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Biogeosciences Discussions

Interactive comment on "Effects of the pH/pCO$_2$ control method in the growth medium of phytoplankton" *by* D. Shi et al.

Anonymous Referee #2

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Shi et al. address a number of very central questions in the field of ocean acidification (OA) research: How can carbonate chemistry be manipulated and do these different perturbations alter the responses of phytoplankton to pH/pCO2? To answer these questions the authors have described three types of CO2 perturbations, two of which have been commonly used in OA research (acid/base addition, bubbling with different pCO2) and as a third option they propose the use of organic buffers. The authors also point out the effect of biomass accumulation as it can significantly offset the desired conditions and discuss the advantages and disadvantages of the different approaches. To judge possible differential responses, the diatom Thalassiosira weissflogii and two strains of the coccolithophore Emiliania huxleyi (calcifying and a non-calcifying) have been incubated under different pH/pCO2 manipulations. Responses were assessed primarily based on growth rate measurements at two pH/pCO2 levels. In some yet not

all of these manipulations also POC and PIC quotas and respective production rates have been determined.

The paper discusses important, although not novel but often overlooked aspects of the carbonate system and proposes new approaches for future research as the use of buffers. The description of experiments and the overall presentation of data, especially regarding the structure, could be significantly improved in some parts. It took me a while to understand what data was obtained for which treatment and/or species and where such data was lacking. Despite several criticisms, I do agree with most of the interpretations and conclusions presented here and therefore recommend the publication after addressing the points below.

General points:

1) The discussion on carbonate chemistry manipulations and the effect on biomass accumulation and/or calcification (Fig. 1 and 3) address one of the most fundamental aspects of OA studies. Although some of these considerations/calculations are not new (see Rost et al. 2008, MEPS 373: 227-237), such detailed comparison is necessary to address the potential for differential responses in the different manipulations and to visualize the effect of biomass.

2) Regarding the responses in these manipulations, the method comparison appears a bit limited to me in the sense that responses have been studied i) only at two pH/pCO2 levels and ii) the interpretations are in most cases based on growth rates only:

Some of the current controversy in OA research relates to the question how phytoplankton like E. huxleyi respond to pH/pCO2 (cf. Iglesias-Rodriguez et al. 2008, Riebesell et al. 2008). In other words, what is the pH/pCO2-dependency of photosynthesis or calcification and does this relationship change with the mode of manipulation? Such question can only thoroughly be answered if the responses are measured at more than two pH/pCO2 levels.

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As most species are able to keep their division rate constant despite changes in pCO2, growth rate is not the best measure to answer whether there are differential responses in different manipulations. This is even more critical when considering that we are really interested in physiological processes like photosynthesis or calcification, which can be uncoupled from cell division. The authors are aware of this and therefore have additionally measured POC and PIC in one of the E. huxleyi strains. Unfortunately, they were unable to yield trustworthy production rates for the "bubbling" treatment, which makes this comparison incomplete.

3) The suggested use of organic buffers in the attempt to reach high(er) biomass without detrimental effects on carbonate chemistry is, as far as I am concerned, not the right way to go for the following reasons:

First of all, even though a buffer can keep the pH and thus the ratio of DIC species constant, it will not avoid a general drawdown of DIC. This point should be stressed more clearly and earlier in the manuscript. Moreover, problems arising from the slow kinetics in the carbonate chemistry are not compensated by buffers. In the current version, all this is only mentioned in the conclusions.

Secondly, organic buffers artificially increase total alkalinity (TA) and therefore preclude using this parameter for the calculation of carbonate chemistry. All the calculation programs "assume" a certain carbonate alkalinity associated to TA. If in the case of buffer addition, this assumption is not met.

Moreover, TA and changes therein have been used to estimate calcification rates (e.g. Smith & Key 1975, L&O 20: 493-495; Gattuso et al. 1998, Global and Planetary Change 18(1) 37-36). In the case of buffer addition, this approach can also not be used.

