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Effects of carbon dioxide on the coccolithophorid *Pleurochrysis carterae* in incubation experiments

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ABSTRACT: Production (abundance and biomass) and net calcification rates of the coccolithophorid *Pleurochrysis carterae* under different partial pressures of CO_2 (pCO_2) were examined using short (15, 24 and 39 h), long (7 d) and dark (7 d) incubation experiments. Short incubations were conducted at ambient, 500 and 820 ppm pCO_2 levels in natural seawater that was enriched with nutrients and inoculated with *P. carterae*. Long incubations were conducted at ambient and 1200 ppm pCO_2 levels in natural seawater (0.2 µm filtered as well as unfiltered) that was enriched with nutrients and inoculated with *P. carterae*. Dark incubations were conducted at ambient and 1200 ppm pCO_2 in unfiltered seawater that was inoculated with *P. carterae*. The abundance and biomass of coccolithophorids increased with pCO_2 and time. The abundance and biomass of most noncalcifying phytoplankton also increased, and were hardly affected by CO_2 inputs. Net calcification rates were negative in short incubations during the pre-bloom phase regardless of pCO_2 levels, indicating dissolution of calcium carbonate. Further, the negative values of net calcification in short incubations became less negative with time. Net calcification rates were positive in long incubations during blooms regardless of pCO_2 level, and the rate of calcification increased with pCO_2. Our results show that *P. carterae* may adapt to increased (~1200 ppm) pCO_2 level with time, and such increase has little effect on the ecology of noncalcifying groups and hence in ecosystem dynamics. In dark incubations, net calcification rates were negative, with the magnitude being dependent on pCO_2 levels.

KEY WORDS: pCO_2 · *Pleurochrysis carterae* · Noncalcifying phytoplankton · Biomass · Net calcification rates · Adaptation

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INTRODUCTION

Increased human activities (e.g. burning of fossil fuels) have resulted in elevated levels of carbon dioxide (CO_2) in the atmosphere. The global atmospheric concentration of CO_2 increased from a pre-industrial value of ~280 to 379 ppm in 2005 (IPCC 2007). Modeling experiments show that atmospheric CO_2 levels continue to increase and may well reach levels exceeding 1000 ppm by 2100 unless emissions are substantially reduced (The Royal Society 2005). The ocean plays an important role in the global carbon cycle, absorbing ~¼ of the CO_2 emission by anthropogenic activities. However, the hydrolysis of CO_2 is leading to a decrease in ocean pH. Over the past 200 yr since pre-industrial times, ocean pH has already dropped by 0.1 unit to its current value of 8.2. The average pH of the ocean will fall by 0.3 to 0.5 units by the year 2100 if global emissions of CO_2 from human activities continue to rise based on current trends (Caldeira & Wickett 2005, Orr et al. 2005). The acidification of the oceans as a result of increasing CO_2 affects the calcification of corals, mollusks, foraminiferans and coccolithophorids (photosynthetic plankton) that have shells or plates of calcium

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carbonate ($CaCO_3$) (Gattuso et al. 1999, Riebesell et al. 2000, Zondervan et al. 2001, Rost & Riebesell 2004, Kurihara et al. 2007) and leads to dissolution of $CaCO_3$ sediments in reef flats (Yates & Halley 2006). $CaCO_3$ produced by living organisms exists in 2 commonly occurring forms: aragonite (e.g. in corals and pteropods) and calcite (in coccolithophorids and foraminiferans). Although the aragonite form of $CaCO_3$ is more soluble than calcite, organisms having either of these forms of $CaCO_3$ are vulnerable to increasing CO_2 concentrations (The Royal Society 2005). A decrease in marine biogenic calcification causes a negative feedback on rising atmospheric CO_2 (Zondervan et al. 2001). So far, many studies have shown that calcification in coccolithophorid species, such as *Emiliania huxleyi* and *Gephyrocapsa oceanica*, is reduced under high CO_2 partial pressure (pCO_2). However, Iglesias-Rodriguez et al. (2008) recently showed that calcification and net primary production in *E. huxleyi* was significantly increased by high pCO_2. Langer et al. (2006) showed that 2 different species of coccolithophorids responded differently, i.e. showed non-uniform physiological responses to increasing pCO_2. Such difference in the responses of coccolithophorid species to pCO_2 calls for further studies on other species of coccolithophorids to understand whole ecosystem responses to increasing ocean acidification. In the present study, we selected the coastal coccolithophorid species *Pleurochrysis carterae,* which has been used as a model for cellular calcification (Marsh 1999). We analyzed the effects of increased CO_2 concentrations (acidification) on the production (abundance and biomass) and net calcification rates of this species using short and long incubations. In these incubations, we also examined the production of noncalcifying phytoplankton and heterotrophs (bacteria, microzooplankton, nano- and picoflagellates). Additionally, we analyzed the effect of increased CO_2 concentrations on net calcification rates using *P. carterae* in dark incubations. The aim of our study was to assess the time course of changes in the production of noncalcifying phytoplankton and in the production and calcification of *P. carterae* in response to increased concentrations of CO_2. We also wanted to know how *P. carterae* responds to increased CO_2 concentrations in the absence of other phytoplankton species that may compete with it for nutrients, and what changes in net calcification rates will occur when photosynthesis is inhibited.

MATERIALS AND METHODS

Seawater was collected near Bora Bay in Miyako Island, Japan (24° 44′ N, 125° 27′ E) using 10 l Niskin bottles and dispensed into 2.6 l Nalgene bottles for incubation. All the bottles were washed and rinsed with 10% HCl and Milli-Q water before use. All incubations were performed in batches. Short incubations were conducted for 15, 24 and 39 h. For these incubations, natural seawater was enriched with nutrients, inoculated with the coccolithophorid *Pleurochrysis carterae*, and incubated at 3 different CO_2 concentrations (ambient, 500 and 820 ppm pCO_2) (Table 1). Additionally, a ^{13}C tracer solution (with 10 mg $NaH^{13}CO_3$ ml^{-1}) was added to all incubation bottles of short experiments at the rate of 1 ml l^{-1} for measuring primary production rates. The ^{13}C tracer solution was previously purified using Chelex-100 to remove trace metal contaminants.

