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# ***Interactive comment on “Co-variation of metabolic rates and cell-size in coccolithophores” by G. Aloisi***

## **Anonymous Referee #2**

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### GENERAL COMMENTS

The paper by Aloisi presents an important analysis of a database of cell volume and cellular rates of growth, calcification and photosynthesis in terms of examining allometric and ecological patterns in coccolithophore physiology. The insights gained are intriguing and of great interest, as well as being very relevant to current studies on coccolithophore ecophysiology. However, there are a few issues that would improve the paper and avoid any potential misunderstandings.

Firstly, the paper contains a number of sweeping statements (e.g. coccolithophores do x) that are based on our incredibly detailed understanding of *E. huxleyi* physiology, but we lack the same level of understanding across the other 200+ species of coccolithophores with which to expand such statements across the entire group. This E.

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huxleyi bias is evident in the database used in the paper, where 82% of the measurements of cell size come from *E. huxleyi* experiments alone. This bias should be clearly stated, addressed and made very obvious in the paper's abstract, scope and conclusions - leading to a drive for future experimentalists and observationists to collect these types of data in the future in order to further examine the patterns seen here.

Another important caveat is the normalisation of the growth rates across the day-lengths used in the different experiments. Shouldn't the growth rates be normalised to the daily photon flux (i.e. the instantaneous irradiance multiplied by the day length)? Different studies use a wide range of irradiances and day lengths, and there is a strong possibility that many of the low irradiances used and short day lengths lead to under-saturated growth in terms of irradiance. All papers should report the day length and irradiance intensity so this should be an easy correction to apply? Since the early work of Paasche (e.g., 1967 – Marine plankton algae grown under light-dark cycles. 1. *Coccolithus huxleyi*, *Physiol Plantarum* 20, 946-956) there has been an awareness of the impact of day length and irradiance on growth rates.

## SPECIFIC COMMENTS

Throughout:

- *Emiliana huxleyi* is never shorted to typical shorted form of *E. huxleyi*.
- Maranon never appears with its correct accents (Marañón)

pg 6216, Ln 1 – Sweeping statement – many marine organisms are sensitive recorders of environmental change, and arguable many are easier to interpret (and potentially more sensitive).

pg 6216, Ln 1-2 – The second line of the abstract is another sweeping statement with no reference.

pg 6216, Ln 16-19 – Will it ever be possible to untangle the multiple ecological and genetic factors influencing cell size in the natural ocean? Or will it depend on where

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you set your conclusions in terms of coupled factors acting in tandem?

pg 6217, Ln 7 – Spelling of mater in ‘dead organic matter’.

pg 6217, Ln 12-15 – Sweeping statement that “coccolithophores show reduced calcification to photosynthesis ratios when CO<sub>2</sub> is changed...”. All the references used only refer to *Emiliana huxleyi* (and 1 to *G. oceanica*), whereas the effect on the majority of coccolithophore species remains unexamined. See general comment on the danger of making sweeping statements.

pg 6217, Lns 18-22: Not all of these studies examine multiple environmental conditions on (*E. huxleyi*) physiology.

pg 6218, Ln 12-13 – Consider moving “also” to after factors so it reads “... that irradiance, nutrients and temperature also play ...”.

pg 6218, Ln 8 – How is pCO<sub>2</sub> an ecological factor? Ecological factors would include susceptibility to grazing or viral lysis, pCO<sub>2</sub> influences cell physiology.

pg 6218, Ln 23-25: All of these references use *E. huxleyi* only in their experiments, not coccolithophores in general.

pg 6218, Ln 27 – Coccolith volume is indeed related to coccolith calcite content, however the number of coccoliths per cells dictates the cellular calcite content. Considerable variability in coccoliths per cell, with growth conditions, then better dictates the cellular level of calcification than examination of individual coccoliths. Also, *E. huxleyi* is notorious in producing multi-layered coccoliths both in culture and in the field.

pg 6219, Ln 17-18 – This reference (Henderiks et al. 2012) is again only based on *E. huxleyi*, not coccolithophores in general, and coccosphere size in this species is highly dependent on the number of coccolith layers.

pg 6219, Ln 21 – Coccolith size “could be” or is “likely to be” under the control of parameters other than pCO<sub>2</sub>. Consider phrasing.

