Supplement of

Can morphological features of coccolithophores serve as a reliable proxy to reconstruct environmental conditions of the past?

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S1 Geological background

Several authors tried to link nannofossil morphological variations with detected environmental conditions: the process assumes to analyze nannofossil species through a sedimentary succession and to evaluate the presence of shape or size anomalies in the considered interval of time. The eventual detected morphological variations are then linked to independent paleoenvironmental data (e.g. sea surface temperature reconstruction (SST), CO$_2$) to find the environmental driver for the identified morphological variation.

Indeed, the past oceans were characterized by episodes of anomalous or extreme sea-water conditions that could have possibly influenced the phytoplanktonic communities. A good example is the oscillations between “calcite seas” and “aragonite seas” (Sandberg, 1983) that possibly influenced the productivity of calcareous nannoplankton at different times (Erba, 2006). The amount of massive amount of chalk deposited during the Late Cretaceous is a good illustration of a high productivity time for calcareous nannoplankton probably permitted by a shift in seawater chemistry towards a very high level of Ca. In parallel, rising Mg/Ca ratio during the Cenozoic and up to present days is correlated to a reduction in coccolithophore diversity and coccolith thickness (Bown et al., 2004). Also, locally, light could have played a major role for coccolithophore calcification in the past ocean: it was documented that during episodes of intensified continental weathering, more elatic particles were transported into the sea and in the coastal area might have diminished the depth of the photic zone (Lechler et al., 2015). The reduction in light availability was associated with habitat changes of the photoautotrophic primary producers that produced smaller coccoliths to be able to dwell in shallower depth and compensate for the reduction in sunlight (Lübke and Mutterlose, 2016). The calcareous phytoplanktonic communities in past oceans were also disturbed by intervals with excess CO$_2$ concentrations related to intense volcanic activity. Modifications in size and morphology of calcareous nannofossil during times were CO$_2$ reached up to 1000-2000 ppm, were interpreted as a transient response to survive progressively increasing surface-water acidification (Erba et al., 2010, Lübke et al., 2015; Faucher et al., 2017). Besides, environmental constraints for calcareous nannoplankton growth, involve the ocean trophic level: in the fossil record, some authors linked the decrease in nutrient availability, with reductions of abundances and sizes of some calcareous nannofossil species (Linnert and Mutterlose, 2012). Other authors, on the contrary, detected similar size reductions in several ocean areas characterized by both oligotrophic and mesotrophic seawater conditions (Bornemann et al., 2006; Faucher et al., 2017). Finally, the ocean in its history was subjected to variation in temperature: a strong hydrothermal activity on one hand and an intense continental weathering, on the other hand, were the main triggers of respectively CO$_2$ released and CO$_2$ sequestration, that in turns, often produced a concomitant increase or decrease in SST. Episodes characterized by relatively low SST were sometimes related to small coccolith sizes (Bornemann and Mutterlose, 2006). However, opposite behaviors were also observed (size decreases under extremely warm conditions, Erba et al., 2010; Lübke et al., 2015) in the same species in different geological intervals.

References:


**S2 Growth rate**

Samples for cell abundance were taken at the end of the experiment with the exception of the nutrient experiments where samples were taken every second day. Incubation bottles were turned to resuspend all cells and to obtain a homogenous suspension of the cells before sampling. Cell numbers were immediately measured three times without addition of preservatives using a Beckman coulter Multisizer. Specific daily growth rates ($\mu$) were calculated as:

$$\mu = \frac{\ln c_1 - \ln c_0}{t_1 - t_0}$$
where $c_0$ and $c_1$ are the cell concentrations at the beginning ($t_0$) and at the end of the incubation period ($t_1$), respectively. Growth rate data were used to check the growth phase of the species.

S2.1 Light

*Emiliania huxleyi* growth rates followed an optimum curve response pattern along the light gradient and the optimum growth rate was recorded at 450 µmol photons m\(^{-2}\) s\(^{-1}\) (Fig. S1; Table 2). *Gephyrocapsa oceanica, C. braarudii* and *P. carterae* growth weren’t influenced by light intensity. *P. carterae* displayed increasing growth rates at higher light intensities. All data are reported in Table 2 and shown in Fig. S1.

