricher (CE100C, RuIHua). The pCO₂ level of the HCs treatment (1200 µatm) is based on the higher end of predicted values in the Representative Concentration Pathway 8.5 (RCP8.5) emission scenario (IPCC, 2014), and this pCO₂ level also emerges periodically at present in coastal regions (Vargas et al., 2017). The fluctuating pCO₂ regime was obtained by setting different pCO₂ levels at different time periods in the CO₂ enricher. To be specific, we changed the output pCO₂ level every 3 h (1500 µatm for 0–3 h, 1200 µatm for 3–6 h, 900 µatm for 6–9 h, 600 µatm for 9–12 h after the onset of light and 900 µatm for 0–3 h, 1200 µatm for 3–6 h, 1500 µatm for 6–9 h, and 1800 µatm for 9–12 h after the onset of the dark period, Figure 1), so that pH gradually increased during the photoperiod and decreased at night, similar to a typical natural diurnal cycle. The pH/pCO₂ variation amplitudes of the HCf treatment (0.45 units for pH changes; 1200 µatm for pCO₂ changes) are typical for coastal waters (Duarte et al., 2013). Cultures were diluted in the middle of the photoperiod (6 h after the onset of light), and the replaced media with cells were used for measurement of physiological parameters. On the last day of the experiment, we tested the circadian rhythm of some parameters (mean cell size, seawater carbonate chemistry, maximum and effective photochemical quantum yields, photosynthesis, and calcification rates); subsamples were taken at five time points in the light period, and the culture volume was refilled to 500 ml in the middle of the photoperiod.

**Carbonate chemistry sampling and measurements**

Samples for total alkalinity (TA) measurements were filtered through cellulose acetate membranes (0.45 µm), poisoned with a saturated HgCl₂ solution, and stored at 4°C. TA was determined by Gran acidimetric titration with a TA analyser (AS-Alk1, Apollo SciTech). HCl (~0.1 mol l⁻¹) was used to titrate the samples. Certified references (Batch no. 162) from the Scripps Institution of Oceanography were used to assure the precision of the TA measurements (± 2 µmol kg⁻¹). TA was determined before and after the culture dilutions to investigate the influence of cell metabolism. In addition, samples were collected every 3 h during the light period for the fluctuating pCO₂ regime, and TA was not measured during the dark period. The pH₅BS was measured every 3 h during the light period by a pH meter (Orion 2 STAR, Thermo Scientific) calibrated with standard National Bureau of Standards (NBS) buffers. CO₂SYS software was used to convert pH₅BS values to pH₅ and calculate other carbonate chemistry parameters based on TA and pH data with the input values of phosphate concentration and salinity being 10 µmol l⁻¹ and 35, respectively. Dissociation constants for carbonic acid of Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and the dissociation constants for sulphuric acid from Dickson (1990) were chosen for calculations.

**Specific growth rate and mean cell size determination**

Samples (20 ml) were taken from culture bottles to determine cell abundance and mean cell size using a Coulter Particle Count and Size Analyser (Z2, Beckman Coulter). Specific growth rate was calculated according to the equation: \( \mu = (\ln N_1 - \ln N_0)/(t_1 - t_0) \), in which \( N_1 \) and \( N_0 \) represent cell abundances at \( t_1 \) and \( t_0 \).

**Chlorophyll and carotenoid contents**

Samples for determination of pigment content were filtered onto GF/F filters (25 mm, Whatman) and then extracted overnight in 5 ml of absolute methanol at 4°C in darkness. After centrifugation (5000g for 10 min), the absorption values of the sample supernatants were analysed by a UV–VIS spectrophotometer (DU800, Beckman Coulter). The concentrations of chlorophyll a and c were calculated according to Ritchie (2006), and carotenoid concentration was determined by the equation of Strickland and Parsons (1972).

