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

Giulia Faucher[1], Ulf Riebesell[2], Lennart Thomas Bach[3]

[1]Dipartimento di Scienze della Terra "Ardito Desio", Università degli Studi di Milano, Milano, 20133 Italia
[2]Biological Oceanography, GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel 24105, Germany
[3]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia

Correspondence to: Giulia Faucher (giulia.faucher@unimi.it)

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 hundred thousand to 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 (Tyrell and Young, 2010). They produce single calcitic platelets named coccoliths and nannoliths. Due to their ability to calcify, 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 environmental drivers such as temperature, salinity, nutrient concentration, light, and carbonate chemistry that can modify abundance and morphology of certain taxa.

Due to their sensitivities to environmental changes fossil remains (coccoliths and nannoliths) of coccolithophores, have often been used as paleo-proxies to reconstruct past physical and chemical conditions in the surface ocean of local or global significance (e.g. Erba, 1994; Lees et al., 2005; Tiraboschi et al., 2009; Erba et al., 2010; Lübke and Mutterlose, 2016; Faucher et al., 2017a; Erba et al., 2019; supplementary S1). However, for the fossil record, it is extremely difficult to disentangle the individual factor(s) that drove changes in coccolith morphology. Therefore, it is not surprising that studies occasionally come to different conclusions about what environmental factor drove a morphological change in the paleo-record. For example, Erba et al., (2010), detected the reduction in size and variation in shape of some nannofossil species during a time of excess volcanogenic CO_2 emissions. They explained their trend with detrimental carbonate chemistry conditions based on physiological incubation studies by Riebesell et al., (2000) who found decreasing calcification rates under increasing CO_2. Conversely, Bornemann and Mutterlose, (2006) explained decreasing coccolith size with decreasing sea surface temperature, a conclusion that was also based on incubation experiments with living coccolithophore species (Renaud and Klaas, 2001; Renaud et al., 2002). These examples illustrate that there is considerable uncertainty when trying to reconstruct paleo-environmental conditions based on coccolith morphology. This in itself is not surprising considering that there are millions of years of evolution between the time when the fossil coccolithophores lived and when the physiological experiments were done (Bown, 2005; De Vargas et al., 2007).

Therefore, the primary goal of our study was to understand if physiological experiments with contemporary species are a valid tool to reconstruct responses of ancient coccolithophores to environmental change in the geological record. The assumption that this approach is valid, is implicit in many studies (Giraud et al., 2006; Erba et al., 2010; Faucher et al., 2017a) but, to the best of our knowledge, not been further tested so far. To test this assumption, we did a series of identical stress test experiments with four selected modern species that have been evolutionarily distinct since hundred thousand to millions of years (Fig.1). Our hypothesis was: 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 physiologically conserved over geological timescales. In other words, if species conserve a similar response to certain types of environmental change for geological timescales, despite very different evolutionary trajectories, then this would strengthen our confidence that responses recorded for modern species also apply for the geological past.

For our experiments we selected four different coccolithophore species: *Emiliania huxleyi* (morphotype R*), 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* that diverged ~ 290 *Kya;* Liu et al., 2010; Bendif et al., 2014). 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 if 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 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 limitations (N or P limitations), Mg/Ca ratio, temperature, carbonate chemistry. Monospecific cultures of the coccolithophores *Emiliania huxleyi* (strain RCC 1216, pelagic, from the Tasmanian sea), *Gephyrocapsa oceanica* (strain RCC 1303, pelagic, from the France coast of the Atlantic Ocean), *Coccolithus pelagicus subsp. braarudii* (strain PLY182G, it will be called hereafter *C. braarudii*, pelagic, from the English channel, Atlantic Ocean), and *Pleurochrysis carterae* (unknown strain number, coastal species) were grown in artificial seawater (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) 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 beginning of the experiments, coccolithophore cultures were acclimated for about 7-10 generations to each of the experimental conditions. 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. Sampling were conducted at the same time for every experiment to avoid changes in cell diameter/volume that occurs within less than an hour (Müller et al., 2012; Sheward et al., 2017).

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 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 (Pierrot et al., 2006) 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) following 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 et al., (2007) and then recalculated to in-situ temperature (15°C) using CO2SYS as is described by 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).

2.3 Scanning Electron Microscopy (SEM)

Sample for SEM analysis were filtered by gravity onto polycarbonate filters (0.2 µm pore size). For every sample, 5-10 ml water was used. Filters were subsequently dried 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 experiment, in all treatments and replicates 50 specimens for each species were analyzed. 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 inner tube thickness, the number distal shield elements and the distal shield elements 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): morphologies were grouped following Langer et al., (2006) and Langer et al., (2010) categories.

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 the four species selected, coccolithophore, cell and coccolith sizes didn't show any distinct trend with variable 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 distal shield elements with light intensities above 400 µmol photons m^{-2} s^{-1}. *Gephyrocapsa 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

Emiliania 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 $[Ca^{2+}]$ 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}. *Gephyrocapsa 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). *Emiliania huxleyi, G. oceanica* and *C. braarudii* coccolith sizes were not affected by changing $[Ca^{2+}]$. *Pleurochrysis carterae* coccoliths were smaller at the highest $[Ca^{2+}]$ concentrations than in the control (Fig. 3; Table 2). *Emiliania 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

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

Emiliania huxleyi and *G. oceanica* coccoliths were larger under P limitation, while there was no significant difference between N limitation and the control. *Emiliania huxleyi* coccoliths had a higher number of distal shield elements under P limitations while the inner tube was thinner in N and P limited treatments compared to the control. *Gephyrocapsa oceanica* produced

thicker inner tube under N and P limitation. *Coccolithus braarudii* was less elliptical under P limitation and *P. carterae* was less elliptical under N limitation. Furthermore, *E. huxleyi* and *G. oceanica*, produced relatively more malformed coccoliths under P limitation (Fig. 4). *Coccolithus braarudii* and *P. carterae* coccolith sizes remained unaffected with no sign of malformation by nutrient limitation (Fig. 3; Supplementary, plate 3).

