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Effect of varying calcium concentrations and light intensities on calcification and photosynthesis in *Emiliania huxleyi*

Scarlett Trimborn, Gerald Langer and Björn Rost
Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27515 Bremerhaven, Germany, scarlett.trimborn@awi.de

Abstract

Various protective and metabolic functions for coccolithophore calcification have been proposed such as providing a means to supply CO_2 for photosynthesis. It has also been speculated that calcification helps to dissipate excess energy under high irradiance, thereby circumventing photoinhibition. To address these questions, cells of a calcifying strain of *Emiliania huxleyi* were grown at three irradiances (30, 300, and 800 μmol photons m^{-2} s^{-1}) in combination with four calcium (Ca) concentrations (0.1, 1, 2.5, and 10 mmol L^{-1}) leading to different degrees of calcification in the same strain. Growth rates (μ), particulate organic carbon (POC), and inorganic carbon (PIC) production as well as carbon isotope fractionation (ε_p) were determined. Photosynthetic O_2 evolution and CO_2 and HCO_3^- uptake rates were measured by membrane inlet mass spectrometry (MIMS). The application of this multimethod approach provides new information on the role of calcification in *E. huxleyi*. Noncalcifying cells showed POC production rates as high as calcifying ones. No differences in ε_p were observed under different Ca concentrations. MIMS measurements indicate that noncalcifying cells can photosynthesize as efficiently as, or even more efficiently than, calcifying ones and that both use HCO_3^- as the main carbon source. The ratio of photosynthetic HCO_3^- uptake relative to net fixation did not differ among cells acclimated to 10 mmol L^{-1} or to 0.1 mmol L^{-1} Ca. These results indicate that (1) calcification is not involved in photosynthetic carbon acquisition, and (2) calcification does not provide a means of energy dissipation under high irradiances.

Marine phytoplankton are a key player in driving the biological carbon pumps. Whereas diatoms only affect the organic carbon pump through the process of photosynthesis, calcifiers like *Emiliania huxleyi* additionally influence the carbonate pump by producing calcium carbonate. *E. huxleyi* is the most abundant coccolithophore in the oceans, distributed worldwide apart from the polar regions (Winter et al. 1994). It is the best-studied coccolithophore species, although it cannot be regarded as a typical coccolithophore in terms of phylogeny (Sáez et al. 2003). An uncommon trait of *E. huxleyi* is its ability to form extensive blooms with densities up to 10^7 cells L^{-1} (Holligan et al. 1993). Such blooms can be observed mainly in early summertime (Balch et al. 1991) when the water column becomes stratified and the mixed water layer depth is 30 m at most (e.g., Nanninga and Tyrrell 1996). This is in contrast to diatom blooms, which mainly form in springtime when the upper ocean is deeply mixed.

Light saturation for growth is comparably high in *E. huxleyi* compared with diatoms (Richardson et al. 1983). Exposed to high irradiances, diatoms are often photoinhibited, whereas *E. huxleyi* appears to be resistant to photoinhibition (Nielsen 1997). Only a few studies have investigated this unusual tolerance for high irradiances in *E. huxleyi* (e.g., Nanninga and Tyrell 1996; Harris et al. 2005). According to their findings, coccoliths do not act as "protective light screens." An alternative, yet not rigorously tested, explanation for the lack of photoinhibition has been proposed, i.e., calcification may serve as a means to dissipate excess energy when exposed to high light levels (Paasche 2001). In addition to this, other metabolic functions of calcification have been hypothesized for *E. huxleyi* (Young 1994; Paasche 2001).

Sikes et al. (1980) proposed that the process of calcification is involved in photosynthetic carbon acquisition. Provided that HCO_3^- is the carbon source, calcification could promote photosynthesis by supplying CO_2 or protons H^+ according to the following reactions:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O \quad (1)$$

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+ \quad (2)$$

CO_2 could then be used directly in photosynthesis or protons could be used in the conversion of HCO_3^- to CO_2. Supported by the observation that the carbon isotope composition of coccoliths is similar to the carbon isotope composition of HCO_3^- (Sikes and Wilbur 1982; Rost et al. 2002), it is generally accepted that HCO_3^- is the carbon source for calcification (*see* Paasche 2001). Such functional coupling would therefore allow the cell to access HCO_3^-, which represents the largest pool of inorganic carbon in seawater, providing an advantage especially under low CO_2 concentrations.

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According to Anning et al. (1996) this mechanism could represent a cost-efficient alternative to a classical carbon concentrating mechanism (CCM) found in microalgae (*see* Badger et al. 1998). CCMs involve active uptake of CO_2 or HCO_3^- (or both) to enhance the CO_2 concentration within the cell, thereby compensating for the low CO_2 affinities of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). On the basis of low affinities for inorganic carbon in *E. huxleyi*, which were only slightly higher than those observed for RubisCO, it has been assumed that this species takes up CO_2 for photosynthesis only via diffusion (Raven and Johnston 1991). Sekino and Shiraiwa (1994) demonstrated, however, that *E. huxleyi* is able to accumulate dissolved inorganic carbon (DIC) to more than 10-fold the ambient seawater concentrations. This is in accordance with Rost et al. (2003), who found that *E. huxleyi* operates a rather inefficient yet actively regulated carbon acquisition.

