

Answer to reviewers comments to ms “Co-variation of metabolic rates and cell size in coccolithophores”, submitted by G. Aloisi to Biogeosciences.

Both reviewers make useful comments that improve the manuscript. I have included the great majority of their suggestions in the revised manuscript. In a limited number of cases I prefer to keep the original text. I explain why in my response below.

Reviewer 1

Reviewer 1 makes useful comments that improve the manuscript. I have included his suggestions in the revised manuscript.

- The reviewer points out that natural assemblages of coccolithophores in marine sediments contain organisms from different stages of the cell cycle, and that this natural “sampling” makes sedimentary assemblages similar to laboratory cultures conducted in continuous light, where the cell cycle is de-synchronized. This is an interesting point. I added the following text at the end of section 2.2:

“Interestingly, fossil coccolithophores represent an integrated sample over the whole light:dark cycle and thus should be more comparable to laboratory samples from desynchronizes cultures – something to keep in mind as the amount of morphological data of coccolithophores from marine sediments is growing (Beaufort et al., 2011; Grelaud et al., 2009).”

- The reviewer suggests I cite a recent paper by Bach et al. (2015) presenting a simplified equation modeling how the calcification rate responds to changes in DIC system parameters. I was not aware of this publication that is in fact very relevant. I added the reference and the following sentence in section 4.1:

“This complex reaction of calcification to changes in the DIC system has been elegantly captured in a recent model equation developed by (Bach et al., 2015).”

- page 6216, L.14: the corresponding sentence in the abstract has been changed to (in bold the changes to the original text):

“An increase in phosphate or temperature (**below the optimum temperature for growth**) produces the opposite effect”.

- page 6217, L.7: I have checked in the coccolithophore literature and found that the coccosphere is often referred to as an “exoskeleton” e.g. (de Vargas et al., 2007). I thus left this term in the paper.

Reviewer 2

First important point

The referee states that the bias of the coccolithophore database towards experiments with *Emiliana huxleyi* should be declared more clearly throughout the manuscript, so that it is

clear that the majority of the conclusions I draw refer to *E. huxleyi* and not the coccolithophores in general. I proceeded to make the following changes:

- In the abstract I added this statement:

“The coccolithophore database is strongly biased in favor of experiments with the coccolithophore *E. huxleyi* (82% of database entries), and more experiments with other species are needed to understand if these observations can be extended to coccolithophores in general.”.

- Also in the abstract, I specify that my model results are capable of reproducing the changes in growth rate and cell size observed in experiments with *E. huxleyi*:

“By considering a simple rule that allocates the energy flow from nutrient acquisition to cell structure (biomass) and cell maturity (biological complexity, eventually leading to cell division), the model is able to reproduce the co-variation of growth rate and cell size observed **in laboratory experiments with *E. huxleyi*** when these nutrients become limiting.”

- At the beginning of section 4, where the original text states that most of the experiments in the database were carried out with *E. huxleyi*, I specify in brackets that *E. huxleyi* experiments account for 82% of database entries.

- At the end of section 4, where I sum up the effect of changes of environmental parameters on cell size and metabolic rates, I added this statement:

“It should be noted that the coccolithophore database is strongly biased in favor of experiments with the coccolithophore *E. huxleyi* (82% of database entries), and more experiments with other species are needed to understand if the above relations between environment, cell size and metabolic rates can be extended to coccolithophores in general.”

Second important point

The reviewer is right in stating that I normalize the experimental physiological rates only with respect to the day-length and not with respect to the instantaneous photon flux, which also has a large influence on metabolic rates. I do not normalize to the photon flux because the photon flux is one of the environmental parameters (with pCO₂, nutrients etc) that vary considerably in the ocean and that force metabolism and cell-size changes. I thus included it in my analysis of forcing parameters and observed that it indeed has a large effect on metabolic rates and cell-size (see figure 6). Model studies suggest that climate change may increase the stratification of the surface ocean, resulting in phytoplankton communities experiencing higher irradiance levels (Bopp et al., 2001). I thus think that the choice of considering irradiance as a forcing parameter is justified.

