https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019

Climate of the Past Discussions

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

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Abstract. Morphological changes in coccoliths, tiny calcite platelets covering the outer surface of coccolithophores, can be the result of physiological responses to environmental changes. Coccoliths recovered from sedimentary successions may therefore provide information on paleo-environmental conditions prevailing at the time when the coccolithophores were alive. To calibrate the biomineralization responses of ancient coccolithophore to climatic changes studies often compared the biological responses of living coccolithophore species with paleo-data from calcareous nannofossils. However, there is uncertainty whether the morphological responses of living coccolithophores are representative for those of the fossilized ancestors. To investigate this, we cultured four living coccolithophore species (*Emiliania huxleyi, Gephyrocapsa oceanica*, *Coccolithus pelagicus subsp. braarudii,* and *Pleurochrysis carterae)* that have been evolutionarily distinct for millions of years, exposed them to changing environmental conditions (i.e. changing light intensity, Mg/Ca ratio, nutrient availability, temperature and carbonate chemistry) and evaluated their responses in coccolith morphology (i.e. size, length, width, malformation). The motivation for this study was that if the species show the same morphological response to changes in any of the tested abiotic environmental factors, then there is a reason to assume that this response is conserved over geological timescales and that coccolith morphology can serve as a paleo-proxy for that specific factor. In contrast with this concept, we found that the four species responded differently to changing light intensity, Mg/Ca ratio, nutrient availability and temperature in terms of coccolith morphology. The lack of a common response reveals the difficulties in using coccolith morphology as a proxy for paleo-environmental conditions. However, a common response was observed under changing seawater carbonate chemistry (i.e. rising CO_2) which consistently induced malformations. This commonality provides some confidence that malformations found in the sedimentary record could be indicative for high CO_2 levels.

1 Introduction

Coccolithophores are calcifying marine phytoplankton and are among the most important calcite producers on Earth. They cover their outer surface with a calcitic exoskeleton (coccosphere) composed of single platelets called coccoliths and nannoliths. Due to their ability to precipitate calcium carbonate, coccolithophores played an important role in rock-formation during the Jurassic and Cretaceous as well as through the Cenozoic (e.g. Erba, 2006). They are directly affected by

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019


Climate of the Past Discussions

environmental drivers such as temperature, salinity, nutrient concentration, light, and carbonate chemistry. Thus, fossil remains (coccoliths and nannoliths) have often been used as paleo-proxies to reconstruct past physical and chemical conditions in the surface ocean (e.g. Erba 1994; Tiraboschi et al., 2009; Erba et al., 2010; Lübke and Mutterlose, 2016; Faucher et al., 2017a). So far, however, it is extremely difficult to disentangle the individual factors, that singularly or combined, affected calcareous nannoplankton abundance and or size variations in the geological record. Therefore, to calibrate the responses of ancient coccolithophore to environmental changes studies often compared the biological responses of living coccolithophore species with paleo-data from calcareous nannofossil. However, there is considerable uncertainty when using fossil coccolith remains to reconstruct paleo-conditions: there are millions of years of evolution between the time when we do physiological experiments and the geologic past. Addressing this uncertainty is difficult since phytoplankton can evolve quickly (Bown et al., 2005; De Vargas et al., 2007). We therefore chose a different approach and did a series of identical stress test experiments with four different modern species that have been evolutionarily distinct since millions of years (Fig.1). In case that coccolith morphology responses to a changing environmental driver are similar in the four species this could be indicative of a response pattern that was conserved over geological timescales. The presence of a conserved response pattern would strengthen our confidence that our findings made with modern species are transferable to the geological past, although even then uncertainties remain due to the possibility of convergent evolution of the species through time.

For our experiments we selected four different coccolithophore species: *Emiliania huxleyi, Gephyrocapsa oceanica*, *Coccolithus pelagicus subsp. braarudii,* and *Pleurochrysis carterae*. According to "molecular-clock-data" they are evolutionarily distinct since the Triassic or the Jurassic (with the exception of *G. oceanica* and *E. huxleyi*; Liu et al., 2010). We present data on how coccolith size and morphology change in response to a suite of different environmental drivers and explore whether there is a common response to any of these drivers among the different species. Based on this we discuss whether morphological features of coccoliths have the potential to serve as paleo-proxies.

2 Material and Methods

2.1 Experimental setup

Five experiments are presented in this study with generally similar design. Every experiment tested the influence of one abiotic parameter on four different coccolithophore species which were cultured individually (i.e. in separate bottles). The tested abiotic factors were: light intensity, nutrient (N or P) limitations, Mg/Ca ratio, temperature, carbonate chemistry. Monospecific cultures of the coccolithophores *Emiliania huxleyi* (strain RCC 1216), *Gephyrocapsa oceanica* (strain RCC 1303), *Coccolithus pelagicus subsp. braarudii* (strain PLY182G, it will be called hereafter *C. braarudii*), and *Pleurochrysis carterae* (unknown strain number) were grown in artificial seawater (ASW; Kester et al., 1967) under dilute batch culture conditions (LaRoche et al., 2010). The artificial seawater medium was enriched with 64 µmol kg^{-1} nitrate, 4 µmol kg^{-1} phosphate to avoid nutrient limitations with the exception of the nutrient limitation experiment (see section 2.1.4). In all experiments we added f/8 concentrations of vitamins and trace metals (Guillard and Ryther, 1962), 10 nmol kg^{-1} of SeO_2 (Danbara and Shiraiwa, 1999)

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019


Climate of the Past Discussions

and 2 ml kg^{-1} of natural North Sea water to provide potential nutrients which were not added with the nutrient cocktail (Bach et al., 2011). The medium was sterile-filtered (0.2 µm). The carbonate chemistry was adjusted with aeration for 24 h using a controlled CO_2 gas mixing system reaching the treatment levels of 400 (total alkalinity, TA, 2302 µmol kg^{-1}) with the exception of the carbonate chemistry experiment (see section 2.1.5).

The medium was then transferred into 0.5 L NalgeneTM bottles. Cultures were incubated in a thermo constant climate chamber (Rubarth Apparate GmbH) at a constant temperature of 15°C, (with the exception of the temperature experiment; see section 2.1.4), with a 16:8 [hour:hour] light/dark cycle, at a photon flux density of 150 µmol photons $m^{-2}s^{-1}$ (with the exception of the light experiment; see section 2.1.1). Before the start of the experiments, coccolithophore cultures were acclimated for about 7-10 generations to each of the experimental conditions. It was assured that all cultures were in the exponential growth phase at the initiation of the experiments (also in the nutrient limitation experiment; see section 2.1.3). All culture bottles were manually and carefully rotated three times a day, each time with 20 rotations in order to reduce sedimentation bias. Final samples were taken when cells were exponentially growing (except for nutrient limitation experiments; see section 2.1.3) but cell numbers were still low enough to limit their influence on the chemical conditions of the growth medium.