The involvement of calcification in photosynthetic carbon acquisition has been discussed controversially (e.g., Young 1994; Paasche 2001; Rost and Riebesell 2004); however, there is increasing evidence that the two processes are not coupled (Paasche 1964; Balch et al. 1996; Herfort et al. 2002, 2004). Paasche (1964) demonstrated that photosynthesis is not affected when the cells are transferred to Ca-free medium, a finding that was confirmed more recently by Herfort et al. (2002). These studies stand in contrast to those of Nimer et al. (1996) who found reduced photosynthetic rates by removal of external calcium.

In the present study two issues are addressed: (1) does calcification play a role in the high light tolerance of *E. huxleyi*? and (2) does photosynthesis benefit from calcification? To address these two questions, cells of a calcifying strain of *E. huxleyi* were grown under three light intensities (30, 300, and 800 μmol photons m^{-2} s^{-1}) in combination with four calcium (Ca) concentrations (0.1, 1, 2.5, and 10 mmol L^{-1}). This experimental setup allowed new information to be obtained on the role of calcification in a calcifying strain, in which both the degree of calcification (via Ca concentration) and photosynthesis (via light intensity) were varied. We assessed the relation between photosynthesis and calcification by determining growth rates, particulate organic carbon (POC) and calcite (PIC) production, as well as ^{13}C fractionation. Scanning electron microscopy (SEM) allowed us to examine the relation between the calcification rates and the morphology of the cells. The carbon sources taken up for photosynthesis were determined by membrane-inlet mass spectrometric (MIMS) measurements.

Material and methods

Culture conditions and sampling—A coccolith-bearing strain of *E. huxleyi* (B92/11) was grown in dilute batch cultures in sterile-filtered (0.2 μm) artificial seawater with four different Ca concentrations and three different incident photon flux densities (PFDs). The Ca concentrations, ranging from 0.1 to 10 mmol L^{-1}, were adjusted by addition of $CaCl_2$. The growth medium was enriched with trace metals and vitamins according to f/2 medium (Guillard and Ryther 1962) and nitrate and phosphate concentrations of 100 and 6.25 μmol L^{-1}, respectively. The detailed composition of the seawater is given in Langer et al. (2006). The pH was adjusted to a value of 8.24 by the addition of 0.5 NaOH (mol L^{-1}). Experiments were carried out under a light : dark (LD) cycle of 16 : 8 h at a constant temperature of 15°C. Three photon flux densities (30, 300, and 800 μmol photons m^{-2} s^{-1}) were applied in an incubator (Rubarth Apparate) with daylight fluorescence lamps providing a spectrum similar to that of sunlight. Each treatment was incubated in triplicate in HCl-rinsed polycarbonate flasks. Gentle rotation of the culture flasks three times a day ensured that the cells were kept in suspension.

Cells were acclimated to experimental conditions (including respective Ca concentrations) for at least 10 d, ensuring exponential growth before inoculation. To prevent consumption of more than 7% DIC during the experiment, cells were harvested at low cell densities of around 60,000 cells mL^{-1}. The experiments lasted between 5 and 10 d depending on the experimental conditions. Alkalinity samples were taken from the filtrate (Whatman GFF filter; ~0.6 μm), stored in 300-mL borosilicate flasks at 4°C, and measured in triplicate by potentiometric titration with an average precision of ±8 μeq kg^{-1} (Brewer et al. 1986). Total alkalinity was calculated from linear Gran plots (Gran 1952). DIC samples were sterile-filtered (0.2 μm) and stored in 13-mL borosilicate flasks free of air bubbles at 4°C until they were measured with a Shimadzu TOC 5050A with an average precision of ±17 μmol kg^{-1}. The carbonate system was calculated from alkalinity, DIC, phosphate, temperature, and salinity using the program CO2Sys (Lewis and Wallace 1998). Equilibrium constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987) were chosen. Detailed information on the parameters of the carbonate system is given in Table 1. The carbonate chemistry did not vary significantly between different flasks. In our experiments the calcite saturation product Ω was changed via the Ca concentration and not through the CO_3^{2-} concentration; the latter one as well as the pH were kept constant (see Table 1). Hence, the seawater carbonate chemistry was not changed in our experimental setup and is similar to values obtained in natural seawater.

