

Dear Editor,

thank you for your decision regarding the manuscript "*Impact of trace metal concentrations on coccolithophore growth and morphology: laboratory simulations of Cretaceous stress*".

In this revised version of the ms, as you can see in our point-by-point replies, we followed the suggestions of the two reviewers.

Furthermore, we modified Fig. 4, 5 and 6 where we calculated and represented the average from the three biological replicates with the standard deviation as error bars. We think that these representations are more consistent with the statistical approach and more intuitive.

Best Regards

Giulia Faucher

*We greatly appreciate the valuable comments and critical reading of the manuscript made by L.J. de Nooijer and a second anonymous reviewer which were useful to improve the scientific quality of the manuscript. Please find below our answers to the **Reviewers** comments.*

Dear editor,

After careful assessment of the manuscript BG-2017-138 by Faucher and co-workers on the effect of trace metals on coccolith growth, I recommend it for publication in Biogeosciences after moderate revisions. It is well-written and reports results that will be of interest to the audience of Biogeosciences. Below, I have listed my minor comments that I hope will help improving the manuscript.

There is one more serious issue I have with the content, which is the absence of data on the actual metal concentrations in the treatments. Why were those concentrations not determined after preparing the culture media? It may have been that the concentrations did not vary much between treatments due to sorption of ions and this may therefore have important consequences for the interpretation of the data. I suggest that the authors either determine trace metal concentrations from the stock solutions, or explicitly report that the difference between treatments is inferred from the recipe that was used to make the culture media.

Authors reply: We have not analysed the metal concentration in our media. The difference between treatments was insured by adding adequate amounts of EDTA to the culture media to avoid precipitation of metal ions. It is, therefore, safe to consider that the concentrations of metals added to the culture media represent their dissolved ion concentrations. The ratio of EDTA to metals in our “high” treatment, for example, was well within the range of EDTA to the metal ratio used in other studies which test the effect of V (partly in combination with Mo and Fe) concentrations on phytoplankton species (Bellenger et al. 2008a, Bellenger et al. 2008b).

Abstract

Reviewers’ comment: Page 1, line 16: what does ‘phylogenetically linked’ mean?

Authors reply: We improved the text in order to make it clearer. The phylogenetic history of coccolithophores shows that the selected/investigated living species are linked to Mesozoic species showing dwarfism under excess metal concentrations.

Introduction

Reviewers’ comment: Page 2, line 4: are there references to support this statement?

Authors reply: References added

Reviewers’ comment: Page 2, line 5: ‘affect’ here probably means ‘negatively affect’, consider replacing by e.g. ‘hamper’. Is there a reference that has reported this?

Authors reply: References added and text modified accordingly

Reviewers’ comment: Page 2, line 31: please add one/ a few references on the evolutionary relation between the studied species. Moreover, it is now suggested that the species themselves have separated from each other in the late Cretaceous, whereas the extant species are likely much younger: the groups to which they belong may have separated in the late Cretaceous.

Authors reply: Text revised and references added.

Reviewers’ comment: Page 3, line 6: no such studies

Authors reply: Text modified accordingly

Reviewers’ comment: Page 3, line 9: metals

Authors reply: Text modified accordingly

Methods

Reviewers' comment: Page 3, line 30: what was the concentration of the EDTA in the trace metal stock solutions?

Authors reply: The EDTA concentration in our culture media was 11.71 μM . We added this information to table 1.

Reviewers' comment: Page 4, line 3: what is meant by 'experimental conditions'? These are the conditions without the trace metals added, I assume.

Authors reply: Cultures were pre-exposed to the four-experimental conditions (normal, low, medium, high and extreme), considering an acclimation period of some generations. The text was modified accordingly.

Reviewers' comment: Page 4, line 23: for how long were the samples incubated in 0.1 M HCl? Was this sufficient to dissolve all CaCO_3 ?

Authors reply: The samples of the present study were acidified and directly measured (within minutes). The coulter counter measurements evidence the disappearance of all free coccoliths (Fig. 1) after the treatment with acid. Furthermore, samples were analyzed with a cross-polarizing microscope and, after the treatment with HCl, no coccoliths were left.

Reviewers' comment: Page 5, line 4: analysis

Authors reply: We made this change

Reviewers' comment: Page 5, line 17: comparison

Authors reply: We made this change

Results

Reviewers' comment: Page 6, line 5: although likely true, this is technically speaking an interpretation and should belong in the discussion.

Authors reply: The text was revised and modified following the **Reviewer** suggestion.

Reviewers' comment: Page 6, line 20: I don't understand the definition of coccosphere volume. Isn't the coccosphere simply the cell volume + the coccolith volume?

Authors reply: We change the name "coccosphere volume" as "volume of the calcitic portion of the coccosphere" (VCP) (see page 4).

The volume of the calcitic portion of the coccosphere" (VCP) was estimated as:

$$\text{Volume of the calcitic portion of the coccosphere (VCP)} = \text{coccosphere volume} - \text{cell volume}$$

The coccosphere volume is the coccolith-bearing cell volume, while the cell volume is the coccolith-free cell volume.

Reviewers' comment: Page 6, line 26: 'cells' should be 'cell'

Authors reply: We made this change

Reviewers' comment: Page 7, line 9: 'the cells' should probably be 'cell volumes'

Authors reply: We made this change

Discussion

Reviewers' comment: Page 9, line 15: should be 'trace metals'

Authors reply: We made this change

Reviewers' comment: Page 9, line 17: should be hand

Authors reply: We made this change

Figures

Reviewers' comment: Please add to the caption what the individual dots and error bars represent.

Authors reply: Information added in the captions

Bellenger, J. P., T. Wichard, and A. M. L. Kraepiel. 2008a. Vanadium Requirements and Uptake Kinetics in the Dinitrogen-Fixing Bacterium *Azotobacter vinelandii*. *Applied and Environmental Microbiology* **74**:1478-1484.

Bellenger, J. P., T. Wichard, A. B. Kustka, and A. M. L. Kraepiel. 2008b. Uptake of molybdenum and vanadium by a nitrogen-fixing soil bacterium using siderophores. *Nature Geoscience* **1**:243-246.

*We greatly appreciate the valuable comments and critical reading of the manuscript made by L.J. de Nooijer and a second anonymous reviewer which were useful to improve the scientific quality of the manuscript. Please find below our answers to the **Reviewers** comments.*

General comments:

In this paper, Faucher et al. investigate the effect of various trace metal concentrations on the growth and morphology of four different coccolithophore species. Using laboratory experiments, the authors simulate the environmental stress identified in Mesozoic geological records and use four coccolithophore species phylogenetically related to Mesozoic species, for comparison with the fossil record. Based on the results obtained, the authors emphasize that each coccolithophore species responds differently to metal availability and that such species-specific response should be taken in to account when coccolithophore morphological characteristics are used to reconstruct seawater chemistry in the geological past. I read the review posted by Dr. L. J. de Nooijer and I agree with his assessment. The manuscript is well written and the results presented can be of interest to a broad audience. However, there are some changes that I recommend the authors to consider in order to improve their manuscript. Overall, I recommend this article for publication in "Biogeosciences" after a minor to moderate revision.

Specific comments:

I agree with the comments already provided by Dr. L. J. de Nooijer. Below are few additional suggestions.

Abstract.

Reviewers' comment: Page 1, line 20. The authors do not really discuss the changes in coccolithophore algae production as consequence of elevated trace metal concentrations in their experiments. Please, delete.

Authors reply: We made this change

Introduction.

Reviewers' comment: Page 2, line 19. "During the latest Cenomanian OAE 2 (. . .), increased by about 8-20 times the background level". Is this "seawater background level"? Please, specify.

Authors reply: Yes, the text was modified accordingly.

Reviewers' comment: Page 2, line 24. Which coccolithophore species?

Authors reply: We added the nannofossil species. Although, size changes during Cretaceous OAEs are further examined in the discussion.

Reviewers' comment: Page 2, lines 26-28. It is likely that this paper will be read by scientists, who might not be familiar with morphological phylogeny. I recommend adding few sentences to explain what morphological phylogeny is, its implications, and its relevance in this study.

Authors reply: We have improved/modified the text following the suggestions of the Reviewer.

Reviewers' comment: Page 3, line 4. It might be worth to explain why *E. huxleyi* is so widely studied compared to other coccolithophore species.

Authors reply: We have improved the text following the suggestions of the Reviewer.

Reviewers' comment: Page 3, line 9. The trace metals tested – which ones?

Authors reply: The four trace metals tested (Ni, Zn, V and Pb) are listed in the material and method paragraph.

Material and Methods.

Reviewers' comment Page 4, line 8. Please, provide the range of duration of each experimental treatment.

Authors reply: Text modified accordingly.

