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Biogeosciences Discussions

Interactive comment on "Phosphorus limitation and heat stress decrease calcification in *Emiliania huxleyi*" *by* Andrea C. Gerecht et al.

Anonymous Referee #4

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General Comments:

Gerecht and co-authors present an interesting study that investigates a two-stressor 'future ocean' scenario of phosphorus limitation combined with beyond thermal optimum temperatures. Concurrently manipulating two primary controls on physiology is a much need step beyond understanding how variability in single conditions affect phytoplankton and is of suitable scope for publication in Biogeosciences. However, I feel that the discussion is weakened by an emphasis on comparing two culturing methods rather than comparing individual vs. interactive effects of the stressors in question. The specific choice of experimental conditions is also poorly justified and not placed into context. I feel that once the points raised by myself and the other reviewers are addressed, this manuscript will be suitable for publication in Biogeosciences.

Specific Comments:

1. In the broader interpretations of their calcification results, the authors state in the Abstract, Introduction and Conclusions that decreases in calcification rates in E. huxleyi could "lessen the ballasting effect of coccoliths and weaken carbon export out of the photic zone", or similar wording. In support, they reference Ziveri et al. (2007), who conclude that, despite the high abundance of Emiliania relative to other coccolithophore species, the small size and very low species-specific carbonate mass of their coccoliths means that they consequently export far less carbonate than expected. Baumann et al. (2004) similarly concluded that Emiliania plays only a relatively minor role in carbonate export in the Equatorial and South Atlantic. Can the authors support their statements of alterations to the carbon cycle more quantitatively using their PIC production values and abundances of Emiliania in the field? Or provide references of studies that better support these statements?

2. Considerable emphasis is made on the short-comings of the batch culturing technique compared to the semi-continuous culture technique. Comparison of these two methods is present throughout the results and discussion, and, in my opinion, obscures a clear and explicit evaluation of contrasting individual (warming only, P-limitation only) vs. interactive (warming and P-limitation) effects and evidence (or not) for positive interactions. It is stated that batch culture experiments can only represent a severely nutrient depleted scenario whereas a semi-continuous set-up provides an acclimated low-nutrient population (p3, ln 7; p7 lns 12-17). I think that these statements are somewhat misleading for the following reason: A batch culture experiment experiences exponential growth at whatever the starting nutrient concentrations until these nutrients become sufficiently depleted that exponential rates of growth can no longer be maintained and growth rate rapidly falls to zero. A semi-continuous culture is just a batch culture that is subcultured/diluted (typically) around mid-exponential-phase cell concentrations several times. I therefore find it strange that the authors did not just sample their P-limited batch culture experiment at mid-exponential phase (as they did with the

control experiments) well before the 'severe' nutrient depletion of stationary phase began, which based on Fig. 4 would have meant sampling on day 3 or 4. Would this not have been a more realistic comparison of control and P-limited conditions during exponential phase in all experiments? It would also allow a comparison of multiple generations of growth (semi-continuous) with far fewer generations of growth (batch to mid-exponential phase). It would also have removed the issues with calculating production rates, as only exponential growth phase populations would have been sampled.

3. I would like to see a broader context of the area where a similar degree of temperature and phosphorus stress is predicted to be experienced in the context of this strain isolated from a Norwegian fjord. Similarly, there is no discussion of the fact that physiological stress experience by one strain of this species under climate change is as likely to lead to its ecological replacement by another, more tolerant strain given recent studies presenting the large genetic pool of Emiliania (e.g., Read et al., 2013, which also discusses differences in genes for tolerance of low phosphorus conditions between strains).

4. I was surprised to see that no figures of any POC, PIC, or POP data were presented, only data in the tables. Was there a reason for this? Given the two experimental approaches, two temperatures and two nutrient states, it made it difficult to quickly visualise the dataset.

5. The strain used seems to be a new isolate – do the authors intend to deposit this strain into a culture collection for use by other researchers? Given it is not held in a culture collection, the authors must provide the essential ancillary information on the isolate and its maintenance in culture.

6. It is stated deep into the discussion that the temperature at the isolation location does not exceed 21 degrees and this is presumably how the authors decided that a temperature of 24 degrees was beyond the thermal optimum. Did the authors perform a systematic temperature optimum assessment by determining growth rates at a range

of temperatures? Given that the exponential growth rates were not substantially different between temperature treatments (semi-continuous) and in fact were higher in the 24 degree treatment for the batch culture approach, this would perhaps suggest that (using exponential growth rate as a physiological indicator) this isolate has a relatively broad thermal tolerance.

7. The authors do not state whether there was any period of acclimation for populations experiencing low phosphate or high temperature treatments.

8. How did the authors account for the tendency of Emiliania to form multi-layer coccospheres when counting the number of coccoliths from SEM images? Comparing the coccosphere size from CASY with the cell size from light microscopy (back calculated from the volume data) and considering the thickness of Emiliania coccoliths, would suggest that coccospheres were not mono-layer.

9. Is there any reason why the authors refer to both light microscope and CASY cell size measurements as "cell size" when CASY gives coccosphere size measurements? There are huge differences in volumes between the two methods due to the coccosphere and whilst cell size directly relates to cell carbon, coccosphere size does not and therefore this unnecessarily confuses the reading in parts.

10. The discussion would benefit from a discussion of the physiological mechanisms behind the observed response to P-limitation and heat stress singularly and combined, and there is considerable literature on this species, other coccolithophore species and other phytoplankton groups that would support such a discussion.

p6, ln 37-39 – referred to changes in size but "twice as large in stationary phase" refers to cell volume, so this should be changed to reflect this.

p8, ln 7-9 – The reference to Sheward et al. (2016) on line 7 should be changed to Gibbs et al. (2013) as the latter presented Emiliania data. This sentence could also include C. braarudii, Calcidiscus and Helicosphaera from Sheward et al. (2017). The

Sheward et al. (2016) at the end of line 9 should be changed to 2017 (I think you have referred to the discussion paper rather than the finally-published article).

Technical Corrections:

Throughout the paper, there are inconsistencies with the author order in your references. Sometimes they are ordered by date, other times alphabetically, and several times I can find no logic to the order! (e.g., p2, ln 37-38).

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