Samples for total particulate carbon (TPC) and POC were filtered onto precombusted (500°C; 12 h) GFF filters and stored in precombusted petri dishes (500°C; 12 h) at −20°C. Before analysis, POC filters were treated with 200 μL of HCl (0.1 mol L^{-1}) to remove all the inorganic carbon. TPC, POC, and related $\delta^{13}C$ values were subsequently measured in duplicate on an elemental analyzer mass spectrometer (ANCA-SL 2020, Sercon) with a precision of ±1.5 μg C and ±0.5‰, respectively. PIC was calculated as the difference between TPC and POC. Cell count samples were fixed with formalin (0.4% final concentration, buffered with hexamethylenetetramine). Cell densities were determined daily or every other day immediately after sampling using a Coulter Multisizer III. Cell-specific growth rate (μ, unit d^{-1}) was calculated as

$$\mu = (\ln N_{\text{fin}} - \ln N_0)/\Delta t \quad (3)$$

Table 1. Parameters of the seawater carbonate system. Samples for total alkalinity and DIC were taken at the beginning (t_0) and the end (t_{fin}) of the experiment. pH was calculated from alkalinity, DIC, phosphate, temperature, and salinity using the program CO2Sys (Lewis and Wallace 1998).

	Beginning of experiments (t_0)	End of experiments (t_{fin})
Total alkalinity (μeq kg^{-1})	2,572 ($\pm$ 34)	2,534 ($\pm$ 45)
DIC (μmol kg^{-1})	2,239 ($\pm$ 49)	2,219 ($\pm$ 41)
pH (total scale)	8.24 ($\pm$ 0.03)	8.22 ($\pm$ 0.07)

where N_0 and N_{fin} denote the cell concentrations at the beginning and the end of the experiments, respectively, and Δt is the corresponding duration of incubation in days. PIC and POC production rates were calculated from cellular inorganic and organic carbon content and cell-specific growth rates according to the following Eqs. 4 and 5.

$$\text{PIC production} = (\text{PIC/cell})\mu \quad (4)$$

$$\text{POC production} = (\text{POC/cell})\mu \quad (5)$$

To investigate the coccolith morphology in the different calcium treatments, 10 mL of culture were filtered onto cellulose–nitrate filters (Sartorius, 0.2 μm), dried, and stored in a desiccator. These samples were finally sputter-coated and examined by means of a field emission SEM.

To determine isotopic composition of DIC ($\delta^{13}C_{DIC}$), 8 mL of culture were fixed with $HgCl_2$ (140 mg final concentration), stored at 4°C, and measured on a Finnegan mass spectrometer (MAT 252) with an average precision of $\delta^{13}C = \pm 0.05‰$. The isotopic composition of CO_2 ($\delta^{13}C_{CO2}$) was calculated from $\delta^{13}C_{DIC}$ using the equation by Rau et al. (1996) on the basis of Mook et al. (1974):

$$\delta^{13}C_{CO_2} = \delta^{13}C_{DIC} + 23.644 - (9701.5/T_K) \quad (6)$$

where T_K is the absolute temperature in Kelvin. The isotopic composition is reported relative to the PeeDee belemnite standard:

$$\delta^{13}C_{Sample} = \left[\frac{(^{13}C/^{12}C)_{Sample}}{(^{13}C/^{12}C)_{PDB}} - 1\right] 1000 \quad (7)$$

Isotope fractionation during POC formation (ε_p) was calculated relative to the isotopic composition of CO_2 in the medium (Freeman and Hayes 1992):

$$\varepsilon_p = \frac{\delta^{13}C_{CO_2} - \delta^{13}C_{POC}}{1 + \frac{\delta^{13}C_{POC}}{1000}} \quad (8)$$

Determination of photosynthesis, CO_2, and HCO_3^- uptake—To investigate inorganic carbon (C_i) fluxes during steady-state photosynthesis, 1000 mL of culture were concentrated by gentle filtration over a 3-μm membrane filter (Isopore, Millipore). The artificial culture media was hereby stepwise exchanged with CO_2-free artificial culture medium, buffered with 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES, 50 mmol L^{-1}, pH 8.0). The carbon flux measurements were performed with a MIMS (Isoprime; GV Instruments). The method established by Badger et al. (1994) is based on simultaneous measurements of O_2 and CO_2 during consecutive light and dark intervals. Briefly, rates of O_2 consumption in the dark and O_2 production in the light are used as direct estimates of respiration and net C_i fixation. While CO_2 uptake is calculated from the steady-state rate of CO_2 depletion at the end of the light period, the HCO_3^- uptake is derived by a mass balance equation, i.e., the difference of net C_i fixation and net CO_2 uptake. In the present study we largely followed the protocol described by Rost et al. (2006). All measurements were performed in artificial culture medium with the respective Ca concentration at 15°C. Dextran-bound sulfonamide (DBS), an inhibitor of external carbonic anhydrase, was added to the cuvette to get a final concentration of 100 μmol L^{-1}. Light and dark intervals during the assay lasted 6 min. The incident PFDs in the assays were 300 and 800 μmol photons m^{-2} s^{-1}, respectively. No C_i flux measurements could be performed for the 30 PFD treatment because the C_i fluxes were too small to create a chemical disequilibrium between CO_2 and HCO_3^- during the light phase, a prerequisite for this technique. For further details on the method and calculation we refer to Badger et al. (1994) and Rost et al. (2006). Samples for the determination of chlorophyll *a* concentration (Chl *a*) were taken after the measurements and stored at −80°C. Chl *a* was subsequently extracted in 10 mL of acetone (overnight in darkness, at 4°C) and determined with a fluorometer (Turner Designs). Chl *a* concentrations in the assay ranged from 0.18 to 0.88 μg mL^{-1}.