Long incubations were conducted for 7 d. In long incubations, seawater (0.2 µm filtered as well as unfiltered) was enriched with nutrients, inoculated with *Pleurochrysis carterae* and incubated at ambient and 1200 ppm pCO_2 levels (Table 1). Filtered seawater was obtained by sequential filtration of natural seawater with Isopore membrane filter (Millipore, 2.0 and 0.2 µm). Dark incubations were conducted for 7 d. In the dark incubations, unfiltered seawater was inoculated with *P. carterae* and incubated at 2 different CO_2 concentrations (ambient and 1200 ppm pCO_2) (Table 1). For the short, long and dark incubations, *P. carterae* were inoculated by adding 1 ml of pure (axenic) culture (obtained from the laboratory of Microalgae Corporation, Miyako Island, Japan) containing ~6×10^5 cells ml^{-1} to each incubation bottle. Seawater was enriched with 4 µM of potassium nitrate (KNO_3) and 0.4 µM of potassium hydrogen phosphate (KH_2PO_4) solutions (N:P = 10:1). Before addition, each nutrient solution was treated with Chelex-100 to remove trace metals. The different levels of pCO_2 in the incubation bottles were adjusted by injecting CO_2 saturated (100%) seawater into the bottles at different volumes until pH values equivalent to the desired pCO_2 levels were obtained (Table 2). CO_2 saturated seawater was prepared by bubbling pure CO_2 gas into natural seawater for 1 h. The pH was measured using a pH meter (ORION 290 A plus ion meter and 9172BNWP electrode) calibrated with NIST (NBS)-scaled buffer solutions (Mettler pH 6.865 and 4.008 buffers) at 25°C in a temperature controlled water bath (Yamato Coolnics circulator CTA400). The slope of the electromotive force (emf) to pH was 99.7 to 100.0% of the theoretical Nernst value at every instance of calibration. Precision of pH measurement was ±0.005 pH unit (1σ, n = 10). The carbonate system in seawater was calculated using the apparent equilibrium constants K'_0 from Weiss (1974), K'_1 and K'_2 from Mehrbach et al. (1973) as described by Millero (1979). The employed constants are among the suitable combinations for the calculation of carbonate systems with the NBS pH scale. Recently, the

Table 1. Experimental design for (short, long and dark) incubations. NCN: incubation of unfiltered seawater with the addition of *Pleurochrysis carterae* and nutrients; FCN: incubation of filtered (0.2 µm) seawater with the addition of *P. carterae* and nutrients; NCD: dark incubation of unfiltered seawater with the addition of *P. carterae*

		pCO_2			
Incubation	Duration (h)	Ambient	500 ppm	820 ppm	1200 ppm
Short	15 h	NCN	NCN	NCN	
	24 h	NCN	NCN	NCN	
	39 h	NCN	NCN	NCN	
Long	7 d	NCN, FCN			NCN, FCN
Dark	7 d	NCD			NCD

Tris buffer pH scale with an acid dissociation constant for total hydrogen ion concentration has been recommended to precisely calculate the whole carbonate system in seawater (Dickson et al. 2007). However, it has been demonstrated that the pCO_2 value from the NBS scale is nearly the same as that from the Tris scale when an appropriate combination of equilibrium constants is used (Millero 1979). Both short and long incubations were performed at a light:dark cycle of 15:9 h, in a lighted and temperature controlled room at the laboratory of Microalgae Corporation. Temperature and light were monitored over the duration of the experiments using *in situ* sensors (MDS-MkV/T and MDS-MkV/L, Alec Electronics). All incubations were conducted at 21 to 24°C. The short and long incubations were conducted at the maximum light intensity of 400 µE m^{-2} s^{-1}. After the completion of incubations, sampling for several parameters was carried out for each incubation bottle. Duplicate samples for pH and total alkalinity (TA) were taken from each incubation bottle and placed in 125 ml Nalgene bottles. pH was measured immediately. For TA, seawater was filtered with a syringe filter (0.45 µm) to remove coccolithophorids and other particles, and kept cool until analysis. TA was measured by potentiometric titration (Radiometer TIM850 and GK2401C pH electrode) with computation using the Gran plot method (Stumm & Morgan 1981). Reproducibility of the TA measurement was ±2 µmol kg^{-1} (1σ, n = 10). A working seawater standard was used for the calibration of the TA measurement. This seawater was taken from the East China Sea (26° 40.660′ N, 127° 04.200′ E) where there was no effect of coastal contamination, and analyzed precisely for TA with a certified reference material for oceanic CO_2 measurement (CRM Batch # 50) distributed by A. Dickson of the Marine Physical Laboratory, University of California, San Diego. The accuracy of the TA measurement was confirmed to be within 2 µmol kg^{-1} of the true value obtained at the technical inter-comparison workshop of the working group (WG13) of the North Pacific Marine Science Organization (PICES) held in 2000 (Feely et al. 2003, Pavlova et al. 2008). Measured TA from initial samples of short incubations was also corrected for addition of ^{13}C (as $NaH^{13}CO_3$) as:

$$TA_{\text{corrected for primary production}} = TA_{\text{measured}} + \text{amount of } NaH^{13}CO_3 \text{ added in } \mu\text{mol kg}^{-1}$$

Measured TA from the incubated samples from both short and long incubations was corrected for nutrient depletion (NO_3 and PO_4) by phytoplankton as:

$$TA_{\text{corrected}} = TA_{\text{measured}} - \Delta NO_3 - \Delta PO_4$$

where ΔNO_3 and ΔPO_4 represent decreases in NO_3 and PO_4. The effect of the ^{13}C addition on pH was a decrease of ~0.010 pH unit. However, this was not significant compared to the SDs of pH (see Table 3). pCO_2 was calculated using pH and TA according to the carbonate equilibrium in seawater described earlier (Millero 1979, Fujimura et al. 2001). Saturation degree for the calcite form of $CaCO_3$ (Ω_{cal}) was calculated from the calcium (Ca^+) and carbonate (CO_3^{2-}) concentrations as: $\Omega_{cal} = [Ca^{2+}]\,[CO_3^{2-}] / K'_{sp}$, where K'_{sp} is the stoichiometric solubility product of calcite derived from a function of salinity and temperature (Mucci 1983).

Net calcification rates (i.e. increases or decreases in calcite production rates) were calculated using the alkalinity anomaly method (Smith 1973, Gattuso et al. 1997, Fujimura et al. 2001) as: $CaCO_3$ (µmol l^{-1} h^{-1}) = $\frac{1}{2}\,\Delta TA/\Delta t$, where ΔTA represents the difference in TA ($TA_{initial} - TA_{final}$) at the beginning and end of each incubation period, and Δt represents time in hour. The alkalinity anomaly method actually yields an estimate of the net value of the $CaCO_3$ precipitation/dissolution balance (Smith 1978, Bensoussan & Gattuso 2007). Positive values indicate $CaCO_3$ precipitation (i.e. calcification) while negative values indicate net $CaCO_3$ dissolution (Leclercq et al. 2002, Schneider & Erez 2006).

