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Interactive comment on “Short-term response of the coccolithophore *Emiliana huxleyi* to abrupt changes in seawater carbon dioxide concentrations” by J. Barcelos e Ramos et al.

Anonymous Referee #1

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This article presents results of a laboratory study examining the short-term responses of *E. Huxleyi* to CO₂ manipulations. The results indicate that many of the CO₂-dependent responses observed in previous studies using longer-term acclimation of *E. Huxleyi* are also observed in short-term experiments. The authors discuss these results in the context of time-scale dependent CO₂ responses.

While I believe that the results of this study are interesting and warrant publication, I feel that there are number of areas in which the manuscript can be significantly improved. These are outlined below.

As has been done in many previous studies, the authors manipulate the carbonate

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system by a large and instantaneous perturbation ('abrupt and drastic', in their words). This is a largely artificial experiment conducted more out of convenience than in an attempt to mimic reality. Certainly in terms of global change (one of the stated motivations of this study), anticipated changes in seawater $p\text{CO}_2$ will occur at the rate of ppm per year, nothing close to the huge and sudden changes imposed here. Even during large phytoplankton blooms, seawater $p\text{CO}_2$ drops by hundreds of ppm on the time scale of several weeks. I am not suggesting that the authors attempt to replicate these kinds of slow perturbations, but I believe that these issues deserve discussion. The authors should be clear in stating that they are observing physiological changes to abrupt artificial carbonate system changes, something that is quite different from responses we might expect on century-scale changes.

If the cultures are aerated, the $p\text{CO}_2$ in the bottles should be set by the $p\text{CO}_2$ of the air that is being equilibrated with the water. Even if the alkalinity changes, the $p\text{CO}_2$ should remain constant as long as the water remains in equilibrium with the gas phase. The $p\text{CO}_2$ in room air can be highly variable depending on how well the room is ventilated and how much people are coming and going. But from the authors' description, the $p\text{CO}_2$ of the bottles seems to be set by alkalinity changes rather than gas-liquid equilibration. This doesn't make sense to me.

I think that this study would have been significantly strengthened by including a control treatment in which cells were maintained in the 'pre-culture' medium. This treatment could have been used to estimate the 'background' change in various physiological parameters over the diel cycle. In general, a more robust description of the cell's physiological circadian rhythm would have been helpful. This could have been used to normalize all parameters to a time-dependent change, thus minimizing diel cycle changes and highlighting CO_2 -dependent effects.

The assumptions outlined in the caption of Table 1 regarding calculation of DIC concentrations are not clear to me. Perhaps this text should be expanded and put in the materials and methods.

p. 4744, last para. The time-scale of the C-fixation experiments is not clear. Was the label added to all bottles at the beginning of the experiment and filtration conducted after 4, 8, 16 etc. hours? Or was label added at 4 h with a filtration at 8 h. etc. My point is that it's not clear to me how long the ^{14}C incubations were conducted for. As the length of ^{14}C experiments increasing, the measurements reflect different things. Short experiments reflect gross primary productivity while longer measurements approach net primary productivity. Please expand this section and clarify the methodology.

p. 4745, 2nd para. As with 14c, it's not clear to me over what time interval the growth rates were calculated. Were the growth rates calculated on the slope measured between each successive sampling time? If so, then only two points are used to calculate the slope of a line with large potential errors resulting. Please expand and clarify. I also think the figure legend needs to be changed as well. In its present form, I am not able to understand what is being said.

I think there needs to be more information and background references on the Fv/Fm measurements. For example, were cells concentrated prior to analysis?

One major limitation of the results is that there is not a single statistical test reported. The authors provide no information what so ever on the statistical significance of their CO_2 -dependent differences. Which parameters show statistically-significant CO_2 differences, and which differences could occur by chance? Every statement reporting a difference (or lack thereof) should be supported by the results of an appropriate statistical test. It surprising that this wasn't done in the first place.

An alternative way to present the data in Fig. 1 would be to compute the slopes of the lines (with standard errors), and use a bar graph (or equivalent) to show the slopes and errors, indicating which treatments show statistically significant differences.

p. 4746, l. 12. The change in POC production was mostly due to a low value in the lowest CO_2 treatment. This is not really clear from the description of the figure.

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While I am willing to believe that there was an increase in the number of malformed coccoliths under high CO₂, I found that Fig. 3 did not provide compelling evidence for this. I don't think that the figure adds much to support this statement. Is there some quantitative measure which could be applied?

Is the presence of malformed coccoliths really remarkable? What is the turnover rate of the coccoliths? If there is a short-term decrease in calcification shouldn't this be immediately translated into malformed coccoliths?

Was there really a statistically significant trend in Fv/Fm related to CO₂. From the figure, I can see some apparent differences in this parameter, but the position of the grey and blue lines makes me wonder whether these differences (if indeed statistically significant) were related to CO₂. A more careful analysis is needed here.

p. 4750, 2nd para. I don't see how a change in cellular carbon quotas necessarily has a direct effect on cell volumes since it's possible to change the carbon content per unit volume.

p. 4751, last para: I think the authors should explicitly define the terms 'acclimation' and 'adaptation' to clarify the subsequent discussion. Adaptation seems to be used in the sense of genetic changes whereas acclimation is being used to convey physiological / homeostatic regulation. This can be discussed more explicitly.

p. 4752, l. 9: I think the authors should give a fuller description of projected changes in the carbonate system. What is meant by 'abrupt changes through time'? Are there any scenarios in which phytoplankton will be subjected to the kind of perturbations simulated in these experiments? The authors use the example of daily / seasonal CO₂ changes, but these are clearly of a very different magnitude and temporal scale than those studied here.

Minor points:

p. 4740, l 21. change 'until' to 'by'.

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p. 4727 l. 3. change 'neither if its' to 'or whether'

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