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Interactive comment on "Environmental controls on the *Emiliania huxleyi* calcite mass" *by* M. T. Horigome et al.

J. Henderiks (Referee)

jorijntje.henderiks@geo.uu.se

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This review benefited from discussions with Manuela Bordiga (Uppsala University). Note also that at the time this review was requested, two comments were already posted in Open Discussion (A. Poulton et al., Interactive Comment 29 July 2013; Anonymous Referee, 5 September 2013), and I take the opportunity to refer to some points of view I share, or can add to.

GENERAL COMMENTS

The manuscript by Horigome et al. addresses an important, and still largely unresolved issue: how should we interpret the variation in coccolith mass [including regional differences and temporal changes] as measured in fossil [and modern] assemblages?

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Interactive Discussion
Discussion Paper

The authors present this issue in light of ongoing ocean acidification and the suite of experimental results that have revealed species-specific and strain-specific calcification responses to a range of environmental variables. Experimental set-ups tend to be restricted to one or few strains of individual species, and specifically test calcification (and other physiological) responses to one or few selected variables – for which statistical tests then help identify possible causal links between the targeted biological process(es) and environmental variable(s). However, in natural settings the challenge is much greater, if we want a process-based understanding of the observed relationships/correlations between coccolith mass and environmental factors, rather than focusing on a transfer-function approach* alone.

This paper appears to be geared towards the former objective, to explain "environmental controls" on "Emiliania huxleyi calcite mass", but despite an impressive dataset (possible caveats aside), it doesn't completely deliver on that specific objective: identifying "what controls Emiliania huxleyi calcite mass". The authors conclude: "the combined effects of nutrients, temperature, and secondarily seawater carbonate chemistry control coccolithophore calcification" and "the balance among various environmental factors makes singular cause-effect relations difficult to be conclusively determined" (p. 9299), which I can agree with as general (and not so surprising) statements. However, why this may be the case remains under-discussed. Here, hypotheses include phenotypic plasticity and/or varying E. huxleyi morphotype composition. But first and foremost, the statistical treatment and interpretation of the data need to be clarified (and/or revised).

*For transfer functions used in paleoclimate research, a "true" process-based linkage may not be required; e.g. using the mean annual SST and/or summer SST for calibration to surface sediment foraminifer assemblages; or focusing on any of the seawater carbonate chemistry parameters that happen to correlate best with coccolith mass (cf. Beaufort et al., 2011).

SPECIFIC COMMENTS

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1. The multivariate challenge: statistical methods

Part of the difficulty in identifying "what controls Emiliania huxleyi calcite mass" is the inherent covariation between oceanographic physicochemical parameters, but multivariate statistical methods could summarize that to few(er) factors/components.

The authors present cluster analyses of (a) the mean coccolith mass in each sample (70 samples) [as a Supplement Figure S2] and (b) oceanographic variables (temperature, salinity, phosphate, carbonate chemistry parameters) INCLUDING mean coccolith mass for each sample (Fig. 3A). Then they resort to Principle Component analysis of the (b) dataset, but split into two subsets based on the cluster analysis (Fig. 3C, D).

Cluster analysis is a helpful tool to explore data, but is not a formal statistical tool as it is difficult to assign any significance level to the clusters. Cluster analysis will place all entries (70 samples) within clusters, no matter how small (or, biologically or oceanographically insignificant) the differences ("distance") between samples.

Figure S2 suggests that the samples, when based on coccolith mass alone, group into three main groups, clusters 1+2+3 (blue-purple colors), 4+5+6 (green-yellow) and 7+8 (yellow-orange-red). It would have been informative to transfer the "cluster colors" onto Figure 2b, for us to see how these groupings reflect the range of size (length) and mass (pg) in your 70 surface sediments. Such presentation would illustrate whether, based on size alone, it is reasonable (or not. . .) to subdivide the data in up to 8 clusters. We suspect 3 groupings could be argued for – which would indicate a (bio)geographical distribution that could be discussed (E. huxleyi (morphotype) abundance and other topics of interest).

By contrast, as the Poulton team also observed, the cluster analysis in Fig. 3A reveals a very different sample composition in a total of 7 clusters. We also suspect that this cluster analysis is mainly driven by the oceanographic parameters. However, our main point of critique is the fact that you include both the supposed "forcing" variables (oceanography) and the supposed "response" variable (coccolith weight) [and

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any other biological response variable, such as Chl a] in your Principle Component analyses.

In addition, the sub-selection of clusters for Principle Component analyses (Fig. 3C and 3D) is puzzling. Fig 3A clusters 5 and 6, yellow and orange, are treated separately; why was cluster 7 (red) not included, as it appears to be "closer" to the orange cluster than the orange is to the yellow?

We recommend that instead of the methodology presented here, the authors apply Canonical Correspondence analysis (same method used by e.g. Boeckel and Baumann, 2004; Marine Micropaleontology 51, 301-320; on similar samples), which is "a direct gradient analysis, where the gradient in environmental variables is known a priori and the [ecological/biological parameters] are considered to be a response to this gradient" (see e.g. http://folk.uio.no/ohammer/past/past3manual.pdf).

We recommend that you treat your dataset as one.

If you keep your (re-interpreted) cluster analysis of coccolith mass, present it in the main article, not in the Supplement.