Table 2. Equivalent values of pCO_2 (ppm) at different pH levels

pH	pCO_2 (ppm)
8.200	390
8.110	500
8.100	520
8.000	680
7.930	820
7.900	890
7.800	1150
7.780	1200
7.700	1480

For primary production measurement in short incubations, 250 ml of water (in duplicate) from each incubation bottle was GF/F filtered (the GF/F was previously burnt at 550°C for 4 h). The filters were kept frozen at –30°C until analysis. Particulate organic carbon (POC) and ^{13}C isotopes in POC filters (samples) for short-term experiments were measured using a mass spectrometer (DELTA plus Advantage, Thermofinigan) equipped with elemental analyzer EA1110. Primary production rate was determined using the ^{13}C tracer technique according to Hama et al. (1993). For long incubations, POC and particulate carbon (PC) were collected separately on GF/F filters by filtering 200 ml samples (in triplicate) from each incubation bottle. Filters were kept frozen at –30°C until analysis. Particulate matter for long experiments was measured using a N/C analyzer (Sumi-Graph NC-90A). The analytical precision (SD) of POC and ^{13}C measurements was <±3%. Prior to analysis, POC filters were dried, acidified using HCl fumes and dried again to remove inorganic carbon from the filters, while PC filters were only dried (i.e. not treated with HCl fumes) to retain organic as well as inorganic carbon on the filter. Particulate inorganic carbon (PIC) was determined by subtracting the values of POC from the PC. Samples for scanning electron microscopy (SEM) of coccolithophorids were collected by filtering 100 to 200 ml of water from each incubation bottle on a 0.8 µm membrane filter (Millipore). SEM photographs of coccoliths were taken using a scanning electron microscope (Quanta Series, FEI). More than 20 photographs of coccoliths were taken from different areas of each filter (sample). For studies of pico and nanoplankton (including bacteria, cyanobacteria, pico- and nanoflagellates), duplicate samples from each incubation bottle were placed in sterilized 50 ml tubes, fixed using glutaraldehyde (2% final concentration) and kept cool. Heterotrophic bacteria and picoplankton were collected on 0.2 µm black polycarbonate filters by filtering 10 to 15 ml aliquots that were previously stained with DAPI. Counting was done under an epifluorescence microscope (Nikon-Eclipse), using a UV-filter for bacteria and a B-filter (blue light) for picophytoplankton. Coccolithophorids and other hetero- and autotrophic nanoflagellates (HNF and ANF) were collected on 0.8 µm black polycarbonate filters by filtering 30 ml aliquots that were previously stained with DAPI, and counting under an epifluorescence microscope using a B-filter. For microplankton (phytoplankton and zooplankton), the remaining water of each incubation bottle was transferred into a clean plastic bottle, and fixed using buffered formalin (4% final concentration). Microphytoplankton and microzooplankton were counted and classified using an inverted microscope (Nikon, Bk-201) according to Chihara & Murano (1997). Carbon biomasses of bacteria and picocyanobacteria were calculated using biovolume to biomass conversion factors, as C cell^{-1} in pg = 0.38 pg C µm^{-3} for bacteria (Lee & Fuhrman 1987) and C cell^{-1} in pg = 0.45 pg C µm^{-3} for picocyanobacteria (Casareto et al. 2000). Carbon biomasses of micro- and nanophytoplankton (including coccolithophorids) and zooplankton were calculated using the relationships given by Menden-Deuer & Lessard (2000): for diatoms: C cell^{-1} in pg = biovolume$^{0.811}$ × 0.288; for other phytoplankton (including coccolithophorids) and zooplankton: C cell^{-1} in pg = biovolume$^{0.939}$ × 0.216. Biovolume was determined by measuring the size of >30 ind. using a graduated scale fitted into the ocular lens and assuming the closest approximation of geometric shape.

RESULTS

Plankton biomass

The initial abundance of *Pleurochrysis carterae* (after inoculation) in each incubation bottle was ~$2.3 \times 10^5 \pm 1.4 \times 10^4$ cells l^{-1}. However, the cell sizes were different; they were smaller in long incubations than in short ones, therefore their initial biomass varied (Figs. 1 & 2), and was 6.6 µg C l^{-1} in short incubations, and 5.9 and 6.4 µg C l^{-1} in unfiltered and filtered seawater of long incubations respectively. The abundance and biomass of *P. carterae* increased in both long and short incubations. In short incubations, the biomass of *P. carterae* increased with pCO_2 and time ($p < 0.01$, 2-factor ANOVA with replication, n = 2 replicates each) (Fig. 1). In long incubations, the biomass of *P. carterae* increased with pCO_2 ($p < 0.01$, 2-factor ANOVA with replication, n = 2 replicates each) (Fig. 2). Further, the increase in the biomass of *P. carterae* was much higher in the incubations in which they were alone than in those in which they were incubated with other plankton species for each pCO_2 level ($p < 0.01$, 2-factor ANOVA with replication, n = 2 replicates each) (Fig. 2). The biomass of noncalcifying micro and nanophytoplankton (e.g. diatoms, dinoflagellates and nanoflagellates) also increased with increasing CO_2 in both short and long incubations of unfiltered seawater ($p < 0.01$, 2-factor ANOVA with replication, n = 2 replicates each for short incubations; and $p < 0.05$, unpaired *t*-test, n = 2 replicates each for long incubations) (Figs. 1 & 2). However, the biomass of picocyanobacteria increased in long incubations only. Consistent with the increase in biomass, the rate of primary production in short incubations also increased with increasing pCO_2 (Fig. 3). The rate of primary production was highest for the incubations that lasted for 39 h and lowest in the incubations that lasted for 24 h, indicating the effect of

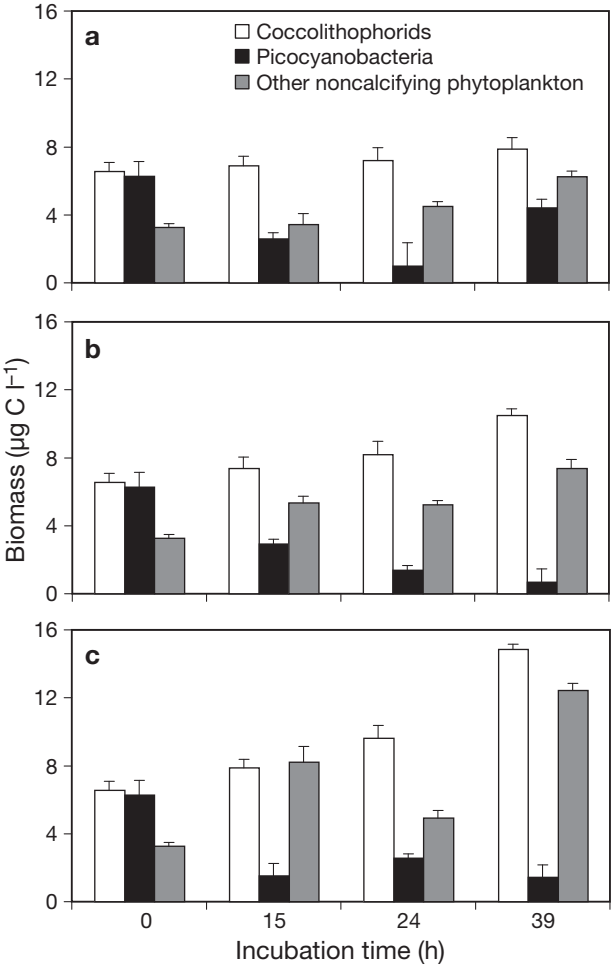

Fig. 1. Carbon biomass (+SD) of different phytoplankton groups at different pCO_2 levels in short incubations. (a) Ambient pCO_2, (b) 500 ppm pCO_2, and (c) 820 ppm pCO_2

