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Increased CO_2 and the effect of pH on growth and calcification of *Pleurochrysis carterae* and *Emiliania huxleyi* (Haptophyta) in semicontinuous cultures

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Abstract The effects of changes in CO_2 and pH on biomass productivity and carbon uptake of *Pleurochrysis carterae* and *Emiliania huxleyi* in open raceway ponds and a plate photobioreactor were studied. The pH of *P. carterae* cultures increased during day and decreased at night, whereas the pH of *E. huxleyi* cultures showed no significant diurnal changes. *P. carterae* coccolith production occurs during the dark period, whereas in *E. huxleyi*, coccolith production is mainly during the day. Addition of CO_2 at constant pH (pH-stat) resulted in an increase in *P. carterae* biomass and coccolith productivity, while CO_2 addition lowered *E. huxleyi* biomass and coccolith production. Neither of these algae could grow at less than pH 7.5. Species-specific diurnal pH and pCO_2 variations could be indicative of significant differences in carbon uptake between these two species. While *E. huxleyi* has been suggested to be predominantly a bicarbonate user, our results indicate that *P. carterae* may be using CO_2 as the main C source for photosynthesis and calcification.

Keywords *Pleurochrysis carterae* · *Emiliania huxleyi* · Raceway pond · Plate photobioreactor · pH · CO_2

Introduction

The large-scale culture of microalgae as a CO_2 "sink" for bioremediation of increased atmospheric CO_2 levels has been proposed by several workers (Benemann 1997; Herzog and Drake 1996). Microalgae have a higher productivity than other photosynthetic organisms such as trees and also have the potential to be grown using saline water and on land which cannot be used for agriculture. The coccolithophorid algae (Haptophyta) have the further potential advantage in that they fix carbon not only into organic biomass high in lipids and hydrocarbons (Fernandez et al. 1994; Riebesell et al. 2000) but also produce $CaCO_3$ in the form of small plates called coccoliths (Paasche 2002). This would allow the fixed C to be buried ("fossilized") or, alternatively, the lipids and hydrocarbons can be used as a renewable fuel or as an energy source by direct co-firing (Wu et al. 1999).

Large-scale algae cultures are generally carbon limited, and the addition of CO_2 enhances growth and productivity (Borowitzka 1998). However, the addition of CO_2 also causes acidification of the medium. Recently, several studies have examined the effects of increased CO_2 on *Emiliania huxleyi* (Leonardos and Geider 2005; Nielsen 1995; Riebesell et al. 2000; Zondervan et al. 2002). Leonardos and Geider (2005) found that elevated CO_2 can result in increasing organic carbon fixation by *E. huxleyi* while grown at low N:P and in high light. Feng et al. (2008) also showed that doubling the pCO_2 can result in reduced particulate inorganic carbon (PIC) in *E. huxleyi* at 400 μmol photons m^{-2} s^{-1}. However, they did not detect any change in the particulate organic carbon (POC) between high and low pCO_2. Thus, increasing CO_2 was found to result in a decrease in calcification and an increase in organic carbon, at least in nutrient-limited *E. huxleyi*. While there have been extensive studies on the effect of pH and elevated pCO_2 on *E. huxleyi*, there is very limited data on the effect of elevated pCO_2 on the productivity and calcification of *Pleurochrysis carterae*.

We have demonstrated that the coccolithophore *P. carterae* can be reliably grown outdoors in open raceway ponds for

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extended periods of up to at least 1 year (Moheimani and Borowitzka 2006a). We have also studied the limits to growth and productivity of this alga when grown outdoors in raceway ponds (Moheimani and Borowitzka 2006b). As part of an examination of the factors limiting growth and coccolith formation in the outdoor cultures of *P. carterae*, we examined the effects of CO_2 addition and compared *P. carterae* with *E. huxleyi*.

Materials and methods

The coccolithophorid algae *P. carterae* Braarud et Fagerland CCMP 647 and *E. huxleyi* Lohmann CCMP 371 were obtained from the Centre for Culture of Marine Phytoplankton, Bigelow Laboratory, Boothbay Harbor, ME, USA. *P. carterae* and *E. huxleyi* cultures were maintained in modified f/2 and f/50 medium, respectively (Guillard and Ryther 1962). The media were modified by omitting Mo and Si from the original recipe and by adding 0.06 μM SeO_2.