3.4 Temperature

Emiliania huxleyi and *G. oceanica* coccospheres and cell sizes were smaller at 22.5 °C. *Pleurochrysis carterae* coccosphere and cell sizes remained unaffected (Table 4). *Emiliania huxleyi* coccoliths were smaller at high temperatures. Furthermore, *E. huxleyi* had less distal shield elements and a thinner inner tube when grown at 22.5°C. *Gephyrocapsa oceanica* and *P. carterae* coccolith size remained largely unaffected by changing temperature but *G. oceanica* produced thicker inner tubes under high temperature. *Pleurochrysis carterae* coccoliths were less elliptical when grown at 22.5°C (Fig. 3; Table 4; supplementary, plate 3). *Coccolithus braarudii* didn't survive under 22.5°C condition.

3.5 Carbonate chemistry parameters

Emiliania huxleyi coccospheres and cells were largest in the OA treatment and smallest in the CS2 treatment. *Gephyrocapsa oceanica* and *C. braarudii* coccospheres were largest in the control and smallest in CS2 treatment. *Gephyrocapsa 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. *Pleurochrysis carterae* coccosphere and cell size were unaffected by changing carbonate chemistry (Table 5).

Emiliania huxleyi formed significantly bigger coccoliths in the OA treatment compared to the control and the CS2 treatment (Fig. 3; table 5). Furthermore, the inner tubes were thicker in the OA and CS1 treatments compared to the control and the CS2 treatments. Malformations were 20% more frequent in the OA, CS1 and CS2 treatments than in the control (Fig. 4; Supplementary, plate 4). *Gephyrocapsa oceanica* generated 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. 2). In the CS1 treatment, coccoliths were slightly smaller compared to the control with a thinner inner tube. *Coccolithus 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. *Pleurochrysis carterae* coccolith size remained unaffected by carbonate chemistry variations but coccoliths are less elliptical under OA, CS1 and CS2 compared to the control.

4 Discussion

Coccolithophores started to calcify in the late Triassic and this biological innovation appeared in a period of strong climatic and biotic pressure (De Vargas et al., 2007). The earliest coccoliths had very simple morphologies and small sizes (2-3 µm; Bown et al., 2004). Calcareous nannoplankton underwent a major diversification 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 coccolithophore 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 (Bown et al., 2004). 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 ranging from protection against excess sun light and/or against grazing (Monteiro et al., 2016). Accordingly, coccolith morphologies are likely only indirectly linked to environmental conditions such as temperature or CO_2 but may rather reflect their adaptation to a specific, yet unknown ecological function (De Vargas et al., 2007; Aloisi, 2015). 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 (Aloisi, 2015). 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 and Mutterlose, 2012; O'Dea et al., 2014; Lübke et al., 2015; Gibbs et al., 2016; Faucher et al., 2017a; Faucher et al., 2017b). Here, the approach is to analyze nannofossil species through a sedimentary succession and link detected shape or size anomalies to morphological responses observed in incubation experiments with living species to find the environmental driver for the identified morphological variation.

This approach depends on the assumption 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 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.

With our experiments, we tried to find a common response of evolutionarily distinct coccolithophore species in response to five different environmental drivers. Interestingly, none of the five tested variable*s* induced a consistent response of coccolith size and shape across all four species. For example, under excess CO_2, *E. huxleyi* formed larger coccoliths while *C. braarudii* formed smaller coccoliths than under optimal conditions parameters. While, *G. oceanica* was the most sensitive species to carbonate chemistry stress and produced under-calcified coccoliths with thinner tube. Another example can be observed in the experiment where algae are grown under N and P limitations: *G. oceanica* produced bigger coccoliths, while *E. huxleyi* coccoliths are smaller under the same conditions.

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 Oceanic Anoxic Event (OAE) 1a, OAE 1b, OAE 2; Bornemann et al., 2006; Erba et al., 2010; Lübcke et al., 2015; Faucher et al., 2017a; 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. Indeed, the lack of a common response to environmental drivers among the tested species suggests that coccolith shape and size are unreliable proxis to reconstruct paleo-environmental conditions.

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 tested species. Malformations are generally considered as an 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 high CO_2 concentrations provides some evidence that at least this response variable could be used as paleo-proxy for episodes of acute carbonate chemistry perturbations.

In the fossil record there are several examples of intervals characterized by high abundances of malformed specimens, linked to the low calcite saturation state of the ocean (Jiang and Wise, 2006; Raffi and De Bernardi, 2008; Agnini et al., 2007; Erba et al., 2010; Bralower and Self Trail, 2016). Different authors argued for high pCO_2 influence on causing these malformations during the Mesozoic OAEs, Paleocene-Eocene Thermal Maximum (PETM) and Eocene Thermal Maximum 2. All these intervals were characterized by excess CO_2 concentrations and/or slightly reduced pH. Malformations were expressed in different ways: it was represented by variation in ellipticity of coccoliths (Erba et al., 2010), asymmetry (Agnini et al, 2007), irregular arrangement and length of their rays and diminished calcification in some nannoliths, (Jiang and Wise, 2006;

Mutterlose et al., 2007; Raffi and De Bernardi, 2008; Bralower and Self Trail, 2016). The short stratigraphic ranges where these malformations occurred, during the core of ocean perturbations, indicated that pH played a role in inducing the production of these aberrant specimens (Mutterlose et al., 2007; Erba et al., 2010). There is still not a clear explanation of the reason why only some species of calcareous nannoplankton were producing aberrant specimens, and there is not a general consensus on the role of carbonate chemistry on coccolithophore biomineralization (Gibbs et al., 2010; Gibbs et al., 2016). However, a more recent work, provides a plausible explanation of what might have happened during the PETM, where only some species moved and inhabited the deep part of the photic zone, to possibly refuge from stressful warm and eutrophic conditions of the surface water, but had to deal with lower saturation conditions that induced the detected malformations for these taxa (Bralower and Self Trail, 2016).

