

# Effect of CO<sub>2</sub> concentration on the PIC/POC ratio in the coccolithophore *Emiliana huxleyi* grown under light-limiting conditions and different daylengths

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## Abstract

We compared the effect of CO<sub>2</sub> concentration ([CO<sub>2</sub>], ranging from ~ 5 to ~ 34 μmol l<sup>-1</sup>) at four different photon flux densities (PFD = 15, 30, 80 and 150 μmol m<sup>-2</sup> s<sup>-1</sup>) and two light/dark (L/D) cycles (16/8 and 24/0 h) on the coccolithophore *Emiliana huxleyi*. With increasing [CO<sub>2</sub>], a decrease in the particulate inorganic carbon to particulate organic carbon (PIC/POC) ratio was observed at all light intensities and L/D cycles tested. The individual response in cellular PIC and POC to [CO<sub>2</sub>] depended strongly on the PFD. POC production increased with rising [CO<sub>2</sub>], irrespective of the light intensity, and PIC production decreased with increasing [CO<sub>2</sub>] at a PFD of 150 μmol m<sup>-2</sup> s<sup>-1</sup>, whereas below this light level it was unaffected by [CO<sub>2</sub>]. Cell growth rate decreased with decreasing PFD, but was largely independent of ambient [CO<sub>2</sub>]. The diurnal variation in PIC and POC content, monitored over a 38-h period (16/8 h L/D, PFD = 150 μmol m<sup>-2</sup> s<sup>-1</sup>), exceeded the difference in carbon content between cells grown at high (~ 29 μmol l<sup>-1</sup>) and low (~ 4 μmol l<sup>-1</sup>) [CO<sub>2</sub>]. However, consistent with the results described above, cellular POC content was higher and PIC content lower at high [CO<sub>2</sub>], compared to the values at low [CO<sub>2</sub>], and the offset was observed throughout the day. It is suggested that the observed sensitivity of POC production for ambient [CO<sub>2</sub>] may be of importance in regulating species-specific primary production and species composition. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Calcification; CO<sub>2</sub>; Coccolithophores; Light; Organic carbon fixation

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## 1. Introduction

Several studies have shown that natural blooms of the coccolithophore *Emiliania huxleyi* occur in highly stratified waters where the mixed layer depth is usually  $\sim 10$ – $20$  m, and is always  $\leq 30$  m (Balch et al., 1991; Robertson et al., 1994; Tyrrell and Taylor, 1996; Nanninga and Tyrrell, 1996; Ziveri et al., 2000). This finding, as well as the timing of the blooms, often in mid-summer (Balch et al., 1991; Fernández et al., 1993) when surface irradiances are high, suggests that these algae preferably grow at high light intensities (Baumann et al., 2000; Ziveri and Thunell, 2000).

Blooms of *E. huxleyi* are unique in their optical properties (Balch et al., 1991). This algal species precipitates  $\text{CaCO}_3$  in the form of calcite platelets, which are subsequently shedded from the surface of the cells when a bloom progresses. These so-called coccoliths scatter the incoming light, which makes the surface water appear brighter and these blooms visible from ships and through remote sensing instrumentation (Holligan et al., 1983; Balch et al., 1991). While in the top few meters of the surface water light intensity increases at higher coccolith concentrations, the extinction of light with depth also increases (Balch et al., 1991; Holligan et al., 1993; Tyrrell et al., 1999). This causes a decrease in the depth of the euphotic zone (Balch et al., 1991; Tyrrell et al., 1999).

Since calcification is an energy-requiring process (Brownlee et al., 1995), it is light-dependent. Several laboratory studies have shown that light intensity affects the production of particulate inorganic carbon in *E. huxleyi* (Balch et al., 1992; Holligan et al., 1993; Bleijswijk et al., 1994). Saturating light intensities reported for *E. huxleyi* range from 72 to  $>500 \mu\text{mol m}^{-2} \text{s}^{-1}$  for calcification (Paasche, 1964: 72–120  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Balch et al., 1992: 539–586  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and from 72 to  $>600 \mu\text{mol m}^{-2} \text{s}^{-1}$  for photosynthesis (Paasche, 1964: 107  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Nielsen, 1995: 72–114  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; Nielsen, 1997: 117–299  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (depending on the acclimation light intensity); Balch et al., 1992: 351–1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (depending on temperature and  $\text{NO}_3^-$  concentration)). Assuming a mixed layer of 30 m in a coccolithophore bloom, according to the model of Tyrrell et al. (1999) cells would experience light levels below 35  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during more than 50% of the time. This suggests that in a natural bloom situation *E. huxleyi* cells grow under light-limiting conditions when growing below the top few meters. It is likely, therefore, that calcite and particulate organic carbon production are to a great extent limited by the light regime under natural conditions.

In a previous study, it was shown that calcite production in *E. huxleyi* and *Gephyrocapsa oceanica* decreases with increasing  $\text{CO}_2$  concentrations in laboratory experiments (Riebesell et al., 2000). These findings were confirmed by a field study, where calcification by a natural plankton community, was low when incubated under high  $\text{CO}_2$  partial pressures ( $p\text{CO}_2$ ), relative to calcification under low  $p\text{CO}_2$  (Riebesell et al., 2000). If this response to increasing  $\text{CO}_2$  concentrations is a general phenomenon in marine planktonic calcifiers, this could significantly change processes in the ocean such as ecosystem functioning and vertical particle flux, which may have important consequences for global biogeochemical cycling (see for discussion, Zondervan et al., 2001).