Last but not least, organic buffers stick to filters and cause high carbon background, hence they preclude POC or TPC sampling, a method widely use in OA research.

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One point I am less certain off but nevertheless troubles me. Can we preclude an effect of organic buffers like EPPS on calcium concentrations and thus Omega? As weak acids, EPPS can form complexes with trace metals, could they also do this with other cations? Substances like EDTA have been used to "bind" Ca2+ in seawater and thereby dissolve CaCO3+ (McEnery & Lee 1970, L&O, 15(2): 173-182, McEnery & Lee 1981, Micropaleontology 21(1): 71-83).

4) It is not clear to me whether two parameters of the carbonate system have always been measured in this study. Assuming the authors did, what methods were employed? I could not find any information on precision and accuracy on estimates on TA, DIC or pH in the text. Such information is fundamental to judge the quality of the carbonate chemistry data and should nowadays be standard in OA studies. I have the feeling that only one parameter has been measured and a second one, for instance the aquatic PCO2 level, was assumed to be in equilibrium with the pCO2 levels in the air. This assumption is certainly not always valid, especially at higher biomass.

Specific points:

Abstract:

From reading the abstract it is not clear what manipulation approach have been used. Neither is any information given on mode of incubation or species used. The abstract should certainly provide more specific information, similar for instance to the last sentence in the introduction.

P. 2416; line 11-12: The sentence "The quantification of these changes is..." is not easy to understand.

Introduction:

P. 2417; line 2: Strictly speaking, the addition of buffer does not "continuously readjust the concentration of dissolved CO2". As buffers keep the pH and thus speciation constant, it prevents strong changes in CO2 but cannot preclude decreasing CO2 owing to

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decreasing DIC. And significant DIC drawdown is likely here as buffers are used in the attempt to reach higher biomass. This problem also applies for the pH-stat systems mentioned (line 23-24).

Material & Methods:

P. 2419; line 3-4: What other parameter than pH was measured? Two are required to calculate carbonate chemistry and pH is usually not the most reliable one. Please provide more information on this, including instrumentation and respective precision!

P. 2419; line 9: Has the air-CO2 mixtures been humidified to reduce evaporation and thus changes in media volume and salinity? As compressed gas mixtures are completely dry, bubbling can significantly change conditions over time!

P. 2419: line 10: Why was the bubbling stopped upon inoculation? The pre-acclimation should have been cultured under the same conditions and thus bubbling should not be "new" to them! Have the cells been pre-acclimated to experimental conditions and for how many generations? If not, a significant lag phase can be expected which alters the responses in the real experiment.

P. 2419; line 15-16: Perhaps it would be good to add some information on how the "target pH" was determined. I assume you took the "bubbling approach" as a reference for that?

Why was 5 mM and 8 mM EPPS buffer concentration chosen for T. weissflogii and E. huxleyi, respectively? In the cultures for T. weissflogii nutrient concentrations where higher and as E. huxleyi calcifies the pH/pCO2 drift in that culture can be expected to be smaller.

P. 2419; line 19-21: It should perhaps be highlighted that NaHCO3/HCl addition cause identical carbonate chemistry to the bubbled treatment as it increases DIC at constant TA. "Acid/base adjustments", on the other hand, are manipulations of TA while DIC is kept constant.

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I assume that all approaches other than the bubbling treatment were done in closed bottles? How were they sealed? Was any headspace avoided?

P. 2420; line 4-7: Measuring responses with 14C incorporation over 2-4h in one treatment (bubbling) and over 24h in the other treatments (buffered, acid/base) can itself cause different results for two reasons. First, short-term incubation tend to provide estimates on gross rates of C fixation whereas longer incubations yield net rates. Secondly, cells may have a diurnal rhythm (despite growing under continuous light) and thus the timing of sampling can yield quite different results.

P. 2420; line 20-21: Are the DIC and TA values assumed? In line 23 one can read that measured pH and known DIC and TA were used to constrain carbonate chemistry. This paragraph is not clear to me.