The increase in the percentage of malformed coccoliths observed in our experiments, suggests a more universal occurrence of malformation in modern coccolithophore species under low pH. 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). 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 Conclusions

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 temperature, light, nutrient, and Mg/Ca variations. 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. Indeed, it will be crucial to evaluate whether malformations remain 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 are indebted to the Editor Erin McClymont, to Mariem Saavedra-Pellitero and an anonymous reviewer for their fruitful comments that greatly improved the quality of this manuscript. A special note of thank goes to Elisabetta Erba for sharing ideas and huge support. We acknowledge Agostino Rizzi for assistance during the never-ending SEM analyses. 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.

References

Agnini, C., Fornaciari, E., Rio, D., Tateo, F., Backman, J., Giusberti, L.: Responses of calcareous nannofossil assemblages, mineralogy and geochemistry to the environmental perturbations across the Paleocene/Eocene boundary in the Venetian Pre-Alps. Marine Micropaleontology, 63(1-2), 19-38, https://doi.org/10.1016/j.marmicro.2006.10.002, 2007.

Aloisi, G.: Covariation of metabolic rates and cell size in coccolithophores, Biogeosciences, https://doi.org/10.5194/bg-12-4665-2015, 2015.

Arbuckle, K., Bennett, C. M., Speed, M. P.: A simple measure of the strength of convergent evolution, Methods in Ecology and Evolution, 5(7), 685-693, https://doi.org/10.1111/2041-210X.12195, 2014.

Bach, L. T., Riebesell, U., Schulz, K. G: Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi*, Limnology and Oceanography, 56(6), 2040-2050, https://doi.org/10.4319/lo.2011.56.6.2040, 2011.

Bach, L. T., Lohbeck, K. T., Reusch, T. B., Riebesell, U.: Rapid evolution of highly variable competitive abilities in a key phytoplankton species, Nature ecology & evolution, 2(4), 611, 2018.

Bendif, E. M., Probert, I., Carmichael, M., Romac, S., Hagino, K., de Vargas, C.: Genetic delineation between and within the widespread coccolithophore morpho-species Emiliania huxleyi and Gephyrocapsa oceanica (Haptophyta). Journal of phycology, 50(1), 140-148, https://doi.org/10.1111/jpy.12147, 2014.

Bornemann, A., Mutterlose J.: Size analyses of the coccolith species *Biscutum constans* and *Watznaueria barnesiae* from the Late Albian "Niveau Breistroffer"(SE France): taxonomic and palaeoecological implications, Geobios, 39(5), 599-615, https://doi.org/10.1016/j.geobios.2005.05.005, 2006.

Bown P. R.: Calcareous nannoplankton evolution: a tale of two oceans, Micropaleontology, 51(4), 299-308, 2005.

Bown, P. R., Lees, J. A., Young, J. R.: Calcareous nannoplankton evolution and diversity through time: In Coccolithophores, pp. 481-508. Springer Berlin Heidelberg, 2004.

Bralower, T. J., Self-Trail, J. M.: Nannoplankton malformation during the Paleocene-Eocene Thermal Maximum and its paleoecological and paleoceanographic significance. Paleoceanography, 31(10), 1423-1439, https://doi.org/10.1002/2016PA002980, 2016.

Cottingham, K. L., Lennon, J. T., Brown, B. L.: Knowing when to draw the line: designing more informative ecological experiments, Frontiers in Ecology and the Environment, 3(3), 145-152, https://doi.org/10.1890/1540-9295(2005)003[0145:KWTDTL]2.0.CO;2, 2005.

Danbara, A., Shiraiwa, Y.: The requirement of selenium for the growth of marine coccolithophorids, *Emiliania huxleyi, Gephyrocapsa oceanica* and Helladosphaera sp.(Prymnesiophyceae), Plant and cell physiology, 40(7), 762-766, https://doi.org/10.1093/oxfordjournals.pcp.a029603, 1999.

De Vargas, C., Aubry, M. P., Probert, I. A. N., Young, J.: Origin and evolution of coccolithophores: from coastal hunters to oceanic farmers, In Evolution of primary producers in the sea (pp. 251-285) Academic Press, 2007.

Dickson, A. G., Afghan, J. D., Anderson, G. C.: . Reference materials for oceanic CO2 analysis: a method for the certification of total alkalinity. Marine Chemistry, 80(2-3), 185-197, https://doi.org/10.1016/S0304-4203(02)00133-0, 2003.

Dickson, A. G., Sabine, C. L., Christian, J. R. Guide to best practices for ocean CO2 measurements. North Pacific Marine Science Organization, 2007.

Erba, E.: Nannofossils and superplumes: the early Aptian "nannoconid crisis", Paleoceanography, 9(3), 483-501, https://doi.org/10.1029/94PA00258, 1994.

Erba, E.: The first 150 million years history of calcareous nannoplankton: biosphere–geosphere interactions, Palaeogeography, Paleoclimatology, Paleoecology, 232, 2, 237-250, https://doi.org/10.1016/j.palaeo.2005.09.013, 2006.

Erba, E., Bottini, C., Weissert, H. J., Keller, C. E.: Calcareous nannoplankton response to surface-water acidification around Oceanic Anoxic Event 1a, Science, 329(5990), 428-432, https://doi.org/10.1126/science.1188886, 2010.

Erba, E., Bottini, C., Faucher, G., Gambacorta, G., Visentin, S.: The response of calcareous nannoplankton to Oceanic Anoxic Events: The Italian pelagic record, Bollettino della Società Paleontologica Italiana 58, 51-71, 2019.

Faucher, G., Erba, E., Bottini, C., Gambacorta, G.: Calcareous nannoplankton response to the latest Cenomanian Oceanic Anoxic Event 2 perturbation, Rivista Italiana di Paleontologia e Stratigrafia (Research In Paleontology and Stratigraphy), 123(1), 2017a.

Faucher, G., Hoffmann, L. J., Bach, L. T., Bottini, C., Erba, E., Riebesell, U.: Impact of trace metal concentrations on coccolithophore growth and morphology: laboratory simulations of Cretaceous stress, Biogeosciences (BG), 14(14), 3603-3613, https://doi.org/10.5194/bg-14-3603-2017, 2017b.

