Biogeosciences Discuss., 6, C4610–C4613, 2010
www.biogeosciences-discuss.net/6/C4610/2010/

Biogeosciences Discussions

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

M. N. Müller et al.

mnmueller@ifm-geomar.de

Received and published: 2 March 2010

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 3 (**bold font**); changes in the manuscript are written in *italics*:

Global warming and ocean acidification are considerable concern to all people. Longterm exposure experiments of coccolithophores held by authors are worthy approach for understanding the response of marine calcareous phytoplankton

against ocean acidification. However, I do not understand why authors adopted 'sub-continuous batch culture' for this research. Authors wrote that they controlled pCO2, but not mentioned how they controlled nutrient concentration for 66 and 98 days. So here I write my following comment under the understanding that authors did not control nutrient concentration during their experiments.
In our experiment we did not measure nutrient concentrations directly but we assured by the experimental set up that at any given time point, conditions were nutrient replete and hence cells growing exponentially. We clarified and explained the experimental set up in more detail to avoid confusion about nutrient availability throughout the course of the experiment (for details, please see below).

In my understanding, physiological and morphological experiments of coccolithophores are usually done while the culture strain is in exponential growth phase to collect healthy cells, and many papers reported culture of E. huxleyi reaches to stationary phases within 7-15 days in their experiments.
We completely agree and therefore, we diluted the exponentially growing cultures of *E. huxleyi* and *C. braarudii* with fresh medium (f/20 nutrients conditions) every 5-11 generations, which means that cultures of *E. huxleyi* were diluted every 5-7 days.
We added now more information about the culture transfer into fresh media:

2.2 Experimental setup:
Cultures were allowed to grow for about 5-11 generations corresponding to a dissolved inorganic carbon (DIC) and nitrate consumption of maximal 10% and 23%, respectively. At this stage exponentially growing cultures were sampled for DIC, pH, cell number, total particulate and particulate organic carbon (TPC and POC), and total particulate nitrogen (TPN) before being transferred into fresh media (f/20 nutrient conditions and carbonate system already adjusted) to a concentration of 100 and $50\,\mathrm{cells\,ml^{-1}}$*, corresponding to a minimum population size of 28,000 and 14,000 cells*

(E. huxleyi and C. braarudii, respectively).This culture dilution and sampling protocol was continuously repeated throughout the course of the experiment.

In this paper, authors wrote that nitrate limitation did not occurred during 98-days experiments, but it seems unusual that culture conditions including nutrient concentration were satisfactory through 98-days-long experiments if the strain is enough healthy.
Yes, nitrate limitation did not occur during the course of the experiment since new nutrients (in f/20 concentration) were added with every dilution step. Our 98-days experiment could be thought of fourteen 7-days experiments in a row. See also paragraph above.

Authors wrote that cell density of culture, which has strain started from 100 cells per 0.1 ml, never reached to 5×10^8 cells per L(-1) level in their 98-day experiments. If nutrient concentration was enough, this result suggests another unknown factor limited healthy exponential growth of the strains.
We hope that we could clarify the experimental setup with the paragraph above and think that it is now clear that the cell concentration never reached a density of 5×10^8 cells per liter because exponentially growing cells were diluted with fresh media when reaching more than approx. 50.000 cells/ml (*E. huxleyi*) or 5.000 cells/ml (*C. braarudii*). See paragraph above.

In my opinion, continuous culture system with nutrient control was more appropriate for the purpose of this study. Or, did authors control other factors (nutrient etc.) in this study although it was not mentioned in the method? If it is so, I should change my comments.
See above.

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