**Chlorophyll a fluorescence**

In vivo chlorophyll a fluorescence parameters were determined using a multi-colour pulse amplitude modulated fluorometer (MULTI-COLOR-PAM, Walz). Maximum and effective photochemical quantum yields were determined according to the equations given by Genty et al. (1989): maximum photochemical quantum yield, \( \Phi_{PSII,max} = (F_{m'} - F_o)/F_{m'} \) for dark-adapted (10 min) samples; effective photochemical quantum yield, \( \Phi_{PSII,eff} = (F_{m'} - F')/F_{m'} \) for light-adapted samples. \( F_o \) and \( F' \) indicate the maximum chlorophyll fluorescence of dark and growth-light-adapted samples, respectively; \( F_o \) is the minimum chlorophyll fluorescence of dark-treated cells; and \( F' \) is the steady-state chlorophyll fluorescence of light exposed samples. \( \Phi_{PSII,eff} \) was measured under an actinic light intensity (~180 µmol photons m⁻² s⁻¹) similar to culture light levels. The saturation pulse was set at 5000 µmol photons m⁻² s⁻¹ for 0.8 s. Given the changing pH/pCO₂ under the fluctuating regime, maximum and effective photochemical quantum yields were measured every 2 or 3 h, respectively, in the light period.

In addition, rapid light curves were determined in the middle of the light period with 10 progressively increasing actinic light intensities (32, 49, 100, 187, 340, 616, 948, 1330, 1816, 2463 µmol photons m⁻² s⁻¹) for 20 s. Maximal relative electron transport rates (rETRₘₐₓₜ₉), apparent photon transfer efficiency (α), and the light saturation point (lₛ) were fitted according the equation of Eilers and Peeters (1988). Since coccoliths of *E. huxleyi* were shown to play an important role in mitigating high light stress to

![Figure 1](https://academic.oup.com/icesjms/advance-article-doi/10.1093/icesjms/fsab036/6158504) Target pCO₂ levels at different time points in the light and dark period under different carbonate chemistry conditions (LCs, open triangle; HCs, closed circle; HCf, half open/closed circles).
The carbonate chemistry was relatively stable under LCs and HCs treatments where pH$_T$ values were 8.00 ± 0.01 and 7.54 ± 0.01, respectively (Table 1). In the steady regimes, changes in DIC and TA caused by biological activities were <3% before and after the culture dilution, with the pH change being <0.03 units. The pH variation range in the HCf treatment was 0.45. At the beginning of the light period, the pH$_T$ was the lowest, with a value of 7.36 ± 0.01, which increased to 7.54 ± 0.01 at 6 h after the onset of the light, similar to the value in the HCs regime. After that, the pH reached its highest value at the end of the photoperiod, at around pH$_T$ 7.81 and then gradually decreased in the dark period, finally completing a fluctuation cycle. During the photoperiod, decreasing DIC and CO$_2$ in the HCf culture was observed over time as expected from the treatement regimes.

### Specific growth rate, cell size, and pigment content
For the steady regime, elevated pCO$_2$ did not show any effects on specific growth rate ($p = 0.945$). The fluctuating carbonate chemistry enhanced growth rates of cells compared with the steady regime, including both LCs and HCs treatments (one-way ANOVA, $p = 0.018$, Figure 2). The growth rate under the HCf treatment was 32% higher than that under HCs.

An increased trend of mean cell size in all treatments was observed from the beginning to the end of the light period (Figure 3). While there were no differences between the two steady treatments, HCf cells were smaller compared with LCs and HCs cells except for the time point at the end of light period ($p = 0.009, 0.033, 0.041$, and 0.007 for the four earlier time points, respectively). There were no significant differences in pigment content, including Chl $a$, Chl $c$, and carotenoids, across the different pCO$_2$ treatments ($p = 0.497, 0.498$, and 0.163 for Chl $a$, Chl $c$, and carotenoids respectively, Table 2).