In their laboratory studies Riebesell et al. (2000) and Zondervan et al. (2001) tested the response of two coccolithophore species to increasing  $\text{CO}_2$  concentrations grown

under a photon flux density (PFD) of  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Since light intensities experienced by *E. huxleyi* cells in a natural field situation are probably below this level for most of the time, in the present study the effect of  $\text{CO}_2$  was examined at different irradiances.

## 2. Methods

*E. huxleyi* (strain PML B92/11) was grown in filtered ( $0.2 \mu\text{m}$ ) natural seawater enriched with nitrate and phosphate to concentrations of 100 and  $6.25 \mu\text{mol l}^{-1}$ , respectively, and with metals and vitamins according to f/2 (Guillard and Ryther, 1962), in Rumed 1200 light-thermostats. In the first experiment, incident photon flux densities (PFD) were 15, 30 and  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ , the temperature was  $15^\circ\text{C}$ . Except at a PFD of  $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ , where cells were grown only under continuous light, at PFD 30 and  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$  cells were grown at a 24/0 as well as a 16/8 L/D cycle. In the L/D treatments, samples were taken during the light period. Five different  $\text{CO}_2$  levels, ranging from 5 to  $34 \mu\text{M CO}_2$ , were adjusted by adding 1 N HCl or 1 N NaOH to the medium. Cultures were preadapted to the experimental conditions for  $>7$  generations and maintained dilute by starting the experiment with low cell concentrations. Samples were taken after eight generations, when the cells were still in the exponential growth phase and cell numbers per ml were in general less than 60000. At this point in time, less than 4% of the dissolved inorganic carbon (DIC) in the medium had been taken up by the cells, causing a shift in pH of not more than 0.15 units. After inoculation in triplicate 2.4-l borosilicate bottles, the bottles were closed immediately with teflon lined screw caps without head space to avoid  $\text{CO}_2$  exchange with the atmosphere.

In a second experiment, the diurnal variation in cellular particulate inorganic carbon (PIC) and particulate organic carbon (POC) content was monitored over a 38-h period. Medium composition and incubation bottles were as described above. *E. huxleyi* cells were grown at 2  $\text{CO}_2$  concentrations of  $\sim 4$  and  $\sim 29 \mu\text{mol l}^{-1}$  and a PFD of  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The cultures were maintained dilute, so that after at least six generations less than 4% of the DIC was taken up by the cells. Samples were taken at the beginning and in the middle of the light period and two hours prior to the onset of the dark phase.

Samples for DIC and alkalinity measurements were taken in 300 ml borosilicate bottles, fixed with 1 ml of a  $\text{HgCl}_2$  solution ( $35 \text{ g l}^{-1}$ ) and stored, in case of DIC samples free of air bubbles, at  $4^\circ\text{C}$ . DIC was measured coulometrically (Johnson et al., 1985) in duplicate and alkalinity was calculated from linear Gran plots (Gran, 1952) after duplicate potentiometric titration (Bradshaw and Brewer, 1988). Concentrations of dissolved  $\text{CO}_2$ ,  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  were calculated from temperature, salinity, and the concentrations of DIC, alkalinity, and phosphate, using equilibrium constants of Goyet and Poisson (1989). Subsamples for the determination of total particulate carbon (TPC) and POC were filtered on precombusted (12 h,  $500^\circ\text{C}$ ) QM-A filters (pore width  $\sim 0.6 \mu\text{m}$ ) and stored at  $-20^\circ\text{C}$ . Prior to analysis, the POC filters were fumed for 2 h with a saturated HCl solution to remove all inorganic C. TPC and POC were subsequently measured on an ANCA-SL 20–20 mass spectrometer. PIC was calculated as the difference between TPC and POC. Samples for cell counts were fixed with 400  $\mu\text{l}/20 \text{ ml}$  sample of a 20%

formaldehyde solution buffered with hexamethylenetetramine, and counted by means of a Coulter “Multisizer II”. The growth rate ( $\mu$ ) was calculated as

$$\mu = \frac{(\ln c_1 - \ln c_0)}{\Delta t} \quad (1)$$

where  $c_0$  and  $c_1$  are the cell concentrations at the beginning and the end of the experiment, respectively, and  $\Delta t$  is the duration of the incubation in days.

Inorganic and organic carbon production ( $P$ , pg C cell<sup>-1</sup> day<sup>-1</sup>) was calculated according to

$$P = \mu \times \text{cellular carbon content} \quad (2)$$

### 3. Results

In the following, we present the results of the present study, and combine these data with observations by Riebesell et al. (2000) and Zondervan et al. (2001) for comparison (data obtained at PFD 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , Figs. 1–4). With increasing  $[\text{CO}_2]$  in the growth medium and corresponding changes in  $p\text{CO}_2$ , pH, and  $[\text{CO}_3^{2-}]$  (not shown), cell growth rate of *E. huxleyi* changed little in all treatments (Fig. 1). Growth rate strongly depended on the light intensity, however, and was about twice as high at PFD of 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  compared to PFD of 15  $\mu\text{mol m}^{-2} \text{s}^{-1}$  under continuous light (Fig. 1). The growth rates of 1.11 and 1.02 for high and low  $[\text{CO}_2]$ , respectively (data not shown), found for the time series experiment agree very well with the data at PFD 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  shown in Fig. 1. There is no significant difference between the growth rate of cells grown at PFD 80 or 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Irrespective of an 8 h dark phase  $\mu$  remained nearly identical over a 24-h period in the two L/D cycles.