The algae were grown either in a plate-type photobioreactor (Fig. 1) or an outdoor raceway pond (Moheimani and Borowitzka 2006a). The plate photobioreactor had a culture volume of 6 L (W×H×L (cm)=10×35×26). The base of the reactor was V-shaped with an air tube at the bottom of the V to promote the suspension or flotation of the relatively heavy coccolithophorid cells. The plate photobioreactor was chemically sterilized by using 12% sodium hypochlorite, for 2 h, rinsed 12 times in sterile deionized water, and dried in a 70 °C oven. The cultures were grown in semicontinuous mode with light provided by 12 cool white fluorescent tubes arranged at both sides of the reactor giving an average irradiance of 320 μmol photons m^{-2} s^{-1} (measured at 24 spots on the surface of reactor) with a 12:12-h light:dark cycle. The growth temperature was 23±1.5 °C.

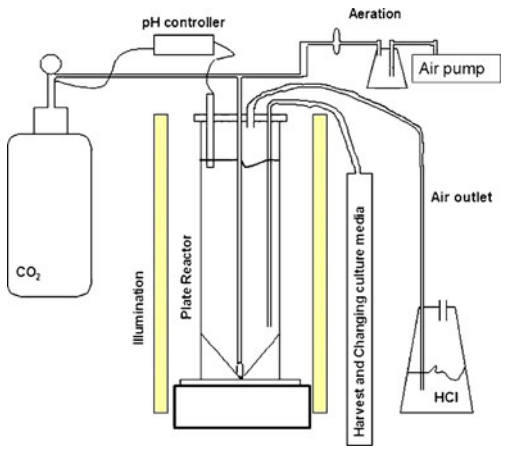

Fig. 1 Schematic diagram of plate photobioreactor

The outdoor cultures were carried out in September 2003 in two 1-m^2 surface area fiberglass paddle wheel raceway ponds operated at 16 cm depth. The four-paddle paddlewheel, operating at a rotation speed of about 28 rpm, generated a flow rate of 20 cm s^{-1}. The cultures were maintained in semicontinuous mode by daily harvesting of a part of the biomass and replacing the harvested medium with fresh medium. The ponds were located at Murdoch University, Perth, Western Australia (31°57 S; 115°52 E). The culture medium for the ponds was chemically sanitized (Moheimani and Borowitzka 2006a). CO_2 was added to the pond using a 0.06-m^2 floating CO_2 injector, based on the design of Becker (1994), and positioned 10 cm downstream from the paddlewheel. In both systems, pH was controlled (±0.4 pH units) by CO_2 addition using a pH controller and a solenoid switch connected to a CO_2 gas cylinder. The carbon chemistry of the cultures, grown in the plate photobioreactor, was calculated from temperature, salinity, phosphate, total alkalinity, and pH of the medium using CO_2sys software (Lewis and Wallace 1998). For total alkalinity, medium was filtered with syringe filter (0.45 μm) to remove cells and other particles. Total alkalinity was determined according to Strickland and Parsons (1972) . Media for the plate reactor were buffered to pH 7.50 (total alkalinity, pCO_2, and total carbon were 2,453.8 μmol kg^{-1}, 1,756.9 μatm, and 2,378.6 μmol kg^{-1}, respectively). Samples were taken daily for measuring growth rates, organic biomass, lipid content, and calcium carbonate production using the methods described in Moheimani and Borowitzka (2006a).

Results

Preliminary experiments in batch cultures showed that in 300 mL cultures of *P. carterae* CCMP 647 and another *Pleurochrysis* sp., the medium pH rose from pH 8.2 to pH 9.5 by the end of the exponential phase, and then declined to pH 8.2 after about 5 days in stationary phase. In contrast, pH in *E. huxleyi* culture did not change throughout the growth period (data not shown). The effects of CO_2 addition and pH were then examined in plate photobioreactor under controlled conditions of light and temperature and then also outdoors in open raceway ponds.

Plate photobioreactors

The pH of the cultures was initially unregulated, i.e., no CO_2 was added. In the *P. carterae* culture, this resulted in a pH increase from pH 8.3 to pH 9.5 during the light period and then a decrease to pH 8.3 by the end of the subsequent dark period (Fig. 2a). In the *E. huxleyi* culture, the pH remained between pH 8.1 and 8.4 in both the light and dark

