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Ocean acidification exacerbates the effect of UV radiation on the calcifying phytoplankter *Emiliania huxleyi*

Kunshan Gao,[a,*] Zuoxi Ruan,[b] Virginia E. Villafañe,[c] Jean-Pierre Gattuso,[d] and E. Walter Helbling[c]

[a] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, China
[b] Marine Biology Institute, Science Center, Shantou University, Shantou, China
[c] Estación de Fotobiología Playa Unión, Playa Unión, Rawson, Chubut, Argentina; Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[d] CNRS, Laboratoire d'Océanographie de Villefranche, Villefranche-sur-Mer, France; Université Pierre et Marie Curie-Paris 6, Laboratoire d'Océanographie de Villefranche, Villefranche-sur-Mer, France

Abstract

Increasing atmospheric CO_2 concentration affects calcification in most planktonic calcifiers. Both reduced or stimulated calcification under high CO_2 have been reported in the widespread coccolithophore *Emiliania huxleyi*. This might affect the response of cells to photosynthetically active radiation (PAR; 400–700 nm) and ultraviolet radiation (UVR; 280–400 nm) by altering the thickness of the coccolith layer. Here we show that in the absence of UVR, the calcification rates in *E. huxleyi* decrease under lowered pH levels (pH_{NBS} of 7.9 and 7.6; pCO_2 of 81 and 178 Pa or 804 and 1759 ppmv, respectively) leading to thinned coccolith layers, whereas photosynthetic carbon fixation was slightly enhanced at pH 7.9 but remained unaffected at pH 7.6. Exposure to UVR (UV-A 19.5 W m^{-2}, UV-B 0.67 W m^{-2}) in addition to PAR (88.5 W m^{-2}), however, results in significant inhibition of both photosynthesis and calcification, and these rates are further inhibited with increasing acidification. The combined effects of UVR and seawater acidification resulted in the inhibition of calcification rates by 96% and 99% and that of photosynthesis by 6% and 15%, at pH 7.9 and 7.6, respectively. This differential inhibition of calcification and photosynthesis leads to significant reduction of the ratio of calcification to photosynthesis. Seawater acidification enhanced the transmission of harmful UVR by about 26% through a reduction of the coccolith layer of 31%. Our data indicate that the effect of a high-CO_2 and low-pH ocean on *E. huxleyi* (because of reduced calcification associated with changes in the carbonate system) enhances the detrimental effects of UVR on the main pelagic calcifier.

The oceans have absorbed one third of the anthropogenic CO_2 released to the atmosphere since the industrial revolution (Sabine et al. 2004). Increased dissolution of CO_2 into seawater leads to an increase in the concentration of pCO_2, HCO_3^-, and H^+ and to a decrease in the concentration of CO_3^{2-} and saturation state of calcium carbonate (Feely et al. 2004). The surface waters of the ocean have already been acidified by 0.1 pH units (corresponding to a 30% increase of H^+) since 1800 and will further decrease by another 0.3–0.4 units (about 100–150% increase of H^+) by 2100 under a "business-as-usual" emission scenario (Brewer 1997; Caldeira and Wickett 2003). A change of this magnitude and at such a fast pace has not occurred in the past 300 million yr (Caldeira and Wickett 2003). Ocean acidification has been suggested to harm marine calcifying organisms by reducing the rate of calcification of their skeletons or shells (Gao et al. 1993; Riebesell et al. 2000; Gazeau et al. 2007).

Coccolithophores, as key $CaCO_3$ producers, form extensive blooms, and most studies carried out with these organisms have demonstrated a decline of calcification rates at low pH (Riebesell et al. 2000; Sciandra et al. 2003; Delille et al. 2005). However, some species do not seem to be affected and others exhibit an optimum rate of calcification (Langer et al. 2006), whereas calcification of an *E. huxleyi* strain seems to be stimulated at low pH (Iglesias-Rodriguez et al. 2008). Nevertheless, these results are still debated (U. Riebesell pers. comm.; C. Turley pers. comm.). On the other hand, higher pCO_2 in seawater was suggested to enhance photosynthesis in *E. huxleyi* (Riebesell et al. 2000; Iglesias-Rodriguez et al. 2008). In most of these studies, however, cells were grown under low photosynthetically active radiation (PAR; 15–170 μmol photons m^{-2} s^{-1}; 3.2–36.5 W m^{-2}) (Riebesell et al. 2000; Sciandra et al. 2003; Langer et al. 2006) and the effect of solar ultraviolet radiation (UVR) was not considered. Enrichment of CO_2 under low PAR levels might have led to enhanced rates of photosynthesis and calcification because of the energy saved because of increased levels of pCO_2 and bicarbonate for their transport (i.e., less energy competition for transport of inorganic carbon for photosynthesis and calcification). On the other hand, increased ocean stratification (shallower upper mixed layer) due to global warming is likely to enhance the already high mean daily solar irradiance received by coccolithophores (Nanninga and Tyrrell 1996; Boyd and Doney 2002) that mainly live in temperate areas.

