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Interactive comment on “CO₂ perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations” by K. G. Schulz et al.

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Received and published: 20 June 2009

Schulz et al compare changes in carbon chemistry using the CO₂ bubbling versus the HCl/NaOH addition method and compare experiments using the two approaches for the coccolithophore *Emiliana huxleyi*. The aim of the ms is to provide advice on the debate on which methods are most appropriate, and while the goal is admirable and the facts raised in the ms are important to bring to the attention of the readers, it is our opinion that some their conclusion does not adequately discuss the biological implications of differences in seawater carbon chemistry. The authors conclude on page 4452 lines 10-11 that: “both CO₂ manipulation methods (variation in TA or DIC)

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change carbon chemistry in a similar way". However, their evidence, and the evidence of past work, does not support this statement. Discussion of the biological implications of any changes in carbonate chemistry is absent. In light of this, we would not support the acceptance of this ms unless major alterations are made to the final conclusions and discussion of the implications of these results. Major points that can be addressed are discussed below.

It is implied that changes in the carbonate chemistry are similar using the two methods (page 442, line 19), even though their calculations show there is a large change in the $[\text{HCO}_3^-]$ (~ 150 and $\sim 250 \mu\text{mol kg}^{-1}$ at pH 7.8 and 7.5 respectively). This increase in $[\text{HCO}_3^-]$ can result in different, and possibly interacting, biological responses depending on the physiology of the organism that is under examination.

Importantly, the authors need to discuss the fact that different organisms utilize carbon species differently. If the organism in question is only capable of using CO_2 (not HCO_3^-) via diffusive uptake, then as the pH of the ocean decreases, the photosynthesis of the organism in question should increase. As there are little differences in carbon chemistry between the two methods, using either method should provide similar results for CO_2 only using organisms.

Alternatively, if the organisms in question are HCO_3^- users, such as *E. huxleyi*, then we need to know at what concentration photosynthesis/organic growth is saturated for HCO_3^- . This is important, because if it is never saturated, or if it is saturated at higher levels than today's concentrations, then as $[\text{HCO}_3^-]$ increases, photosynthesis should also increase. Therefore, if one method results in higher $[\text{HCO}_3^-]$ then the two methods may result in different rates of photosynthesis/organic growth. However, if $[\text{HCO}_3^-]$ is already saturating for the organism in question at the current pH, then there should be no effect of enhanced photosynthesis/growth with decreasing pH. This needs to be discussed by the authors, as it appears from the current literature that $[\text{HCO}_3^-]$ saturation for growth/photosynthesis in present day seawater is species-specific (e.g. Huertas and Lubián 1998; Israel et al 1999; Clark and Flynn 2000; Rost et al 2003).

Additionally, some photosynthetic organisms do not calcify, while those that do use a variety of different methods of calcification. For those that do calcify, the way in which they calcify is of extreme importance. For example, *E. huxleyi* and other coccolithophores are thought to calcify internally. This means that rates of photosynthesis and, hence, $[\text{HCO}_3^-]$, may not have an effect on calcification, as it is probably biologically mediated (Borowitzka 1987). Alternatively, for certain organisms, such as coralline algae, calcification is mediated by photosynthetic increase of pH at the algal surface. Therefore, theoretically, an increase in photosynthesis for species that calcify externally, could equate to an increase in calcification over the short term. For corals, calcification increases as $[\text{HCO}_3^-]$ increases and saturates at around 6-8 mmol kg⁻¹ (Herfort et al 2008). Hence, the authors need to consider the biology of all photosynthetic organisms when making their conclusions, not only the biology of select few.

Most importantly, these differences in carbon chemistry between the two methods would be exacerbated in instances where $[\text{HCO}_3^-]$ is saturated for photosynthesis and/or calcification at a given future seawater pH/ $[\text{CO}_2]$ for an organism using the CO₂ bubbling method but not the HCl addition method. This would result in one method finding completely contrasting results than if the experiment was conducted using the alternative method, contradicting the authors conclusions on page 4452 line 21-22 and page 4442 line 19-20.

Finally, conclusions based on differences between the methods of published results for *Emiliana Huxleyi* (pg 4442, ln 17; pg 4451, ln 28) should be altered. The authors claim that there is no difference in POC and PIC when comparing the CO₂ gas or HCl addition methods. However, with so few experiments (Table 2), they do not have a large enough sample size (3-7) to detect meaningful differences. Comparing these studies is also difficult, as they use different strains, PAR levels, nutrients, culture types, and CO₂ levels. Also, the methodologies in the DIC-manipulations vary substantially between each other and the TA-manipulation approach. These differences would confound any conclusions made from a meta-analysis and should further limit any conclusions drawn

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from Table 2.

Other minor issues are that:

Figures 2 and 4 are too small, making it difficult to directly compare values from the different aspects of these figures, especially for figure 4.

Page 4443 line 6, needs a reference in regards to the term “ocean carbonation”.

Page 4443 line 11, either cite with a link, such as: <http://www.epoca-project.eu/index.php/Home/Guide-to-OA-Research/>, or insert an acceptable alternative, such as:

Hurd, C.A., Hepburn, C.D., Currie, K.I., Raven, J.A. and Hunter, K.A. Testing methods of ocean acidification on algal metabolism: consideration of methods. *J Phycol* 45(6): In press
Rost, B., Zondervan, I., and Wolf-Gladrow, D. 2008. Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions. *Mar Ecol Prog Ser* 373:227-237.

Page 4457 Table 2, Papers cited in this table do not appear in the references section.

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Interactive comment on *Biogeosciences Discuss.*, 6, 4441, 2009.

BGD

6, C888–C891, 2009

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