Results

Growth rates, PIC, and POC production—Light intensity had a strong effect on growth rates (μ), i.e., increasing rates with increasing PFDs (Fig. 1a; ANOVA, *F*-test: $p < 0.0001$). Growth rates of *E. huxleyi* ranged from 0.42 to 1.12 d^{-1}. Within the respective PFD treatment, variations in growth rates among the different Ca concentrations were small (ANOVA, *F*-test: $p > 0.05$ for 30, 800 PFD treatments; $p < 0.0001$ for 300 PFD treatments) with the exception of the 0.1 mmol L^{-1} Ca treatment (0.1 Ca treatment), in which μ was slightly reduced (0.42 d^{-1} compared with 0.5 d^{-1}).

With the exception of the 0.1 Ca treatment, the PIC production correlated positively with light intensity (Fig. 1b; ANOVA, *F*-test: $p < 0.0001$ for 1, 2.5, 10 Ca

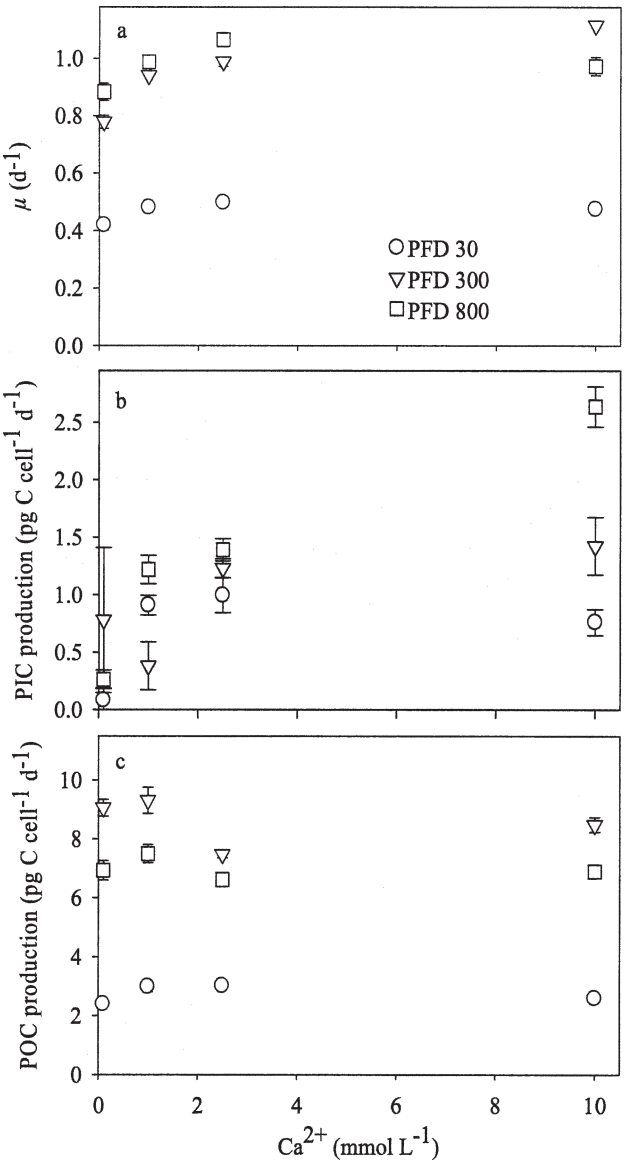

Fig. 1. Effects of Ca concentrations on (a) growth rates (μ), (b) PIC, and (c) POC production at different photon flux densities (PFDs). Symbols denote PFDs in μmol m^{-2} s^{-1}. Values represent the means of triplicate incubations (± SD).

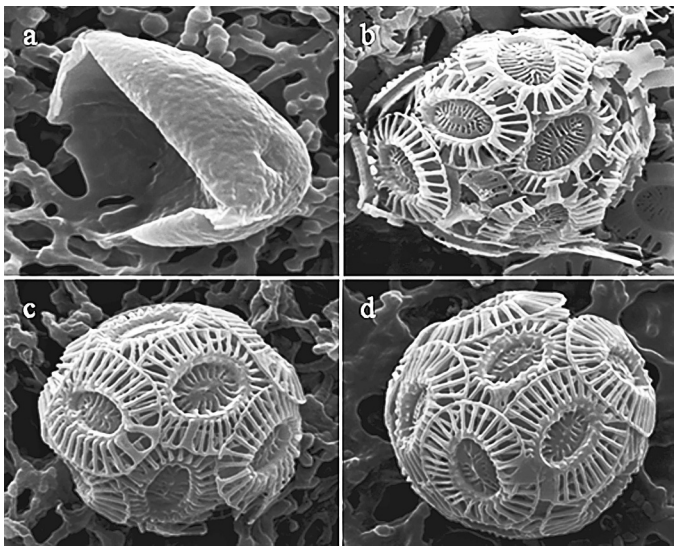

Fig. 2. Different degrees of calcification as depicted by SEM images of cells grown in artificial seawater containing (a) 0.1 mmol L^{-1}; (b) 1 mmol L^{-1}, (c) 2.5 mmol L^{-1}, and (d) 10 mmol L^{-1} Ca. Images show cells acclimated to 300 μmol photons m^{-2} s^{-1}.