Gattuso, J. P., Lee, K., Rost, B., Schulz, K.: Approaches and tools to manipulate the carbonate chemistry: Publications Office of the European Union, 2010.

Gibbs, S. J., Stoll, H. M., Bown, P. R., Bralower, T. J.: Ocean acidification and surface water carbonate production across the Paleocene–Eocene thermal maximum. Earth and Planetary Science Letters, 295(3-4), 583-592, https://doi.org/10.1016/j.epsl.2010.04.044, 2010.

Gibbs, S. J., Bown, P. R., Ridgwell, A., Young, J. R., Poulton, A. J., O'Dea, S. A.: Ocean warming, not acidification, controlled coccolithophore response during past greenhouse climate change, Geology, 44(1), 59-62, https://doi.org/10.1130/G37273.1, 2016.

Giraud, F., Pittet, B., Mattioli, E., Audouin, V.: Paleoenvironmental controls on the morphology and abundance of the coccolith Watznaueria britannica (Late Jurassic, southern Germany). Marine Micropaleontology, 60(3), 205-225, https://doi.org/10.1016/j.marmicro.2006.04.004, 2006.

Guillard, R. R. L., Ryther, J. H.: Studies of marine planktonic diatoms: I. Cyclotella Nana Hustedt, and Detonula Confervacea (CLEVE) Gran, Canadian journal of microbiology 8.2: 229-239, 1962.

Jiang, S., Wise Jr, S. W.: Surface-water chemistry and fertility variations in the tropical Atlantic across the Paleocene/Eocene Thermal Maximum as evidenced by calcareous nannoplankton from ODP Leg 207, Hole 1259B. Revue de micropaléontologie, 49(4), 227-244, https://doi.org/10.1016/j.revmic.2006.10.002, 2006.

Kester, D. R., Duedall, I. W., Connors, D. N., Pytkowicz, R. M.: Preparation of artificial seawater, Limnology and oceanography 12(1) 176-179, 1967.

Langer, G., Geisen, M., Baumann, K. H., Kläs, J., Riebesell, U., Thoms, S., Young, J. R.: Species-specific responses of calcifying algae to changing seawater carbonate chemistry. Geochemistry, Geophysics, Geosystems, 7(9), https://doi.org/10.1029/2005GC001227, 2006.

Langer, G., De Nooijer, L. J., Oetjen, K.: On the role of the cytoskeleton in coccolith morphogenesis: the effect of cytoskeleton inhibitors1, Journal of Phycology 46(6) 1252-1256, https://doi.org/10.1111/j.1529-8817.2010.00916.x, 2010.

LaRoche, J., Rost, B., Engel, A.: Bioassays, batch culture and chemostat experimentation, In Approaches and tools to manipulate the carbonate chemistry, Guide for Best Practices in Ocean Acidification Research and Data Reporting. In: Riebesell U., Fabry VJ, Hansson L., Gattuso J.-P.(Eds.), pp. (pp. 81-94), 2010.

Lees, J. A., Bown, P. R., Mattioli, E.:. Problems with proxies? Cautionary tales of calcareous nannofossil paleoenvironmental indicators. Micropaleontology, 51(4), 333-343, https://doi.org/10.2113/gsmicropal.51.4.333, 2005.

Linnert, C., Mutterlose, J.: Biometry of Cenomanian–Turonian placoliths: a proxy for changes of fertility and surface-water temperature?. Lethaia, 46(1), 82-97, https://doi.org/10.1111/j.1502-3931.2012.00323.x, 2012.

Liu, H., Aris-Brosou, S., Probert, I., de Vargas, C.: A time line of the environmental genetics of the haptophytes, Molecular biology and evolution, 27(1), 161-176, https://doi.org/10.1093/molbev/msp222, 2010.

Lohbeck, K. T., Riebesell, U., Reusch, T. B.: Adaptive evolution of a key phytoplankton species to ocean acidification. Nature Geoscience, 5(5), 346, https://doi.org/10.1038/ngeo1441, 2012.

Lübke, N., Mutterlose, J.: The impact of OAE 1a on marine biota deciphered by size variations of coccoliths, Cretaceous Research, 61, 169-179, https://doi.org/10.1016/j.cretres.2016.01.006, 2016.

Lübke, N., Mutterlose, J., Bottini, C.: Size variations of coccoliths in Cretaceous oceans, a result of preservation, genetics and ecology? Marine Micropaleontology, 117, 25-39, https://doi.org/10.1016/j.marmicro.2015.03.002, 2015.

Monteiro, F. M., Bach, L. T., Brownlee, C., Bown, P., Rickaby, R. E., Poulton, A. J., Tyrrel, T., Beaufort, L., Dutkiewicz, S., Gibbs, S., Gutowska, M. A., Lee, R., Riebesell, U., Young, J., Ridgwell, A.: Why marine phytoplankton calcify, Science Advances, 2(7), e1501822, https://doi.org/10.1126/sciadv.1501822, 2016.

Müller, M. N., Beaufort, L., Bernard, O., Pedrotti, M. L., Talec, A., Sciandra, A.: Influence of CO_2 and nitrogen limitation on the coccolith volume of Emiliania huxleyi (Haptophyta), Biogeosciences, 9(10), 4155-4167, https://doi.org/10.5194/bg-9-4155-2012, 2012.

Mutterlose, J., Linnert, C., Norris, R.: Calcareous nannofossils from the Paleocene–Eocene Thermal Maximum of the equatorial Atlantic (ODP Site 1260B): evidence for tropical warming. Marine Micropaleontology, 65(1-2), 13-31, https://doi.org/10.1016/j.marmicro.2007.05.004, 2007.

O'Dea, S. A., Gibbs, S. J., Bown, P. R., Young, J. R., Poulton, A. J., Newsam, C., Wilson, P. A.: Coccolithophore calcification response to past ocean acidification and climate change, Nature communications, 5, 5363, https://doi.org/10.1038/ncomms6363, 2014.

