

We greatly appreciate the valuable comments and critical reading of the manuscript made by the two anonymous reviewers, which were useful in improving the scientific quality of the manuscript. Please find below our answers to the Reviewers' comments. For clarity, the lines mentioned in the rebuttal referred to the reviewed version of the manuscript.

Kind regards,

Giulia Faucher and co-authors

General comments

Overall quality of the preprint

*The manuscript by Faucher et al. is an interesting and well written study on how the globally important pelagic calcifying algae, *Emiliana huxleyi*, will respond to OAE with NaOH in lab experiments. This is an important study to understand the physiological response of individual and critical taxa to increased alkalinity. Many studies have examined the response of *E. huxleyi* to ocean acidification, so it is a great model species to study the response to alkalinity enhancement also.*

*One of the major findings of all the ocean acidification studies on *E. huxleyi* has been a strain specific response to acidification (e.g., Langer et al., 2009 Strain-specific responses of *Emiliana huxleyi* to changing seawater carbonate chemistry, *Biogeosciences*, 6, 2637–2646). Could it be possible that similar findings could be observed for OAE? This study is only using 1 strain of *E. huxleyi*, so perhaps using a number of other strains might provide different responses? I think it would be good if the authors can make a comment about this, and also in the discussion of the manuscript.*

In the last part of the discussion, the possible species-specific and even strain-specific responses towards OAE for coccolithophore algae were already mentioned. It also emphasised the urgency for new physiological data on the perturbation induced by OAE. Following the reviewer's suggestion, a few more references were added for clarity (line 251).

Specific comments

Line 45: do they mean ocean alkalinity or acidification studies?

If the reviewer refers to the paper by Bednaršek et al. (2024), they mean ocean acidification studies, as mentioned in the text

Line 87: State this is a calcifying strain of *E. huxleyi* and where the strain was obtained from (i.e. culture collection)

This information has been added to the text.

Line 90: "*Monospecific cultures of *Emiliana huxleyi* (B92/11; Plymouth Marine Laboratory)*".

Line 106-107: was there a change in growth rate during the acclimation period? Especially given that growth rate showed a decrease with TA increase. Was this a gradual decline over the acclimation time or an immediate reduction?

Yes, the growth rate differed already during the acclimation phase at different alkalinity levels. The cells were acclimated in all treatments for 7-9 generations to the experimental conditions. Due to varying growth rates, the acclimation period ranged from a few days to 10-12 days. This information is available in the text (Line 102).

Unfortunately, we cannot reply to the second question posed by the reviewer. Although the cell concentrations were regularly measured during the acclimation phase, we did not record daily values that allowed us to back-calculate the growth rate trend during this phase.

Line 108: define what is meant by "low" biomass (i.e. did you target a particular cell abundance or part of the growth curve?)

It's good practice for batch culture experiments with phytoplankton that the phytoplankton biomass at the harvest time consumes less than 5% of the total dissolved inorganic carbon. Cell concentrations were therefore kept lower

than 60.000 cells/ml. We added a sentence in the manuscript (line 90). We added a second reference to the text (Zondervan et al., 2002).

Zondervan, I., Rost, B., and Riebesell, U.: *Effect of CO₂ concentration on the PIC/POC ratio in the coccolithophore *Emiliana huxleyi* grown under light-limiting conditions and different daylengths*, *Journal of Experimental Marine Biology and Ecology*, 272, 55–70, [https://doi.org/10.1016/S0022-0981\(02\)00037-0](https://doi.org/10.1016/S0022-0981(02)00037-0), 2002.

Line 133: what cellular concentration of PIC and POC did you use? There can be some variability between strains of *E. huxleyi* (e.g., see Harvey et al., 2015. Consequences of strain variability and calcification in *Emiliana huxleyi* on microzooplankton grazing; Daniels et al., 2014, Biogeochemical implications of comparative growth rates of *Emiliana huxleyi* and *Coccolithus* species). Did you use an average or the values specific for this strain?

If we interpret correctly the request from the reviewer, the POC and PIC production rates were calculated for each sample by multiplying growth rates with the cellular POC or PIC contents. This information is given in lines 138-134. We rephrased the text to make it more explicit.

“The amount of PIC was determined as the difference between TPC and POC. PIC and POC production rates were calculated for each sample by multiplying the μ with the cellular POC or PIC contents.”

Line 143: what is R in this context, the level of alkalinity?

R is fCO₂. The specifics are given in the caption of Figure 4.

Line 168: Is PIC not also a function of u ?

If we interpret correctly the question raised by the reviewer, at line 168, POC production is defined as a function of μ . The same is the case for PIC production since it is calculated by multiplying growth rates with the cellular PIC contents. As mentioned previously, it has been made more explicit in the material and method chapter.

Figure 3c and 3d: what is driving the low PIC production rate (and hence PIC:POC ratio) at 2.5 mmol kg⁻¹ of TA? It stands out as quite the outlier.

We agree with the reviewer and presume the low PIC at 2.5 mmol kg⁻¹ of TA (specifically at 2499 $\mu\text{mol kg}^{-1}$) is probably an outlier. Since we haven't seen any variation from the growth rate trends and/or in the carbonate chemistry values, we presume there was a mistake during the filtration process of TPC. We decided to keep this value in the graph to give the full experiment overview (and maintain the resolution). The analyses in Figure 4 were performed considering or excluding this value and didn't change the outcome.

We added a sentence (lines 176-1179)

*“The low PIC (0.11 pg cell⁻¹ d⁻¹) value obtained at TA 2499 $\mu\text{mol kg}^{-1}$ is possibly an outlier. We hypothesise that there was an error in reporting the filtration me for the TPC filter for this sample. The sample was retained because no other anomalies were observed in the carbonate chemistry values or the growth of *E. huxleyi*. The statistical analyses were performed both including and excluding the sample, with no variations in the final results.”*

Technical corrections

Line 88-89: second reference to *E. huxleyi*, so can abbreviate.

If the name of a species is at the beginning of the sentence, it should keep the full name. We didn't change the text.