The cellular content of PIC and POC depended strongly on the PFD, and increased by ~32% and 56%, respectively, from PFD 15 to 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at a 24:0 L/D cycle. Whereas under continuous light there was no difference in cellular PIC and POC content between PFD 80 and 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Figs. 2B and 3B), under a 16:8 L/D cycle cellular carbon contents were generally lower at PFD 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  than at 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Figs. 2A and 3A). Both cellular PIC and POC content were nearly identical for the two different light/dark cycles (Figs. 2 and 3, Table 1).

With increasing  $[\text{CO}_2]$  a slight decrease in the cellular PIC content could be observed in several light treatments. This is statistically significant (ANOVA  $F$ -test, see Table 1) in cells grown at PFDs of 80 (24:0 L/D) and 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (16:8 and 24:0 L/D), but not significant at a PFD of 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (16:8 L/D), 30  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (24:0 L/D), and 15  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (24:0 L/D) (Fig. 2, Table 1). With the exception of the PFD treatments of 15 and 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (24:0 L/D), all other treatments yielded a significant increase in POC content with increasing  $[\text{CO}_2]$  (Fig. 3, Table 1). Slopes varied for different light intensities.

By multiplying growth rates with cellular carbon content, we obtained a carbon specific growth rate (=POC or PIC production). POC production showed a similar response to changing  $[\text{CO}_2]$  as observed in the cellular POC content, due to the more pronounced variation in cellular POC content with  $[\text{CO}_2]$  than in growth rate. With increasing  $[\text{CO}_2]$ ,

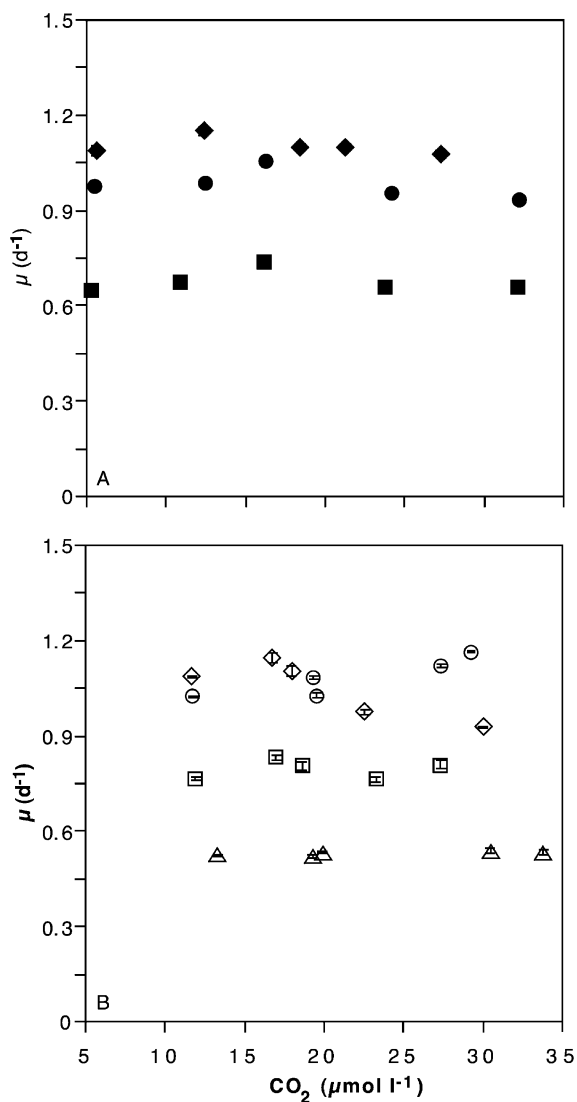


Fig. 1. Growth rate ( $\mu$ ) at different PFD (in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and two L/D cycles: 16:8 (A, closed symbols) and 24:0 (B, open symbols), plotted vs. ambient  $[\text{CO}_2]$ . The symbols denote PFD 150 (diamonds) (Riebesell et al., 2000; Zondervan et al., 2001), 80 (circles), 30 (squares), and 15 (triangles). Error bars represent 1 SD ( $n=3$ ).

POC production increased significantly at all light intensities (Table 1). Whereas PIC production significantly decreased with increasing  $[\text{CO}_2]$  at a PFD of  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ , it remained more or less constant below this light intensity (Table 1). Since both growth rate and cellular carbon content showed light dependency, PIC and POC production rates also increased with increasing light intensity.

