Biogeosciences Discuss., 11, 1021–1051, 2014
www.biogeosciences-discuss.net/11/1021/2014/
doi:10.5194/bgd-11-1021-2014

Biogeosciences Discussions Open Access

High temperature decreases the PIC / POC ratio and increases phosphorus requirements in *Coccolithus pelagicus* (Haptophyta)

A. C. Gerecht[1], L. Šupraha[2], B. Edvardsen[3], I. Probert[4], and J. Henderiks[1,2]

[1]CEES, Dept. of Biosciences, University of Oslo, P.O. Box 1066 Blindern, 0316 Oslo, Norway
[2]Palaeobiology, Dept. of Earth Sciences, Uppsala University, Villavägen 16, 75236 Uppsala, Sweden
[3]Marine Biology, Dept. of Biosciences, University of Oslo, P.O. Box 1066 Blindern, 0316 Oslo, Norway
[4]Station Biologique de Roscoff, CNRS/UPMC, Place G. Teissier, 29680 Roscoff, France

Received: 22 November 2013 – Accepted: 20 December 2013 – Published: 16 January 2014

Correspondence to: A. C. Gerecht (a.c.gerecht@ibv.uio.no)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Rising ocean temperatures will likely increase stratification of the water column and reduce nutrient input into the photic zone. This will increase the likelihood of nutrient limitation in marine microalgae, leading to changes in the abundance and composition of phytoplankton communities, which in turn will affect global biogeochemical cycles. Calcifying algae, such as coccolithophores, influence the carbon cycle by fixing CO_2 into particulate organic carbon (POC) through photosynthesis and into particulate inorganic carbon (PIC) through calcification. As calcification produces a net release of CO_2, the ratio of PIC / POC determines whether coccolithophores act as a source (PIC / POC > 1) or a sink (PIC / POC < 1) of atmospheric CO_2. We studied the effect of phosphorus (P-) limitation and temperature stress on the physiology and PIC / POC ratios of two subspecies of *Coccolithus pelagicus*. This large and heavily calcified species (PIC / POC generally > 1.5) is a major contributor to calcite export from the photic zone into deep-sea reservoirs. Phosphorus limitation did not influence exponential growth rates in either subspecies, but P-limited cells had significantly lower cellular P-content. A 5 °C temperature increase did not affect exponential growth rates either, but nearly doubled cellular P-content under both high and low phosphate availability. The PIC / POC ratios did not differ between P-limited and nutrient-replete cultures, but at elevated temperature (from 10 to 15 °C) PIC / POC ratios decreased by 40–60 %. Our results suggest that elevated temperature may intensify P-limitation due to a higher P-requirement to maintain growth and POC production rates, possibly reducing abundances in a warmer ocean. Under such a scenario *C. pelagicus* may decrease its calcification rate relative to photosynthesis, resulting in PIC / POC ratios < 1 and favouring CO_2-sequestration over release. Phosphorus limitation by itself is unlikely to cause changes in the PIC / POC ratio in this species.

BGD
11, 1021–1051, 2014

1 Introduction

Coccolithophores represent a prominent functional group of marine phytoplankton and are major contributors to the carbon cycle. These eukaryotic microalgae fix CO_2 into particulate organic carbon (POC) through photosynthesis and into particulate inorganic carbon (PIC) through calcification. Although removing carbon from seawater, the production of calcite scales (coccoliths) is a net source of CO_2 to the environment (Gattuso et al., 1995; Rost and Riebsell, 2004). Therefore, the ratio of calcification to photosynthesis (PIC/POC ratio) determines whether coccolithophores act as a source or sink of atmospheric CO_2 (Balch et al., 1991; Holligan et al., 1993; Buitenhuis et al., 1996). The ballasting of organic matter (POC) by coccoliths (PIC) is believed to be an efficient way of transporting carbon out of the photic zone (Armstrong et al., 2002; Klaas and Archer, 2002), contributing to the drawdown of atmospheric CO_2 on longer time scales. In carbon cycle models, the PIC/POC ratio is therefore used as a measure for carbon export into sedimentary reservoirs (Archer, 1991; Ridgwell et al., 2009).

Various environmental factors affect POC and PIC production in coccolithophores. Elevated CO_2 may increase photosynthesis (Beardall and Raven, 2004) whereas calcification is generally reduced at higher CO_2-concentrations (Riebesell et al., 2000; Feng et al., 2008; Findlay et al., 2011; Krug et al., 2011). Rising ocean temperatures may directly impact POC and PIC production in coccolithophores (Paasche, 2002) and increase the likelihood of nutrient limitation via a more stratified water column (Sarmiento et al., 2004). To accurately model the effect of a changing climate on ocean-atmosphere CO_2-exchange, it is important to constrain the PIC/POC ratio of coccolithophores. In the cosmopolitan species *Emiliania huxleyi*, nutrient limitation has been shown to increase PIC/POC ratios (e.g. Paasche and Brubak, 1994; Paasche, 1998; Riegman et al., 2000), whereas a recent study by Langer et al. (2012) showed PIC/POC ratios in *Calcidiscus leptoporus* to be insensitive to nutrient limitation. Given the species-specific response to ocean acidification (Langer et al., 2006; Krug et al., 2011), it is necessary to examine more species and lineages of coccolithophores to gain a more

BGD
11, 1021–1051, 2014

general view of the possible responses of coccolithophores to changing nutrient availability.

Coccolithus pelagicus (Wallich) Schiller, 1930 is one of the largest and most heavily calcified extant coccolithophores with PIC/POC ratios generally > 1.5 (Langer et al., 2006; Krug et al., 2011) and calcite weights of individual coccoliths at least two orders of magnitude larger than those of coccoliths produced by the smaller *E. huxleyi* (Beaufort and Heussner, 1999; Young and Ziveri, 2000; Cubillos et al., 2012). Hence, this species is an important contributor to calcite export into the sediments (Broerse et al., 2000). As *C. pelagicus* calcifies above unity (PIC/POC ratio > 1), CO_2-release to the environment should be favoured over CO_2-sequestration and blooms of this species may be a potential source of CO_2 to the atmosphere. We investigated the combined effect of phosphorus (P-) limitation and elevated temperature on the physiology, elemental quotas, and PIC/POC ratio of this species. *Coccolithus pelagicus* is divided into two morphotypes on the basis of heterococcolith size (Geisen et al., 2002; Sáez et al., 2003). We here use the division of the two morphotypes into subspecies, *Coccolithus pelagicus* ssp. *braarudii* (Gaarder) Geisen et al., 2002 and *Coccolithus pelagicus* ssp. *pelagicus*, according to Geisen et al. (2002), but genetic infomation suggests that they are separated at the species level (Sáez et al., 2003). The subarctic morphotype *C. pelagicus* ssp. *pelagicus* can form substantial blooms in the North Atlantic and North Pacific regions (e.g. Winter et al., 1994; Ziveri et al., 2004), while the temperate morphotype *C. pelagicus* ssp. *braarudii* is common in coastal upwelling regions of the Northeast and Southeast Atlantic (e.g. Cachão and Moita, 2000; Henderiks et al., 2012). One strain of each subspecies was grown in batch culture under phosphate-limited and nutrient-replete conditions and at two temperatures for ssp. *pelagicus* to test for physiological differences between the two subspecies. Calcification was evaluated by PIC quota of cells, individual coccolith volumes and the occurrence of coccolith malformations. Growth rate and POC quota of cells were determined to monitor POC production.

BGD
11, 1021–1051, 2014

2 Methods

2.1 Experimental design

Two strains of *C. pelagicus* were obtained from the Roscoff Culture Collection. Strain RCC1200 is a clone of *C. pelagicus* ssp. *braarudii* and was isolated from the South Atlantic offshore Namibia. Strain J23 is a clone of *C. pelagicus* ssp. *pelagicus* and was isolated from the North Atlantic near Scotland. Clonal batch cultures were grown in triplicate in sterile-filtered $K/2$ (–Tris, –Si) medium (Keller et al., 1987; with modifications recommended by I. Probert) at two initial phosphate concentrations. Aged natural sea water from the Oslofjord was enriched with 160 µM nitrate and phosphate concentrations of either 10 µM (high-P treatment) or 1 µM (low-P treatment). Low-P cultures were expected to become P-limited, whereas the high-P control treatment ensured exponential nutrient-replete growth throughout the experiment. Trace metal and chelator enrichments corresponded to $K/2$ medium with the following modifications: omission of Tris and Si, addition of $NiCl_2 \bullet 6H_2O$ (3.14 nM), and increase of EDTA-concentrations (5.85 µM). Vitamins were added according to $f/2$ medium (Guillard, 1975). Each subspecies was cultured near its respective isolation temperature, ssp. *braarudii* at 15 °C and spp. *pelagicus* at 10 °C. The latter was also subjected to high-P and low-P treatments at elevated temperature (15 °C).