Reviewers' comment Page 4, line 10. What is meant by "main experiment"?

Authors reply: We delated "main" in the text.

Reviewers' comment: Page 5, section 2.4.2. Please, specify why these analyses were done only on *E. huxleyi*.

Authors reply: We added a comment in the text.

Results.

Reviewers' comment: Page 6, lines 7-8. "On the other hand, *E. huxleyi*, *G. oceanica*, *C. pelagicus* and *P. carterae* survived in L, M and H". I suggest adding the word "treatments" (or equivalent) at the end of the sentence.

Authors reply: Text modified accordingly.

Reviewers' comment: Page 6, lines 28-29. "The coccosphere volume was significantly reduced under increased trace metal concentrations compared to control conditions (Fig 6b) with similar coccosphere volumes recorded in both L, M and H". Can the coccosphere volume be really defined "similar" in L, M, and H?

Authors reply: We checked the results and we modified the text accordingly.

Discussion.

Reviewers' comment: As a general comment, there is no discussion of *E. huxleyi* coccolith malformations (Figure 3).

Authors reply: The Reviewer is correct. We improved the text following the suggestions of the Reviewer (page 7).

Reviewers' comment: Figure 1. The meaning of the grey line is a little bit foggy – what does it represent? Does it represent the coccosphere volume? Please, specify.

Authors reply: The grey line represents coccolith-free cell volume of *C. pelagicus* after acidification with HCl. The caption was modified.

Reviewers' comment: Figure 2. I would recommend moving the column "Control" prior the columns "Low", "Medium", and "High", for consistency with Tables 1-4 and the other Figures.

Authors reply: The figure was modified accordingly.

Reviewers' comment: Table 2. Growth rate, coccosphere diameter, cell diameter, and coccosphere volume are reported either as (almost) fully spelled name or as symbol. Please, be consistent and revise the table caption accordingly

Authors reply: Table and caption modified.

Impact of trace metal concentrations on coccolithophore growth and morphology: laboratory simulations of Cretaceous stress.

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10 **Abstract.** The Cretaceous ocean witnessed intervals of profound perturbations such as volcanic input of large amounts of CO₂, anoxia, eutrophication, and introduction of biologically relevant metals. Some of these extreme events were characterized by size reduction and/or morphological changes of a few calcareous nannofossil species. The correspondence between intervals of high trace metal concentrations and coccolith dwarfism suggests a negative effect of these elements on nannoplankton biocalcification processes in past oceans. In order to test this hypothesis, we explored the potential effect of a mixture of trace
15 metals on growth and morphology of four living coccolithophore species, namely *Emiliania huxleyi*, *Gephyrocapsa oceanica*, *Pleurochrysis carterae* and *Coccolithus pelagicus*. **The phylogenetic history of coccolithophores shows that the selected living species are linked to Mesozoic species showing dwarfism under excess metal concentrations.** The trace metals tested were chosen to simulate the environmental stress identified in the geological record and upon known trace metal interaction with living coccolithophore algae.

20 Our laboratory experiments demonstrated that elevated trace metal concentrations, similarly to the fossil record, affect coccolithophore algae **size and/or weight**. Smaller coccoliths were detected in *E. huxleyi* and *C. pelagicus*, while coccoliths of *G. oceanica* showed a decrease in size only at the highest trace metal concentrations. *P. carterae* coccolith size was unresponsive to changing trace metal concentrations. These differences among species allow to discriminate most- (*P. carterae*), intermediate- (*E. huxleyi* and *G. oceanica*), and least- (*C. pelagicus*) tolerant taxa. The fossil record and the
25 experimental results converge on a selective response of coccolithophores to metal availability. These species-specific differences must be considered before morphological features of coccoliths are used to reconstruct paleo-chemical conditions.

1 Introduction

Trace metal concentrations influence the productivity and species composition of marine algae communities (Bruland et al., 1991; Sunda and Huntsman, 1998). A number of trace metals are important micronutrients (e.g. zinc, iron, copper, nickel) but some of them can become toxic and inhibit marine algal productivity at elevated concentrations (Brand et al., 1986; Sunda and Huntsman, 1992). Others like lead and mercury have no known metabolic functions and can hamper marine phytoplankton growth already at low concentrations (Sunda, 1989; Sunda et al., 2005).

The geological record offers the opportunity to investigate past case histories marked by profound changes in the ocean, such as volcanic injection of large amounts of CO₂, ocean anoxia, eutrophication and introduction of biologically relevant metals (e.g. Larson and Erba, 1999; Erba, 2004; Jenkyns, 2010; Erba et al., 2015). These events can be seen as “natural experiments” useful to decrypt the ecosystem response to major perturbations at time scales longer than current modifications. Finding out how the changes in seawater composition affect marine biota requires the integration of a long-term and large-scale geological perspective that has been recognized as an essential ingredient for more coherent predictions of how marine organisms might react to future environmental changes. Insights on ocean/atmosphere dynamics under warmer-than-present-day conditions predicted for the end of this century can be obtained by including geological data of past ecosystems, especially those derived from cases of extreme conditions. Well-known perturbations were the “Oceanic Anoxic Events” (OAEs) which took place during the Mesozoic. These events were caused by intense volcanism that produced large igneous provinces (LIPs) (Snow et al., 2005; Neal et al., 2008; Pearce et al., 2009; Erba et al., 2015) that released magmatic fluids delivering metals, mixed with warmed ambient seawater that had enough buoyancy to rise to the surface (Snow et al., 2005; Erba et al., 2015). During the latest Cenomanian OAE 2 for example, less volatile elements, such as nickel and iron (released during the formation of the Caribbean LIP), increased by 8-20 times above seawater background levels while more volatile elements like lead and cadmium (derived from water-rock exchange reactions), increased by about 4-8 times above background level (Orth et al., 1993; Snow et al., 2005). Entering the ocean environment, more and less volatile elements became biologically relevant as evidenced by changes and turnover in marine plankton communities (Leckie, 1985; Leckie et al., 1998; Erba, 2004; Erba et al., 2015).

Studies on calcareous nannofossils documented a size reduction of some coccolithophore species (*Biscutum constans*, *Zeugrhabdotus erectus* and *Discorhabdus rotatorius*) coeval with trace metal concentration peaks across both the early Aptian OAE 1a and latest Cenomanian OAE 2 (Erba et al., 2015; Faucher et al., 2017). The fossil record shows that, although most of the Mesozoic nannoplankton taxa didn't survive the mass extinction event at the end of the Cretaceous, reconstructed phylogenetic trees (Bown et al., 2004), based on morphological observations of coccolith (shape and ultrastructure), and molecular trees, mostly based on rDNA (de Vargas and Probert, 2004) indicate a link between selected Mesozoic groups and some living coccolithophores. The group which the four species tested here, namely *Emiliania huxleyi*, *Gephyrocapsa oceanica*, *Coccolithus pelagicus* and *Pleurochrysis carterae*, belong to, evolutionarily diverged from one another since the Late Cretaceous, with the exception of *E. huxleyi* and *G. oceanica* that are separated since 250,000 years ago (De Vargas et

al., 2007; Liu et al., 2010). Specifically, genera *Emiliana* and *Gephyrocapsa* belong to the Cenozoic family Noelaerhabdaceae derived from the extinct Prinsiaceae that, in turn, branched off the Mesozoic family Biscutaceae. Indeed, coccolith dwarfism was observed in genus *Biscutum* during times of high CO₂ and metal concentrations in both OAE 1a and OAE 2 (Erba et al., 2010; Faucher et al., 2017). It is challenging to unambiguously disentangle the cause/s of such changes in the fossil record but evidence of a correspondence between intervals of high trace metal concentrations and coccolith dwarfism suggest a negative effect of these elements on nannoplankton biocalcification processes.

Previous work on the response of living coccolithophores to trace metal concentrations focused on *Emiliana huxleyi*, one of the most abundant species in the world ocean with a nearly global distribution (Westbroek et al., 1989; Winter et al., 2014).

Experiments documented a decreasing growth rate under high trace metal concentrations (Vasconcelos et al., 2001; Hoffmann et al., 2012, Santomauro et al., 2016). So far, no such studies have been performed on other coccolithophore species. Furthermore, to our knowledge, this is the first study investigating the effect of high trace metal concentrations on coccolithophore and coccolith morphology and size. The trace metals tested (Zn, V, Ni, Pb) were chosen based on peaks identified in the geological record (Snow et al., 2005) and known trace metal interactions with living coccolithophores to simulate the environmental conditions during OAEs. The main goal of this study is to understand if, similarly to the fossil record, anomalously high quantities of essential and/or toxic metal induce changes in coccolith shape and size and cause coccolith dwarfism in coccolithophore species.