2.1.1 Specifics in the light experiment

The light setup was adjusted to test the response of the four species to a gradient of photon flux densities (PFD). Because light intensities are difficult to replicate we chose a gradient design in this experiment at the expense of replication (Cottingham et al., 2005). Therefore, the light was set to the highest possible intensity in the light chamber and the bottles were placed at different positions so that 12 different PFDs were established (50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 µmol photons $m^{-2}s^{-1}$). Light intensities were measured at every treatment position in the light chamber, using a Li-250A light meter (Li-Cor, Heinz Walz GmbH, Effeltrich).

2.1.2 Specifics in the Mg/Ca experiment

This experiment was designed to test the physiological response of coccolithophore algae to changing $[Ca^{2+}]$ while keeping $[Mg^{2+}]$ constant at the modern seawater value. In the control, the Mg/Ca ratio was set to simulate the modern ocean values (Mg/Ca = 5.2) with $[Ca^{2+}]$ = 9.8 mmol L^{-1} and $[Mg^{2+}]$ = 50 mmol L^{-1}. The low Mg/Ca treatments were set by increasing $[Ca^{2+}]$ to 25 and 50 mmol L^{-1}, respectively. The control and both treatments were replicated three times.

2.1.3 Specifics in the nutrient experiment

Batch cultures were grown under N or P limitations. For N-limitation, all cultures were run into N-limitation during the acclimation phase but care was taken that this occurred at low cell densities so that the chemical conditions in the seawater (apart from nutrients) remained largely unaffected. During the main experiment, cell concentrations were counted every other day and 0.14 pmol N $cell^{-1}$ (as $NaNO_3$) was added to the medium when cultures reached the stationary phase (i.e. they stopped dividing). The same was done in P-limitation experiments except that 0.01 pmol P $cell^{-1}$ (as NaH_2PO_4) was added when

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019


Climate of the Past Discussions

reaching the stationary phase. As control, we used exponentially growing cells which were replete in both N and P. Nutrient concentrations were not measured but limitations were assured by measuring and comparing growth rates which were much lower than in the nutrient replete controls. Controls and both treatments were replicated three times.

2.1.4 Specifics in the temperature experiment

The experiments were carried out in two temperature-controlled light chambers in order to test the response of the coccolithophores to increased temperature. Batch cultures were grown at 15°C and 22.5°C. Both temperature treatments were replicated three times.

2.1.5 Specifics in the carbonate chemistry experiment

In the ocean acidification (OA) treatment, TA was kept constant (2348 µmol kg^{-1}) whereas fCO_2 was increased to 1020.5 µatm. In the Cretaceous scenario1 (CS1) treatment, fCO_2 was kept constant at 1020.5 µatm, while TA was increased to 3729 µmol kg^{-1}. In the Cretaceous scenario2 (CS2) treatment, fCO_2 was increased up to 3061 µatm and TA up to 4978 µmol kg^{-1}. Carbonate chemistry parameters (pHf (free scale), HCO_3^-, CO_3^{2-}, CO_2) were calculated using the program CO2SYS (Lewis and Wallace, 1998) from measured TA, and calculated estimated DIC, temperature, salinity and [PO_4], and the dissociation constants determined by Roy et al., 1993. In the OA, CS1 and CS2 treatments, DIC and TA levels were adjusted by adding calculated amounts of Na_2CO_3 (Merck, Suprapur quality and dried for 12 hours at 500°C) and hydrochloric acid (3.571 mol L^{-1}, certified by Merck) (Gattuso et al., 2010).

Samples for pH and TA analyses were taken at the beginning and at the end of the experiments. Samples were filtered (0.7 µm) and stored at 4°C until measurements that were performed within 2 days for pH measurements and 14 days for TA. pH was measured spectrophotometrically with Varian Cary 100 in 10 cm cuvette at 25°C as described in Dickson (2010) and then recalculated to in-situ temperature (15°C) using CO2SYS (Schulz et al., 2017). Every sample was measured 3 times. Samples for TA were measured in duplicate with Metrohm 862 Compact Tritino Sampler device following Dickson, 2003. TA data were accuracy controlled with certified reference material (A. Dickson, La Jolla, CA).

2.2 Cell abundance, coccosphere and cell size

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.

After the abundance measurements, samples were acidified with 0.1 mmol L^{-1} HCl to dissolve all free and attached coccoliths and subsequently measured another 3 times each in order to obtain cell diameters and volumes (Müller et al., 2012).

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019


Climate of the Past Discussions

2.3 Scanning Electron Microscopy (SEM)

5-10 ml of sample were filtered by gravity on polycarbonate filters (0.2 µm pore size) and dried directly after filtration at 60°C for two days. Samples were sputtered with gold-palladium. SEM analysis was performed at the Earth Sciences department of the University of Milan with SEM Cambridge Stereoscope 360. All pictures were taken with the same magnification (5000x) and the scale bar given on SEM pictures was used for calibration. For every coccolith the length (DSL) and the width (DSW) of the coccolith distal shield were manually measured using the public domain program Fiji distributed by ImageJ software (Schindelin et al., 2012). For *E. huxleyi*, the tube thickness, the number of rays and the ray thickness were also measured. For *G. oceanica* the tube thickness and the bridge orientations were measured. Moreover, the presence of malformations was quantified by visual inspection (Fig. 2; Langer et al., 2006; Langer et al., 2010)

2.4 Statistics

Data were tested for normality and homogeneity of variances (Bartlett and Fligner-Killeen tests). To test the null hypothesis that differences in growth rates and sizes among treatments are the same, the average values of parameters from triplicate cultures were compared between treatments. A one-way analysis of variance was used to determine statistical significance of the main effect of the different parameters tested on the variables. A Tukey post-hoc test was used to assess whether differences between treatments or the control were statistically significant. Statistical treatments of data were performed using R software. Statistical significance was accepted for $p < 0.05$. For the light experiment a non-linear regression was used to explore the relationship between light and coccolithophore parameters (growth and sizes).

3 Results

3.1 Light

In all the four species, coccolithophore, cell and coccolith sizes didn't show any distinct trend with light intensity. Data are reported in Table 1.

E. huxleyi coccoliths were less elliptical with light intensities above 400 µmol photons m^{-2} s^{-1} and characterized by a higher number of rays with light intensities above 400 µmol photons m^{-2} s^{-1}. *G. oceanica* and *C. braarudii* coccolith size and shape did not change with light intensity. Finally, *P. carterae* coccoliths were less elliptical only at irradiances of 350 µmol photons m^{-2} s^{-1}. (Fig. 3; Supplementary, plate 1). Malformed coccoliths increased in percentage only in *E. huxleyi* at 500 µmol photons m^{-2} s^{-1} and in *G. oceanica* at 200 µmol photons m^{-2} s^{-1} (Fig. 4).