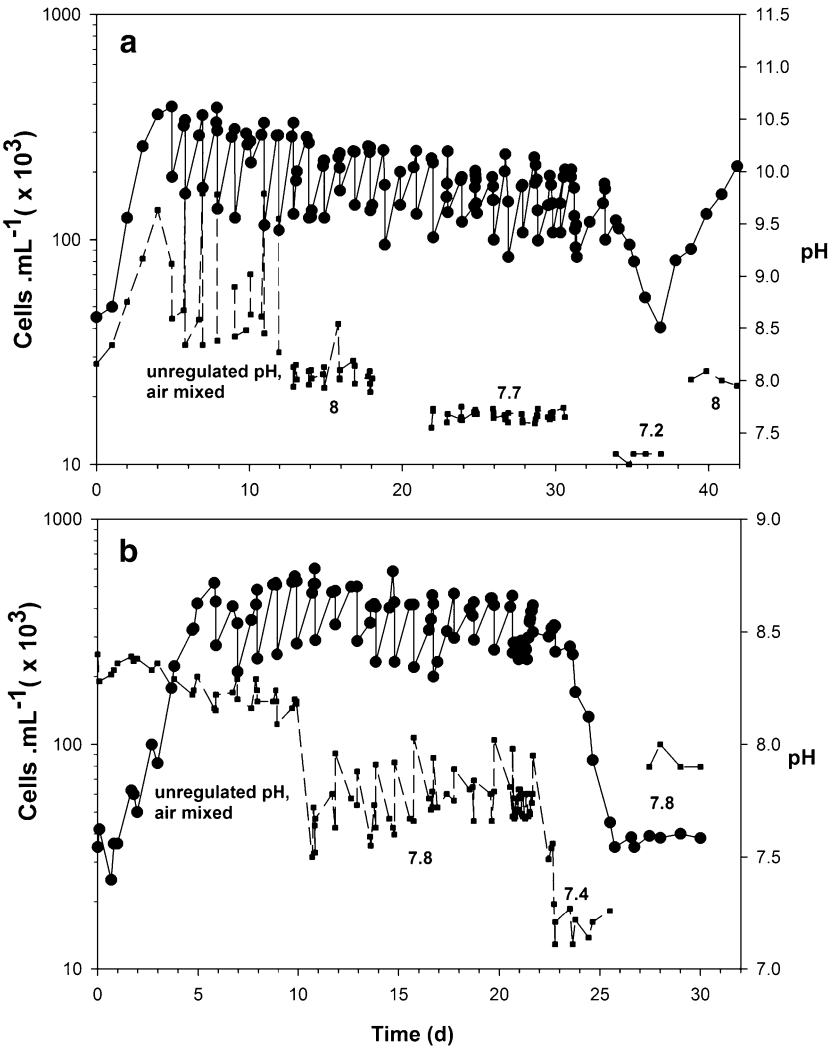

Fig. 2 Growth (*circles*) and medium pH (*squares*) of **a** *Pleurochrysis carterae* and **b** *Emiliania huxleyi* grown in a plate-type photobioreactor under different pH conditions controlled by a pH-stat system with CO_2 addition

periods (Fig. 2b). Culturing *P. carterae* under unregulated pH resulted in a reduction in pCO_2, whereas *E. huxleyi* grown under the same unregulated condition increased the pCO_2 (Table 1).

The pH of the culture medium was then regulated by addition of CO_2 using the pH stat system. Between pH 7.9–8.1 and pH 7.6–7.9 for *P. carterae* and between pH 7.7 and 7.9 for *E. huxleyi*, the cultures continued to grow well (Fig. 2). When the pH was reduced to pH 7.4 for *P. carterae* and to pH 7.2 for *E. huxleyi*, the algal cells started sticking to the photobioreactor walls and also began to clump, and semicontinuous culture could not be maintained. Increasing the pH of the culture medium to the previous higher value significantly reduced clumping in both species.

The growth rate and productivities of both species at the different pH values are shown in Table 1. *P. carterae* showed the highest specific growth rate of 0.76 day^{-1} and maximum dry weight productivity of 0.51 gL^{-1} day^{-1} at pH 8. The growth rate and dry weight productivities of *P. carterae* were greater in pH 8 than pH 7.7, and unregulated pH and the total lipid and $CaCO_3$ content also followed the same pattern. In *P. carterae*, pCO_2 was higher at pH 7.7 than pH 8 and unregulated pH (Table 1). When grown at controlled pH, total alkalinity, pCO_2, and total carbon declined in both strains in the afternoon (Table 1). In *E. huxleyi*, growth rate and all productivities were highest in the unregulated pH treatment, even though less pCO_2 and total carbon was available to the cells when grown at pH 7.8 (Table 1).

Cell lipid per total dry weight remained constant between 21% and 24% of dry weight in *P. carterae*, whereas in *E. huxleyi*, the lipid content increase from 19% to 26% of total dry weight between the unregulated pH (pH 8.1–8.3) and pH 7.8. The highest amount of $CaCO_3$ per total dry weight (11%) was at pH 8 in *P. carterae*, whereas in *E. huxleyi*, $CaCO_3$ per total dry weight remained at 12% in both the unregulated pH and pH 7.8 cultures.