The covering coccoliths may protect the cells from being harmed by high levels of PAR as well as UVR, and this is important because UVR can damage coccolithophores' DNA, and moreover, stabilization of the water column strongly promoted this damage in surface seawater (Buma et at. 2000, 2001). Hence, ocean acidification could threaten the survival of coccolithophores by exposing less-protected

* Corresponding author: ksgao@xmu.edu.cn

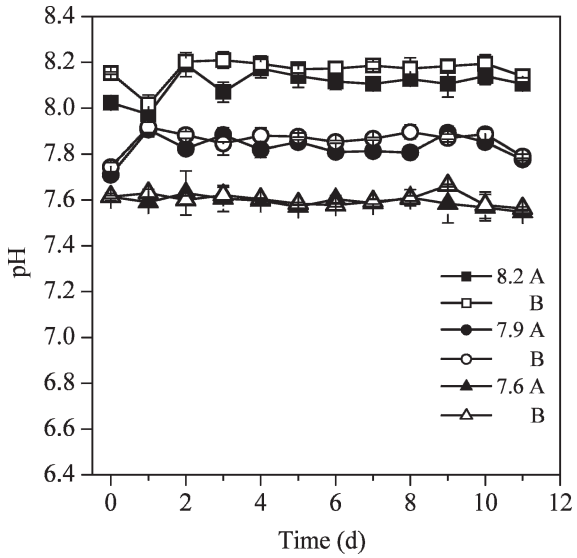

Fig. 1. Changes in pH_{NBS} levels during the semi-continuous cultures of *E. huxleyi* at cell concentrations ranging from 1.3 to 4.0 $\times 10^5$ cells mL^{-1}. pH was monitored every 24 h either (A) before or (B) immediately after renewing medium. Data are means ± SD for triplicate cultures at each treatment.

cells to harmful levels of solar radiation. In spite of this, and to the best of our knowledge, few previous studies have examined the effects of ocean acidification in combination with UVR (Sobrino et al. 2008). Here, we test two hypotheses: that the coccoliths provide protection against photodamage or photoinhibition by shielding off significant amounts of UVR and PAR and that ocean acidification is likely to enhance the processes involved in photodamage and photoinhibition by reducing the calcified coverage of the cells.

Methods

Culture media—Sterilized seawater (salinity = 31) was enriched with K stock medium (Keller 1987). Reduction of pH to 7.9 or 7.6 was performed prior to cell inoculation by aerating the medium with air enriched either with 81 Pa (804 ppmv, pH_{NBS} 7.9 level) or 178 Pa (1759 ppmv, pH_{NBS} 7.6 level) CO_2 through a filter (0.22 μm, Whatman). The pH level of 8.2 was set as control (present-day level).

Culture—Emiliania huxleyi (CS369, isolated from Pipe Clay Lagoon, Tasmania, Australia) was obtained from the Collection of Living Microalgae at Commonwealth Scientific and Industrial Research Organisation. Cells were grown at 18°C, 425 μmol photons m^{-2} s^{-1} (14 : 10 light : dark) of PAR (irradiance saturating growth and photosynthesis) (Nanninga and Tyrrell 1996) for 10 d (at least 10 generations) before being used to inoculate in the acidified and the control media. Transfusion bags (500 mL) made of non-PVC medicine multilayer co-extrusion infusion film (styrene-ethene-butylene-styrene copolymer and polypropylene, Southwest Pharmaceutical) were used as culture vessels for easy sampling without any loss of CO_2. These bags transmit about 85% of PAR (400–700 nm), which was 500 μmol photons m^{-2} s^{-1} at the surface of the bag; therefore, the cells inside the bag were exposed to 425 μmol photons m^{-2} s^{-1} of PAR while growing at different pH levels. The cultures were run semi-continuously by partially renewing the medium every 24 h to restore the initial cell concentration at 1.3 $\times 10^5$ cells mL^{-1}, so that pH levels were maintained within a daily variation of less than 0.08 (Fig. 1). The bags were gently agitated three times a day to ensure homogenous distribution of cells in the medium. Triplicate cultures were set at each pH treatment.

Estimation of changes in the carbonate chemistry—pH_{NBS} and dissolved inorganic carbon were measured in *E. huxleyi* cultures, and other related parameters for the carbonate chemistry were estimated according to these values using the software CO2SYS (Lewis and Wallace 1998) (Table 1). The equilibrium constants K_1 and K_2 for carbonic acid dissociation were determined after Roy et al. (1993), K_B for boric acid was obtained after Dickson (1990), and Ksp for calcite saturate state was obtained after Mucci (1983). The levels of pH_{NBS} were monitored daily before (2 h later in the light period) and after (4 h later in the light period) the renewal of the medium using a pH meter (Mettler Toledo Seveneasy) calibrated with standard National Bureau of Standards buffer solution (Merck) (Fig. 1). Total alkalinity of the culture media was 2150 μeq kg^{-1} and its decrease during the 24 h (between renewing medium) was determined to be about 90 μeq kg^{-1} from the calcification rate. Therefore, its effect on calcite saturation state ($\Omega_{calcite}$) was negligible (less than 5%).

