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

**Anonymous Referee #3**

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Review of Gerecht et al. for Biogeosciences (2017)

In general the article is a worthwhile contribution. They find that P-limitation can actually decrease the PIC quota, PIC production, and PIC/POC ratios in E. huxleyi, which is opposite that which has been most commonly reported in earlier literature, although some more recent studies are cited to report similar results. This contrast is little discussed. Neither the P-stress nor the heat stress used is very clearly justified. Where are such changes predicted to occur? Why is P-stress chosen instead of N-stress, when much more of the world's ocean is thought to show N-limitation of primary production? It is especially not clear to me what natural conditions are mimicked in the P-limited batch cultures. Do E. huxleyi blooms naturally experience these chemical conditions (e.g., such low DIC and omega-calcite values)? If these are conditions that



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only arise in batch cultures at very high cell densities only reachable in lab monocultures, perhaps they must be more careful of extrapolating their results from stationary phase cultures to changes in carbon export.

In terms of heat stress, it's not made very clear why the temperatures of 19ËŽ and 24ËŽ were chosen, although there is some justification given in the Discussion. Is 19ËŽ a typical SST in the North Sea (assuming the clone here represents a North Sea population) or typical of the Oslo Fjord? With global warming is it expected to reach 24ËŽ regularly? What about E. huxleyi populations currently found in 8-12ËŽ waters, would the same tendencies occur if grown at from 13ËŽ to 19ËŽ?

It's not necessary for all studies to try to replicate specific environmental conditions (often impossible), perhaps especially when the goal is to understand physiological limits or to take a first approximation. However, considering this lack of grounding of experimental design within an explicit environmental context, it appears that the Conclusions should be more cautious in extrapolating to biogeochemical effects.

There is a focus on biogeochemical effects, but nothing on ecological effects of the documented changes in PIC and coccoliths. What function do they serve? I might suggest the review by Monteiro et al. to look at, and think of some of the consequences.

Finally, I'm not so sure of the extent of the novelty of this study. They say "To our knowledge, this study is the first to specifically test the impact of heat stress. . .", but then there actually are a few quite relevant studies (it depends on how "heat stress" is defined), some of which they cite. For that reason, a more rigorous study design in an explicit environmental context would have been much stronger.

Considering the careful criticism, I think it will be worth accepting the paper once the issues I have identified have been addressed. I do not foresee that the authors will have trouble resolving these issues.

I also have several issues, discussed point-by-point below, about the details (or the de-





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tails they provided) about experimental design, analyses, and some of their introduction or discussion of the relevant literature. These must be resolved.

p. 1 Line 26, should probably cite something more recent as well, such as the meta-analysis by Meyer and Riebesell 2015.

I don't understand the justification of P-stress, as opposed to perhaps N-stress, considering that much more of the ocean is thought to be N-limited than P-limited.

p. 2 Lines 6-9 : "Batch culture on the other hand represents an end-of-bloom scenario in which the lack of nutrients limits further cell division... production cannot be determined in the batch approach ". That's only true if the last part of a batch culture is analyzed, as growth becomes limited due to exhaustion of nutrients, build-up of metabolites, shading, limited gas exchange, etc. In fact, there are many published experiments where production rates were determined in dilute batch culture, in the early exponential phase of growth before DIC consumption or nutrient consumption was substantial. Line 29: "None of these studies, however, tested the effect of above-optimum temperature ". I don't understand this unless one defines what is "above-optimum temperature". In the study presented here the temperatures used are 19ËŽ C and 24ËŽ C. The study of Feng et al. (2008) (which they cite) used 20ËŽ C and 24ËŽ C, so if 24ËŽ C is an above-optimum temperature in the present study, why wasn't it in Feng et al.? Rosas-Navarro et al. 2016), which they also cite, used 25ËŽ as the highest temperature. How is "above-optimum temperature" defined, and wouldn't it possibly depend on the origin of the cultures? For example, E. huxleyi from lat. 50ËŽ in the North Atlantic probably does not experience such temperature, E. huxleyi in the Mediterranean Sea will rarely experience temperatures that high, and those may be unexceptional surface temperatures for the tropical Pacific, where E. huxleyi can also be found. This is discussed much later on p. 8, lines 20-26, but I am not so convinced how relevant this temperature range is. Lines 36-37: Should consider (and cite) also work of van Bleiswijk et al. 1994 and Rokitta et al. 2016 very relevant for theme of E. huxleyi response to P-limitation. p. 3 I have a problem with the use of K medium for nutrient