### Chlorophyll $a$ fluorescence
While there were no significant differences in relative photoinhibitory ratio of rETR among the three treatments, cells grown under the LCs condition showed significantly higher rETR$_{max}$ and $\alpha$ than HCs and HCf grown ones. The fluctuating regime stimulated the electron transport rate compared with the steady regime under HC (Figure 4). The rETR$_{max}$ of cells grown under the HCf treatment was 11% higher than that of the HCs treatment ($p < 0.0018$, Figure 2). The growth rate under the HCf treatment was 32% higher than that under HCs. The time courses of $\Phi_{PSII}$ and $\Phi_{PSII_{eff}}$ during the light period were different among the different pCO$_2$ treatments (Figure 5). The values of $\Phi_{PSII_{max}}$ under the LCs and HCf treatments were relatively constant in the first half of the light period, then increased to around 0.57 at the end of the photoperiod. In contrast, HCs cells showed the highest $\Phi_{PSII_{max}}$ in the middle of the light period and the values were generally lower than those for cells under the other two treatments. For effective photochemical quantum yield, LCs cells always showed the highest values and HCs consistently had the lowest values among the three treatments. A generally increased trend of $\Phi_{PSII_{eff}}$ Values over the light period was observed for HCf cells, although cells in the other two treatments showed peak values in the middle of light period.

### Statistical analyses
Data were analysed using SPSS statistics 22 and reported as means ± SD. Homogeneity of variance and normality of data was tested before performing parametric tests. One-way ANOVA and post hoc Tukey’s tests were used to determine differences among pCO$_2$ treatments with a significance level of $p < 0.05$. The carbonate chemistry in the experimental regimes

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Fluctuating pCO₂ enhances growth of Emiliania huxleyi

Table 1. Carbonate chemistry parameters of culture media under steady ambient pCO₂ (LCs), steady elevated pCO₂ (HCs), and diurnally fluctuating elevated pCO₂ (HCF).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>TA (µmol kg⁻¹)</th>
<th>DIC (µmol kg⁻¹)</th>
<th>HCO₃⁻ (µmol kg⁻¹)</th>
<th>CO₂⁻/C₀ (µmol kg⁻¹)</th>
<th>CO₂ (µmol kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCs</td>
<td>8.00 ± 0.01ab</td>
<td>1 939.95 ± 2.78ab</td>
<td>1 751.01 ± 2.62ab</td>
<td>1 615.07 ± 2.42ab</td>
<td>122.06 ± 0.18ab</td>
<td>13.88 ± 0.02ab</td>
</tr>
<tr>
<td>HCs</td>
<td>7.54 ± 0.01bc</td>
<td>2 009.45 ± 6.34bc</td>
<td>1 971.60 ± 6.65bc</td>
<td>1 875.96 ± 6.33bc</td>
<td>48.61 ± 0.61bc</td>
<td>47.22 ± 0.69bc</td>
</tr>
<tr>
<td>HCF 0 h</td>
<td>7.36 ± 0.01de</td>
<td>1 982.59 ± 28.02de</td>
<td>1 992.78 ± 28.57de</td>
<td>1 889.20 ± 27.08de</td>
<td>32.71 ± 0.47de</td>
<td>70.88 ± 1.02de</td>
</tr>
<tr>
<td>HCF 3 h</td>
<td>7.45 ± 0.01ef</td>
<td>1 994.52 ± 8.83ef</td>
<td>1 978.79 ± 7.32ef</td>
<td>1 881.46 ± 7.17ef</td>
<td>40.39 ± 0.69ef</td>
<td>56.94 ± 0.54ef</td>
</tr>
<tr>
<td>HCF 6 h</td>
<td>7.54 ± 0.02gh</td>
<td>2 006.45 ± 21.54gh</td>
<td>1 967.64 ± 17.27gh</td>
<td>1 872.16 ± 16.39gh</td>
<td>48.71 ± 2.13gh</td>
<td>46.77 ± 1.24gh</td>
</tr>
<tr>
<td>HCF 9 h</td>
<td>7.72 ± 0.01ij</td>
<td>2 004.01 ± 14.70ij</td>
<td>1 911.89 ± 14.38ij</td>
<td>1 810.43 ± 13.62ij</td>
<td>71.81 ± 0.54ij</td>
<td>29.65 ± 0.22ij</td>
</tr>
<tr>
<td>HCF 12 h</td>
<td>7.81 ± 0.01kl</td>
<td>2 011.37 ± 16.12kl</td>
<td>1 891.08 ± 15.72kl</td>
<td>1 780.95 ± 14.92kl</td>
<td>86.24 ± 1.24kl</td>
<td>23.89 ± 0.40kl</td>
</tr>
</tbody>
</table>