Growth rates—Growth rate of *E. huxleyi* was monitored every day. Samples of 10–15 mL were withdrawn before and after the medium renewal. The coccoliths were removed by bubbling with CO_2 (about 99.9%) for 30 s. Naked cells were counted with a particle counter (Coulter Z1, Beckman) and the specific growth rate (μ) was calculated as follows:

Table 1. Carbonate chemistry of *E. huxleyi* cultures grown under pH levels of 8.2, 7.9, and 7.6 adjusted by bubbling ambient or CO_2-enriched air.*

pCO_2(Pa)	pH_{NBS}	DIC (μmol L^{-1})	TA (μeq L^{-1})	CO_2 (μmol L^{-1})	HCO_3^- (μmol L^{-1})	CO_3^{2-} (μmol L^{-1})	$\Omega_{calcite}$
35.4±3.0	8.22±0.02	1926±95	2165±95	12.2±1.0	1737.7±90.4	176.0±4.0	4.32±0.10
81.5±4.5	7.89±0.01	2021±84	2124±84	28.1±1.5	1901.3±79.7	91.7±2.5	2.24±0.07
178.2±4.4	7.58±0.01	2147±25	2154±24	61.4±1.5	2037.3±23.7	48.0±0.0	1.18±0.01

* Data are means ± SD for triplicate cultures at each treatment. DIC, dissolved inorganic carbon; TA, total alkalinity.

$$\mu = (\ln C_1 - \ln C_0)/(t_1 - t_0),$$

where C_0 and C_1 represent the cell concentration at t_0 (initial or just after the dilution) and t_1 (before the dilution), respectively.

Cell size and amount of coccoliths—Cells grown under different pH levels were sampled and examined under a microscope (Zeiss Axioplan 2 Imaging); photo images of cells were taken from each culture. The diameter of cells with or without coccoliths was measured with the photo processing program Auxio (Zeiss). The thickness of the coccolith layer was estimated as half the difference between coccolith-covered and naked (coccolith-removed) cells.

Radiation treatments and measurements—To determine photosynthesis and calcification of *E. huxleyi* and their response to UVR, cells grown at different levels of pH were exposed to three radiation treatments: (1) PAR + UV-A + UV-B (PAB) in quartz tubes covered with 295-nm cut-off film (Ultraphan, Digefra), transmitting irradiances above 295 nm; (2) PAR + UV-A (PA) in quartz tubes covered with 320-nm cutoff film (Montagefolie, Folex), transmitting irradiances above 320 nm; and (3) PAR, in quartz tubes covered with 395-nm cutoff film (Ultraphan UV Opak, Digefra), transmitting irradiances above 395 nm. Triplicate samples from each treatment were exposed under a solar simulator (Hönle UV Tech.) with irradiance levels of 88.5 W m^{-2} (420 μmol photons m^{-2} s^{-1}), 19.5 W m^{-2}, and 0.67 W m^{-2} for PAR, UV-A, and UV-B, respectively. The biologically weighted UV-B irradiance was 0.07 W m^{-2} (normalized at 300 nm) (Setlow 1974), estimated on the basis of irradiances reaching the cells in the quartz tubes covered with a 295-nm cutoff filter.

Simulated solar radiation was measured with a broadband filter radiometer (ELDONET, Real Time Computer Inc.) that has channels for UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm). This instrument has been calibrated every year and has an error of less than 0.5% in comparison with the most accurate instrument (certificate No. 2006/BB14/1).

Determination of photosynthetic and calcification rates—After cells were grown at different levels of pH for at least 11 generations, they were dispensed into quartz tubes (13 mL) and inoculated with 5 μCi (0.185 MBq) of labeled sodium bicarbonate (Amersham). The tubes were placed in a water bath connected to a cooling circulator (Cole Palmer, Model 01268-16) for temperature control (18 ± 0.5°C) under the radiation treatments described above. After 2 h of incubation, samples were immediately filtered under dim light onto Whatman GF/F glass fiber filters (25 mm), rinsed with unlabeled medium, put in 20-mL scintillation vials, and then dried (45°C, 4 h) before counting in a liquid scintillation counter (Beckman, LS6500) with 3 mL scintillation cocktail (Perkin Elmer). These measurements provided information on the total ^{14}C incorporated (inorganic and organic production). Photosynthetic ^{14}C fixation was measured after removal of coccoliths. Two methods were tested to gain the highest accuracy cells on GF/F filters fumed with HCl overnight to expel non-fixed ^{14}C and cells exposed to pure CO_2 for 30 s to remove the cocolith coverage before being filtrated onto GF/F filters. Both sets of filters were dried (45°C for 4 h) before measuring radioactivity. Photosynthetic carbon fixation rates obtained by these two approaches were not significantly different (*t*-test, $t = 1.520$, $p = 0.189$, df = 5, $n = 6$). The rate of calcification of *E. huxleyi* was estimated as the difference between the total and the photosynthetic carbon fixation determined after the coccoliths were removed by the acidification (Paasche 1963).

