**Biogeosciences
Discussions**

Comment on "Effects of long-term high CO$_2$ exposure on two species of coccolithophores" by Müller et al. (2010)

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**Comment on Müller
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Abstract

I briefly discuss how tools from experimental microbial evolution may be used to measure evolutionary responses in marine phytoplankton grown in high CO_2 environments. I outline why the particular biology of marine microbes makes conventional experimental evolution difficult, and suggest that "black box" frameworks that focus on partitioning phenotypic change, such as the Price equation, may be used instead.

One of the major tasks faced by biologists today is to understand how future populations of marine phytoplankton may differ from contemporary ones. But how far away are these future populations? The answer must be decades, since most DIC manipulation experiments use projected CO_2 levels from about the turn of the next century (Barry et al., 2010). Microbes have large population sizes and reproduce quickly, which ensures a more than adequate supply of mutations for evolutionary change over decades. If some component of global change exerts selection pressure, there is also scope for adaptation by natural selection in phytoplankton. That genetic change will occur is inevitable; the question is whether evolutionary change will be an important contributor to phenotypic shifts that arise in marine algae during long-term responses to ocean acidification. This question is being addressed by at least two separate groups of researchers working in two separate paradigms, with surprisingly little dialogue between them: biological oceanographers and microbial experimental evolutionary biologists (for examples see Falkowski and Oliver, 2007; Rost et al., 2008; Bell and Collins, 2008).

The study by Müller et al. (2010) marks an important step towards explicitly incorporating the possibility of genetic evolution into empirical biological oceanography. As an evolutionary biologist, I read this paper with great interest, though probably with a different agenda than the intended audience. Since Müller and colleagues set up their

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cultures as a mini selection experiment (long term growth of replicate populations that were initially genetically identical under novel and control environmental conditions), I read it looking for indications of evolutionary change after a few dozen generations of growth under rising pCO_2.

5 The goal of the experiment by Müller et al. (2010) was not to measure evolutionary change, but to ask whether or not short-term physiological responses scale up. While the data show convincingly that physiological responses to DIC enrichment measured over a few generations in two coccolithophore species predict the physiology seen after tens or hundreds of generations, the authors note that the possibility of genetic change
10 cannot be ruled out. The usual way to check for an evolutionary response to selection is to either measure the ability of an evolved genotype to outcompete its own ancestor (Elena and Lenski, 2003), or to compare components of fitness, such as growth rates, in both high pCO_2 and low pCO_2 of strains grown under both long-term high pCO_2 and long-term low pCO_2 (Collins and Bell, 2004). While the first comparison is impossible
15 since the genetic tools to transform coccolithophores are not yet available, the second could be carried out by simply returning the high pCO_2 selection lines to low pCO_2 at the end of the experiment, and doing the opposite with the low pCO_2 selection lines. A second tool used in experimental evolution is to measure correlated responses (Travisano et al., 1995), which are heritable changes in fitness or physiology in non-selected
20 environments (corresponding to the phenotype of the high pCO_2 selected cultures under any conditions other than the ones they experienced during selection). Correlated responses would be expected if, for example, long-term growth at high pCO_2 favoured types that were somehow specialized for growth at high pCO_2. For instance, long-term growth at elevated pCO_2 may affect the ability of strains to deal with nutrient limitation
25 or changes in temperature. Although the authors never claim to be doing experimental evolution, basic tools from experimental evolution could be applied in this type of experiment to support or disprove the conclusion that primarily a sustained physiological response is being observed, and to verify whether or not a systematic genetic response has occurred.