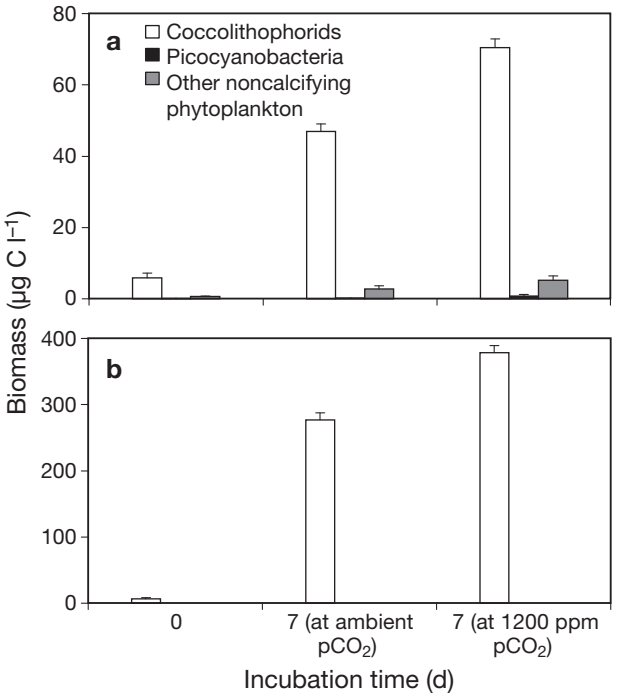

Fig. 2. Carbon biomass (+SD) of different phytoplankton groups at different pCO_2 levels in long incubations. Incubations of (a) unfiltered and (b) filtered (0.2 µm) seawater, with the addition of *Pleurochrysis carterae* and nutrients

respiration during 9 h dark periods. The biomass of heterotrophs (zooplankton, heterotrophic nano- and picoflagellates and bacteria) also increased in both short and long incubations. In short incubations, biomass increased with time but not with increasing CO_2 in most of the cases (Fig. 4); however, in long incubations of unfiltered seawater, biomass increased with pCO_2 (Fig. 5a).

pH and calcite saturation state (Ω_{cal})

The initial pH of the seawater in the incubation bottles varied from 8.194 to 8.20 (at ambient pCO_2 levels) to 7.78 (at 1200 ppm pCO_2) (Table 3, Fig. 6a,b). The initial saturation state for calcite (Ω_{cal}) varied from 5.1 (at ambient pCO_2 levels) to 2.3 (at 1200 ppm pCO_2 levels) (Table 3). Small changes in pH and Ω_{cal} occurred during incubations.

Net calcification rates

Net calcification rates were negative in short incubations irrespective of pCO_2 levels, indicating a net decrease in $CaCO_3$ production (or $CaCO_3$ dissolution) in these incubations. However, the negative values of net calcification in these incubations became less negative with time (Fig. 7a). In long incubations, net calcification rates were positive at all pCO_2 levels, indicating calcification (or precipitation of $CaCO_3$). Further, net calcifications rates in long incubations increased with pCO_2. Net calcification in long incubations were higher in those in

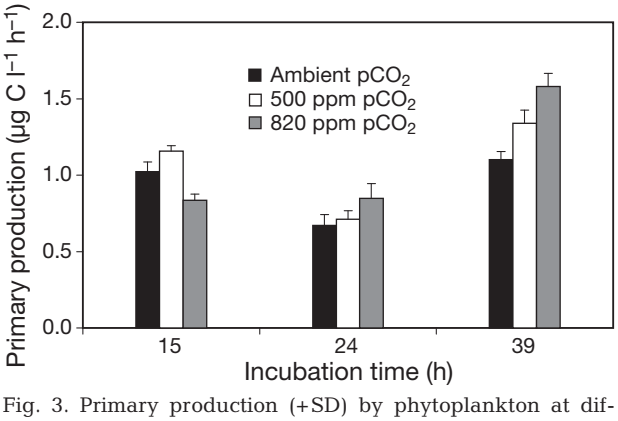

Fig. 3. Primary production (+SD) by phytoplankton at different pCO_2 levels in short incubations

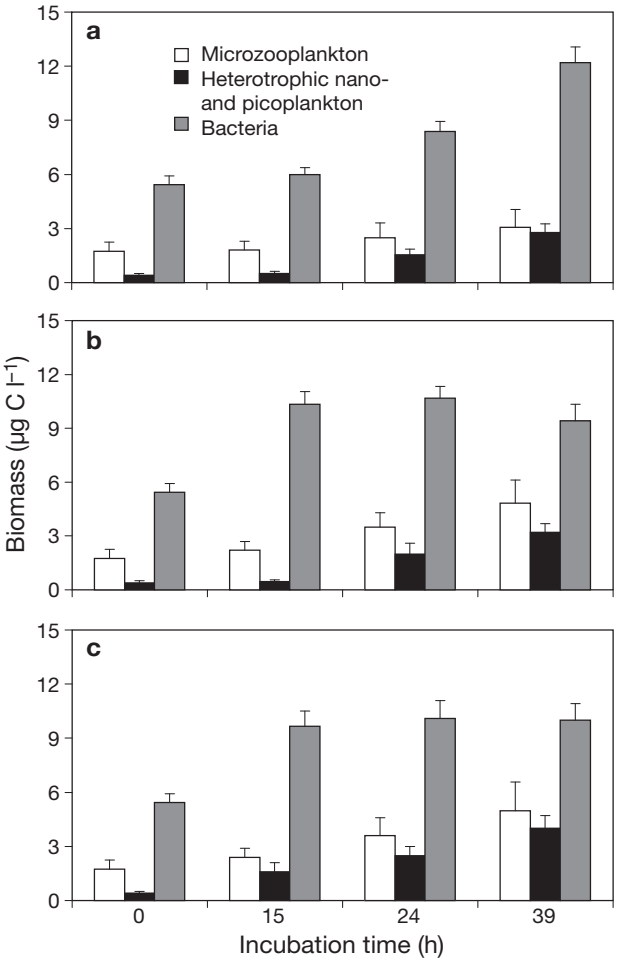

Fig. 4. Carbon biomass (+SD) of different heterotrophs at different pCO_2 levels in short incubations. (a) Ambient pCO_2, (b) 500 ppm pCO_2, and (c) 820 ppm pCO_2