Cells were acclimated to culture conditions for at least 10 generations before starting the experiment. Cultures were kept in culture flasks (350 mL, BD Biosciences, USA) in an environmental test chamber (MLR-350, Panasonic, Japan) on a 12:12 h light:dark cycle at an irradiance of ~ 100 µmol photons $m^{-2} s^{-1}$. Culture flasks were agitated manually on a daily basis. Two paired experiments (high-P and low-P treatments) of three replicate cultures each were carried out for ssp. *braarudii* (15 °C) and ssp. *pelagicus* (10 °C). Additionally, one paired high-P and low-P experiment was performed in triplicate with ssp. *pelagicus* at 15 °C. Low-P cultures were sampled upon reaching stationary phase, here defined as zero growth rate, and high-P cultures were sampled at similar cell densities while still in exponential phase. This sampling method ensured

BGD
11, 1021–1051, 2014

similar carbonate chemistry between the treatments. Cell densities were determined daily on an electronic particle counter (CASY, Roche Diagnostics, Switzerland) two hours after the onset of the light phase. Exponential growth rates (μ_{max}) were calculated by linear regression of cell densities in exponential phase over time.

High-P and low-P culture media were sampled for initial chemistry (T_0, Table 1). Upon sampling the cultures, growth media were again sampled to analyse carbonate chemistry parameters and residual phosphate concentrations at time of sampling (T_{sample}, Table 1). Culture samples were collected for elemental analysis (particulate organic phosphorus (POP), particulate nitrogen (PN), particulate organic carbon (POC), and total particulate carbon (TPC)), for morphological analysis of coccoliths by polarized light (POL), and for measuring coccosphere diameters, coccolith coverage, and coccolith malformations by scanning electron microscopy (SEM).

2.2 Medium chemistry

2.2.1 Residual phosphate

Culture medium was sterile filtered (0.2 µm, Filtropur) into plastic scintillation vials (Kartell, Germany) and stored at −20 °C until analysis. Orthophosphate concentrations were determined colorimetrically on a spectrophotometer (UV 2550, Shimadzu, Japan) as molybdate reactive phosphate following Murphy and Riley (1962) with a precision of ±4 %. Residual phosphate concentrations near or below the detection limit (~ 0.05 µM) confirmed that low-P cultures were P-limited upon sampling (T_{sample}, Table 1), whereas phosphate concentrations of high-P cultures remained > 6 µM.

2.2.2 Carbonate chemistry

Samples for total alkalinity (A_T) and pH were filtered through GF/F-filters (Whatman, GE Healthcare, UK), stored airtight at 4 °C and analysed within 24 h. A_T was calculated from Gran plots (Gran, 1952) after duplicate potentiometric titration using an

BGD
11, 1021–1051, 2014

automatic titrator (TTA 80 Titration assembly, Radiometer, Denmark) or manual titration with a precision of ±5 %. The pH was measured by a combined electrode (Red Rod, Radiometer, Denmark) which was two-point calibrated to NBS scale (precision ±0.4 %). The carbonate system was calculated using the program CO2sys (version 2.1 developed for MS Excel by D. Pierrot from E. Lewis and D. W. R. Wallace) using the dissociation constants for carbonic acid of Roy et al. (1993).

The initial carbonate chemistry of the medium (T_0, Table 1) was modified by culture growth in both P-treatments with a drawdown of alkalinity by 31–36 % in ssp. *braarudii* and of 22–35 % in ssp. *pelagicus* (T_{sample}, Table 1). The availability of dissolved inorganic carbon (DIC) was reduced by up to 37 % and there was a shift towards lower pH-values in all cultures from an initial pH = 8.09 ± 0.07 to a minimum pH = 7.79. One high-P treatment (three replicate cultures) of ssp. *pelagicus* grown at 10 °C showed a strong shift in the system towards pH = 7.23. This resulted in an omega-value below one whereas all other treatments were saturated in carbonate (omega > 1, Table 1). This low pH-value likely represents a technical error (no other samples were measured on that same day) rather than an actual shift in the system because the culture parameters of this high-P treatment were otherwise very similar to the other high-P treatment of ssp. *pelagicus* at 10 °C. The carbonate system parameters measured in these three replicates are therefore not included in Table 1.

2.3 Elemental quotas and ratios

2.3.1 Particulate organic phosphorus

Samples for POP were filtered onto precombusted (500 °C, ~ 2 h) GF/C-filters (Whatman) and stored at −20 °C. They were converted to orthophosphate by oxidative hydrolysis with potassium persulfate under high pressure and temperature in an autoclave (3150EL, Tuttnauer, Netherlands) according to Menzel and Corwin (1965). They were then quantified as molybdate reactive phosphate as described in Sect. 2.2.1.

BGD
11, 1021–1051, 2014

2.3.2 Particulate total and organic carbon

Samples for TPC, POC and PN were filtered onto precombusted GF/C-filters and dried at 60 °C overnight in a drying oven and stored in a desiccator until analysis on an elemental analyser (Flash 1112, Thermo Finnegan, USA, detection limit 2 µg) with a precision of ±8 %. For POC filters PIC was removed by pipetting 230 µL 2M HCl onto the filters before analysis (Langer et al., 2009) and PIC was calculated as the difference between TPC and POC. Data was only available for one paired experiment of high-P and low-P cultures of each subspecies (n=3). Elemental quotas were compared using a *t* test and one-way ANOVA with GraphPad Prism version 6 for Windows (GraphPad Software, USA).

For high-P cultures (sampled in exponential phase), cellular production rates were calculated from exponential growth rates (μ_{max}) and their respective cellular quota. This was not done for low-P cultures as cells were sampled in stationary phase (zero growth rate). Growth rate (μ_{max}), measured in exponential phase, can therefore not be directly coupled with cellular quota, measured in stationary phase (see Langer et al., 2012 for a deeper discussion). Cellular quotas and ratios were therefore used in our comparisons between high-P and low-P cultures.

2.4 Coccosphere and coccolith morphology

2.4.1 Coccolith dimensions

Samples for POL were filtered onto cellulose nitrate filters (0.8 µm, Whatman) after dispersing coccoliths by a Triton-NaOCl treatment (Paasche et al., 1996). Filters were dried at 60 °C in a drying oven, mounted with Canada Balsam (Merck, USA) on microscope slides and viewed under crossed polarizers with a DM6000B Leica microscope, which had a modified turret accommodating polarizing filters at different angles (Beaufort et al., 2014). Individual coccoliths were randomly selected and captured at 1000× magnification with a SPOT Flex colour digital camera (Diagnostic Instruments Inc.,

BGD
11, 1021–1051, 2014

USA) and a compound 8-bit greyscale image created within SYRACO software (Beaufort et al., 2014). A custom-made macro in ImageJ freeware measured coccolith length through an ellipse approximation, as well as mean grey level (MGL) of the selected area measured in intensity values of 0 (black) to 255 (white). MGL was used as a proxy for a change in coccolith thickness based on the birefringence of calcite (Beaufort, 2005). Coccolith length measured by this method considers the proximal shield only as the larger distal shield is non-birefringent under crossed polarizers (e.g. Cubillos et al., 2012).

2.4.2 Coccolith malformations and coccosphere dimensions

Samples for SEM were filtered onto polycarbonate filters (0.8 µm, Cyclopore, Whatman), air-dried and sputter-coated with gold-palladium. Imaging was performed with an S-4800 field emission scanning electron microscope (Hitachi, Japan) at 2500× magnification. Coccolith morphology was classified into four categories: normal, incomplete (normal, but incomplete distal shield elements), malformed (more than one malformed distal shield element), and very malformed (blocky structures or holes). Coccosphere diameter and the number of coccoliths covering one sphere were estimated from the same calibrated SEM images.

