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Biogeosciences Discussions

Interactive comment on "Influence of CO_2 and nitrogen limitation on the coccolith volume of *Emiliania huxleyi* (Haptophyta)" *by* M. N. Müller et al.

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General comments

The authors report on experimental results of the coccolithophore Emiliania huxleyi that was grown under nutrient replete (+N) and nitrogen limited (-N) conditions, both under a similar range of pCO2 levels / CO2 concentrations in the culture media. Each set of experiments was subjected to three concentrations of CO2 (corresponding to a "pre-industrial" lower-than-modern pCO2 of ∼200-280 μatm, and two higher-than-modern levels, ∼450 and 1000 μatm).

In their Introduction, the authors mention the fact that most "ocean acidification" ex-

periments to date have been done under nutrient-replete conditions, and that E. huxleyi's physiological response is rather uniform, despite strain-specific differences (e.g. Langer et al., 2009; Findlay et al., 2011). Fewer physiology studies exist on the same species (and strains) grown under nutrient-limitation in combination with variable/elevated CO2. Here, we are thus offered a "direct" comparison between the two (or, rather, 6) scenarios - on one single strain. Still, some fundamental differences are inherent to comparing batch and chemostat cultures, both methodologically and in terms of the physiological state of the algae.

Another interesting aspect of this study is the data on coccosphere/cell diameter (μm) and individual coccolith volumes (μm3), as calculated from Coulter Counter analysis. The rationale to look at these parameters appears fuelled by the fact that paleoceanographic studies, that aim to reconstruct past changes in coccolithophore productivity and calcification, primarily rely on the calcite fossils of this group, which are generally single coccoliths and rarely fossilized intact coccospheres. Since coccolith size and its volume (and thus weight, if multiplied by the density of calcite) does not necessarily reflect calcification rate (which can only be estimated through growth experiments; PIC production rates), valuable insights should be gained by the presented approach. The authors conclude that coccolith volume is best correlated with the coccosphere/cell diameter, but that coccolith volume is not correlated to PIC production rate. To explain variations in sphere/cell diameter, the authors argue that these variations are "presumably related to lengthening and/or compression of certain phases in the cellular division cycle (Müller et al., 2008)." But it's rather disappointing that this is not further explained or discussed - in light of the different phyisological states that the batch (exponential growth) and chemostat (stationary growth) cultures represent.

Specific comments

1. Batch vs. chemostat cultures

If I understand correctly, the batch and chemostat culture vessels were identical (filled

to 1.8 litre) (p. 4983, l. 13-14), and the culture media prepared in the same way to attain the three different CO2 target values (aeration or "bubbling" with mixtures of pure CO2 and CO2-free air during 4 days, prior to inoculation of E. huxleyi cultures). The pre-cultures were not acclimated to the CO2 concentrations before inoculation; but the authors are quick to state that this is not an issue, that "over the course of 5 or more generations" the algae would be fully acclimated to the conditions at the time of sampling (p. 4983, l.5-11). It is not made clear if, nor how, the pre-cultures for the N-limited chemostats were treated (did acclimation also start first after inoculation? If so, the algae has to acclimate to both -N and different CO2, any comments on the rationale here would be informative).

Nutrient-replete batch cultures were conducted in triplicate, whereas the nitrogen limited continuous/chemostat experiments appear to not have been replicated (as indicated by lack of SD values in Tables 1-3). Therefore, statistical power is much weaker for the chemostat series. Also, some cellular production rates and ratios data are lacking for C1, but why is not mentioned in Table 2 - what happened?

p. 4990, lines 6-8; Given Fig. 3 and Table 4, I cannot judge whether the Coulter Counter data reported for the chemostats reveal significant "trends" within the range of tested CO2, or not. Without replicates, I'd be very reluctant to state, as the authors do, that such trends exist under N-limitations.

