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

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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 through photosynthesis (POC production) and into particulate inorganic carbon through calcification (PIC production). As calcification produces a net release of CO_2, the ratio of PIC to POC production determines whether coccolithophores act as a source (high PIC / POC) or a sink (low PIC / POC) of atmospheric CO_2. We studied the effect of phosphorus (P-) limitation and high temperature on the physiology and the PIC / POC ratio of two subspecies of *Coccolithus pelagicus*. This large and heavily calcified species 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. One of the subspecies was subjected to a 5 °C temperature increase from 10 °C to 15 °C, which did not affect exponential growth rates either, but nearly doubled cellular P-content under both high and low phosphate availability. This temperature increase reduced the PIC / POC ratio by 40–60 %, whereas the PIC / POC ratio did not differ between P-limited and nutrient-replete cultures when the subspecies were grown near their respective isolation temperature. Both P-limitation and elevated temperature significantly increased coccolith malformations. Our results suggest that a temperature increase 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, thus favouring CO_2 sequestration over release. It seems unlikely that P-limitation by itself causes changes in the PIC / POC ratio in this species.

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 through photosynthesis (POC production) and into particulate inorganic carbon through calcification (PIC production). 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 Riebesell, 2004). Therefore, the ratio of calcification to photosynthesis (PIC / POC production ratio) determines whether coccolithophores act as a source or a 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). To accurately model the effect of a changing climate on ocean–atmosphere CO_2 exchange, it is important to constrain the PIC / POC production ratio of coccolithophores.

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). In some cases, nutrient limitation has been shown to increase the ratio of PIC to POC production in the cosmopolitan species *Emiliania huxleyi* (Paasche and Brubak, 1994; Paasche, 1998; Riegman et al., 2000). In these studies, POC production rates decreased due to decreasing nutrient availability whereas PIC production rates remained unaffected, leading to an increase in the ratio of PIC to POC. In the field, the increased ratio of loose coccoliths to coccospheres has also been ascribed to decreasing nutrient availability (Balch et al., 1991; Fernández et al., 1993; van der Wal et al., 1995) and mesocosm studies have shown that calcification continues after POC fixation ceases due to nutrient exhaustion (van Bleijswijk et al., 1994). On the other hand, several other studies on *E. huxleyi* have reported stable PIC / POC ratios due to a decrease in both POC and PIC production rates with decreasing nutrient availability (e.g. Fritz, 1999; Borchard et al., 2011) and a decrease in the PIC / POC ratio under nutrient limitation has also been reported (Langer et al., 2013a). A recent study by Langer et al. (2012) examined the effect of P- and nitrogen (N-) limitation on the PIC / POC ratio of *Calcidiscus leptoporus* and showed this ratio to be insensitive to limitation by these macronutrients. Given the species- and strain-specific response to ocean acidification (Langer et al., 2006, 2009; Krug et al., 2011), it is not surprising that the response to macronutrient limitation is not uniform among strains and species. The response may also depend on the experimental method, i.e. batch vs. (semi-) continuous cultures (Langer et al., 2013a). In addition, only a few species have been studied to date. It is therefore necessary to examine more species and lineages of coccolithophores to gain a more 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 a PIC / POC ratio of generally > 1.5 (Langer et al., 2006; Krug et al., 2011). The calcite weights of individual coccoliths are at least two orders of magnitude higher than the average weight 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). *Coccolithus pelagicus* is divided into at least two morphotypes on the basis of heterococcolith size and distribution (Geisen et al., 2002; Sáez et al., 2003). Although genetic information suggests that they are separated at the species level (Sáez et al., 2003), we here use the division of the two morphotypes into subspecies, *C. pelagicus* subsp. *braarudii* (Gaarder) Geisen et al., 2002 and *C. pelagicus* subsp. *pelagicus*, according to Geisen et al. (2002) as the genetic and morphological differentiations are slight (Jordan et al., 2004). The subarctic morphotype *C. pelagicus* subsp. *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* subsp. *braarudii* is common in coastal upwelling regions of the Northeast and Southeast Atlantic (e.g. Cachão and Moita, 2000; Henderiks et al., 2012).

We investigated the combined effect of P-limitation and elevated temperature on the physiology, elemental quotas, and the PIC / POC ratio of this species. One strain of each subspecies was grown in batch culture under P-limited and nutrient-replete conditions to test for physiological differences between the two subspecies. Subspecies *pelagicus* was grown in P-limited and nutrient-replete batch culture at two temperatures to test the effect of a temperature increase. Calcification was evaluated by the PIC quota of cells, individual coccolith volumes and the occurrence of coccolith malformations. Whereas altered carbonate chemistry has been shown to increase the malformation of coccoliths (e.g. Langer et al., 2006, 2011; Rickaby et al., 2010), Langer et al. (2012) concluded that nutrient limitation does not significantly affect coccolith morphology. Particulate organic carbon production was monitored by growth rate and the POC quota of the cells.

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* subsp. *braarudii* and was isolated from the South Atlantic offshore Namibia. Strain RCC3776 is a clone of *C. pelagicus* subsp. *pelagicus* and was isolated from the North Atlantic near Scotland. Clonal batch cultures were grown in triplicate in sterile-filtered modified K/2 medium (Keller et al., 1987; with the following modifications: omission of Tris and Si, addition of $NiCl_2 \times 6H_2O$ (3.14 nM), increase of EDTA concentrations (5.85 µM)) at two initial phosphate concentrations. Vitamins were added according to f/2 medium (Guillard, 1975). Aged natural sea water from the Oslo Fjord 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 nutrient-replete exponential growth throughout the

experiment. Each subspecies was cultured near its respective isolation temperature, subsp. *braarudii* at 15 °C and subsp. *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 experimental light and temperature conditions for at least 10 generations before starting the experiments. 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 ca. 100 µmol photons m^{-2} s^{-1}. Culture flasks were agitated manually on a daily basis. Two experiments of paired high-P and low-P treatments (main and additional experiments; see Tables 1–3) were carried out for subsp. *braarudii* (15 °C) and subsp. *pelagicus* (10 °C). Each high-P and low-P treatment consisted of three replicate cultures. Additionally, one paired high-P and low-P experiment was performed in triplicate with subsp. *pelagicus* at 15 °C. Cell densities were determined daily on an electronic particle counter (CASY, Roche Diagnostics, Switzerland) 2 hours after the onset of the light phase. Exponential growth rates (μ_{max}) were calculated by linear regression of log-transformed cell densities in exponential phase over time. Low-P cultures were sampled upon reaching stationary phase (on the day that cell densities had increased not more than 5 % compared to the previous day). High-P cultures were sampled at similar cell densities while still in exponential phase to ensure similar carbonate chemistry between the treatments.