3.2 Mg/Ca

E. huxleyi, coccosphere and cell sizes were influenced by changes in seawater $[Ca^{2+}]$. Elevating seawater $[Ca^{2+}]$ to ≈ 25 mmol L^{-1} and 50 mmol L^{-1} resulted in a significant increase in the coccosphere and cell diameters ($p < 0.05$). Increased $[Ca^{2+}]$

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019


Climate of the Past Discussions

concentrations impacted *G. oceanica, C. braarudii* and *P. carterae* cell sizes with a reduction in size in comparison to[Ca^{2+}] of 9.8 mmol L^{-1}, when seawater [Ca^{2+}] was elevated to ≈ 25 mmol L^{-1} and 50 mmol L^{-1}. *G. oceanica* and *P. carterae* coccosphere diameters were unaffected while the *C. braarudii* coccosphere was smaller when grown under [Ca^{2+}] of 50 mmol L^{-1} (Table 2). *E. huxleyi, G. oceanica* and *C. braarudii* coccolith sizes were not affected by changing [Ca^{2+}]. *P. carterae* coccoliths were smaller at the highest [Ca^{2+}] concentrations than in the control (Fig. 3; Table 2). *E. huxleyi* produced a higher percentage of malformed and/or incomplete coccoliths with increasing calcium concentrations (Fig. 4; Supplementary, plate 2) while no increased malformation was observed in the other species.

3.3 Nutrient limitation

E. huxleyi and *C. braarudii* coccospheres were larger under P-limitation than under N-limitation and the control. *G. oceanica* coccospheres were larger under N-limitation than under P-limitation and the control. *P. carterae* coccospheres were larger under N-limitation compared to the control. Cell size remained unaffected in *E. huxleyi* by nutrient limitation. *G. oceanica* cell and *C. braarudii* cells were larger under P-limitation compared to the control and N limitation. *P. carterae* cells were larger under N limitation compared to the control (Table 3).

E. huxleyi and *G. oceanica* coccoliths were larger under P limitation, while there was no significant difference between N limitation and the control. *E. huxleyi* coccoliths had a higher number of ray elements under P limitations. The tube was thinner in N and P limited treatments compared to the control and the central area was therefore wider. Furthermore, *E. huxleyi* and *G. oceanica*, produced relatively more malformed coccoliths under P limitation (Fig. 4). *C. braarudii* and *P. carterae* coccolith sizes remained unaffected with any sign of malformation by nutrient limitation (Fig. 3; Supplementary, plate 3).

3.4 Temperature

E. huxleyi and *G. oceanica* coccospheres and cell sizes were smaller at 22.5 °C. *P. carterae* coccosphere and cell sizes remained unaffected (Table 4).

E. huxleyi coccolith were smaller under high temperatures. Furthermore, *E. huxleyi* had less rays and a thinner tube when grown at 22.5°C. *G. oceanica* and *P. carterae* coccolith size remained largely unaffected by changing temperature. *P. carterae* coccoliths were less elliptical when grown at 22.5°C (Fig. 3; Table 4; Supplementary, plate 3).

3.5 Carbonate chemistry

E. huxleyi coccospheres and cells were largest in the OA treatment and smallest in the CS2 treatment. *G. oceanica* and *C. braarudii* coccospheres were largest in the control and smallest in CS2 treatment. *G. oceanica* cell size was lower in the CS2 treatment than in the control, as well as the OA and CS1 treatments. The cell size of *C. braarudii* was smaller in the OA, CS1 and CS2 treatments compared to the control. *P. carterae* coccosphere and cell size were unaffected by changing carbonate chemistry (Table 5)

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019


Climate of the Past Discussions

E. huxleyi formed significantly bigger coccoliths in the OA treatment compared to the control and the CS2 treatment (Fig. 3; Table 5) Furthermore, the tubes were thicker in the OA and CS1 treatments compared to the control and the CS2 treatment. Malformations were 20% more frequent in the OA, CS1 and CS2 treatments than in the control (Fig. 4; Supplementary, plate 4). *G. oceanica* formed a high number of malformed coccoliths in the OA and CS2 treatments. For *G. oceanica*, under OA and CS2 conditions morphometric analyses were not performed because a large majority of the coccoliths were extremely malformed and it wasn't possible to measure the shape of the specimens (Fig. 4). In the CS1 treatment, coccoliths were slightly smaller compared to the control. *C. braarudii* coccoliths were smaller in the OA and CS2 treatments compared to the control and the CS1. In the OA and CS1 treatments 40% of the *C. braarudii* coccoliths were malformed and ~ 10 % were incomplete. In the CS2 treatment 97% of coccoliths were malformed or incomplete. *P. carterae* coccoliths remained unaffected by carbonate chemistry.

4 Discussion

Coccolithophores started to calcify in the late Triassic and this biological innovation appeared in a period of strong climatic and biotic pressure. The earliest coccoliths had very simple morphologies and small sizes (2-3 µm; Bown et al., 2004). A great diversification in morphologies occurred in the Mesozoic and Paleocene where many new morphologies occurred. The appearance of new coccolith shapes followed the main geological events, at the K/Pg boundary, and the P/E boundary, and these big reorganizations suggest that certain kind of morphologies might have been no longer advantageous for coccolithophores algae under the new ecological circumstances. The evolution of calcareous nannoplankton through ~220 Ma documents a remarkable morphological diversity within the group and in the last 30 Ma there has been a loss of species that produced large and heavily calcified coccoliths but an increase in the modern community of coccolith architectures (Monteiro et al., 2016). The cause of this impressive number of structures is unknown but there might be a reason connected to the function of coccoliths for the different species to produce such different shapes. Accordingly, coccolith morphologies are likely only indirectly linked to environmental conditions but may reflect their adaptation to a specific, yet unknown function.

If morphological changes in coccoliths are the result of a physiological response to environmental variations (e.g. CO_2, nutrient, temperature), coccoliths recovered from marine sediments could potentially conserve paleo-environmental information prevailing when the coccolithophore was alive. Indeed, many studies on geological records calibrated biomineralization responses of ancient species to environmental drivers with experiments with modern species (e.g. Bornemann et al., 2006; Erba et al., 2010; Suchéras-Marx et al., 2010; Linnert et al., 2014; O'Dea et al., 2014; Lübke et al., 2015; Gibbs et al., 2016; Faucher et al., 2017a; Faucher et al., 2017b). This builds on the concept that coccolithophores conserved a certain response to certain environmental parameters over geological timescales. However, fossils and living coccolithophores diverged a long time ago, have a different genetic background and therefore, calcareous nannoplankton in the past and nowadays did and do not necessarily act in the same way to external stress. Furthermore, morphology may not only depend on abiotic environmental conditions but could perhaps also be the result of evolutionary development induced through ecological interactions. For

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019

example, if a coccolithophore genotype which forms larger coccoliths is better suited to protect a cell against prevalent grazers then these genotypes will likely proliferate whereas related genotypes forming smaller variants could eventually go extinct. The geological record would not easily allow us to distinguish if morphological changes are caused by physiological or ecological drivers as it is difficult enough to reconstruct abiotic paleo-environmental conditions but almost impossible to unravel relevant processes in the food web of the geologic past. Therefore, the fundamental question we asked ourselves was whether morphological features observed in living coccolithophores under specific environmental parameters could help to build reliable proxy for abiotic paleo-environmental conditions.