Table 1 Carbon chemistry, mean growth rates, and productivities of *P. carterae* and *E. huxleyi* grown in a plate photobioreactor at different pH

	pH	Total alkalinity (μmol kg^{-1})	pCO_2 (μatm)	Total inorganic carbon (μmol kg^{-1})	*n*	Specific growth rate (day^{-1})	Productivity (mg L^{-1} day^{-1})		
							Dry weight	Lipid	$CaCO_3$
P. carterae	Unregulated								
	Morning (8.3)	3,176.6	267.3	2,589.1	10	0.62±0.00	290±17	71±14	24.1±13.11
	Afternoon (9.5)	2,905.0	2.6	1,344.2					
	pH 8								
	Morning	2,703.4	603.2	2,703.4	8	0.76±0.00	510±13	121±20	61.0±17.27
	Afternoon	2,064.6	460.6	2,064.6					
	pH 7.7								
	Morning	3,090.4	1,350.2	2,921.2	10	0.50±0.01	410±9	88±19	40.9±12.08
	Afternoon	1,940.3	834.6	1,805.7					
E. huxleyi	Unregulated								
	Morning (8.4)	2,509.3	152.1	1,934.3	7	0.98±0.01	310±23	61±13	40.0±1.90
	Afternoon (8.0)	1,522.3	290.1	1,300.4					
	pH 7.8								
	Morning	2,485.2	834.6	2,991.6	10	0.76±0.00	230±16	59±13	28.7±0.81
	Afternoon	2,289.5	765.9	2,104.4					

Raceway pond

Two raceway ponds of *P. carterae* operated in parallel were set up and starting on 28 September 2003. One pond had no pH control, and in the other, the pH was controlled by CO_2 addition using a pH-stat. The light profile and medium temperature of cultures grown in raceway ponds have been reported previously by Moheimani and Borowitzka (2006a). The changes in cell density and pH variations in the raceway ponds are shown in Fig. 3, and the effects of

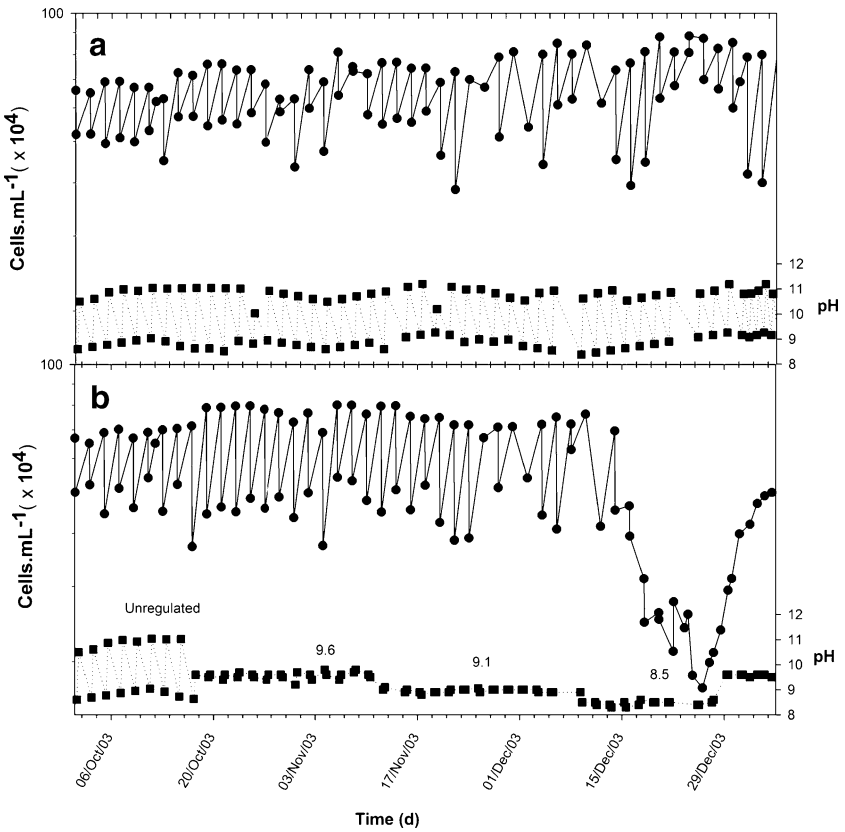

Fig. 3 *Pleurochrysis carterae* growth (*circles*) and daily medium pH range (*squares*) in outdoor raceway ponds under **a** unregulated pH (control) condition and **b** under different pH conditions controlled by a pH-stat using CO_2 addition

different pH on growth rates and productivities are summarized in Table 2. The pH in both ponds was unregulated between 1 and 15 October 2003 (see Fig. 3). During this period, the pH increased during the day from pH 8.3 to pH 10.9 and decreased to the initial pH of 8.3 during the night. The pH decreased 2 pH units after each dilution and then reached the maximum daily pH less than 2 h thereafter. There was no difference in maximum cell concentration, growth rate, and productivity between the control and experimental raceway ponds during the unregulated pH period (see Table 2).