More specifically, we address the following questions: i) does coccolithophore growth change in response to increasing trace metal concentrations? ii) does coccolith size and morphology, as well as coccolithophore size, change in response to high and anomalous trace metal concentrations? iii) do trace metal combinations, which mimic OAEs conditions, lead to a uniform response among species or to species-specific responses on morphological features? iv) do coccolith morphometrical features have a potential to serve as a proxy to reconstruct paleo-ocean trace metal concentrations?

2 Material and Methods

2.1 Culture conditions

Monospecific cultures of the coccolithophores *Emiliana huxleyi* (strain RCC 1216), *Gephyrocapsa oceanica* (strain RCC 1303), *Coccolithus pelagicus* (strain PLY182G), and *Pleurochrysis carterae* (no known strain number) were grown as batch cultures in artificial seawater (ASW) produced as described by Kester et al., (1967). The artificial seawater medium was enriched with 64 µmol kg⁻¹ nitrate, 4 µmol kg⁻¹ phosphate to avoid nutrient limitations, f/8 concentrations for vitamins (Guillard and Ryther, 1962), 10 nmol kg⁻¹ of SeO₂ and 2 ml kg⁻¹ of natural North Sea water (Bach et al., 2011). The carbonate chemistry was adjusted by bubbling with CO₂-enriched air overnight to reintroduce inorganic carbon thereby reaching atmospheric CO₂ partial pressure (~400 µatm). All culture bottles were manually and carefully rotated three times a day, each time with 20

rotations in order to avoid cell settling. In the control treatment, the medium was enriched with f/8 concentrations for trace metals (Guillard and Ryther, 1962).

Pb, Zn, Ni and V concentrations were added in low (L), medium (M), high (H) and extreme treatments because of their high concentrations identified in the Aptian OAE 1a (Erba et al., 2015) and Cenomanian-Turonian OAE 2 (Snow et al., 2005).

5 (Table 1). The trace metal chelator EDTA (ethylenediamine tetraacetic acid) was added to the trace metal stock solutions in order to guarantee a constant level of bioavailable trace metals for phytoplankton and prevent metal precipitation. The cultures were incubated in a thermo constant climate chamber (Rubarth Apparate GmbH) at a constant temperature of 15° C, a 16:8 [hour:hour] light/dark cycle, at an photon flux density of 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

10 The cultures were pre-exposed to the different treatment conditions (acclimation period) for 7-10 generations, which varied between 6-10 days depending on the species-specific cell division rates. All cultures were incubated in autoclaved 500 mL square glass bottles (Schott Duran). The initial cell density was relatively low with $\sim 50 \text{ cells ml}^{-1}$. Final samples were taken when cells were still in their exponential growth phase and cell numbers were low enough to avoid a strong change in the chemical conditions of the growth medium. Therefore, the experimental duration differed among species and among treatments (between 6-10 days) due to the different growth rates. Each treatment was replicated three times. Final cell densities in the
15 experiment didn't exceed 50000 cells ml^{-1} in *E. huxleyi*, 20000 cells ml^{-1} in *G. oceanica*, 3000 cells ml^{-1} in *C. pelagicus* and *P. carterae*.

2.2 Cell abundance and growth rate

20 Samples for cell abundance were taken every second day with the exception of the control treatment where samples were only taken at the end of the experiment. Incubation bottles were gently turned 10 times in order to obtain a homogenous suspension of the cells before sampling. Cell numbers were immediately measured three times without addition of preservatives using a Beckman coulter Multisizer. Specific daily growth rates (μ) were calculated from the least-squares regression of cell counts versus time during exponential growth (Eq. 1)

25
$$\mu = \frac{\ln c_1 - \ln c_0}{t_1 - t_0} \quad (1)$$

were c_0 and c_1 are the cell concentrations at the beginning (t_0) and at the end of the incubation period (t_1), respectively.

2.3 Coccosphere and cell sizes

Cell abundance samples were acidified with 0.1 mmol L^{-1} HCl to dissolve all free and attached coccoliths and subsequently measured 3 times in order to obtain cell diameters and volumes (Müller et al., 2012). In this study, we define the coccosphere
30 volume as the coccolith-bearing cell volume, and the cell volume as the coccolith-free cell volume. Therefore, the volume of

the calcitic portion of the coccosphere (VCP) was estimated as:

$$\text{Volume of the calcitic portion of the coccosphere (VCP)} = \text{coccosphere volume} - \text{cell volume}$$

Coccolith volume and free coccolith concentrations were also determined for *C. pelagicus* (Fig. 1).

5 2.4 Coccolith dimensions and malformations

2.4.1 Scanning Electron Microscopy (SEM)

10 Samples were taken from each of the 48 incubation bottles. 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. 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. For each sample, 50 specimens were digitally captured and subsequently analyzed with Image J software. All pictures were taken with the same magnification (5000x) and the scale bar given on SEM pictures was used for calibration (Fig 2).

2.4.2 Coccolith dimensions and *E. huxleyi* malformation

15 We measured the length of distal shield (DSL) and the width of the distal shield (DSW) manually using the public domain program Fiji distributed by ImageJ software (Schindelin et al., 2012). The distal shield area (DSA) was calculated, assuming an elliptical shape of the coccolith, as (Eq. 2):

$$DSA = \pi \frac{DSL \times DSW}{4} \quad (2)$$

20 Assuming elliptical shape has been shown to yield near identical results to direct measurements of DSA in *E. huxleyi* (Bach et al., 2012).

Malformations were determined for *E. huxleyi* since in the filters analyzed with SEM coccoliths were very abundant and allow a visual comparison of 100 individual coccoliths for every sample. We sorted the degree of malformation in several categories: our categories were used to describe the morphology of *E. huxleyi* as “normal”, “malformed”, “incomplete” and “incomplete and malformed” coccoliths (Langer et al., 2010; Langer et al., 2011) (for reference images for the categories, see Fig 3). We considered normal *E. huxleyi* coccolith with regular shape and well-formed distal shield elements forming a symmetric rim; malformed: malformed coccolith shape or malformed shape of individual elements; incomplete: coccolith with variations in its degree of completion; malformed and incomplete: coccolith with malformed shape and variations in its degree of completion.

2.5 Statistics

Prior to statistical analyses, data were tested for normality and homogeneity of variances. To test the null hypothesis the average value of parameters from triplicate cultures were compared between treatments. Mean μ values, coccosphere and cell diameters, VCP and coccolith sizes of each treatment were compared to the control and among each other. A one-way analysis of variance was used to determine statistical significance of the main effect of trace metals on the variables. A Tukey post-hoc test was used to identify the source of the main effect determined by ANOVA to assess whether differences in μ and sizes between trace metal treatments were statistically significant. Statistical treatments of data were performed using R software. Statistical significance was accepted for $p < 0.05$.

3 Results

3.1 Growth rate

In the treatment with extreme trace metal concentrations up to $8 \mu\text{mol L}^{-1}$, none of the four species tested survived the acclimation phase. In L, M and H treatments, on the other hand, *E. huxleyi*, *G. oceanica*, *C. pelagicus* and *P. carterae* all survived. However, the addition of trace metals decreased the growth rate of *E. huxleyi*, *G. oceanica*, and *C. pelagicus* compared to the control treatment. *E. huxleyi* growth rate was 1.22 d^{-1} in the controls and 1.12, 1.16 and 1.10 d^{-1} in L, M, H trace metal concentration treatments respectively (Table 2; Fig 4a). *G. oceanica* growth rate was 0.66 d^{-1} in the controls. In L, M and H the growth rate was significantly lower compared to the control at values of 0.58 d^{-1} in L, 0.60 d^{-1} in M and 0.58 d^{-1} in H. (Table 2; Fig 4b). *C. pelagicus* had an average growth rate in the control experiment of 0.56 d^{-1} . The growth rate was significantly lower in trace metal treatments compared to the control, with values of 0.42 d^{-1} in L and 0.43 d^{-1} in M and H in average (Table 2; Fig 4c). Contrarily, *P. carterae* showed an increase in growth rate with trace metal addition compared to the control. In the control treatment, the growth rate was 0.52 d^{-1} and is significantly lower compared to L, M and H treatments with growth rates of 0.57, 0.56 and 0.57 d^{-1} , respectively (Table 2; Fig 4d).

3.2 Coccosphere and cell sizes and volume of the calcitic part of the coccosphere

In *E. huxleyi*, the mean coccosphere diameters were significantly lower in the L, M, and H treatments compared to the control (mean diameter $4.88 \mu\text{m}$; Table 2, Fig 5a). The VCP was reduced under all increased trace metal treatments compared to the control conditions (Table 2; Fig 6a) with lowest VCP recorded in the M treatment.