High-P and low-P culture media were sampled for initial chemistry (T_0, Table 1). Upon sampling the cultures, the growth media were again sampled to analyse carbonate chemistry parameters and residual phosphate concentrations at the time of sampling (T_{sample}, Table 1). Culture samples were collected for elemental analysis (particulate organic phosphorus (POP), particulate nitrogen (PN), 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 media were sterile filtered (0.2 µm) 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 (ca. 0.05 µM) confirmed that low-P cultures had taken up all available phosphate from the medium by the time of sampling (T_{sample}, Table 1), whereas phosphate concentrations of high-P cultures remained > 6.4 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. Total alkalinity was calculated from Gran plots (Gran, 1952) after duplicate potentiometric titration using an automatic titrator (TTA 80 Titration assembly, Radiometer, Denmark) or manual titration with a precision of ±50 µmol kg^{-1}. The pH was measured with a combined electrode (Red Rod, Radiometer) 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).

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. Particulate organic phosphorus was 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). This was then quantified as molybdate reactive phosphate as described in Sect. 2.2.1.

2.3.2 Particulate total and organic carbon and nitrogen

Samples for TPC, POC and PN were filtered onto precombusted GF/C filters, dried at 60 °C overnight in a drying oven and stored in a desiccator until analysis on an elemental analyser (Flash 1112, Thermo Finnigan, USA; detection limit 2 µg) with a precision of ±8 %. Particulate inorganic carbon was removed from POC filters by pipetting 230 µL of 2 M HCl onto the filters before analysis (Langer et al., 2009) and PIC calculated as the difference between TPC and POC. Data for PIC was available only for the main experiments of subsp. *braarudii* (15 °C) and subsp. *pelagicus* (10 °C and 15 °C). As production rates cannot be accurately calculated in batch cultures going into stationary phase due to discontinuous growth rates (Langer et al., 2012, 2013a), we compare elemental ratios of P-limited and nutrient-replete cultures using cellular quotas, rather than production rates. These were compared using a *t*-test and one-way analysis of variance (ANOVA) in GraphPad Prism version 6 for Windows (GraphPad Software, USA). For high-P cultures (sampled in exponential phase), production rates were calculated from exponential growth rates (μ_{max}) and the respective cellular quota.

Table 1. Initial (T_0) and residual (T_{sample}) medium chemistry: phosphate concentrations and carbonate chemistry parameters in paired high-P and low-P media batch experiments with *Coccolithus pelagicus* subsp. *braarudii* (RCC1200) grown at 15 °C and subsp. *pelagicus* (RCC3776) grown at 10 and 15 °C. Initial values (T_0, $n = 2$) were measured directly from the high-P and low-P medium. The residual medium chemistry represents the average of triplicate batch cultures (T_{sample}, $n = 3$) with standard deviation (SD) in brackets.

C. pelagicus	subsp. *braarudii* (RCC1200)				subsp. *pelagicus* (RCC3776)							
	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}
(a) Main experiments												
PO_4^{3-} (µM)	7.96	7.10	1.05	0.00	9.75	9.28	1.16	0.08	9.55	6.40	1.05	0.03
(SD)		(0.32)		(0.00)		(0.33)		(0.08)		(0.91)		(0.05)
A_T (µmol kg^{-1})	2000	1400	2000	1500	2050	1650	2050	1350	1950	1400	2000	1550
(SD)		(50)		(50)		(50)		(50)		(50)		(50)
pH, NBS	8.12	7.81	7.98	7.92	8.15	7.84	8.20	7.91	8.18	7.81	7.99	7.95
(SD)		(0.02)		(0.01)		(0.03)		(0.06)		(0.02)		(0.03)
pCO_2 (µatm)	293	488	433	369	219	415	190	285	240	477	421	367
(SD)		(13)		(2)		(18)		(34)		(11)		(27)
CO_2 (µmol kg^{-1})	11.0	18.3	16.3	13.9	9.6	18.2	8.4	12.5	9.0	17.9	15.8	13.8
(SD)		(0.5)		(0.1)		(0.8)		(1.5)		(0.4)		(1.0)
HCO_3^- (µmol kg^{-1})	1600	1250	1700	1300	1600	1450	1550	1150	1500	1250	1700	1350
(SD)		(50)		(50)		(50)		(50)		(50)		(50)
CO_3^{2-} (µmol kg^{-1})	161	61	123	81	171	76	189	69	175	60	126	92
(SD)		(6)		(3)		(9)		(7)		(5)		(2)
DIC (µmol kg^{-1})	1750	1350	1850	1350	1800	1550	1750	1250	1700	1300	1850	1450
(SD)		(100)		(0)		(50)		(50)		(50)		(50)
Ω_c	3.9	1.5	3.0	2.0	4.1	1.8	4.5	1.7	4.2	1.4	3.0	2.2
(SD)		(0.2)		(0.1)		(0.2)		(0.2)		(0.2)		(0.1)
(b) Additional experiments												
PO_4^{3-} (µM)	10.2	7.08	1.14	0.06	8.90	7.70	1.11	0.08				
(SD)		(0.85)		(0.06)		(0.19)		(0.07)				
A_T (µmol kg^{-1})	2200	1300	2100	1350	2050	1400	2000	1350				
(SD)		(100)		(50)		(50)		(50)				
pH, NBS	8.15	7.90	8.15	8.06	8.15	7.23	7.91	7.86				
(SD)		(0.01)		(0.01)		(0.01)		(0.01)				
pCO_2 (µatm)	296	347	284	227	219	1751	418	315				
(SD)		(21)		(2)		(81)		(14)				
CO_2 (µmol kg^{-1})	11.1	13.0	10.7	8.5	9.6	77.0	18.4	13.9				
(SD)		(0.8)		(0.1)		(3.6)		(0.6)				
HCO_3^- (µmol kg^{-1})	1750	1100	1650	1100	1600	1350	1750	1150				
(SD)		(100)		(50)		(50)		(50)				
CO_3^{2-} (µmol kg^{-1})	188	66	180	95	171	15	104	62				
(SD)		(5)		(4)		(1)		(3)				
DIC (µmol kg^{-1})	1900	1200	1850	1200	1800	1450	1850	1250				
(SD)		(100)		(50)		(50)		(50)				
Ω_c	4.5	1.6	4.3	2.3	4.1	0.4	2.5	1.5				
(SD)		(0.1)		(0.1)		(0.0)		(0.1)				

