

Dear Mariem Saavedra-Pellittero, thank you for the constructive review that was very helpful to improve our manuscript.

Please find our point-by-point reply in the pdf file too.

Below we respond to all points raised (summarized, see original comment by Dr. Mariem Saavedra- Pellittero for details).

Note: the pages and lines are from the original file.

Please find our point-by-point reply in the pdf file too.

Kind regards

Giulia Faucher

Page 1

Abstract

- ***L 15-16: Rephrase; “evolutionarily distinct for millions of years” is misleading and not the case for *E. huxleyi* and *Gephyrocapsa*”.***

We rephrased as suggested: “to investigate this, we cultured four living coccolithophore species (*Emiliana huxleyi*, *Gephyrocapsa oceanica*, *Coccolithus pelagicus subsp. braarudii*, and *Pleurochrysis carterae*) that have been evolutionarily distinct **for hundred thousand to millions of years...**”

- ***L 18-20 and L 41-43 (P2) and 210-212 (P-8): I understand that this is one of the main motivations of this piece of research, but the authors are jumping here from hours/days to Millions of years. They need to be more careful linking present-day changes to past evolution.***

The idea behind this approach was to use species that diverged with each other many million years ago. If these species would have responded in the same way to one of the parameters that we tested, there could be a good chance that this physiological response was genetically conserved through time. This assumption comes from the idea that the chosen species share common ancestors but followed separate diversification processes and transitions for millions of years (Liu et al., 2010). Furthermore, fossil and living organisms have millions of years in between and therefore, it's true that we are comparing different time scale. But there are millions of years of divergence time among the tested living coccolithophore too and among living and fossil species. To this end, from our point of view, it is possible with caution, to make a comparison between fossil species and living algae. Finally, it's true that *E. huxleyi* and *G. oceanica* diverged with each other kyrs ago. We decided, however, to use both species to check whether the response among these two taxa would have been more similar under the tested environmental parameters and if this could be due to their closer genetic relatedness.

We changed the text: “... *that have been evolutionarily distinct for hundred thousand to millions of years...*”

- ***L 22-25. This conclusion needs to be discussed in more detail (not here, but in section 4).***

We thank Dr. Mariem Saavedra- Pellittero for this comment that gave us the chance to dig deeper into malformations in the fossil record. We added a new paragraph in the discussion. See below

Introduction

- ***L27 Add reference after “producers on Earth.***

Reference added: Tyrrell and Young, 2010.

Tyrrell, T. and 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

- **L28-29: “...exoskeleton (coccosphere) composed of single platelets called coccoliths and nannoliths”. Rephrase, being more accurate. If I am not mistaken, some nannoliths can be internal (e.g., *Ceratholithus*).**

We checked and rephrased accordingly. Yes, *Ceratholithus* is characterized by both nannolith (ceratholith) inside but also outside the cell

Cros, L., Kleijne, A., Zeltner, A., Billard, C., & Young, J. R., 2000. New examples of holococcolith–heterococcolith combination coccospheres and their implications for coccolithophorid biology. *Marine Micropaleontology*, 39(1-4), 1-34.

- **L29 “...ability to precipitate calcium carbonate” = to calcify?**

Rephrased accordingly and changed as suggested by the referee.

Page 2

- **L34-39 Reword, because this part is a bit confusing for the reader and it sounds contradictory to sentence 39-40.**

We added a sentence that could help to understand the aim of our work and the chose approach.

“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”.

- **L41-43. In case that coccolith morphology responses to a changing environmental driver are similar in the four species this could be indicative of a response pattern that was conserved over geological timescales”. This is a general assumption that the authors do for all the 4 taxa. Can the authors add some reference(s) backing up this? (I.e. explaining why these taxa would have to behave in the same way, I could imagine that would be the case for *E. huxleyi* and *Gephyrocapsa*, but it might be worthy to additional information in that regard).**

We modified the text to clarify our approach and we replied to this comment in the line above.

“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. 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).”

- **L 47-48. They way this sentence is written is tricky/misleading, because *E. huxleyi* and *Gephyrocapsa* are already half of the taxa considered. Also, despite the long evolutionary history mentioned, no traces of the delicate coccoliths of the calcifying species within the family *Pleurochrysidaceae* have been observed in the fossil record (e.g. De Vargas et al., 2007). Therefore Faucher et al should be careful how they objectively show the state of the art information).**

We changed the sentence to be clearer on the hypothesis of our experiments.