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experiments. K medium contains a mix of ammonium and nitrate as N-source, and it contains glycerophosphate as a P source. It's not clear from Gerecht et al. (2014) if they modified these components. They need to give the basal medium composition they used. What volume were cultures? p.4 For semi-continuous cultures, what was the dilution rate or growth rate? How was it determined or confirmed that the cultures in fact were limited by P-limitation in the semi-continuous cultures? How could maximum cell concentrations have reached 170000 cells/ml if cultures were diluted back to 10000 cells/ml every second day? To have reached 170000 cells/ml from 10000 cells/ml in only 2 days would require aprox. 4 cell divisions per day, which has never been reported for this species. Is this due to experimental or measurement errors? Line 7: "P-limited cultures were harvested in stationary phase, . . ." for how long in stationary phase? This is not clear from Fig. 4. p. 5 Line 10: Give manufacturer & city for "CASY" Line 30: "Average values were compared by a t-test". Was this pairwise test performed after the two-way ANOVA? If so, with what correction for multiple comparison? They are testing two factors (T and P-limitation) so should be doing a two-way ANOVA, not t-tests. p. 6 Line 24 and Table 3: What limited the growth of control batch cultures?

p. 8 Lines 10-12: "These large "ready-to-divide" cells (Gibbs et al., 2013) not only accumulate POC, but also accumulate PIC, leading to the 2-3-fold increase in coccolith number per cell observed in stationary phase cultures (Fig. 2c,d)." How do you know these cells are "ready-to-divide"? If they really are "ready-to-divide", do you mean they are blocked in G2 or M phase of the cell cycle? That doesn't make much sense.

Lines 10-12: This is an important justification for their selection of temperatures. Nevertheless, I'm not very convinced about how these temperatures aare reoe. I would prefer them to explicitly give the range of temperatures experienced in the North Sea as well as the fjord. Why is 19ËŽ C a "normal temperature"? What does that mean?

Lines 12-13: "Stationary phase can be likened to an end-of-bloom scenario in nature, during which E. huxleyi sheds numerous coccoliths, leading to the characteristic milky color of coccolithophore blooms". Maybe, but it's also well know that the end of E.





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huxleyi blooms involves infection by the virus EHV.

p. 9 Line 10 “The percentage of partially dissolved coccoliths was higher at normal temperature than under heat” Where is this shown? Data is presented on “incomplete”, “malformed”, and “normal” coccoliths in Fig. 3. They state “The high numbers of incomplete coccoliths observed in P-limited batch cultures were likely a result of secondary dissolution (Fig. 1d; Langer et al., 2007) due to the low calcite saturation state reached in stationary phase cultures.” I would like to see more examples of incomplete coccoliths. Perhaps they can show that the type of incompleteness that appears in P-limited batch cultures (when omega-calcite is less than 1) is distinct from what appears when omega-calcite is greater than one?

Overall responses: 1. Does the paper address relevant scientific questions within the scope of BG? Yes. 2. Does the paper present novel concepts, ideas, tools, or data? Some novelty. 3. Are substantial conclusions reached? Somewhat. 4. Are the scientific methods and assumptions valid and clearly outlined? I have outlined some places where further details or considerations are needed. 5. Are the results sufficient to support the interpretations and conclusions? Mostly. 6. Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)? I have detailed several points that need to be corrected, but I anticipate the authors will have no problem responding. 7. Do the authors give proper credit to related work and clearly indicate their own new/original contribution? Mostly. I indicate a few places where they might consider or cite other works. 8. Does the title clearly reflect the contents of the paper? Yes. 9. Does the abstract provide a concise and complete summary? Yes. 10. Is the overall presentation well structured and clear? Yes. 11. Is the language fluent and precise? Yes. 12. Are mathematical formulae, symbols, abbreviations, and units correctly defined and used? Yes. 13. Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated? I mention several points that need clarification, but I do not specify exactly how they can do this. They could, for instance, use more images of





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coccoliths in different states to show how they have classified. 14. Are the number and quality of references appropriate? Yes, but I suggest a couple more. 15. Is the amount and quality of supplementary material appropriate?

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