Absorptivity of coccolith coverage—Absorption spectra of cells with or without coccoliths were examined. For these measurements, cells (3.73×10^7 cells in 25 mL) were filtered onto Whatman GF/F glass fiber filters (25 mm), and the filters placed at the window near the detector of a UV-VIS recording spectrophotometer (Shimadzu UV2501PC) (Kishino et al. 1985). A filter soaked with the culture medium was used as control.

Statistics—Data were analyzed using the statistical package SPSS. Before performing parametric tests, data were tested for homogeneity of variance and normality. A *t*-test and one-way analysis of variance (ANOVA) were used at a significance level of $p < 0.05$. When necessary, the post hoc Duncan test was used to determine differences between individual means. Data are reported as mean ± SD. For the ratio of growth rate at lowered pH levels to that at pH 8.2, data were randomly paired to establish the SD.

Results

Growth rate—The specific growth rate (μ) ranged between 0.6 and 1.1 depending on time and pH treatment (Fig. 2). During the initial period, days 2–4, μ was significantly lower at pH 7.6 than at pH 8.2 (*t*-test, $t = 6.020$, $p < 0.001$, df = 16, $n = 18$); and was higher at pH 8.2 than at pH 7.9 except on day 2 (but the differences were not significant). There were no significant differences among the pH regimes on days 5 and 6, and growth at pH 8.2 was different from that at pH 7.9 and 7.6 on day 8. Overall, the differences were significant only at pH 7.6 during the first week and became nonsignificant during the last 3 d of the culture.

Cell size and coccolith shell—Analysis of at least 100 diameter measurements showed that lowering pH resulted in a significant (ANOVA, $p < 0.001$) reduction of the cell size and coccolith thickness in *E. huxleyi* (Fig. 3). The cell diameter at day 11 became smaller by 0.5 μm (about 7% as compared to that of pH 8.2) in the presence of coccoliths at pH 7.9. However, no significant difference (*t*-test, $t = 0.671$, $p = 0.503$, df = 438, $n = 440$) was found between pH 8.2 and pH 7.6. When the cells were made naked by removing their coccoliths (by high CO_2) they were bigger by about 0.9 μm (*t*-test, $t = 10.466$, $p < 0.001$, df = 201, $n = 203$) at pH 7.6 in contrast to those grown at the higher

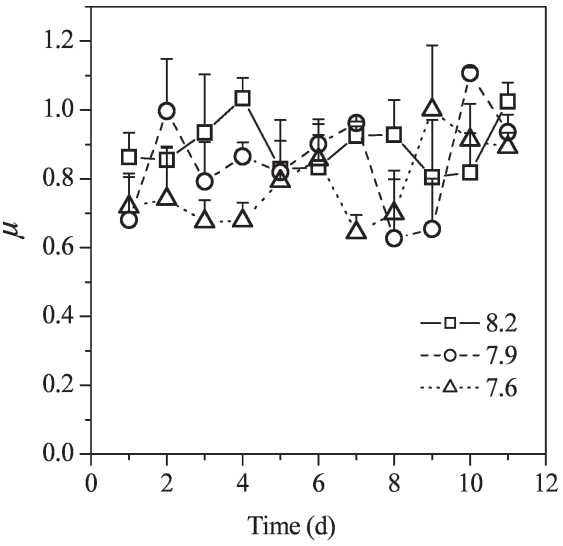

Fig. 2. Specific growth rate (μ) of *E. huxleyi* grown at pH_{NBS} levels of 8.2, 7.9, and 7.6, respectively. Triplicate cultures at each pH level and values are the means for triplicate cultures at each treatment ± SD.

pH levels, at which the naked cells were identical in diameter. The thickness of the coccolith layer, derived from half of the difference between the cell sizes with and without coccoliths, showed a significant decrease from 1.3 to 0.9 μm with the pH decline (ANOVA, $p < 0.001$) (Fig. 3B). The mean thickness of the coccolith layer of each cell was 1.3 μm at pH 8.2 and decreased by 23% and 31% (to 1.0 and 0.9 μm) at pH 7.9 and 7.6, respectively.