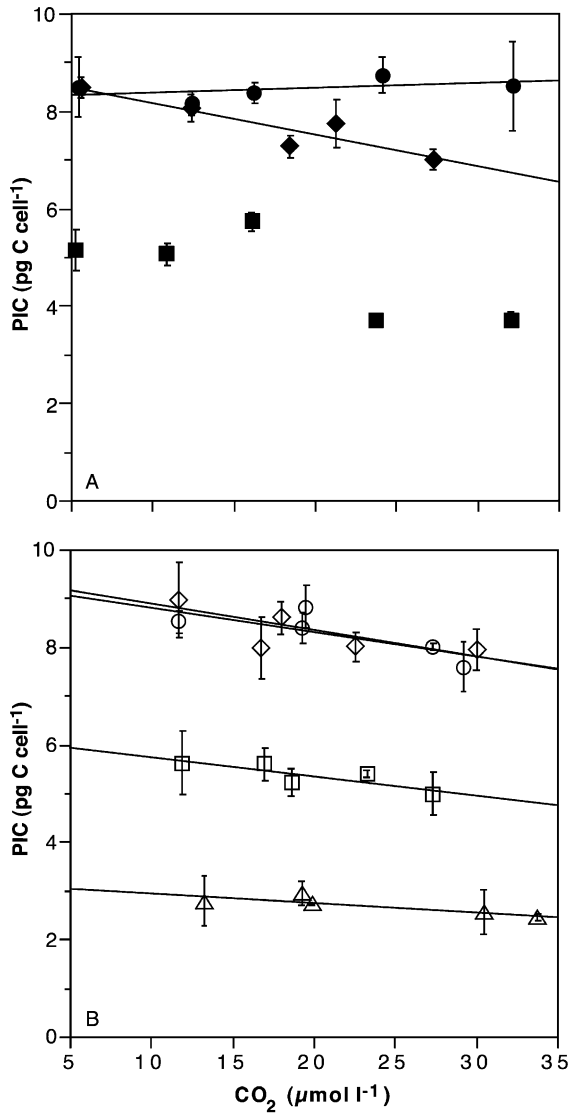


Fig. 2. Cellular content of particulate inorganic carbon (PIC) at different PFD (in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and two L/D cycles: 16/8 (A, closed symbols) and 24/0 (B, open symbols), plotted vs. ambient  $[\text{CO}_2]$ . Symbols as in Fig. 1. Error bars represent 1 SD ( $n=3$ ).

With the exception of the  $15 \mu\text{mol m}^{-2} \text{s}^{-1}$  PFD, the PIC/POC ratio showed a pronounced and highly significant decrease with increasing  $[\text{CO}_2]$  (Figs. 4 and 5; Table 1). Moreover, the PIC/POC ratios of the different treatments had very similar PIC/POC values at the various  $[\text{CO}_2]$ , with the exception of the  $15 \mu\text{mol m}^{-2} \text{s}^{-1}$  PFD treatment, for which the PIC/POC ratio was  $\sim 40\%$  lower (Fig. 4).

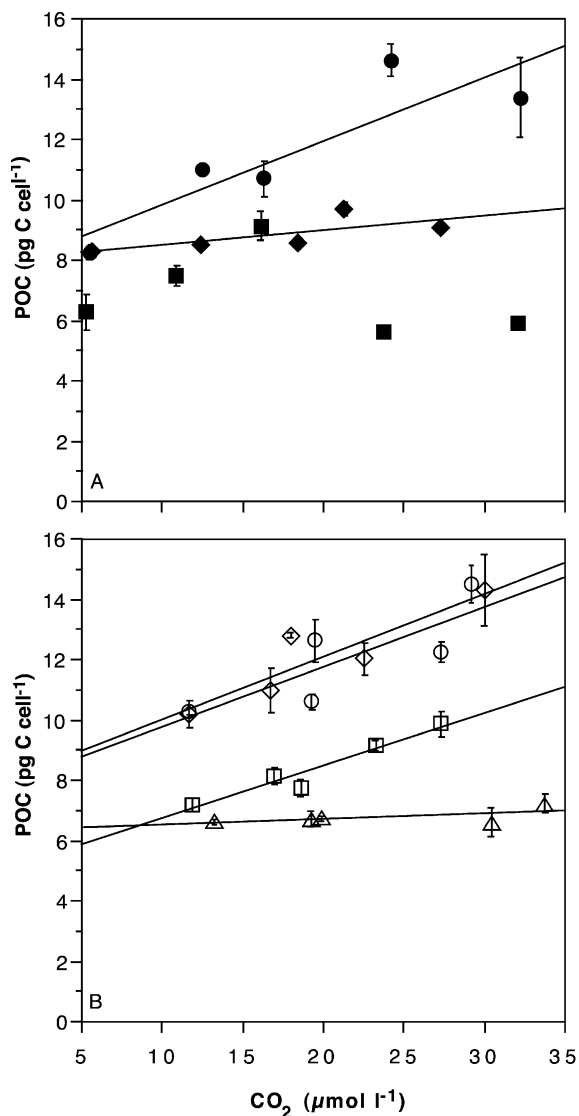


Fig. 3. Cellular content of particulate organic carbon (POC) at different PFD (in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and two L/D cycles: 16:8 (A, closed symbols) and 24:0 (B, open symbols), plotted vs. ambient  $[\text{CO}_2]$ . Symbols as in Fig. 1. Error bars represent 1 SD ( $n=3$ ).

A pronounced diurnal variation in PIC and POC content was observed (Fig. 5). When grown under high  $[\text{CO}_2]$ , the cellular content at the beginning of the light period was  $\sim 55\%$  and  $48\%$  of the PIC and POC content at the beginning of the dark phase, respectively. In the low  $\text{CO}_2$  treatments, cellular PIC and POC contents at the end of the dark phase were  $59\%$  and  $43\%$  of those at the end of the light period, respectively (Fig. 5).

Table 1

Cellular content of particulate organic carbon (POC, pg C cell<sup>-1</sup>) and particulate inorganic carbon (PIC, pg C cell<sup>-1</sup>), production of POC (pg C cell<sup>-1</sup> day<sup>-1</sup>) and PIC (pg C cell<sup>-1</sup> day<sup>-1</sup>), and the ratio of PIC/POC at different photon flux densities (PFD, μmol photons m<sup>-2</sup> s<sup>-1</sup>), L/D cycles (h/h), DIC, alkalinity, and CO<sub>2</sub> concentrations (μmol l<sup>-1</sup>), and pH