In *G. oceanica*, the coccosphere diameters were largest in the control treatment (mean diameter $7.25 \mu\text{m}$; Table 2; Fig 5b). L, M and H coccosphere diameters were significantly smaller compared to the control. Specifically, L and M show similar values of $6.58 \mu\text{m}$ and $6.60 \mu\text{m}$, respectively, while H shows the lowest coccosphere diameter of $6.14 \mu\text{m}$ (Table 2). Similarly, cell

diameters were significantly larger in the control treatment (5.45 μm), intermediate in L and M (L= 5.18 μm ; M=5.19 μm) and smaller in H (4.74 μm). Furthermore, the coccosphere and cell diameters were significantly smaller in H compared to L and M. The VCP was significantly reduced under increased trace metal concentrations compared to control conditions (Fig 6b) with similar coccosphere VCP recorded in both L, M and H.

- 5 *C. pelagicus* coccosphere and cell diameters were significantly larger in the control (19.82 μm and 15.65 μm , respectively) compared to L (17.12 μm and 10.10 μm , respectively), M (17.05 μm and 10.46 μm , respectively) and H (16.85 μm and 10.38 μm , respectively) (Table 2.; Fig 5c). However, a significant increase in coccosphere VCP was observed from 1760 in the control to 2102, 2036, and 1954 in the L, M and H treatments, respectively. (Fig 6c, Table 2).

- 10 *P. carterae* showed a smaller coccosphere diameter in the control compared to the L, M and H treatments. The coccosphere diameter in L and H (L= 12.11 μm ; H=11.99 μm) is significantly bigger compared to the control (coccosphere diameter 11.70 μm). In M, the coccosphere has a mean diameter of M=11.88 μm (Fig 5d). On the other hand, the cell diameters were very similar among all treatments. The VCP was slightly lower in the control compared to the other three treatments (L, M and H; Table 2.; Fig 6d).

3.3 Coccolith size and *C. pelagicus* coccolith concentrations

- 15 *E. huxleyi* coccoliths were longer and wider in the control treatment compared to the other three treatments (Table 3.). Increasing trace metal content reduced coccolith length and width significantly ($p < 0.05$) and the high trace metal treatment showed the lowest distal shield length (DSL) and distal shield width (DSW) coccolith size. In *G. oceanica* coccoliths were longer and wider in the controls compared to the other three treatments (Table 3). However only in H, coccolith were significantly smaller ($p < 0.05$) compared to the control treatment.
- 20 *C. pelagicus* coccoliths in the L, M and H trace metal treatments were significantly smaller compared to the control replicates (Table 3). A higher number of free coccoliths was present in the trace metal treatments compared to the control replicates (Table 4). Free coccoliths progressively increased with increasing trace metal content.
- P. carterae* coccoliths showed very similar sizes in all the four treatments (Table 3).

3.4 *Emiliana huxleyi* coccolith malformation

- 25 Scanning electron microscope analyses of *E. huxleyi* coccoliths showed changes in the proportion of malformed and incomplete coccoliths. Specifically, malformations and incomplete coccoliths of *E. huxleyi* increased in all trace metal treatments (L, M and H concentrations) by about 20-35 % compared to the control treatment (Fig 7).

4 Discussion

4.1 Growth rate

Whereas in the extreme trace metal treatment none of the species survived the acclimation phase, revealing that the conditions were apparently poisonous, in L, M and H treatments the four test species responded in different ways to trace metal additions.

- 5 Previous studies on *E. huxleyi* responses to trace metal enrichment resulting from volcanic ash showed no significant effects on growth rate for most ashes tested (Hoffmann et al., 2012). Only the addition of pumice, which released low concentration of trace metals, had a beneficial effect and increased *E. huxleyi* growth. In one case, however, progressively increased ash content strongly suppressed the growth rate of *E. huxleyi* in the volcanic ash which contained the highest trace metal concentrations (e.g. Pb from 0.5 up to 2.6 nM L⁻¹; Ni from 12 up to 60 nM L⁻¹) (Hoffmann et al., 2012). Vasconcelos et al.
10 (2001), report a 10-20 % growth rate reduction of *E. huxleyi* with increasing Pb up to 0.25 µmol L⁻¹ without additions of EDTA.

In our study, *E. huxleyi* growth decreased with increasing Pb, Zn, Ni, and V concentrations, whereby the highest concentration of trace metals up to 0.8 µmol L⁻¹ slowed down *E. huxleyi* growth by 10% (Fig 4a).

- Trace metal effects on *G. oceanica* have not been tested before. We observed a decrease of *G. oceanica* growth of 12% under
15 the highest trace metal concentrations (Fig 4b). A similar **negative** response to elevated trace metal concentration was observed for *C. pelagicus* where the growth rate decreased by 31% in each of the trace metal treatment compared to the control (Fig 4c). The bigger growth rate reduction observed for *C. pelagicus* suggests a **comparatively** high sensitivity of this species to trace metals enrichment. A stepwise increase in trace metal concentration did not induce any progressive growth rate reduction attesting a strong sensitivity of both *G. oceanica* and *C. pelagicus* already at low trace metal concentrations.
- 20 *P. carterae* growth rate generally increased with trace metal concentration (Fig 4d). This beneficial effect of high trace metal quantities (L, M and H) on *P. carterae* growth rate might be due to the preferred habitat of this species in eutrophic lagoons and estuaries (Heimdal, 1993), where trace metal concentrations are generally much higher (Sunda and Hunstman, 2005) than in open waters.

4.2 Morphometrical analyses

- 25 The coccolithophore species tested evidenced a detrimental effect of trace metals on coccosphere, cell and coccolith sizes. Indeed, three species, *E. huxleyi*, *G. oceanica* and *C. pelagicus*, displayed significant size reductions when grown under anomalously high trace metal concentrations. However, the morphometrical responses are highly variable among species (Fig 8): 1) *E. huxleyi* reduced its coccolith sizes under high trace metal concentration. This reduction can explain the coccosphere diameter decrease and the concomitant stable cell sizes. **Additionally, an increase in the percentage of malformed and/or**
30 **incomplete coccoliths were observed under high trace metal concentrations.** 2) Trace metal concentration **also** influenced *G.*

oceanica coccosphere and cell sizes. Furthermore, an extra size reduction of both parameters, that goes along with coccolith size decrease, occurred at the highest trace metal concentration tested. This implies a particularly noxious effect of very high trace metal concentration on *G. oceanica* growth. 3) *C. pelagicus* coccosphere, cell diameters and coccolith sizes were negatively influenced by higher trace metal quantities. Increased trace metal induced a reduction of coccosphere, cell and coccolith sizes in *C. pelagicus* at all the concentrations tested. However, the volume of the calcitic portion of the coccosphere significantly increased under high trace metal concentrations. A plausible explanation is that the size decline of the cell goes hand in hand with an increase in the coccolith numbers that cover the cell. Indeed, progressively increased numbers of free/detached *C. pelagicus* coccoliths go together with a gradual increase in trace metal concentrations. (Table 4). This hints at a beneficial effect of trace metal on the number of *C. pelagicus* coccolith produced per cell (Paasche et al., 1998; Müller et al., 2012). 4) On the contrary, *P. carterae* doesn't show any sensitivity to trace metal concentration since coccosphere, cell and coccolith sizes remain stable in all treatments tested. Trace metal concentration in coastal areas are commonly much higher compared to the open ocean.

Coccolithophore algae therefore, respond differently to changes in trace metal concentrations. This species-specific sensitivity suggests a different degree of adaptation of the species tested.

4.3 Analogy and contrast with the fossil record

The trace metals tested here, were chosen based on metal peaks identified in the Aptian OAE 1a (Erba et al., 2015) and latest Cenomanian OAE 2 (Snow et al., 2005). Zn and Pb are more volatile elements that are concentrated in magmatically degassed fluids. On the other hand, Ni and V are found in higher concentrations in water-rock exchange reactions of typical steady-state hydrothermal vents (Rubin, 1997). Therefore, the composition of trace metal tested was intended to simulate OAE conditions. We emphasize that the coccolithophore species chosen for this experiment are linked to the Mesozoic family Biscutaceae based on the fossil record tracing their biocalcification history back to ~200 million years ago (Bown et al., 2004). When genomic data-sets are considered for reconstruction of coccolithophore evolution, it appears that the selected Coccolithales order (*C. pelagicus* and *P. carterae*) diverged from the Isochrysidales order (*E. huxleyi* and *G. oceanica*) in the earliest Triassic (De Vargas et al., 2007) or even in the latest Permian (Liu et al., 2010), some 300 million years ago.

Considering that the species tested in this study have a long evolutionary history, it may be justified to conduct a comparison among fossil and living coccolithophore responses.