Between 16 October and 9 November 2003, the pH was set to pH 9.6 in the experimental pond, while the pH in the control pond remained unregulated (Fig. 3). This resulted in a significantly higher growth rate, total dry weight productivity, lipid productivity, and $CaCO_3$ productivity in the pH-regulated pond compared to the control pond (one-way ANOVA, $P<0.05$; Table 2). There was no difference between the maximum cell density between the two ponds at this pH (one-way ANOVA, $P>0.05$).

Between 10 November and 7 December 2003, the pH in the experimental pond was decreased to pH 9.0 (Fig. 3b). While no difference was observed in growth rate and maximum cell concentration between the two ponds (one-way ANOVA, $P>0.05$), the pH-regulated pond achieved significantly higher dry weight, lipid, and $CaCO_3$ productivities (one-way ANOVA, $P<0.05$; Table 2).

Between 8 and 26 December 2003, the pH was further decreased to pH 8.5 in the experimental pond (Fig. 3). Growing *P. carterae* at this pH resulted in reduction of cell number from 7×10^5 to 3×10^5 cells mL^{-1} in less than 12 days (Fig. 3b). Due to this reduction, dilution of the experimental pond was not possible (Fig. 3b). However, the control pond (unregulated pH) could be diluted five times during the same period of time (Fig. 3a). Shifting the pH from 8.5 back to pH 9.6 in the experimental pond resulted in a recovery of growth of *P. carterae* (Fig. 3b).

The pH changes by CO_2 addition did not affect the cell lipid and $CaCO_3$ content. Total lipid was between 32% and 34% of total dry weight, and $CaCO_3$ was between 9.9% and 10.2% of total dry weight in both ponds.

Diurnal cycle (biomass and pH)

Over a diurnal cycle, the pH of the culture medium remained constant in the range of pH 8.3 to 8.5 in the *E. huxleyi* culture (Fig. 4c). On the other hand, in the *P. carterae* culture, the pH of the culture medium increased during the light period from pH 7.8 to 10.1 in the plate photobioreactor and from pH 8.2 to 11 in the raceway pond (Fig. 4a, b). During the dark period, the pH decreased to pH 8.0±0.2 by the end of the night (Fig. 4a, b). In *E. huxleyi*, the coccolith concentration increased from the start of light period, and there appeared to be little decalcification during the night (Fig. 4c). In *P. carterae* cultures, the coccolith concentration increased from 2×10^5 to 9×10^5 coccoliths mL^{-1} during the light period (Fig. 4a, b). During the first 2 h of the dark period, coccolith number declined to 3×10^5 coccoliths mL^{-1} (Fig. 4a, b). This loss in the number of coccoliths was most likely due to decalcification in the first 5 h of the dark period together with a decrease in the pH of the culture medium (Fig. 4a, b). Coccolith numbers then started to increase so that by sunrise, the coccolith number was >50% of that reached during the day. In contrast, there was a very much smaller decline in coccolith numbers in the dark period in *E. huxleyi*, and coccolith numbers only increased after the onset of light (Fig. 4c). The cell dry weight of both *P.*

Table 2 Mean growth rates and productivities of control and experimental raceway ponds at different pH

Cultivation period		*n*	pH range	Specific growth rate (day^{-1})	Maximum cell concentration ($cells\times10^5\ mL^{-1}$)	Productivity ($mg\ L^{-1}\ day^{-1}$)		
						Dry weigh	Lipid	$CaCO_3$
Control pond	1 Oct 2003–15 Oct 2003	10	8.3–10.9	0.532	6.2	170	56.1	16.8
	16 Oct 2003–9/1 Nov 2003	13	8.3–10.9	0.545	7.9	180	59.4	17.5
	10 Nov 2003–7 Dec 2003	12	8.3–10.9	0.591	8.02	180	63.0	17.7
	8/1 Dec 2003–26 Dec 2003	10	8.3–10.9	0.518	8.1	190	64.6	18.9
	27 Dec 2003–5 Jan 2004	5	8.3–10.9	0.528	7.9	170	56.1	16.3
Experimental pond	1 Oct 2003–15 Oct 2003	10	8.3–10.9	0.539	6.3	170	56.1	16.8
	16 Oct 2003–9/1 Nov 2003	13	9.6±0.2	0.592	7.8	230	78.2	22.7
	10 Nov 2003–7 Dec 2003	12	9.1±0.2	0.593	7.1	210	69.3	21.0
	8/1 Dec 2003–26 Dec 2003	10	8.5±0.2					
	27 Dec 2003–5 Jan 2004	5	9.6±0.2	0.391				

