

All referee comments are in **bold** and our answers in normal font.  
*Changes in the manuscript text are kept in italics.*

**Referee Jeremy Young**

#### **General comments**

**This is a very intriguing set of data. As the authors note, the hypothesis that coccolith calcification may have evolved as a mechanism for removing Ca<sup>2+</sup> ions from cells has been postulated a few times. It is also trivially easy to see that this is an absurd suggestion since, as the authors again note, all unicellular organisms living in seawater have this problem and the ancestor of coccolithophores would certainly have had effective mechanisms for removing Ca from the cell - as indeed must do the numerous modern haptophytes not belonging to the Calcihaptophyte clade. Moreover the fact that in culture coccolithophores often produce mutant cells which do not calcify clearly shows that calcification does not have an essential physiological role such as Ca regulation. Indeed intra-cellular calcification involves introducing vast amounts of Ca into the cell and so would appear to exacerbate rather than solve the problem of Ca toxicity. Given this background the experimental results presented here are undeniably intriguing. They clearly show that coccolithophores are more tolerant of elevated Ca concentrations than other, non-calcifying algae and it is hard to dispute the inference that this is likely to be because calcification has given coccolithophores a more sophisticated and higher capacity Ca handling system than non-coccolithophores. In this context the evidence that calcification can be stimulated in coccolithophores by elevated Ca concentrations is even more intriguing. This result applies only to under-calcifying *E. huxleyi* strains, as in regularly calcifying *E. huxleyi* cellular PIC production rates were not enhanced at elevated Ca levels. Nonetheless the clear evidence that calcification was enhanced in low calcifying strains suggests that modern Ca levels may be near the tolerance levels of *E. huxleyi*, even though it is a highly successful species. So the hypothesis that Ca levels may have played a major role in coccolithophores evolutionary success on geological timescales appears reasonable and well-worth exploring.**

**The authors present data on coccolithophore diversity as an index of evolutionary success but in parallel with these broad trends in diversity there are also trends in coccolith size, degree of calcification (e.g. reducing number of rays in discoasters) and total coccolithophore calcification all broadly paralleling the decline in diversity. A driver for these parallel trends has previously been elusive so Ca concentration is certainly intriguing and well-worth exploring.**

**Finally it has often been noted that planktonic foraminifera and coccolithophores seem to follow broadly parallel macroevolutionary trajectories, so again Ca concentrations maybe pertinent in considering the evolution of planktonic foraminifera. The work may also have some more practical applications, many coccolithophores are both hard to culture and/or prone to calcify poorly in culture. This study suggests that elevating Ca concentrations may be a profitable mechanism for encouraging calcification in cultures. I believe this will be a very stimulating and much cited paper and am happy to recommend it for publication.**

We thank J. Young for his positive evaluation and encouraging comments regarding our manuscript and the importance of seawater Ca concentrations on the evolutionary trajectory of planktonic calcifiers.

#### Notes on some specific aspects

**Title - the current title is “Phytoplankton calcification as an effective mechanism to prevent cellular calcium poisoning”, it is easy to misread this title as suggesting that calcification evolved as a functional adaptation to prevent Ca poisoning which is clearly neither logical nor the conclusion of the paper. It should be changed.**

We understand the concerns of J. Young regarding a misinterpretation of the title. However, we think that the title reflects very nicely the main message of the manuscript (if not misunderstood as the evolutionary trigger of calcification).

We will therefore make this differentiation very clear in the abstract of the revised manuscript and additionally change the title to "Phytoplankton calcification as an effective mechanism to alleviate cellular calcium poisoning" because calcifying coccolithophores were indeed negatively affected by high Ca concentrations (negative slope in Figure 3).

#### Changed part of the abstract:

*"We hypothesize that the process of calcification in coccolithophores provides an efficient mechanism to alleviate cellular calcium poisoning and thereby offered a potential key evolutionary advantage, responsible for the proliferation of coccolithophores during times of high seawater calcium concentrations. The exact function of calcification and the reason behind the highly-ornate physical structures of coccoliths remain elusive."*

**page 5 lines 10-12: The life cycle of *E. huxleyi* is characterized by three distinct different stages: (a) the coccolith carrying non-motile diploid form (C-cell), (b) the naked non-motile diploid form (N-cell) and (c) the scaly motile haploid form (S-cell).**

**Comment: This is incorrect - the life cycle has two stages haploid and diploid whilst N cells are aberrant diploid cells, not a discrete part of the life-cycle.**

We completely agree and will change the statement.

#### In the revised manuscript the above statement will be changed to:

*"Emiliana huxleyi is characterized by three distinct different cell forms: (a) the coccolith carrying non-motile diploid form (C-cell), (b) the naked non-motile diploid form (N-cell) and (c) the scaly motile haploid form (S-cell). The latter haploid form possesses organic body scales covering the cell and two flagellates that enable motion (Paasche, 2002). The life cycle of *E. huxleyi* consists of C- and S-cells whereas N-cells are mostly observed in the laboratory after extended culture periods (Paasche, 2002) or under unfavourable culture conditions (Müller et al. 2015). This study investigated only the diploid coccolith carrying (C-cell) and the naked (N-cell) cell forms of *E. huxleyi*. Our observations and the presence of N- and S-cells in laboratory cultures and natural populations (Paasche, 2002; Frada et al., 2012; Müller et al., 2015) indicate that *E. huxleyi* cells have the ability to control intracellular  $Ca^{2+}$  homeostasis at modern  $Ca^{2+}$  concentrations without the need of biomineralization."*

**page11 lines 26 to 31: On the other hand, seawater Ca<sup>2+</sup> concentrations might have been an important factor enhancing coccolithophore extinction related to past geological ocean acidification events (e.g. Paleocene-Eocene Thermal Maximum and the Cretaceous Mass Extinction Event) where the impediment of calcification in coccolithophores might have increased the potential for cellular calcium poisoning at elevated seawater Ca<sup>2+</sup> concentrations.**

**Comment: There is little evidence that the end Cretaceous mass extinction was related to ocean acidification and during the PETM there is only a slight increase in extinction rates.**

We will remove this statement in the revised version of the manuscript as it is clearly too far fetched and not supported by sufficient evidence (see also comment of T. Tyrell).

**page12 lines 1-2: Coccolith formation has presumably been reinvented throughout the evolutionary history of coccolithophores (De Vargas et al. 2007)**

**Comment: This hypothesis has very little support - molecular genetics has shown that all coccolithophores belong to a single clade, and heterococcolith calcification has highly distinctive features indicating that it only evolved once.**

We agree with J. Young and are happy to remove this statement from the manuscript.

*“... and may have provided an evolutionary advantage to coccolithophores over non-calcareous phytoplankton during the Jurassic and Cretaceous period (Fig. 1). However, secondary benefits of calcification are likely responsible for its continued operation under modern ocean Ca<sup>2+</sup> concentrations.”*