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Comment

## ***Interactive comment on “Phytoplankton calcification as an effective mechanism to prevent cellular calcium poisoning” by M. N. Müller et al.***

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(Please note: These comments reflect discussions from a research group meeting; several people contributed to this comment, including: Lucie Munns, Manon Duret, Chris Daniels, Kyle Mayers, Alex Poulton, Rosie Sheward).

GENERAL COMMENTS The manuscript by Müller et al. examines the potential for high external calcium (Ca) to be inhibitory or toxic to a number of phytoplankton grown in laboratory conditions. The outcome is that calcifying organisms (i.e. coccolithophores) show little or no response to high medium Ca concentrations in terms of reduced growth, calcification rates or photosynthetic rates. An interesting side-line from the work is that a non-calcifying ‘mutant’ strain begins to calcify at extremely high Ca (relative to present day oceanic Ca concentrations). The paper shows that coccol-

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ithophores can regulate their intracellular Ca concentration to a greater degree than the other phytoplankton groups tested and avoid Ca poisoning. It should be noted that the Ca concentrations, which are inhibitory to the other phytoplankton groups examined, are in excess of current levels seen in the modern ocean.

The paper is nicely written, however the discussion leaps straight into the bigger picture, without fully discussing the results, which are very interesting in themselves, in sufficient context and depth to support the wider conclusions. The Results section is very short and could be enhanced by more emphasis being placed on the interesting trends seen in the data (e.g., growth rate and Fv/Fm).

As the authors state in the Discussion (pg 12701: In 13), it is likely that the ability of the coccolithophores to tightly control internal Ca<sup>2+</sup> transport allows them to withstand high Ca<sup>2+</sup> concentrations. This point could more clearly be defined as the outcome of the study and the reader is potentially left with the question – did the paper actually address what the title states? Indeed the calcifying types of phytoplankton assessed in this study showed no depression of growth rate at high Ca concentrations, but is it the process of calcification (i.e. combining Ca with bicarbonate, strictly regulating crystal growth and extrusion of the end result from the cell) or the strong control on Ca fluxes into the cell required to regulate the process of calcification that alleviates Ca poisoning? – Simply put, is it the whole process of coccolith formation or just the control of Ca influxes that prevents this group from being influenced by Ca toxicity? We are not sure that the paper really addresses this and an alternative title could be, for example, ‘Superior ability of coccolithophores to maintain their fitness in high Ca<sup>2+</sup> concentrations’.

Indeed the inclusion of a non-calcifying coccolithophore could indicate that the process of calcification itself is critical – however non-calcifying diploid coccolithophores are ‘mutants’ not original organisms and the mutation that occurred to prevent them calcifying could, in theory, happen anywhere on the cellular pathways to coccolith production – the fact that the non-calcifying strain did begin to calcify at high Ca after a

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prolonged period could suggest that the mutation occurred somewhere along its Ca uptake pathway (i.e. it could only take up Ca successfully and form coccoliths at 3 times the ambient Ca concentration – note that this information is only found in the figure 4 legend, not the paper). Some of this is semantics, but part of it is experimental design and interpretation and generally we remain unconvinced that phytoplankton calcification is an effective mechanism to prevent cellular calcium poisoning – although the paper does make a convincing case that it is the coccolithophores ability to tightly control internal  $\text{Ca}^{2+}$  transport that allows them to withstand high  $\text{Ca}^{2+}$  concentrations.

**SPECIFIC COMMENTS** - Methods and quantitative results from the second experiment must be shown (e.g. state how many cells coccolith counts were taken from under the SEM and show the data that the statistics are based on).

- The limitations of using *E. huxleyi* and *G. oceanica* should be discussed in the context of the geological record - i.e. both are recently evolved species ( $\sim 0.3$  and 1.9 Ma respectively), and are genetically very closely related. These species are often selected for laboratory experiments due to their tolerance to artificial conditions and are perhaps not very representative of all coccolithophores, particularly given the palaeo-ecological emphasis of the introduction and discussion.

- It should also be acknowledged that the high abundance of coccolithophore fossils from sediments of Cretaceous age additionally represents favourable conditions for  $\text{CaCO}_3$  export and preservation (e.g. extensive warm shallow shelf seas as favourable depositional environments) and not exclusively  $\text{CaCO}_3$  production or coccolithophore dominance relative to other phytoplankton groups.

- There is no conclusive evidence that naked strains form a third part of the *E. huxleyi* natural life cycle (pg. 12701: In 23 and see comments from other reviewers). Such non-calcifying strains are most widely considered to be the artefacts of mutation in culture, in which any number of 'faults' could be present in the calcium metabolism or coccolith production and exocytosis pathway. It is hard to interpret the results from a strain that

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is not well understood (e.g. It spontaneously begins calcifying? 1296: In 17) - It would be beneficial to have a clear discussion early in the paper of what calcium poisoning is and how the authors would define it. For example, why would we expect cells to suffer at high  $\text{Ca}^{2+}$ ? What do we know about how single celled organisms avoid this? This introduction to Ca poisoning could then be used to inform the conclusions drawn.

- Results from other studies (e.g. *C. braarudii* results) shouldn't be included in the results section, but be brought into the discussion section only.

- Cellular PIC and POC quotas and growth rates are meaningful independent of each other and show interesting trends in their own right, whereas PIC and POC production can be misleading as they are heavily affected by growth rate – i.e. they are the combination of growth rates (which change in this study) and cellular carbon inventories (which don't). For example, pg 12699, In 6 - Significantly lower PIC production by *E. huxleyi* at low  $\text{Ca}^{2+}$  is caused entirely by a decrease in growth rate, as cellular PIC quota remains the same at both  $\text{Ca}^{2+}$  levels.

- Fig 2: The note about different methodology for *C. clostridium* should be in the methods section instead of the figure caption.

- pg 12703, In 15: There is little evidence that the extinction events referred to are related to ocean acidification (temperature and nutrients are also strong controls on fitness for example), and furthermore, it is also highly debated in recent literature whether ocean acidification on geologically-relevant timescales (i.e. hundreds to thousands of years) actually prevents intracellular calcification in coccolithophores.

- pg 12703, In 27: Please explain further the sentence 'this let us suggest that these two species in the modern ocean don't rely on cellular  $\text{Ca}^{2+}$  detoxification by mineralization'. How does this fit with the title or that current ocean Ca concentrations are not toxic to any of the phytoplankton examined?

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