3 Results

3.1 Phosphorus limited growth

Division rates during exponential growth were comparable between high-P and low-P treatments for both subspecies (Table 2). The temperate morphotype ssp. *braarudii* had higher maximum growth rates ($0.44 \pm 0.06\,d^{-1}$) than the subarctic morphotype ssp. *pelagicus* ($0.32 \pm 0.05\,d^{-1}$). In low-P medium ssp. *braarudii* (RCC1200) grew to final cell concentrations of $1.3 \pm 0.2 \times 10^4$ cells mL^{-1} (Table 2). Subspecies *pelagicus* (J23) grew

BGD
11, 1021–1051, 2014

to similar final cell concentrations of $1.3 \pm 0.1 \times 10^4$ cells mL^{-1} at 10 °C. At 15 °C, however, stationary phase was reached at lower cell densities ($1.0 \pm 0.1 \times 10^4$ cells mL^{-1}) even though exponential growth rates were the same between the two temperatures. All low-P cultures were P-limited at the time of sampling as demonstrated by lower particulate organic phosphorus (POP) quotas and higher C/P and N/P ratios compared to high-P treatments (Table 2, Fig. 1a). The two subspecies had the same POP quotas when comparing low-P and high-P cultures, respectively. POP production, however, was lower in ssp. *pelagicus* than in ssp. *braarudii* because of lower growth rates (Table 2, Fig. 1b). Most striking is the high POP quota of ssp. *pelagicus* cultures grown at high temperature (15 °C). POP quotas almost doubled in both low-P and high-P treatments and POP production rates in high-P cultures were more than doubled at 15 °C, indicating a higher requirement for phosphorus at elevated temperature.

3.2 Cellular carbon quotas and ratios

Particulate organic carbon (POC) quotas of cells were similar between low-P and high-P treatments for both subspecies (*t* test: $p > 0.05$, Table 2, Fig. 2a). In ssp. *pelagicus*, temperature did not have a significant effect on POC quota either (*t* test: $p > 0.05$). Interestingly, ssp. *pelagicus* cells contained ca. 25 % more POC than cells of ssp. *braarudii* which were larger in terms of coccosphere size (Fig. 4). Particulate inorganic carbon (PIC) quotas were not affected either by P-limitation (*t* test: $p > 0.05$). However, there was a significant reduction in PIC quota in ssp. *pelagicus* at high temperature under both P-treatments (*t* test: $p < 0.001$, Fig. 2b) and in consequence, PIC/POC ratios were below unity (PIC/POC < 1) at 10 °C compared to PIC/POC ratios of 1.4 ± 0.2 at 15 °C (Fig. 2c). The decrease was strongest (*t* test, $p = 0.009$) in exponentially growing high-P cultures which had a reduced PIC/POC ratio of 0.5 ± 0.2 compared to a PIC/POC ratio of 0.9 ± 0.1 in P-limited cultures (*t* test, $p = 0.003$). Reduced PIC/POC ratios at high temperature were due to a decrease in PIC production rates whereas POC production rates were unaffected (Fig. 3).

BGD
11, 1021–1051, 2014

3.3 Coccosphere and coccolith dimensions

The average coccosphere diameter of ssp. *pelagicus* was smaller (18.1 ± 1.9 µm) than that of ssp. *braarudii* (19.9 ± 2.0 µm) (Fig. 4). Phosphorus limitation led to an increase in coccosphere size in ssp. *pelagicus*, but not ssp. *braarudii*. Temperature, on the other hand, decreased coccosphere size in ssp. *pelagicus*. These smaller spheres were composed of fewer coccoliths (12 ± 3 liths sphere^{-1} in high-P cultures) compared to spheres of cultures grown at 10 °C (15 ± 3 liths sphere^{-1} in high-P cultures).

In both subspecies, the mean length of the proximal shield (PSL) was unaffected by changes in P-availability. Temperature did not change mean PSL in ssp. *pelagicus* either (*t* test: $p > 0.05$, Fig. 5). Yet, PSL showed a wide range of 6.4 to 14 µm in ssp. *braarudii* and 5.8–12 µm in ssp. *pelagicus* at 10 °C. Mean grey level (MGL), a proxy for coccolith thickness, did not change due to P-availability or temperature (*t* test, $p > 0.05$). Coccoliths produced by ssp. *pelagicus* were significantly smaller than those produced by ssp. *braarudii* (*t* test, $p < 0.001$).

3.4 Coccolith malformations

Scanning electron microscopy revealed a background percentage of malformed coccoliths in high-P cultures of almost 35 % in ssp. *braarudii* (Fig. 6). This percentage increased to almost 50 % under P-limitation, mainly due to an increased presence of coccoliths with blocky structures or holes, classified as "very malformed" (Fig. 6). In ssp. *pelagicus*, the background percentage of malformed coccoliths in high-P cultures grown at 10 °C was ca. 40 % with a lower percentage of incomplete coccoliths than ssp. *braarudii* (Fig. 6). Malformations increased to ca. 50 % under P-limitation and to ca. 55 % due to elevated temperature (15 °C). The highest abundance of malformed coccoliths (ca. 2/3 of all coccoliths) was observed in ssp. *pelagicus* cultures grown under P-limitation and high temperature with ca. 1/3 of coccoliths classified as "very malformed".

BGD
11, 1021–1051, 2014

4 Discussion

Laboratory experiments are useful to systematically test the short-term physiological (plastic) responses of coccolithophores to various environmental factors and could constrain how PIC/POC ratios are likely to change under future climate scenarios. In *Emiliania huxleyi*, nutrient limitation has been shown to generally increase PIC/POC ratios (Paasche and Brubak, 1994; Riegman et al., 2000) by increasing coccolith production (Balch et al., 1993) and inducing the formation of multiple coccolith layers around the cell (Linschooten et al., 1991; Paasche, 1998). In the field, increased ratios of loose coccoliths to coccospheres have been ascribed to decreasing nutrient availability (Balch et al., 1991; Fernández et al., 1993; van der Wal et al., 1994) and mesocosm studies have shown that calcification continues after POC fixation ceases due to nutrient exhaustion (van Bleijswijk et al., 1994). However, little is known about the effect of nutrient limitation on other species and the present data set is the first to specifically test P-limitation and temperature for their effect on the PIC/POC ratio of *C. pelagicus*.

4.1 Effect of P-limitation and temperature on culture growth and phosphorus quota

Initial phosphate concentrations of the growth medium did not influence cell division in exponential phase in either subspecies. The exponential growth rate of ssp. *braarudii* was similar to those determined previously under similar experimental conditions (Taylor et al., 2007; Buitenhuis et al., 2008). Although other studies have observed higher exponential growth rates (up to 0.7 d^{-1}) for the same strain (Langer et al., 2006; Krug et al., 2011), these authors used higher irradiance and a longer light phase which probably increased cell division rates. The subarctic subspecies (ssp. *pelagicus*) had a lower exponential growth rate than the temperate ssp. *braarudii*, even when these two subspecies were grown at the same temperature (15 °C). This may be due to subspecies or strain-specific differences as have been described for different strains of *E. huxleyi* (Langer et al., 2009).

BGD
11, 1021–1051, 2014

We found clear evidence for P-limitation with significantly lower POP quota and higher C/P- and N/P-ratios in low-P cultures. The doubled POP quota of ssp. *pelagicus*, grown at 15 instead of 10 °C, points towards higher phosphorus requirements at elevated temperature. Because of this, the same phosphate supply (1 µM) in low-P cultures supported lower final cell densities at 15 °C although exponential growth rates were unaffected. This increased requirement for phosphorus under temperature stress may be linked to increased energy demands and deserves further study as an increased requirement for phosphorus at high temperature could exacerbate nutrient limitation in a warmer ocean (Sarmiento et al., 2004).