Morphometric analyses of selected nannofossil taxa across Cretaceous OAEs in various geological settings, revealed differential species-specific patterns. *Biscutum constans*, a cosmopolitan coccolithophore species of the Cretaceous ocean, evidenced size variations in times of environmental change. Specifically, coccolith dwarfism (*sensu* Erba et al., 2010) occurred at intervals characterized by high trace metal concentrations (Erba et al., 2010; Erba et al., 2016; Faucher et al., 2017). On the other hand, *Watzanaueria barnesiae*, a cosmopolitan species described as a r-selected opportunistic species (Hardas et al., 2007) did not change in size across OAEs (Erba et al., 2010; Bornemann and Mutterlose, 2006; Lübke and Mutterlose, 2016,

Faucher et al., 2017). Indeed, a more pronounced ellipticity, interpreted as evidence of malformation, was documented by Erba et al., (2010) during OAE 1a, but not during other times of global anoxia (Bornemann and Mutterlose, 2006; Faucher et al., 2017). The very low variability in *W. barnesiae* size indicates that this taxon was most adaptable and only marginally affected by the paleoenvironmental stress characterizing Cretaceous OAEs. Finally, *D. rotatorius* and *Z. erectus*, species with a meso-eutrophic preference, evidenced inconsistent size trends without a distinct relationship between size and metal peaks (Faucher et al., 2017).

Our laboratory experiments on living coccolithophore algae demonstrate that elevated trace metal concentrations affect coccolith size and/or weight similar to the fossil record. Moreover, as quantified in nannofossil assemblages, our results reveal species-specific responses. In fact, large differences were observed between species and we could identify the most- (*P. carterae*), intermediate- (*E. huxleyi*, *G. oceanica*), and the least- (*C. pelagicus*) tolerant taxa to increasing trace concentrations. Parallel changes among fossil and living coccolithophores suggest that trace metal concentrations have the potential to influence coccolith production and sizes.

We stress the fact that both the fossil record and the experimental results converge on a species-specific response of coccolithophores to metal availability. Consequently, the indiscriminate use of coccolith sizes as a proxy of trace metal concentration in seawater should be avoided. Instead, it is crucial to identify the species index/indices that better trace paleoenvironmental stress induced by high (selected) metal concentrations.

5 Conclusion

With this study, we demonstrated for the first time that a mixture of trace metals affected growth and morphology of all of the coccolithophore species. A size reduction of the coccosphere and cell diameters has been observed in three of the analyzed species. Furthermore, we **observed** the production of dwarf coccoliths (*sensu* Erba et al., 2010) under high trace metal concentrations. Our data show a species-specific sensitivity of coccolithophores to trace metal concentration, allowing the recognition of most- (*P. carterae*), intermediate- (*E. huxleyi* and *G. oceanica*), and least- (*C. pelagicus*) tolerant taxa.

The comparison of data on living coccolithophores and Mesozoic calcareous nannofossils shows strong similarities, suggesting that laboratory simulations of past extreme conditions are viable when extant taxa are phylogenetically linked to extinct fossil species. Our study supports the hypothesis that anomalous trace metal conditions in the past oceans significantly contributed to the morphological coccolith changes during Cretaceous OAEs.

Laboratory experiments on modern coccolithophore species remain the only means to quantitatively assess the individual or combined role of environmental parameters (e.g. trace metal **availability**) on coccolith secretion. Our results emphasize the need to consider species-specific differences where coccolith morphological features are used to reconstruct paleo-chemical conditions.

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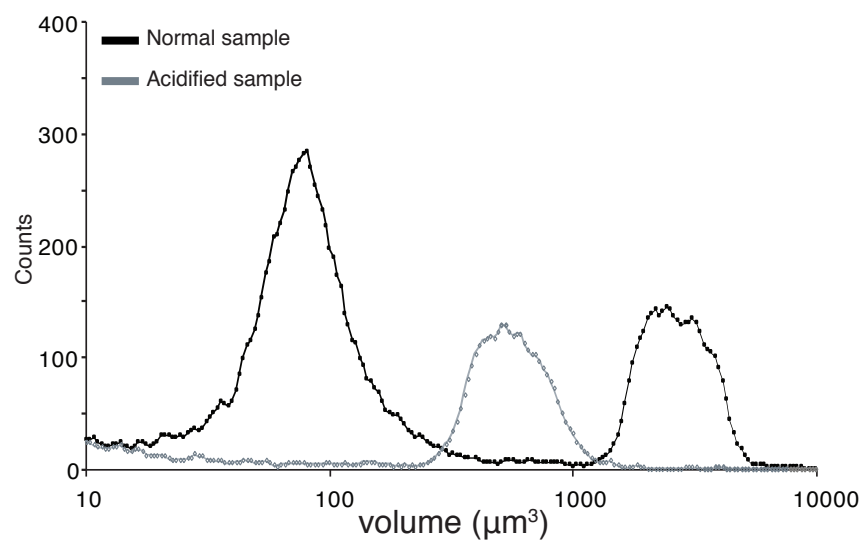
5 References

- Bach, L. T., Riebesell, U., Schulz, K. G.: Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliana huxleyi*. *Limnology and Oceanography*, 56(6), 2040-2050, 2011.
- Bach, L. T., Bauke, C., Meier, K. J. S., Riebesell, U., & Schulz, K. G.: Influence of changing carbonate chemistry on morphology and weight of coccoliths formed by *Emiliana huxleyi*. *Biogeosciences (BG)*, 9(8), 3449-3463, 2012.
- 10 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, 2006.
- 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.
- Bruland, K. W., Donut, J.R., Hutchins, D. A.: Interactive influences of bioactive trace metals on biological production in
15 oceanic waters. *Limnol. Oceanogr*, 36(8), 1555-1577, 1991.
- De Vargas, C., Probert, I.: New keys to the Past: Current and future DNA studies in Coccolithophores. *Micropaleontology*, 45-54, 2004.
- De Vargas, C., Aubry, M., P., Probert, I., and Young, J.: Origin and evolution of coccolithophores: from coastal hunters to
20 oceanic farmers. *Evolution of primary producers in the sea*, 12, 251-285, 2007.
- Erba E (2004) Calcareous nanofossils and Mesozoic oceanic anoxic events. *Marine Micropaleontology*, 52, 1, 85-106, doi:10.1016/j.marmicro.2004.04.007, 2004.
- 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, doi: 10.1126/science.1188886, 2010.
- 25 Erba E, Duncan RA, Bottini C, Tiraboschi D, Weissert H, Jenkyns HC et al.: Environmental Consequences of Ontong Java Plateau and Kerguelen Plateau Volcanism. *GSA Special Paper* 511 doi:10.1130/2015.2511, 2015.
- 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), 2017.
- 30 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.

- Hardas, P., Mutterlose, J.: Calcareous nannofossil assemblages of Oceanic Anoxic Event 2 in the equatorial Atlantic: Evidence of an eutrophication event. *Marine Micropaleontology* 66(1) 52-69, <http://dx.doi.org/10.1016/j.marmicro.2007.07.007>, 2007.
- Heimdal, B. R.: Modern coccolithophorids. CR Tomas. Academic Press, San Diego, CA, 731-831, 1993
- Hoffmann, L. J., Breitbarth, E., Ardelan, M. V., Duggen, S., Olgun, N., Hassellöv, M., Wängberg, S. Å.: Influence of trace metal release from volcanic ash on growth of *Thalassiosira pseudonana* and *Emiliana huxleyi*. *Marine Chemistry* 132 28-33., 2012.
- Jenkyns, H.C.: Geochemistry of oceanic anoxic events. *Geochemistry, Geophysics Geosystems* 11 Q03004 doi:10.1029/2009GC002788, 2010.
- 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., 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 doi: 0.1111/j.1529-8817.2010.00916.x, 2010.
- Langer, G., Bode, M.: CO₂ mediation of adverse effects of seawater acidification in *Calcidiscus leptoporus*. *Geochemistry, Geophysics, Geosystems*, 12(5) doi:10.1029/2010GC003393, 2011.
- Larson, R. L. and Erba, E.: Onset of the Mid-Cretaceous greenhouse in the Barremian-Aptian: igneous events and the biological, sedimentary, and geochemical responses. *Paleoceanography*, 14(6), 663-678, doi:10.1029/1999PA900040, 1999.
- Leckie, R. M.: Foraminifera of the Cenomanian-Turonian Boundary Interval, Greenhorn Formation, Rock Canyon Anticline, Pueblo, Colorado. In: Society of Economic Paleontologists and Mineralogists Field Trip Guidebook 4, 139-155. Midyear Meeting, Golden, Colorado, 1985.
- Leckie, R. M., Yuretich, R. F., West, O. L. O., Finkelstein, D., Schmidt, M.: Paleooceanography of the southwestern Western Interior Sea during the time of the Cenomanian-Turonian boundary (Late Cretaceous), in *Stratigraphy and Paleoenvironments of the Cretaceous Western Interior Seaway, USA*, Concepts in Sedimentol. Paleontol. vol. 6, edited by Dean WE and Arthur MA pp. 101–126, Soc. Sediment. Geol., Tulsa, Okla, 1998.
- Liu, H., Aris-Brosou, S., Probert, I., and 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.
- Lübke, N. and Mutterlose, J.: The impact of OAE 1a on marine biota deciphered by size variations of coccoliths. *Cretaceous Research*, 61, 169-179, 2016.
- Müller, M. N., Beaufort, L., Bernard, O., Pedrotti, M. L., Talec, A., Sciandra, A.: Influence of CO₂ and nitrogen limitation on the coccolith volume of *Emiliana huxleyi* (Haptophyta). *Biogeosciences (BG)* 9(10) 4155-4167, 2012.
- Neal, C. R., Coffin, M. F., Arndt, N. T., Duncan, R. A., Eldholm, O., Erba, E., et al. (2008). Investigating large igneous province formation and associated paleoenvironmental events: a white paper for scientific drilling. *Scientific Drilling*, 6, 4-18, 2008.
- Orth, C. J., Attrep, M., Quintana, L. R., Elder, W. P., Kauffman, E. G., Diner, Villamil, T.: Elemental abundance anomalies in the late Cenomanian extinction interval: a search for the source(s). *Earth and Planetary Science Letters* 117(1) 189-204, 1993.

