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

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We wish to thank Jorijntje Henderiks and Manuela Bordiga for the detailed analysis of our paper which have been very helpful and greatly improved the quality of this manuscript. A detailed reply to each comment follows below:

1. The multivariate challenge: statistical methods

Rev. Comment: 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

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(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.

Author Response: This is an excellent remark, on which basis we decide to revise our approach: in the revision the cluster analysis will only be conducted on the environmental parameters (temperature, salinity, nitrate, phosphate, Chl-a, pH, pCO2 and [CO32-]). This first analysis will help us to highlight 3 main areas (clusters) with different physicochemical properties. We decided to remove from the manuscript the cluster analysis performed on the mass of E. huxleyi since the classification only proposed to group the mass to different classes. Then the results did not allow any interpretation regarding the influence that may have the environmental parameters on the mass of E. huxleyi.

Rev. Comment: 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).

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Author Response: We agree with this comment and we decided to remove from the revised manuscript the cluster analysis performed on E. huxleyi mass.

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

Author Response: We performed cluster analysis on the environmental parameters and the E. huxleyi mass. Now, we will present the results of the cluster analysis performed only on the environmental parameters (we keep Chl-a concentrations as an indicator of the surface productivity). The results show that the samples can be divided into 3 groups (clusters): cluster #3 corresponds to the Subantarctic region, cluster #2 to the south Atlantic gyre and cluster #1 to the regions outside of these two structures and including the Agulhas Current.

Rev. Comment: 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?

Author Response: In the revised version of the manuscript the 70 samples will be divided into 3 clusters. The PCA will be conducted i) first on the entire dataset, to have an overview of the different parameters that prevail in this region and ii) secondly on the samples of the 3 clusters.

Rev. Comment: 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),

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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).

Author Response: This method may not be applicable to our purpose. Indeed, we compare 1 variable (mass of E. huxleyi) to the environmental parameters. The Canonical Correspondence analysis requires at least 2 variables to be tested against the environmental parameters.

Rev. Comment: We recommend that you treat your dataset as one.

Author Response: This will be done at least for the 1st PCA analysis conducted on the 70 samples.

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

Author Response: The results of the cluster analysis performed on the mass of E. huxleyi will not be presented/discussed in the revised version of the manuscript.

Rev. Comment: 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?)

Author Response: With respect to the modern values, the pre-anthropogenic [CO32-] are on average 32.2 units higher, the pH is on average 0.096 units higher and the pCO2 is on average 80.02 units lower. The fact that we observe a strong correlation between the modern and pre-anthropogenic values (R2=0.994 for [CO32-], R2= 0.925 for pH and R2=0.934 for pCO2) suggests that the adjustment will not lead to inappropriate interpretations.

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

Author Response: In the revised manuscript, the figure 3 will show the comparison between all the environmental parameters and the averaged mass of E. huxleyi. The possible correlations will be discussed in detail. We will add as well a second table (Table 2) showing the coefficients of correlation between the environmental parameters and the averaged mass of E. huxleyi for the 3 clusters and the entire dataset.

Rev. Comment: 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).

Author Response: In the revised manuscript, we will modify this sentence: “Finally, our study suggests that paleorecords of coccolith calcite mass should not be used in a straightforward manner to decipher the response of coccolithophore calcification to past atmospheric CO2 fluctuations” into: “Finally, our study suggests that paleorecords of coccolith calcite mass should not only be interpreted as the response of coccolithophore calcification to past atmospheric CO2 fluctuations since others parameters such as nutrient availability or calcification temperature could affect the coccolith mass. Changes in coccolith mass in the sedimentary record can be a critical factor for understanding responses to climatic change."

Rev. Comment: 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

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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 . . .”

Author Response: We agree about this point and the fact that correlation does not mean causation. In the natural environment correlations within parameters are expected. In the revised text we carefully distinguish between “response” vs. “relationship”.

2. Phenotypic plasticity VERSUS morphotypes

Rev. Comment:- 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.

Author Response: We will clarify this sentence and change it from: “An important factor to be considered when analyzing multiple inter- and intra-specific responses to environmental conditions is the coccolithophore phenotypic plasticity. For instance, distinct morphotypes within the genus Gephyrocapsa are related to surface seawater temperature shown by the distribution of selected morphotypes.” Into: : “An important factor to be considered when analyzing multiple inter- and intra-specific responses to environmental conditions is the coccolithophore phenotypic plasticity. In addition, distinct morphotypes are commonly find within Emiliania huxleyi and the genus Gephyrocapsa.”

Rev. Comment: - 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.

Author Response: The SEM images presented in figure 1 are coccospheres and they

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were added to give a visual example of E. huxleyi coccolith mass variability. We will remove these images from the revised figure 1. As mentioned above, it is almost impossible to accurately distinguish the different E. huxleyi morphotypes by light microscope. Since these samples have been previously studied for coccolithophore assemblages by SEM, we are using these published results to support and help the interpretation of our results.

Rev. Comment: - 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.

Author Response: In the revised manuscript we are referring to existing published data and observations in Boeckel et al., (2008); Baumann et al., 2004a,b.