Effects of UVR on calcification and photosynthetic carbon fixation—Photosynthetic and calcification rates of *E. huxleyi* cells exposed to UVR decreased (as compared to the PAR-only control) with decreasing pH levels (Fig. 4). Cells receiving only PAR had significantly ($df_1 = 8$, $df_2 = 18$, post hoc Duncan test, $p < 0.05$) higher photosynthetic carbon fixation at pH 7.9, but no significant difference was found between pH 7.6 and pH 8.2 (Fig. 4A). The relative inhibition due to UVR increased from high to low pH levels, with values of 4%, 14%, and 16% at pH 8.2, 7.9, and 7.6, respectively (Fig. 4B). Most of the UVR-induced inhibition of photosynthetic carbon fixation was due to UV-A. The rate of calcification was significantly (ANOVA, $p < 0.001$) reduced by both UVR and pH reduction (Fig. 4C). Lowering pH to 7.9 or 7.6 reduced the calcification rate by 43% and 90% under PAR alone, by 61% and 96% under PA, and by 72% and 99% under PAB treatments, respectively, as compared to the values at pH 8.2 (under PAR only). Presence of UVR caused an additional reduction in calcification rates, with decreases of 45%, 52%, and 91% at pH 8.2, 7.9, and 7.6, respectively. Of the total observed inhibition, UV-A accounted for 14%, 32%, and 63%, whereas UV-B accounted for 31%, 20%, and 28% at pH 8.2, 7.9, and 7.6, respectively (Fig. 4D).

Obviously, seawater acidification exacerbated the negative effects caused by UV-A and UV-B, with a greater effect (i.e., more reduction) on calcification than on photosynthesis, thus resulting in a decline of the calcification to photosynthesis (C : P) ratios at the low pH levels (Fig. 5). Under PAR alone, the C : P ratio (as compared to values at pH 8.2) was significantly (*t*-test, $t = 7.197$, $p < 0.001$, df = 16, $n = 18$) reduced by 48% at pH 7.9, and (*t*-test, $t = 15.960$, $p < 0.001$, df = 16, $n = 18$) by 90% at pH 7.6. A reduction in the C : P ratio due to UVR exposure was also observed, i.e., 42%, 43%, and 88% at pH 8.2, 7.9, and 7.6, respectively. In this latter case, most of the observed inhibition was due to UV-B radiation, which accounted for >50% of the total inhibition.

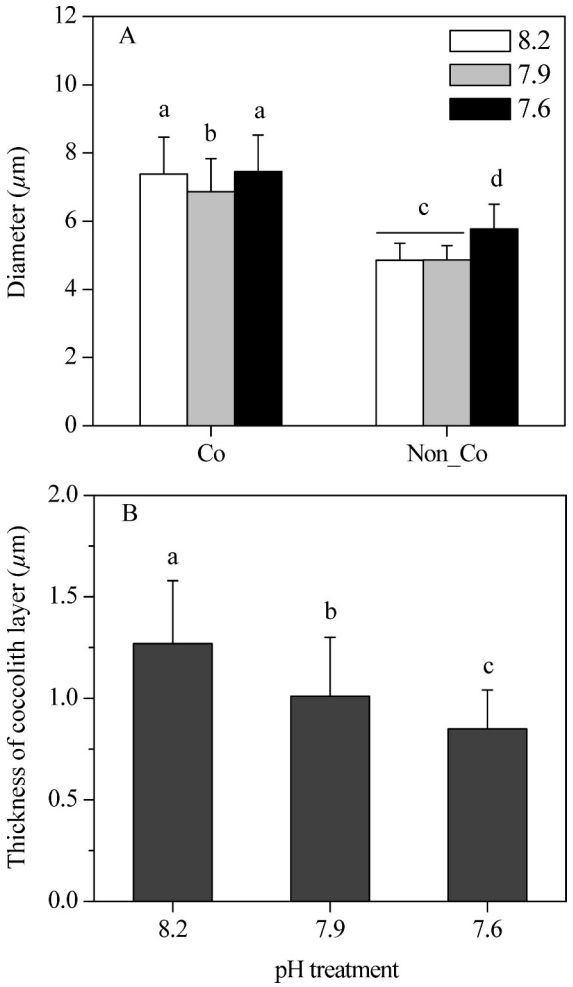

Fig. 3. (A) Diameter of *E. huxleyi* cells grown for 11 d at pH_{NBS} levels of 8.2, 7.9, and 7.6, respectively. Diameters are shown for cells either with (Co) or without coccoliths (Non_Co). (B) Thickness of the coccolith layer, derived from half the difference in the diameter of cells with and without coccoliths. The letters on top of the bars indicate significant difference at $p = 0.05$, so for the same letters there are no significant differences among the data. Values are means ± SD ($n \geq 100$).

Optical characteristic of coccolith shell—The coccolith layer of *E. huxleyi* reduced the transmission of both visible radiation and UVR because of absorption and scattering (Fig. 6). In contrast to naked cells, coccolith-covered cells reduced the transmission of UVR (in the range 300–400 nm) by 20–25% and that of PAR by 10–22%.