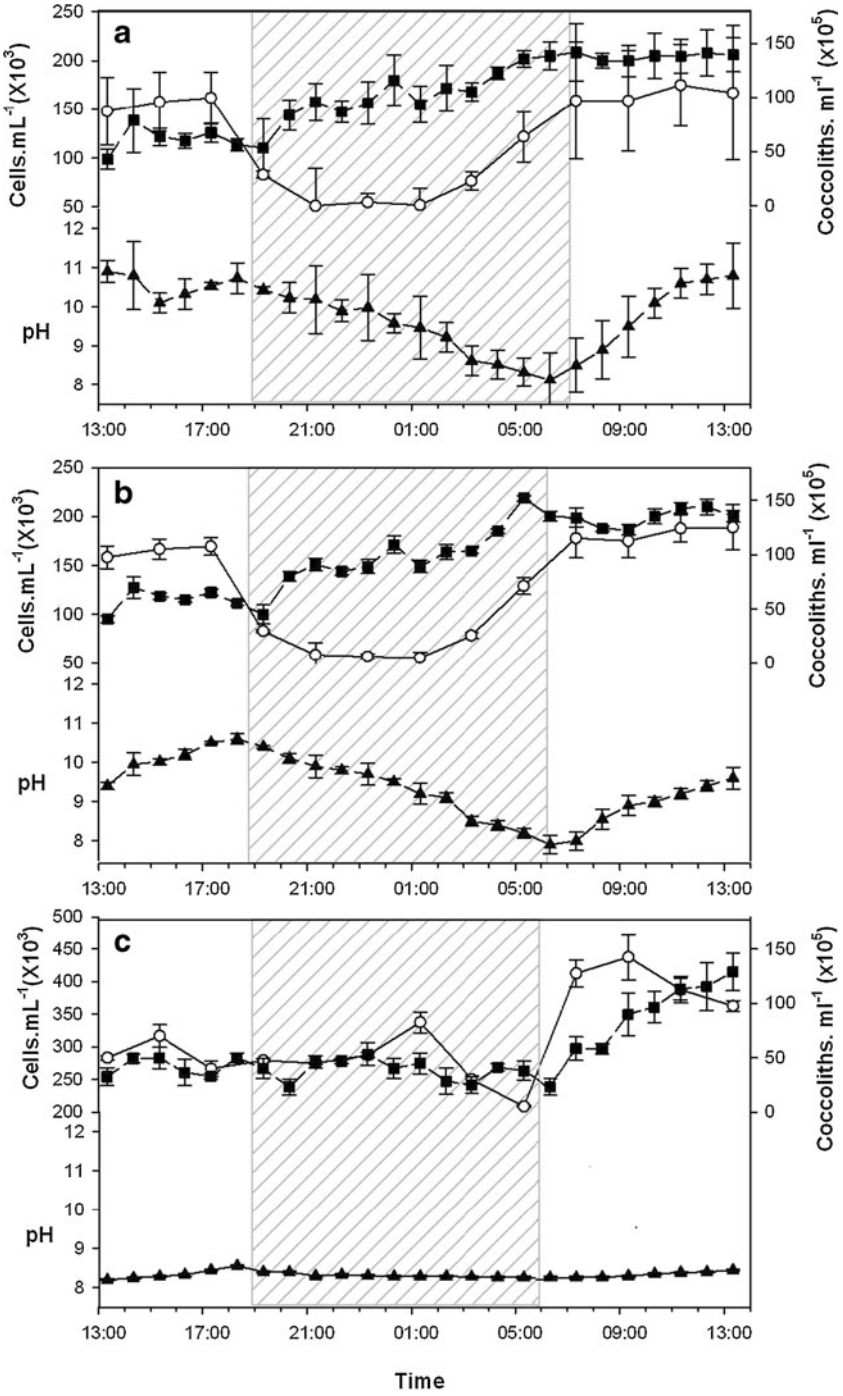

Fig. 4 Changes in cell concentration (*squares*), coccolith concentration (*circles*), and pH of the culture medium (*triangles*) over 24 h for **a** *Pleurochrysis carterae* grown in a raceway pond (mean ±SE, n=5), **b** *P. carterae* grown in a plate photobioreactor (mean ±SE, n=5), and **c** *E. huxleyi* grown in a plate photobioreactor (mean±range, n=3)

carterae and *E. huxleyi* decreased by 55% to 75% during the dark period followed by an increase during the light period (data not shown).