Pierrot, D. E. Lewis, D. W. R. Wallace: MS Excel Program Developed for CO2 System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a, 2006.

Raffi, I., De Bernardi, B.: Response of calcareous nannofossils to the Paleocene–Eocene Thermal Maximum: Observations on composition, preservation and calcification in sediments from ODP Site 1263 (Walvis Ridge—SW Atlantic). Marine Micropaleontology, 69(2), 119-138, https://doi.org/10.1016/j.marmicro.2008.07.002, 2008.

Renaud, S., Klaas, C.: Seasonal variations in the morphology of the coccolithophore Calcidiscus leptoporus off Bermuda (N. Atlantic). Journal of Plankton Research, 23(8), 779-795, https://doi.org/10.1093/plankt/23.8.779, 2001

Renaud, S., Ziveri, P., Broerse, A. T.: Geographical and seasonal differences in morphology and dynamics of the coccolithophore Calcidiscus leptoporus. Marine Micropaleontology, 46(3-4), 363-385, https://doi.org/10.1016/S0377-8398(02)00081-6 , 2002.

Riebesell U., Zondervan I., Rost B., Tortell P. D., Zeebe R. E., Morel, F. M.: Reduced calcification of marine plankton in response to increased atmospheric CO2. Nature, 407(6802), 364-367, https://doi.org/10.1038/35030078, 2000.

Roy R. N., Roy L. N., Vogel K. M., Porter-Moore C., Pearson T., Good C. E., Millero F. J., Campbell, D. M.: The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45 C. Marine Chemistry, 44(2-4), 249-267, https://doi.org/10.1016/0304-4203(93)90207-5, 1993.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J., Y., White, D. J., Hartenstein, V., Eliceiri K., Tomancak, P., Cardona, A.: Fiji: an open-source platform for biological-image analysis, Nature methods, 9(7), 676-682, https://doi.org/10.1038/nmeth.2019, 2012.

Schulz, K. G., Bach, L. T., Bellerby, R. G. J., Bermúdez, R., Büdenbender, J., Boxhammer, T., Czerny, J., Engel., A., Ludwig, A., Meyerhöfer, M., Larsen, A., Paul, A. J., Sswat, Michael, Riebesell, U.: Phytoplankton blooms at increasing levels of

atmospheric carbon dioxide: experimental evidence for negative effects on prymnesiophytes and positive on small picoeukaryotes, Frontiers in Marine Science, 4, 64, https://doi.org/10.3389/fmars.2017.00064, 2017.

Sheward, R. M., Poulton, A. J., Gibbs, S. J., Daniels, C. J., Bown, P. R.:. Physiology regulates the relationship between coccosphere geometry and growth phase in coccolithophores. Biogeosciences, 14(6), 1493-1509, https://doi.org/10.5194/bg-14-1493-2017, 2017.

Suchéras-Marx, B., Mattioli, E., Pittet, B., Escarguel, G., Suan, G.: Astronomically-paced coccolith size variations during the early Pliensbachian (Early Jurassic), Palaeogeography, Palaeoclimatology, Palaeoecology, 295(1-2), 281-292, https://doi.org/10.1016/j.palaeo.2010.06.006, 2010.

Tiraboschi, D., Erba, E., Jenkyns, H. C.: Origin of rhythmic Albian black shales (Piobbico core, central Italy): Calcareous nannofossil quantitative and statistical analyses and paleoceanographic reconstructions, Paleoceanography and Paleoclimatology, 24(2), https://doi.org/10.1029/2008PA001670, 2009.

Tyrrell, T., Young, J. R.: Coccolithophores, in: Encyclopedia of Ocean Sciences, edited by: Steele, J. H., Turekian, K. K., and Thorpe, S. A., Academic Press, San Diego, 3568–3576, https://doi.org/10.1016/B978-012374473-9.00662-7, 2009.