Our experiments did not reveal any common response among the four species with respect to coccolith sizes and shapes, in response to changing environmental drivers, even though variable responses were observed in various cases. For example, under excess CO_2, *E. huxleyi* formed larger coccoliths while *C. braarudii* formed smaller coccoliths than under control conditions. Under N and P limitations *G. oceanica* produced bigger coccoliths, while *E. huxleyi* coccoliths are smaller under the same conditions. The lack of a common response to the environmental drivers among the tested species points towards a high variability when using coccolith morphology as paleo-proxy for the suite of environmental drivers tested herein.

The experimental dataset generated herein is in line with observations from the fossil record. Indeed, there are several observations where just some of the prevailing species showed changes in morphology during intervals characterized by extreme climatic conditions even though all species were exposed to environmental stress. For example, dwarf specimens were recorded for *Biscutum constans* in all Mesozoic episodes characterized by abnormal conditions, during intervals of extreme volcanic activity (e.g. during OAE 1a, OAE 1b, OAE 2; Bornemann et al., 2006; Erba et al., 2010; Lübcke et al., 2015; Faucher et al., 2017; Erba et al., 2019). The inconsistency of morphological responses to changing environmental drivers observed in our experiments and the geological record makes it difficult to use morphological responses of living species as analogues for morphological changes of extinct species.

The one exception in our dataset are the observed responses in malformation to changes in carbonate chemistry where some consistency was observed among the four species. Malformations are generally considered evidence of errors during intracellular coccolith formation so that a disturbance of coccolithogenisis conserved in a malformation could indeed be the consequence of a direct (i.e. physiological) impact. Indeed, malformations are unlikely to be the consequence of an evolutionary (i.e. ecological) adaptation to environmental stress because there seems to be no obvious ecological advantage of producing malformed coccoliths. The high degree of malformation when coccolithophore were grown under excess CO_2 provides some evidence that at least this response variable could be used as paleo-proxy for episodes of acute carbonate chemistry perturbations.

However, it is important to bear in mind that in the geological record critical intervals characterized by excess CO_2 concentrations lasted for some dozen or hundred thousand years, whereas our experiments lasted a few generations (days). Thus, environmental stress on geological timescales may still be long enough for coccolithophores to adapt which can occur within months to years (Lohbeck et al., 2012; Bach et al., 2018).

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019

It also needs to be kept in mind that even if the four coccolithophore species tested here had shown similar morphological responses to changing environmental drivers, it could have not been excluded that this is the result of convergent evolution. Indeed, restriction on biological conditions and adaptation to particular habitats can produce widespread convergence as convergent evolution is often a consequence of adaptation to a similar niche (Arbuckle et al., 2014). Therefore, we want to point out that convergent morphological developments could represent similar adaptations of different species to abiotic parameters that occurred multiple times separate from each other.

4 Conclusion

According to the data provided in this study we come to the following conclusions: 1) sizes and morphologies of the four tested species change differently in response to changing temperature, light, nutrient, and Mg/Ca. In some cases, there were opposing reactions among species under the same abiotic stress; 2) A high number of malformations were detected when coccolithophores were grown under excess CO_2 and this response occurred in all species tested here.

Overall, there is no support for the suitability of coccolith morphometry to serve as proxy for temperature, light, nutrient, and Mg/Ca conditions of the past. However, coccolith malformations could perhaps be useful indicators for carbonate chemistry stress. Although more work is needed, in both living calcareous nannoplankton and in the fossil record, to evaluate whether malformations remains over long time period or if coccolithophores have and had an adaptive potential towards extreme carbonate chemistry conditions that might rapidly eliminate malformation in some generations.

The authors declare that they have no conflict of interest

Acknowledgments

We acknowledge Agostino Rizzi for SEM photography of coccoliths. This research was funded through MIUR-PRIN 2011 (Ministero dell'Istruzione, dell'Università e della Ricerca–Progetti di Ricerca di Interesse Nazionale) to Elisabetta Erba and through SIR-2014 (Ministero dell'Istruzione, dell'Università e della Ricerca–Scientific Independence of young researchers) to Cinzia Bottini.

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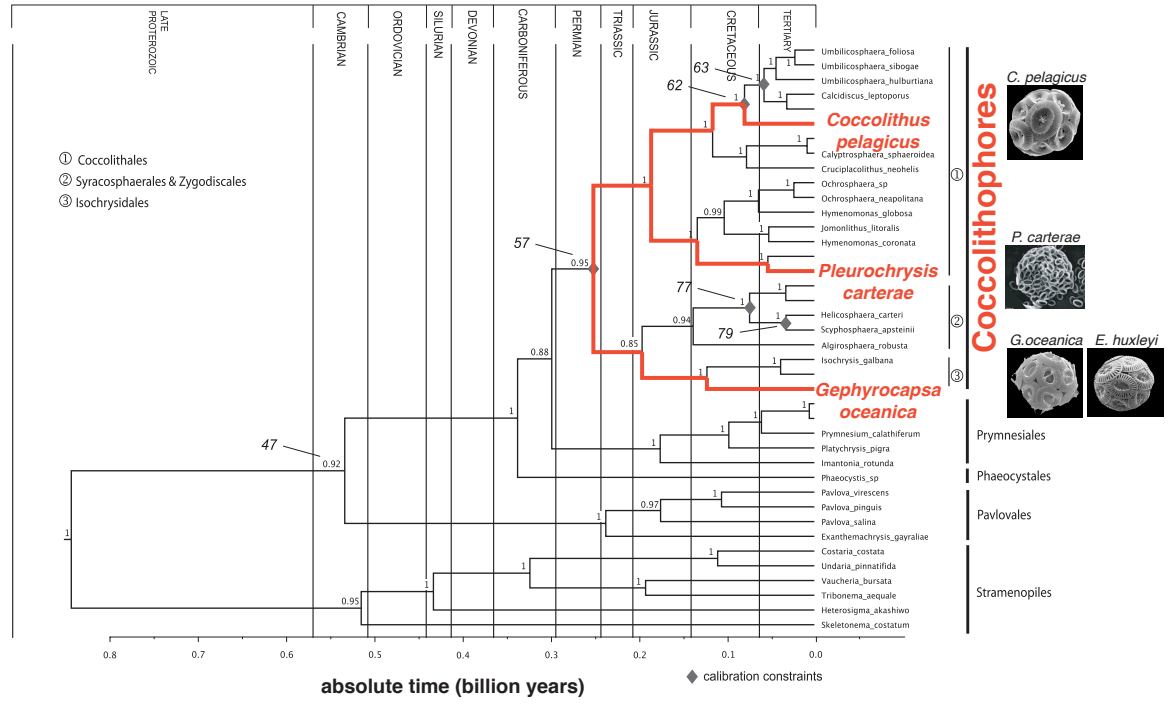


Figure 1: Phylogeny and divergence times of the Haptophytes. From Liu et al., (2010) modified. Times are in billion years. In red the species tested for this study.