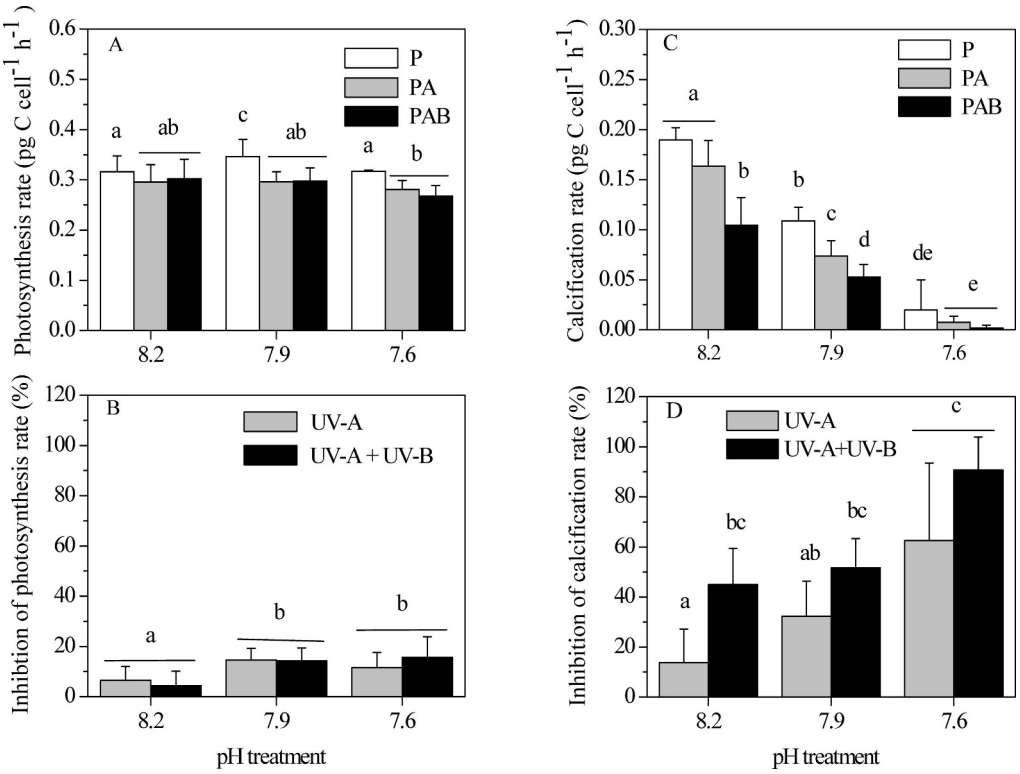

Fig. 4. (A) Effects of UVR on photosynthetic carbon fixation of *E. huxleyi* grown at 8.2, 7.9, and 7.6 pH_{NBS} levels. The cells were exposed to PAR only (P), PAR + UV-A (PA), or PAR + UV-A + UV-B (PAB). (B) UV-A–induced or UV-A + UV-B–induced inhibition of photosynthesis at pH levels of 8.2, 7.9, and 7.6. (C) Effects of UVR on calcification carbon fixation of *E. huxleyi* grown at 8.2, 7.9, and 7.6 pH_{NBS} levels. The cells were exposed to PAR only (P), PAR + UV-A (PA) or PAR + UV-A + UV-B (PAB). (D) UV-A–induced or UV-A + UV-B–induced inhibition of calcification at pH levels of 8.2, 7.9, and 7.6. The letters on top of the bars indicate significant difference at $p = 0.05$, so for the same letters there are no significant differences among the data. The values are means ± SD ($n = 9$; 3 measurements for each of the triplicate cultures at day 11).

Therefore, and because acidification of seawater to pH 7.9 and 7.6 reduced the coccolith layer thickness by 23% and 31%, respectively, the cells could have received additional UVR and PAR by 17% and 26%, correspondingly, as the coccolith thickness and transmission of UVR and PAR were tested to be linearly related.

Discussion

The rate of calcification was reduced and the coccolith layer became significantly thinner in the cells of *E. huxleyi* grown under the high-CO_2-acidified conditions, though their photosynthetic carbon fixation was stimulated at pH 7.9. Cells with fewer coccoliths showed higher inhibition caused by UVR for both photosynthesis and calcification. UVR-induced inhibition increased from about 45% at pH 8.2 to about 91% at pH 7.6 for calcification, but only from about 4% at pH 8.2 to about 16% at pH 7.6 for photosynthesis, leading a large decrease of the C : P ratio. The thinned coccolith layer of cells grown under the acidified conditions shielded off less UVR, thus reflecting the fact that the coccoliths play a protective role in the cellular physiological processes against harmful UVR under normal conditions.