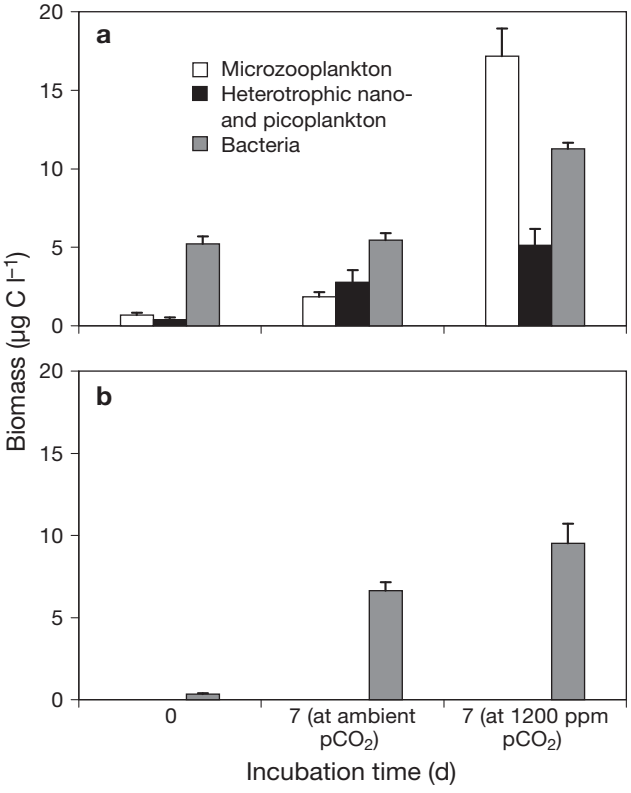

Fig. 5. Carbon biomass (+SD) of different heterotrophs at different pCO_2 levels in long incubations. Incubations of (a) unfiltered and (b) filtered (0.2 µm) seawater, with the addition of *Pleurochrysis carterae* and nutrients

which *Pleurochrysis carterae* were incubated alone (filtered seawater) than in those in which they were incubated with other plankton (unfiltered seawater) for each pCO_2 level. However, the difference in calcification rates between unfiltered and filtered seawater incubations was significant only for 1200 ppm pCO_2 levels ($p < 0.01$, unpaired *t*-test, n = 2 replicates each). In dark incubations, net calcification rates were negative at all pCO_2 levels (Fig. 7b), and values were even more negative for incubations at high pCO_2 level than in those at ambient pCO_2 level ($p < 0.05$, unpaired *t*-test, n = 2 replicates each). Consistent with these results, SEM photographs of *P. carterae* showed that the coccoliths in most of the short incubations were more malformed (Fig. 8b) than the initial ones (Fig. 8a), particularly after the first 15 h of incubation. Moreover, in most of the long incubations (Fig. 9), the coccoliths were nearly similar to the initial ones, irrespectively of pCO_2 levels. In dark incubations, the coccoliths were more malformed than the initial ones, particularly at 1200 ppm pCO_2 (Fig. 9c).

Particulate organic and particulate inorganic carbon

Consistent with the increase in biomass, POC also increased by as much as 2 orders of magnitude in long incubations, and the increase in POC was higher at 1200 ppm than at ambient pCO_2 levels (Fig. 10a). However, the difference in POC build-up (change in standing stock from Day 0 to Day 7) between ambient and 1200 ppm was not significant. Nevertheless, for the

Table 3. Initial values (±SD) of pH and calcite saturation state (Ω_{cal}) at different pCO_2 levels in different incubations

Incubations	pH	Ω_{cal}
Short		
Ambient	8.194 ± 0.007	5.065 ± 0.152
500 ppm	8.110 ± 0.005	4.368 ± 0.130
820 ppm	7.932 ± 0.012	3.097 ± 0.091
Long		
Ambient	8.200 ± 0.008	5.131 ± 0.033
1200 ppm	7.781 ± 0.013	2.323 ± 0.009
Dark		
Ambient	8.198 ± 0.012	5.122 ± 0.020
1200 ppm	7.784 ± 0.006	2.331 ± 0.019

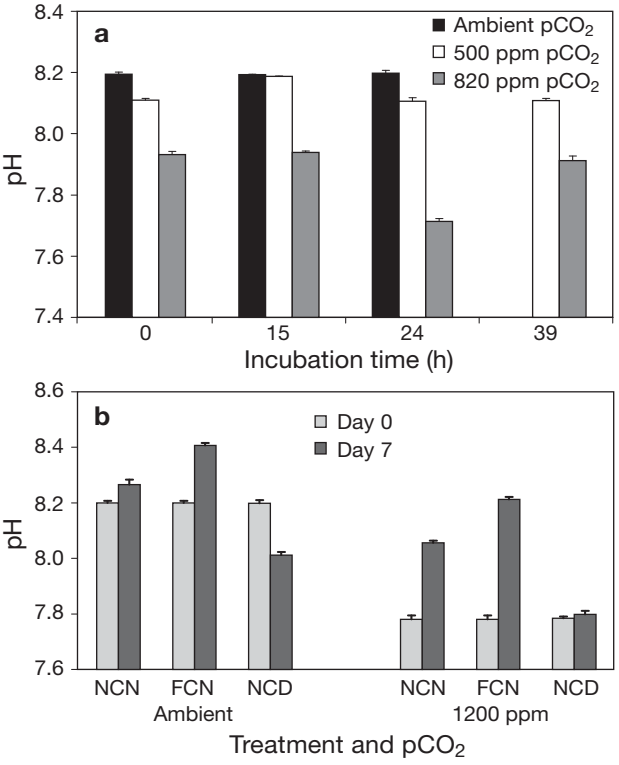

Fig. 6. pH (+SD) in different incubations. (a) Short incubations (samples for pH measurement at ambient pCO_2 after 39 h were lost), (b) long and dark incubations. See Table 1 for abbreviations

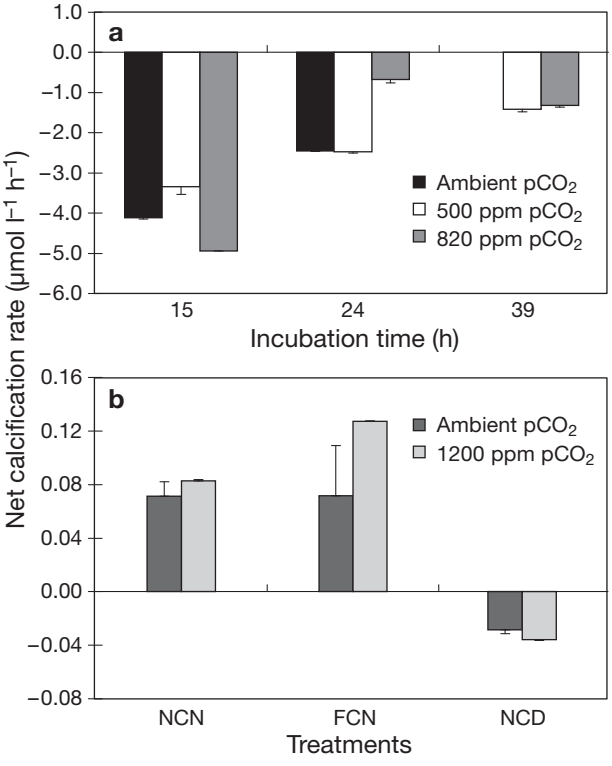

Fig. 7. Net calcification rates (+SD) in different incubations. (a) Short incubations, (b) long and dark incubations. (+, –) Increase or decrease in net calcification rates respectively. See Table 1 for abbreviations