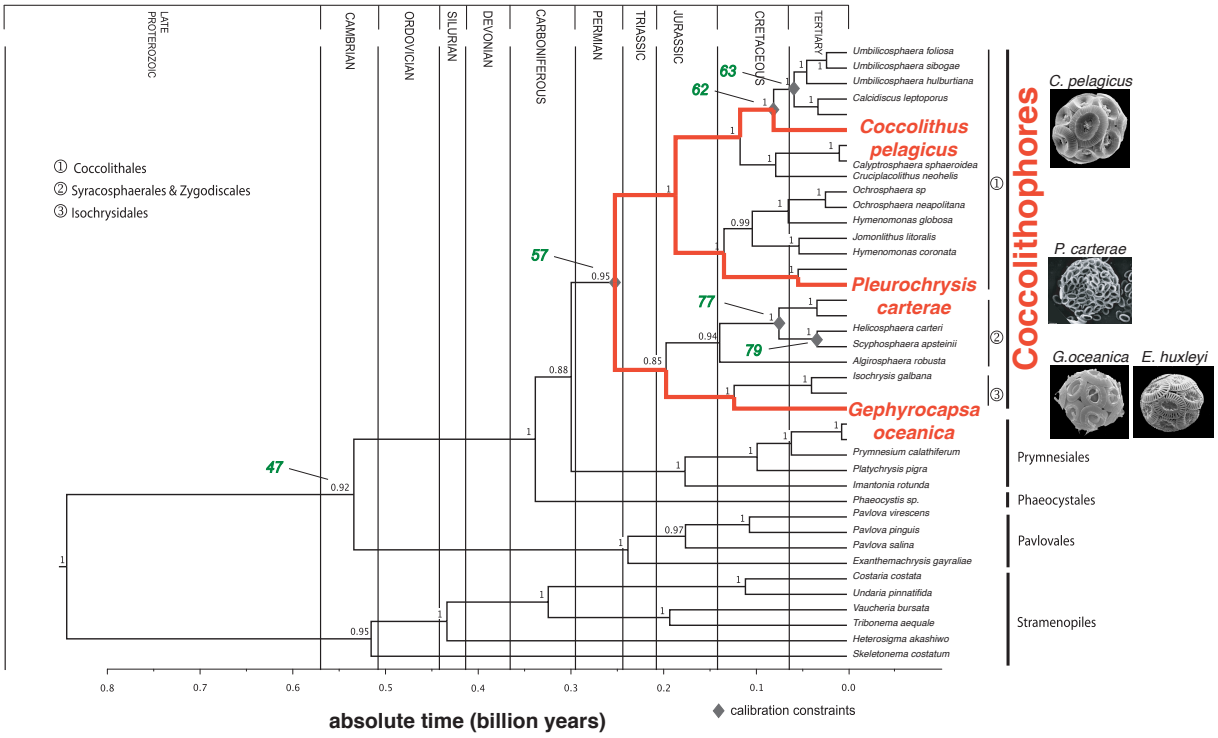

Figure 1: Phylogeny and divergence times of the Haptophytes, modified from Liu et al., (2010). Time is indicated in billion years. The species selected for this study are shown in red. The nodes represent following divergence episodes. The number in green represents specific nodes: node 47: *Exanthemachrysis gayraliae* and *Helicosphaera carteri*; node 57: *Coccolithus pelagicus* and *H. carteri*; node 62: *C. pelagicus* and *Umbilicosphaera hulburtiana*; node 63: *Calcidiscus leptoporus* and *Umbilicosphaera foliosa*; node 77: *Coronosphaera mediterranea* and *Scyphosphaera apsteinii*; node 79: *H. carteri* and *S. apsteinii* (node 79). Numbers are related to calculated divergence times. For further information see Liu et al., 2010.

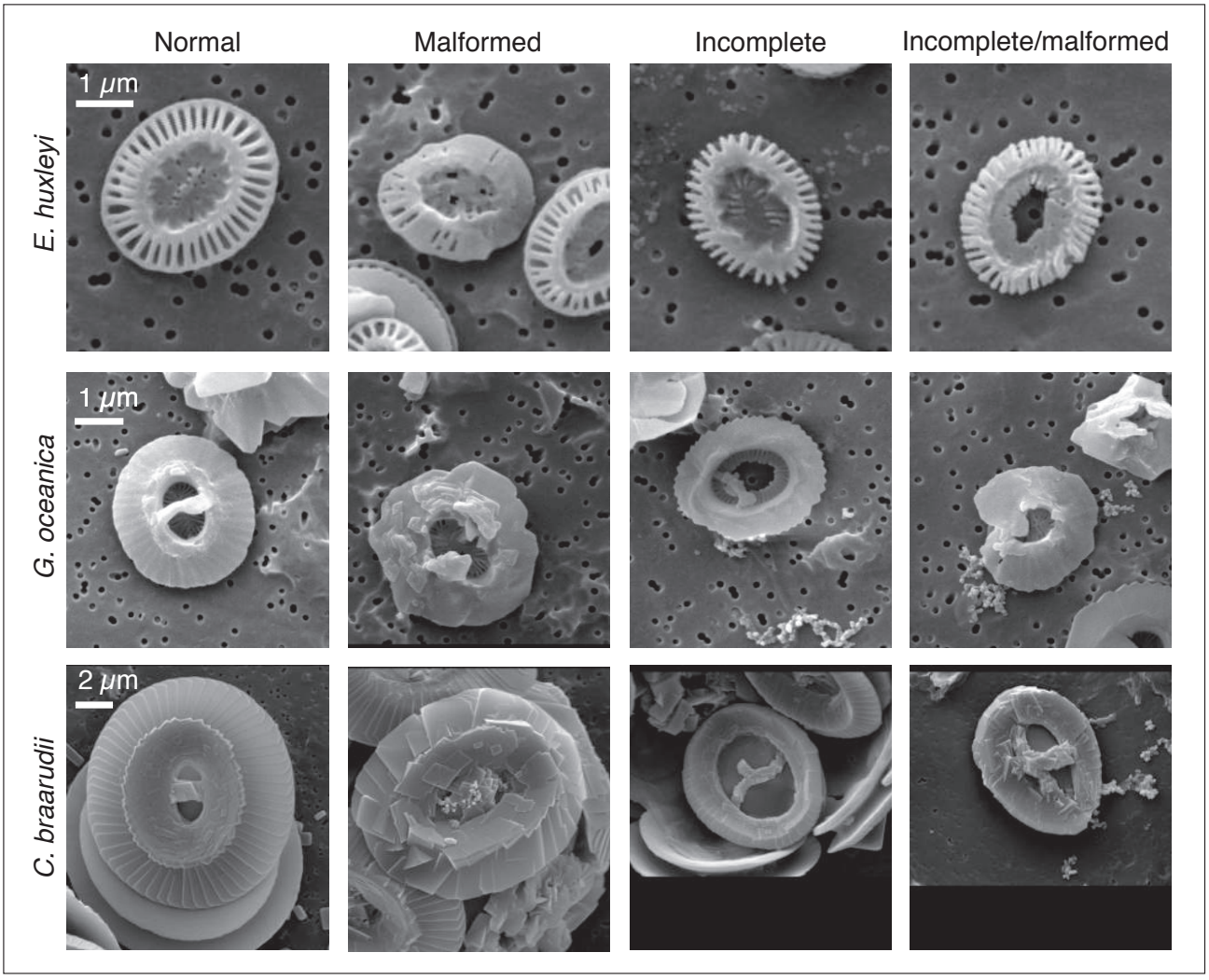

Figure 2: Examples of different morphological categories: normal, malformed, incomplete and incomplete/malformed for *E. huxleyi*, *G. oceanica* and *C. braarudii*.