PFD	L/D	DIC	Alk	CO <sub>2</sub>	pH	POC	POC-prod.	PIC	PIC-prod.	PIC/POC
15	24:0	2102.29	2188.67	33.8	7.84	7.23 (0.30)	3.89 (0.17)	2.46 (0.08)	1.32 (0.05)	0.34
15	24:0	2140.78	2246.83	30.4	7.89	6.60 (0.48)	3.58 (0.26)	2.57 (0.45)	1.39 (0.24)	0.39
15	24:0	2114.44	2291.18	19.2	8.07	6.73 (0.26)	3.54 (0.14)	2.95 (0.25)	1.55 (0.13)	0.44
15	24:0	2120.80	2292.38	19.9	8.00	6.74 (0.10)	3.63 (0.05)	2.73 (0.01)	1.20 (0.01)	0.41
15	24:0	2126.54	2381.77	13.2	8.23	6.63 (0.07)	3.51 (0.04)	2.79 (0.51)	1.77 (0.27)	0.42
						NS	*	NS	NS	NS
30	16:8	1989.93	2069.72	32.1	7.84	5.90 (0.15)	3.91 (0.10)	3.70 (0.16)	2.45 (0.11)	0.63
30	16:8	1996.71	2117.77	23.8	7.97	5.66 (0.16)	3.76 (0.11)	3.71 (0.09)	2.46 (0.06)	0.66
30	16:8	2008.59	2194.62	16.2	8.13	9.15 (0.48)	6.78 (0.36)	5.73 (0.20)	4.24 (0.15)	0.63
30	16:8	2019.13	2287.86	10.9	8.29	7.48 (0.33)	5.075 (0.23)	5.07 (0.22)	3.44 (0.16)	0.68
30	16:8	2024.45	2492.26	5.3	8.57	6.29 (0.60)	4.11 (0.39)	5.14 (0.43)	3.36 (0.28)	0.82
						–	–	–	–	–
30	24:0	2061.87	2174.68	27.3	7.92	9.91 (0.43)	8.02 (0.37)	5.02 (0.43)	4.07 (0.35)	0.51
30	24:0	2071.76	2210.88	23.2	7.99	9.18 (0.15)	7.02 (0.14)	5.43 (0.08)	4.15 (0.08)	0.59
30	24:0	2078.85	2256.83	18.6	8.08	7.79 (0.30)	6.28 (0.26)	5.25 (0.29)	4.23 (0.25)	0.67
30	24:0	2098.04	2296.78	16.9	8.12	8.16 (0.29)	6.80 (0.25)	5.62 (0.33)	4.69 (0.28)	0.69
30	24:0	2105.94	2382.51	11.9	8.27	7.21 (0.24)	5.52 (0.19)	5.65 (0.65)	4.33 (0.50)	0.78
						***	***	NS	NS	***
80	16:8	1984.73	2060.23	32.2	7.84	13.42 (1.33)	12.59 (1.25)	8.51 (0.92)	7.98 (0.87)	0.64
80	16:8	2003.31	2122.13	24.2	7.96	14.67 (0.54)	14.03 (0.52)	8.75 (0.37)	8.37 (0.36)	0.60



80	16:8	2001.72	2185.44	16.3	8.12	10.74 (0.59)	11.34 (0.62)	8.39 (0.21)	8.86 (0.22)	0.78
80	16:8	2017.57	2256.91	12.5	8.23	11.03 (0.17)	10.93 (0.18)	8.18 (0.25)	8.10 (0.25)	0.74
80	16:8	2020.42	2477.37	5.5	8.55	8.29 (0.21)	8.14 (0.21)	8.50 (0.61)	8.33 (0.61)	1.03
						***	***	NS	NS	***
80	24:0	2097.51	2204.07	29.2	7.90	14.55 (0.62)	16.92 (0.72)	7.62 (0.50)	8.86 (0.58)	0.52
80	24:0	2101.27	2218.20	27.3	7.93	12.27 (0.34)	13.72 (0.39)	8.03 (0.06)	8.98 (0.08)	0.65
80	24:0	2113.95	2288.58	19.5	8.07	12.65 (0.70)	12.98 (0.73)	8.85 (0.44)	9.08 (0.46)	0.70
80	24:0	2107.95	2283.33	19.3	8.07	10.63 (0.24)	11.50 (0.26)	8.42 (0.32)	9.11 (0.35)	0.79
80	24:0	2111.22	2392.52	11.7	8.27	10.30 (0.10)	10.53 (0.11)	8.55 (0.23)	8.74 (0.24)	0.83
						***	***	**	NS	***
150	16:8	2098.39	2213.34	27.2	7.93	9.07 (0.12)	9.78 (0.15)	7.01 (0.20)	7.56 (0.23)	0.77
150	16:8	2095.58	2249.45	21.3	8.03	9.69 (0.20)	10.66 (0.24)	7.76 (0.49)	8.53 (0.54)	0.80
150	16:8	2098.48	2278.53	18.4	8.09	8.59 (0.09)	9.44 (0.12)	7.28 (0.22)	8.00 (0.25)	0.85
150	16:8	2102.38	2366.24	12.4	8.25	8.48 (0.13)	9.76 (0.19)	8.08 (0.29)	9.28 (0.35)	0.95
150	16:8	2106.77	2595.34	5.6	8.56	8.28 (0.21)	9.02 (0.26)	8.49 (0.22)	9.24 (0.27)	1.02
						**	*	***	***	***
150	24:0	2059.80	2160.68	30.0	7.88	14.30 (1.18)	13.26 (1.10)	7.96 (0.41)	7.38 (0.38)	0.56
150	24:0	2040.34	2177.95	22.6	8.00	12.04 (0.52)	11.76 (0.52)	8.02 (0.30)	7.83 (0.30)	0.67
150	24:0	2071.82	2254.24	17.9	8.09	12.81 (0.08)	14.03 (0.21)	8.61 (0.33)	9.43 (0.39)	0.67
150	24:0	2085.81	2284.29	16.8	8.12	10.99 (0.72)	12.59 (0.84)	8.00 (0.63)	9.16 (0.73)	0.73
150	24:0	2075.45	2350.13	11.7	8.27	10.20 (0.47)	11.08 (0.51)	8.98 (0.77)	9.75 (0.83)	0.88
						NS	***	***	*	***

Numbers in parentheses 1 SD ( $n=3$ ). Statistical analysis (ANOVA  $F$ -test): \*\*\* $P<0.001$ ; \*\* $P<0.01$ ; \* $P<0.05$ ; NS, not significant; –, no statistical analysis, data ignored in further implications (see text).

