

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

## **Referee 1**

**I found this paper to be enjoyable to read and am glad to see that calcium ion concentrations across geological time have been examined as possible correlates to the evolution of calcifying phytoplankton. Figure 1 is really interesting, especially that it took 50My for the coccolithophores to catch up in terms of diversity at their peak following the peak of seawater Ca. I also found the taxon-specific differences in fitness responses to seawater Ca interesting. That the non-calcifying strains are induced to calcify at higher seawater Ca levels is fascinating.**

We thank referee 1 for his positive review and feedback regarding our manuscript.

We would like to clarify one point that might have been miss-interpreted by referee 1 and the discussion group of the National Oceanographic Centre (short comment in the discussion forum):

In our study we show that under-calcified strains of *E. huxleyi* (< 2 coccoliths per cell) are induced to produce more than 12 coccoliths per cell at elevated seawater Ca concentrations of 36 mmol L<sup>-1</sup>. We don't have any data that indicates that strains with an absent calcification mechanism are induced to calcify at elevated Ca concentrations. Our under-calcified populations consist of cells with no or single attached coccoliths. We do not know if the cells of these populations with no coccolith attached lacked the ability to calcify, lost coccoliths or just had not yet produced coccoliths. We think, however, that it is reasonable to assume that these populations were genetically the same and had the same physiological abilities.

We acknowledge that Fig. 4 probably caused this confusion showing a cell without any coccolith (Fig. 4A). We will rearrange Fig. 4 and only show a picture of under-calcified cell with one coccolith attached to avoid miss-understanding and interpretation. Additionally, we will state this issue more clearly in the revised manuscript.

We will add the following sentence in the materials and methods section:

*"The under-calcified populations (strains SO-5.25 and SO-8.04) consist of cells with no or single attached coccoliths. Cells with no coccoliths attached in these populations either lost their coccoliths, lacked the ability to produce coccoliths or did not yet produce coccoliths."*

**All of the evidence in this paper provides clues for cellular mechanisms that must be involved, that must differ among diatoms and coccolithophores, and that may require cellular energy. Namely, there must be differential regulation of calcium transporters (i.e., Ca ATPases), calcium channels (i.e., voltage-gated or otherwise gated), and/or calcium binding proteins (e.g., calreticulin, myosin) among species and within strains across seawater Ca gradients. Some of those Ca-binding and transporting proteins are known. I think that this paper points the way to cellular physiology hypotheses that should be tested to better understand the cellular regulation of calcification as it relates to seawater Ca levels. I think that the manuscript should include a much more thorough discussion of what is**

**already known about these cellular mechanisms within coccolithophores and across the other phytoplankton taxa. Where there are unknowns, potentially Ca regulation in ossifying tissues such as bone or in tightly regulated cytosolic locations such as mammalian muscle, may provide clues.**

In the revised manuscript version we will extend the discussion regarding cellular Ca regulation in phytoplankton cells, similarities and differences to land plants and mammalian cells.

We will add the following paragraph in the discussion section:

*"Marine phytoplankton presumably operate several mechanisms which contribute to cellular Ca<sup>2+</sup> regulation such as intra and extra cellular enzymatic binding capacities and/or the influx regulation via selective channels (Gadd, 2010). Over the past decade progress has been made in the discovery of cellular compartments (e.g. endoplasmic reticulum, chloroplast, mitochondria) regulating plant Ca<sup>2+</sup> homeostasis and signalling (McAinsh & Pittmann, 2009; Webb, 2008; Brownlee and Hetherington, 2011) and on differences in Ca<sup>2+</sup> channels between eukaryotes and higher plants and mammalian cells (Wheeler and Brownlee, 2008). However, many unknowns remain about phytoplankton intracellular ion regulation and the homeostasis of the major biological active cations like Ca<sup>2+</sup> and Mg<sup>2+</sup> and their interaction and possible influence on each other. For example, Ca<sup>2+</sup> has a higher ion-exchange capacity than Mg<sup>2+</sup> (Harris, 2010) and when present in high concentrations might interfere with enzymatic reactions where Mg<sup>2+</sup> acts as a cofactor (Moore et al., 1960; Legong et al., 2001). However, it remains speculative if this is a possible explanation for the observed reduction in growth rate and Fv/Fm of non-calcifying phytoplankton species (Fig. 2). "*

Additional references:

McAinsh MR, Pittman JK. (2009) Shaping the calcium signature. *New Phytologist* 181: 275–294.

Webb AAR. (2008) The chloroplast as a regulator of Ca<sup>2+</sup> signalling. *New Phytologist* 179: 568–570.

Brownlee C. and Hetherington A. (2011) Introduction to a Virtual Special Issue on calcium signalling in plants. *New Phytologist* 192: 786–789.

Wheeler GL, Brownlee C. (2008) Ca<sup>2+</sup> signalling in plants and green algae--changing channels. *Trends Plant Sci.* 13(9):506-14. doi: 0.1016/j.tplants.2008.06.004. Epub 2008 Aug 12.

Legong L., Tutone A. F., Drummond R. S. M., Gardner R. C. and Luan S. (2001) A novel family of magnesium transport genes in Arabidopsis. *Plant Cell* 13, 2761–2775

Harris, D. C. (2010) *Quantitative Chemical Analysis*, W.H. Freeman; 8th Edition

Moore D. P., Overstreet R. and Jacobson L. (1960) Uptake of magnesium and its interaction with calcium in excised barley roots. *Plant Physiol.* 36, 290–295.