

Interactive comment on “Responses of the diatom *Asterionellopsis glacialis* to increasing sea water CO₂ concentrations and the effect of turbulence” by Francesca Gallo et al.

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Response to Anonymous Referee #1

We would like to thank the opportunity to explain our manuscript and we thank all the constructive criticism. Several changes can be made throughout the manuscript following recommendations in order to make our study clearer. Below, we respond to the various concerns on our manuscript in a point-by-point manner.

Referee #1: This manuscript looks at the combined effect of both ocean acidification and turbulence on the marine diatom *Asterionellopsis glacialis*. The authors found that the response to pCO₂ in terms of growth rate, elemental stoichiometry and chain size

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was different in shaken cultures versus non-shaken cultures. First off, I am excited to see a paper looking at how turbulence can affect cell growth especially as there appears to be a clearly different effect of pCO₂ depending on whether the cells were shaken or not. However, I do not think the authors have adequately controlled pCO₂ in the bottles (leading to uncertainty as to what actual CO₂ concentration is being tested), nor have they quantified how shaking cultures translates into shear stress. I have discussed these concerns in more detail below. I strongly feel these issues need to be addressed before this manuscript is ready for publication. Comments: I would like to see more quantitation of turbulence. How does a shaker at 200 rpm translate into shear stress around the cell? What are the cell size, shape and morphology and how do these factors, combined with the simulated turbulence affect shear stress on the cell? How does the turbulence created by the shaker relate to expected turbulence in the ocean?

Response: We are happy to improve the manuscript by clarifying the methodology used to generate turbulence and its application according to the reviewer's suggestion. It is correct that we did not quantify small-scale turbulence, but an estimation can be added in the revised version. According to Guadayol et al (2009) to correctly assess the effects of small-scale turbulence on plankton, turbulence should be constant, containers size should be adjusted to the organism being study (for phytoplankton turbulence scales are millimeters to centimeters) and there should be no other influence than water motion (our bottles had no headspace). All of these requirements were taken into account in our manuscript and will be made clearer in a revised version. Moreover, in this manuscript we tested the effects of turbulence on the response of one species (*Asterionellopsis glacialis*) to rising CO₂ concentrations. Since the cells are much smaller than the scale of turbulence, their shape and size does not influence the hypothesis proposed in the manuscript, even though they will influence water motion around the cells according to physics. Finally, the constancy of movement and virtually null headspace prevented interrupted turbulent eddies and occasional high energy events. Therefore, the setup mimics open ocean conditions (in opposition to coastal),

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that depending on the meteorological and oceanic variables, and depth at which the phytoplankton cells are, might correspond to a ripple or a storm. Additional information can be added in the revised version.

Referee #1: I am very concerned with how the CO₂ manipulations were monitored. CO₂ concentrations changed significantly between the beginning and end of the experiment (especially for the high CO₂ treatments). Stating that the CO₂ treatment was the average of these two measurements (beginning and end) is not scientifically accurate. First, I doubt there was a linear change in CO₂ over time and second, because CO₂ concentrations are changing over time, it is uncertain what pCO₂ the cells are acclimating/responding to.

Response: The carbonate system was manipulated and monitored following the "Guide to best practices for ocean acidification research and data reporting", edited by Riebesell et al., 2011 (eds. European Commission). In our experiment, we used diluted batch-cultures and phytoplankton biomass at the end of the experiment (time of harvesting) promoted a drawdown lower than 5% the total dissolved inorganic carbon (DIC) in the culture medium (average drawdown of 3.4%) as recommended in the Guide. In accordance with the Referee's concern we added, for each carbonate chemistry parameter the correspondent values at the beginning, at the end and during the experiment (in table 1).

Referee #1: I was not convinced by the justification of this experiment to future-world scenarios, where increased storm/wind events would create a more turbulent environment for diatoms. The paper by Moum and Symth, 2001, is a very general paper about increased wind and storm events. There needs to be a more specific discussion about how the intensity and duration of surface ocean turbulence in regions where chain-forming diatoms are found is predicted to change in the future.

Response: We acknowledge that the manuscript will benefit from additional information concerning the increase of wind events and consequently storms, in future world

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scenarios. Thus, in the revised version of the manuscript, we will elaborate this issue including new references. Particularly, by including a number of studies focused on future climate scenarios which showed that extreme wind speeds and storm events might probably increase in future leading to enhanced surface waves and consequently turbulence in the surface ocean (Rockel and Woth, 2007; Elsner et al., 2008; Garreaud and Falvey, 2009; Landsea et al., 2010).

Referee #1: In addition there needs to be justification for how bottle experiments where phytoplankton have been acclimated to constant conditions for 18 generations translates to the duration and intensity of storm events in the ocean.

Response: When carrying out CO₂ perturbation experiments with all types of cultures, and especially phytoplankton, it is important to ensure good pre-conditioning of the microorganism, especially when considering biomass / bulk parameters. Thus, pre-cultures should grow for more than five generations at the same experimental conditions (light, temperature and nutrients) as the experiment. Furthermore, cells should be kept at low abundance and in the exponential phase until the onset of the experiment. Therefore, before to start the experiment, we grew two pre-cultures (9 generations each) in order to acclimate the cells to experimental conditions. (as described by La Roche et al., 2011 in "Guide to best practice for ocean acidification research and data reporting – Part 2: Experimental design of perturbation experiment –Chapter 5: Bioassay, batch culture and chemostat experimentation"). In relation to the turbulence issue, the first approach to test the potential influence of small-scale turbulence should consider constant conditions which provide information about cells exposed to long (days) lasting surface winds or storms. Moreover, to be able to address the stress response of this or other species of phytoplankton to a strong storm, other parameters should be measured (e.g.: uptake rates, calcification rates, nitrogen fixation rates). Further studies might now build on this knowledge and test more complex setups, as variable turbulence. Our approach was to determine the effects of constant small-scale turbulence on the bulk. The text and hypothesis will be made clearer in the revised

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manuscript.

Referee #1: Also needed is a discussion of how these extremely high CO₂ concentrations (at 3000 uatm pCO₂) is relevant to a future scenario.

Response: In our experiment, we tested three CO₂ levels which are considered extremely relevant in the context of realistic range in future atmospheric carbon dioxide concentration (present day, 780 and 1100 μ atm). Furthermore, we complemented this range testing a less realistic future scenario with CO₂ concentrations of 2800 μ atm in order to better understand the physiological thresholds of the cells.

Referee #1: There were a quite a few spelling mistakes throughout the text that need to be addressed.

Response: We will correct the spelling mistakes according to the suggestions in the revised manuscript.

Referee #1: I think it would be useful to also measure cell size under these different CO₂/turbulence treatments as I think this may help the authors interpretation of results.

Response: Cell sizes were measured, however no remarkable differences were observed between treatments. Thus this parameter was not considered in the manuscript. This information can be added, if considered relevant, in the revised version of the manuscript. Instead, we observed that carbon dioxide concentrations significantly influence the number of cells per chain, as discussed in the manuscript.

Referee #1: The discussion (and references) presents a one-sided argument for diatoms increasing growth in response to elevated CO₂. There is a large body of literature that the authors should acknowledge where no response or a negative response of diatom growth to increasing CO₂ concentrations were found. See Table 1 in Gao and Campbell (2014) *Functional Plant Biology* 41:449 – 459 for a good summary of different CO₂ manipulation experiments on diatoms showing enhanced, no effect and negative effect.

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Response: More references concerning the negative response of diatom growth to increasing CO₂ concentrations can certainly be added in the manuscript considering the study recommended.

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