360

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019

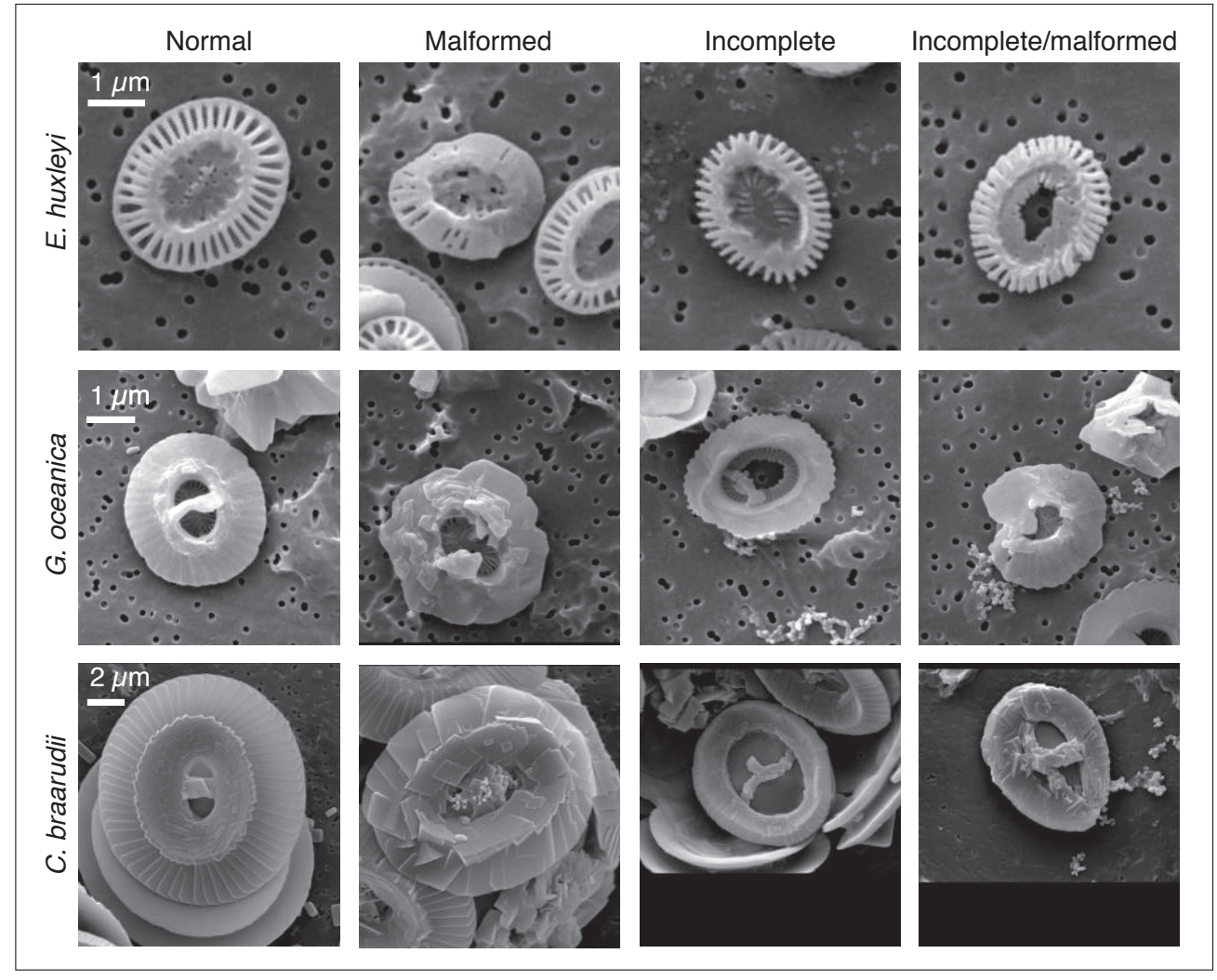
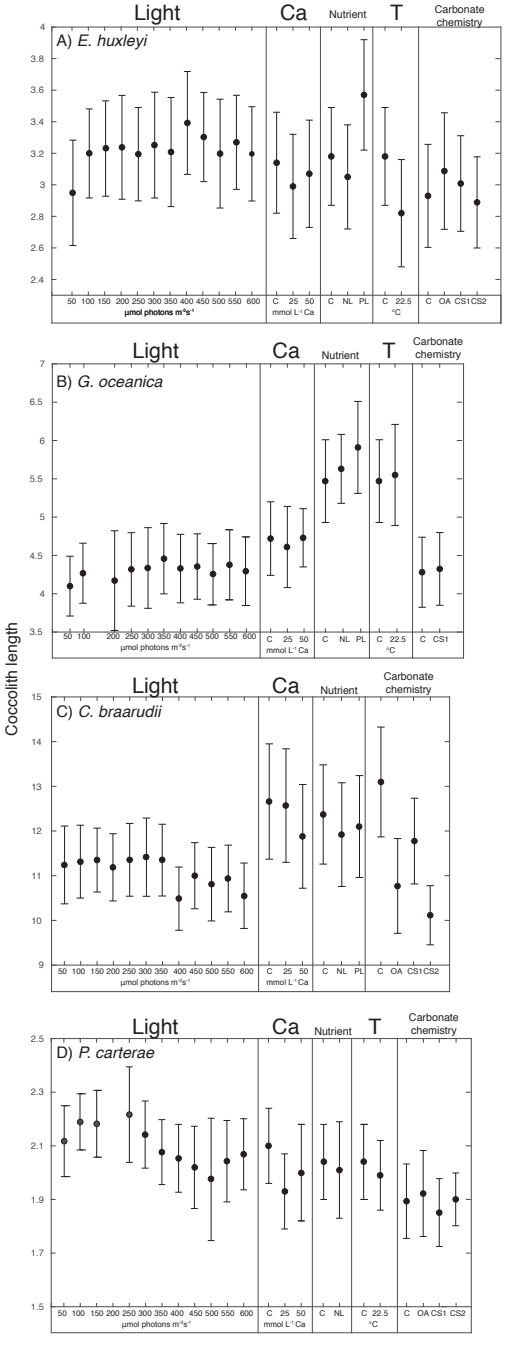


Figure 2: Examples of different morphological categories: normal, malformed, incomplete and malformed and incomplete for *E. huxleyi, G. oceanica* and *C. braarudii*.

