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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.

**M. N. Müller et al.**

mnmueller@ifm-geomar.de

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

**The authors assert several times in the manuscript that they suspect that the end response they see is a sustained physiological response (ie, that the response seen in short term experiments scales up). This can be verified empirically by measuring the growth rate of the end populations in both high**

**pCO2 and in air, as well as the control cultures in both high pCO2 and air. Comparing the plastic response of populations that have lived at high pCO2 for 50 or 150 generations to the plastic responses of naïve (control) populations would allow the authors to verify that the phenotype that they see is entirely due to a sustained plastic response, rather than partially attributable to genetic change. While the dip in pCO2 that occurred halfway through the experiment is consistent with a plastic response, but it is not a conclusive test. Since one of the main conclusions is that 'observed CO2 sensitivities are persistent over multiple generations.' (Abstract, last sentence), the authors should empirically test that they really are looking at a persistent physiological response. I think that these measurements are vital to the conclusions stated in the manuscript. A second option would be to restate the conclusion to say that the phenotypes observes are the same as those seen in short term experiments, though it is not known if this is the result of a sustained acclimation response alone, or some combination of physiological acclimation and genetic change. I think that this uncertainty would detract considerably from the main message of the paper, and strongly suggest that the authors add the necessary measurements.**
Unfortunately, it is not possible anymore to verify a sustained physiological response for this long-term experiment. Therefore, we rephrased the corresponding statement in the abstract and conclusion sections as suggested by the referee:

*Abstract:*
*... These results are consistent with those obtained in shorter-term high* $CO_2$ *exposure experiments following abrupt pertubations of the seawater carbonate system and indicate that for the strains tested here a gradual* $CO_2$ *increase does not alleviate* $CO_2/p$H *sensitivity.*

*Conclusions:*
*... Here, we discussed data from a multiple-generation experiment using two coccol-*



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*ithophore species which generally confirm the observed $CO_2$ sensitivities obtained in short-term experiments, though based on our data it can not be distinguished whether this is the result of a sustained acclimation response alone, or involved genetic change.*
...

**One of the main results of this study is that physiological responses to increased pCO2 from short-term experiments scale up for these two species. This implies that evolutionary change is unimportant (has no effect) or unlikely (does not occur) on this timescale. This is surprising, given that microbes frequently evolve over hundreds of generations. Given the mutational supply in this system (population size x mutation rate), there is certainly enough variance for natural selection to act, at least in principle. Yet it apparently does not. There are several explanations for this that I would like the authors to at least touch on, though an in-depth discussion is beyond the scope of this paper. First, the cultures were grown as vegetative diploids, making the expression of novel genetic variants unlikely because individuals bearing new mutations will be homozygous for them, so that only the subset of novel mutations that are dominant would be detected. However, natural populations presumably have a) sex and b) a haploid phase, both of which would make the expression of new mutations faster by a) creating homozygotes through heterozygotes mating or b) allowing mutations to be expressed in haploids. The experimental setup used here is strongly biased against detecting genetic change. Second, previous work (in a haploid, where it was more likely that novel genetic change would be detected), has shown that evolutionary responses to CO2 enrichment are largely neutral with respect to fitness. Because evolution is not adaptive, the growth rate of populations that have evolved at elevated CO2 for over 1000 generations is the same as that of populations that have only acclimated for a few days, even though the phenotype of the evolved populations is attributable to genetic change (Collins and Bell, 2004).**



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We added a new paragraph dealing with these issues in the discussion (section 4.5).

**Finally, the level of replication in this experiment makes it difficult to measure small changes in fitness (growth rate is usually a reasonably proxy for fitness), so that even if some amount of genetic change is occurring, it would be hard to detect if it is small relative to the acclimation response. The reduction in growth rate for E. huxleyi seems small (0.1) and the error bars for the control and treatment appear to overlap, since both have a s.e. (or s.d.? please clarify) of 0.06. Please add some reassuring statistics, or state that the difference is non-significant. A non-significant difference is not necessarily a problem for the general conclusions, as the replication (and power) in this experiment is fairly low, the change in growth rate is arguably still biologically relevant, and the difference in growth rates for C. braarudii are clearly different.**
We added statistics and clarified the error of the growth rate as the standard deviation (1SD).

**That being said, some sort of statistical testing for differences in all measured parameters (growth rate, PIC:TPN, PIC:POC etc.) is needed, since it is not clear at all whether the the high pCO2 treatment has a small but significant effect in the E. huxleyi populations, or whether E. huxleyi really is almost insensitive to increases in pCO2. For example, in Fig 2, the range of y values occupied by the open and closed symbols appear to overlap for some (or most) of the time points in all of the traits measured.**
We added statistical analysis (binomial test) in the result section.

**Minor comments:**
**Since this work will be of interest to non-oceanogaphers, please add the detail that the species were grown as asexual diploids. Please also state the minimum**



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**population sizes (not just population densities) during the experiment. These details are important in assessing the chances for genetic changes to be expressed and to fix in the populations on this timescale.**
We added details in the methods section:

*2.1 Cultures:*
*...Both cultures were grown as asexual diploids at* $16^{\circ}\mathrm{C}$ *in* $0.2\,\mu\mathrm{m}$ *filtrated North Sea water with a salinity of* $33$ *and f/20 nutrient additions (Guillard, 1975), corresponding to* $88\,\mu\mathrm{mol\,l^{-1}}$ *nitrate and* $3.6\,\mu\mathrm{mol\,l^{-1}}$ *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* $140\,\mu\mathrm{mol\,photons\,m^{-2}\,s^{-1}}$ *(Philips TL-D 90 DeLuxePro, 36W/950) under a 14:10* $\mathrm{h}$ *light:dark cycle.*

*2.2 Experimental setup:*
*... 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 medium (f/20 nutrient conditions and the 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). ...*

**A point that the authors may or may not wish to address is that growth rate (and so presumably fitness) drops in response to increases in pCO2. Though the populations are apparently unable to adapt (increase their growth rate) over the timescale of this experiment, it does suggest that, in theory, there is the possibility for fitness recovery in coccolithophore populations growing at high pCO2, since we know that at high pCO2, the cells are not up against some sort of physical limit of how fast they can divide. Adaptation (and so a return to**



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**higher growth rates) could be possible with a higher mutational supply (larger populations) and/or once sex and a haploid phase (both of which allow natural selection to act more effectively) are taken into account.**
We included discussion on this (section 4.5).

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