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Biogeosciences Discussions

Interactive comment on "Effects of long-term high CO_2 exposure on two species of coccolithophores" *by* M. N. Müller et al.

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We greatly acknowledge the comments made by the anonymous reviewers to improve our manuscript.

Please find below our response (normal font) to the points raised by reviewer 2 (**bold font**); changes in the manuscript are written in *italics*:

They use often 'generation time', but how do they define it? They should define it to gain attention from all scientists in a field of biogeoscience.
The 'generation time' refers to the average time between two cell divisions, however, we did not use the term 'generation time' in the previous version of our manuscript.

We only used the term 'generation', which refers to a new set of descendants, and is a well established expression in natural sience. In the newly written paragraph 4.5 'From short- to long-term pCO_2 response of coccolithophores', we used the term 'generation time' and defined it for clarification.

4.5 From short- to long-term pCO_2 response of coccolithophores:
...Considering the short 'generation time' of coccolithophores (the average time between two cell divisions; ≈1-2 days for coccolithophores), evolutionary change and adaptation may occur during long-term incubations. ...

It may be obvious for algal physiologists but they should give a reason why they used f/20 medium because they discussed nitrate limitation latter in the Discussion.
The use of f/20 medium is a standard protocol in marine algal culturing to assure supply of all essential nutrients for growth. In our semi-continuous dilute batch culture experiment, the f/20 medium was sufficient to assure nutrient replete conditions at all times. We added informations about f/20 media in the method section.

2.1 Cultures:
...Both cultures were grown as asexual diploids at $16\,^{\circ}C$ *in* $0.2\,\mu m$ *filtrated North Sea water with a salinity of* 33 *and f/20 nutrient additions (Guillard, 1975), corresponding to* $88\,\mu mol\,l^{-1}$ *nitrate and* $3.6\,\mu mol\,l^{-1}$ *phosphate, a sufficient supply of macro- and micronutrients for exponential algal growth under semi-continuous culture conditions (see below), at a photon flux density of* $140\,\mu mol\,photons\,m^{-2}\,s^{-1}$ *(Philips TL-D 90 DeLuxePro, 36W/950) under a 14:10* h *light:dark cycle.*

Additionally, we clarified the paragraph in the discussion section about nitrate limitation. Please see paragraph below.

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It may be too detail to mention but it is critical. As algal physiologist, I can not understand what the growth rate means. What time interval do they consider in their calculation in the section of 2.4 Cell counts on p.10968. Without any time unit, nobody understand what does it mean. And suddenly unit of per day appeared in the section of 3.1 Emiliania huxleyi on p.10969!
We added in the section 2.4 the necessary information regarding the unit of the growth rate 'μ'.
See also the next paragraph.

They talked about Fig.2B. They showed 15 data points during the period of 98 days of experiment. Although they mention a duplicate of semi-continuous culture, how did they collect samples to calculate the growth rate?
We added more information about the sampling for cell number and the subsequent calculation of the growth rate.

2.4 Cell counts:
One sub-sample from each flask was taken and the cell number was immediatly determined with a Coulter Counter (Z Series). Samples were measured three times and the mean was used to calculate the growth rate 'μ' (d^{-1}) as
$\mu = \frac{(\ln c_1 - \ln c_0)}{t_1 - t_0}$
where c_0 and c_1 are the cell concentrations at the beginning (t_0) and end (t_1) of the incubation period (expressed in days).

Although they mention a duplicate of semi-continuous culture, why do they have only one data for open circles?
This has been corrected. Duplicate data points were added to Figs. 2 and 3.

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On p.10973 they should provide how to estimate why 88 micro-mol per liter (?) is sufficient for the species of E. huxleyi. If all 88 micro-mol nitrate was converted to cellular nitrogen, how many cells of E. huxleyi can they estimate?
With a particulate cellular nitrogen content of E. huxleyi of $2.6\,\mathrm{pgN\,cell^{-1}}$, as calculated from the mean of all TPN measurements of *E. huxleyi* under constant high pCO_2 (measurements from the continuous high pCO_2 treatment were considerably higher than measurements from the low pCO_2 treatment), $88\,\mu\mathrm{mol}$ nitrate converted to cellular nitrogen correspond to a cell density of approximately $5 \times 10^8\,\mathrm{cells\,l^{-1}}$.

We improved the corresponding paragraph as follows:

4.4 POC:TPN:
... However, we can exclude nitrogen limitation of E. huxleyi in the present study since i) an initial nitrate concentration of $88\,\mu\mathrm{mol\,l^{-1}}$ *would be sufficient to support exponential growth up to a cell density of* $5 \times 10^8\,\mathrm{l^{-1}}$ *(calculated with a TPN content of* $2.6\,\mathrm{pgN\,cell^{-1}}$*) which was never reached during this study, and ii) the measured growth rate of* $1.10 \pm 0.06\,\mathrm{d^{-1}}$ *corresponds to maximal growth rates under nutrient replete conditions for the temperature and light levels applied in our study (Buitenhuis et al., 2008). Therefore, we can rule out nitrogen limitation to be responsible for the higher POC:TPN ratio in the present study.*

The statement of 'POC:TPN ratios of about 10 and higher were observed in E. huxleyi under nitrogen limitation' is conflicting with the statement of 'the maximal growth rates under nutrient replete, similar temperature and light conditions'. They are not talking about the same idea in the two statements.
The statement of 'POC:TPN ratios of about 10 and higher were observed in *E. huxleyi* under nitrogen limitation' refers to results from the literature and not to the current study. We clarified this paragraph to avoid any confusion:

4.4 POC:TPN:

... The measured ratio of $10.0 \pm 1.4\,\mathrm{molC\,molN^{-1}}$ *under long-term cultivation is higher than ratios reported from short-term and mesocosms experiments, which vary between 6 and* $7\,\mathrm{molC\,molN^{-1}}$ *(Engel et al., 2005; Feng et al., 2008). Previous studies reported POC:TPN ratios of* ≈ 10 *and in E. huxleyi only under nitrogen limitation (Engel et al., 2004; Sciandra et al.,2003). ...*

It would be much convincing if they showed the direct measurement of nitrate in f/20 medium.

We did not measure nitrate in the growth medium since we assured by the experimental setup that at any given time the cells were under nutrient replete conditions and were not N-limited in growth. See also response to reviewer 3.

In 5 Conclusions, how can they draw the concluding paragraph at the end from the preceding paragraph? It is too general.

We reformulated and focused the conclusion paragraph.

They should run appropriate statistical analysis to talk about any difference.

We added statistic analysis in the result section.

Interactive comment on Biogeosciences Discuss., 6, 10963, 2009.