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019



365

Figure 3: Coccolith length (µm) versus experiments. The experiments displayed represent light, Mg/Ca (Ca), nutrient, temperature (T), and carbonate chemistry manipulations in A) *E. huxleyi*; B) *G. oceanica*; C) *C. braarudii*; D) *P. carterae*.

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019

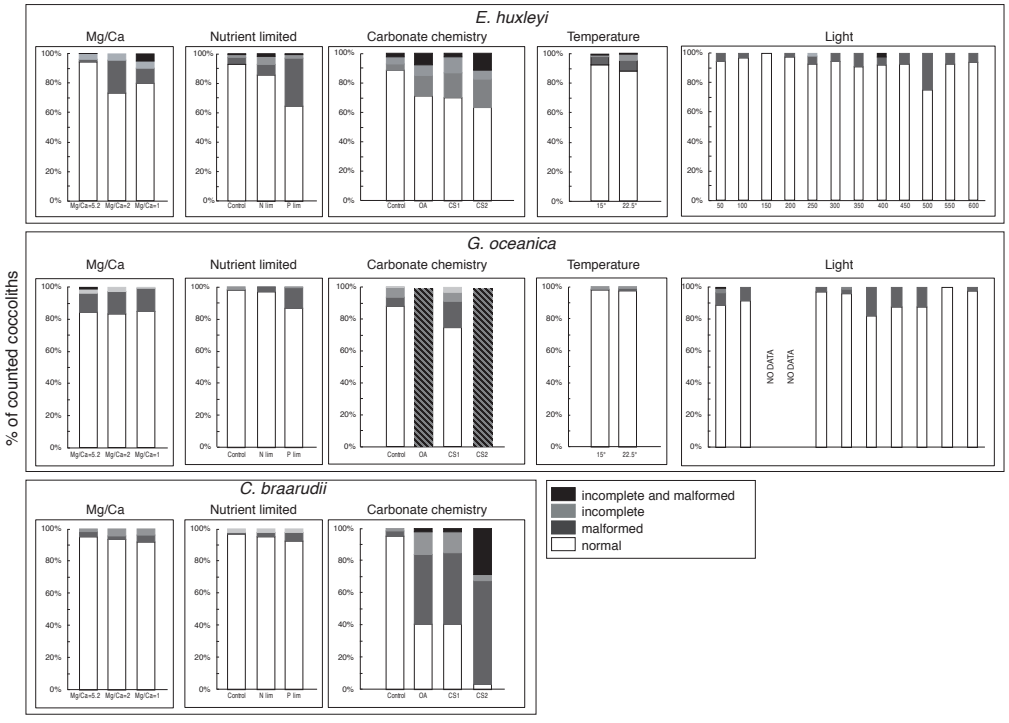


Figure 4: Percentage of normal, malformed, incomplete and malformed and incomplete coccoliths versus experiments. The experiments displayed represent Mg/Ca, nutrient, carbonate chemistry, temperature, and light intensity manipulations. *C. braarudii* didn't survive under high SST (22.5°C) and any malformations were observed under the different light intensities tested; therefore, percentage of malformations are not represented for these experiments for this species. Furthermore, any malformation was observed for *P. carterae* and percentage are not shown.

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019

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Light											
E. huxleyi	*μ*	coccosphere	cell	DSL	d.Std L	DSW	d.Std W	Ellipticity	rays	ray width	tube thick.
50	0.41	4.57	3.94	2.95	0.33	2.43	0.33	1.22	29.3	0.11	0.37
100	0.43	4.57	4.02	3.20	0.28	2.59	0.23	1.23	30.5	0.12	0.32
150	0.74	4.82	4.52	3.23	0.30	2.64	0.27	1.23	31.3	0.10	0.29
200	0.59	5.05	4.46	3.24	0.33	2.66	0.29	1.22	30.5	0.11	0.31
250	1.01	5.01	4.41	3.19	0.30	2.63	0.26	1.22	32.2	0.11	0.32
300	1.03	4.87	4.50	3.25	0.34	2.68	0.32	1.22	33.0	0.11	0.32
350	1.06	5.05	4.39	3.21	0.35	2.64	0.31	1.22	32.7	0.11	0.36
400	1.18	5.02	4.43	3.39	0.33	2.81	0.30	1.21	33.1	0.10	0.33
450	1.20	5.03	4.42	3.30	0.28	2.74	0.26	1.21	33.5	0.11	0.38
500	1.10	5.01	4.38	3.20	0.34	2.64	0.31	1.21	32.7	0.10	0.35
550	0.97	4.85	4.35	3.27	0.30	2.72	0.27	1.21	33.2	0.11	0.33
600	0.87	4.92	4.34	3.20	0.30	2.65	0.27	1.21	33.2	0.11	0.33
G. oceanica	*μ*	coccosphere	cell	DSL	d.Std L	DSW	d.Std W	Ellipticity	tube thick.	angle °	
50	0.52	6.78	5.17	4.10	0.39	3.55	0.36	1.16	1.34	57.41	
100	0.66	7.75	5.55	4.27	0.39	3.69	0.38	1.16	1.32	61.68	
200	0.63	7.22	5.23	4.17	0.65	3.61	0.53	1.16	1.34	61.69	
250	0.67	6.83	5.47	4.32	0.48	3.76	0.46	1.15	1.35	61.88	
300	0.67	6.74	5.27	4.34	0.53	3.71	0.51	1.17	1.40	65.91	
350	0.71	6.74	5.16	4.46	0.46	3.90	0.41	1.14	1.32	58.12	
400	0.66	6.88	5.51	4.33	0.45	3.84	0.40	1.13	1.23	66.67	
450	0.74	6.92	5.60	4.36	0.43	3.76	0.43	1.16	1.29	65.11	
500	0.63	6.57	4.88	4.25	0.40	3.71	0.34	1.15	1.26	62.89	
550	0.71	6.62	4.97	4.38	0.46	3.82	0.43	1.15	1.27	61.29	
600	0.50	6.41	4.92	4.29	0.45	3.72	0.42	1.16	1.25	68.27	
C. braarudii	*μ*	coccosphere	cell	DSL	d.Std L	DSW	d.Std W	Ellipticity			
50	0.40	18.74	13.35	11.24	0.87	9.34	0.83	1.21			
100	0.35	17.58	10.82	11.31	0.82	9.47	0.71	1.20			
150	0.42	17.81	10.72	11.35	0.72	9.46	0.73	1.20			
200	0.44	19.37	11.26	11.19	0.75	9.38	0.72	1.19			
250	0.44	17.80	11.33	11.36	0.81	9.63	0.80	1.18			
300	0.49	17.55	10.94	11.41	0.88	9.54	0.75	1.20			
350	0.52	17.54	10.94	11.35	0.80	9.58	0.78	1.19			
400	0.52	17.35	10.20	10.49	0.71	8.88	0.66	1.18			
450	0.49	18.60	12.33	11.00	0.74	9.42	0.67	1.17			
500	0.49	17.69	10.52	10.81	0.82	9.13	0.72	1.19			
550	0.50	17.59	10.62	10.94	0.75	9.20	0.70	1.19			
600	0.63	17.19	10.95	10.55	0.73	8.91	0.80	1.19			
P. carterae	*μ*	coccosphere	cell	L	d.Std L	W	d.Std W	Ellipticity			
50	0.18	11.02	7.93	2.12	0.13	1.32	0.08	1.61			
100	0.19	11.60	8.91	2.19	0.10	1.36	0.06	1.62			
150	0.17	11.41	8.69	2.18	0.12	1.35	0.09	1.62			
250	0.19	12.16	10.52	2.22	0.18	1.40	0.14	1.61			
300	0.29	13.09	10.28	2.14	0.13	1.34	0.09	1.60			
350	0.28	12.73	10.32	2.08	0.12	1.33	0.11	1.57			
400	0.28	11.84	10.48	2.05	0.13	1.35	0.10	1.58			
450	0.28	11.35	10.73	2.02	0.15	1.31	0.10	1.56			
500	0.28	11.73	10.15	1.97	0.23	1.26	0.17	1.58			
550	0.26	12.71	9.97	2.04	0.15	1.30	0.10	1.58			
600	0.32	12.06	9.82	2.07	0.13	1.32	0.12	1.58			