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 the coccoliths with 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., USA) and subsequently processed to a compound 8 bit greyscale image (Beaufort et al., 2014). A custom-made macro in ImageJ freeware measured coccolith length through an ellipse approximation, as well as mean

Table 2. Cellular quotas and molar ratios derived from paired high-P and low-P media batch experiments ($n = 3$) of *Coccolithus pelagicus* subsp. *braarudii* (RCC1200) grown at 15 °C, and subsp. *pelagicus* (RCC3776) grown at 10 and 15 °C. 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. Reported are the averages of triplicate batch cultures with standard deviation (SD) in brackets.

C. pelagicus	subsp. *braarudii* (RCC1200)		subsp. *pelagicus* (RCC3776)			
	high-P 15 °C	low-P 15 °C	high-P 10 °C	low-P 10 °C	high-P 15 °C	low-P 15 °C
(a) Main experiments						
Cell concentrations (cells mL^{-1})	12750	11 800	10 000	13 200	8450	9650
(SD)	(650)	(500)	(1250)	(400)	(350)	(1200)
μ_{max} (d^{-1})	0.42	0.37	0.24	0.36	0.32	0.34
(SD)	(0.03)	(0.03)	(0.03)	(0.02)	(0.02)	(0.03)
POP (pg cell^{-1})	5.9	2.8	5.9	2.6	10.3	4.4
(SD)	(0.3)	(0.4)	(0.8)	(0.5)	(0.7)	(0.5)
POC (pg cell^{-1})	155	169	245	229	217	212
(SD)	(11)	(9)	(33)	(19)	(14)	(21)
PIC (pg cell^{-1})	208	200	312	334	116	189
(SD)	(24)	(18)	(34)	(52)	(48)	(28)
POC/POP (mol mol^{-1})	66	163	107	229	54	120
(SD)	(6)	(28)	(7)	(25)	(7)	(28)
PN/POP (mol mol^{-1})	7.5	18	9.7	23	6.7	16
(SD)	(0.5)	(3)	(0.4)	(3)	(0.7)	(4)
PIC / POC (mol mol^{-1})	1.34	1.18	1.28	1.45	0.54	0.89
(SD)	(0.05)	(0.08)	(0.19)	(0.15)	(0.19)	(0.04)
(b) Additional experiments						
Cell concentrations (cells mL^{-1})	17 550	15 000	16 400	13 650		
(SD)	(1300)	(1350)	(400)	(300)		
μ_{max} (d^{-1})	0.49	0.52	0.35	0.29		
(SD)	(0.02)	(0.04)	(0.03)	(0.02)		
POP (pg cell^{-1})	4.7	2.3	5.0	2.6		
(SD)	(0.6)	(0.5)	(0.4)	(0.3)		
POC (pg cell^{-1})	207	231	371	437		
(SD)	(9)	(35)	(6)	(29)		
PIC (pg cell^{-1})	n/a	n/a	n/a	n/a		
(SD)						
POC/POP (mol mol^{-1})	114	262	193	433		
(SD)	(8)	(58)	(2)	(37)		
PN/POP (mol mol^{-1})	12	25	22	56		
(SD)	(1)	(5)	(2)	(7)		
PIC / POC (mol mol^{-1})	n/a	n/a	n/a	n/a		
(SD)						

grey level (MGL) of the selected area measured in intensity values of 0 (black) to 255 (white). Mean grey level 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

Table 3. Coccosphere and coccolith size parameters as well as coccolith morphology from paired high-P and low-P media batch experiments ($n = 3$) with *Coccolithus pelagicus* subsp. *braarudii* (RCC1200) grown at 15 °C, and subsp. *pelagicus* (RCC3776) grown at 10 and 15 °C. Reported is sample size (n), average value of pooled triplicate batch cultures with standard deviation (SD) or 95 % confidence interval (CI) in brackets.

C. pelagicus	subsp. *braarudii* (RCC1200)		subsp. *pelagicus* (RCC3776)			
	high-P 15 °C	low-P 15 °C	high-P 10 °C	low-P 10 °C	high-P 15 °C	low-P 15 °C
(a) Main experiments						
			coccosphere size parameters			
n (number of coccospheres)	245	135	165	201	142	91
coccosphere diameter [µm]	19.9	19.7	18.0	20.4	16.6	18.3
(SD)	(1.9)	(2.3)	(1.8)	(1.8)	(1.8)	(1.9)
coccolith number	15	15	15	17	12*	12*
(SD)	(3)	(4)	(3)	(3)	(3)	(3)
			coccolith size parameters			
n (number of coccoliths)	165	166	329	324	164	167
coccolith length [µm]	9.7	9.8	8.8	9.0	8.8	9.0
(SD)	(1.2)	(1.4)	(1.1)	(1.0)	(1.2)	(1.2)
MGL [intensity pixel^{-1}]	113	111	113	123	114	117
(SD)	(14)	(16)	(11)	(12)	(12)	(13)
			coccolith morphology			
n (number of coccoliths)	1706	1215	978	1386	1373	1329
normal [%]	42	29	43	38	28	18
(CI)	(2)	(3)	(3)	(3)	(2)	(2)
incomplete [%]	28	25	16	18	8	5
(CI)	(2)	(2)	(2)	(2)	(1)	(1)
malformed [%]	22	28	34	35	39	35
(CI)	(2)	(3)	(3)	(3)	(3)	(3)
very malformed [%]	8	18	7	9	25	42
(CI)	(1)	(2)	(2)	(2)	(2)	(3)
(b) Additional experiments						
		coccosphere size parameters				
n (number of coccospheres)	180	152	53	77		
coccosphere diameter [µm]	20.0	21.0	18.6	19.3		
(SD)	(2.1)	(2.8)	(2.1)	(2.3)		
coccolith number	16	15	16	18		
(SD)	(3)	(5)	(4)	(4)		
		coccolith size parameters				
n (number of coccoliths)	300	306	166	165		
coccolith length [µm]	9.8	9.9	9.1	8.8		
(SD)	(1.1)	(1.1)	(1.1)	(1.1)		
MGL [intensity pixel^{-1}]	123	126	120	124		
(SD)	(12)	(12)	(12)	(13)		
		coccolith morphology				
n (number of coccoliths)	1412	1568	413	568		
normal [%]	30	30	38	32		
(CI)	(2)	(2)	(5)	(4)		
incomplete [%]	33	22	20	12		
(CI)	(3)	(2)	(4)	(3)		
malformed [%]	27	23	35	39		
(CI)	(2)	(2)	(5)	(4)		
very malformed [%]	10	25	7	17		
(CI)	(2)	(2)	(3)	(3)		

