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Interactive comment on "High temperature decreases the PIC / POC ratio and increases phosphorus requirements in *Coccolithus pelagicus* (Haptophyta)" *by* A. C. Gerecht et al.

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We would like to thank Anonymous referee #2 for the comments on the manuscript. We would like to address the main issues raised and submit a revised Table 1 (Table R1). In Table R1, the data for the two experimental runs for ssp. braarudii at 15°C and ssp. pelagicus at 10°C has been separated (see also Gerecht, Biogeosciences Discuss., 11, C60–C63, 2014).

1) There is a considerable drift between initial and final conditions. Most importantly, there is a different drift between treatments.

Our aim with the present experiments was to test whether phosphorus limitation (and

temperature) would affect the ratio of inorganic to organic carbon production in Coccolithus pelagicus. This hypothesis was tested by running low-P batch cultures into stationary phase at moderate final cell densities (ca. 13.000 cells mL-1). Final cell densities were a result of initial phosphate concentrations which were chosen based on feasibility of culture volumes vs. sampling requirements and taking into account a moderate change in carbonate chemistry.

The considerable – and expected – drift between initial and final carbonate chemistry in all treatments was due to the consumption of carbon for organic matter production and calcification during culture growth. The extent to which dissolved inorganic carbon (DIC) was consumed was dictated primarily by the final cell concentrations reached by the time of sampling. For example, in ssp. braarudii, DIC concentrations were lower in both high-P and low-P cultures of the additional experiment than in high-P and low-P cultures of the main experiment (see Table R1), due to higher cell concentrations in the additional experiment. In general, these differences in final cell concentrations (and thereby carbonate chemistry) were greater between main and additional experiments than between high-P and low-P cultures of the same experiment. Yet, the observed trends, e.g. increased malformation of coccoliths in low-P cultures, were similar between the experiments. Therefore carbonate chemistry is an unlikely candidate for explaining the observed differences.

The pH-values in the cultures decreased down to pH=7.81. Studies by Krug et al. (2011) have shown that PIC production in C. braarudii remains unaffected down to pH-values of ∼7.6. Furthermore, pH-values tended to be lower in high-P than in low-P cultures (Table R1) so that increased malformations in low-P cultures cannot be explained by a relative pH difference between high-P and low-P cultures or an overall decrease in pH.

2) There is a big difference in HCO3- concentrations, a known substrate for calcification.

DIC concentrations decreased by 13-37% from initial (T0) to final (Tsample) conditions, depending on final cell concentrations. As ca. 90% of DIC is in the form of bicarbonate (HCO3-), bicarbonate concentrations showed a similar decrease down to ca. 1100 μmol kg-1. Bicarbonate is an important substrate for both calcification and organic carbon fixation (Rickaby et al. 2010) and could potentially become limiting for either or both processes. Rickaby et al. (2010) grew C. pelagicus (ssp. braarudii RCC1200; the same strain as used in the current study) in dilute batch cultures with varying DIC concentrations. These authors showed that at the lowest DIC concentrations (HCO3- ca. 1000 μmol kg-1), the batch cultures retained similar exponential growth rates as cultures under "normal" conditions (HCO3- ca. 1900 μmol kg-1). This shows that bicarbonate concentrations down to ca. 1000 μmol kg-1, slightly lower than the values reached in the current study, do not limit exponential growth of this strain (see Rickaby et al. 2010, supplementary information). The percentage of malformed coccoliths was lower at these low DIC values compared to the "normal" treatment (10 vs. 17%). There is therefore no evidence that an almost 50% reduction in bicarbonate concentrations influences calcification in this species.

In our study, the strongest effect of culture conditions on calcification (low PIC quota, high percentage of malformed coccoliths) was observed in the high temperature (15°C) low-P treatment of ssp. pelagicus. These cultures, however, had higher final bicarbonate concentrations (ca. 1350 μmol kg-1) than low-P cultures at 10°C (ca. 1150 μmol kg-1). The reduced PIC quota and increased occurrence of malformed coccoliths can therefore not be explained by decreased bicarbonate availability.

3) Initial TA and pH measurements were in some cases quite different and with relatively high standard deviations.

As mentioned in Gerecht (Biogeosciences Discuss., 11, C60–C63, 2014), the original Table 1 shows average values of two experimental runs of triplicate batch cultures each for ssp. braarudii 15°C (high-P and low-P cultures) and ssp. pelagicus 10°C (high-P and low-P cultures). The two experimental runs have now been separated (see Table

R1), which reduces some of the variation.

Table R1 no longer includes a standard variation for initial values (T0) as duplicate measurements for pH and TA were carried out on samples collected directly from the inoculation medium. However, these duplicate measurements varied at most 0.01 units for pH-measurements and 50 μmol kg-1 for TA concentrations. The at times higher variations in average values of final conditions (Tsample) in Table R1 are due to variation among the triplicate cultures.

The high standard deviation reported for TA values (50-100 μmol kg-1) results from the chosen methodology (manual titration) which has a precision of only $\pm$50 μmol kg-1. As the present experiments were aimed at examining the effect of temperature and P-limitation, not the effect of changing carbonate chemistry, this precision was judged as sufficient.

4) Running cells into limitation with relatively unconstrained knowledge if the amount spent in stationary phase is similar between treatments, adds another factor of uncertainty.

All cultures were followed by daily cell counts. Phosphorus limited cultures were sampled on the day the cultures stopped growing (i.e. when the cell counts on that day had increased by max. 5% from the previous day's counts). Therefore, the time spent in stationary phase was minimal and as similar between temperature treatments as was experimentally feasible.

To conclude, although there was a considerable shift in carbonate chemistry parameters due to cultures reaching moderate cell densities, the observed changes are considered to be within a range that should not affect calcification in this species for the reasons outlined above. Furthermore, relative or absolute changes in carbonate chemistry parameters are not correlated to the consistent increases in malformed coccoliths and reduced PIC quota, which are rather explained by P-limitation and increased temperature.

References:

Gerecht, A., Biogeosciences Discuss., 11, C60–C63, 2014.

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, 2011.

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, 2010.

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Table R1. 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* ssp. *braarudii* (RCC1200) grown at 15°C and ssp. *pelagicus* (J23) 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.

a) Main experiments:

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)	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)

Fig. 1.

b) Additional experiments:

C. pelagicus	ssp. *braarudii* (RCC1200)				ssp. *pelagicus* (J23)			
	high-P 15°C		low-P 15°C		high-P 10°C		low-P 10°C	
	T_0	T_{sample}	T_0	T_{sample}	T_0	T_{sample}	T_0	T_{sample}
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_A	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)

Fig. 2.