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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.

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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. Anonymous Referee #2 Received and published: 27 August 2019

Temperature is an important driver regulating phytoplankton physiology. Previous laboratory and field investigations suggest that the trend of global warming may strongly affect future phytoplankton communities and the consequent marine biogeochemistry. Most previous studies of warming effects on phytoplankton were mainly conducted under relatively constant temperature regimes. However, under future climate change

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scenario, in addition to warming (i.e. increasing mean temperature), the magnitude of temperature fluctuation will also be changed. The response pattern of marine phytoplankton to thermal variations/fluctuations is still largely unknown. The present study investigated the physiological response of a well-studied marine coccolithophore species Emiliania huxleyi to not only a broad range of temperature regime, but also two different frequencies (one-day and two-day) of thermal variation. The examined physiological parameters include growth, photosynthetic and calcification rates, and elemental compositions. The results suggest that higher thermal variation frequency (one-day) was less inhibitory on E. huxleyi physiological processes than two-day variations especially under high temperature, indicating that the frequency of temperature fluctuation may be of importance in regulating the impacts of extreme high temperature events on key phytoplankton groups. The conclusions are valuable and help to predict the relevant marine biogeochemistry under a more realistic condition of a complex and changing marine environment. In general, the manuscript is well written and organized; the results are also well explored and discussed. I would suggest the manuscript to be accepted with minor revisions. My detailed comments and suggestions are listed below.

Response: We appreciate the reviewer's thoughtful comments and enthusiasm for our study, and have described our revisions and responses to their helpful comments below.

Line 140: How often were these cultures diluted? Does this mean that steady-state growth was not observed for 28.6_C treatment?

Response: The cultures were diluted every two days the for constant and one-day variation treatments, and every four days for two-day variation treatments. (Methods, Line 173-175). The reviewer is correct, since a negative growth rate was calculated from the decrease of cell numbers at 28.6 oC during cultivation, the coccolithophore was unable to survive at this temperature, and growth was not at steady state- this treatment could not be diluted due to the declining biomass, and thus represents a

batch culture rather than a semi-continuous one. To be certain that 28.6 oC exceeded the upper thermal limit, we repeated the experiment at this temperature several times. We have discussed this with new text on Line 146- 150

Lines 144-148: For the different fluctuation cycles (one-day and two-day), how was the temperature adjusted? Was temperature changed gradually during a one-day or two-day period or the cultures experienced abrupt temperature changes? Was there any lag phase for temperature changes? It would be better to provide the details of temperature fluctuation patterns in different treatments in order to better explain the observed different effects of fluctuation frequencies on Emiliania huxleyi physiology.

Response: The temperature setting of the thermal block setup was switched over fully (not gradually) at each transition between fluctuation cycles, but took about $\frac{1}{2}$ hour to equilibrate to the new temperature after being changed, thus allowing some time for the cells to acclimate to the temperature shift. We did not observe any significant growth rate lag following the thermal shifts, just a rapid transition to a new growth rate. We have provided a detailed description in the manuscript. (Line 158-163)

Lines 152-155: What was the nutrient condition in the culture medium used for dilution? What do you mean by “100 _mol L-1 nitrate and 10 _mol L-1 phosphate was added every two days”? Please clarify.

Response: We adjusted the N and P midway through the 4 day cycle (2 day variation treatment) by adding concentrated Aquil stocks at these final concentrations to make sure nutrients were replete. We have revised this text in the manuscript to better describe this. (Line 168-173)

Line 170: Please delete “GFC”

Response: We have revised in the manuscript as suggested. (Line 189)

Lines 174-176: “Total Particulate Carbon” and “Particulate Organic Carbon/Nitrogen” should all be lowercased.

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Response: We have made this change. (Line 194-196)

Line 206: I found the abbreviation of “TPC” a bit confusing here, since it refers to “total particulate carbon” in the earlier text.

Response: The abbreviation TPC for ‘thermal performance curve’ has been removed here, since the reviewer is correct, it was used earlier in the paper for ‘total particulate carbon’. Thermal performance curve is now written out. (Line 409-413, 417, 441, 653, 1004-1006).

Line 209: misspelling of Emiliania huxleyi

Response: We revised this mis-spelling. (Line 223)

Line 210: Please specify how the equation was modified.

Response: Our approach used for predicting thermal response curves under variable thermal conditions (as opposed to the constant temperatures used in the classic Eppley study) was first published by Bernhardt et al. (2018). It is a non-linear averaging model that incorporates the principle of Jensen’s inequality, and so is based on Eppley’s equation but with these modifications to deal with fluctuating temperatures. It has been applied in published thermal variation studies by Qu et al (2019) and Kling et al. (in press), both cited here. The full derivation of this thermal variation model is too lengthy to give here, but can be obtained by interested readers from the Bernhardt paper. We changed the original confusing wording to more accurately describe this model on Line 252-255.

Line 251: Please rephrase the text to “The growth rates during the cool phase of the one-day variation cycle were lower than those. . .”

Response: We followed this suggestion and have revised the manuscript. (Line 278)

Line 419: should be revised to “ can be influenced. . .”.

Response: We revised the manuscript as suggested. (Line 450)

Line 596 - : In this section, it might be worth to also expand the discussion on how thermal variation would affect the competition advantage of coccolithophores over other phytoplankton functional groups (such as diatoms) in the community level.

Response: Thank you for this good suggestion. We have revised the discussion text accordingly. (Line 656-661)

Fig. 1. The growth rates presented in the figure were supposed to be measured during steady growth phase. However, according the context, the cultures were not able to survive at 28.6_C. I assume the negative growth rate was calculated based on the decreased in-vivo fluorescence values over the consecutive sampling days. I'd suggest using the value 0 instead of negative value for fitting at this data point.

Response: As noted above, the negative growth rate was calculated from the decrease of cell numbers at 28.6 oC during cultivation during a batch culture, an experiment which we repeated several times to robustly verify this result. The magnitude of the negative growth rate here is an expression of the degree of stress the culture experienced at this temperature, and may be useful to some readers for comparison with the other positive growth rate values in the variation experiments. We appreciate the comment, but with the editor's permission would like to keep the negative value here.

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

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