* Many collapsed spheres.

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 subsp. *braarudii* (RCC1200) had higher maximum growth rates (0.44 ± 0.06 d^{-1}) than the subarctic morphotype subsp. *pelagicus* (RCC3776) (0.32 ± 0.05 d^{-1}). In low-P medium subsp. *braarudii* grew to final cell concentrations of $1.3 \pm 0.2 \times 10^4$ cells mL^{-1} (Table 2). Subspecies *pelagicus* grew 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 POP quotas and higher ratios of POC and PN to POP, compared to high-P treatments (Table 2; Fig. 1a). The two subspecies had the same POP quota when comparing low-P and high-P cultures, respectively. Particulate organic phosphorus production, however, was lower in subsp. *pelagicus* than in subsp. *braarudii* because of lower growth rates (Fig. 1b). Most striking is the high POP quota of subsp. *pelagicus* cultures grown at high temperature (15 °C). This almost doubled in both low-P and high-P treatments. Accordingly, POP production rates in high-P cultures of subsp. *pelagicus* were more than twice as high at 15 °C.

The initial carbonate chemistry of the medium (T_0, Table 1) was modified by culture growth in all treatments with a drawdown of alkalinity (A_T) of 25–41 % in subsp. *braarudii* and of 20–34 % in subsp. *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 = 8.10 ± 0.09 to a minimum pH = 7.81. The high-P treatment of the additional experiment of subsp. *pelagicus* grown at 10 °C showed a strong shift in the system towards pH = 7.23 (Table 1b). The saturation state for calcite (Ω_c) calculated for this treatment was below one whereas all other treatments were saturated in calcite ($\Omega_c > 1$, 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 high-P treatment of the main experiment and there was no evidence for coccolith dissolution.

3.2 Cellular carbon quotas and ratios

The cellular POC quota was similar between low-P and high-P treatments for each experiment of the two subspecies (*t*-test: $p > 0.05$; Table 2), except for the additional experiment of subsp. *pelagicus* grown at 10 °C in which the cells of low-P cultures contained significantly more POC than those of high-P cultures (*t*-test: $p = 0.02$; Table 2b). High temperature (15 °C) did not affect POC quotas in either P-treatment of subsp. *pelagicus* when only the main experiments were considered (*t*-test: $p > 0.05$; Table 2a; Fig. 2a). In the additional experiments, POC quotas were higher in both low-P and high-P treatments of both subspecies compared to the main experiments (one-way ANOVA: $p < 0.001$; Table 2b). These differences were not due to changing bacterial abundance as bacteria accounted for < 5 % of total organic carbon in all cultures (data not shown). Interestingly, subsp. *pelagicus* cells contained almost 40 % more POC than cells of subsp. *braarudii*, although subsp. *pelagicus* was smaller in terms of coccosphere size (see Sect. 3.3). Particulate inorganic carbon quotas were not affected either, by P-limitation (*t*-test: $p > 0.05$) when the cultures were grown near their respective isolation temperature. However, there was a significant reduction in the PIC quota of subsp. *pelagicus* at high

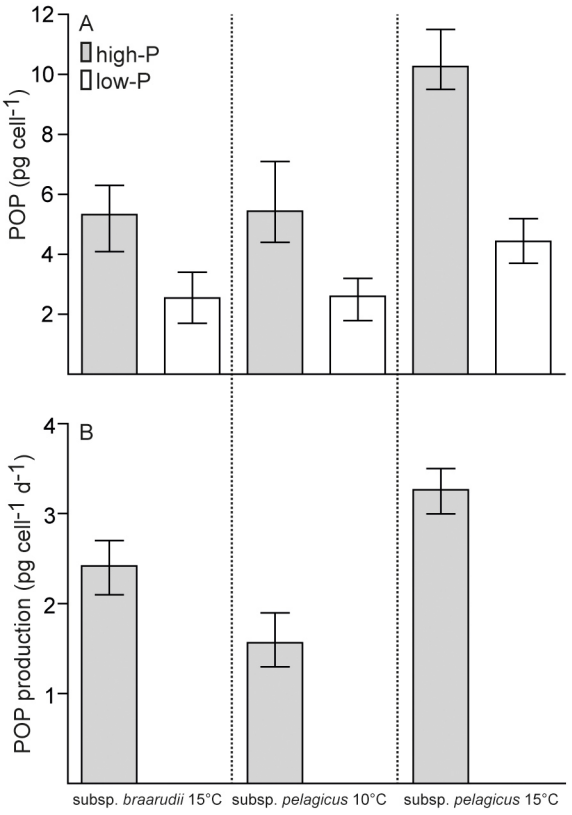

Figure 1. (A) Particulate organic phosphorus (POP) quotas of *Coccolithus pelagicus* subsp. *braarudii* grown at 15 °C and subsp. *pelagicus* grown at 10 and 15 °C, in high-P and low-P medium. **(B)** POP production in high-P cultures. Mean ± min/max; values for subsp. *braarudii* at 15 °C and subsp. *pelagicus* at 10 °C are averages of the main and additional experiments ($n = 6$); for subsp. *pelagicus* at 10 °C, $n = 3$.