Pearson product–moment correlation indicated a significant association between the pH of the culture medium and the coccolith concentration in the culture of *P. carterae* (r=−0.69, df=24, P<0.05), whereas there was no correlation between pH and coccolith concentration in the cultures of *E. huxleyi* (r=−0.31, df=24, P> 0.05).

Discussion

Regulating the pH at pH 8 by the addition of CO_2 increased both the growth rate and organic and $CaCO_3$ productivity in *P. carterae*. Lowering the pH further to pH 7.7 reduced the growth rate, but the organic and $CaCO_3$ productivity remained higher than when the cells were grown under unregulated pH (pH 8.3–9.5) conditions. The *E. huxleyi* cultures, however, had the highest growth rate and organic

and $CaCO_3$ productivities under unregulated pH (pH 8.1–8.3) conditions. These results suggest that the high pH values reached in the unregulated cultures of *P. carterae* lead to carbon limitation, as at pH 10, there is no free CO_2, some HCO_3^-, and the bulk of the C_i is in the form of CO_3^{2-}. The observed pH changes during a diurnal cycle in actively growing cultures of *P carterae* and *E. huxleyi* indicate significant differences in carbon uptake and metabolism between these species. During the light period, *P. carterae* significantly increased the pH of the medium (up to pH 11 in the outdoor cultures), whereas in *E. huxleyi*, the pH did not change. Israel and Gonzalez (1996) and Crenshaw (1964) observed the same differences in the pattern in pH during growth of *P. carterae* and *E. huxleyi*.

Growth of both strains, when grown under controlled pH, was accompanied by a concomitant decrease in total carbon and pCO_2. While total carbon utilization was the same between *P. carterae* and *E. huxleyi* when grown under uncontrolled pH, there was a completely different pattern in pCO_2 and pH for these two strains. *E. huxleyi* decreased the pH and increased the pCO_2 between morning and afternoon, while the opposite was observed in *P. carterae*. The inorganic carbon system is the main buffering system in the ocean. Alkalinization of the medium is observed in many photosynthesizing algae and aquatic plants as a result of either CO_2 uptake (with or without an external carbonic anhydrase) and/or HCO_3^- uptake with concurrent OH^- efflux (Borowitzka 1982; Brewer and Goldman 1976). The precipitation of $CaCO_3$, on the other hand, can lead to acidification (Gattuso et al. 1995), and this was observed in our *E. huxleyi* culture. The interaction between photosynthesis and calcification, and the concomitant C fluxes has been extensively studied in *E. huxleyi* and, to a much lesser extent, in *P. carterae* and not at all in other coccolithophorid algae (Berry et al. 2002; Borowitzka 1989; Brownlee and Taylor 2004; Paasche 2002). There is substantial evidence that the bulk of the carbon for photosynthesis in *E. huxleyi* comes from bicarbonate (Buitenhuis et al. 1999; Sikes and Wheeler 1982), and it has been suggested that the H^+ produced during $CaCO_3$ formation is used to offset any cytoplasmic alkalinization resulting from HCO_3^- utilization for photosynthesis and the action of carbonic anhydrase in the chloroplast (Berry et al. 2002; Quiroga and Gonzalez 1993).

It has been shown that *E. huxleyi* has a membrane anion exchange protein which is involved in active HCO_3^- transport into the cells (Herfort et al. 2002). At low external C_i concentrations and in stationary phase *E. huxleyi* cells, extracellular carbonic anhydrase activity has also been detected (Herfort et al. 2002; Nimer et al. 1994, 1996, 1997). In contrast, Israel and Gonzalez (1996) have demonstrated external carbonic anhydrase activity at both high and low C_i concentrations in a *Pleurochrysis* sp. *E. huxleyi* is also a bicarbonate user which may explain there was no CA activity detection at high C_i concentration by Herfort et al. (2002). On the other hand, *P. carterae*, as inferred from the observed pH shifts in the current study, could be predominantly a CO_2 user and thus requires an active external carbonic anhydrase. However, this hypothesis remains to be tested. The apparent differences in C uptake between *E. huxleyi* and *P. carterae* shown here may also help to explain the differences in the ^{18}O stable isotopic composition of the coccoliths of these algae observed by Dudley et al. (1986). If the C for coccolith formation were not only provided by HCO_3^- taken up from the seawater but were also provided by HCO_3^- derived from CO_2 in the cytoplasm, then this could account for the observed depletion in ^{18}O of the coccolith $CaCO_3$. Carbonic anhydrase, respiration, and other metabolic processes are known to discriminate against ^{18}O (Guy et al. 1989, 1993; Miller et al. 1997).

