Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2019-179-AC2, 2019

Biogeosciences
Discussions

Interactive comment on "How will the key marine calcifier *Emiliania huxleyi* respond to a warmer and more thermally variable ocean?" *by* Xinwei Wang et al.

Xinwei Wang et al.

dahutch@usc.edu

Received and published: 15 September 2019

Response to Interactive comment on "How will the key marine calcifier Emiliania huxleyi respond to a warmer and more thermally variable ocean?" by Anonymous Referee #1

Review on: 'How will the key marine calcifier Emiliania huxleyi respond to a warmer and more thermally variable ocean?' by Wang et al. The experiments are well designed and I have only a couple of smaller questions (see specific comments). The manuscript is well written. Overall, I found the discussion not extremely inspiring because I thought it missed a conceptual framework that helps to arrange the numerous datasets. Nevertheless, some of the key conclusions are interesting and the data is

valuable. I therefore only have 'minor comments' One major issue, however, is that the authors should deposit their data in a publicly accessible data repository and provide the link within the paper. This is important.

Response: The authors would like to thank the anonymous Reviewers for their constructive comments and suggestions to improve the quality of the paper. Those comments are all valuable and very helpful for revising and improving our paper. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper (Please click the supplement to download the revised manuscript). The main corrections in the paper and the responds to the reviewer's comments are as flowing:

Response to Reviewer #1 (highlights): Thank you very much for your helpful comments. Our data from this paper have been submitted to the Biological and Chemical Oceanography Data Management Office (BCO-DMO, bco-dmo.org), as is required by the conditions of our major funding agency (US NSF). The data are currently in the queue to be uploaded, but the data management office is running behind and we have been told that it will be several months more before the data can be quality checked, vetted and formatted, and posted to be made publicly available. When this is finished, the data will be available at our project webpage: www.bco-dmo.org/project/668547. We can provide this link with the paper if the editor agrees, but it will still take some time before the data from this paper are live.

Response to Reviewer #1 (Specific comments):

Line 132: How was light measured and kept identical between treatments? Measuring light in such blocks is challenging and there may be large differences between replicates and treatments. Please provide a detailed description.

Response: We agree that getting the lighting uniform for every replicate within a thermal block is essential but can be difficult, and we went to considerable effort to carefully measure and adjust light levels in each position in the block to be as close to identical

as possible. We followed your suggestion, and now provide a detailed section in the Methods on how we measured and adjusted the light intensity in the thermal-blocks. (Line 133-135)

Line 135: Was the dilution medium also Aquil? Please clarify.

Response: Yes, the Aquil medium was used as the dilution medium, and have now we clarified this in the manuscript. (Line 141)

Line 139: It is unclear to me from this description how negative growth was measured. Wasn't it just the reduction in cell numbers or in your case red fluorescence? Please explain this better.

Response: Yes, the negative growth rate was calculated from the decrease of cell numbers at 28.6 oC during cultivation. In our preliminary experiments, we repeated this process several times to rigorously verify that cultures were unable to grow at this temperature. We have revised and expanded the description of how negative growth rates were measured in our manuscript. (Line 146-150)

Line 146: Please indicate how long it took for the temperature block to reach the new temperature after switching the water bath temperature. Is there a significant time lag? I wonder if this could partially explain the lower response in the one day cycle, as the time lag may have promoted a weaker response.

Response: This is an important point. It took the block about half an hour to re-adjust to the transformed temperature for each growth phase, which shouldn't represent a significant time lag relative to the 24-48 h thermal cycles. The reason for the lower response to the one-day cycle is likely the acclimation characteristics of the coccolithophorid. We have revised and clarified the description of the thermal cycles and their re-adjustment times during transitions in the manuscript. (Line 158-163)

Line 154: Weren't the nutrients already in the dilution medium? Or did you adjust to 100 and 10 _mol/L? This is confusing. Please clarify.

Response: Thanks for pointing this out, we agree this text was unclear and confusing. We did adjust the N and P midway through the 4 day cycle (2 day variation treatment) by adding concentrated Aquil stocks at these concentrations to make sure nutrients remained replete throughout the 4 day cycle. We have revised this text in the manuscript to better describe this. (Line 168-172)

Line 167: It remains unclear if you always measured both fluorescence and cell number or if this varied between treatments? Please clarify and ideally give the reader an idea how similar the growth rates were when determined with these two measurements.

Response: Following your suggestions, we have revised in the manuscript to clarify this. (Line 185-187) Under constant conditions such as in the thermal block and the constant controls of the variation treatment, the cell numbers and the in vivo fluorescence are strongly correlated and relatively invariant (as verified by microscopic counts). So, we used the in vivo fluorescence to calculate the growth rate. However, the cellular in vivo fluorescence (cellular Chl a content) changed during temperature fluctuation, so for these treatments we applied cell counts only to calculate the growth rate.

Line 180: Please provide percentage of the HCl acid. Was it 37%? In this case fuming overnight is fairly extreme and may perhaps breakdown POC?

Response: We revised in the manuscript to provide this information (Line 201) In our experiment, we used the $\sim$37% saturated HCl for fuming overnight to thoroughly remove the inorganic carbon. We are not aware of any published evidence that $\sim$12h of HCl fuming can degrade organic carbon, but we can consider this possibility if the reviewer knows of any. From our results shown in Fig. 2C, the PIC/POC ratio was extremely low ($\sim$0.05), meaning that the POC content was nearly as high as the TPC (PIC+POC) content. This result suggests that the cellular POC is very likely not degraded by our saturated HCl fuming method.