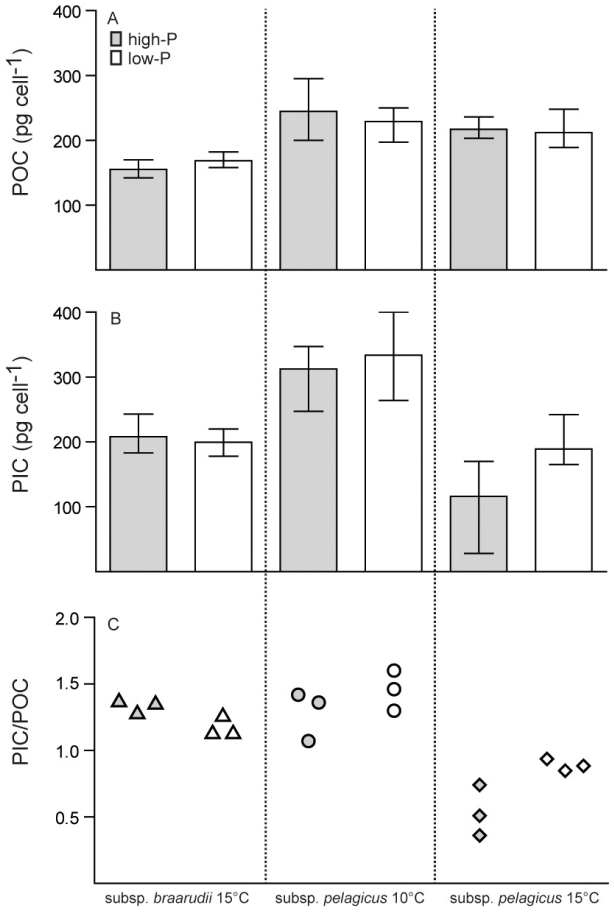

Figure 2. (A) Particulate organic carbon (POC) quotas of *Coccolithus pelagicus* subsp. *braarudii* grown at 15 °C and subsp. *pelagicus* grown at 10 and 15 °C, in high-P and low-P medium. **(B)** Particulate inorganic carbon (PIC) quotas. **(C)** PIC / POC ratios. Mean ± min/max. Values are from main experiments only, $n = 3$.

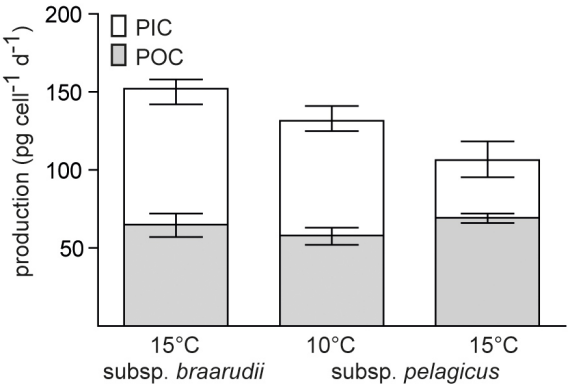

Figure 3. Particulate organic (POC) and particulate inorganic carbon (PIC) production of *Coccolithus pelagicus* subsp. *braarudii* grown at 15 °C and subsp. *pelagicus* grown at 10 and 15 °C in high-P medium. Mean ± min/max. Values are from main experiments only, $n = 3$.

temperature (15 °C) in both P-treatments (t-test: $p < 0.001$; Fig. 2b). In consequence, the PIC / POC ratio of subsp. *pelagicus* was below one at 15 °C compared to PIC / POC ratios of 1.37 ± 0.18 at 10 °C (Fig. 2c). The decrease was strongest (t-test: $p = 0.009$) in exponentially growing high-P cultures. These had a PIC / POC ratio of 0.54 ± 0.19 compared to a significantly higher PIC / POC ratio of 0.89 ± 0.04 in P-limited cultures at this temperature (t-test: $p = 0.003$). The reduced PIC / POC ratio of high-P cultures of subsp. *pelagicus* at high temperature (15 °C) was mainly due to the decreased rate of PIC production, although POC production rate increased by ca. 20 % (Fig. 3).

3.3 Coccosphere and coccolith dimensions

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

In both subspecies, the mean length of the proximal shield (PSL) was unaffected by changes in P-availability (t-test: $p > 0.05$; Fig. 4b). Temperature did not change mean PSL in subsp. *pelagicus* either (one-way ANOVA: $p > 0.05$; Fig. 4b). Coccoliths produced by subsp. *pelagicus* were significantly smaller than those produced by subsp. *braarudii* (t-test: $p < 0.001$). Yet, PSL showed a wide range of 6.4–14 µm in subsp. *braarudii* and 5.8–12 µm in subsp. *pelagicus*. Coccoliths of low-P cultures of subsp. *pelagicus* in the main experiment had a significantly higher mean grey level (MGL), a proxy for coccolith thickness (t-test: $p < 0.0001$; Table 3a). However, this increase in thickness in low-P cultures could not be confirmed in the additional experiment or between P-treatments at high temperature (15 °C). The difference in mean coccolith thickness between P-treatments of subsp. *braarudii* was < 3 %, which is not considered biologically significant (Table 3). There was no effect of temperature on coccolith thickness (t-test: $p > 0.05$).