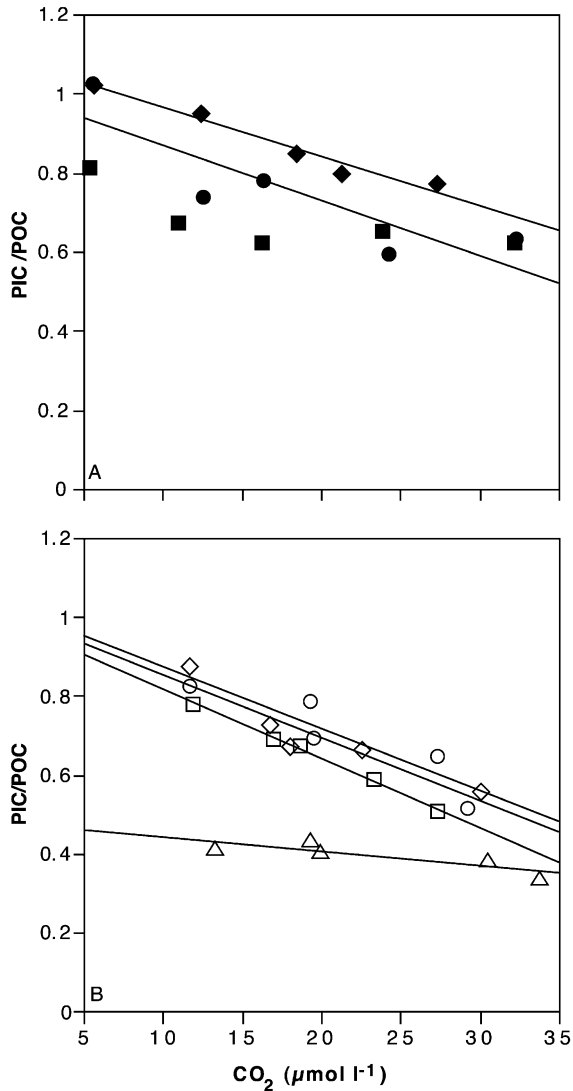


Fig. 4. Ratio of particulate inorganic to particulate organic carbon (PIC/POC) at different PFD (in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and two L/D cycles: 16:8 (A, closed symbols) and 24:0 (B, open symbols), plotted vs. ambient  $[\text{CO}_2]$ . Symbols as in Fig. 1.

Although there was a pronounced diurnal variation, cellular content of PIC and POC was significantly higher and lower, respectively, under  $4 \mu\text{mol CO}_2 \text{l}^{-1}$  relative to  $30 \mu\text{mol CO}_2 \text{l}^{-1}$  (Fig. 5). In both high and low  $\text{CO}_2$  treatments, a weak diurnal variation in the PIC/POC ratio was observed in the time series, where the ratio was lower at the end of the light period compared to after the dark phase (Fig. 5). This is probably due to both respiration and some degree of calcification during the dark period.

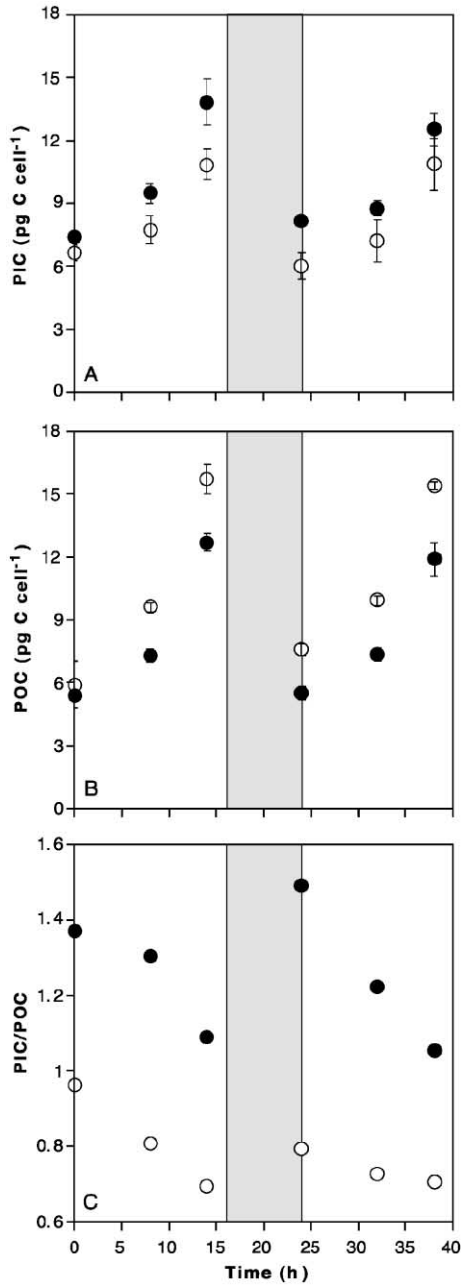


Fig. 5. Diurnal variation in cellular PIC (A) and POC (B) content, and the ratio of PIC/POC (C), monitored over a 38-h period, at high (open symbols) and low (closed symbols) [CO<sub>2</sub>]. Dark period indicated by shaded area. Error bars represent 1 SD (*n* = 3).

