

***Interactive comment on* “Effects of the pH/ $p\text{CO}_2$ control method in the growth medium of phytoplankton” by D. Shi et al.**

Anonymous Referee #1

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I enjoyed reading Shi et al paper and followed their results with interest. Their work is the next logical step after recent discussions regarding which manipulation is appropriate to test the effect of ocean acidification on the growth and physiology of marine biota. The scientific methods and assumptions are valid and clearly outlined. The Authors argue that continuous cultures are the only avenue to control pH/CO₂ in the medium. However, it is unclear to me what method the Authors used to grow the cells (continuous/semicontinuous/batch?). The Authors state “Experimentally we observe that all methods of adjustment of pCO₂/pH can be used, the choice of one or the other depending on the specifics of the experiments”. I assume the Authors mean that the choice of the method depends upon the questions asked, for example, whether the aim is to elucidate the cellular mechanistic response to pH and or CO₂ or the effect of ocean acidification on cell physiology.

The Authors make reference to the mechanical effect of bubbling cultures and argue that this gives more variable results than other methods of pCO₂/pH control. Do they mean in continuous or in semicontinuous cultures? From the results presented here the Authors can only speculate about whether bubbling can be the cause for such variation. The Authors state that if the aim is to assess the response of biota in the surface ocean, the changes in culture media by CO₂ fixation, nutrient utilization and calcification may be allowable or even desirable if they are kept at reasonable levels. The relative effect of bubbling versus that of changes in these variables may be negligible yet it needs to be tested. Finally, the writing is poor in places and could be greatly improved. I have made some suggestions on how to improve clarity and flow. I recommend this manuscript for publication subject to amendments suggested below.

General comments.

This paper addresses scientific questions within the scope of BG. However, the title does not represent what the paper is about – it should be changed to “Effects of the pH/pCO₂ control method on the physiology of phytoplankton”

The Authors should mention in the discussion the likely effect of increasing H⁺ on the catalytic properties of housekeeping enzymes such as carbonic anhydrase, e.g., the acid–base balance homeostasis (see discussion on acid/base manipulation versus bubbling air with different partial pressures). While the concept of alkalinity is useful to us, cellular processes are controlled by sources and products and thus, the Authors should focus on CO₂, carbonate, bicarbonate and protons (for example, affecting proton pump activities and which fluxes are dependent on proton pump efficiency of the cells) rather than alkalinity.

Specific comments.

• Page 2416, line 1. Change “To study” to ‘Studying’.

• Page 2416, line 2. “. . .key chemical parameters of the growth medium. . .” also include nutrients. Do the authors mean ‘carbonate chemistry parameters’?

• Page 2416, lines 8 and 9. Change to

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'For example, in the absence of pH-buffering or CO₂ bubbling, a sizeable decrease in pCO₂ occurs at a biomass concentration as low as 50 μ M C in non-calcifying cultures.' âĀĀ Page 2416, lines 13 and 14. Change to '...the choice of one or the other depending on the aim of the experiment.' âĀĀ Page 2416, lines 14 and 15. I think this statement is speculative as no bubbling test was conducted, e.g., using different aeration rates and investigating the effect on physiological parameters. âĀĀ Page 2418, section 2.1. State whether batch, semicontinuous or continuous culturing was used in the experiments. âĀĀ Page 2418, line 22: add space after "ln" in: "...of ln (cell number)...". âĀĀ Page 2419, line 3. Provide details on how pH was monitored daily, what instrument, and its precision. âĀĀ Page 2419, line 10. This sentence is unclear and confusing. What do the Authors mean by "it was then resumed and continued till the end of the experiment"? âĀĀ Page 2422, line 3. This is inconsistent with the statement on page 2419 (line 6) (adjustment when changes in pH are >0.02 or 0.05 units?; in all treatments?) Please clarify and/or amend. âĀĀ Page 2422, line 17. How is pH maintained constant? âĀĀ Page 2422, line 18. In the following sentence: "The changes in DIC and Ω are similar to those in a bubbled cultures" what treatments are the Author comparing? âĀĀ Page 2422, line 22. Change "object" to 'objective'. âĀĀ Page 2422, line 22. "...sufficiently constant chemistry..." is rather vague. What do the Authors mean? Could they give a value? âĀĀ Page 2423, line 15. It is unclear to me how the changes in dissolved inorganic carbon can be given by the total particulate organic and inorganic carbon in the medium. âĀĀ Page 2424, line 8. Change "whether" to 'regardless of whether...'. âĀĀ Page 2424, line 15. I believe the Authors mean 'a high-calcifier' rather than "an abundant calcifier". âĀĀ Page 2424, section 3.3. Refer to 'Figure 3A' instead of "Figure 3a". âĀĀ Page 2425, line 15. Do the Authors mean 'harvesting' rather than "stopping"? âĀĀ Page 2426, line 8. The citation of Figure 2C is wrong if comparing results at pHs 7.8 and 8.09. Also the slope looks comparable at both pH 7.8 and 8.09 – perhaps the Authors could be a bit more specific instead of stating that the cells "...grew slightly slower than those in which CO₂ was increased by acidification." âĀĀ Page 2426, line 10. The Authors need to be conservative in their

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argument that the lower growth rate “results from the mechanical effect of bubbling”. Since there are no data supporting this statement, this must be written as a speculative statement. I suggest to substitute “results” by ‘may result’. Equally, how does this help in comparing real-world bubbling with the lab observations? From a physiological point of view, likely differences in proton pump activities potentially caused by varying pH manipulations could be the source for the observed differences in physiology. I would expand the discussion to include likely effects of the different treatments. – Page 2426, lines 20-25. Excellent point made. However, the pCO₂ of the cultures is always significantly lower than the pCO₂ of the bubbled gas in an open system. – Page 2428, lines 4-7. This statement is incorrect – from the data presented it is not possible to argue whether the observed differences in organic and inorganic carbon are controlled by cell physiology or by carbonate chemistry or what is the contribution of each. – Page 2430, end of Conclusions section. One aspect the Authors have not discussed is whether one can extrapolate observations to the natural environment based on media with different chemical properties. As stated above, this is more of a philosophical question and the Authors may ask themselves whether some treatments are appropriate or even preferable when investigating the cellular mechanistic responses to specific parameters associated with ocean acidification but not adequate to extrapolate to real case scenarios. – Page 2430, last sentence of Conclusion section. A factor in the surface ocean is gas exchange – how is this taken into account?

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