3.4 Coccolith malformations

Scanning electron microscopy revealed a background percentage of malformed coccoliths in high-P cultures of subsp. *braarudii* of 30 and 37 % in the main and additional experiment, respectively (Table 3; Fig. 5). This percentage increased to 46–48 % under P-limitation, mainly due to an increased presence of coccoliths with blocky structures or holes, classified as "very malformed" (Table 3; Fig. 5). In subsp. *pelagicus*, the background percentage of malformed coccoliths in high-P cultures grown at 10 °C was 42 % in both experiments with a lower percentage of incomplete

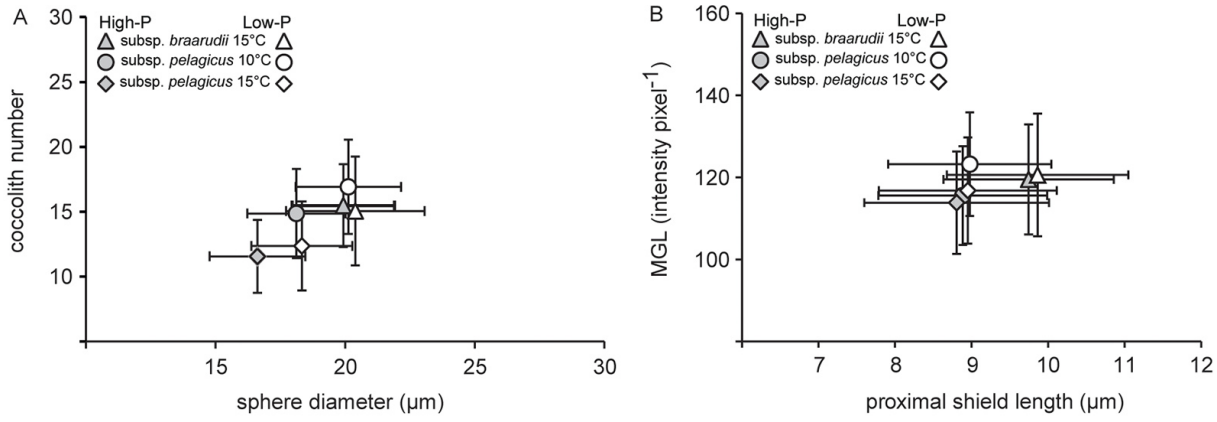

Figure 4. (A) Number of coccoliths per coccosphere plotted against the diameter of that coccosphere produced in high-P and low-P medium by *Coccolithus pelagicus* subsp. *braarudii* grown at 15 °C and subsp. *pelagicus* grown at 10 and 15 °C. **(B)** Mean grey level (MGL) plotted against mean proximal shield length (PSL) of individual coccoliths. Mean ± SD. Values for subsp. *braarudii* at 15 °C and subsp. *pelagicus* at 10 °C are combined for the main and additional experiments.

coccoliths than subsp. *braarudii* (Table 3; Fig. 5). Malformations increased to 56 % under P-limitation in the additional, but not in the main experiment. When subsp. *pelagicus* was grown at elevated temperature (15 °C), malformations increased to 64 % in high-P cultures. The highest abundance of malformed coccoliths (77 %) was observed in subsp. *pelagicus* cultures grown under P-limitation and high temperature with 42 % of coccoliths classified as "very malformed" (Table 3; Fig. 5).

4 Discussion

Laboratory experiments are useful to systematically test the short-term physiological (plastic) response of coccolithophores to various environmental factors and could constrain how the PIC / POC (production) ratio is likely to change under future climate scenarios. The experiments presented here provide the first available data set to test the effect of P-limitation and elevated temperature on *C. pelagicus*. We ran batch cultures of this species into P-limitation at moderate final cell densities (ca. 13 000 cells mL^{-1}) to obtain enough sampling material at feasible culture volumes. The production of both POC and PIC created a drift in the carbonate system during culture growth. The (up to 37 %) consumption of DIC by the time of sampling depended mainly on the final cell densities reached in the culture. These final cell densities varied more strongly between the main and additional experiments of the same treatment (e.g. low-P) than between treatments (high-P vs. low-P). Yet, the observed trends, such as increased coccolith malformations in low-P cultures, were similar between the experiments, making carbonate chemistry an unlikely candidate for explaining the observed differences between treatments. Although bicarbonate, a known substrate for calcification, was reduced down to ca. 1100 µmol kg^{-1}, Rickaby et al. (2010) have shown that a reduction in bicarbonate down to 1000 µmol kg^{-1} had no effect on either culture growth (same growth rate as control) or calcification (low percentage of malformed coccoliths) in *C. pelagicus* subsp. *braarudii*. The changes in calcification observed in the current study (reduced PIC quota and increased coccolith malformations) at high temperature were therefore not due to reduced substrate availability as the cultures were kept above the threshold for bicarbonate limitation. Furthermore, due to lower final cell densities, bicarbonate availability was actually higher in 15 °C than in 10 °C degree cultures of subsp. *pelagicus*. The reduction in PIC quota at 15 °C was therefore not related to bicarbonate availability.

4.1 Effect of phosphorus limitation and temperature on culture growth and phosphorus quotas

Initial phosphate concentrations of the growth medium did not influence cell division in exponential phase in either subspecies. The exponential growth rate of subsp. *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 0.9 d^{-1}) for the same strain (Langer et al., 2006; Krug et al., 2011), these authors used a higher irradiance and a longer light phase which probably increased cell division rates. The subarctic subspecies (subsp. *pelagicus*) had a lower exponential growth rate than the temperate subsp. *braarudii*, even when these two subspecies were grown at the same temperature (15 °C). This may be due to strain-specific differences as have been described for different strains of *E. huxleyi* (Langer et al., 2009).

We found clear evidence for P-limitation with significantly lower POP quotas and higher C/P and N/P ratios in low-P cultures. The doubled POP quota of subsp. *pelagicus* grown at 15 °C instead of 10 °C, points towards higher