2. Size, volume, weight, statistical treatment

The authors conclude that "coccolith volume was found to be primarily a function of the coccosphere/cell diameter both under nutrient replete and nitrogen limited conditions". Given the data in Table 4 and Figure 4, again, I would argue that the relationship is not convincingly demonstrated under N-limitation in the chemostats: Table 4 lacks any significance statistics (because of no replicate experiments) - assuming the p- and F-values given for the batch set is from a one-way ANOVA (as in Table 2)? The lith volume to sphere/cell diameter chemostat triangles look like a flat-liner, non-relationship. (see

also p. 4990, lines 6-8, for similar question marks re. the pCO2 and coccolith volume in chemostats).

A relationship between sphere/cell diameter and coccolith volume seems evident for the batch series (Fig. 4), but by definition it cannot be a linear relationship, since you compare μm-units to μm3-units. Coccoliths are not spherical, but flat elliptical discs, and in case of E. huxleyi the volume will even be less than a "full disc" due to the space between individual coccolith elements - so that the data presented in Figure 4 indeed appear to "fit" a linear regression (as shown). The authors should discuss this (and why it may be the best one can do for a first assessment of the data), and highlight that this relationship is expected to (drastically) change given more data on additional E. huxleyi strains / morphotypes and other species (as, for example, suggested by the data in Beaufort et al., 2011).

Clearly, future studies should also provide biometric data of individual coccoliths (SEM or LM) - so that we can truly test the Equation (2) (recast from that in Young & Ziveri, 2000), in similar fashion as was recently done by Cubillos et al. (2012) on fossil and modern Coccolithus pelagicus (sensu lato). In other words, is coccolith volume primarily affected by changes in size (maximum diameter), or by changes in the morphology/thickness of individual elements? Therefore it's a real pity that there's not more SEM evidence - not only to verify whether the batch cultures rendered lith sizes >4.5μm, but also to verify if the chemostat rendered smaller liths (according to the Young & Ziveri 2000 recast equation, given same shape factor of 0.2 and Table 4 CC volume, they would have been $\sim$3.5μm).

Fig. 5: May we see some SEM images from the chemostat experiments as well? In the debate of size vs. volume (weight), it would be instructive to see how well- or malformed the individual coccoliths were under these growth conditions.

3. Process-based interpretations

As mentioned above, it is a bit disappointing that the physiological reasons behind

the observations remain unexplained. The obvious differences in cell size and growth rate between the batch (+N) and chemostat (-N) would give some hints as to whether "certain phases in the cellular division cycle are lenghtened and/or compressed", or if not, why not? Please expand this discussion.

That said, maybe we should accept these experimental results as what they are: an interesting data set that raises important questions and offers a good starting point to discuss what parameters should be routinely measured, and how, in future. In my view, it's a real shame that the "wrong" filters and storage problems apparently precluded any detailed microscopy (SEM or LM) which is clearly the common methodology that (experimental) marine biologists and paleoceanographers share in their quest to better understand calcification in coccolithophores and other prominent marine calcifiers.

Technical corrections

2.2. Experimental setup You mention "The target pCO2 value (see Table 1)" - where Table 1 gives the final values as attained in each experimental set up. The final pCO2 value may deviate from the target value for several reasons (e.g. the low pCO2 in the chemostat reservoir tanks and C1 appears much lower than that in the batch B1), so I'd suggest to mention the 3 target values in this paragraph (also because it reads nicer), and then report Table 1 under the results section.

The language has certainly improved since the earlier version of this ms. Some textual issues remain, and I recommend a careful read by the authors and a native English speaker. This list of examples is not exhaustive:

p.4980, l. 25: replace "are subsequently" by "have since been" (or similar phrasing)

p. 4981, l. 4: referred to "as" ocean carbonation/acidification ...

p. 4986, l.1: "f/20, excepting the nitrate concentration" - "except for" or rewrite into two sentences.

p.4990, l.9/10: reshuffle sentence structure to "Production rates ... were decreased by

over 50%, under equilibrium .... compared to ..."

Table 3 caption: "phosphor" = "phosphorus"

References

Cubillos, J.C., Henderiks, J., Beaufort, L., Howard, W.R., and Hallegraeff, G.M., 2012, Reconstructing calcification in ancient coccolithophores: Individual coccolith weight and morphology of Coccolithus pelagicus (sensu lato): Marine Micropaleontology, v. 92-93, p. 29-29.

Other cited references as in reviewed manuscript.

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