Morphology of *Emiliania huxleyi* coccoliths on the North West European shelf – is there an influence of carbonate chemistry?

AUTHORS RESPONSE TO REFEREES COMMENTS AND CHANGES WE HAVE MADE TO THE MS.

We have revised the ms in light of the referees comments as explained below. The most substantial changes are modification of figs 2 and 9 and insertion of extra text in section 3.3. We have also added two references. Morphometric measurements. All the alterations we have made are covered in the notes below on our responses to the reviewers.

Sebastian Meier

1) It would be good to see some more images with different relative tube width ratios in order to document the "subjective" variation in degree of calcification that the authors refer to.

- we have added extra images to figure 2

Also, it would be very interesting, how the different relative tube width values would translate into coccolith weight, as this is widely used in other studies as an indicator for coccolith calcification.

- we have added a paragraph to the methods section explaining this.

Coccolith mass has also often been used in studies of the impact of ocean acidification on coccolithophores. Young & Ziveri (2000) showed that the mass (m) of coccoliths could be estimated as $m = 2.7 \times k_s \times l^3$ where l is coccolith length and k_s a shape dependant constant. For normally calcified *E. huxleyi* coccoliths they derived a value of $k_s = 0.02$, if length is given in microns and mass in picogrammes. The profile this is based on (Fig. 3 of Young & Ziveri 2000) has a relative tube thickness of 0.3. Other aspects of degree of calcification, such as ray width appear to broadly co-vary with relative tube width (Figure 2) so we would predict that coccolith mass would be roughly proportional to relative tube width, i.e. it can be used as an estimate of k_s and specifically that $k_s = 0.07 \times rtw$

In this respect, also ray width would be of interest for the degree of calcification as well. Was this measured as well, and is this something the authors would suggest to investigate in the future?

- The relevant part of the methods section has been modified to answer this

A routine was also developed to automatically count the number of rays (elements) and measure their width. However, ray number, along with most other parameters was found to be very strongly correlated with coccolith length (r=0.92, 150 measurements) and so this did not yield useful data. Ray width did appear to be variable but the image resolution was not high enough to reliably record this.

2) On page 4543/4544 the authors state: "The neritic populations tend to be larger (Fig. 9a) and to show a decrease in calcification with size in contrast to the oceanic populations which tend to be smaller and show an increase in degree of calcification with size."

Does this mean that there is an optimum in the degree of calcification in mid-sized E. huxleyi coccoliths, and the difference between the two populations then just would be in size? Again, it would be very interesting how this translates into coccolith weight/the amount of $CaCO_3$ per coccolith.

- this comment is not very logical, there is no reason to infer an "optimum" in calcification in mid-sized coccoliths and rather obviously the combined effect of the variation in size and degree of calcification is to make mass an even less useful parameter.

Minor comment: Introduction:

P4533 L 18-26: I think a short note on how these field studies can be compared to laboratory experiments could be useful, i.e. significance of single strain observations vs. natural assemblage studies with multiple morpho-/genotypes could be useful at this point. There is also a recent study on the Holocene variability in coccolithophore weight in the North Atlantic that might be useful:

Berger, C., Meier, K.J.S., Kinkel, H., Baumann, K.H., 2014. Changes in calcification of coccoliths under stable atmospheric CO2. Biogeosciences 11, 929–944.

We have now mentioned this reference in the introduction

Berger et al. (2014) have shown that coccolith mass during the Holocene varied significantly even though CO_2 concentrations are thought to have been stable

Lennart Bach

1. Significance of results from bioassays E1 and E5 The referee suggested that there is a discrepancy between our light microscope observations which show a stable population of both cells and loose liths and our calcification measurements which indicate significant calcite production. However, the values the reviewer uses are the maximum values rather than the average values for these experiments.

- We have added a new table (table 3) giving the average values of cell numbers and inorganic carbon fixation for each experiment and the derived estimates of inorganic carbon fixation per cell per day and coccolith production per cell per day. It shows that estimated coccolith production rates for E1 and E5 are 4 and 5 liths per cell per day, rather than 20 liths per day as estimated by the reviewer. These lower rates are unexceptional and compatible with our light microscope observations.