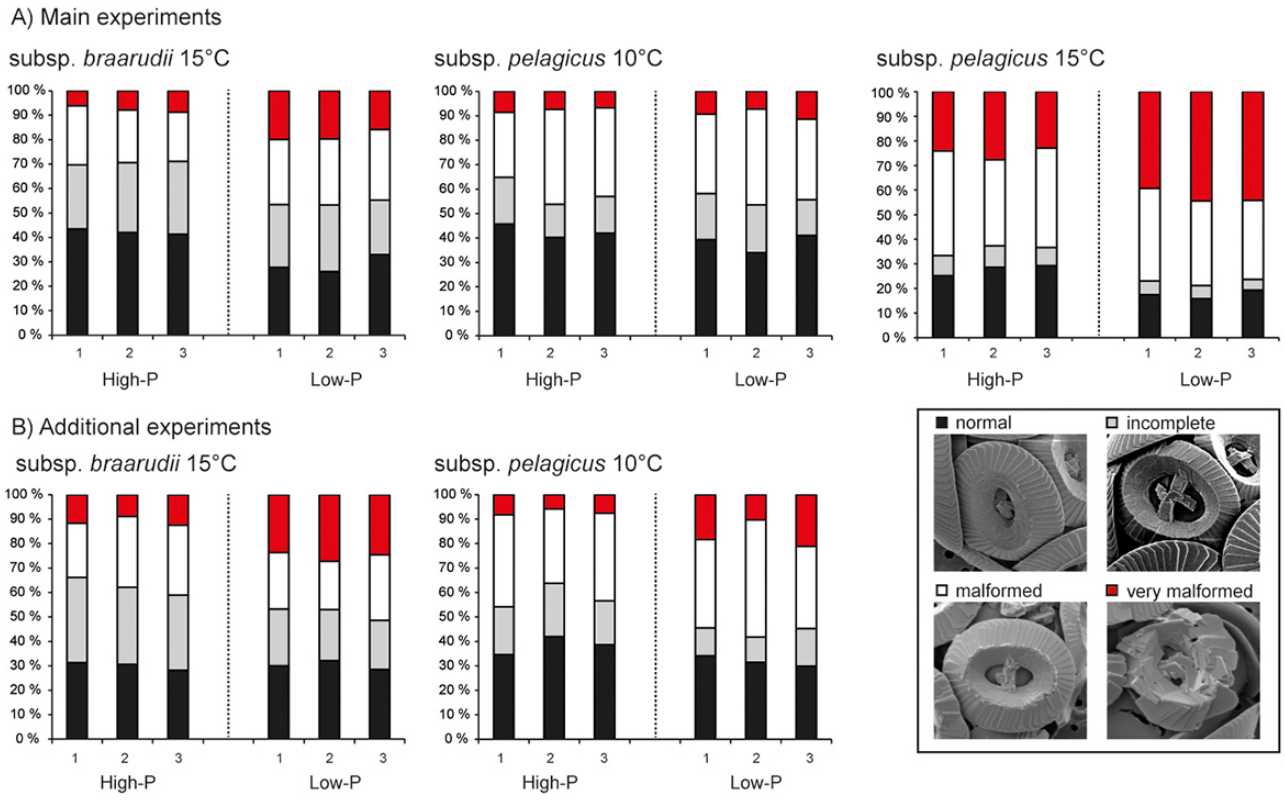

Figure 5. Morphology of coccoliths observed by scanning electron microscopy, shown as the percentage of four categories: normal (black), incomplete (grey), malformed (white) and very malformed (red). **(A)** Main experiments and **(B)** additional experiments of high-P and low-P cultures of *Coccolithus pelagicus* subsp. *braarudii* at 15 °C and subsp. *pelagicus* at 10 and 15 °C. Triplicate batch cultures (1, 2, 3) are presented separately for each experiment.

P-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 P under temperature stress may be linked to increased energy demands and deserves further study as an increased P-requirement at high temperature could exacerbate nutrient limitation in a warmer ocean (Sarmiento et al., 2004). Most of cellular P is to be found in RNA (Geider and La Roche, 2002). The higher POP content of subsp. *pelagicus* grown at high temperature (15 °C) could therefore also be related to an increased RNA content caused by upregulated expression of genes related to a temperature stress response. However, the nitrogen content of the cells did not increase, offering no evidence for an increase in enzyme production.

4.2 Effect of phosphorus limitation and temperature on the PIC / POC ratio

Temperature had a stronger effect on the PIC / POC ratio than P-availability. Whereas P-limitation did not affect the PIC / POC ratio when the subspecies were grown near their respective isolation temperature, elevated temperature decreased the PIC / POC ratio by more than half in high-P cultures of subsp. *pelagicus*. This decrease in the PIC / POC ratio was driven by the 40–60 % decrease in PIC quota whereas POC quotas were similar between the two temperatures. In high-P cultures, PIC production declined by ca. 60 %. This sharp reduction in PIC quota and production in subsp. *pelagicus* at elevated temperature, points towards a lower production of coccoliths or a decreased calcite content of single coccoliths. Although the variation in calcite content of individual coccoliths was high within one strain, the mean coccolith calcite content was unaffected 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 of subsp. *pelagicus* at elevated temperature (15 °C) contained ca. 12 % naked cells whereas no naked cells where observed at 10 °C. The coccolith coverage estimated by SEM 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. Many of the spheres observed in the high-temperature treatment of both high-P and low-P cultures were collapsed and/or incomplete, increasing the uncertainty of the estimate for coccolith coverage. However, total coccolith counts from POL samples confirmed that cultures at 15 °C contained ca. 40 % fewer

coccoliths than cultures at 10 °C. Reduced PIC production at elevated temperature was therefore due to a reduction in coccolith production with cells being covered by fewer coccoliths and an increased percentage of naked cells. This, together with an increased percentage of malformed coccoliths, apparently made the coccospheres unstable.

High temperature (15 °C) changed the response of subsp. *pelagicus* to P-limitation. At 15 °C the PIC / POC ratio, although still lower than at 10 °C, was significantly higher under P-limitation. This was due to a higher PIC quota of low-P compared to high-P cultures. This observation seems counterintuitive considering that at 10 °C there was no effect of P-limitation on the PIC quota in subsp. *pelagicus*. We suggest that this difference lies in high-P and low-P cultures being harvested in different growth phases (exponential vs. stationary phase) and the stronger dependence of POC production on P-availability. We explain this hypothesis in more detail in the following.

Coccolithus pelagicus has so far not been described to produce multiple coccolith layers (this study; Gibbs et al., 2013), whereas *E. huxleyi* can more than double its PIC / POC ratio by forming multiple layers of coccoliths around the cell (Linschooten et al., 1991; Paasche, 1998). It is therefore likely that there is an upper constraint to the PIC / POC ratio in *C. pelagicus* dictated by cell geometry. Without producing multiple layers, this species cannot increase PIC quota without increasing the cell surface area necessary to accommodate further coccoliths. However, increasing the surface area entails an increase in cell volume and thereby POC quota. Under such a scenario, there is a limit as to how freely the PIC / POC ratio can vary. This may explain the lack of increase in the PIC / POC ratio under P-limitation at "normal" temperature (15 °C for subsp. *braarudii*, 10 °C for subsp. *pelagicus*). Similarly, Langer et al. (2012) observed that the PIC / POC ratio of *C. leptoporus* is stable under nutrient limitation. Riegman et al. (2000), on the other hand, reported sustained PIC production in *E. huxleyi* during decreasing nutrient availability in P-limited chemostats in which POC production decreased.

