

## ***Interactive comment on “Dissolution of coccolithophorid calcite by microzooplankton and copepod grazing” by A. N. Antia et al.***

### **Anonymous Referee #3**

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Dissolution of calcite biologically produced in the surface ocean is an important process within the global carbon cycle. Understanding and quantifying coccolithophorid calcite dissolution through grazing by microzooplankton and copepods would therefore be an important contribution to the field of Biogeosciences. This is the aim of the research conducted by Antia and co-workers. Unfortunately, too many questions and issues remain after reading their paper to state that they managed to reach their aim. Overall, the revisions I advice below are beyond the regular and a renewed submission and new independent review should be carried out. If the authors chose to leave the manuscript as it stands, then I advice that the manuscript is rejected. General and some detailed points are given below to support this advice.

Considering the materials and methods section: this should be rewritten with the aim

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that a reader could reproduce the experiments and calculations. It should also be motivated why certain choices were made, such as for the organisms used, experimental conditions (e.g. salinity), etc. and what the limitations are of the methods used. On P4 L11 it is stated that "experiments were always conducted during exponential growth" but this statement is not supported by the control-run results (Figures 1a, c, e). Furthermore, it is unclear where and how the grazers were obtained, if the treatment "to adjust the food vacuoles to the coccolithophorid" (P4 L16) affects the extrapolation to grazing the world's oceans, and what the quality of the population of *Emiliania huxleyi* at the end of all experiments was (more about the latter point below). For the mesocosm experiments, a clear summary should be given in order for the reader to understand the method. Also, results presented elsewhere but relevant for the current paper should be given; which *E. huxleyi* grazers were present in the mesocosm, for example?

Considering the experimental data on the cell counts in Figure 1: firstly, I could not find how the authors can distinguish life and dead algae in the Coulter counts and, if not, how they corrected for this in their calculations on growth (and calcification) rates. The numbers of cells measured at the end of the microzooplankton experiments (when all organisms were dead, P7, L1-4) suggest no distinction could be made. Secondly, the three controls (filled circles in Figures 1a, c and e) should form a triplicate. The control in Figure 1e, however, has 5 times more *E. huxleyi* at the start of the experiment than the other 2 controls and than initially added. This discrepancy is not explained in the paper. It is mentioned that the growth of *E. huxleyi* outcompeted grazing by the copepods to such extent that the flasks of this set of experiments was placed in the dark 48 hrs into the experiments. Assuming that the start of the experiments was when the copepods were added to the flasks with *E. huxleyi* (this is not defined in the Materials Methods section), the outcompeting growth of *E. huxleyi* cannot explain the discrepancy mentioned above. Thirdly, it is unclear to me why 50 *Brachionus plicatilis* that ingest an individual *E. huxleyi* per 2 hours are not outcompeted and 30 copepods that ingest one individual every 20 minutes are (assuming both sets of experiments did start off with a similar number of *E. huxleyi* cells). Moreover, I assume the "residence

time" of *E. huxleyi* in the organisms is an average over the entire experiment (again no explained in the text). Considering the fact that, at the end of the experiments with microzooplankton all organisms were dead (p7, L 1-4; and what about the controls?), this average is meaningless. For illustration: in the first 48 hours of the experiment with *B. plicatilis*, using a 2-hour ingestion time, 1200 individuals should have been ingested, but Figure 2c learns that 2000 individuals were lost, and this number does not include newly grown individuals. Clearly, the reported "residence times" are upper estimates. Lastly, the effect of keeping the flasks of the copepod experiments in the dark on the growth of the algae, and the fact that the control-in-the-dark shows a similar trend as the other controls, is nowhere discussed in the paper.

Considering the calcium data, it is unclear how the measured calcium concentrations are back-calculated to biogenic calcite, and which assumptions were made. The same holds true for the "specific" growth and grazing coefficients. In particular it should be motivated if and why coprophagy can be ignored. Also, error bars should be plotted.

Considering the SEM images: the information the authors claim to see should be more clearly presented in enlarged images and arrows should indicate the details referred to in the text (where's the organic membrane and the inner tube, where are the spines?). I suggest to enlarge the inset in Figure 2f to full size and to zoom in on Figures 2 b and d. Also, the authors mention they observed coccolithophore-filled vacuoles in *B. plicatilis* (P4 L23). It would be most interesting to see images of these vacuoles, also for *Oxyrrhis marina*.

Considering Figure 3, it is impossible to understand what these graphs mean since it is unclear what the coefficients are that are plotted. Furthermore, I do not see the correlation mentioned in the text (P8 L4) in Figure 3a.

Considering Figure 4, the caption mentions that the data for the three mesocosm experiments (at three different CO<sub>2</sub> partial pressures) are plotted, but this is not visible in the plot. Presumably, the peak in the SS<sub>cells</sub> is the bloom referred to in the text? This

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should be specified (in the text). Since the value of  $SS_{cells}$  was obtained using  $10^{-6}$   $\mu\text{mol Ca/cell}$ , it may be worthwhile to mention that using the values for grams of calcite and the number of liths per *E. huxleyi* ([www.noc.soton.ac.uk](http://www.noc.soton.ac.uk), the "Ehux homepage" of Southampton University, UK) gives a value of  $2 \times 10^{-7}$   $\mu\text{mol Ca/cell}$ , a factor 5 less than the value used here.

Considering the discussion, the first paragraph is merely a review; it could be merged with the introduction. In the second paragraph (P10, L4-5), the authors pick and chose to compare their results only to the amounts of dissolution obtained in one model case and ignore literature data that are in disagreement with their results (Harris, 1994; and what about Langer et al., 2007?). The authors should discuss all available data on this subject and discuss the disagreements. Lastly, on P13 L1-12, the discussion from the last paragraph of the results section is unnecessarily repeated. These lines can be deleted and the reference to Paulino et al. moved to P8 L19.

One last point I would like to raise is: what is the expected effect of grazing on a population of *E. huxleyi* (for example, faster growth of the population in response to grazing)? This should be taken into account when using controls without grazing organisms in the calculation of the effect of grazing on a parameter such as calcite loss.

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