- Paasche, E.: Roles of nitrogen and phosphorus in coccolith formation in *Emiliana huxleyi* (Prymnesiophyceae). *European Journal of Phycology* 33(1) 33-42, 1998.
- Pearce, M. A., Jarvis, I., Tocher, B. A.: The Cenomanian–Turonian boundary event, OAE2 and palaeoenvironmental change in epicontinental seas: new insights from the dinocyst and geochemical records. *Palaeogeography, Palaeoclimatology, Palaeoecology* 280(1) 207-234, 2009.
- Rubin, K.: Degassing of metals and metalloids from erupting seamount and mid-ocean ridge volcanoes: observations and predictions. *Geochimica et Cosmochimica Acta* 61(17) 3525-3542, 1997.
- Santomauro, G., Sun, W. L., Brümmer, F., Bill, J.: Incorporation of zinc into the coccoliths of the microalga *Emiliana huxleyi*. *BioMetals* 29(2) 225-234 doi: 10.1007/s10534-015-9908-y, 2016.
- 10 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. and Cardona, A.: Fiji: an open-source platform for biological-image analysis. *Nature methods*, 9(7), 676-682, doi:10.1038/nmeth.2019, 2012.
- Snow, L. J., Duncan, R. A., Bralower, T.J.: Trace element abundances in the Rock Canyon Anticline, Pueblo, Colorado, marine sedimentary section and their relationship to Caribbean plateau construction and oxygen anoxic event 2. *Paleoceanography* doi:10.1029/2004PA001093, 20, 3., 2005.
- 15 Sunda, W.G., Huntsman, S.A.: Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems. *Science of the Total Environment* 219(2) 165-181, 1998.
- Vasconcelos, M. T. S., Leal, M. F. C.: Antagonistic interactions of Pb and Cd on Cu uptake, growth inhibition and chelator release in the marine algae *Emiliana huxleyi*. *Marine Chemistry* 75(1) 123-139, 2001.
- 20 Winter, A., Henderiks, J., Beaufort, L., Rickaby, R. E., & Brown, C. W.: Poleward expansion of the coccolithophore *Emiliana huxleyi*. *Journal of Plankton Research*, 36(2), 316-325, 2014.

Figure 1: Example of multisizer volume spectra. Black line: spectra of *C. pelagicus* population (coccolith spectrum and coccosphere



spectrum); grey line: *C. pelagicus* coccolith-free cell after treatment with HCl.

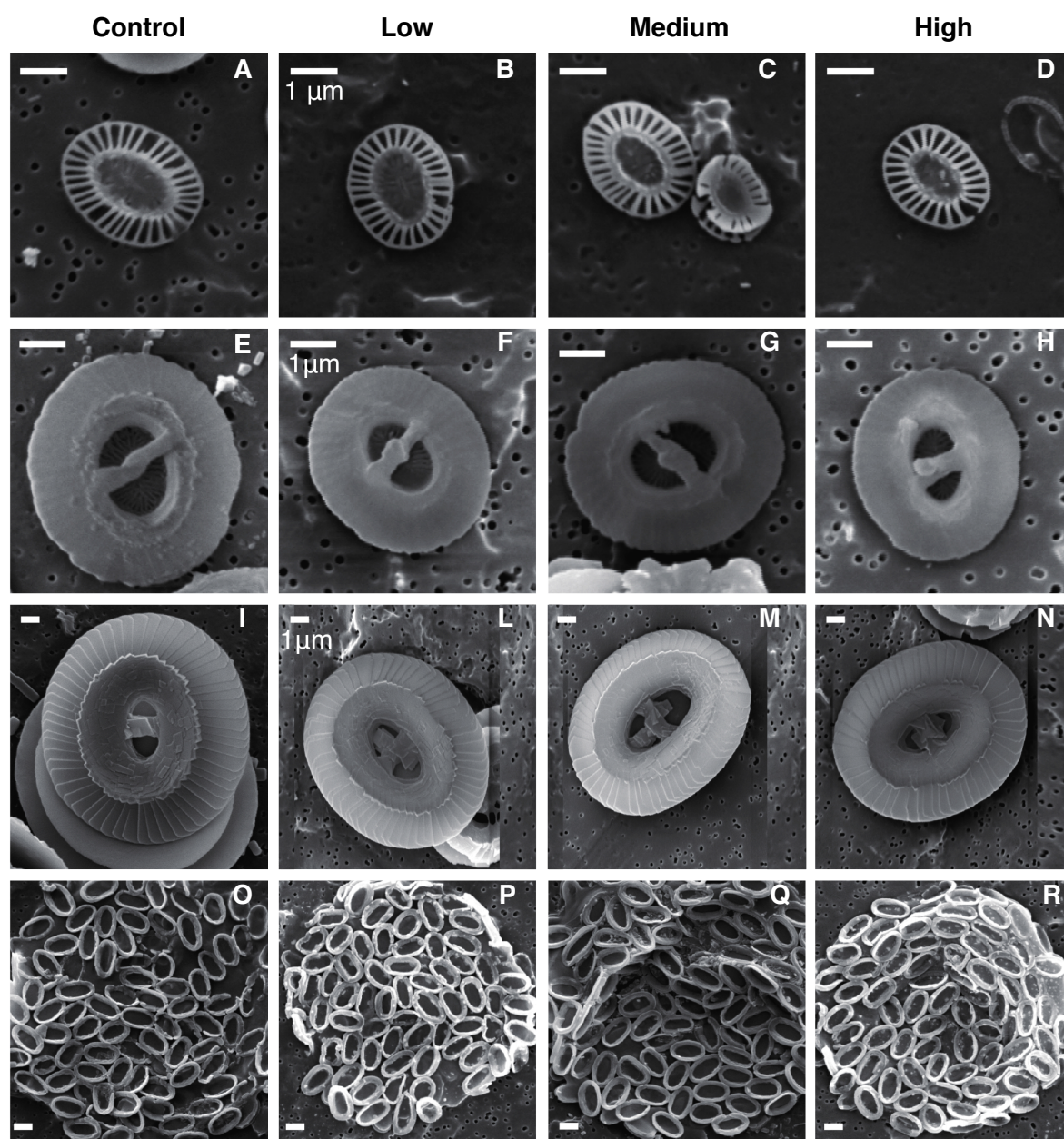


Figure 2: Plate. Example of the coccoliths of the four species tested under different trace metal concentrations. A-D, *E. huxleyi*; E-H, *G. oceanica*; I-L, *C. pelagicus*; M-P, *P. carterae*.