Cells grown under a light/dark cycle at a PFD of  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$  showed a pronounced drop in PIC and POC content at  $[\text{CO}_2] > 16 \mu\text{mol l}^{-1}$  (Figs. 2A and 3A). Bottles of these treatments were sampled at an earlier time of the day—at the end of the dark period—compared to the bottles with low  $\text{CO}_2$  treatments, which were sampled during the photoperiod. The time series experiment showed lower cellular carbon contents after the dark period, due to cell division in the dark (Fig. 5) (see also Linschooten et al., 1991; Bleijswijk et al., 1994). The lower cellular PIC and POC content in the high- $\text{CO}_2$  treatments could thus be explained by the earlier time of sampling. These data will therefore not be considered in the following. Note that this confirms the finding of Bleijswijk et al. (1994), who emphasized the importance to carefully consider the time of sampling when cell division is synchronized by a light/dark cycle, in cultures or in a natural field assemblage.

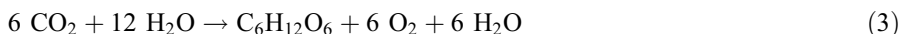
#### 4. Discussion

The ratio of PIC/POC in *E. huxleyi* decreases with increasing  $\text{CO}_2$  concentration, irrespective of light intensity and daylength. However, this study shows that the individual response in cellular PIC and POC production of *E. huxleyi* to  $[\text{CO}_2]$  strongly depends on the light intensity under which the cells grow. Whereas POC production increases significantly with increasing  $[\text{CO}_2]$ , independent of light intensity and daylength, PIC production remains constant with  $[\text{CO}_2]$  at PFDs ranging from 80 down to  $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

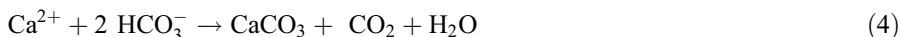
It has been shown in previous studies that the cellular carbon content varies strongly when cell division is synchronized by a light/dark cycle (i.e., Bleijswijk et al., 1994). We therefore examined whether the offset in cellular carbon content between cells grown at high and low  $\text{CO}_2$  concentrations occurs throughout a diurnal cycle. In agreement with the findings of Riebesell et al. (2000) and Zondervan et al. (2001), we observed in the time series experiment at a PFD of  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  a lower calcite and higher POC content at elevated  $[\text{CO}_2]$ . The diurnal variation in cellular carbon content (PIC and POC) in a L/D cycle is much larger than the difference in carbon content between cells grown at high and low  $[\text{CO}_2]$ . However, the  $\text{CO}_2$ -related offset in PIC and POC content is observed throughout the day. Thus, the effect of  $\text{CO}_2$  on the production of PIC and POC occurs in addition to the daily amplitude of carbon production, suggesting that the  $\text{CO}_2$  effect is not an artifact due to the time of sampling.

The increase in cellular carbon production by *E. huxleyi* with increasing PFD appears to level off above  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$  in this study (Table 1), which is at the lower end of saturating light intensities reported in previous laboratory studies (see Paasche, 1964; Balch et al., 1992; Nielsen, 1995, 1997). In fact, whereas PIC and POC production under continuous light conditions show very similar rates (Table 1), carbon production at a 16:8 L/D cycle is somewhat lower at PFD  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  than at  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$ . It is unlikely that this can be ascribed to photoinhibition at PFD  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ , since this effect is not observed under continuous light conditions. Also, several previous studies on *E. huxleyi* have shown that this species does not show photoinhibition at much higher light intensities (e.g. Nanninga and Tyrrell, 1996). Unfortunately, presently we are unable to give a mechanistic explanation for these observations.

Particulate organic carbon production and biogenic calcification are generally described by the overall reactions



and



respectively. It has been suggested in previous studies that calcification in *E. huxleyi* promotes POC production by providing  $\text{CO}_2$  or protons for organic carbon fixation (Sikes et al., 1980; Nielsen, 1995). The trend in the PIC/POC ratio in this study indicates high PIC relative to POC production when cells are grown under low  $[\text{CO}_2]$  concentrations. This would support the idea that calcification supplies  $\text{CO}_2$  (or protons) for photosynthesis, especially when the external  $[\text{CO}_2]$  is limiting for organic carbon fixation. However, except for the cells grown at a PFD of  $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , this trend in PIC/POC is mainly due to changing POC production, i.e. POC production increases with increasing  $[\text{CO}_2]$ , whereas PIC production remains more or less constant over the  $\text{CO}_2$  range tested. Only when *E. huxleyi* cells grow under replete light conditions PIC production increases under  $\text{CO}_2$  limiting conditions, which may partly compensate  $\text{CO}_2$  limitation in organic carbon production.

It has been reported that *E. huxleyi* has a low affinity for external  $\text{CO}_2$  (for review, see Raven and Johnston, 1991; Badger et al., 1998) and also has a low cellular carbon concentrating factor (Tortell, 2000). Our results agree with these findings, as POC production is highly sensitive to the external  $[\text{CO}_2]$ . However, on the basis of stable carbon isotope data, Rost et al. (2002) found evidence for operation of a  $\text{CO}_2$  concentrating mechanism (CCM) in *E. huxleyi*. This indicates that *E. huxleyi* may make use of a CCM not readily visible by an internal inorganic carbon pool (Badger et al., 1998), but that the CCM does not support maximum organic carbon fixation at typical seawater  $[\text{CO}_2]$ . It is possible that a relationship between photosynthesis and calcification exists, but our data do not support the idea of a close coupling between these two processes under the conditions studied here for this high calcifying strain of *E. huxleyi* (see also Rost et al., 2002), as suggested in previous studies (Sikes et al., 1980; Brownlee et al., 1995; Buitenhuis et al., 1999). This was also concluded by Paasche (1964), who found calcification in *E. huxleyi* in the absence of photosynthesis.