P. 2420; line 25-26: None of the buffer factors are defined; instead it is referred to an unpublished manuscript. Some more details, at least the definitions, have to be presented here so the reader is not lost.

Results & Discussion:

P. 2422; line 4-6: Does this mean that 2 mM buffer is sufficient to keep pH constant within 0.05 units when biomass attains 100 mM C if the buffer works at its pKa? And therefore twice as much buffer is required if it is used away from the pKa by about 0.5 units? Please clarify.

P. 2422; line 10-12: The statement that changes in pCO2 and pH are smaller in calcifying cultures is mostly true (at least when compared to non-calcifiers) but strongly depends on the assumption of PIC/POC ratio (here assumed to be 1), which differ strongly between species, strains and growth conditions. I would therefore suggest mentioning that, depending on the PIC/POC ratio, the pCO2 can decrease, stay constant or even increase with biomass accumulation, but and this is important always at reduced DIC concentrations! Under these conditions pCO2 cannot be used as a

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"proxy" for the rest of the carbonate chemistry.

P. 2422; line 13-16: The first sentence is difficult to understand. The second statement, that pH and Omega changes are small critically depends on PIC/POC ratio.

P. 2422; line 18-19: It is true that the changes are "...similar to those in the bubbled culture" but perhaps it would be more specific to state that "...the changes are slightly larger than in the bubbled approach"?

P. 2422; line 23: "In cultures where the object is to maintain sufficiently constant carbonate chemistry, the changes in DIC and Alk resulting from the growth of phytoplankton must be kept relatively small and a reasonable accurate and simple method is provided by the use of buffers capacities". Strictly speaking, buffers do not prevent changes in DIC and TA at all, they keep pH and therefore pCO2 rather constant compared to a non-buffered approach.

Using of buffers requires "tedious" calculations (Page 2422; line 21) and may have further disadvantages (see general points above). To me it seems as if buffers are used to treat the "symptoms" of too high biomass and it alleviate some of the "distress", this is the reason why they are widely used by microbiologist, but the best way to prevent that the system gets "sick" is by working with low biomass! The authors come to the same conclusion on page 2429, line 19-20 where they state "The simplest method is to limit the experiments to sufficiently low cell concentrations..." which makes the very detailed discussion on buffers appear a bit lengthy in the end (at least to me).

P. 2424; line 1: "According to the values in Table 1, if PIC/POC=1, the relative decrease in pCO2 is slightly smaller in acidified cultures, while the increase in pH is slightly larger." I have problems to vision that the shift in carbonate system is smaller in an acidified system from Table 1, especially when one needs to consider PIC/POC = 1 at the same time! Perhaps it would be better to refer to Fig 1 a and b (or Fig 3 b and c) for this statement?

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P. 2424; line 9: Here and elsewhere in the manuscript, bubbling was presented as a way to "fix" pCO2. This assumption about instantaneous equilibration is also implicit in Fig. 3D. Owing to kinetic aspects of the carbonate system, including the slow air-water gas exchange, this assumption is not always valid and certainly not when cell densities > 105 have been attained. I would therefore be careful with the term "fix", especially as it apparently has not been determined whether carbonate chemistry was equilibrated to the target pCO2. I would suggest using "manipulated" instead.

P. 2424; line 10 and Fig 2a: It is difficult to judge differences (or similarities) in growth rates shown in a log scale graph only. In order to show that responses were in fact identical in all manipulations, the full data and some statistics should be provided.

P. 2424; line 22: Where C quota and C:N ratio assumed or measured? If measured, please provide more details. If not, please provide reference.

Table 2: Where are the data on bubbled cultures? Why are they on page 2427, line 27 but not in the table? It is important that the responses obtained in all tree manipulations are shown here.

Page 2426; line 5-6: "The data of Figs. 2a–c show no difference in growth rates among cultures where pCO2/pH are controlled by different methods." Again, it is very difficult to judge growth rate (differences) from a log plot. Please provide mean values and standard deviation for all manipulations in Table 1.