“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 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. The assumption that this approach is valid has often made but, to the best of our knowledge, not been further tested so far.”

- **L47 Double check if *Pleurochrysis carterae* is the more adequate name for this taxa or if it should be changed to *Chrysotila carterae*.**

<http://www.mikrotax.org/system/index.php?dir=Coccolithophores/Coccolithales/Pleurochrysidaceae/Chrysotila>

Chrysotila carterae and *Pleurochrysis carterae* are synonyms. We left the name *Pleurochrysis carterae* since this is the name labeled in the stock culture and it was already used for previous work (see Faucher et al., 2017b).

<http://www.mikrotax.org/Nannotax3/index.php?id=290>.

- **L 50-51. Change one of the words “whether” to avoid repetition.**
Changed

Material and Methods

- **L54 Delete “generally modified**
Modified
- **L56 Do you mean nutrient limitations or content? If the authors are referring to limitation, maybe it would be worthy to add a bit more information regarding this.**

We mean nutrient limitation that was added to the text. More information about this experiment is written in paragraph 2.1.3. Therefore, we didn’t change this part.

- **L56 Carbonate chemistry: : parameters?**

Carbonate chemistry: four different conditions were tested and we listed the information in the paragraph with the specifics about this experiment 2.1.5. Therefore, we didn’t change this part.

Page 3

- **L57 Which *E. huxleyi* morphotype? Do the authors have information about it? There might be different responses by different morphotypes.**
L 57-59. It might be worthy to specify where all those strains originally come from.

Information added in the text: “*Emiliana 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)”

- **L59 Delete ASW,**
ASW deleted.
- **L70 Change “start” for “beginning”.**
Changed accordingly.
- **L71 “It was assured” sounds odd to the reader**
Changed and deleted.

Page 4

- **L106-113 Why there are two different references for the program CO2SYS (Lewis and Wallace, 1998) Schulz et al., 2017)? Why the authors did not use Pierrot et al. (2006) instead of Lewis and Wallace (1998) that seems to be a more recent program?**

We checked and the right reference was added to the text which is, Pierrot et al., (2006).
In line 113, Schulz et al., (2007) is referred to the recalculation of pH.

- **L107 parentheses added.**

- L109 rephrased

Page 5

- L124 *I sentence should not start in that way. Rephrase or write “Fifteen to ten ml: : :” P*

The sentence was rephrased.

- L130-131 *What those references (Langer et al., 2006; Langer et al., 2010) refer to? The way that the malformations were quantified?*

It was added to the text that we followed Langer et al., (2006 and 2010) to group normal, malformed, incomplete and incomplete/malformed coccoliths.

- L138. *Why a non-linear regression? Elaborate.*

We used the nonlinear regression because for the light experiment we decided to perform an experiment with a high number of treatment levels but at the expense of replication (one replicate per sample).

Results

- L142 *Delete “all”, add “selected” after species and add “variable” before light intensity.*

Changed accordingly.

- L144 *No need for a new paragraph after only one sentence.*

Paragraph deleted.

- L145 *Here and elsewhere in the manuscript. A sentence should not start with an abbreviation or a number, therefore “G. oceanica” should be “Gephyrocapsa oceanica” here. Please, double check this throughout the manuscript (and supplementary material). There are plenty of abreviations at the beginning of sentences, especially in section 3 (L. 153, 155, 156, 160, 161, 162, 163: : :).*

Abbreviation checked and modified through the text.

Page 6

- L166 *Inner tube?*

Inner tube, changed in the text.

- L169 *Replace “any” by “no”. L 173. No need for a new paragraph after only one sentence. L 173. Replace “under” by “at”.*

Modified as requested.

- L173 *What do the authors mean by rays? T-elements? Revise this and use the adequate nomenclature.*

Rays are now called **distal shield elements**.

- L176 *I suggest to change the title of this section to “Carbonate chemistry parameters” or something along those lines. L 183. A dot is missing after (Table 5).*

Title modified accordingly and dot added.

Page 7

- **L185** *“oceanica formed a high number of malformed coccoliths” sounds odd. Reword if possible.*

Text modified.