Higher CO_2 availability at lowered pH levels could differentiate the sensitivity of photosynthesis and calcification to UVR. There was about a 10% increase in the photosynthetic carbon fixation at pH 7.9 as compared to pH 8.2, but no effect was observed at pH 7.6. CO_2 levels at pH 7.9 and 7.6 were 28.1 and 61.4 μmol L^{-1}, respectively, being much higher than the level at pH 8.2 (12.2 μmol L^{-1}). Increased supply of CO_2 can enhance the photosynthesis of *E. huxleyi* (Riebesell et al. 2000; Zondervan et al. 2002). However, a decrease of pH can also result in negative effects on photosynthesis and other physiological processes (Coleman and Colman 1981; Nimer et al. 1994). The balance between the effects caused by low pH and high CO_2 can affect the rate of photosynthetic carbon fixation. At pH 7.9, the CO_2 enhancement could have exceeded the effects caused by low pH, whereas at pH 7.6, the high CO_2 and low pH effects might have been balanced, thus resulting in even photosynthetic carbon fixation. The POC production of this species increased at 60 Pa (pH 7.95) but not at 78 Pa CO_2 (pH 7.80) in contrast to the current CO_2 level (pH 8.2) (Riebesell et al. 2000). Lowering the pH to 7.0 inhibited the photosynthetic oxygen evolution of *E. huxleyi* (Nimer and Merrett 1992). In the present study, lowering the pH from 8.2 to 7.9 slightly enhanced photosynthesis, but further acidification to pH 7.6 did not affect photosynthesis

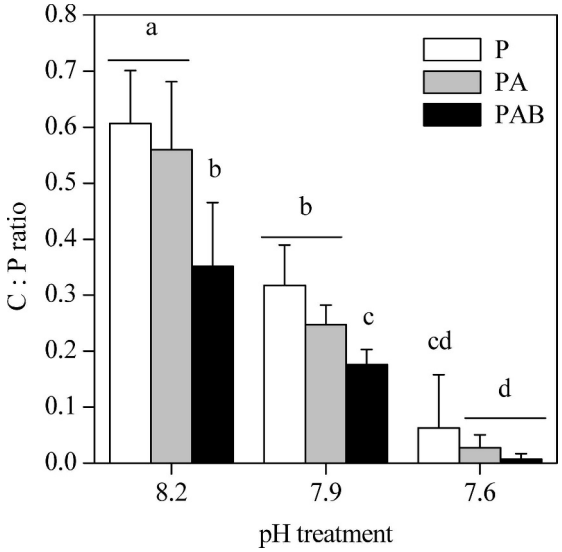

Fig. 5. Effects of UVR on the carbon fixation ratio of calcification (C) to photosynthesis (P) grown at 8.2, 7.9, and 7.6 pH_{NBS} levels. The cells were exposed to PAR only (P), PAR + UV-A (PA), or PAR + UV-A + UV-B (PAB). The letters on top of the bars indicate significant difference at $p = 0.05$, so for the same letters there are no significant differences among the data. The values are means ± SD ($n = 9$; 3 measurements for each of the triplicate cultures at day 11).

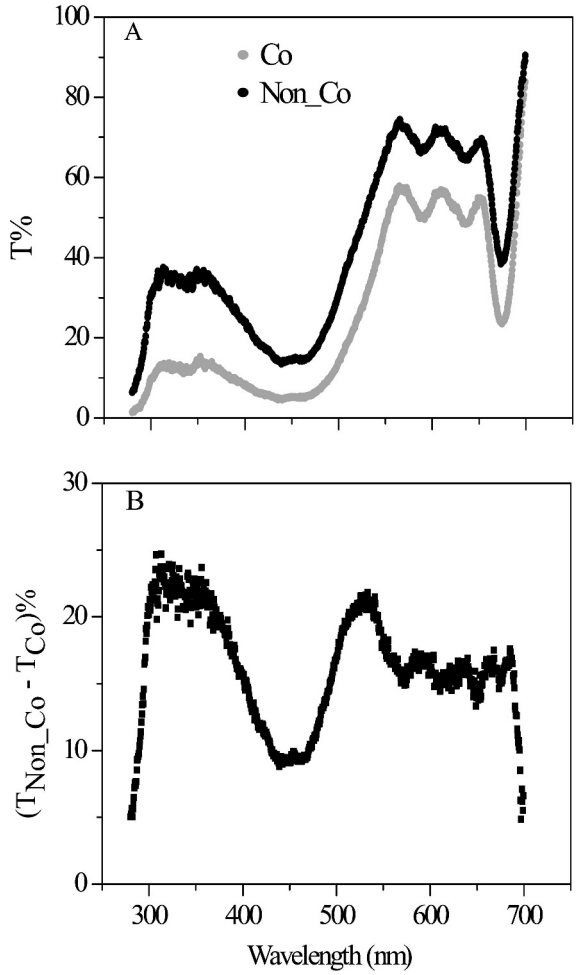

Fig. 6. (A) Transmission spectra of cells with (Co) and without (Non_Co) coccolith cover. (B) Reduction of the transmission by the coccolith layer.

