

## ***Interactive comment on “Responses of the diatom *Asterionellopsis glacialis* to increasing sea water CO<sub>2</sub> concentrations and the effect of turbulence” by Francesca Gallo et al.***

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Response to Anonymous Referee #2

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 explain in detail how we can respond to the various concerns on our manuscript in a point-by-point manner.

Referee #2: This manuscript addresses an interesting and important question- ‘how do synergistic changes in pCO<sub>2</sub> and turbulence impact diatom growth?’. While this is an

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important question that deserves enhanced attention in the literature, I have some concerns about the execution of these experiments and their interpretation, as described below: 1) How frequently were the cultures diluted and by what factor? How was this dilution rate selected (which measurements?), and when were the growth rates deemed to be in steady state? For instance, one criteria for determining this is to assume growth rates to be in steady state when they did not change by more than 10% between dilutions for some set number of generations or to use a statistical test to determine that the growth rates are not changing over some set period of semicontinuous manipulation. See, for example, Fu et al 2007 J. Phycol 10.1111/j.1529-8817.2007.00355.x This is incompletely described in this manuscript and thus leaves the reader with some difficulty interpreting the results. Other important experimental details are also missing, such as growth temperature.

Response: Two pre-cultures (9 generations each) were grown in dilute batch-cultures under the same conditions of the experiment in order to acclimate the cells to experimental conditions. Batch culture were diluted at steady state (difference in growth rate between the 2 pre-cultures and the experiment was always lower than 10%) and their abundance never exceed 15000 cells/ml, thus keeping DIC consumption by photosynthesis below 5% (in accordance to “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”, edited by Riebesell et al., 2011, eds. European Commission). These information can be added in the manuscript to make clearer the interpretation of the results.

Referee #2: 2) There is not enough detail provided to determine whether the carbonate system manipulations were effective and properly controlled. The bicarbonate and strong acid manipulations are only appropriate in closed system settings, eg: [www.biogeosciences.net/6/2121/2009/](http://www.biogeosciences.net/6/2121/2009/) and it is not clear that this was maintained. In fact, the data in Table 1 suggest it was not.

Response: We acknowledge that data concerning DIC drawdown of each treatment

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and replicate were not clearly expressed in the manuscript and will be added in the Result session and in Table 1 of the revised manuscript. The carbonate system was manipulated and controlled following the “Guide to best practice for ocean acidification research and data reporting” through addition of HCl and NaHCO<sub>3</sub> and we will add more information about the manipulation methods in the Material and Methods section (namely reference to the lack of head space of our closed bottles) The DIC drawdown between the beginning and the end of the experiment was less than 5% as recommended in the Guide, indicating that the carbonate system was properly manipulated.

Referee #2: 3) It's also not clear whether the system was monitored frequently enough. It seems that these measurements were made simply at the beginning and “end” of the experiment. It's not only not clear how this is defined- how many generations did it take the semi-continuous cultures to reach steady state growth rates (end?)?, suggesting that these parameters “during” the experiment were simply and average of beginning and end values in not defensible (Table 1).

Response: During the experiment, cells were kept at low density and diluted at steady state (no changes in cell growth rates) which always kept DIC consumption below 5% (in accordance to “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”, edited by Riebesell et al., 2011, eds. European Commission). For this reason, the carbonate system in our experiment is considered stable throughout the experiment.

Referee #2: 4) Cell size is discussed in the text as a way to understand when turbulence might be favourable or unfavourable, but not data is reported on cell size. From the cell quotas, we can make inferences, but measuring cell size would allow the authors to more specifically address their own question and test the models and mechanisms for turbulence impacts that they describe here (line 104). Hopefully the authors have retained images from their culture monitoring that could be used to address this. It would strengthen the interpretation involving chain length changes as well.

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Response: We agree with the referee that cell size could have been affected by our treatments. However, we did not observe remarkable differences between treatments. In the manuscript, when we discussed the effect of enhanced turbulence on large and small cells, we were actually referring to different phytoplankton species, with substantial differences in size. This can be made cleared in the revised version of the manuscript.

Referee #2: 5) Table 1: In addition to the problems identified above, the authors should also indicate measured vs calculated values more clearly here.

Response: We agree with the reviewer and we will add this information in both table and table legend.

Referee #2: 6) Figure 1: the fits of these lines are not good, and not described. This should be excluded or explained and justified in much more detail.

Response: We agree with the reviewer and will modify to a more appropriate curve as described in Megard 1984. This curve has been developed to describe the stimulating and inhibiting effects of light on growth rate and can be applied here in a similar fashion as in a coupled (TA constant at varying DIC) carbonate system with stimulating effects due to increasing substrate (CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup>) and inhibiting effects due to increasing H<sup>+</sup> concentrations.

Referee #2: 7) I agree with anon. reviewer 1 that the turbulence imposed here needs to be put in much better context in order to justify extending the results to expectations in a future stormier ocean.

Response: We will improve the explanation concerning this issue including new references about future wind events scenarios (Rockel and Woth, 2007; Elsner et al., 2008; Garreaud and Falvey, 2009; Landsea et al., 2010). Moreover, we will improve our text to tone down direct extrapolation to the future stormier ocean.

Referee #2: 8) There are many spelling and grammar mistakes in the manuscript. If it

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is to move forward, this should be much more carefully addressed by the authors. In particular, the Abstract has many problematic sentences.

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

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