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Interactive comment on “Individual and interacting effects of $p\text{CO}_2$ and temperature on *Emiliana huxleyi* calcification: study of the calcite production, the coccolith morphology and the coccosphere size” by C. De Bodt et al.

Anonymous Referee #2

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De Bodt et al., have conducted a series of culture experiments to investigate the combined effects of temperature and CO_2 on the calcification and organic carbon fixation in *E. huxleyi*, the most prevalent coccolithophore of the modern ocean. They conclude, like other studies that there is a decrease in the calcification ability with increased CO_2 , and that coccolithophore size is most sensitive to temperature, becoming smaller at higher temperature.

Whilst the manuscript is largely well written, I found that a number of details about the manipulation of the data were not particularly clear and are essential to be provided

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before publication. Perhaps my biggest concern is that it is unclear which days results are used to compile each of the figures. The methods state, that samples were taken every 2-3 days for analysis of PIC and POC, chl-a, and cell density etc but in the results, and for the rest of the figures it is not clear which data from which sample are used in the figures. It is well documented that the PIC/POC ratio of cells can evolve throughout a batch culture, and so therefore it is essential that like be compared with like i.e. samples at e.g. 30% and 60% of the maximum population should be compared, or even samples with similar growth rates. Samples taken at different times from the batch culture and particularly throughout the stationary phase cannot be compared as ‘replicates’ in any realistic way. It is essential that this sort of data be presented and discussed before this manuscript can be finally published.

I also found a lot of the discussion of the PIC and POC concentrations and chl-a concentrations very unhelpful. The actual data of interest should be presented as cell-normalised in terms of C fixation rates per cell per day (either for PIC or POC). There is quite a lot of unnecessary discussion about increasing POC, PIC and chl-a concentrations through an exponentially growing culture!

A few smaller comments:

I think it would be very helpful to include a table which details the carbonate chemistry parameters both at the beginning and the end of the experiments (i.e. DIC, TA, pH, pCO₂, CO₃²⁻, HCO₃⁻ and saturation state) just so that the reader is able to see what changes in saturation state occur with temperature etc and one can independently see how these parameters change throughout the experiment.

I am interested in what was done regarding agitation of the cultures. Mixing of the cultures is essential both during the experiment and at a minimum before sampling, but there are little details regarding what sort of shaking or disturbances were applied.

On Figure 2, I think that cell normalised chl-a should be added as a series of panels.

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Section 3.4 The comparison of coccolith morphology is interesting. I wonder if there is a compilation of the standard “expected” rates of malformation in a laboratory culture. Certainly these liths can be produced in the field and seem to be a standard feature of coccolithophores not always getting it right. I also wonder about the degree of subjectivity in the 4 categories of malformation which are presented.

Section 4.3: The authors have some apparently robust differences between the size of the cells in each of the treatments particularly with respect to temperature. However, it appears they have not really explored their data to the full here. Do the values from the Coulter Counter sizer agree with what can be measured on SEM. Did they attempt to add a few drops of acid to the cultures being counted and sized which can eliminate the liths and yield and lith-free cell size? This would allow us to know really whether the changes in cell size reported are due to changing lith numbers on the cells, or due to a fundamental change in the organic cell size.

Section 4.4 The authors still talk about the apparently contradictory results from Riebesell et al., 2000 versus Iglesias-Rodriguez, 2008. There is an additional paper from Langer et al., 2009 which addresses strain-specific affects of different *E. huxleyi* strains under different CO₂ conditions and it appears that strain selection in culture may go some way to reconciling some of these differences. I would suggest that a reference to this paper is useful here.

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