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pg 6219, Ln 28-29 – First mention of metabolic rates. Metabolic rates should be defined earlier in the introduction– metabolic rates could mean many things, but herein are growth rate, photosynthetic rate and calcification rate. Also, with so little data from species other than *E. huxleyi* have you truly investigated how coccolithophore metabolic rates scale with cell-size in five species of coccolithophore?

pg 6221, Ln 6-8 – Cocosphere size carried out with counter counters, flow cytometers and optical and scanning electron microscopes (SEM) . Severe bias in this data, plus none measure the inner cell diameter.

pg 6221, Lns 11-12 – Determining growth rates via cell counts in batch cultures do not yield erroneous results IF the growth rates prior to complete nutrient depletion are examined (i.e. growth rates from changes in cell numbers during the (shortened) exponential part of the growth curve are calculated). Please rephrase.

pg 6223, Ln 24-25 – It should be highlighted in the text that, of this third of data with coccosphere data, 82 are data for *E. huxleyi* and only 1 for *G. oceanica*, 10 for *C. braarudii*, 3 for *C. leptopus* and 4 for *S. pulchra*. *E. huxleyi* therefore represents >80% of the database and there is a strong bias.

pg 6224, Ln 2-3 – full derivation for cell size is in Appendix A2, not A1. Also needs correcting on pg 6225, ln 6.

pg 6224, Ln 15 – It would be good to detail the taxa that the values from Walsby & Reynolds (1980) are from.

pg 6224, Lns 16-26 – The shield thickness / coccosphere thickness in *E. huxleyi* is going to be strongly driven by the characteristic of this species to produce multiple layers of coccoliths. The relative size of the cell volume to coccosphere volume in individual cells, and comparatively across and between populations, is therefore not likely to increase linearly relatively to each other as they are decoupled to a far greater extent in this species than in all other (single-layer) species. Cell size can remain fairly

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constant as the coccosphere volume increases as more layers of the coccosphere are constructed during growth. The SI table does not report any experimental or field measured cell diameters, only some coccosphere diameters. Henderiks et al. (2012) also only report coccosphere diameters, not cell diameters. So where do you reported the cell:coccosphere volume comparisons to really justify your choices of a fixed fCY and fSH and it is unclear where the measured culture and field cell diameters plotted in Fig. 2b and 2d come from. Or have these been calculated from the coccosphere diameters?

pg 6224, Ln 23 – Wrong appendix referenced – A2 has the details on the coccosphere diameter. Reference to the appendixes throughout the paper need to be checked, several are incorrect.

pg 6225, Ln 14 – Although there is much discussion in A2, the relevant references about Coulter Counters failing to detect the coccosphere should be listed here (e.g. Oviedo et al. 2014). Also see Franklin et al. 2010 – Dimethylsulphoniopionate (DMSP), DMSPlyase activity (DLA) and dimethylsulphide (DMS) in 10 species of coccolithophore, Mar. Ecol. Prog. Ser., 410, 13-23 – their Figure 2 clearly shows that in this case the Coulter counter identifies strongly calcified (i.e. coccosphere) and decalcified (i.e. cell) size differences in *C. leptoporus*. They also report in the text that this was similar in other species, though not shown in the paper.

pg 6225, Ln 27 – The reported data in Henderiks (2008) is based on fossil *C. pelagicus*, not modern *C. braarudii* so the size comparison is not directly appropriate. However, their Fig. 7 does plot the range of *C. braarudii* data of Henderiks, though it is unpublished. Which data is being referred to? Should probably be unpublished if it is their Fig. 7 data?

pg 6225, Ln 28-30 – Can you cite any other evidence for multiple coccolith layers in *C. braarudii*?

