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Biogeosciences Discussions

Interactive comment on "Individual and interacting effects of pCO_2 and temperature on *Emiliania huxleyi* calcification: study of the calcite production, the coccolith morphology and the coccosphere size" *by* C. De Bodt et al.

Anonymous Referee #1

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General comments

De Bodt et al. present results on the physiological response of the coccolithophore Emiliania huxleyi to changes in the seawater CO2 concentration and temperature condition. The experiment was conducted with monospecific batch cultures under two temperatures and two, resp. three, CO2 concentrations achieved by continuous aeration with the target CO2 value. Growth and bloom development was allowed freely to evolve till the end of the experiment, whereby samples for physiological parameters and coccolith morphology were repeatedly taken. In general, the results of this study confirm

previously published data on the response of E. huxleyi to elevated CO2. Additionally, this investigation provides a new laboratory data set on the coccolith morphology in response to CO2 and temperature. However, even though the authors provide a new data set on coccolith morphology, I am afraid that the current state of the manuscript does not justify publication in BG due to some major concerns, including some regarding the experimental setup and data analyses. Unless the authors can provide additional data on the carbonate system and invalidate my concerns (listed below) I recommend rejecting the present manuscript.

Specific comments

Carbonate System: As I guess, the cultures were continuously aerated with the target CO2 value throughout the experiment. This is not clearly stated in the method section and no carbonate system parameters are reported. Additionally, please report the flow rate of the CO2 gas stream. It is essential and necessary to report at least two carbonate system parameters! The authors measured total alkalinity and acknowledged for DIC and pH measurements, therefore these data should be available and reported. Especially, since the cell densities were high and capable to change the seawater carbonate chemistry.

Statistics and data analyses: I was left wondering why the low pCO2 / 18°C condition was not included into the study design. Such a full 3x2 design is desirable for several reasons. The most prominent one would be that all data can be analyzed by means of a two-factorial ANOVA, instead of the multiple (and sometimes unnecessary and even unwarranted) testing procedures on the same data points. As a negative consequence, the result section in its present state is a tedious reading task. At the very least, the authors need to rework the whole section more systematically and add an argument for why the design was incomplete.

The statistics lack details necessary for evaluation: Please report degrees of freedom plus the associated test values (t-values or F-values, respectively).

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For each treatment (total of five) duplicates provide data from a “starting day” x to an “ending day” z. Thus, analyses are based on y=2*(z-x) data points (per treatment). At first glance this may (or may not) justify the use of parametric tests (like the t-test or ANOVAs). However, due to the necessary dependence of data points, aggregating them and using non-parametric tests might be the better way.

Page 11138, lines 11-15: I assume that in line 14 “low” should be “future” (otherwise the same difference would be significant and non-significant). However, the significant effect of the one-way ANOVA is pretty unsurprising given the differences already evaluated with the t-tests. In fact, this analysis does not provide any news. This is an instance where the opposite way is of more value: first the ANOVA, second (if the ANOVA was significant) follow-up tests to explore the source of the ANOVA (main) effect.

Page 9, line4-7: I do not understand what the authors mean “by comparing the slope of the significant linear regression”.

Growth and ‘health’ of Emiliania huxleyi: I have doubts regarding the ‘health’ status of E. huxleyi since the growth rates indicated in Table 1 (max. μ = 0.1 d-1) are far to low for the experimental temperature and light conditions. At similar conditions Buitenhuis et al. (2008) report growth rates of approx. 0.8 d-1 at 13°C and 1.2 d-1 at 18°C under nutrient replete conditions. “Aged surface post-bloom seawater” without addition of micronutrients was used as growth media, could this have lead to a limitation be one or several micronutrients? Possibilities leading to this low growth rate should be discussed as well as the effects of phophate limitation during the stationary growth phase.

Definition of the exponential growth phase and the ‘calcification phase’: How was the exponential growth phase defined and on what basis where the data points pooled to calculate the regressions of POC and PIC per cell in Table 2? There are contrary statements since on page 11135, line 20, it is written that: “The duration of the exponential

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growth phase varied between 7 (low CO2/13°C treatment) and 15 d (present and future CO2/18°C treatment) from one culture experiment to another." but Table 2 indicates a exponential growth phase for the low CO2/13°C treatment of 26 days (d8 to d34); there are similar contraries for the other treatments.

The 'calcification phase' was defined when the calcite saturation state (Omega) was above one (Table 2 caption). Does that mean that on the other days (which are not included in the calculation of PIC per cell) Omega was below one?

Nutrients and total alkalinity: Nutrient consumption was used for alkalinity correction. How were the nutrients measured and can you provide data on the nitrate and phosphate consumption under the different CO2 levels and temperatures.

Figure 7 and calcification rates: The reported calcification rates of <1.1 pgPIC per cell and day (derived from Fig. 6) are far lower than commonly reported in the literature ($\sim$10pgPIC per cell and day). Unfortunately, the authors provide no discussion point on that, rather for the concomintant low POC production rates (since a PIC:POC ratio of $\sim$ 2 is reported). Please plot the error bars (1SD) for the individual data points in Fig. 7.

Page 11140, line 23: "The smaller size of coccosphere at 18°C (Fig. 6) is likely to be at the origin of the lower chl-a per cell ratio at higher temperature." Why should lower chla content per cell lead to a smaller size of the coccosphere. What about the organic and inorganic carbon content of the cell?

Page 11142, line 22:"Lower PIC levels at high pCO2 could be explained by: (1) a lower calcite content per coccolith (2) a decrease in coccolith number per coccolithophore cell or (3) a decrease in coccolith production rate, all of them not mutually exclusive." All three points can be summarized as a decrease in the calcite production rate, what leads to 'lower PIC levels'.

Page 11141, line 19: "Nevertheless, in some treatments corresponding to future con-

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ditions (future CO2/13°C, present CO2/18°C, future CO2/18°C), higher POC concentrations than expected from the Redfield stoichiometry were measured. Indeed, a consumption of 32 μmol L$-$1 of nitrates induces a production of 212 μmol L$-$1 of POC (or 2.5mgL$-$1 POC). This suggests the occurrence during these experiments of carbon overconsumption, which refers to a continuous uptake of DIC by phytoplankton after nutrient exhaustion (Banse, 1994). Carbon overconsumption could lead to the exudation of carbon-rich dissolved organic matter (DOM) which can aggregate to form extracellular particulate organic matter (POM) (Schartau et al., 2007). This implies an increase in extracellular release of primary production at the expense of cellular biomass due to increased CO2 levels." This paragraph is very confusing and it took me some time to understand it. Please clarify this paragraph. Anyway, what is meant be the "extracellular release of primary production"?

Technical comments

Report the salinity

Be consistent in reporting the unit of POC and PIC, it switches between 'gram' and 'mol'.

Fig. 2: Clarify the legend, diamonds are missing.

Fig. 2 caption: "Squares and diamonds represent the duplicate culture experiment". There are no suares in this figure.

Page 11139, line 9: "No interactive effect of pCO2 and the temperature was..." Change 'interactive' to 'interaction'.

Fig. 6 caption: What is meant by the maximum and minimum mean values (how are they calculated)?

Cited literature: Buitenhuis et al.: Growth rates of six coccolithophorid strains as a function of temperature. Limnol. Oceanogr., 53(3), 2008, 1181–1185.

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