treatments; $p > 0.05$ for 0.1 Ca treatments), and also showed a strong dependency on ambient Ca concentrations (ANOVA, *F*-test: $p < 0.0001$), whereby PIC production rates declined with decreasing Ca concentrations. Pictures taken by SEM of the 0.1 Ca treatment revealed that the cells were noncalcified; all other Ca treatments showed coccolith-bearing cells (Fig. 2). In the 1.0 Ca treatment, coccoliths and coccospheres were present, but the coccoliths displayed dissolution effects.

The POC production varied between 2.4 and 9.3 pg cell^{-1} d^{-1} (Fig. 1c). At PFD 30 μmol photons m^{-2} s^{-1}, POC production rates were about 2.4 to 3.0 pg cell^{-1} d^{-1} and increased by up to threefold under higher light intensities (ANOVA, *F*-test: $p < 0.0001$). The 300 PFD treatment obtained higher POC production, with values between 7.5 and 9.3 pg cell^{-1} d^{-1} in comparison with 800 PFD treatments, ranging from 6.6 to 7.5 pg cell^{-1} d^{-1}. Within the respective light treatment, variations in POC production between the 0.1 and the 10 Ca treatments were small (ANOVA, Bonferroni's multiple comparison test: $p < 0.0001$ for 30, 300 PFD treatments; $p > 0.05$ for 800 PFD treatments). At high PFDs (300 and 800 μmol photons m^{-2} s^{-1}), POC production rates of cells acclimated to 0.1 mmol L^{-1} Ca were slightly higher compared with cells grown under 10 mmol L^{-1} Ca (ANOVA, Bonferroni's multiple comparison test: $p < 0.0001$ for 300 PFD treatments; $p > 0.05$ for 800 PFD treatments).

Photosynthesis and C_i fluxes—Net photosynthesis was measured by simultaneously monitoring O_2 and CO_2 concentrations over consecutive LD intervals. Maximum rates of photosynthesis (V_{max}) were calculated from a Michaelis–Menten fit and are shown in Fig. 3a. The 800 PFD treatments obtained higher V_{max} with values, between 740 and 900 μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1}, in comparison with 300 PFD treatments ranging from 290 to 470 μmol O_2 (mg Chl *a*)$^{-1}$ h^{-1}. Over the investigated Ca range, no clear trend was observed within a respective PFD treatment (ANOVA, *F*-test: $p > 0.05$). At 300 PFD, the 10 Ca treatment had the lowest V_{max} of all Ca treatments. Acclimation to 800 PFD under different Ca concentrations caused V_{max} to be highest within the 2.5 Ca treatments and equally low at Ca concentrations of 0.1 and 10 mmol L^{-1}.

The C_i flux measurements enabled us to estimate CO_2 and HCO_3^- uptake rates following equations by Badger et al. (1994). During steady-state photosynthesis, *E. huxleyi* grown under different Ca concentrations used CO_2 as well as HCO_3^- as carbon source. Figure 3b shows the contribution of HCO_3^- uptake relative to total carbon

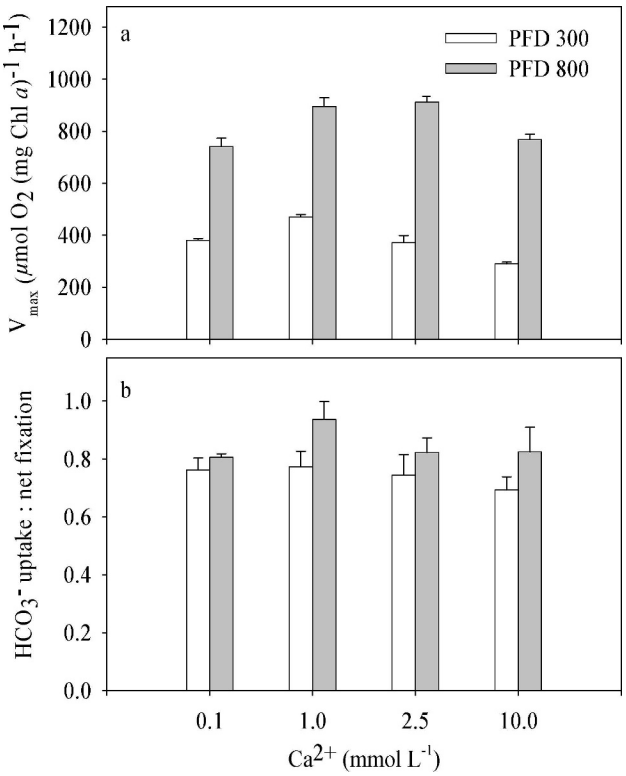

Fig. 3. (a) V_{max} and (b) ratios of HCO_3^- uptake : net photosynthesis of cells aclimated to 300 μmol photons m^{-2} s^{-1} and to 800 μmol photons m^{-2} s^{-1} as a function of Ca concentrations. V_{max} was calculated from a Michaelis–Menten fit. Ratios were based on the rates obtained at C_i concentrations of about 2 mmol L^{-1}. Error bars denote ± SD (n = 3).