- **L187** *Do the authors mean Figure 2 or Figure 4?*
It refers to Fig. 2. The text was changed accordingly
- **L191:** add “variations” after “carbonate chemistry”
Text modified.

Discussion

- **L193:** *How do we know it is a biological innovation? Any reference for that?*
A reference was added and text modified.

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.

- **L194-195** *“A great diversification in morphologies occurred in the Mesozoic and Paleocene where many new morphologies occurred.” Rephrase. It sounds like circular reasoning.*

Rephrased

- **P 7, L 198.** *Coccolithophore algae (without s)*
s deleted
- **L 201-202** *“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.” This sounds very vague.*

We modified the sentence in order to be less vague and better express our statement.

“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).”

- **L202:** *I miss a reference here.*
Reference added

Page 8

- **210-212.** *“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”. This sentence kind of undermines some of the author’s basic assumption(s) for this study, but I like that they mention this kind information. I even suggest to discuss this more in detail. Maybe it would be worthy also to add something along those lines in the introduction/state of the art (this applies to information provided from L210 to L220) to outline better the main goals, but also limitations, of this research work.*

We replied to this referee comment, above.

- **235-242.** *This is one of the main conclusions drawn from this research, but somehow the authors only mention it superficially, and do not even discuss it. Discussion needs to be added, especially considering existing literature (already cited by the authors!) regarding relatively well preserved nannofossils in high pCO₂ past environments;*

e.g., during Paleocene-Eocene Thermal Maximum, O’Dea et al., 2015, Gibbs et al., 2016: :). In my opinion this part needs to be improved.

The fact that coccolithophores are generating malformed coccoliths in extreme/” harsh” boundary conditions (high CO₂ concentrations in this very specific case) is something that has been previously noted or at least mentioned by other authors. Still Faucher et al. miss the chance to discuss it in section 4. I recommend the authors to add some more references to make stronger the discussion.

We thank Dr. Saavedra-Pellittero for this comment and for the opportunity she gave us to dig into malformation in the fossil record. We added a paragraph to the text.

“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 pCO₂ 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 CO₂ 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.”

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.

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

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.

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.

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.

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.

Page 9

- **L253: Change “excess” to “high concentration”**
Modified accordingly

Conclusion

- **L255:** I would rewrite this sentence as: “: :in response to temperature, light, nutrient, and Mg/Ca variations”. **260-262.** Rephrase this sentence. Make it simpler or split into two sentences.

L255 Sentence was rephrased

L260-262 The sentence was rephrased.

6. References:

Check the way dois are cited, e.g., L271 vs L274.

Checked and modified as requested.

Figures and tables

- **Figure 1: Is it possible to use italics for the name of the species? What is the meaning of the numbers at the nodes (47, 57: : :)? Maybe it is worthy to specify what they mean in the caption**
L 360. Caption: I suggest to reword it as follows: “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.”

Caption and Fig.1 modified accordingly.

Caption:

“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. Nodes, representing following divergence, used for calibrating the tree with dates from the fossil record, are shown in green.*”

- **Figure 2. Caption: Based on the (very nice) figure itself, I would change this sentence to “: : incomplete and incomplete / malformed for *E. huxleyi*: : :”**

Modified accordingly

- **Figure 3 Is it possible to add the chemistry parameters (i.e., pH, TA: : :)? I would also recommend to use the same (or similar) size font. The authors can always use abbreviations if the whole word does not fit.**

Fig. 3 Modified accordingly. The chemistry parameters are added in the caption.

- **Figure 4 Caption and figure. Use incomplete / malformed, as in figure 2.**

Fig. 4 Modified accordingly.

- **Table 1. Use the same font size (This applies to all the tables). L 390. Caption (here and elsewhere): double check that these units are correct: “Growth rate (□)”. L 392. Here and elsewhere: “E. huxleyi ray number (rays)” Do you mean T-elements?**

Table 1: Rays were intended to be the distal shield elements. They are now named “distal shield elements” and the abbreviation are SE for the mean number of distal shield elements and SEW for the average width. The font size, growth rate units are changed in all tables.

- **I would also recommend the authors to re-structure all the tables. Do not mix different parameters in the same column (e.g., rays and tube thick. In Table 1). I think it would be better to leave gaps or specify that there is no data (as in Table 5) rather than mixing different measurements. It is confusing for the reader.**

We followed the advice for the tables and re-structured them.