same pCO_2 levels, the increase in POC was much higher in the incubations with *Pleurochrysis carterae* alone than in those with other plankton species ($p < 0.01$, unpaired *t*-test, n = 2 replicates each). PIC increased by 1 order of magnitude at both ambient and 1200 ppm pCO_2 levels (Fig. 10b). PIC build-up was higher for the incubations at 1200 ppm pCO_2 than in those at ambient pCO_2, and was also higher in the incubations in which *P. carterae* were incubated alone than in those with other phytoplankton species. However,

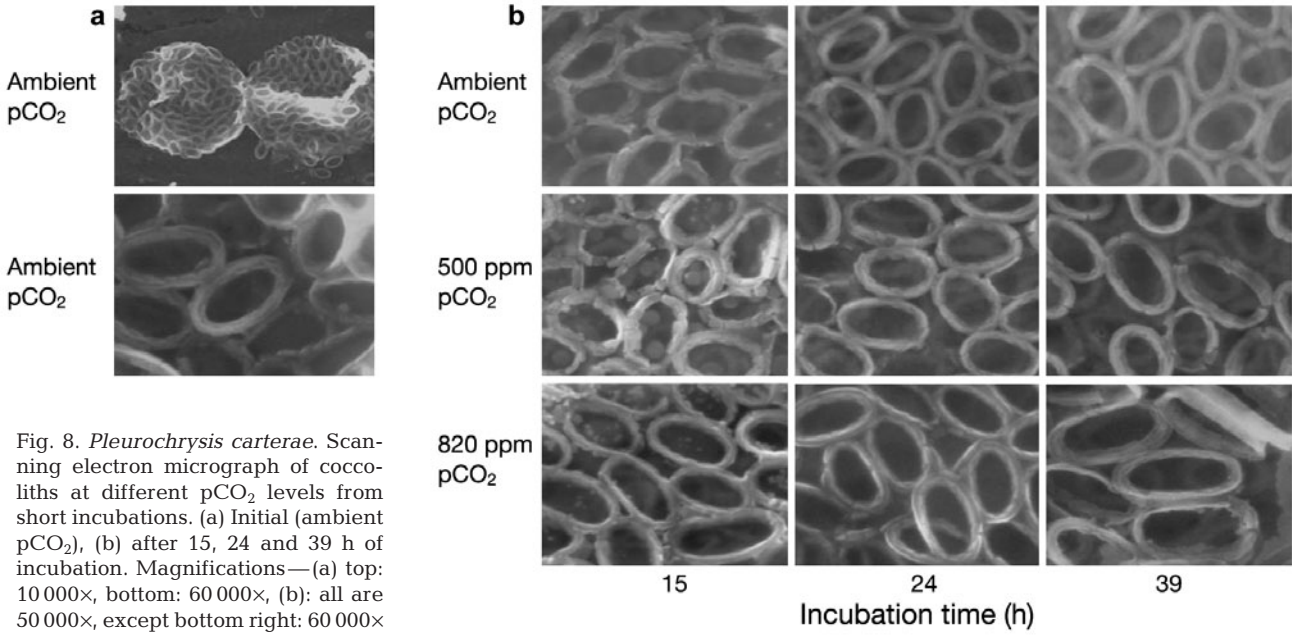

Fig. 8. *Pleurochrysis carterae*. Scanning electron micrograph of coccoliths at different pCO_2 levels from short incubations. (a) Initial (ambient pCO_2), (b) after 15, 24 and 39 h of incubation. Magnifications—(a) top: 10 000×, bottom: 60 000×, (b): all are 50 000×, except bottom right: 60 000×

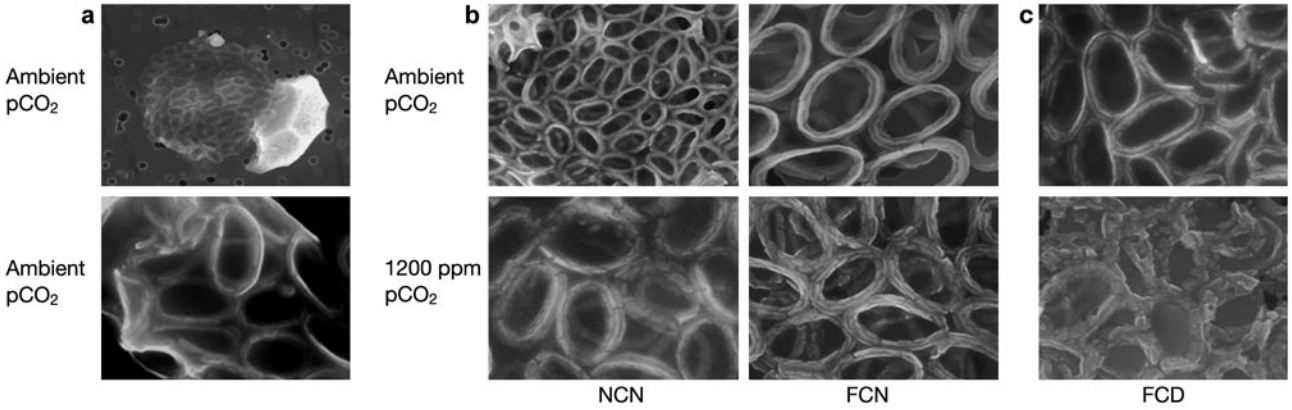

Fig. 9. *Pleurochrysis carterae*. Scanning electron micrograph of coccoliths at different pCO_2 levels from long and dark incubations. (a) Initial, (b) on Day 7 in long incubations, (c) on Day 7 in dark incubations. Magnification—(a) top: 10 000×, bottom: 50 000×; (b) top left: 20 000×, other panels: 50 000×; (c) top, bottom: 50 000×. See Table 1 for abbreviations