2. Possibility of an acclimation effect in Bioassay E4. The referee suggests that the lower cell numbers in the high CO2 conditions in bioassay E4 may be due to an acclimation effect.

- we have mentioned this possibility in the revised ms

In this bioassay there is a clear inhibition of coccolithophore growth at the highest CO_2 conditions, suggesting that elevated CO_2 levels are detrimental to the growth of

Emiliania huxleyi. However this might be a short term acclimation effect and similar effects of inhibition of growth rates at high CO_2 treatments were shown by other phytoplankton during the experiments (Richier et al. this volume)

MINOR ASPECTS

1) Title: Why do you ask the question: "Is there an influence of carbonate chemistry?" when you can answer the question? Maybe it would be nicer to answer the question in the title already. E.g. "No detectable influence of ocean acidification on morphology of Emiliania huxleyi coccoliths on the North-West European shelf. I think it would be also better to call it "ocean acidification" because we have shown that there is an influence of carbonate chemistry on morphology, if conditions are manipulated extremely enough (Bach et al., 2012).

Reply: we prefer the existing title, since it succinctly describes the purpose of the study. We prefer to use carbonate chemistry rather than ocean acidification in the title since much of our data relates to natural variation in carbonate chemistry rather than ocean acidification

2) Page 4532 L. 7: It may be better not to call it "E4" because the reader does not know what that means.

- we have left the reference to E4 here (the abstract) since it may be of value to readers of the special issue who may know what E4 refers to and it does not impair the understanding of readers who do not know.

3) Page 4532 L. 22 and elsewhere: "Calcification" is a vague term. Here you probably mean calcification rates. In other cases (e.g. Page 4533 L. 20) you may mean coccolith size. It would be easier to understand what you mean if you were precise on this.

- we have reworded the txt in varios places to avoid this type of ambiguity.

4) Page 4532 L. 23-26: I do not understand this sentence. How could growth rates obscure these response? Calcification rates are the product of CaCO3 cell-1 and growth rates.

We have modified the text in this section by changing "obscure" to

“complicate”.

5) Page 4533 L. 28-29: What do you mean by “such issues”?

we have rewored this: "a project aimed at investigating the likely effects of ocean acidification in the surface ocean via cruise-based research”

6) Page 4537 L. 18-20: Would you get more useful results if you had normalized number of rays on coccolith size?

We have added the following text:

However, ray number, along with most other parameters was found to be very strongly correlated with coccolith length (r=0.92, 150 measurements) and so this did not yield useful data.

7) Page 4538 L. 24: Do you mean x-axis?

We have corrected this

8) Page 4542 L. 5-9: I do not really understand why you selected the upper 25%. It would be great if you could explain this in more detail.

We have added an extra sentence here to make this clearer.

This indicates that there is a weak tendency for an increase in calcification with size in the oceanic populations, but a decrease in calcification with size in the neritic populations. This should mean that the difference between the populations will be most apparent in the larger coccoliths. To test this the populations in each sample were sorted by size, the largest 25% (upper quartile) selected and means of coccolith length and relative tube width for these sub-samples were calculated (Fig. 10C).

9) Page 4542 L. 12: One “E” too much.

- corrected

10) Page 4543 L. 23-25: What do you mean by “muted”? By what could it be muted?

We have changed that to low

11) Page 4545 L. 1-2: What is the difference between the “net effect of ocean acidification” and the “actual effect”?

We have reworded this “whilst the net effect of ocean acidification on *Emiliania huxleyi* is likely to be detrimental the magnitude of this effect is likely to be low,”

12) Figure 3: Legend and X-axis label are missing.

we have corrected this

13) Figure 8: “Samples” is written twice

we have corrected this

14) Figure 9: I know it could be quite some work but it would look great if you could show individual symbol sizes which are related to the given numbers. That way you would immediately see where you can find large coccoliths.

We have redrafted Fig. 9 in this way