For HCF treatment, TA and pH were determined at different time points in the light period (0, 3, 6, 9, and 12 h after the onset of light). The pHNBS values were converted to pH with the CO2SYS software. Values are the means ± SD of triplicate cultures. Different superscripted letters indicate significant (p < 0.05) differences among treatments.

Photosynthesis and calcification rates

After the onset of light, the photosynthesis rate of cells grown under the HCF treatment increased with time and reached a maximum (1.09 ± 0.27 pg C cell⁻¹ h⁻¹) in the middle of the light period and was maintained at this rate until the light was turned off. LGS and HCS cells showed a similar trend to that of HCF cultures, though lower photosynthetic rates than those of HCF cells were found at two time points during the light period (Figure 6a).

No significant time-related trends in the calcification rates could be observed for HCS and HCF cells as a result of quite large standard deviation values, while an increase in calcification rate over time was found for LGCs cells (Figure 6b). The calcification rate reached a maximum at the end of the photoperiod under the LGCs treatment, attaining a value of 1.66 ± 0.34 pg C cell⁻¹ h⁻¹. Overall, there were no significant differences in calcification rates among three pCO₂ treatments during the photoperiod with the exceptions that cells grown under the HCS condition had a higher rate in the middle of the light period and LGCs cells showed maximum calcification rate at the end of the light period.

A fluctuating regime at elevated pCO₂ enhanced the mean value of daily photosynthesis rate, although the increase was not statistically significant (p = 0.062, Figure 7a). In contrast, LGCs cells had the highest daily calcification rate (11.8 ± 0.7 pg C cell⁻¹ d⁻¹), which was 36% (p = 0.003) and 46% (p = 0.001) higher than that of cells under the HCS and HCF treatments, respectively (Figure 7b). Therefore, the highest C/P ratio was observed under the LGCs treatment, with a value about 1.5, and no significant differences in the ratio were detected for the steady and fluctuating regimes (Figure 7c).

Discussion

Our findings demonstrated that fluctuations in pCO₂ had a positive effect on the growth of E. huxleyi, with enhanced photosynthetic rates in the middle and at the end of the light period along with stimulated (by about 11%) energy transfer as reflected in the electron transport rate. These results indicate that this E. huxleyi strain can increase energy supply to cope with diurnal pH changes and benefit from the elevated CO₂ concentration for growth.

Emiliania huxleyi strains are known to be capable of establishing a new physiological equilibrium through changes in rates of various essential processes in <24 h when exposed to contrastingly different environmental changes (Barcelos e Ramos et al., 2012).
Table 2. Pigment contents of E. huxleyi cells grown under different carbonate chemistry conditions [LCs, steady ambient pCO2 (LCs); HCs, steady elevated pCO2; HCF, diurnally fluctuating elevated pCO2].

<table>
<thead>
<tr>
<th>pCO2 treatment</th>
<th>Chlorophyll a content (pg cell⁻¹)</th>
<th>Chlorophyll c content (pg cell⁻¹)</th>
<th>Carotenoid content (pg cell⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCs</td>
<td>0.12 ± 0.02±</td>
<td>0.04 ± 0.01±</td>
<td>0.13 ± 0.01±</td>
</tr>
<tr>
<td>HCs</td>
<td>0.12 ± 0.01±</td>
<td>0.04 ± 0.01±</td>
<td>0.14 ± 0.01±</td>
</tr>
<tr>
<td>HCF</td>
<td>0.13 ± 0.01±</td>
<td>0.03 ± 0.01±</td>
<td>0.15 ± 0.01±</td>
</tr>
</tbody>
</table>

Values are the means ± SD of triplicate cultures. Different superscripted letters indicate significant (p < 0.05) differences among treatments.