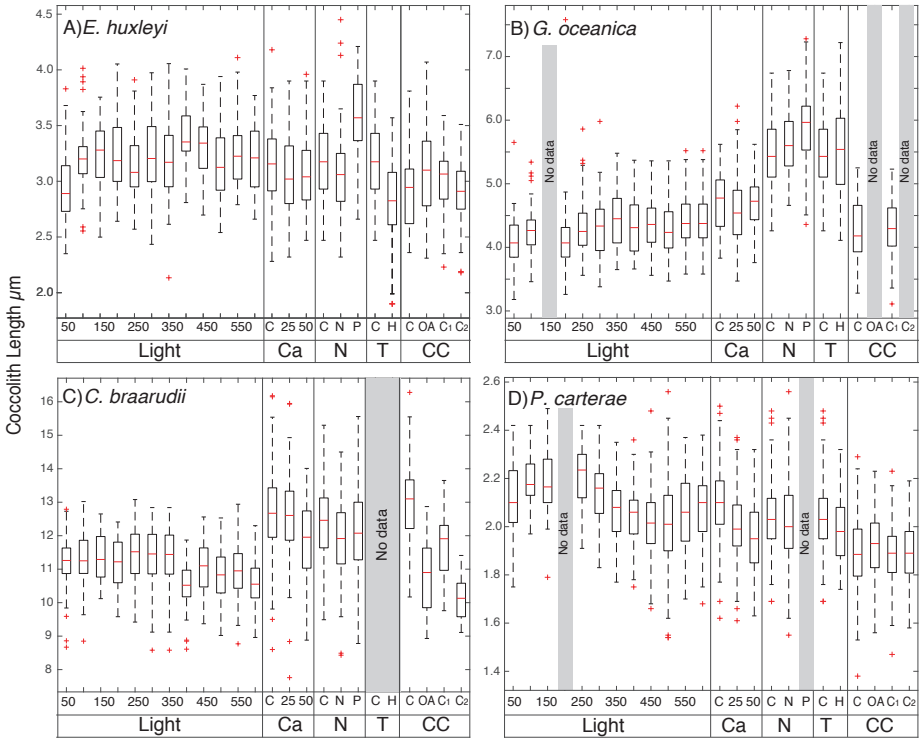

Fig. 3 Box plots of coccolith length from the different experiments. In A) *E. huxleyi*; B) *G. oceanica*; C) *C. braarudii*; D) *P. carterae*. C= control treatment for every experiment. Light: experiment with 12 different light intensities from 50 to 600 µm photons m^{-2} s^{-1}; Ca: calcium manipulation experiment, 25, [Ca^{2+}] =25 mmol L^{-1}; 50, [Ca^{2+}]= 50 mmol L^{-1}. N: nutrient limitation experiment, N= nitrogen limited condition; P= phosphate limited condition. T: temperature experiment, H = 22.5°C; CC: carbonate chemistry experiment; theoretical CO_2 values: C= 400 ppm, OA, ocean acidification=1000 ppm, C1, cretaceous scenario1= 1000 ppm, C2 cretaceous scenario2= 3000 ppm (for further information see paragraph 3.5). The tops and bottoms of each "box" are the 25th and 75th percentiles of the samples respectively. The red line in the middle of each box is the median. The whiskers, extending above and below each box, represent the furthest observations. Observations beyond the whisker length are marked as outliers (red cross). For the light experiment, 50 specimens were considered for every treatment. For Mg/Ca experiment (Ca), nutrient experiment (N), temperature (T) and carbonate chemistry manipulations (CC) experiments, every box plot represents 150 measurements in total, (50 measurements for each replicate). The Light experiment was performed in December 2013; the Ca experiment was performed in June 2014; the N experiment was performed in December 2017; the T experiment was performed in October 2017; the CC experiment was performed in August 2014.