Specific comments:

- The short form *E. huxleyi* is used throughout the manuscript instead of the long form *Emiliana huxleyi*.

- The correct spelling of the surnames Marañón and Cermeño is used throughout the manuscript and references.

- Page 6216, Ln 1: I prefer to leave the two starting sentences of the abstract unchanged because they have the merit of putting the reader in the general context of changing climate and the effect on marine organisms. I trust sufficient detail comes early on in the rest of the abstract and, with numerous literature references, in the introduction, so that the reader quickly understands the specific focus of my analysis.

- Page 6216, Ln, 16-19:

“If the biological reasons behind the environment-metabolism-size link are understood, it will be possible to use coccosphere size changes in the modern ocean and in marine sediments to investigate the fate of coccolithophores in the future ocean.”

I cannot say at the moment to which extent this statement will be confirmed by future studies. Some clues from column water investigations of coccolithophores where the size of single morphotypes is considered (using Scanning Electron Microscope observations) (Henderiks et al., 2012) suggests that changes in cell size of a given morphotype are consistent with cell-size trends seen in the dataset (I discuss this in section 5).

- Page 6217, Lines 12-15, it is now stated that the decrease in calcification to photosynthesis ratios has been observed in *E. Huxleyi*.

- Page 6218, Line 8. “physiological” is used instead of “ecological”.

- Page 6218, line 23-25. It is now stated that the experiments in question refer to *E. huxleyi*.

- Page 6218, line 27. My statement that coccolith mass is related to the mass of coccospheres refers to the observations of Beaufort et al 2011 (their Supplementary figure 1). These show a positive correlation between these two parameters in the field. The reviewer is right, however, that multiple layers of coccolithophores may complicate this simple picture in other areas of the ocean. I thus added a comment in the sentence in question, that now reads:

“Coccolith size (length, volume) and weight are used as proxies for coccolithophore calcification because they are related to the total mass of calcite in the cell (Beaufort et al., 2011) **(although multiple layers of coccoliths around cells may complicate this simple picture).**”

- Page 6219, lines 17-18. It is now specified that we are talking about *E. huxleyi* and not coccolithophores in general.

- 6219, lines 28-29. I defined the rates I deal with in the introduction with the following sentence:

“The metabolic rates considered are the growth rate (in units of day^{-1}), the rate of photosynthesis (in units of $\text{pg}_C \text{ cell}^{-1} \text{ day}^{-1}$) and the rate of calcification (in units of $\text{pg}_C \text{ cell}^{-1} \text{ day}^{-1}$).”

- Page 6221, lines 6-8. There indeed is bias introduced by some methods of measuring cell and/or coccosphere size. I discuss this in section 2.4.

- Page 6221, lines 11-12:

I do not agree with the statement of the reviewer. The growth rate can be deduced from plots of cell number vs. time on an ln scale only if nutrient limitation is not present. Graphically, the growth rate is the slope of the tangent to the cell abundance data. In the exponential

growth phase cell abundance data lie along a line, and the growth rate is the slope of this line. But as soon as nutrient limitation starts to kick in, the cell abundance data points plot below the linear regression through the exponential growth data. Graphically, the instantaneous growth rate (the growth rate which we are interested in) is still the tangent to the cell abundance data, but the growth rate decreases in time until it reaches zero in the stationary phase (when the tangent has a slope of zero). So, estimating growth rates via cell counts on an ln scale in batch cultures is ok if nutrient limitation is absent. As soon as limitation starts to decrease the instantaneous growth rate, the growth rate estimated via linear regression through the cell count data will overestimate the instantaneous growth rates. This is nicely explained in (Langer et al., 2013).