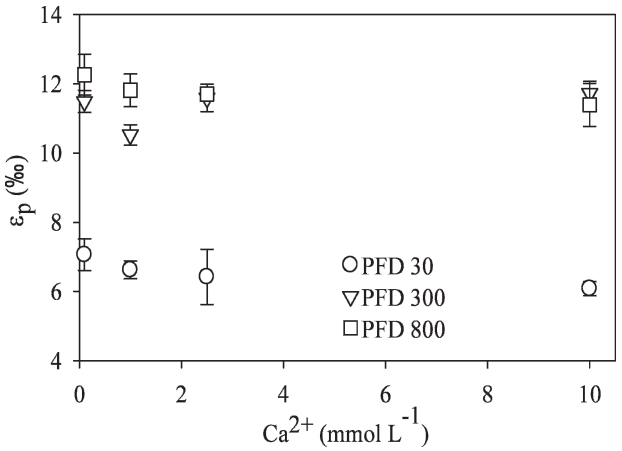

Fig. 4. Isotope fractionation (ε_p) as a function of Ca concentrations, calculated from the $^{13}C_{CO2}$ and $^{13}C_{POC}$ in the respective acclimations. Symbols denote photon flux densities (PFDs) in μmol m^{-2} s^{-1}. Values represent the means of triplicate incubations (± SD).

fixation for the conditions of the respective incubations. With values ≥0.7 the preference for HCO_3^- in *E. huxleyi* is high and further increases with light intensity. Within PFD treatments, HCO_3^- contribution was similarly high in all Ca concentrations (ANOVA, *F*-test: $p > 0.05$).

Carbon isotope fractionation—Carbon isotope fractionation showed a positive correlation with light intensity (Fig. 4; ANOVA, *F*-test: $p < 0.0001$). At high PFDs (300 and 800 μmol photons m^{-2} s^{-1}), ε_p values ranged between 10.5‰ and 12.3‰, whereas at 30 μmol photons m^{-2} s^{-1} carbon isotope fractionation was reduced to values ranging from 6.1‰ to 7.1‰. Variations in isotope fractionation with Ca concentration were small, less than 1.2‰, and showed no apparent trend over the investigated Ca range (Fig. 4; ANOVA, *F*-test: $p > 0.05$).

Discussion

The present study investigates the combined effects of varying Ca concentrations and light intensities in a calcifying strain of *E. huxleyi*. Acclimation of calcifying cells to different Ca concentrations led to different degrees of calcification within the same strain. The application of this multimethod approach provides new information on the role of calcification in *E. huxleyi*. Here we discuss the effects of varying light intensities and different Ca concentrations on calcification and photosynthesis before considering the role of calcification in photosynthetic carbon acquisition.

Growth—Cell division rates of *E. huxleyi* increased with increasing light intensity (Fig. 1a). Light-saturated growth irradiances found for *E. huxleyi* reported in Nielsen (1997) and Harris et al. (2005) are ~200 μmol photons m^{-2} s^{-1}. In accordance with these findings, we observed that cells acclimated to 300 and 800 μmol photons m^{-2} s^{-1} showed similar growth rates. This is also consistent with the previous observation that very high irradiances do not reduce growth rates in *E. huxleyi* (Nielsen 1997; Harris et al. 2005). With the exception of the 0.1 Ca treatment, Ca concentration had no effect on growth rates (Fig. 1a). Although growth rates in our study were similar to those observed in experiments that were carried out in natural seawater with the same strain of *E. huxleyi* (Rost et al. 2002; Zondervan et al. 2002), the PIC/POC ratio in the 10 Ca treatment was comparably low, with measured values of about 0.3 (Fig. 1b,c). Zondervan et al. (2002) observed PIC/POC ratios to vary between 0.3 and 1.0 under nutrient-replete conditions in this strain. We suggest that the use of artificial seawater might be a potential reason for the lower rates in our experiments, but also point out that the SEM analysis indicates normally calcified cells at high Ca concentrations.

Effect of Ca on photosynthesis—The acclimation of calcifying cells to low Ca concentrations (0.1 mmol L^{-1} in our lowest Ca concentration) did not affect their POC production (Fig. 1c), a finding that has been observed in previous investigations either by transferring decalcified cells to low Ca concentrations such as 0.1 and 1 mmol L^{-1} (Paasche 1964), by the acclimation of calcifying cells to Ca-free medium (Herfort et al. 2002), or by growing them in seawater that contains different Ca concentrations (0–

20 mmol L^{-1}; Herfort et al. 2004). These findings stand in contrast to observations by Nimer et al. (1996), who found reduced photosynthetic rates by removal of external Ca. Possible reasons for these contrasting results are difficult to assess here but may be associated with the lack of acclimation to the respective Ca concentrations in these experiments (Brownlee pers. com.) or the low temperature during centrifugation of cells (15 min at 4°C despite a growth temperature of 15°C).