Line 185: Not 100% sure but I assume Fu et al., 2007 did not invent this protocol.

Please provide original papers here and also for POC, PON above.

Response: We gave our own references for these methods because in our lab over the years we have made minor modifications to these classic protocols, and this allows readers to look up the exact procedures we used if desired. However, in response to this suggestion we have revised the manuscript by adding the original citations as well for all of these methods (Line 203, 205-207)

Line 188: See previous comment.

Response: As noted above, we have now added citations to the original protocols preceding the citations of our slightly modified versions of the techniques. (Line 210-211)

Line 217: The description of the applied statistical tests needs a better description. Perhaps briefly go through the consecutive steps. Just for completeness. Only mentioning which tests were done may raise some eye brows.

Response: We followed this suggestion and have revised in the manuscript to include a better and more in-depth description of the statistical methods. (Line 240-245)

Line 227: What is the rationale behind showing the TPC/PON ratio? What meaning does it have and why is it important? I would intuitively say that this dataset could be removed from the results but I am of course interested what the authors think.

Response: We understand that many coccolithophore studies don't present the TPC/PON ratio, but we feel it is worth presenting as it encompasses all of the C fixed (into both POC and PIC) relative to all of the cellular N quota. We also of course present the more traditional POC:PON and PIC:POC ratios as well.

Line 266: This may indicate a time lag until the high temperature was established so that the warm period was shorter than indicated by assuming an instant change in temperature. Please provide a retention time for how long it lasted until the new temperature was reached within the bottles.

Response: The time for the thermal block to re-equilibrate the experimental bottles after temperatures were switched was only half an hour, which we suggest is too short to significantly affect the overall growth rates in either the one day or two day thermal variation treatments. We have revised in the manuscript with new text to point this out. (Line 294-296)

Line 267: This comment basically addresses all quota measurements and ratios. When you look at e.g. PIC/POC and do this for a one day period in the cycled experiments. To what extent is the response you measure and report here 'diluted' by the PIC/POC that manifested during the previous temperature that prevailed before? Is there a carry-over to the next day that needs to be accounted for?

Response: We have considered this phenomenon during our experiment, so during dilutions we replaced a large proportion of the culture with fresh medium (up to 80-90%) to avoid significant carry-over from the old growth phase. The ideal condition of course would be to switch from cool phase to warm phase and then cycle without dilution. However, this is impossible as dilution with fresh medium is necessary to avoid nutrient limitation setting in and confounding our results. In addition, volume removed for sampling needs to be replaced with fresh medium in our relatively small volume experimental thermal block setup.

Line 380: The abbreviation TPCs is not ideal because it can be confused with total particulate carbon. I would suggest to use no abbreviation here.

Response: We followed this suggestion and have revised the manuscript to avoid using the abbreviation TPC here, as indeed we had already used to stand for total particulate carbon. Instead, here we now write out the words 'temperature performance curve' (Line 409-413, 417, 441, 653, 1004-1006).

Line 406: A particularly comprehensive assessment was done by Zhang et al., 2014 from the Reusch group. This should definitely be considered here.

Response: We followed this suggestion and have revised in the manuscript to include the Zhang et al. reference. (Line: 437)

Line 409: The Zhang et al., paper seems an overlooked but important paper here.

Response: As noted above, we have revised in the manuscript to include consideration of the Zhang et al. 2014 study. (Line: 440)

Line 417: Schlueter et al., 2014 (also Reusch group) have shown that Ehux can quickly adapt to warming. Should be mentioned here, perhaps.

Response: We talk about rapid adaptation to warming in the following section, and we have already cited the Schlueter et al. 2014 study in this context. (Line: 668)

Line 476: I don't understand how this trend can suggest these things. Isn't the damage of biochemical mechanisms simply your interpretation of what may have happened. Should be rephrased.

Response: We agree that we should be more specific and support our suggestion with evidence from the literature. Accordingly, we have revised in the manuscript to point out that energetic and material investments in cellular repair machinery such as heat shock proteins are needed to deal with stressfully high temperatures, and supported this statement with a new reference (O'Donnell et al. 2018). (Line: 511-515)

Line 607: 'ectothermic' refers to animals or also plants/microbes? Please specify.

Response: We have now stated that we are specifically referring to ectothermic animals at this point in the manuscript. We agree that even though plants and microbes can't control their body temperature either, the term ectotherm is usually reserved for animals. (Line: 645)

Fig. 2 shows that the plasticity in PIC/POC is much larger than in the other ratios in this figure. I find this very interesting. Maybe it would be worth discussing this issue.

Response: This is an insightful comment, and so we have added new text to the Dis-

cussion to point out the large plasticity in PIC:POC ratios with temperature changes, and to discuss this observation in terms of a prior study by Krumhardt et al. (2017), as well as pointing out potential implications for ballasting of sinking particles. (Line 478-484)

Fig 6B: y-axis incomplete.

Response: The Y-axis scale in Fig 6 has now been extended to 1.0 in order to encompass all of the data points, thanks for pointing this out.

I hope my suggestions help the authors to improve their manuscript.

We do appreciate the constructive comments of the reviewer, and they have indeed improved the paper.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2019-179/bg-2019-179-AC2-supplement.pdf

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2019-179, 2019.