- Page 6223, lines 24-25. The sentence now reads:

“Coccosphere size data is reported only in a third of the experiments included in the dataset **(of which more than 80% of measurements are for *E. huxleyi*), ...**”

- Page 6224, lines 16-26:

The reviewer is correct in saying that multiple layers of coccoliths around a coccolithophore cell may increase the coccosphere diameter while the cell diameter does not change (in any case does not change proportionally to the diameter of the coccosphere). In the simple geometrical model I propose in equation 7, I calculate the diameter of cells and coccospheres based on POC and PIC data measured in culture experiments (no field data is used in this exercise). Therefore, the calculated cell and coccosphere diameters are free to change independently from one another based on the measured values of POC and PIC. Indeed, as the cell diameter changes smoothly with POC (this simple model assumes that the density of carbon in biomass is constant) (figures 2a and 2c), the coccosphere diameter varies irregularly because it depends on measured PIC quota. I then compare the calculated coccosphere diameters using equation 7 with the available coccosphere measurement in the database in figures 2b and 2d and discuss the origin of the discrepancy observed.

To avoid misunderstanding, I made it clear that the database does not include cell-size data (there is no such data in figures 2b and 2d) by modifying the first sentence of section 2.4:

“Coccosphere size data is reported only in a third of the experiments included in the dataset (of which more than 80% of measurements are for *E. huxleyi*), **while no cell-size measurements are included in the database.**”

- Page 6625, line 14. I added the relevant references in the text.

-Page 6225, lines 27. The morphological data for *C. braarudii* is indeed the unpublished data appearing as shaded area in a figure of Henderiks (2008). I thus changed the corresponding reference.

-Page 6227, lines 10-15. The sentence has been modified to : “These plots show that, **for coccolithophores grown in optimum conditions,**...”

- Page 6229, lines 6-7. The sentence has been changes to: “**Some** coccolithophores posses CCMs...”

- Page 6233, line 26. The sentence was modified to:

“Iron is a key component of carbon concentrating mechanisms (CCMs) that increase the rate of import of inorganic carbon (CO_2 and HCO_3^-) for photosynthesis, **and of chlorophyll**; thus,

under iron-limiting conditions, the decrease in metabolic rates is produced by carbon-limitation (Schulz et al., 2007).”

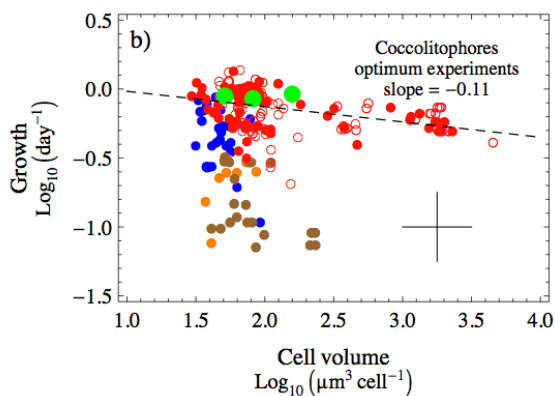
- Page 6236, lines 13-15. The reviewer is correct, conditions in the DCM are far from being optimal! I rephrased like this:

“The largest coccospheres occurred at the depth of the deep chlorophyll maximum (DCM) – where growth conditions **can be assumed to have been more favorable than in the overlying and underlying water masses** - whereas coccospheres above and below the DCM were significantly smaller.”

Page 6245, line 6. The sentence has been changed to:

“In addition cell size in *E. huxleyi* depends on environmental conditions.”

SI material – Indeed I incorrectly reported volumes and not cell diameters. I changed this in the SI material. Concerning the attribution of those three measurements to the three species in question, I had an email exchange with E. Marañón. He checked his dataset and did not find any evident erroneous data swaps. I then highlighted how the three datapoints show on a log-log plot with the growth rates (green dots in the figure below), and decided to keep the data as it is.



Note: the data from E. Marañón appears in green only in the figure above, in the manuscript it appears as red dots like the rest of the coccolithophores grown in optimal conditions.

References

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