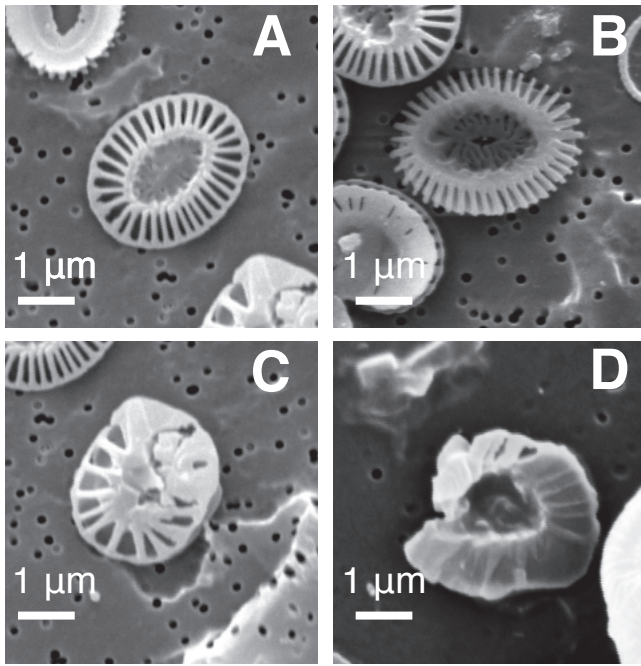


Figure 3: SEM images of *Emiliana huxleyi* coccoliths. A. Normal coccolith; B. Incomplete coccolith; C. Malformed coccolith; D. Malformed and incomplete coccolith.

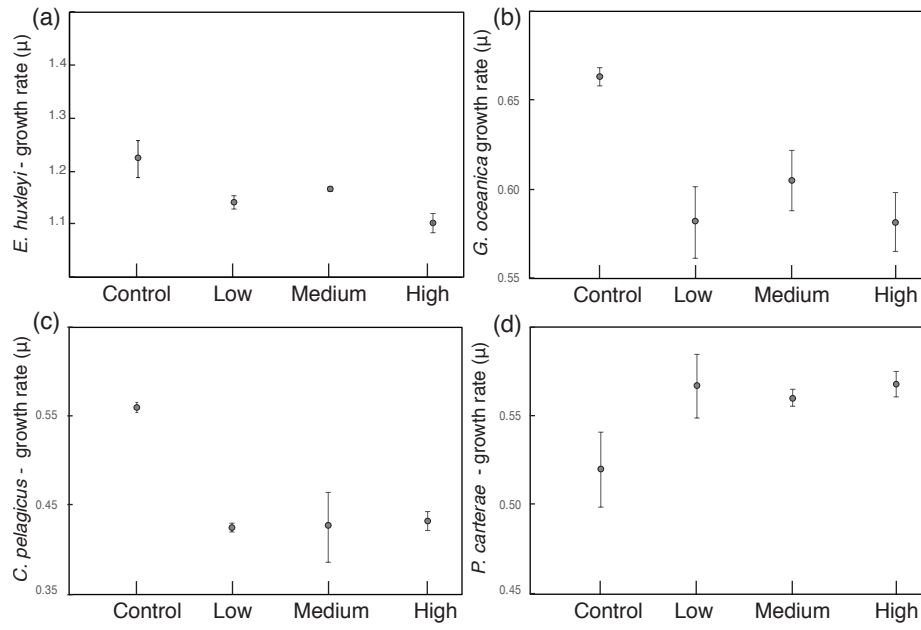


Figure 4: Average growth rate; all measurements are done in triplicates; error bars denote standard deviation. If not visible, error bars are smaller than symbols. A) *E. huxleyi*; B) *G. oceanica*; C) *C. pelagicus*; D) *P. carterae*. Note the different scales on the y-axis.

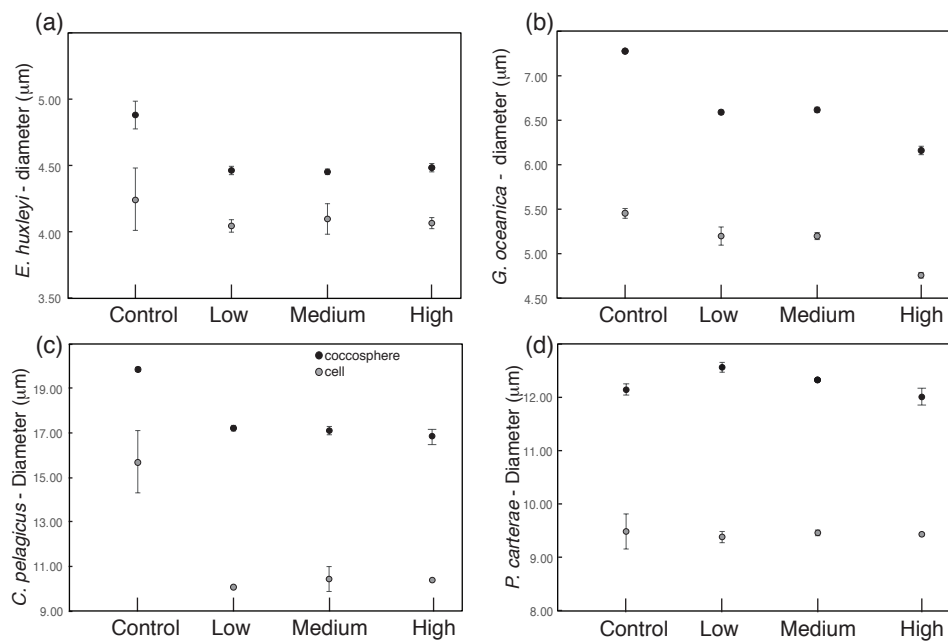


Figure 5: Average values of the coccolithophore diameters (black dots) and cell diameters (grey dots). All measurements were done in triplicates, error bars denote standard deviation. If not visible, error bars are smaller than symbols. A) *E. huxleyi*; B) *G. oceanica*; C) *C. pelagicus*; D) *P. carterae*. Note the different scales on the y-axis.

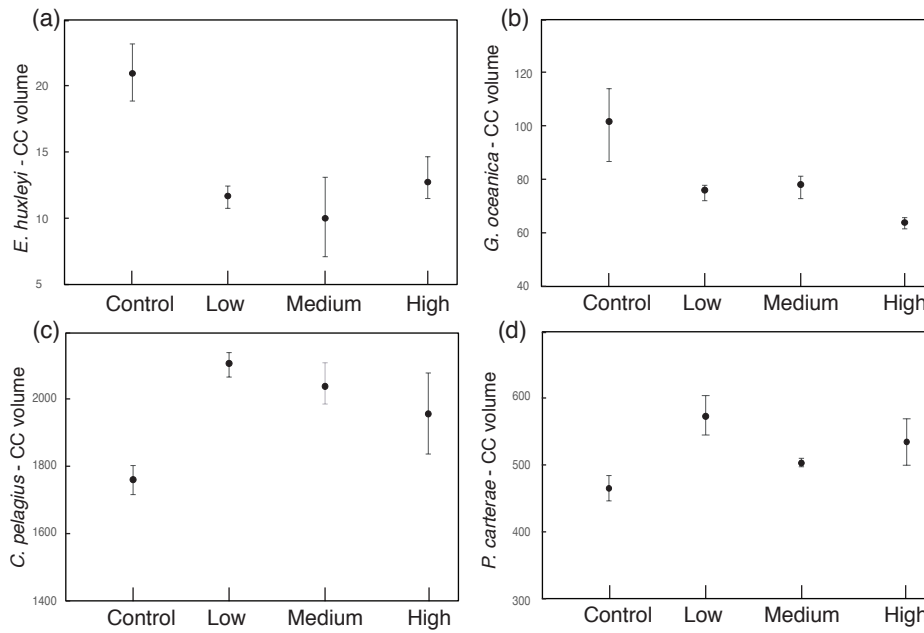


Figure 6: Average volume of the calcitic portion of the coccosphere (VCP). All measurements were done in triplicates; error bars denote standard deviation. If not visible, error bars are smaller than symbols measured for each replicate: A) *E. huxleyi*; B) *G. oceanica*; C) *C. pelagicus*; D) *P. carterae*. Note the different scales on the y-axis.

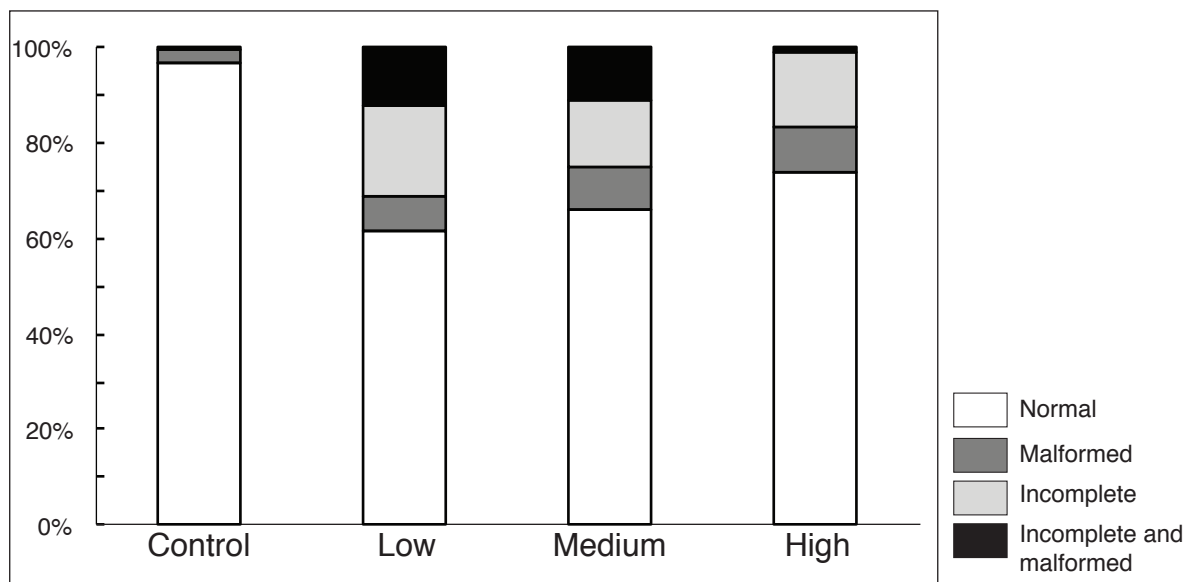


Figure 7: Malformation percentage. Percentages of normal, malformed, incomplete and malformed and incomplete coccoliths of *E. huxleyi* versus trace metal concentrations.

