

## ***Interactive comment on “Phosphorus limitation and heat stress decrease calcification in *Emiliana huxleyi*” by Andrea C. Gerech et al.***

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The culture study by Andrea Gerech and collaborators addresses the fate of coccolithophore algae in our Anthropocene ocean. The Authors did not directly examine the effect of increased ocean carbonation and decrease oceanic pH, but rather their laboratory culture study tackles more indirect, yet important, ancillary environmental change by seeking to determine how calcification by this biological group will evolve with rise in temperature and nutrient limitation in the oceans. Their experiments are based on one single strain of *Emiliana huxleyi* originating from a fjord in Norway. The authors also compare the results obtained from batch vs semi-continuous cultures and highlight significant discrepancies between the two techniques, which is of potential interest from a more methodological perspective. The take-home message

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claimed by the team is that limiting phosphorus availability and increasing temperature (the latter being introduced as a stressor) will likely reduce carbon fixation by *Emiliana huxleyi* and diminish mineral-ballasting export of organic carbon to the deep ocean.

I am generally supportive of publication of this work in Biogeosciences. I have, however, a number of comments and questions, which I hope the Authors will find useful to prepare their revisions.

### General comments

(1) More information is needed on the cultured strain of *Emiliana huxleyi*, including (where possible) the date of isolation, the morphotype of coccoliths, whether the strain is deposited in a Culture Collection (or in the process to be), and the conditions under which the stock culture is maintained in the laboratory (temperature, light irradiance, etc), is the strain axenic?

(2) There are no details given of the culture technique *per se* apart from strategy (batch vs semi-continuous) adopted. Were the cells acclimated to the target phosphorus concs and temperature conditions when proper experiments began? This is all the more important as changing temperature is conceived a stressing factor in the study. We know that coccolithophores, and singularly *E. huxleyi* is fast adapting to changing environments so this methodological aspect is crucial for the implications of culture study to wild specimens and at timescales compatible with adaptation in the natural environment.

(3) I would be valuable to elaborate on the malformations of the coccospheres/coccoliths observed by the Authors. The rationale behind the discrete

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class of malformation features and the implications for biomineralisation are elusive in the manuscript. On this note the argument that 24 °C represents a heat stress for this strain as it was isolated from waters measured at 21 °C or lower, and that more malformations were observed (p. 8 lines 23-25) does not appear as a strong argument to me. Likewise, it is not entirely clear to me how the Authors are able to distinguish between malformation and dissolution features.

(4) I feel that at places the discussion is too descriptive and lacks a better attempt to understand the cellular mechanisms at play for the environmentally-driven change in carbon fixation. An integration of P acquisition strategy by *E. huxleyi* (a species with the ability to excrete ligands to increase P supply to the cell) with growth dynamics and organic and inorganic carbon fixation for each condition would be extremely useful and add value to the paper.

### Specific comments

- Page 3 Lines 6-7: In my opinion, looking at Table 3 it is not nutrient limitation that limits further algal growth in thus set-up, but rather the drift in the carbonate chemistry of the medium (see e.g. Hermoso 2014 in *Cryptogamie*, *Algologie* 35(4):323-351). More broadly, I do not believe that the stationary phase represents an end-of-bloom scenario.

- Page 3 Line 19: inter alia?

- Page 3 Line 22: Carbon "fixation" rather than "production"

- Page 3 Lines 22-24: There a many other references (Bollmann et al. 2010 in *Protist*

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161:78–90; McClelland et al. 2016 in SciReport 6:34263 *etc etc* of which some cited at the end of the discussion should be also mentioned here).

- Page 3 Line 30: I still think that "heat stress" is not appropriate here for the reasons outlined in general comments. The effect of changing temperature from 19 to 24 °C on growth rate is not very detrimental (by 10 percent) compared to the effect of other manipulations of the culture medium in literature. *E. huxleyi* has a broad tolerance and adaptability to temperature change compared other taxa, such as *C. pelagicus*.
- Page 6 Line 26-27: I disagree with this statement. Also Table 3 should be given the starting conditions.
- Page 7 Line 1-2: How about the number of layers of coccoliths forming the spheres? This could be useful to put in the context of the dynamics of cell division.
- Page 7 Lines 35-36: The Authors should add a discussion on the mechanisms for this observation. There are a few studies on cells being stuck in the haploid phase due to the lack of N and P provision to replicate DNA and allow further division.
- Page 8 Lines 8-9: Please refer to recent study on this particular point by Aloisi in Bio-geosciences 15: 4665-4692, and incorporate suitable discussion on the mechanisms.
- Page 9 Lines 6-7: I do not follow the argument being made here. Please clarify.
- Page 10 Lines 1-2: I recommend that the Authors tone this down, as we know that such a conclusion at the scale of the global biogeochemical cycle requires longer-term

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and multi-strain investigation although I appreciate the "may" being used here.

Sincerely,

Michael Hermoso

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