(Fig. 4), and the growth of the acclimated cells was not affected (Fig. 2) under PAR treatment alone. Insignificant influences of seawater acidification on the growth rate of coccolithophores have also been reported elsewhere (Zondervan et al. 2002; Delille et al. 2005; Engel et al. 2005). However, in the present study, photosynthetic rate was significantly reduced by the lowered pH in the presence of UVR (Fig. 4), reflecting a synergistic effect of UVR with reduced pH. Growth rates of *E. huxleyi* have been shown not to differ at reduced pH levels either under nitrogen-, phosphorus-, or light-limited conditions (Zondervan et al. 2002; Sciandra et al. 2003; Leonardos and Geider 2005) or under nutrient- and light-saturated conditions (Engel et al. 2005; this study); however, they are very sensitive to UVR. The growth rate of *E. huxleyi* decreased by UVB at only 1/10 of its common incident biological effective dose (100 J m^{-2} d^{-1}) (Gieskes and Buma 1997), and even halted at higher levels of UVB (above 400 J m^{-2} d^{-1}) (Buma et al. 2000). Such inhibition of growth caused by UVR was proportional to DNA damage evaluated as cyclobutane pyrimidine dimer formation (Gieskes and Buma 1997; Buma et al. 2000; Garde and Cailliau 2000). Our preliminary results showed that the growth rate of *E. huxleyi* (CS369) was reduced by about 12% in the presence of UVB at the biological effective dose of 289 J m^{-2} d^{-1} at pH 7.9 (data not shown).

In the present study, the calcification rate was reduced at the lower levels of pH, and the coccolith layer became thinner. However, even the cells grown at pCO_2 of 178 Pa (61.4 μmol L^{-1}) still had coccoliths. Lowering pH might have not affected the number of the attached or free coccoliths per coccosphere (Riebesell et al. 2000). Decreased calcification at lowered pH may be because of a lower saturating state of calcite in the coccolith vesicle and subsequently disturbed nucleation and formation of the crystallization (Bilan and Usov 2001; Zondervan et al. 2002). The presence of UVR inhibited the calcification rate when the cells were exposed to PAR + UVR, and the inhibition became larger at reduced pH levels. Because UVR is known to damage DNA and proteins (Buma et al. 2000, 2001; Garde and Cailliau 2000), the molecular mechanisms for calcification of *E. huxleyi* must have been negatively affected by UVR. The thinner coccolith layer at the lower pH levels allowed more UVR to go through and therefore caused more intracellular damage and led to much less calcification, which implies a self-destructive feedback strategy. Addition of UV stress might have further stimulated such strategy for much less calcification. The calcification of *E. huxleyi* reduces its intracellular alkalinity and pH; therefore, it is crucial for the cells to maintain its intracellular pH stability by sacrificing calcification.

In the present study, the thickness of the coccolith layer decreased at decreasing pH, whereas the size of the naked

cell increased significantly at pH 7.6 as compared to pH 8.2 when the photosynthetic and growth rates were almost identical. Whether the decrease in the shell thickness promoted the increase in the cell size or the thickness of the shell alone exerts some constraint on the cell size needs to be tested. Nevertheless, reduced thickness of the coccoliths at the lowered pH levels did result in higher inhibition of both photosynthesis and calcification caused by UVR, reflecting that coccoliths act as a filter and protect the cells from being harmed by UVR. Quintero-Torres et al. (2006) assessed by modeling that coccolith structures can backscatter as much as 50% UVR. The coccoliths were found to shield off about 25% of UVR because of absorption and reflection (Gordon and Du 2001). In this study, the coccolith-covered cells received 20–25% less UVR and 10–22% less PAR compared with the naked cells.

E. huxleyi blooms have been observed when surface solar PAR varied between 115 and 200 W m^{-2} (about 530 and 921 μmol photons m^{-2} s^{-1}) (Nanninga and Tyrrell 1996; Giekes and Buma 1997). Such a PAR range corresponds to 17.4–30.3 W m^{-2} of UV-A and 0.55–0.96 W m^{-2} of UV-B in offshore waters. In the present study, the UV-A and UV-B levels (UV-A 19.5 W m^{-2}, UV-B 0.67 W m^{-2}) to which the cells were exposed reflect the levels of UVR in the natural habitats of *E. huxleyi*. Considering the slow mixing of the upper layer during daytime, *E. huxleyi* cells can experience higher UVR exposures than that set in the present study. Although the response of coccolithophores to ocean acidification appears to be species- or strain-specific (Langer et al. 2006; Iglesias-Rodriguez et al. 2008), the thinning of the coccolith layer increases their sensitivity to solar UVR. Because UVR reduced inorganic production to a larger extent than it did organic production, the C : P ratio is expected to decrease more if UVR is taken into consideration. Decreased C : P ratio may decrease cell density and weaken downward carbon fluxes (Delille et al. 2005). On the other hand, increased stratification of surface waters in the ocean because of global warming will expose phytoplankton cells to higher solar radiation (Nanninga and Tyrrell 1996; Boyd and Doney 2002) than at present because of reduced mixing depth (Young 1994). Therefore, solar UVR will lead to more reduction of the calcification and a much lower C : P ratio of coccolithophores with increasing levels of CO_2 and temperature in surface ocean waters. In future oceans, blooms of *E. huxleyi* may be less frequent and of smaller magnitude as a result of higher photoinhibition caused by ocean acidification and stratification.

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