4.2 Effect of P-limitation and temperature on PIC/POC ratios

Temperature had a stronger effect on PIC/POC ratios than P-availability. Whereas P-limitation did not affect PIC/POC ratios in either subspecies, elevated temperature decreased PIC/POC ratios by almost 2/3 in ssp. *pelagicus*. PIC/POC ratios were below unity (< 1) in both high-P and low-P treatments at high temperature (15 °C). This decrease in PIC/POC ratios was driven by the 40–60 % decrease in PIC quota whereas POC quotas were similar between the two temperatures. In high-P cultures, POC production was sustained at elevated temperature whereas PIC production declined by ca. 50 %. This sharp reduction in PIC quota and production at elevated temperature in ssp. *pelagicus* points towards either a lower production of coccoliths or a decreased calcite content of individual coccoliths. Although the variation in coccolith calcite content was high within one strain, the mean calcite content was not affected by the tested environmental parameters (see also Sect. 4.3) and can therefore not explain the reduced PIC quota. Light microscope images showed that high-P cultures from ssp. *pelagicus* at elevated temperature contained ca. 12 % naked cells whereas no naked cells where observed at 10 °C. The coccolith coverage estimated by scanning electron microscopy was 12 coccoliths sphere^{-1} at 15 °C compared to 15 coccoliths sphere^{-1} at 10 °C, indicating reduced coccolith coverage in the high temperature treatment. Total coccolith counts from POL samples confirmed that cultures at 15 °C contained ca. 40 % fewer

BGD
11, 1021–1051, 2014

coccoliths than cultures at 10 °C which explains at least partly the ca. 60 % reduction in PIC quotas at high temperature. It therefore seems that reduced PIC production at elevated temperature led to cells being covered by fewer coccoliths with an increased percentage of naked cells.

Within the temperature-stress treatment, PIC/POC ratios, although still below unity (< 1), were significantly higher under P-limitation due to higher PIC quota. This seems counterintuitive considering that at 10 °C there was no effect of P-limitation on PIC quota in ssp. *pelagicus*. However, the offset between high-P and low-P cultures may be due to these treatments being sampled in different growth phases. High-P cultures were sampled in exponential phase when resources were unlimited and likely relegated to both POC production and, albeit to a reduced extent at this temperature, to PIC production. Low-P cultures on the other hand were sampled in stationary phase when cell division and POC production had stopped due to the depletion of phosphate from the medium. Assuming a stronger constraint on POC production by phosphorus than on PIC production, cells probably continued producing coccoliths in stationary phase increasing coccolith coverage of the cells and increasing PIC quota. Accordingly, Riegman et al. (2000) showed that increased PIC/POC ratios of *E. huxleyi* growing in P-limited continuous culture were not due to a positive enhancement of calcification, but rather due to a decrease in POC production. Calculating POC and PIC production rates requires a continuous or semi-continuous culturing setup as production rates cannot be calculated in batch culture due to the changing division rates as the cultures go through different growth phases (Langer et al., 2012).

Phosphorus limitation did not affect PIC/POC ratios in *C. pelagicus*. This is in sharp contrast to the described increase in PIC/POC ratios in *E. huxleyi* under nutrient limitation (e.g. Paasche and Brubak, 1994; Riegman et al., 2000). The maximum PIC/POC ratios reported in this study are close to the maximum values (~ 1.7) reported in the literature for this species (Langer et al., 2006; Krug et al., 2011) and are well below maximum values described for *E. huxleyi* (up to 2.3, van Bleijswijk et al., 1994). However, *C. pelagicus* has so far not been described to produce multiple

BGD
11, 1021–1051, 2014

coccolith layers (this study; Gibbs et al., 2013), whereas *E. huxleyi* can more than double its PIC/POC ratio by forming multiple coccolith layers (Linschooten et al., 1991; Paasche, 1998). There may therefore be an upper constraint on PIC/POC ratios in species such as *C. pelagicus* dictated by cell geometry. This species cannot increase PIC quota without increasing the surface area necessary to accommodate new coccoliths. However, increasing surface area would entail an increase in cell volume and POC quota, likely stabilizing maximum PIC/POC ratios. It is interesting to note that under ocean acidification scenarios, PIC/POC ratios in *C. pelagicus* also remained unaffected (Langer et al., 2006), unless very high CO_2-concentrations (~ 1800 µatm) were applied which led to a decrease in PIC/POC ratios driven by decreased PIC quota (Krug et al., 2011), similar to the effect of elevated temperature observed in this study.

4.3 Effect of P-limitation and temperature on coccolith morphology

The two subspecies overlapped in their size distribution, both in regard to coccosphere diameters and coccolith length. Overlapping coccolith size ranges have been previously described for these two subspecies, and our results confirm that ssp. *pelagicus* is the smaller morphotype of the two (Baumann et al., 2000; Geisen et al., 2002; Cubillos et al., 2012). There was no effect of either temperature or P-limitation on the mean coccolith volume and the same amount of calcite was fixed into individual coccoliths independent of the tested abiotic factors. Gibbs et al. (2013) have shown that mean coccolith size in *C. pelagicus* ssp. *braarudii* does not change during different growth phases and in *E. huxleyi*, coccolith dimensions have been described as remaining constant among different dilution rates in N-limited cyclostats (Fritz, 1999). It therefore seems that within one strain, mean coccolith volume is not a plastic trait.

Using a conversion factor for mean grey level (MGL) to coccolith thickness (Beaufort, 2014), we estimated mean coccolith calcite weight for the two subspecies. Subspecies *braarudii* produced coccoliths with an average weight of 117 ± 34 pg calcite whereas ssp. *pelagicus* coccoliths were lighter at 100 ± 27 pg calcite. These estimates are lower

BGD
11, 1021–1051, 2014

than the estimates obtained when using the shape factor of Young and Ziveri (2000). By means of a species-specific shape factor (0.06 for *C. pelagicus*), mean coccolith weight is estimated from the proximal shield length (PSL), rendering values of 159 ± 54 pg calcite for ssp. *braarudii* and 120 ± 44 pg calcite for ssp. *pelagicus*. The main reason for this offset is that *Coccolithus* coccoliths are not entirely birefringent (Beaufort, 2005; Cubillos et al., 2012). Therefore calculations based on birefringence will underestimate absolute weight but are useful for comparative purposes.

Malformation of coccoliths was generally high in all cultures (~ 40 % in high-P cultures) and incomplete coccoliths also occurred frequently, especially in ssp. *braarudii* (~ 30 % in high-P cultures). The presence of malformations in control cultures can probably be ascribed to as yet unknown culture artefacts (Langer and Benner, 2009). These authors described a high degree of malformation in control cultures for *E. huxleyi* while Langer et al. (2012) reported up to 53 % malformed coccoliths in control cultures of *Calcidiscus leptoporus*. Although Rickaby et al. (2010) observed low levels of background malformation (~ 20 %) in the same ssp. *braarudii* strain as used in this study (RCC1200), Langer et al. (2006) described only 50 % of coccoliths to be normal in this strain, similar to our findings. Phosphorus limitation increased the percentage of malformed coccoliths further which has also been described for *E. huxleyi* (Båtvik et al., 1997; Paasche, 1998). In ssp. *pelagicus*, elevated temperature had a stronger effect than P-limitation on coccolith morphology. The percentage of normal coccoliths in high-P cultures of ssp. *pelagicus* went down to ca. 30 % at 15 °C. This temperature was probably outside of the optimal temperature range for this subarctic strain and increased malformations could have been related to a stress response to high temperature. The highest degree of malformation was observed in ssp. *pelagicus* cultures faced with both P-limitation and temperature stress indicating that several environmental stressors can add up to compromise coccolith morphology.

BGD
11, 1021–1051, 2014

5 Conclusions

The two strains of *C. pelagicus*, representing the two recognized subspecies, responded in the same manner to P-limitation, although exponential growth rates differed. Phosphorus limited cells of *C. pelagicus* had consistently lower phosphorus quota, but showed no change in PIC/POC ratios when grown near the subspecies' respective isolation temperature. This is in contrast to *E. huxleyi* in which nutrient limitation increases PIC/POC ratios. Experimental conditions did not affect mean coccolith calcite content and as *C. pelagicus* does not produce multiple coccolith layers, there is an upper constraint to the PIC/POC ratio. Coccoliths exhibited increased malformations under both P-limitation and elevated temperature, with the highest percentage of malformed coccoliths observed under combined P-limitation and temperature stress. Temperature stress had a strong impact on PIC/POC ratios which decreased by 40–60 %, most likely due to a reduced production of coccoliths. Elevated temperature also doubled phosphorus quota indicating an increased P-requirement to maintain similar growth and POC production rates. Thus, warmer ocean temperatures may reinforce P-limitation in natural populations, potentially leading to lower cell densities as species- and even strain-specific sensitivities to climate change will contribute to changes in the abundance and composition of phytoplankton communities (Thomas et al., 2012; Barnard et al., 2004), affecting global biogeochemical cycles. Species-specific differences, as those described here for the PIC/POC ratios in *C. pelagicus* and *E. huxleyi* need to be taken into consideration when predicting general responses of coccolithophores to changing environmental parameters and possible changes in CO_2-feedback to the atmosphere. It is unlikely that a more nutrient poor ocean will increase CO_2-release by *C. pelagicus* as this species does not increase calcification over POC production when faced by nutrient limitation. However, as PIC production and CO_2-release are already favoured in this species (PIC/POC > 1), changes in the abundance of this species may be of greater importance to feedback mechanisms of ocean-atmosphere CO_2-exchange than changes in the PIC/POC ratio.