Effect of Ca on calcite production—PIC production is strongly dependent on irradiance in *E. huxleyi* (e.g., Balch et al. 1992; Holligan et al. 1993; Zondervan et al. 2002). In general, light saturation irradiances for PIC production of *E. huxleyi* range from 72 to >500 μmol photons m^{-2} s^{-1} (Paasche 1964; Balch et al. 1992), which is consistent with the results of our study (Fig. 1b). PIC production of *E. huxleyi* decreased with decreasing Ca concentrations in the medium (Fig. 1b). Given the standard deviation, the PIC production can be considered to be almost nil in all 0.1 Ca treatments. The high variation in PIC production, especially under 300 μmol photons m^{-2} s^{-1}, might be due to filtration artifacts. The notion of PIC production lacking in all 0.1 Ca treatments is further supported by SEM analysis (Fig. 2a), whereas at highest Ca levels SEM analysis verified normally calcified cells.

Some response in calcification may, however, also be related to dissolution after the coccolith is extruded by the cells. Calcite dissolution can occur when the calcite saturation state (Ω) is below 1. While for the 2.5 Ca treatment Ω is greater than 1, in the 0.1 Ca treatments dissolution after calcite production is not inconceivable since Ω is 0.04. However, several lines of evidence strongly support our conclusion that reduced calcite quotas under lower Ca concentrations are the result of decreasing production rates rather than increasing dissolution. First, ^{14}C incorporation experiments by Paasche (1964) demonstrated that the low measured calcification rates under low calcium concentrations were not due to dissolution effects, but to a minimized calcification process. Even under the assumption that calcification would still occur and that every coccolith extruded would dissolve instantaneously, this has to result in a PIC content at least equivalent to the coccolith currently produced inside the coccolith vesicle. However, Paasche (1964) found that the PIC content is much lower than the equivalence of one coccolith, confirming that the low calcification rates were not due to dissolution. Moreover, SEM analysis displayed only noncalcified cells at 0.1 mmol L^{-1} Ca (Fig. 2a). If these cells were naked because of dissolution, some coccolith residues would have been visible on the filter, but this was not the case. SEM images of the 1 Ca treatment (Ω = 0.4) revealed not only that cells were fully covered with coccoliths, but also overcalcified coccospheres (images not shown) despite Ca undersaturation. This observation can be explained by the fact that coccoliths dispose of an organic membrane sheath that stabilizes the calcite against dissolution (Paasche 2001). Hence, we conclude that the cells did not calcify at 0.1 mmol Ca L^{-1} and that this finding was predominantly due to a biological effect rather than calcite dissolution.

Role of calcification under high irradiances—Various authors report that *E. huxleyi* lacks photoinhibition, even when cells are exposed to irradiances up to 1,500 μmol photons m^{-2} s^{-1} (Nielsen 1995; Nanninga and Tyrell 1996; Harris et al. 2005). To explain this unusual tolerance to high irradiance it has been proposed that calcification might serve as a metabolic protection in *E. huxleyi* when suddenly exposed to high light levels (Paasche 2001). As an energy-requiring process, calcification could provide a means to dissipate excess light energy. In the case of such a mechanism occurring under high light conditions, photoinhibition should arise when calcification ceases.

In our experiments, POC production was higher under 300 than 800 μmol photons m^{-2} s^{-1}, which indicates photoinhibition (Fig. 1c). In contrast, C_i flux assays yielded highest maximal photosynthetic rates at 800 μmol photons m^{-2} s^{-1} in all Ca treatments (Fig. 3a). These apparently conflicting results are likely to reflect the properties of the different methods applied. The C_i flux assays yield instantaneous rates of photosynthetic O_2 evolution, whereas the POC production shows integrated responses over the duration of the experiments, hence, i.e., over many days, including dark phases. Our results therefore suggest that under short-term exposure to high irradiances, *E. huxleyi* was able to maintain maximal photosynthetic rates and thus avoid photoinhibition, but over long-term exposure to high irradiances photosynthetic activity decreased.

Our results from the C_i flux assays are in agreement with various studies that observe a lack of photoinhibition under short-term exposure to high irradiances (Nielsen 1995; Nanninga and Tyrell 1996; Houdan et al. 2005). On the long-term exposure of *E. huxleyi* to high irradiances, however, only few data exist. Harris et al. (2005) showed that the POC production remained constant when cells of a noncalcifying strain of *E. huxleyi* were acclimated to high light intensities. The apparent differences from our results (Fig. 1c) may be explained by strain-specific differences, but cannot be answered conclusively here.