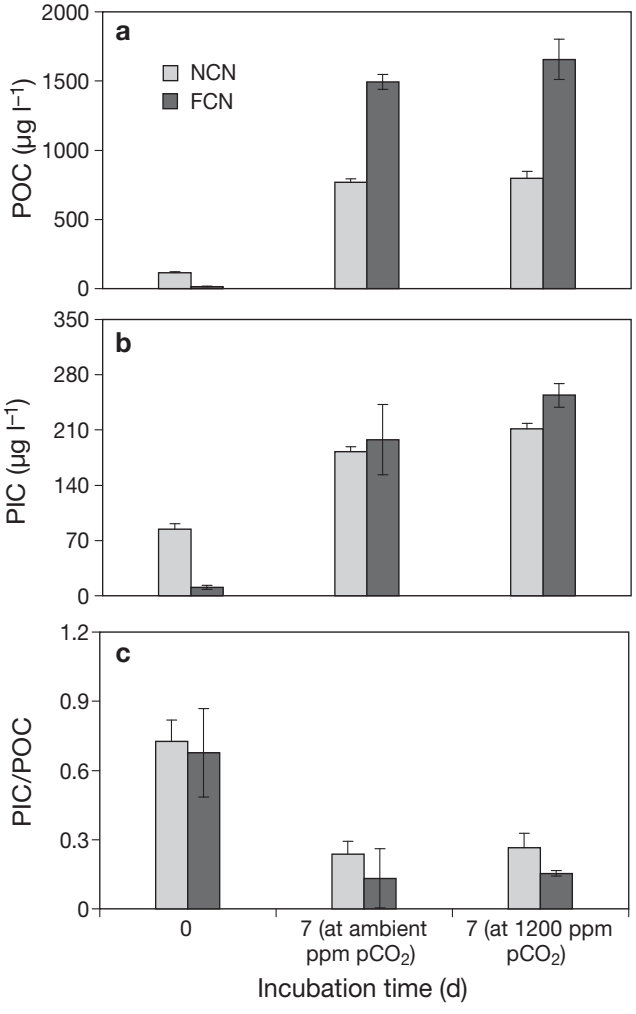

Fig. 10. (a) Particulate organic carbon (POC), (b) particulate inorganic carbon (PIC) and (c) PIC/POC ratio (+SD) at different pCO_2 levels in long incubations

the difference in PIC build-up was significant only between the incubations of unfiltered and filtered seawater ($p < 0.05$, unpaired *t*-test, n = 2 replicates each). Despite the increases in both POC and PIC, the PIC/POC ratio decreased in all these incubations (Fig. 10c).

DISCUSSION

Plankton biomass

Changes in seawater CO_2 concentration may influence phytoplankton species composition (Tortell et al. 2002), and the relative abundance of calcifying versus noncalcifying phytoplankton (Rost et al. 2003). An increase in CO_2 has only a small direct effect on the rate of photosynthesis in most noncalcifying phytoplankton species (The Royal Society 2005 and references therein) but increases the rate of photosynthesis in coccolithophorid species such as *Emiliania huxleyi* and *Gephyrocapsa oceanica* (Riebesell et al. 2000, Zondervan et al. 2001, Iglesias-Rodriguez et al. 2008). It has been shown that owing to the operation of carbon concentrating mechanisms (CCM, a process in which phytoplankton actively take up CO_2 or HCO_3^- to overcome carbon deficiency during carboxylation; Riebesell 2004), noncalcifying phytoplankton (e.g. diatoms and the prymnesiophyte species *Phaeocystis globosa*) have high affinities for inorganic carbon and are at or close to CO_2 saturation at present day CO_2 levels (Burkhardt et al. 2001, Riebesell 2004, Rost & Riebesell 2004). In contrast, coccolithophorid species such as *E. huxleyi* and *G. oceanica* have comparatively low affinities for inorganic carbon because of less efficient CCMs than other phytoplankton (Burkhardt et al.

2001, Rost et al. 2003), and are carbon-limited in today's ocean (Rost et al. 2003, Rost & Riebesell 2004). However, others have shown that the organic carbon production of the coccolithophorid species *E. huxleyi* remains constant (Delille et al. 2005, Engel et al. 2005) or even decreases (Nimer et al. 1994, Riebesell et al. 2000), while that of diatoms (Tortell et al. 2002, 2008, Kim et al. 2006), ANF (Hare et al. 2007), the picocyanobacteria *Synechococcus* (Fu et al. 2007) and natural plankton communities (Riebesell et al. 2007) increases with increasing CO_2. In our incubations in which we inoculated *Pleurochrysis carterae* in both natural and filtered (0.2 µm) seawater at different pCO_2 levels (ambient, 500, 820 and 1200 ppm pCO_2), the abundance and biomass of this species increased with pCO_2. We also found that their abundance and biomass increased as the duration of incubations increased from 15 h to 7 d. Further, coccolithophorids grew even more at increased CO_2 concentration (1200 ppm) in the absence of competing phytoplankton species and bacteria, and zooplankton grazers (in the case of filtered seawater). Our results show that coccolithophorids might benefit from increased concentrations of CO_2 (1200 ppm pCO_2) in water. In addition to the enhancement of coccolithophorids, the biomass of noncalcifying phytoplankton (e.g. diatoms, dinoflagellaes) also increased with increasing CO_2 in both short and long incubations. However, the biomass of picocyanobacteria showed some fluctuation; their biomass decreased in short incubations and increased in long incubations. Studies have shown that CCMs require additional energy for their operation regardless of their efficiency (Riebesell 2004). However, the energy available for active transport of inorganic carbon is often limited in the marine environment because of light limitation. Consequently, phytoplankton do not rely entirely on the active uptake of inorganic carbon from the abundant pool of HCO_3^- but may also rely on the small and variable pool of CO_2 (Riebesell 2004, Rost & Riebesell 2004). Therefore, it seems that the elevated CO_2 concentrations enhanced the photosynthetic carbon fixation of phytoplankton by reducing the energetic costs of CCMs (Fridlyand et al. 1996).

Consistent with the increase in phytoplankton biomass, the concentrations of nutrients also decreased rapidly in long incubations. The concentrations of dissolved inorganic nitrogen decreased from ~5.20 µM (at the initial stage) to 0.01 – 0.08 µM at the end of these incubations. PO_4 also decreased from 0.50 – 0.61 µM (at the initial stage) to 0.08 – 0.11 µM at the end of the incubations. However, the rapid increase in the biomass of phytoplankton indicates that phytoplankton growth was aided by recycled nutrients. Previous studies have discussed the growth of phytoplankton based on recycling of nutrients mediated by heterotrophs (Carlsson & Granéli 1999, Gaul et al. 1999, Niraula et al. 2007). Moreover, POC increased much more rapidly relative to PON in our incubations. The POC/PON molar ratio in unfiltered seawater increased from an initial value of 7.3 to values of 18.6 at ambient pCO_2 and 18.0 at 1200 ppm pCO_2, and from an initial value of 3.8 to values of 26.4 at ambient pCO_2 and 31.7 at 1200 ppm pCO_2 in filtered seawater incubations (Table 4). This indicates that carbon fixation by phytoplankton exceeded that of nitrogen assimilation in these incubations, i.e. more carbon was fixed than can be predicted by the Redfield ratio of carbon to nitrogen consumption (C/N: 6.6). Previous studies have also shown excess carbon fixation relative to nitrogen assimilation (Granéli et al. 1999, Wetz & Wheeler 2003, Riebesell et al. 2007). In our incubations, the C/N ratio seemed higher, although the ratios are below the values reported by others. For example, Gilpin et al. (2004) showed an increase in POC/PON ratio of ~40 under nitrogen deficient conditions.