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019

Climate of the Past Discussions
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Table 1 Light experiments data. Growth rate (µ); coccosphere, cell diameters (µm); coccolith morphometric analysis were performed on 50 specimens for every treatment: average of coccolith distal shield length (DSL; µm) and coccolith distal shield width (DSW; µm); ellipticity (L/W); average *E. huxleyi* ray number (rays) and average mean ray width (µm, ray width); average *E. huxleyi* tube thickness (µm, tube thick); *G. oceanica* tube thickness (µm, tube thick); *G. oceanica* bridge angle (angle°). For *G. oceanica* and *P. carterae*, data from 150 and 200 µmol photons $m^{-2}s^{-1}$ are missing due to errors on light intensity inside the light cabinet.

E. huxleyi	*µ*	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity	rays	ray width	tube thick.
Control	0.97	0.01	4.69	0.01	4.08	0.00	3.14	0.32	2.57	0.29	1.22	30	0.13	0.37
Mg/Ca=2	0.92	0.01	4.84	0.06	4.22	0.03	2.99	0.33	2.44	0.28	1.20	30	0.11	0.35
Mg/Ca=1	0.85	0.00	4.92	0.02	4.33	0.02	3.07	0.34	2.50	0.30	1.23	31	0.11	0.35
G. oceanica	*µ*	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity	tube thick.	angle °	
Control	0.65	0.00	6.86	0.09	5.29	0.03	4.72	0.48	4.14	0.50	1.15	1.43	66.49	
Mg/Ca=2	0.60	0.01	6.82	0.04	5.16	0.01	4.61	0.53	4.01	0.46	1.15	1.40	68.27	
Mg/Ca=1	0.55	0.00	6.83	0.04	5.15	0.03	4.73	0.38	4.18	0.40	1.14	1.46	71.75	
C. braarudii	*µ*	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity			
Control	0.56	0.01	19.82	0.11	15.65	1.39	12.66	1.29	11.00	1.21	1.16			
Mg/Ca=2	0.39	0.01	19.44	0.19	12.08	0.39	12.57	1.27	10.79	1.19	1.17			
Mg/Ca=1	0.48	0.01	18.74	0.09	11.59	0.40	11.88	1.16	10.63	0.72	1.16			
P. carterae	*µ*	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity			
Control	0.35	0.04	11.63	0.22	9.56	0.21	2.10	0.14	1.34	0.10	1.57			
Mg/Ca=2	0.39	0.04	11.09	0.15	8.73	0.03	1.93	0.14	1.30	0.09	1.54			
Mg/Ca=1	0.41	0.01	11.39	0.34	9.00	0.16	2.00	0.18	1.24	0.12	1.56			

Table 2 Mg/Ca experiment data. Data presented are the average of three replicates. Growth rate (µ); coccosphere, cell diameters (µm); coccolith morphometric analysis were performed on 50 specimens for every treatment: average of coccolith distal shield length (DSL; µm) and coccolith distal shield width (DSW; µm); ellipticity (L/W) diameter; average *E. huxleyi* ray number (rays) and average mean width (µm, ray width); average *E. huxleyi* tube thickness (µm, tube thick), *G. oceanica* bridge angle (angle°).

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019

Climate of the Past Discussions
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415

E. huxleyi	μ	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity	rays	ray width	tube thick.
Control	0.94	0.01	4.94	0.07	4.28	0.03	3.18	0.31	2.61	0.28	1.22	32	0.12	0.48
N limited	0.40	0.02	5.08	0.00	4.17	0.23	3.05	0.33	2.49	0.28	1.23	31	0.12	0.37
P limited	0.31	0.06	7.28	0.00	3.97	0.18	3.57	0.35	2.96	0.32	1.21	35	0.12	0.41
G. oceanica	μ	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity	tube thick.	angle °	
Control	0.63	0.07	8.01	0.14	6.29	0.09	5.47	0.54	4.66	0.48	1.18	1.48	64.58	
N limited	0.15	0.09	10.27	0.40	8.21	0.70	5.63	0.45	4.75	0.41	1.19	1.54	63.80	
P limited	0.36	0.02	9.20	0.75	7.51	0.53	5.91	0.60	5.03	0.53	1.18	1.60	65.06	
C. braarudii	μ	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity			
Control	0.54	0.01	18.86	0.03	11.48	0.14	12.37	1.11	10.60	0.97	1.17			
N limited	0.10	0.00	19.83	0.10	10.69	0.09	11.92	1.16	10.11	1.05	1.18			
P limited	0.32	0.02	28.89	2.02	13.53	0.94	12.10	1.14	10.58	1.11	1.15			
P. carterae	μ	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity			
Control	0.44	0.04	11.38	0.25	8.94	0.64	2.04	0.14	1.27	0.10	1.60			
N limited	0.20	0.04	13.15	0.54	11.01	0.73	2.01	0.18	1.29	0.13	1.57			