The reason for the reduction in coccolith coverage in subsp. *pelagicus* at elevated temperature (15 °C) is unclear. However, at this temperature the coccolith coverage of the cells was overall reduced, alleviating the spatial constraint to the production of new coccoliths. High-P cultures were harvested in exponential phase, in which cells were dividing and POC production was relatively constant. Low-P cultures, on the other hand, were sampled in stationary phase when cell division and POC production had declined due to the depletion of phosphate from the medium. If we assume PIC production to be less dependent on P-availability than POC production, as suggested by Riegman et al. (2000) for one particular strain of *E. huxleyi*, low-P cultures could have continued producing coccoliths while cell division was slowing down. Sustained PIC production while POC production was decreasing in cultures going into stationary phase would explain the observed increase in the PIC / POC ratio in low-P cultures.

4.3 Effect of phosphorus limitation and temperature on coccolith morphology

The two subspecies overlapped in their size distribution, both in regard to coccosphere diameter and coccolith length. Overlapping coccolith size ranges have been previously described for these two subspecies, and our results confirm that subsp. *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 independently of the tested abiotic factors. Gibbs et al. (2013) have shown that mean coccolith size in *C. pelagicus* subsp. *braarudii* does not change during different growth phases and in *E. huxleyi*, coccolith dimensions have also 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 et al., 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 subsp. *pelagicus* coccoliths were lighter at 100 ± 27 pg calcite. These estimates are lower than the estimates obtained using the shape factor of Young and Ziveri (2000). By means of this species-specific shape factor (0.06 for *C. pelagicus*), mean coccolith weight was estimated from the proximal shield length (PSL), rendering values of 159 ± 54 pg calcite for subsp. *braarudii* and 120 ± 44 pg calcite for subsp. *pelagicus*. The main reason for this difference in calcite content estimates 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 (ca. 40 % in high-P cultures at "normal" temperature) and incomplete coccoliths also occurred frequently, especially in subsp. *braarudii* (ca. 30 % in high-P cultures). The presence of malformations in control cultures can probably be ascribed to culture artefacts which are not yet completely understood (Langer and Benner, 2009; Langer et al., 2013b). These authors described a high degree of malformation in control cultures of *E. huxleyi* while Langer et al. (2012) reported up to 53 % malformed coccoliths in control cultures of *C. leptoporus*. Although Rickaby et al. (2010) observed low levels of background malformations (ca. 20 %) in the same strain of subsp. *braarudii* as was used in this study (RCC1200), Langer et al. (2006) described only 50 % of coccoliths as normal in this strain, similar to our findings. Malformations in control cultures may arise from high cell

densities (Langer et al., 2013b). This may explain the low level of malformations observed by Rickaby et al. (2010) in highly dilute batch cultures ($< 2\,300$ cells mL^{-1}) compared to our higher cell concentrations (ca. 13 000 cells mL^{-1}). However, the 50 % malformed coccoliths reported by Langer et al. (2006) were observed in batch cultures at cell concentrations of ca. 6 000 cells mL^{-1}, indicating that the percentage of malformations may not be constant over time (Langer et al., 2013b). Another factor leading to malformations in culture is a lack of mixing (Langer et al., 2013b). Our cultures were agitated only once a day which may have contributed to the high degree of malformations observed in our control cultures.

Phosphorus limitation increased the percentage of malformed coccoliths further by ca. 14 %. Nutrient limitation has previously been suggested to increase malformations in *E. huxleyi* in both mesocosm and laboratory experiments (e.g. Båtvik et al., 1997; Paasche, 1998). However, the malformation increases described by these authors are slight and/or poorly quantified and Langer et al. (2012) argued that a difference of less than 10 % in malformations may be within the range of natural variation. Also, these authors failed to observe an effect of nutrient availability on coccolith morphology in *C. leptoporus* and concluded that the influence of nutrient availability on the coccolith machinery is negligible. Our study is therefore the first to show that coccolith morphology can be significantly (> 10 %) modified by limitation by a macronutrient, in this case phosphate. In subsp. *pelagicus*, elevated temperature had an even stronger effect than P-limitation on coccolith morphology. The percentage of normal coccoliths in high-P cultures of subsp. *pelagicus* went down to ca. 30 % at 15 °C. This temperature was probably above the temperature tolerance of this subarctic strain and increased malformations could have been related to a stress response to high temperature. Similarly, Langer et al. (2010) observed a decrease of normal coccoliths in *E. huxleyi* from 81 % at 10 °C to only 29 % at 25 °C. The highest degree of malformation in this study was observed in subsp. *pelagicus* cultures faced with both P-limitation and temperature stress indicating that several environmental stressors can add up to compromise coccolith morphology.

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 P-quotas, but showed no change in the PIC / POC ratio when grown near the subspecies' respective isolation temperature. As *C. pelagicus* does not produce multiple coccolith layers, there is likely an upper constraint to the PIC / POC ratio in this species. Temperature stress, on the other hand, had a strong impact on the PIC / POC ratio which decreased by 40–60 %. This was due to a reduced production of coccoliths, whereas the mean calcite content of individual coccoliths was not affected by experimental conditions. However, malformations of coccoliths increased under both P-limitation and elevated temperature, with the highest percentage of malformed coccoliths observed under a combination of P-limitation and high temperature stress. Although increased malformations under nutrient limitation have been previously suggested (e.g. Båtvik et al., 1997; Paasche, 1998), the evidence has so far been poor (Langer et al., 2012). This study is therefore the first to show that limitation by a macronutrient can indeed impact coccolith morphology.

Elevated temperature doubled P-quotas, indicating an increased P-requirement to maintain similar growth and POC production rates at higher temperature. 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). This in turn will affect global biogeochemical cycles. Species- and strain-specific differences in physiology 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, a decrease in PIC production at elevated temperature and changes in the abundance of this species may influence feedback mechanisms of ocean–atmosphere CO_2 exchange.

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