BGD
11, 1021–1051, 2014

Acknowledgements. This research was funded by the Research Council of Norway (FRIMED-BIO project 197823) and the Royal Swedish Academy of Sciences through a grant from the Knut and Alice Wallenberg Foundation (KAW 2009.0287). Preliminary experiments at the Biological Station Roscoff were supported by an FP7 ASSEMBLE research grant. We thank Sissel Brubak, Vladislava Hostyeva, and Viljar Skylstad for laboratory support and Berit Kaasa for running CN-analyses.

References

Archer, D.: Modeling the calcite lysocline, J. Geophys. Res., 96, 17037–17050, 1991.

Armstrong, R. A., Lee, C., Hedges, J. I., Honjo, S., and Wakeham, S. G.: A new, mechanistic model for organic carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals, Deep-Sea Res. Pt. II, 49, 219–236, 2002.

Balch, W. M., Holligan, P. M., and Ackleson, S. G.: Biological and optical properties of mesoscale coccolithophore blooms in the Gulf of Maine, Limnol. Oceanogr., 36, 629–643, 1991.

Balch, W. M., Kilpatrick, K. A., and Holligan, P. M.: Coccolith production and detachment by *Emiliania huxleyi* (Prymnesiophyceae), J. Phycol., 29, 566–575, 1993.

Barnard, R., Batten, S. D., Beaugrand, G., Buckland, C., Conway, D. V. P., Edwards, M., Finlayson, J., Gregory, L. W., Halliday, N. C., John, A. W. G., Johns, D. G., Johnson, A. D., Jonas, T. D., Lindley, J. A., Nyman, J., Pritchard, P., Reid, P. C., Richardson, A. J., Saxby, R. E., Sidey, J., Smith, M. A., Stevens, D. P., Taylor, C. M., Tranter, P. R. G., Walne, A. W., Wootton, M., Wotton, C. O. M., and Wright, J. C.: Continuous plankton records: Plankton atlas of the North Atlantic Ocean (1958–1999), II. Biogeographical charts, Mar. Ecol.-Prog. Ser., Supplement, 11–75, 2004.

Baumann, K.-H., Young, J. R., Cachão, M., and Ziveri, P.: Biometric study of *Coccolithus pelagicus* and its palaeoenvironmental utility, J. Nannoplankton Res., 22, p. 82, 2000.

Beardall, J. and Raven, J. A.: The potential effects of global climate change on microalgal photosynthesis, growth and ecology, Phycologia, 43, 26–40, 2004.

Beaufort, L.: Weight estimates of coccoliths using the optical properties (birefringence) of calcite, Micropaleontology, 51, 289–297, 2005.

BGD
11, 1021–1051, 2014

Beaufort, L. and Heussner, S.: Coccolithophorids on the continental slope of the Bay of Biscay – production, transport and contribution to mass fluxes, Deep-Sea Res. Pt. II, 46, 2147–2174, 1999.

Beaufort, L., Barbarin, N., and Gally, Y.: Protocol for optical measurements of calcite crystal thickness and mass of thin (< 4.5 µm) carbonate particles such as coccoliths, Nat. Protoc., in press, 2014.

Broerse, A. T. C., Ziveri, P., and Honjo, S.: Coccolithophore (-$CaCO_3$) flux in the Sea of Okhotsk: seasonality, settling and alteration processes, Mar. Micropaleontol., 39, 179–200, 2000.

Buitenhuis, E. T., van Bleijswijk, J., Bakker, D., and Veldhuis, M.: Trends in inorganic and organic carbon in a bloom of *Emiliania huxleyi* in the North Sea, Mar. Ecol.-Prog. Ser., 143, 271–282, 1996.

Buitenhuis, E. T., Pangerc, T., Franklin, D. J., Le Quere, C., Malin, G.: Growth rates of six coccolithophorid strains as a function of temperature, Limnol. Oceanogr., 53, 1181–1185, 2008.

Båtvik, H., Heimdal, B. R., Fagerbakke, K. M., and Green, J. C.: Effects of unbalanced nutrient regime on coccolith morphology and size in *Emiliania huxleyi* (Prymnesiophyceae), Eur. J. Phycol., 32, 155–165, 1997.

Cachão, M. and Moita, M. T.: *Coccolithus pelagicus*, a productivity proxy related to moderate fronts off Western Iberia, Mar. Micropaleontol., 39, 131–155, 2000.

Cubillos, J. C., Henderiks, J., Beaufort, L., Howard, W. R., and Hallegraeff, G. M.: Reconstructing calcification in ancient coccolithophores: Individual coccolith weight and morphology of *Coccolithus pelagicus* (sensu lato), Mar. Micropaleontol., 92–93, 29–39, 2012.

Feng, Y., Warner, M. E., Zhang, Y., Sun, J., Fu, F.-X., Rose, J. M., and Hutchins, D. A.: Interactive effects of increased pCO_2, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae), Eur. J. Phycol., 43, 87–98, 2008.

Fernández, E., Boyd, P., Holligan, P. M., and Harbour, D. S.: Production of organic and inorganic carbon within a large-scale coccolithophore bloom in the northeast Atlantic Ocean, Mar. Ecol.-Prog. Ser., 97, 271–285, 1993.

Findlay, H. S., Calosi, P., and Crawfurd, K.: Determinants of the PIC:POC response in the coccolithophore *Emiliania huxleyi* under future ocean acidification scenarios, Limnol. Oceanogr., 56, 1168–1178, 2011.

Fritz, J. J.: Carbon fixation and coccolith detachment in the coccolithophore *Emiliania huxleyi* in nitrate-limited cyclostats, Mar. Biol., 133, 509–518, 1999.

BGD
11, 1021–1051, 2014

Gattuso, J.-P., Pichon, M., Frankignoulle, M.: Biological control of air-sea CO_2 fluxes: effect of photosynthetic and calcifying marine organisms and ecosystems, Mar. Ecol.-Prog. Ser., 129, 307–312, 1995.
Geisen, M., Billard, C., Broerse, A. T. C., Cros, L., Probert, I., and Young, J. R.: Life-cycle associations involving pairs of holococcolithophorid species: intraspecific variation or cryptic speciation?, Eur. J. Phycol., 37, 531–550, 2002.
Gibbs, S. J., Poulton, A. J., Bown, P. R., Daniels, C. J., Hopkins, J., Young, J. R., Jones, H. L., Thiemann, G. J., O'Dea, S. A., and Newsam, C.: Species-specific growth response of coccolithophores to Palaeocene-Eocene environmental change, Nat. Geosci., 6, 218–222, 2013.
Gran, G.: Determination of the equivalence point in potentiometric titrations of seawater with hydrochloric acid, Oceanol. Acta, 5, 209–218, 1952.
Guillard, R. R. L.: Culture of phytoplankton for feeding marine invertebrates, in: Culture of Marine Invertebrates, edited by: Smith, W. and Chanley, M., Plenum, 29–60, 1975.
Henderiks, J., Winter, A., Elbrächter, M., Feistel, R., van der Plas, A., Nausch, G., and Barlow, R.: Environmental controls on *Emiliania huxleyi* morphotypes in the Benguela coastal upwelling system (SE Atlantic), Mar. Ecol.-Prog. Ser., 448, 51–66, 2012.
Holligan, P. M., Fernández, E., Aiken, J., Balch, W. M., Boyd, P., Burkill, P. H., Finch, M., Groom, S. B., Malin, G., Muller, K., Purdie, D. A., Robinson, C., Trees, C. C., Turner, S. M., and van der Wal, P.: A biogeochemical study of the coccolithophore, *Emiliania huxleyi*, in the North Atlantic, Global Biogeochem. Cy., 7, 879–900, 1993.
Klaas, C. and Archer, D. E.: Association of sinking organic matter with various types of mineral ballast in the deep sea: Implications for the rain ratio, Global Biogeochem. Cy., 16, 1116, doi:10.1029/2001GB001765, 2002.
Keller, M. D., Selvin, R. C., Claus, W., and Guillard, R. R. L.: Media for the culture of oceanic ultraphytoplankton, J. Phycol., 23, 633–638, 1987.
Krug, S. A., Schulz, K. G., and Riebesell, U.: Effects of changes in carbonate chemistry speciation on *Coccolithus braarudii*: a discussion of coccolithophorid sensitivities, Biogeosciences, 8, 771–777, doi:10.5194/bg-8-771-2011, 2011.
Langer, G. and Benner, I.: Effect of elevated nitrate concentration on calcification in *Emiliania huxleyi*, J. Nannoplankton Res., 30, 77–80, 2009.