Table 3 Nutrient limited condition experiment data. Data presented are the average of three replicates. Growth rate (μ); coccosphere, cell diameters (μm); coccolith morphometric analysis were performed on 50 specimens for every treatment: average of coccolith distal shield length (DSL; μm) and coccolith distal shield width (DSW; μm); ellipticity (L/W) diameter; average *E. huxleyi* ray
420 **number (rays) and average mean width (μm, ray width); average *E. huxleyi* tube thickness (μm, tube thick), *G. oceanica* bridge angle (angle°).**

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019

Climate of the Past Discussions
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E. huxleyi	μ	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity	rays	ray width	tube thick.
Control	0.94	0.01	4.94	0.07	4.28	0.03	3.18	0.31	2.61	0.28	1.22	32	0.12	0.48
High	1.47	0.01	3.70	0.10	3.18	0.03	2.82	0.34	2.33	0.29	1.21	29	0.11	0.27
G. oceanica	μ	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity	tube thick.	angle °	
Control	0.63	0.07	8.01	0.14	6.29	0.09	5.47	0.54	4.66	0.48	1.18	1.48	64.58	
High	1.11	0.08	7.21	0.06	5.51	0.02	5.55	0.66	4.74	0.62	1.17	1.59	67.65	
P. carterae	μ	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.Std L	DSW	d.Std W	Ellipticity			
Control	0.44	0.04	11.38	0.25	8.94	0.64	2.04	0.14	1.27	0.10	1.60			
High	0.31	0.04	11.82	0.23	9.05	0.23	1.99	0.13	1.37	0.10	1.45			

Table 4 Temperature experiment data. Data presented are the average of three replicates. Growth rate (µ) coccosphere, cell diameters (µm); coccolith morphometric analysis were performed on 50 specimens for every treatment: average of coccolith distal shield length (DSL; µm) and coccolith distal shield width (DSW; µm); ellipticity (L/W) diameter; average *E. huxleyi* ray number (rays) and average mean width (µm, ray width); average *E. huxleyi* tube thickness (µm, tube thick), *G. oceanica* bridge angle (angle°). *C. braarudii* didn't grow at 22.5°C and therefore, any data is presented.

https://doi.org/10.5194/cp-2019-84
Preprint. Discussion started: 15 July 2019

Climate of the Past Discussions

		Control	OA	CS1	CS2			Control	OA	CS1	CS2
E. huxleyi	pH	7.99	7.65	7.81	7.53	*G. oceanica*	pH	7.91	7.79	7.79	7.53
	TA	2302	2302	3611	4931		TA	2172	2303	3611	4933
	fCO2	460	1068	1178	3142		fCO2	570	1366	1256	3142
	HCO3-	1958.58	2114	3289	4714		HCO3-	1945	2164	3303	4714
	Ca out	3.50	1.74	3.88	2.93		Ca out	3.01	1.52	3.74	2.93
	μ	1.05	0.76	1.12	0.52		*μ*	0.66	0.27	0.57	0.15
	dev. Std	0.03	0.08	0.11	0.00		dev. Std	0.00	0.01	0.04	0.00
	coccosphere	4.88	5.09	4.94	4.7		coccosphere	7.25	6.24	6.51	5.44
	dev. Std	0.11	0.11	0.08	0.01		dev. Std	0.01	0.12	0.25	0.01
	cell	4.23	4.59	4.35	4.44		cell	5.45	5.40	5.31	4.83
	dev. Std	0.24	0.01	0.03	0.00		dev. Std	0.06	0.13	0.03	0.01
	DSL	2.93	3.09	3.01	2.89		DSL	4.28	No data	4.32	No data
	dev. Std	0.33	0.37	0.3	0.29		dev. Std	0.46		0.47	
	DSW	2.39	2.53	2.44	2.38		DSW	3.73		3.71	
	dev. Std	0.28	0.33	0.27	0.24		dev. Std	0.42		0.40	
	ellipticity	1.23	1.23	1.24	1.21		ellipticity	1.15		1.17	
	ray n°	28	33	30	30		tube	1.32		1.23	
	ray width	0.11	0.11	0.12	0.1		angle	63.81		70.29	
	tube	0.25	0.29	0.31	0.23						
		Control	OA	CS1	CS2			Control	OA	CS1	CS2
C. braarudii	pH	7.86	7.60	7.78	7.51	*P. carterae*	pH	8.03	7.82	7.88	7.61
	TA	2170	1994	3234	4895		TA	2351	2313	3722	4984
	fCO2	591	1142	1190	3356		fCO2	409	697	968	2782
	HCO3-	1800	2009	3123	4711		HCO3-	1946	2027	3271	4700
	Ca out	2.68	1.52	3.54	2.77		Ca out	3.83	2.46	2.46	4.58
	μ	0.56	0.43	0.42	0.23		*μ*	0.52	0.53	0.51	0.52
	dev. Std	0.01	0.00	0.01	0.02		dev. Std	0.02	0.02	0.02	0.00
	coccosphere	19.82	18.15	18.15	16.78		coccosphere	11.70	11.39	11.52	11.56
	dev. Std	0.11	0.07	0.49	0.69		dev. Std	0.10	0.32	0.09	0.44
	cell	15.65	11.76	12.91	12.81		cell	9.03	9.16	9.35	9.77
	dev. Std	1.39	0.29	0.82	0.91		dev. Std	0.32	1.11	0.59	0.08
	DSL	13.10	10.77	11.78	10.12		DSL	1.89	1.92	1.84	1.90
	dev. Std	1.23	1.06	0.96	0.66		dev. Std	0.14	0.16	0.13	0.10
	DSW	11.43	9.11	10.10	8.61		DSW	1.17	1.22	1.22	1.19
	dev. Std	1.16	0.99	0.96	0.55		dev. Std	0.10	0.10	0.10	0.08
	ellipticity	1.15	1.19	1.17	1.18		ellipticity	1.63	1.58	1.52	1.60

Table 5 Carbonate chemistry experiment data. Data presented are the average of three replicates. Growth rate (μ); coccosphere, cell diameters (μm); coccolith morphometric analysis were performed on 50 specimens for every treatment: average of coccolith distal shield length (DSL; μm) and coccolith distal shield width (DSW; μm); ellipticity (L/W) diameter; average *E. huxleyi* ray number (rays) and average mean width (μm, ray width); average *E. huxleyi* tube thickness (μm, tube thick), *G. oceanica* bridge angle (angle°). Carbon chemistry speciation calculated as the mean of start and end values of measured pH and TA.