Figure 4. Rapid light curves of E. huxleyi cells grown under different carbonate chemistry conditions (LCs, open triangle; HCs, closed circle; HCF, half open/closed circle). Values are the means ± SD of triplicate cultures.

2010). Additionally, an E. huxleyi strain isolated from a Norwegian fjord has been shown to be more tolerant to low pH than strains isolated from waters near the Azores and Canary Islands (Zhang et al., 2018), which was attributed to the larger pCO2 and pH variability in coastal waters off Bergen compared with the rather stable oceanic conditions at the other two sites (Omar et al., 2010). These results are consistent with the reported results for a coastal diatom, T. weissflogii (Li et al., 2016). As mirrored in the present study and other works (Li et al., 2016; White et al., 2018), coastal phytoplankton isolates may be less sensitive to, and even benefit from, dynamic carbonate chemistry compared with oceanic strains. The findings, reported in this work, that growth of E. huxleyi was enhanced under diel fluctuation of elevated pCO2 might be a partial explanation for the high abundance of coccolithophores in coastal sediments (Cheng and Wang, 1997). However, some field studies have shown that coccolithophores do not necessarily exhibit adaptations to naturally high CO2 waters (Dassow et al., 2018). Thus, more studies are needed to clarify the adaptations of coccolithophores to OA and fluctuating pH.

The pH12 under the fluctuating regime gradually decreased to around 7.36 before the onset of the light period (Table 1), an acidified environment would promote respiration (Bach et al., 2011; Jin et al., 2013; Meyer and Riebesell, 2015). This could further lower pH within the DBL surrounding the cells (Flynn et al., 2012). Energy generated from enhanced respiration at decreased pH might be re-allocated to maintain intracellular acid-base homeostasis, considering that the intracellular pH of E. huxleyi decreases as seawater pH decreases (Suffrian et al., 2011). The enhanced respiration could also be related to the higher growth under the fluctuating regime, as this process generates ATP and carbon skeletons for growth (Raven and Beardall, 2005).

Many coastal calcifying organisms could adapt to large pH fluctuations in their habitats in ways that mitigate the negative effects on calcification of lowered pH under OA conditions (Hendriks et al., 2015). For example, the bivalve Pinna nobilis inhabits Mediterranean seagrass meadows, where pH inside the meadow is 0.3–0.5 units higher than the surrounding seawater during the daytime, and this is beneficial for its calcification (Hendriks et al., 2014). Other bivalves, such as the mussel Mytilus edulis, could utilize their body fluids such as extrapallial fluid or haemolymph, a set of internal buffering systems, to avoid lower pH at calcification sites (Heinemann et al., 2013). Scleractinian corals could manipulate the pH of the calcifying fluid by Ca²⁺-ATPase pumping of calcium ions in exchange for protons, and this acts as a form of extracellular regulation of pH (Allemand et al., 2004). This mechanism is beneficial to calcification of corals, as shown by Dufault et al. (2012), who found that a fluctuating pCO2 regime could enhance the calcification of the coral Seriatopora caliendrum.