BGD
11, 1021–1051, 2014

Langer, G., Geisen, M., Baumann, K.-H., Klaes, J., Riebesell, U., Thomas, S., and Young, J. R.: Species-specific responses of calcifying algae to changing seawater carbonate chemistry, Geochem. Geophy. Geosy., 7, 1–12, 2006.

Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry, Biogeosciences, 6, 2637–2646, doi:10.5194/bg-6-2637-2009, 2009.

Langer, G., Oetjen, K., and Brenneis, T.: Calcification of *Calcidiscus leptoporus* under nitrogen and phosphorus limitation, J. Exp. Mar. Biol. Ecol., 413, 131–137, 2012.

Linschooten, C., van Bleijswijk, J. D. L., van Emburg, P. R., de Vrind, J. P. M., Kempers, E. S., Westbroek, P., and de Vrind-de Jong, E. W.: Role of the light-dark cycle and medium composition on the production of coccoliths by *Emiliania huxleyi* (Haptophyceae), J. Phycol., 27, 82–86, 1991.

Menzel, D. H. and Corwin, N.: The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation, Limnol. Oceanogr., 10, 280–282, 1965.

Murphy, J. and Riley, J. P.: A modified single solution method for the determination of phosphate in natural waters, Anal. Chim. Acta, 27, 31–36, 1962.

Paasche, E.: Roles of nitrogen and phosphorus in coccolith formation in *Emiliania huxleyi* (Prymnesiophyceae), Eur. J. Phycol., 33, 33–42, 1998.

Paasche, E.: A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions, Phycologia, 40, 503–529, 2002.

Paasche, E. and Brubak, S.: Enhanced calcification in the coccolithophorid *Emiliania huxleyi* (Haptophyceae) under phosphorus limitation, Phycologia, 33, 324–330, 1994.

Paasche, E., Brubak, S., Skattebøl, S., Young, J. R., and Green, J. C.: Growth and calcification in the coccolithophorid *Emiliania huxleyi* (Haptophyceae) at low salinities, Phycologia, 35, 394–403, 1996.

Rickaby, R. E. M., Henderiks, J., and Young, J. N.: Perturbing phytoplankton: response and isotopic fractionation with changing carbonate chemistry in two coccolithophore species, Clim. Past, 6, 771–785, doi:10.5194/cp-6-771-2010, 2010.

Ridgwell, A., Schmidt, D. N., Turley, C., Brownlee, C., Maldonado, M. T., Tortell, P., and Young, J. R.: From laboratory manipulations to Earth system models: scaling calcification

BGD
11, 1021–1051, 2014

impacts of ocean acidification, Biogeosciences, 6, 2611–2623, doi:10.5194/bg-6-2611-2009, 2009.
Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E., and Morel, F. M. M.: Reduced calcification of marine plankton in response to increased atmospheric CO_2, Nature, 407, 364–367, 2000.
Riegman, R., Stolte, W., Noordeloos, A. A. M., and Slezak, D.: Nutrient uptake and alkaline phosphatase (EC 3 : 1 : 3 : 1) activity of *Emiliania huxleyi* (Prymnesiophyceae) during growth under N and P limitation in continuous cultures, J. Phycol., 36, 87–96, 2000.
Rost, B. and Riebesell, U.: Coccolithophores and the biological pump: responses to environmental changes, in: Coccolithophores – from molecular processes to global impact, edited by: Thierstein, H. R. and Young, J. R., Springer, Heidelberg, Germany, 99–126. 2004.
Roy, R., Roy, L., Vogel, K., Porter-Moore, C., Pearson, T., Good, C., Millero, F., and Campbell, D.: The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 °C, Mar. Chem., 44, 249–267, 1993.
Sáez, A. G., Probert, I., Geisen, M., Quinn, P., Young, J. R., and Medlin, L. K.: Pseudo-cryptic speciation in coccolithophores, P. Natl. Acad. Sci. USA, 100, 7163–7168, 2003.
Sarmiento, J. L., Slater, R., Barber, R., Bopp, L., Doney, S. C., Hirst, A. C., Kleypas, J., Matear, R., Mikolajewicz, U., Monfray, P., Soldatov, V., Spall, S. A., and Stouffer, R.: Response of ocean ecosystems to climate warming, Global Biogeochem. Cy., 18, GB3003, doi:10.1029/2003GB002134, 2004.
Schiller, J.: Coccolithineae, in: Kryptogamen-Flora von Deutschland, Österreich und der Schweiz, Akademische Verlagsgesellschaft, Leipzig, Germany, 10, 89–226, 1930.
Taylor, A. R., Russel, M. A., Harper, G. M., Collins, T., and Brownlee, C.: Dynamics of formation and secretion of heterococcoliths by *Coccolithus pelagicus* ssp. *braarudii*, Eur. J. Phycol., 42, 125–136, 2007.
Thomas, M. K., Kremer, C. T., Klausmeier, C. A., and Litchman, E.: A global pattern of thermal adaptation in marine phytoplankton, Science, 338, 1085–1088, 2012.
Van Bleijswijk, J. D. L., Kempers, R. S., van der Wal, P., Westbroek, P., Egge, J. K., and Lukk, T.: Standing stocks of PIC, POC, PON and *Emiliania huxleyi* coccospheres and liths in sea water enclosures with different phosphate loadings, Sarsia, 79, 307–317, 1994.
Van der Wal, P., Kempers, R. S., and Veldhuis, M. J. W.: Production and downward flux of organic matter and calcite in a North Sea bloom of the coccolithophore *Emiliania huxleyi*, Mar. Ecol.-Prog. Ser., 126, 247–265, 1994.

BGD
11, 1021–1051, 2014

Winter, A., Jordan, R., and Roth, P.: Biogeography of living coccolithophores in ocean waters, in: Coccolithophores, edited by: Winter, A. and Siesser, W., Cambridge University Press, UK, 161–177, 1994.

Young, J. R. and Ziveri, P.: Calculation of coccolith volume and its use in calibration of carbonate flux estimates, Deep-Sea Res. Pt. II, 47, 1679–1700, 2000.

Ziveri, P., Baumann, K.-H., Boeckel, B., Bollmann, J., Young, J. R.: Biogeography of selected Holocene coccoliths in the Atlantic Ocean, in: Coccolithophores – from molecular processes to global impact, edited by: Thierstein, H. R. and Young, J. R., Springer, Heidelberg, Germany, 403–428, 2004.

BGD
11, 1021–1051, 2014

Table 1. Initial (T_0, $n = 4$) and residual (T_{sample}, $n = 6$) medium chemistry: phosphate concentrations and carbonate chemistry parameters (mean ± SD) in high-P and low-P media batch cultures of *Coccolithus pelagicus* ssp. *braarudii* (RCC1200) grown at 15 °C, and ssp. *pelagicus* (J23) grown at 10 and 15 °C. For *C. pelagicus* ssp. *pelagicus* at 15 °C only mean presented (T_0, $n = 2$; T_{sample}, $n = 3$).