Page 2426; line 6-7: "The cultures of E. huxleyi strain PLY M219 showed a small but systematic increase in growth rate at pH=7.8 compared to pH=8.1 in acidified cultures, with or without buffer (Fig. 2c)." I find the observed stimulation under low pH in growth, PIC and POC production quite significant and interesting but this information is given in Table 2 and not Fig. 2.

Page 2426; line 13-16: "...our experiments with bubbled cultures have yielded more variable results than those in which we used other methods to adjust pH/pCO2. Oth-

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ers have also obtained results with a high degree of variability in bubbled cultures of strain PLY M219 (Iglesias-Rodriguez et al., 2008b)." First of all, what specific results are more variable and where is that data shown? Some of that variability in responses may be attributable to the fact that 14C incubations were performed over shorter time (2-4h), in contrast to those of manipulation in closed systems where incubation covered 24h. Also, it was not clear from the text whether cells have been pre-acclimated to the respective conditions (see question above)? If not, inoculation into bubbled media may induce a lag phase that may further increase the variability in responses (see comments above). And was the gas mixture humidified prior to bubbling the media? If not, significant evaporation takes place and may further increase the apparent variability in these approaches. Anyhow, such interpretation can only be followed by the reader when the full data is given.

Page 2426; line 21-23: "It should be also noted that when cultures (of any organism) reach high cell concentrations, it becomes difficult to supply enough CO2 through bubbling to keep up with the rate of CO2 fixation by the cells." This point is very important as I believe a lot of the calculations in the bubbled treatment have been based on the assumption of full equilibration with the target pCO2. It would be important to not assume but measure this!

Interesting and not stressed enough is that Shi et al. observed stimulation in growth, POC and PIC production in closed systems while it was stated that no such response was observed in the bubbled approach. These findings contrast those of Riebesell et al. 2000, who observed no effect on growth, increasing POC and generally decreasing rates in PIC production in a closed system. These findings however also contradict Iglesias-Rodriguez et al. (2008), who observed stimulation in POC and PIC production with pCO2 in a bubbled approach.

Page 2428; line 1-4: "... PIC/POC ratio of PLY M219 decreased slightly with increasing pCO2, making it likely that a change in calcification in the nutrient limited Surface Ocean will provide..." Please change to "changes in calcification to photosynthesis" as

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the change in PIC/POC is the result of both processes and the later is, at least under some conditions, even more pronounced (Zondervan et al. 2002).

Page 2428; line 9: "... methodological point of view we observed no significant differences in growth or photosynthetic rates, or in PIC:POC ratios between the different methods used to control pCO2/pH, aside from the slightly lower growth rates of bubbled cultures". Please provide the mentioned data for the bubbled cultures in a table (and not just the log plot).

Page 2428; line 11-13: "... As shown in Fig.1, the presence of EPPS in the medium has no significant effect on the growth of nutrient-replete phytoplankton, and thus, presumably, no direct physiological effects on the organisms." It is quite a vague assumption that if there is no effect on growth, there are no physiological effects. As mentioned here and elsewhere, strong pCO2 effects were observed for instance in POC and PIC production without any measurable effect on growth. Besides, please change to Fig. 2 (as Fig. 1 shows no growth rates).

Results & Discussion:

Page 2429; line 23-25: "... The decrease in Alk that results from precipitation of CaCO3 partly compensates for the effects of decreasing DIC, and, as a result, pCO2 and pH are less variable in calcifying than in non-calcifying cultures." It should be mentioned somewhere that under those conditions pH/pCO2 values are not the best representatives for the carbonate system. Depending on the PIC/POC ratio pH or pCO2 can decrease, increase or even stays constant (see comment above).

Page 2430; line 3-5: "... Presumably as a result of the mechanical effect of bubbling, we have found it more difficult to obtain reproducible results in bubbled cultures than in cultures with other methods of pCO2/pH control." What is this statement based on, fixation rates by 14C or growth rates? Please see comments above!

Interactive comment on Biogeosciences Discuss., 6, 2415, 2009.