In contrast, E. huxleyi, as a unicellular phytoplankter without a host, lacks complex structures such as the extrapallium of bivalves or the blastophyllum of corals. Moreover, the surface conditions of nano-phytoplankton like E. huxleyi, with an equivalent spherical diameter <5 μm, are close to bulk-water conditions, due to the positive relationship between cell size and the thickness of the DBL (Wolf-Gladrow and Riebesell, 1997; Flynn et al., 2012). That is to say, E. huxleyi cells distributed in the coastal seawater are directly exposed to the fluctuating carbonate chemistry environment with little buffering effect of a DBL. Thus, E. huxleyi could adopt different strategies in calcification to respond to fluctuating pH. As shown in the present study, seawater acidification treatment depressed the daily calcification rates regardless of the steady or fluctuating regime (Figure 7b). In accordance with the calcification rate, lower C/P (calcification to photosynthesis) was also detected at elevated pCO2 level (Figure 7c). These results are consistent with a large body of studies (Meyer and Riebesell, 2015) and the pattern of decreasing calcification with increasing pCO2 and concomitant decreasing CO2 regime could enhance the calcification of the coral Seriatopora caliendrum.
calcified less and produced similar amounts of organic carbon as the cells were grown at ambient pCO₂ level, which might lead to decreased sinking rate for coccolithophores is positively related to the ratio of particulate inorganic carbon to organic carbon (Hoffmann et al., 2015). Nevertheless, it should be noted that the results obtained from laboratory studies cannot be simply extrapolated to the real ocean. In contrast to results from most culture studies, the percentage and abundance of over-calcified morphotype of *E. huxleyi* increased in the Bay of Biscay when pH and CaCO₃ saturation are lowest (Smith et al., 2012).

The cell size of *E. huxleyi* cells increased with time irrespective of the CO₂ treatments (Figure 3) and has little to do with changes in the calcified layer thickness. Although there were significant differences among the treatments in the early phase of the light period, with the HCf-grown cells being of larger size, the difference becomes smaller and insignificant at the end of light period, implying that the POC quota increased with time during the daytime. Faster assimilation to generate more organic matter under the fluctuating high CO₂ due to enhanced energy transfer is likely to be responsible for the “catch-up” in cell size. Nevertheless, cell division under fluctuating high CO₂ must have resulted in the smaller cells at the time when the light was turned on (Figure 3).

The dynamic nature of coastal waters, especially as regards seawater carbonate chemistry, makes them a special, though generally overlooked, environment. Our findings in the present work,

<table>
<thead>
<tr>
<th>pCO₂ treatment</th>
<th>rETR&lt;sub&gt;max&lt;/sub&gt;</th>
<th>α</th>
<th>I&lt;sub&gt;k&lt;/sub&gt; (µmol photons m&lt;sup&gt;−2&lt;/sup&gt; s&lt;sup&gt;−1&lt;/sup&gt;)</th>
<th>Inhibition ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCs</td>
<td>93.40 ± 3.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.243 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>385 ± 20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCs</td>
<td>76.08 ± 1.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.230 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>331 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCf</td>
<td>84.68 ± 1.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.221 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>383 ± 10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17 ± 2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The relative photoinhibition ratio for rETR was evaluated here by the following equation: Inh (%) = (rETR<sub>max</sub> - rETRx)/rETR<sub>max</sub> × 100%. Values are the means ± SD of triplicate cultures. Different superscripted letters indicate significant (p < 0.05) differences among treatments.
Along with others (Li et al. 2016), imply that coastal phytoplankton species or strains show relatively more resilience to reduced pH under OA conditions. With progressive OA, the buffering capacity of seawater decreases as dissolved inorganic carbon increases, which would enhance the amplitude of pH variation in coastal waters (Egleston et al. 2010). More energy for coastal phytoplankton cells to maintain intracellular pH homeostasis would be required, especially during night time when respiration exacerbates acidification. However, the beneficial effects from elevated CO$_2$ during daytime might counteract the acidification stress, as shown in our work with Emiliania huxleyi. It should be kept in mind that these results were based on a short-term experiment (~14 generations) and a single strain culture. The species, and even strain, specificity (Langer et al. 2009) and adaptive evolution (Lohbeck et al., 2012) need to be taken into account when predicating the overall responses of coccolithophores to OA. Nevertheless, basic investigations such as the present study are the first step to understand the effects of fluctuating seawater carbonate chemistry, and there is an urgent need for studies on more species.

**Supplementary data**

Supplementary material is available at the ICESJMS online version of the manuscript.

**Data availability**

The data underlying this article will be shared on reasonable request to the corresponding author.

**Acknowledgements**

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**References**


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