S2.2 Mg/Ca

*Emiliania huxleyi, G. oceanica* and *C. braarudii* growth rates were negatively influenced by increasing [Ca\(^{2+}\)] while no effect was observed on *P. carterae* growth (Fig. S2; Table 3).

S2.3 Nutrient

In the N and P limited treatments, *E. huxleyi* growth rate was 58% and 71% lower than in the (nutrient replete) control treatment (Fig. S3; Table 4). *Gephyrocapsa oceanica* growth rate in N and P limited treatments was 76% and 43% lower (Fig. S3). *Coccolithus braarudii* growth rate was 82% and 69% lower. *Pleurochrysis carterae* did not grow in the P limited conditions. N-limitation reduced growth rate compared to the control by 50%.

S2.4 Temperature

Elevated temperature, accelerated growth rates in *E. huxleyi* and *G. oceanica* by 50 and 75%, respectively. *Pleurochrysis carterae* growth rates declined by about 30% at 22.5°C relative to 15°C. *Coccolithus braarudii* did not grow at 22.5°C (Fig. S4; Table 5).

S2.5 Carbonate chemistry

*Emiliania huxleyi* growth rate was significantly lower in the OA and CS2 treatments compared to the control and the CS1 treatment. (Fig. S5, Table 6). *Gephyrocapsa oceanica* growth rate was reduced in the OA, CS1 and CS2 treatment compared to the control with the lowest rate observed in the CS2 treatment. *Coccolithus braarudii* growth was reduced in the OA, CS1 and CS2 treatments compared to the control. *Pleurochrysis carterae* growth rate was unaffected by changing carbonate chemistry.
Figure S1: Average growth rate under different irradiances. Square: *E. huxleyi*; dot: *G. oceanica*; triangle: *C. braarudii*; diamond: *P. carterae*.

Figure S2: Average growth rate under different Mg/Ca conditions; all measurements were done in triplicates; error bars denote standard deviations. If not visible, error bars are smaller than symbols. Symbols: square: *E. huxleyi*; dot: *G. oceanica*; triangle: *C. braarudii*; diamond: *P. carterae*.

Figure S3: Average growth rate under different nutrient conditions; all measurements were done in triplicates; error bars denote standard deviations. If not visible, error bars are smaller than symbols. Symbols: square: *E. huxleyi*; dot: *G. oceanica*; triangle: *C. braarudii*; diamond: *P. carterae*; *P. carterae* didn’t grow under P limited regime.
Figure S4: Average growth rate under different temperature conditions; all measurements were done in triplicates; error bars denote standard deviations. If not visible, error bars are smaller than symbols. Symbols: square: *E. huxleyi*; dot: *G. oceanica*; diamond: *P. carterae*.

Figure S5: Average growth rate under different carbonate chemistry conditions; all measurements were done in triplicates; error bars denote standard deviations. If not visible, error bars are smaller than symbols. Symbols: square: *E. huxleyi*; dot: *G. oceanica*; triangle: *C. braarudii*; diamond: *P. carterae*. 
Plate 1: Example of coccoliths of the four tested species under different light intensities

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>[Ca(^{2+})] 25</th>
<th>[Ca(^{2+})] 50</th>
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</thead>
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<td><img src="image2" alt="Image of E. huxleyi coccoliths" /></td>
<td><img src="image3" alt="Image of E. huxleyi coccoliths" /></td>
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<td><img src="image12" alt="Image of P. carterae coccoliths" /></td>
</tr>
</tbody>
</table>

Plate 2: Example of coccoliths of the four tested species under different Ca concentrations
Plate 3: Example of coccoliths of the four tested species under different nutrient conditions and temperature values.
Plate 4: Example of coccoliths of the four tested species under different CO$_2$ concentrations