Changes in net calcification rates

Shiraiwa (2003) showed that cell growth and calcification in coccolithophorids proceed apparently independently at different phases; firstly, an increase in cell population may be triggered by an adequate supply of nutrients, while an increase in calcification may occur when nutrients become depleted by substantial algal growth. Müller et al. (2008) reported that under nitrogen and phosphorus limited conditions, coccolithophorid species *Emiliania huxleyi* showed an increase in cell specific calcite content. In our short incubations, net calcification rates were also negative when the abundance and biomass of coccolithophorids were increasing and nutrients were replete at all pCO_2 levels. Negative values of net calcification rates became less negative as the production (abundance and biomass) increased with time (from 15 to 39 h) in these incubations. By Day 7 (long incubations) when blooms of coccolithophorids developed and nutrients were rapidly consumed, net calcification rates became posi-

Table 4. Particulate organic carbon (POC), particulate organic nitrogen (PON) and POC/PON ratio in long incubations. See Table 1 for abbreviations

	Day	POC (µM)	PON (µM)	POC/PON
NCN	0	9.69	1.33	7.3
	7 (ambient ppm)	64.18	3.44	18.6
	7 (1200 ppm)	66.44	3.70	18.0
FCN	0	1.31	0.35	3.8
	7 (ambient ppm)	124.52	4.71	26.4
	7 (1200 ppm)	137.96	4.36	31.7

tive (calcification was resumed) in all incubations irrespective of pCO_2 levels. Net calcification rates were negative even by Day 7 only in the dark incubations in which photosynthesis was inhibited, and the rate became higher with pCO_2.

Consistent with these results, SEM photographs also showed deformed coccoliths in short and dark incubations and intact coccoliths in long incubations. The carbonate ion (CO_3^{2-}) is an important buffer to neutralize CO_2 entering seawater (Gattuso & Buddemeier 2000, Feely et al. 2004, The Royal Society 2005). Acidification of the oceans as a result of increasing CO_2 is reducing the CO_3^{2-} concentration (Gattuso et al. 1999, Kleypas et al. 1999) and thus the level of $CaCO_3$ saturation (Ω) (Gattuso & Buddemeier 2000, Orr et al. 2005). A Ω value of unity indicates saturation equilibrium (100% saturation) and that >1 indicates supersaturation (Gattuso et al. 1999). An undersaturated Ω caused by increasing CO_2 concentration leads to dissolution of $CaCO_3$ present in living and dead calcified organisms to neutralize CO_2 entering the water (The Royal Society 2005), and also decreases biologically mediated calcification (Wolf-Gladrow et al. 1999, Riebesell et al. 2000, Merico et al. 2006). However, a number of studies have shown that the rate of calcification could decline even when surface waters remain supersaturated ($\Omega_{cal} > 1$) (Kleypas et al. 1999, Feely et al. 2004). These studies have shown that the degree of supersaturation with respect to $CaCO_3$ is important in determining the calcification rates of individual species and communities. The degree of supersaturation ranges from 5 to 6 for calcite (Gattuso et al. 1999, Kleypas et al. 1999); reduction of the Ω_{cal} below these values may lead to large decreases in calcification rates (Kleypas et al. 1999, Feely et al. 2004), implying that these values act as threshold levels for determining increase or decrease in calcification (Feely et al. 2004). Consistent with these findings, our results also showed negative values of net calcification in short and dark incubations in which Ω_{cal} was <6. However, in long incubations, net calcification rates were positive (calcification occurred) even when initial Ω_{cal} was ~2.3. Our results show that an increase or decrease in net calcification in *Pleurochrysis carterae* depends on biological processes (the growth phase) and the time scale if initial Ω_{cal} in seawater is ~2 to 5 (supersaturated but below the threshold value). Net calcite production decreases (i.e. dissolution of $CaCO_3$ occurs) during the early growth phase (pre-bloom phase) of *P. carterae* even if Ω_{cal} is ~5 and pCO_2 is at ambient state, and net calcite production increases during or after blooms, even if Ω_{cal} is ~2 and pCO_2 is ~1200 ppm. Our results further show that increases in the organic production and calcification of coccolithophorids are even more pronounced in the absence of competition from other phytoplankton groups or grazing by zooplankton. However, in the dark when phytoplankton cannot grow, dissolution of $CaCO_3$ occurs and this effect is more noticeable with increasing pCO_2 levels and decreasing Ω_{cal}.

PIC/POC ratio

Apart from effects on calcification, increased CO_2 has also been shown to affect the PIC/POC production ratio of coccolithophorids. The PIC/POC ratio in the coccolithophorid *Emiliania huxleyi* decreases with increasing pCO_2 levels either due to an increase in POC and a decrease in PIC production (Riebesell et al. 2000, Zondervan et al. 2001, Sciandra et al. 2003), or only to a decrease in PIC production (Delille et al. 2005, Engel et al. 2005). However, Langer et al. (2006) recently showed that both PIC and POC remained constant with increasing pCO_2 in the coccolithophorid species *Coccolithus pelagicus*, resulting in a constant PIC/POC ratio; in *Calcidiscus leptoporus*, PIC changed but POC remained constant with pCO_2, which they attributed to the effects of specific variation in the PIC/POC ratio. In our long incubations using *Pleurochrysis carterae*, the PIC/POC (standing stock) ratio decreased. However, the decrease in the PIC/POC ratio occurred through an increase in both POC and PIC with increasing pCO_2. Our results show that the PIC/POC ratio decreased even with increase in calcification provided that the magnitude of increase in POC was much higher than the magnitude of increase in PIC.

CONCLUSIONS

Our results demonstrate that *Pleurochrysis carterae* adapt with time to changing carbonate chemistry or CO_2 concentrations. The results also show that growth phase and time play important roles in determining the calcification and dissolution of $CaCO_3$ in *P. carterae*. Net calcification rate is negative, i.e. dissolution of $CaCO_3$ occurs during the initial growth phase and becomes positive (i.e. calcification resumes) with time towards bloom conditions. Positive values of net calcification in long incubations and negative values in dark incubations indicate that biological processes (photosynthesis or organic carbon production) are more important than physico-chemical reactions in determining calcification and dissolution in coccolithophorids. Apart from *P. carterae*, noncalcifying phytoplankton also showed a positive response to increasing pCO_2 levels, indicating that even increases in seawater CO_2 concentrations to 1200 ppm may have little or no impact on ecosystem dynamics.

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