C. pelagicus	ssp. *braarudii* (RCC1200)						ssp. *pelagicus* (J23)					
	high-P 15 °C		low-P 15 °C		high-P 10 °C		low-P 10 °C		high-P 15 °C		low-P 15 °C	
	T_0	T_{sample}	T_0	T_{sample}	T_0	T_{sample}*	T_0	T_{sample}	T_0	T_{sample}	T_0	T_{sample}
PO_4^{3-} (µM)	8.9 ± 1.4	7.1 ± 0.6	1.09 ± 0.09	0.03 ± 0.05	8.9 ± 1.4	8.5 ± 0.9	1.13 ± 0.11	0.08 ± 0.05	9.6	6.4	1.1	0.03
A_T (µmol kg^{-1})	2100 ± 100	1350 ± 100	2050 ± 50	1400 ± 100	2050 ± 100	1550	2050 ± 50	1350 ± 50	1950	1400	2000	1600
pH, NBS	8.14 ± 0.02	7.85 ± 0.05	8.03 ± 0.08	8.00 ± 0.08	8.14 ± 0.02	7.85	8.06 ± 0.18	7.89 ± 0.04	8.18	7.81	7.99	7.95
pCO_2 (µatm)	294 ± 2	412 ± 78	384 ± 68	296 ± 78	258 ± 40	410	311 ± 162	296 ± 29	244	476	15.9	363
CO_2 (µmol kg^{-1})	11.1 ± 0.1	15.4 ± 2.9	14.5 ± 2.5	11.1 ± 2.9	10.4 ± 0.8	18.1	13.7 ± 7.1	13.0 ± 1.3	9.2	17.9	422	13.7
HCO_3^- (µmol kg^{-1})	1660 ± 80	1170 ± 100	1670 ± 20	1160 ± 110	1620 ± 80	1400	1650 ± 110	1140 ± 40	1500	1250	1680	1340
CO_3^{2-} (µmol kg^{-1})	175 ± 16	62.6 ± 5.3	140 ± 27	87.2 ± 7.8	169 ± 11	75.4	150 ± 50	64.4 ± 5.4	173	59.3	126	90.5
DIC (µmol kg^{-1})	1840 ± 100	1250 ± 100	1830 ± 10	1260 ± 100	1800 ± 90	1490	1810 ± 70	1220 ± 40	1680	1300	1830	1430
omega	4.2 ± 0.3	1.5 ± 0.1	3.4 ± 0.6	2.1 ± 0.2	4.1 ± 0.2	1.8	3.5 ± 1.2	1.5 ± 0.1	4.2	1.4	3.1	2.2

* for carbonate system parameters, $n = 3$ (only mean presented)

BGD
11, 1021–1051, 2014

Table 2. Cellular quotas and elemental ratios derived from high-P and low-P media batch experiments with *Coccolithus pelagicus* ssp. *braarudii* (RCC1200) grown at 15 °C ($n = 6$), and ssp. *pelagicus* (J23) grown at 10 °C ($n = 6$) and 15 °C ($n = 3$). Note that cell concentrations reflect those at time of sampling, and that maximum growth rate (μ_{max}) was calculated during exponential growth phase. Low-P cultures were in stationary phase at time of harvest.

C. pelagicus	ssp. *braarudii* (RCC1200)			ssp. *pelagicus* (J23)		
	high-P 15 °C	low-P 15 °C	high-P 10 °C	low-P 10 °C	high-P 15 °C	low-P 15 °C
Cell concentrations (cells mL^{-1})	15 169 ± 2789	13 397 ± 1985	13 211 ± 3585	13 425 ± 416	8442	9640
μ_{max} (d^{-1})	0.45 ± 0.05	0.43 ± 0.07	0.29 ± 0.06	0.33 ± 0.04	0.32	0.34
POP (pg $cell^{-1}$)	5.3 ± 0.7	2.6 ± 0.5	5.5 ± 0.6	2.6 ± 0.3	10.3	4.4
POC (pg $cell^{-1}$)	181 ± 30	200 ± 41	308 ± 70	333 ± 115	217	212
PIC (pg $cell^{-1}$)*	208	199	312	334	119	189
POC/POP	91 ± 26	208 ± 67	150 ± 47	331 ± 115	55	125
PN/POP	10 ± 3	21 ± 6	16 ± 7	40 ± 19	7	17
PIC/POC*	1.3	1.2	1.3	1.5	0.5	0.9

* for PIC, $n = 3$ (only mean presented)

BGD
11, 1021–1051, 2014

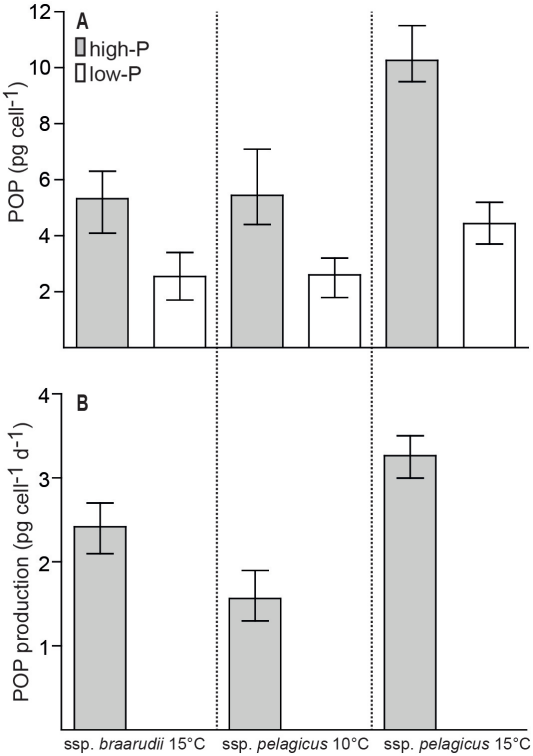

Fig. 1. (A) Particulate organic phosphorus (POP) quota of *Coccolithus pelagicus* ssp.*braarudii* grown at 15 °C ($n = 6$), and ssp. *pelagicus* grown at 10 °C ($n = 6$) and 15 °C ($n = 3$) in high-P and low-P medium. **(B)** POP production in high-P cultures. Mean ± min/max.

BGD
11, 1021–1051, 2014

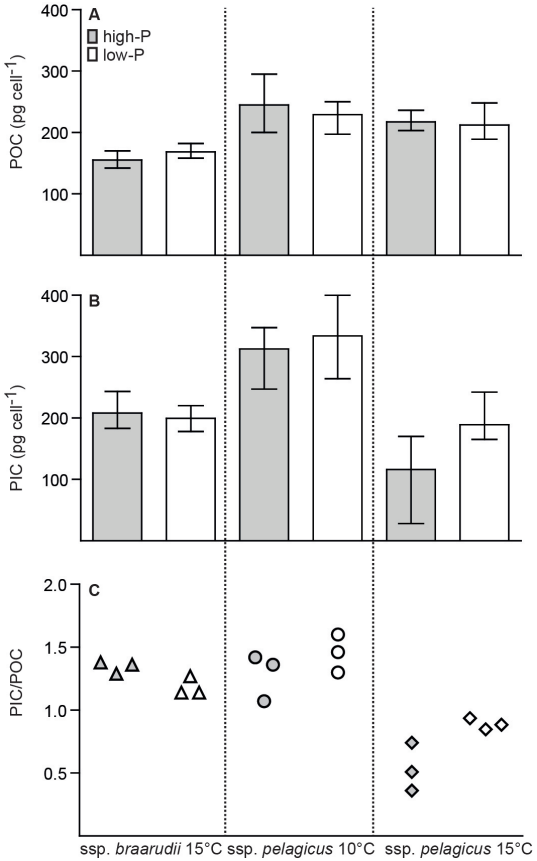

Fig. 2. (A) Particulate organic carbon (POC) quota of *Coccolithus pelagicus* ssp.*braarudii* grown at 15 °C, and ssp. *pelagicus* grown at 10 and 15 °C in high-P and low-P medium. **(B)** Particulate inorganic carbon (PIC) quota. **(C)** PIC/POC ratios. Mean ± min/max, $n = 3$.