pg 6227, Lns 10-15 – Would be useful to point out that all these conclusions relate to

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growth under optimum conditions. Also, due to the format of equation 3 and the Log10 scale, many of your growth rates in Fig 3 are negative (which could be associated with no growth) – some note of this in the paper would avoid any potential confusion.

pg 6227, Lns 20 – Missing accents on Cermeño et al. 2006.

pg 6229, Lns 6-7 – Do all coccolithophores possess carbon concentrating mechanisms?

pg 6229, Ln 10 – Spelling of ‘from’ rather than ‘form’.

pg 6229, Ln 14-15 – The isometrical scaling of growth rates with cell size also has important (and interesting) implications for nutrient demand and cellular utilization across the size range of coccolithophores. Probably worth mentioning?

pg 6229, Ln 23 – “... Most of the data comes from cultures of *E. huxleyi* ...”. In fact 82% comes from *E. huxleyi* based studies and it would be good to make this point here (eg. by using the percentages).

pg 6232, Ln 3 – missing “to” in “seem to point”.

pg 6233, Ln 26 – Iron is also a key component in photosynthetic pigments (chlorophyll), hence the decrease in metabolic rates could also be related to energy-limitation due to ineffective light harvesting.

pg 6235, Ln 1 – The order Isochrysidales is composed of the families Gephyrocapsaceae, Isochrysidaceae and Noelaerhabdaceae, with the genera *Emiliania*, *Gephyrocapsa* and *Crenalithus* only some of the modern genera in these three families. Consider rephrasing.

pg 6236, Lns 13-15 – Why would growth in the DCM, which often represents only 1% of surface irradiance (e.g., if irradiance at sea surface was  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$  then this would be  $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) be optimal growth conditions? Optimal nutrient conditions indeed, but not irradiance. Wouldn't the low irradiance cause larger cells (see fig. 8a)?

pg 6237, Ln 19-22 – Arguably what is needed alongside field observations of coccolith

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size frequency along environmental gradients is molecular details of the population structure.

pg 6242, Ln 6 – Typo, should be 10  $\mu\text{m}^3$  not 10 mm<sup>3</sup>.

pg 6245, Ln 6 – When you say taxonomy, you mean morphotype. Would be good to make this clearer in the conclusions.

Tables

Tables 4 – Units in column “Max cell (sphere) diameter change ( $\mu\text{m}^3$ )” should be  $\mu\text{m}$  not  $\mu\text{m}^3$ .

Figures

Fig. 2. – Species names should be italicised.

Fig. 3. – Coccolithophores and phytoplankton are misspelt and the species names should be italicised. Of a minor point, the order of panels is different from last figure (e.g. going a-b-c-d in a N-pattern rather than a Z-pattern as in the last).

Fig.4. – Again coccolithophores and phytoplankton are misspelt. Also pCO<sub>2</sub> should be written as pCO<sub>2</sub> with the 2 as subscript and N-limités should read N-limited.

SI material

The database includes data from Marañón et al. 2013. There appears to be a problem with this data – *C. leptoporus* is reported as having a coccosphere diameter of 51  $\mu\text{m}$ , *E. huxleyi* 158  $\mu\text{m}$  and *G. oceanica* 82  $\mu\text{m}$ . Firstly, if you refer to the paper the values you have reported are actually coccosphere volume and not coccosphere diameter. The correct values reported in Marañón et al. 2013 should then be *C. leptoporus* 4.6  $\mu\text{m}$ , *E. huxleyi* 6.7  $\mu\text{m}$  and *G. oceanica* 5.4  $\mu\text{m}$ . Secondly, these diameters must be the wrong way around, and a mistake in the original Marañón et al. 2013 reference – cell sizes are more likely to be *E. huxleyi* as 4.6  $\mu\text{m}$ , *G. oceanica* as 5.4  $\mu\text{m}$  and *C. leptoporus* as 6.7  $\mu\text{m}$ .

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