In *E. huxleyi*, various studies have found that bicarbonate is used for calcification (Paasche 1964; Sikes et al. 1980) and that CO_2 from intracellularly converted bicarbonate is the major "C" source for photosynthesis (Dong et al. 1993; Nimer and Merrett 1992; Sikes et al. 1980). The net change in the inorganic carbon in the medium is the product of inorganic "C" uptake by coccolithophorids subtracted from the respiratory CO_2 excreted by cell. Nimer and Merrett (1993) showed that in *E. huxleyi*, when bicarbonate is the main "C" source in media, the stoichemistry between photosynthesis and calcification is 1:1 (measured using $^{14}CO_2$). This means that the same amount of "C" is used for calcification and photosynthesis. The

Table 3 Summary of P:C ratios for different strains of *P. carterae* and *E. huxleyi*

Species	Strain	P:C ratio	Reference	Comments
P. carterae	CCAP961/2	22.25±9.94	Seki et al. 1995	P:C measured under several nitrate concentrations (n=12)
	CCMP645	24.66±6.24	Fabry 2007	P:C measured under several light conditions (n=9)
E. huxleyi	SMBA279	1.5	Nimer and Merret 1992	
	88E	1.25±0.21	Nimer et al. 1996	P:C measured under several nutrient conditions (n=12)
	PCC.B11	0.66±0.16	Herfort et al. 2002	P:C measured under several nutrient conditions (n=10)

photosynthesis to calcification ratios (P:C) of *P. carterae* and *E. huxleyi* are summarized in Table 3. For *E. huxleyi*, the P:C ratio is between 0.52 to 1.53, while this ratio is at least tenfold higher in *P. carterae.* This is compatible with the observation of an absence of pH changes in the medium of actively growing *E. huxleyi* observed by us in this study. However, the large alkalinization of the medium in actively photosynthesizing *P. carterae* cultures means that CO_2 uptake must significantly exceed HCO_3^- uptake and implies that this CO_2 is the main C source for photosynthesis, and possibly also for calcification, in this species. Comparative studies of carbon uptake and use in photosynthesis and calcification in *Pleurochrysis* and other coccolithophorid algae and compared with the extensively studied *Emiliania* are clearly required.

Neither *P. carterae* nor *E. huxleyi* could grow at a pH of less than about pH 7.5, with *E. huxleyi* appearing to be slightly more sensitive to low pH. This inhibition could be due to the inability of these algae to generate sufficient OH^- to neutralize the H^+ produced by calcification (Nimer and Merrett 1993; Sciandra et al. 2003) and thus prevent acidification of the cytoplasm or due to a direct effect of a more acidic cytoplasm.

Apparent decalcification during the night as observed in this study in *P. carterae* has also been reported for *E. huxleyi* (Balch et al. 1996; Linschooten et al. 1991; Paasche 1964; Sekino and Shiraiwa 1994). This decalcification is probably due to localized acidification caused by respiratory CO_2 production, resulting in a partial dissolution of the coccoliths.

This study was part of a larger study examining the suitability of large-scale cultures of coccolithophorid algae for CO_2 bioremediation (Moheimani and Borowitzka 2006a, b). This study showed that *P. carterae* CCMP647 could be grown in outdoor raceway cultures for periods of up to 10 months in semicontinuous culture, whereas *E. huxleyi* cultures could not be maintained in this system (Moheimani and Borowitzka 2006a). The results presented here show that CO_2 addition used to maintain the culture pH between pH 8.1 and 9.3 increases the specific growth rate and productivity of *P. carterae. P. carterae* has shown to be a reliable microalga when grown in a semicontinuous mode and at a constant pH in both plate photobioreactor and raceway ponds. The ability to grow the alga in semicontinuous culture is very important as this reduces the overall cost of producing the algae (Borowitzka 1999).

This study has also provided some evidence that *P. carterae* seems to markedly differ from *E. huxleyi* in its carbon uptake system and carbon concentrating mechanism. An interesting question raised here is whether this difference is reflected in the evolution of the Haptophyta (*Pleurochrysis* is classified in the Coccolithales whereas *Emiliania* is in the Isochrydales (Edvardsen et al. 2000)) or in structural differences in coccolithogenesis (Hawkins and Lee 2001; Paasche 2002). The implication of this to our understanding of coccolithophorid calcification and photosynthesis and to the potential effects of ocean acidification due to increases in atmospheric CO_2 requires further study.

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