BGD
11, 1021–1051, 2014

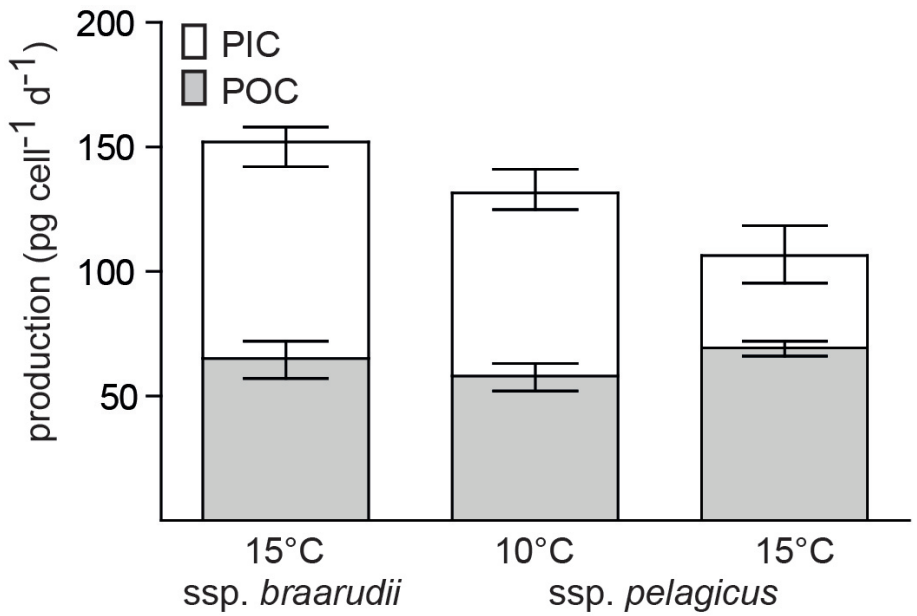

Fig. 3. Particulate organic carbon (POC) and particulate inorganic carbon (PIC) production of *Coccolithus pelagicus* ssp. *braarudii* grown at 15 °C, and ssp. *pelagicus* grown at 10 and 15 °C. Mean ± min/max, $n = 3$.

BGD
11, 1021–1051, 2014

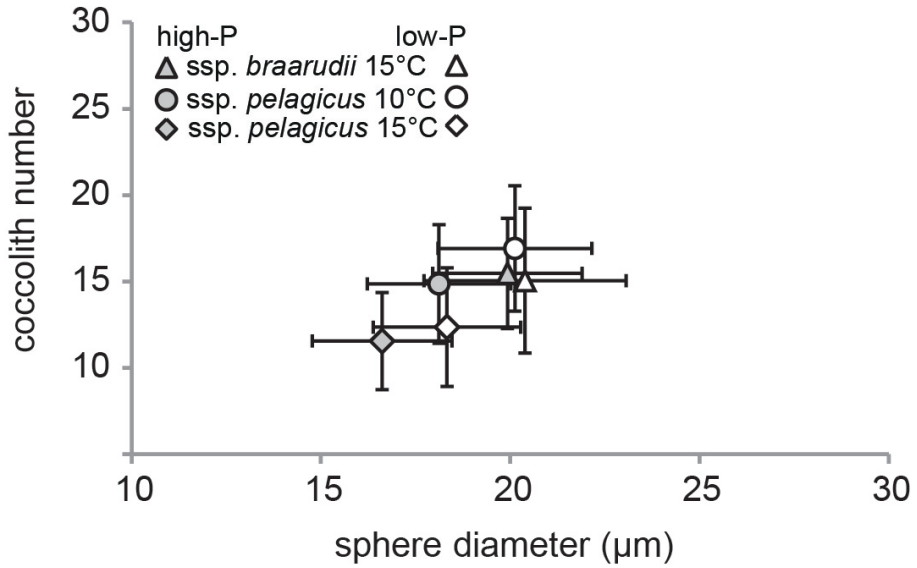

Fig. 4. Number of coccoliths per coccosphere plotted against the diameter of that coccosphere produced in high-P and low-P medium. *Coccolithus pelagicus* ssp. *braarudii* at 15 °C (high-P: n = 503, low-P: n = 420) and ssp. *pelagicus* at 10 °C (high-P: n = 236, low-P: n = 303) and 15 °C (high-P: n = 204, low-P: n = 181). Mean ± SD.

BGD
11, 1021–1051, 2014

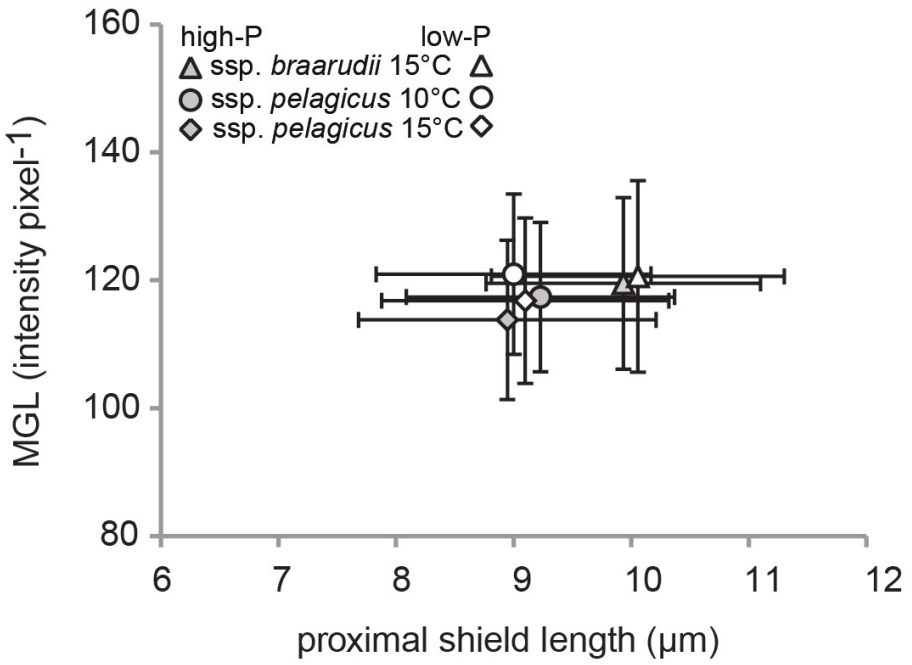

Fig. 5. Mean grey level (MGL) plotted against mean proximal shield length (PSL) of individual coccoliths produced in high-P and low-P medium. *Coccolithus pelagicus* ssp. *braarudii* at 15 °C (high-P: n = 465, low-P: n = 472) and ssp. *pelagicus* at 10 °C (high-P: n = 495, low-P: n = 489) and 15 °C (high-P: n = 164, low-P: n = 167). Mean ± SD.

BGD
11, 1021–1051, 2014

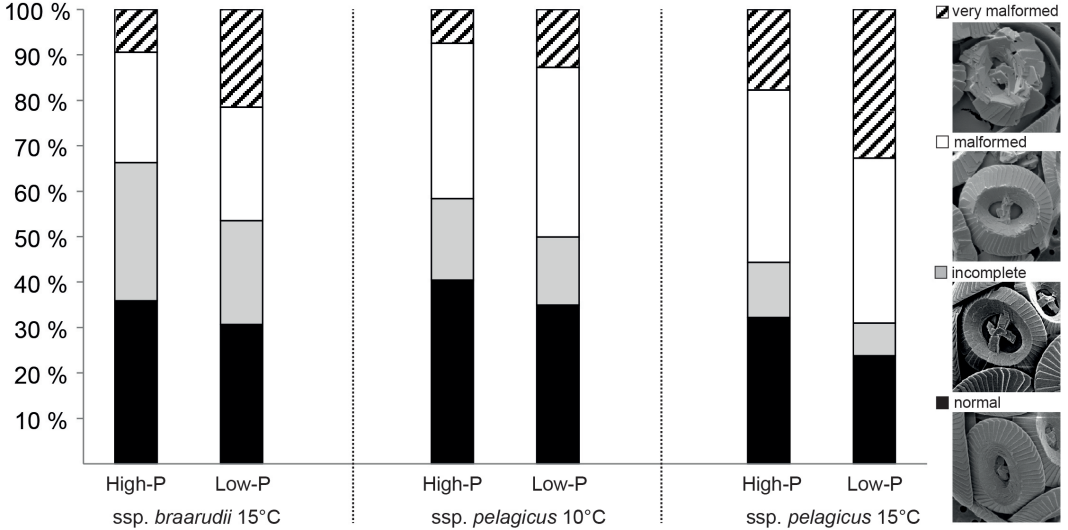

Fig. 6. Morphology of coccoliths observed by scanning electron microscopy of *Coccolithus pelagicus* ssp. *braarudii* at 15 °C (high-P: $n = 3118$, low-P: $n = 2783$), and ssp. *pelagicus* at 10 °C (high-P: $n = 1389$, low-P: $n = 1954$) and 15 °C (high-P: $n = 1373$, low-P: $n = 1329$).

BGD
11, 1021–1051, 2014