How, then, is the observed light-dependence of *E. huxleyi* correlated with calcification? At 800 μmol photons m^{-2} s^{-1} noncalcifying and calcifying cells showed similar POC production (Fig. 1c). Likewise, photosynthetic O_2 evolution was similar at 0.1 mmol L^{-1} and 10 mmol L^{-1} Ca (Fig. 3a). Therefore, our data do not support the hypothesis that calcification provides a means of energy dissipation under high irradiances in *E. huxleyi*. This interpretation is further supported by the observation that both low- and high-calcifying strains of *Pleurochrysis* spp. are insensitive to photoinhibition (Israel and Gonzalez 1996). Since coccospheres were lacking under 0.1 mmol L^{-1} Ca, our data further support previous studies that negated a direct role of coccoliths in photoprotection (Nanninga and Tyrell 1996; Harris et al. 2005).

Role of calcification in photosynthetic carbon acquisition—The hypothesis that photosynthesis benefits from cal-

cification by providing CO_2 is still under debate (Rost and Riebesell 2004 and references therein). Although there is increasing evidence against such a mechanism, this hypothesis seems to be widely accepted and even found its way into textbooks. We tested this theory experimentally by modifying rates of calcification and photosynthesis in *E. huxleyi* while keeping CO_2 supply constant.

Rost and Riebesell (2004) show that a calcifying and a noncalcifying strain of *E. huxleyi* are able to photosynthesize as efficiently as, or even more efficiently than, calcifying ones. Additionally, C_i flux measurements indicated that HCO_3^- uptake rates of the noncalcifying strain were even higher than in the calcifying one; thus the authors conclude that HCO_3^- utilization is not tied to calcification. Some of these findings could, however, have been associated with the different strains used. Our results were obtained from cells of the same strain and clearly show that calcification does not promote photosynthesis. Three key findings support this: (1) POC production in noncalcifying cells in comparison with calcifying cells was similar or elevated (Fig. 1c), an observation confirmed by the C_i flux assays (Fig. 3a), which yield similar or even higher maximum rates of photosynthetic O_2 evolution in noncalcifying cells. (2) According to the assumption that calcification promotes photosynthesis by providing a mechanism to access HCO_3^-, it follows that calcifying cells should have higher HCO_3^- uptake rates than noncalcifying ones. However, independent of the Ca treatments and hence the degree of calcification, the contribution of HCO_3^- uptake relative to carbon net fixation was constant (Fig. 3b). (3) Carbon isotope fractionation was used as a tool to investigate whether calcification is channeling inorganic carbon to photosynthesis. The carbon source for calcification, HCO_3^-, is about 8‰ to 10‰ enriched in ^{13}C compared with CO_2 (*see* Zeebe and Wolf-Gladrow 2001). Assuming CO_2 is produced internally via calcification (Eq. 1), it should have the same isotopic signature as the HCO_3^- previously taken up. In the case of the suggested coupling, higher fractionation values should be expected under low Ca concentrations when supposedly less HCO_3^- is used for photosynthesis. Fractionation within light levels did not, however, differ despite the large differences in Ca concentration (Fig. 4), indicating no effect of calcification on HCO_3^- use in *E. huxleyi*.

Our findings are in accordance with Paasche (1964) and Herfort et al. (2002, 2004), who performed ^{14}C incorporation experiments with *E. huxleyi*. They observed that photosynthesis in *E. huxleyi* remained constant when calcification ceased. These results bear an interesting resemblance to hermatypic corals (Gattuso et al. 2000), in which photosynthesis was also not found to be coupled to calcification. Further evidence supporting our results comes from Balch et al. (1996), who demonstrated that calcification is decoupled from photosynthesis under steady-state light-limited growth in *E. huxelyi*. Finally, the relatively high pH required for calcite precipitation represents another argument against the hypothesis that photosynthesis benefits from calcification. The pH in the coccolith vesicle of *Coccolithus pelagicus* was up to 8.3 while the pH in the cytosol was measured to be only 7.0 (Anning et al. 1996). As a consequence of this large pH difference, the coccolith vesicle would rather act as a CO_2 sink as opposed to supplying photosynthesis with additional inorganic carbon. Overall, on the basis of our data and that of previous investigations, we conclude that calcification is not involved in photosynthetic carbon acquisition of *E. huxleyi*.

In conclusion, the results of this study provide new information on the role of calcification in *E. huxleyi* and confirm findings of earlier work: (1) calcification does not provide a means of energy dissipation under high irradiances, and (2) calcification is not involved in photosynthetic carbon acquisition. These findings prompt further investigations, especially with regard to the light dependence of *E. huxleyi* and how it is affected by long- and short-term exposure to high irradiances. Furthermore, it is important to test other coccolithophore species in this respect.

The data presented here have interesting implications concerning the effects of ocean acidification on coccolithophores. It has been suggested that calcification will decrease under elevated atmospheric CO_2 levels as a result of altered carbonate chemistry (Wolf-Gladrow et al. 1999; Riebesell et al. 2000). A reduction in the degree of calcification is assumed to put coccolithophores at an ecological disadvantage, suggesting a rather "grim future" for this group of phytoplankton. However, our data demonstrate that cells perform equally well in terms of growth rate or photosynthesis despite different degrees of calcification. Therefore an understanding of calcification will be key to assessing the effects of global change on the ecological dynamics of coccolithophore communities in the future.

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