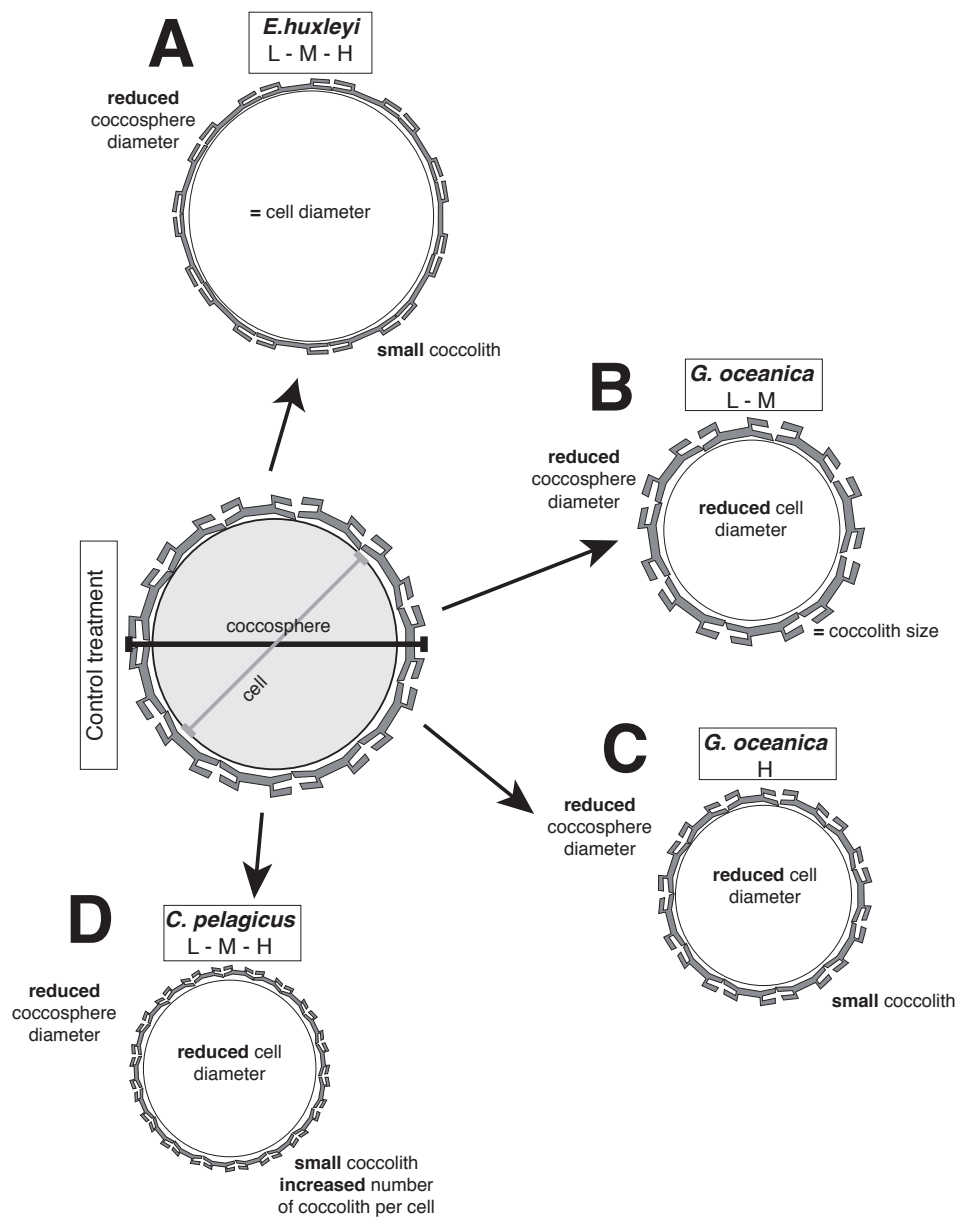


Figure 8: Species-specific responses to trace metal enrichment

	Control	Low	Medium	High	Extreme
	$\mu\text{mol L}^{-1}$				
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	11.7	11.7	11.7	11.7	11.7
$\text{Na}_2 \cdot 2\text{H}_2\text{O}$	11.7	11.7	11.7	11.7	11.7
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.04	0.04	0.04	0.04	0.04
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.03	0.03	0.03	0.03	0.03
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.04	0.04	0.04	0.04	0.04
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.08	0.16	0.16	0.96	8.08
Pb	0.01	0.08	0.8	8.00
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.08	0.08	0.8	8.00
VOSO_4	0.08	0.08	0.8	8.00
EDTA	11.7	11.7	11.7	11.7	11.7

Table 1. Trace metal concentrations in the growth medium of the different treatments.

	<i>E. huxleyi</i>				<i>G. oceanica</i>			
	Control	Low	Medium	High	Control	Low	Medium	High
μ	1.22	1.12*	1.16	1.10*	0.66	0.58*	0.60*	0.58*
Coccosphere D	4.88	4.45*	4.44*	4.48*	7.25	6.58*	6.60*	6.14*
Cell D	4.22	4.04	4.08	4.05	5.45	5.18*	5.19*	4.74*
VCP	20.98	11.78*	10.01*	12.83*	101.02	75.48*	77.41*	62.96*
	<i>C. pelagicus</i>				<i>P. carterae</i>			
	Control	Low	Medium	High	Control	Low	Medium	High
μ	0.56	0.42*	0.43*	0.43*	0.52	0.57*	0.56*	0.57*
Coccosphere D	19.82	17.12*	17.05*	16.85*	11.70	12.11*	11.88	11.99*
Cell D	15.65	10.10*	10.46*	10.38*	9.03	8.93	9.02	8.98
VCP	1760	2102*	2036*	1954*	463	570*	500	533*

Table 2. μ = growth rate, coccosphere D (μm) = coccosphere diameter, cell D = cell diameters (μm) and VCP = volume of the calcitic portion of the coccosphere (μm^3). Significance was tested using an ANOVA and a Tukey post-hoc test ($p < 0.05$). Asterisks indicate significant difference from the control treatment.

	Control			Low			Medium			High		
	DSL	DSW	DSA	DSL	DSW	DSA	DSL	DSW	DSA	DSL	DSW	DSA
<i>E. huxleyi</i>	2.98	2.44	5.71	2.66*	2.11*	4.42	2.65*	2.06*	4.29	2.59*	1.98*	4.03
<i>G. oceanica</i>	4.31	3.77	12.78	4.10	3.57	11.47	4.15	3.58	11.69	3.97*	3.37*	10.49
<i>C. pelagicus</i>	12.76	11.08	111.08	10.43*	8.43*	69.11	10.03*	8.17*	64.38	10.47*	8.50*	69.87
<i>P. carterae</i>	1.90	1.18	1.75	1.68	1.05	1.38	1.87	1.16	1.70	1.90	1.19	1.78

Table 3. Coccolith distal shield length (DSL, μm) and distal shield width (DSW; μm) average values and calculated distal shield area (DSA; μm^2) for all experiments. Asterisks indicate significant difference from the control treatment. Significance of DSL and DSW was tested using an ANOVA and a Tukey post-hoc test ($p < 0.05$). Asterisks indicate significant difference from the control treatment

	replicate	free coccolith number		replicate	free coccolith number
Control	r1	2112	Medium	r1	9017
	r2	2297		r2	100425
	r3	2972		r3	12325
Low	r1	8876	High	r1	13089
	r2	7734		r2	11523
	r3	8358		r3	11350

Table 4. Free *C. pelagicus* coccolith concentration measured with the coulter counter; r= replicates.