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# Comment on “Phytoplankton Calcification in a High-CO<sub>2</sub> World”

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Iglesias-Rodriguez *et al.* (Research Articles, 18 April 2008, p. 336) reported that the coccolithophore *Emiliania huxleyi* doubles its organic matter production and calcification in response to high carbon dioxide partial pressures, contrary to previous laboratory and field studies. We argue that shortcomings in their experimental protocol compromise the interpretation of their data and the resulting conclusions.

The uptake of anthropogenic CO<sub>2</sub> by the ocean is projected to drive seawater pH in the course of this century to levels lower than have occurred over the past 20 million years (1). Despite much uncertainty about the resulting impacts on marine biota, there will be both winners and losers of ocean carbonation and acidification. Calcareous organisms will for the most part be on the losing side, as increasing seawater acidification (decreasing pH) incurs a greater metabolic energy requirement to precipitate calcium carbonate (2). Some photoautotrophic groups are likely to be on the winning side as increasing ocean carbonation [increasing CO<sub>2</sub> partial pressure (*P*CO<sub>2</sub>)] makes it energetically less expensive to obtain the CO<sub>2</sub> required for photosynthesis (3). But what about organisms that perform both photosynthesis and calcification, such as the coccolithophores? Studies conducted over the past 8 years indicate marked differences in CO<sub>2</sub>/pH sensitivities at the species level and possibly also at the strain level. In the range of *P*CO<sub>2</sub> changes projected for this century, calcification in *Coccolithus pelagicus* appears almost insensitive to seawater acidification, *Emiliania huxleyi* and *Calcidiscus leptoporus* show a moderate decline in calcification, and *Gephyrocapsa oceanica* shows a strong decline (4–11). In terms of photosynthesis, these species were either insensitive or responded to a doubling of present-day *P*CO<sub>2</sub> with a moderate increase of 5 to 15%.

In contrast, Iglesias-Rodriguez *et al.* (12) suggest that in a single strain of the most abundant coccolithophore, *E. huxleyi*, both photosynthesis and calcification increased by 100 to 150% over a CO<sub>2</sub> range from 280 to 750 μatm. This is an

order of magnitude larger than previously observed responses of marine phytoplankton to rising CO<sub>2</sub> and, in terms of the change in calcification, opposite in sign to the earlier studies, many of which manipulated the CO<sub>2</sub> system similar to Iglesias-Rodriguez *et al.* by bubbling with CO<sub>2</sub>-enriched air. As discussed below, we believe that shortcomings in the experimental protocol compromise the interpretation of these data and raise doubts about the conclusions drawn from them.

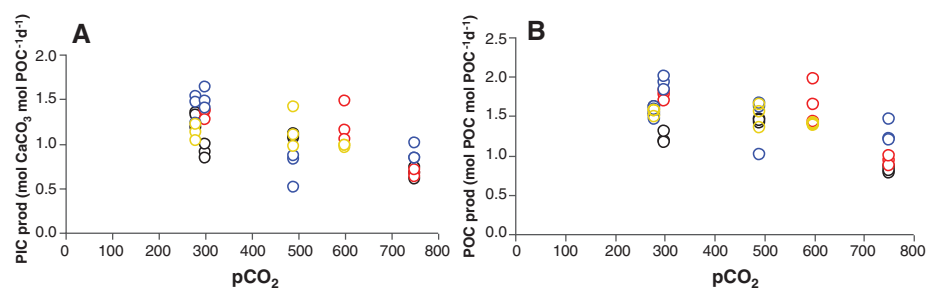
First, precultures in (12) were grown to densities of up to 500,000 cells mL<sup>-1</sup>, that is, 5 to 10 times as high as in the actual experiments (13). This large difference in cell concentrations can be expected to cause strong divergence in growth conditions between precultures and experimental incubations. For example, with a 6 to 13% drawdown of alkalinity in high-CO<sub>2</sub> experimental treatments [table 2 in (12)], a five-fold higher cell density in precultures is expected to result in an alkalinity drawdown of 30 to 65%. This would cause a severe drift in the preculture’s carbonate system, including shifts in CO<sub>2</sub> concentration and carbonate saturation. Under these circumstances, it is questionable whether preculturing allowed true acclimatization of cells to the experimental conditions.

Second, some of the precultures used by Iglesias-Rodriguez *et al.*, particularly those in

high-CO<sub>2</sub> treatments, may have experienced nutrient limitation at the time of transfer to the experimental flasks. This is suggested by an estimation of nitrate drawdown based on a cell density of 500,000 cells mL<sup>-1</sup> and values from figure 1, B and E, in (12) (1.8 pmol C cell<sup>-1</sup> and cellular C:N ~7). The calculated nitrogen demand of 128.6 μmol N L<sup>-1</sup> exceeds the initial 100 μmol nitrate L<sup>-1</sup> applied in the precultures. Nitrate limitation in *E. huxleyi* is known to increase cell size and carbon quota (14) and may also explain the larger size and carbon quota of the high-CO<sub>2</sub>-grown cells.

Third, experimental incubations lasted for only 1.5 to 3 days, allowing for about 1 to 2 cell generations. With incubation times this short, there can be little certainty that cells were actually in steady-state exponential growth, and the outcome of the experiments depended to a large extent on the cells’ preconditioning. Because growth conditions in the precultures were not monitored, but likely continued to have an effect during experimental incubations, it is uncertain which growth factors have led to the observed physiological responses. Previous studies with coccolithophores have generally allowed for a minimum of 8 to 10 cell generations under experimental conditions.

Finally, cells grown at high CO<sub>2</sub> had a carbon quota (cellular biomass) two to three times greater than low-CO<sub>2</sub>-grown cells [figure 1, A and B, in (12)]. As the experiments allowed for only 1 to 2 cell generations, the strong difference in biomass could not have developed during the experimental run, but could be attributable to the preculturing. Although the experiment was set out with two test variables, cellular biomass and CO<sub>2</sub> concentration, the former was not treated as a variable. When expressing the data on a per cell basis, as in (12), the observed trends may be related to the CO<sub>2</sub> treatment, or to the difference in cellular biomass between treatments, or both. Correcting for a possible biomass effect, for example, by normalizing the data to algal biomass, reverses the trends in calcification and primary production rates with *P*CO<sub>2</sub> reported in (12) (see Fig. 1), making their results entirely consistent



**Fig. 1.** Daily production of (A) particulate inorganic carbon (PIC) and (B) particulate organic carbon (POC) normalized to POC biomass for *E. huxleyi* cultures under different *P*CO<sub>2</sub>. Each color represents one independent experiment in (12). Calculations are based on cell numbers at the beginning and end of the experiments and cellular PIC and POC content at the time of incubation (data provided by M. D. Iglesias-Rodriguez).

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with previous studies on the CO<sub>2</sub> sensitivity of *E. huxleyi* (4–10).

Understanding what will happen to the ocean biota over the next century in response to global change is important to humanity. All efforts to augment the relatively meager experimental information on the response of marine organisms to acidification are to be encouraged, particularly if they put into question the current wisdom, but these reports must be based on sound interpretations of the available data. We contend that, to date, there is no unequivocal laboratory or field

study showing that increasing CO<sub>2</sub> causes an increase in coccolithophore calcification.

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