The decrease in the PIC/POC ratio with increasing  $[\text{CO}_2]$  observed under high light intensities could also be caused by an inhibition of calcification by the high ambient  $[\text{CO}_2]$  or the low pH or  $[\text{CO}_3^{2-}]$  in the medium. A high intracellular concentration of protons or  $\text{CO}_2$ , caused by high extracellular concentrations might have an adverse effect on calcite precipitation (Paasche, 1964). Assuming that maintenance of a high pH inside the coccolith producing vesicle (CPV) is required for  $\text{CaCO}_3$  precipitation (Brownlee et al., 1995), calcification may become more costly in terms of energy when protons or  $\text{CO}_2$  molecules are pumped against a higher gradient from the CPV to the cytoplasm.

Our results indicate that below  $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$  calcification is limited by light, and remains unaffected by external  $[\text{CO}_2]$ . In contrast, POC production is co-limited by  $[\text{CO}_2]$  and light at intensities below  $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Since POC production did not saturate

over the  $\text{CO}_2$  range tested here, organic carbon fixation seems to be carbon limited even at the highest  $[\text{CO}_2]$  applied in our experiments. This was reported in previous studies, where at low light and natural seawater DIC levels of photosynthesis of *E. huxleyi* was both light- and carbon-limited (Paasche, 1964; Nielsen, 1995). At a PFD of  $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ , both calcification and photosynthesis are severely light-limited, and POC production is not stimulated by higher ambient  $[\text{CO}_2]$ . At this light intensity, the production of PIC is more affected than of POC, which can be deduced from the low PIC/POC ratio compared to that at higher light intensities. The response in PIC and POC content to light intensity found in the present study is in close agreement with observations by Paasche (1999), who found an increase in calcium and organic carbon content, and a higher calcium/organic carbon ratio in *E. huxleyi* with increasing irradiance, covering the range of light intensities in the present study.

It is unclear whether changes in PIC production are in all cases due to less  $\text{CaCO}_3$  precipitation per coccolith (the coccoliths become thinner), or to a decrease in the number of coccoliths produced per cell. Riebesell et al. (2000) reported malformation of coccoliths and incomplete coccospheres under high  $[\text{CO}_2]$  in *E. huxleyi* and *G. oceanica* cultured under a PFD of  $150 \mu\text{mol m}^{-1} \text{s}^{-1}$ , and no consistent trend was obtained in the number of attached or free coccoliths per coccosphere. This points to a lower  $\text{CaCO}_3$  content per coccolith, also shown previously by Paasche (1999) at low irradiance.

Riebesell et al. (2000) and Zondervan et al. (2001) stated on the basis of results from laboratory and field experiments that the current rise of atmospheric  $\text{CO}_2$  (Houghton et al., 1995) may slow down marine planktonic calcification. If the observed response of *E. huxleyi* to changes in carbonate chemistry caused by rising atmospheric  $\text{CO}_2$  is a general phenomenon in the oceans, a decrease in the PIC/POC ratio could significantly modify the environment in areas where coccolithophores play a dominant role. This could include changes in surface ocean stratification, light penetration and particle sinking rate. Moreover, enhanced POC production in *E. huxleyi* due to the current increase in atmospheric  $[\text{CO}_2]$  could have implications for its ecological success, and has the potential to significantly change the structure and functioning of marine ecosystems. This was also mentioned by Paasche in his study of 1964: "In view of the relative constancy of the inorganic carbon concentration in sea water, it is difficult to see that any great ecological advantage would be gained from carbon subsaturation. Nevertheless the present observations raise the possibility, largely neglected by marine plankton ecologists, that if inorganic carbon is a "limiting factor" for the growth of some phytoplankton species in sea water, the variations in the carbon dioxide system that do occur in the sea may be of direct importance in regulating primary production". What the ultimate effect will be of the current increase in atmospheric  $p\text{CO}_2$  on the regulation of the production of POC and PIC in *E. huxleyi*, and primary production in general, is still an unresolved, but fascinating and important question.

This study shows that the response of *E. huxleyi* to increasing  $[\text{CO}_2]$  depends on the light conditions it experiences. Light intensity in blooms varies considerably with depth, where light penetration depends on absorption and scattering by algal cells and other particulate and dissolved matter, and scattering by detached coccoliths in blooms of *E. huxleyi* (Balch et al., 1991; Holligan et al., 1993). Therefore, different responses in cellular carbon content, especially in PIC, to changing  $[\text{CO}_2]$  are to be expected in the field depending on the depth of the mixed layer.

Production of organic matter and calcification have the opposite effect on CO<sub>2</sub> partial pressure in seawater (Eqs. (3) and (4)). Organic carbon production reduces the [CO<sub>2</sub>] in the surrounding medium due to CO<sub>2</sub> fixation (Eq. (3)). POC production therefore is a sink for CO<sub>2</sub>. From Eq. (4), it can be seen that calcification shifts the equilibrium of the carbonate system in the direction of CO<sub>2</sub>, which is then potentially released to the environment. Calcification therefore is a source for CO<sub>2</sub>. Thus, both an increase in POC production and a decrease in PIC production lowers the CO<sub>2</sub> partial pressure in seawater. A decrease in the ratio of PIC/POC due to an increase in the POC production or a decrease in the PIC production therefore have the same significance in terms of potential CO<sub>2</sub> storage capacity by the surface ocean.

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