Rev. Comment: - 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!)

Author Response: The results obtained in our study (mainly E. huxleyi mass and length) cannot be directly compared with detailed E. huxleyi morphotype abundance. We are, however, making use of existing E. huxleyi morphotype data and observations from the study region (when possible from the same set of samples) to better understand the results.

3. Weighing coccoliths

Rev. Comment:- 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

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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 smear slides (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.

Author Response: We propose to revise the section of the manuscript concerning the mass estimation as follows: Smear slides of surface sediment samples were prepared following standard procedures. We used a Leica DM6000B cross-polarized light microscope with $\times$1000 magnification fitted with a Leica DDC12DMC camera. For each sample, we took on average 50 pictures that were analyzed with SYRACO, an automated system of coccolith recognition (SYstème de Reconnaissance Automatique de COccolithes, Dollfus and Beaufort, 1999; Beaufort and Dollfus, 2004), which is able to make the distinction between the different species composing the assemblages. A morphological study was performed on Emiliania huxleyi and Gephyrocapsa oceanica. The coccolith length and width in relation to the distal shield were converted from pixels to micrometers: the pictures having a resolution of 832x832 pixels, 1 pixel $\sim$0.15 μm. The masses of single coccolith were estimated using the method developed by Beaufort (2005) based on the brightness properties of calcite particles (with a thickness <1.57 μm) when viewed in cross-polarized light.

A total of 9 calibration slides were prepared with known amounts of pure crystalline calcite particles, the same as used by Beaufort (2005). Those particles have an elon-

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gated shape with a length ranging from 1 to 5 μm and a thickness compatible with our purpose (<1.55 μm). We used cellulose acetate membrane filters and a low pressure vacuum pomp to have an even distribution of the particles. A total of 100 pictures in grey level (GL) were taken for each calibration slide. Then, for each amount of calcite, we estimated the averaged GL for 1 pixel and compared it to the averaged mass of calcite for 1 pixel (Figure 2a). It was then possible to calculate the mass of a single coccolith as follows: Mcoc = ΣGLcoc/2275.14 (1) Where Mcoc is the mass of a coccolith in pg and ΣGLcoc is the sum of the GL composing the picture of this coccolith. The constant 2275.14 is the slope of the linear regression presented in figure 2a. The high correlation between length and mass indicates a positive relation among size and mass (Fig. 2b).

As the two methods (SYRACO and calcite mass estimation) are related to the brightness of the coccoliths when viewed in cross-polarized light, a tight control of the luminosity of the microscope is necessary. Indeed, the luminosity tends to decrease as the light bulb ages. At the time of the calibration and data acquisition, the luminosity of the microscope was set to 255 for a slide with filter and 198 for smear slides. Those two values represent the optimal luminosity for which SYRACO provides the most accurate results, i.e. the specimen recognized from a filter or a smear slide present very comparable GL.

Rev. Comment: - 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.

Author Response: In order to compare our results to previously published global record by Beaufort et al. (2011), we added the G. oceanica mass results to the E. huxleyi dataset and make the average with the mass of E. huxleyi. However, in their study,

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Beaufort and colleagues presented the coccolith mass as an average of the combined E. huxleyi, the gephyrocapsids and the small placolith mass. In the revised manuscript, to make the comparison more robust, we decided to add the measurements of the gephyrocapsids and the small placoliths and average them with the mass of E. huxleyi with the data produces by Beaufort et al. (2011)(figure 4 of the revised manuscript). Those data will be provide or in the table 1 or in the supplemental material

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

Author Response: In the first version of the manuscript we present only few samples with G. oceanica results. It does not mean that this species is absent from the other samples. We just decided to limit the results to few selected samples for each cluster. This will be changed in the revised manuscript since we will add the gephyrocapsids and the small placoliths to our results instead of only adding the results obtained on G. oceanica.

4. Surface sediment samples

Rev. Comment: 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).

Author Response: One of our prime assumptions is that the age of the studied sediments range between modern (pre-industrial) and Late Holocene, thus, making the generated sedimentary record directly comparable with pre-industrial surface ocean physicochemical properties. We appreciate the remarks of Poulton and colleagues that prompted us to realize our rather informal treatment of this issue. However, we are convinced of pre-industrial age for all of the investigated material, despite not having direct age control on most of the samples analysed. For those samples in