The experiment by Müller et al. (2010) raises the question of how evolutionary effects might be measured in marine phytoplankton. Marine microbes are far from ideal organisms for experimental evolution. If one were to seek out an experimental system where the cards were stacked against detecting evolutionary change in the laboratory, you could take your pick of any of the major groups of eukaryotic marine algae. Coccolithophores, for example, are grown in culture as asexual diploids, making it unlikely that novel or rare mutations will be selected unless they are expressed in heterozygotes. Recessive mutations cannot be brought together in homozygotes since we do not know how to mate coccolithophores in culture. This impedes the action of natural selection in laboratory cultures (Colegrave, 2002; Zeyl et al., 2003). In addition, marine algal cultures must be grown at relatively low cell densities, and it is logistically difficult to grow the number of independent replicate cultures usually used in microbial experimental evolution to gain the statistical power to detect small changes in fitness – typically tens of cultures in each environment. For example, a fitness difference on the order of 0.01 between populations that have been exposed to high pCO_2 over the short vs. long times could easily be ecologically important, and is probably a reasonable effect size for selection in a relatively benign environment, including elevated CO_2 (Kassen and Bataillon, 2006; Perfeito et al., 2007). Growth rate is often a reasonable proxy for fitness in batch culture experiments such as the one done by Müller et al. (2010), so a fitness difference of 0.01 would be measured as a 1% difference in growth rates. However, the number of replicates needed to detect such a difference even given a very small standard deviation in fitness between replicate populations (say 0.001) is 17 independent replicates per group for a simple t-test. Furthermore, much of the power of traditional microbial experimental evolution lies in being able to have a living "fossil record" of evolving populations where samples are placed in suspended animation (usually in a freezer or on a petri dish where growth is minimal) at several timepoints during the experiment. This allows the fitness (either growth rate or competitive ability) and phenotypes of the same evolving strain at several timepoints (including the ancestral version) to be measured at exactly the same time under common conditions,

allowing direct comparisons (Elena and Lenski, 2003; for an extensive treatment of microbial selection experiments see Bell, 2008). Finally, high DIC alone probably does not impose strong selection on marine algae – when a decrease in growth rate is seen, it is small, indicating that adaptive evolution may be slow, or that neutral evolutionary
5   change may be responsible for phenotypic shifts (Collins and Bell, 2004). Because of these limitations, detecting evolutionary effects seems unlikely in marine microbes.

I do not intend to paint a grim picture or to discourage attempts to detect evolutionary change in marine algae. Tools that use "black box" approaches, such as the Price equation (Price, 1970; see partition by Collins and Gardner, 2009) or discrimi-
10   nant analysis (Okasha, 2006) could be used in marine microbes. These methods can be used to partition change in some character of a community into contributions from a sustained physiological response, interactions between lineages, and evolutionary change within lineages. Here, the outcome of competition between strains, measured as differences in community composition, could be used to uncover evolution within
15   strains, as was done in the worked example in Collins and Gardner (2009) examining the appearance of diuron resistance reported by McClellan et al. (2008). There is some evidence that selection on interactions between strains or species can act in the opposite direction from selection within strains or species, causing the effects to roughly cancel out, as was the case with the appearance of diuron resistance. This same pat-
20   tern appears in some cases of multi-strain communities of a single algal species grown under long-term CO_2 enrichment (Collins, unpublished data). Here, if only a physiological response is measured, it will apparently account for the full change in community phenotype, even though evolutionary change has occurred and must be taken into account to correctly understand the underlying basis for observed changes in community
25   function (such as biomass production, carbon uptake or PIC:POC ratios). To use either of these approaches, DIC manipulation experiments could be started with diverse communities, though these could be several different strains of a single species such as *E. huxleyii*, so long as the strains could be reliably distinguished one from another in mixed culture and changes in strain frequency could be quantified alongside the usual

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measurements of changes in physiological characters and growth rates. In this way, evolutionary effects that occur in communities could be indirectly measured.

The time is ripe for collaborations between experimental evolution and biological oceanography, two fields that have developed in near-complete isolation. Collaboration between biological oceanographers and evolutionary biologists on experimental design and data reporting could allow information and understanding from each field to be used more effectively by the other. This would improve investigations in both fields, allow us to build on each other's knowledge, and give us a better chance at answering the question of how and why phytoplankton may respond to global change over the coming decades.

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