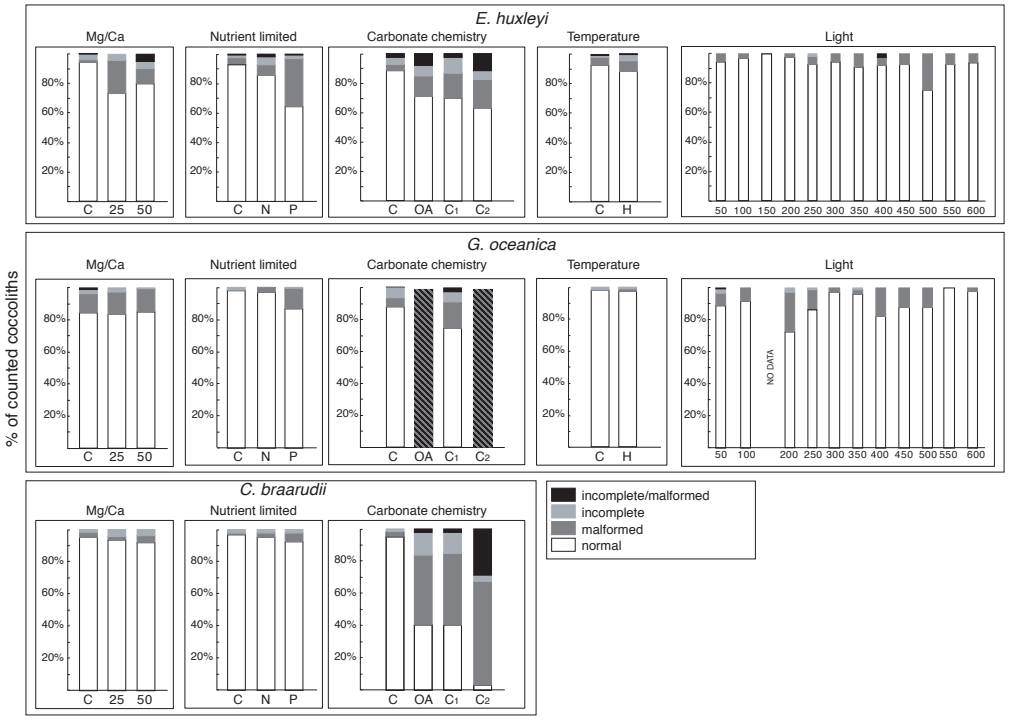

Figure 4: Percentage of normal, malformed, incomplete and incomplete/malformed coccoliths versus experiments. The experiments displayed represent Mg/Ca, nutrient limitation, carbonate chemistry, temperature, and light intensity manipulations. *C. braarudii* didn't survive under high temperature (22.5°C) and no malformations were observed under the different light intensities tested; therefore, percentage of malformations are not represented for these experiments for this species. Furthermore, no malformation was observed for *P. carterae* and percentage are not shown. C= control treatment. Mg/Ca: calcium manipulation experiment, 25, $[Ca^{2+}]$ =25 mmol L^{-1}; 50, $[Ca^{2+}]$= 50 mmol L^{-1}; N: nutrient limitation experiment, N= nitrogen limited condition; P= phosphate limited condition. Temperature experiment, H = 22.5°C; Carbonate chemistry experiment; theoretical CO_2 values: C= 400 ppm, OA, ocean acidification=1000 ppm, C1, cretaceous scenario1= 1000 ppm, C2 cretaceous scenario2= 3000 ppm. Light: 12 different light intensities from 50 to 600 µm photons m^{-2} s^{-1}. For every treatment and for every replicate 100 specimens were considered.

Light												
E. huxleyi	*µ*	coccosphere	cell	DSL	d.StdL	DSW	d.StdW	Ellipticity	SE	SEW	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.StdL	DSW	d.StdW	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.StdL	DSW	d.StdW	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	DSL	d.StdL	W	d.StdW	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				

Table 1 Light experiments data. Growth rate (µ, cell d^{-1}); 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* distal shield elements number (SE) and average distal shield elements width (µm, SEW); average *E. huxleyi* inner 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; dStd= standard deviation.

E. huxleyi	μ	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.StdL	DSW	d.StdW	Ellipticity	SE	SEW	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	
$[Ca^{2+}]$ = 25 mmol L^{-1}	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	
$[Ca^{2+}]$ = 50 mmol L^{-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.StdL	DSW	d.StdW	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
$[Ca^{2+}]$ = 25 mmol L^{-1}	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
$[Ca^{2+}]$ = 50 mmol L^{-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.StdL	DSW	d.StdW	Ellipticity				
Control	0.56	0.01	19.82	0.11	15.65	1.39	12.66	1.29	11.00	1.21	1.16				
$[Ca^{2+}]$ = 25 mmol L^{-1}	0.39	0.01	19.44	0.19	12.08	0.39	12.57	1.27	10.79	1.19	1.17				
$[Ca^{2+}]$ = 50 mmol L^{-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.StdL	DSW	d.StdW	Ellipticity				
Control	0.35	0.04	11.63	0.22	9.56	0.21	2.10	0.14	1.34	0.10	1.57				
$[Ca^{2+}]$ = 25 mmol L^{-1}	0.39	0.04	11.09	0.15	8.73	0.03	1.93	0.14	1.30	0.09	1.54				
$[Ca^{2+}]$ = 50 mmol L^{-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, cell d^{-1}); coccosphere, cell diameters (μm); coccolith morphometric analysis were performed on 50 specimens for every treatment and for every replicate. Data represent the average of three replicates: average of coccolith distal shield length (DSL; μm) and coccolith distal shield width (DSW; μm); ellipticity (L/W) diameter; average *E. huxleyi* distal shield elements number (SE) and average distal shield elements width (μm, SEW); average *E. huxleyi* inner tube thickness (μm, tube thick.), *G. oceanica* bridge angle (angle°); dStd= standard deviation.

E. huxleyi	μ	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.StdL	DSW	d.StdW	Ellipticity	SE	SEW	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.StdL	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.StdL	DSW	d.StdW	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, cell d^{-1}); coccosphere, cell diameters (µm); coccolith morphometric analysis were performed on 50 specimens for every treatment and for every replicate. Data represent the average of three replicates: average of coccolith distal shield length (DSL; µm) and coccolith distal shield width (DSW; µm); ellipticity (L/W) diameter; average *E. huxleyi* distal shield elements number (SE), and average distal shield elements width (µm, SEW); average *E. huxleyi* inner tube thickness (µm, tube thick.), *G. oceanica* bridge angle (angle°); dStd= standard deviation.

E. huxleyi	*μ*	d.Std	coccosphere	d.Std	cell	d.Std	DSL	d.StdL	DSW	d.StdW	Ellipticity	SE	SEW	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.StdL	DSW	d.StdW	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.StdL	DSW	d.StdW	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, cell d^{-1}) coccosphere, cell diameters (μm); coccolith morphometric analysis were performed on 50 specimens for every treatment and for every replicate. Data represent the average of three replicates: average of coccolith distal shield length (DSL; μm) and coccolith distal shield width (DSW; μm); ellipticity (L/W) diameter; average *E. huxleyi* distal shield elements number (SE), and average distal shield elements width (μm, SEW); average *E. huxleyi* inner 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, dStd= standard deviation.

E. huxleyi		Control	OA	CS1	CS2	*G. oceanica*		Control	OA	CS1	CS2
	pH	7.99	7.65	7.81	7.53		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
	d.StdL	0.33	0.37	0.3	0.29		d.StdL	0.46		0.47	
	DSW	2.39	2.53	2.44	2.38		DSW	3.73		3.71	
	d.StdW	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	
	SE	28	33	30	30						
	SEW	0.11	0.11	0.12	0.1						
	tube thick.	0.25	0.29	0.31	0.23		tube thick.	1.32		1.23	
							angle	63.81		70.29	
C. braarudii		Control	OA	CS1	CS2	*P. carterae*		Control	OA	CS1	CS2
	pH	7.86	7.60	7.78	7.51		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
	d.StdL	1.23	1.06	0.96	0.66		d.StdL	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
	d.StdW	1.16	0.99	0.96	0.55		d.StdW	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, cell d^{-1}); coccosphere, cell diameters (μm); coccolith morphometric analysis were performed on 50 specimens for every treatment and for every replicate. Data represent the average of three replicates: average of coccolith distal shield length (DSL; μm) and coccolith distal shield width (DSW; μm); ellipticity (L/W) diameter; average *E. huxleyi* distal shield elements number (SE) and average distal shield elements width (μm, SEW); average *E. huxleyi* inner 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.