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which 14C and/or 210Pb data are available (i.e., 1413-2, 1414-2, 1415-1, 1417-1, CD154-01-01K, CD154-02-03K, CD154-03-05K, CD154-05-07K, CD154-10-10K, and MD02-2594) modern to late Holocene ages are confirmed (Mollenhauer et al., 2004; Martinez-Mendez et al., 2010; Negre et al., 2010; Jonkers et al., 2012). In addition, the basic evidence that the remaining samples from the wider South Atlantic region are at least of Late Holocene age (and not older) comes from a couple of (nearby) dated core-tops in the South Atlantic (Mollenhauer, 2002, Mollenhauer et al., 2003, 2004, 2006, 2007), and from a number of investigated sediment cores from the entire study region (e.g., various articles in Wefer et al., 2004). The available core-top radiocarbon ages display a large variability ranging mostly between modern and about 3500 14C years (e.g., 2730 – 3470 14C years for GeoB 1112-3; Mollenhauer, 2002). A few "old" core top dates up to 8000 radiocarbon years (Mollenhauer et al., 2006) are from piston cores and probably reflect the loss of sediment during the coring process. Despite the relatively large variability in age, the available data collectively rule out the possibility that our dataset is influenced by glacial sediments (and coccoliths), which calcified under physicochemical conditions in both surface- and deep-ocean that were substantially different from the modern/Holocene (e.g., Hönisch & Hemming, 2005; Foster, 2008; Yu et al., 2010). In Table 1 of the revised manuscript, we will provide the sedimentation rates (from the nearest radiometrically dated records, or from the same sites considered in this study) from previously published studies.

We also appreciate the concern of Poulton and colleagues about potential contamination by post-industrial material. We stress, however, that ages containing bomb-radiocarbon (i.e., younger than 1950s), in contrast, were only observed in the South Atlantic in a few areas with extremely high sedimentation rates (up to > 20 cm/kyr; see compilation in Baumann et al., 2004, Mollenhauer et al., 2004). Since those areas are limited to the uppermost continental margins and shelf areas (see Fig. 4 in Mollenhauer et al., 2004), we expressly decided not to sample these sectors of the South Atlantic for our study. Hence we are confident that we only chose pre-industrial Holocene sediments. Sedimentation rates in the areas selected for the present study

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are between about 1 cm/kyr up to about 4-6 cm/kyr, which de facto rule out a verifiable influence of post-industrial sediments.

Vertical displacement by bioturbation has been extensively discussed in the literature (e.g., Trauth et al., 1997, Thomson et al., 2000, Anderson et al., 2001, Bard, 2001). If bioturbation is assumed to create a homogenized mixed layer in the sediment, the potential effect on core-top radiocarbon ages can been estimated (as has been done by Mollenhauer et al., 2007): The radiocarbon age of a homogenized layer of 10 cm thickness would be approximately 5970 14C years, if the sedimentation rate were 1 cm kyr-1. At sedimentation rates of 3, or 5 cm kyr-1, the age of the 10 cm-thick homogenized mixed layer would be 2650, or 1940 14C years, respectively. The available core-top ages up to 3500 14C years fall within the range of calculated values for sedimentation rates between 1 and 3 cm kyr-1 and would thus imply that these "old" core-top ages were the result of homogenization of the upper 10 cm of the sediment. However, these are maximum ages, indicating that bioturbation is not mixing up "much older" (i.e., glacial) sediments to the sediment-water interface.

We acknowledge that the sinking of particles is not vertically downward, because of the ocean currents. However, the flux of materials to the deep-sea is dominated by larger, organic-rich particles with sinking rates up to several hundred meters per day (e.g. Fischer & Karakas, 2009). Fecal pellets are thought to be the major carrier for coccolithophores to the deep ocean (e.g., de La Rocha and Passow, 2007). Such particles containing high amounts of coccoliths can reach sinking rates of up to several hundreds meters per day (Ploug et al., 2008). Therefore it is not surprising that comparisons of coccolithophore plankton communities with those of underlying sediments (Baumann et al., 2000, Boeckel & Baumann, 2008) have shown that the distribution of the water column coccolithophores is well reflected in the sedimentary archive, thus proving their potential as paleoecological archive/proxy systems. Hence, we conclude that it is suitable to that point X on the sea floor either correlates with a source region directly above it (if no lateral displacement is considered) or integrates the "informa-

tion" over a slightly larger area of the surface ocean (if small lateral displacement is considered).

Rev. Comment:- 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.

Author Response: The modern hydrographic lysocline is around 4300-4400 m in the South Atlantic (Broecker and Peng, 1982). Moreover, it has been shown that extremely high coccolith carbonate contents exist even down to 4700 m in the South Atlantic (Baumann et al., 2004a; Baumann et al., 2004b)! Thus, coccoliths - or at least some species as E. huxleyi or the Gephyrocapsids - seem to be very resistant to dissolution (as already observed by Schneidermann, 1973 or by Berger, 1973). The preservation of the selected samples is generally good and has been documented by SEM work in Boeckel et al., 2006 and Boeckel and Baumann (2008). Assemblages preserved in samples from water depths less than 4000 m of course might be affected by dissolution, in areas of high TOC content, as at the continental margin of SW-Africa and thus in the periphery of the Namibian Upwelling. But in the present study most of the samples are from areas far away from the upwelling-influenced high-productive areas.

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.

On the figure 2b, the linear regression is not forced through zero. Then it is true that on this scale one could "approach it" as linear.

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- 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?

The figure 4 will be revised so the data from Beaufort et al. (2011) will be clearly distinct from our dataset. The fact that the data we used from Beaufort et al. (2011) are water samples will appear clearly in the figure caption.

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

The figure S2 will be revised.

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