Manipulation of the input data: How large is the difference (and statistical outcome) between the original modern carbonate chemistry and your pre-industrial adjusted dataset? Could this operation mislead interpretations in any way, also considering that pre-industrial temperatures may have been cooler than today's (and assuming that you used the modern temperature and phosphate concentrations to derive to your pre-industrial carbonate chemistry in CO2sys?)

Overall, we agree with "show and discuss all parameters" raised by the Poulton team, their points 6 and 7 (p. C3809-10).

It is not clear how the authors derive the following conclusion: p. 9299, line 9: "it appears clear that combined these changes [not sure what's meant with "these changes"?] can have profound impact [you mean significant correlation? or via

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what process?] on coccolithophore calcification, the balance [you mean covariation?] among various environmental factors makes singular cause-effect relations difficult to be conclusively determined."

In light of transfer-function applications, rephrase: "paleorecords of coccolith calcite mass should not ONLY BE INTERPRETED AS the response of coccolithophore calcification to past atmospheric CO2 fluctuations" (p. 9299, line 17-19).

Any of the relationships/correlations that can be detected have the potential to be informative, but the cautionary note should be towards the notion "correlation does not mean causation". Therefore, the authors need to carefully distinguish between "response" vs. "relationship" when discussing their results and interpretations. See e.g. p. 9298, line 13-16: "Our study provides a picture of the E. huxleyi calcification response to changing seawater physicochemical properties . . ."

2. Phenotypic plasticity VERSUS morphotypes

- Phenotypic plasticity (of single genotypes) is not the same thing as changing morphotypes (which are genetically distinct; morphotypes remain stable in culture): p. 9297, lines 24-28 seem to suggest you equate the two.

- Morphotypes cannot (or are hard to) be detected under LM, and as I understand it, you made no SEM investigations of the sediment samples. I agree with other Commenters that the coccosphere images (=plankton samples, which the Fig. caption fails to mention) illustrated next to Fig. 1 (results of sediment samples) are therefore misleading, because you don't discuss the morphotype composition of each sediment sample.

- Nevertheless, morphotype composition remains the first-order and most plausible hypothesis to explain the change in size and mass you record with the SYRACO image analysis. You need to discuss how morphotypes link with mass (i.e. both size and degree of calcification count), and discuss how you could test this hypothesis with the data you do have available.

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- Lots more literature is available on the topic of morphotype abundance relating to environmental factors (see also Poulton team's point 4, p. C3808; note Cubollis = Cubillos, Hendericks = Henderiks . . .OOPS!)

3. Weighing coccoliths

- Instead of repeating the principles of Beaufort's (2005) birefringence methodology and calibration, focus your methodology section on: why your calibration factor (assuming that is "2275.14", eq. (1), p. 9291) is different from Beaufort's (∼1000, in the 2005 publication): e.g., you have a different camera (light sensitivity, camera pixel resolution Leica DDC12DMC vs. SPOT-Flex), what are light settings, and how did you calibrate your light settings during analysis (bulb aging), what other differences matter?

- As long as your calibration and microcopy settings were consistent throughout the study, I don't share the other Commenters' views that your calibration technique could be called into question. However:

- I wonder about the fact that you used a "cellulose acetate membrane" (p. 9291, line5-6) to prepare the calibration slides (with known amounts of "pure crystalline calcite", please also comment on size and shape of used particles), but that you used smearslides (i.e. no filters) to prepare your sediment samples. How did you correct for differences in background GL between your calibration filter-background and that of glass-slide-only background for the analyses? Arguably, this could create a systematic offset between fossil GL and calibration GL, and thus in your calcite mass conversion.

- Statistically, it would be of interest if you could add two columns (one for E. huxleyi and one for G. oceanica) to Table 1, listing the total number of coccoliths measured in each sample, I assume all liths that were encountered in 50 FOVs? Ideally, you would also report on mean mass and standard deviation for each.

- The latter could also clarify how you "mix in" G. oceanica to make your comparison with the Beaufort et al., 2011 data somewhat more comparable.

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- Not every sample contains G. oceanica, but are the size/mass trends between samples similar/comparable between G. oceanica and E. huxleyi?

4. Surface sediment samples

Finally I agree with comments already raised by the other Commenters re. the possible caveats of using surface sediments:

- Poulton's team point 1), in that the authors should explain better the reasons for assuming the database holds Holocene assemblages, and what the (on average) expected sedimentation rates are in the area (and what that implies in terms of age averaging within one sample).

- Preservation: SEM evidence and other arguments would strengthen your case, see e.g. Boeckel et al. 2006 (DSR-I) and Boeckel & Baumann 2004 (MarMic) who did all coccolith counts with SEM and discuss preservational indices; assuming many of the samples used here are the same?

- Preservation/Lysocline: not all samples are "lying well above the depth of the modern lysocline" (p. 9288, line 12-13), according to your Table 1.

TECHNICAL COMMENTS

- Figure 2: include transfer functions (linear regression forced through zero also in (b)?). Arguably, the regression between size (length) and mass (volume) is not linear, but on this scale one could "approach it" as linear.

- Figure 4: Legend Beaufort et al. 2011 data – grey point could look like it's part of the data cloud; clearly separate. State what data are included in the Beaufort et al. 2011 data cloud: only plankton?

- Figure S2: note that the sample labels are shifted w/ respect to the x-axis.

Interactive comment on Biogeosciences Discuss., 10, 9285, 2013.