

Interactive comment on “Influence of changing carbonate chemistry on morphology and weight of coccoliths formed by *Emiliana huxleyi*” by L. T. Bach et al.

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REPLY to reviewer's' comments:

We would like to thank both anonymous reviewers for their valuable comments which helped to improve the manuscript. Please find our replies to the reviewer's' comments in the following.

1) REVIEWER #1: Page 5850, lines 5-6: This is vague and misleading. Vague, because it is unclear what you mean by “understood”, and misleading, because the phrase “...how these changes are...” suggests that it is unknown whether morphogenesis is affected at all. But you tackle the more specific, and more challenging, question

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which parameter affects morphogenesis.

REPLY: We changed this sentence to: “Calcification rates of *E. huxleyi* are known to be sensitive to changes in seawater carbonate chemistry. It has, however, not yet been clearly determined how these changes are reflected in the size and weight of individual coccoliths and which specific parameter(s) of the carbonate chemistry drive morphological modifications”.

2) REVIEWER #1: Page 5851, lines 26-27: In your particular strain. With respect to *E. huxleyi*, this is not the first study to look into this question.

REPLY: We will add the strain specification to this sentence.

3) REVIEWER #1: Page 5852, line 13: What was the number of replicates, if any?

REPLY: We analysed the data using regression analysis and therefore do not combine treatment levels to replicates. In order to clarify we will change the sentence: “For an overview of the carbonate chemistry in the experiments see table 1” to “An overview of carbonate chemistry conditions in all treatments is given in Fig. 1 and table 1. Note that each culture bottle is considered as individual treatment in our data analysis and the error bars given in Fig. 1 denotes the change of the carbonate chemistry within the culture bottle from the beginning to the end of the experiment”.

4) REVIEWER #1: Page 5852, lines 16-19: You did not add borate? If there's no borate CO₂sys will assume the wrong borate alkalinity. If you did, the TA prior to addition of bicarbonate was not zero.

REPLY: Borate was added as boric acid (H₃BO₃) as described in Kester et al. (1967). In water, H₃BO₃ reacts with H₂O to form B(OH)₄⁻ and H⁺. According to the chemical definition of total alkalinity given in Dickson (1981) equimolar amounts of B(OH)₄⁻ and H⁺ balance each other leading to no effect on total alkalinity. Thus, TA was zero prior to bicarbonate/carbonate addition even though borate was present in the culture medium.

5) REVIEWER #1: Page 5852, line 19: 2 ml of NSW per what?

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REPLY: 2 mL kg⁻¹ artificial seawater. We thank REVIEWER #1 for spotting this mistake and will correct accordingly.

6) REVIEWER #1: Page 5856, lines 6-7: Why different input parameters? Did the choice of the input parameter affect the calculated CO₂?

REPLY: We had to use different input parameters because it is not possible to measure the same input parameters in all experiments. For example TA is not measurable in constant pH experiments where organic buffer (HEPES) was used. Since we did not over-determine the carbonate system we cannot say whether the choice of input parameters affected the calculated carbonate chemistry parameters.

7) REVIEWER #1: Page 5858, lines 1-2: This number is useless. Please state how many were analysed per sample.

REPLY: On average, 82 coccoliths per sample were investigated for DSA, DSL and DSW and 36 for CAL and CAW. We will add this information to the revised version of the manuscript.

8) REVIEWER #1: Page 5858, line 23: Does that equal "per sample"? It is unclear because you do not state whether there were replicates.

REPLY: In this data analysis each data point reflects a treatment (see also REPLY to comment 3). Therefore, "per treatment" equals "per sample". We will change the sentence to: "On average, 500 coccoliths were evaluated for coccolith weight per sample" to avoid confusion.

9) REVIEWER #1: Page 5860, line 8: How many liths were analysed per sample?

REPLY: We analysed on average 27 coccoliths per sample for malformation. This information will be added to a revised version of the manuscript.

10) REVIEWER #1: Page 5863, line 23: What means "more representative"? Give numbers, ie typical sample size used in visual analyses as opposed to your new

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method.

REPLY: The typical sample size in visual comparisons is approximately 350 coccoliths per sample (Langer et al., 2011) whereas we evaluated on average 27 coccoliths per sample. This information will be added to the revised version of the manuscript.

11) REVIEWER #1: Page 5863, line 27: Why not possible for type R? Please mention this.

REPLY: The Reticulofenestra-like morphotype R has usually no slits between two adjacent distal shield elements. Therefore it is impossible to perform the described measurements of fine-scale morphological structures on this morphotype. Using the malformation equation for morphotype R would require measurement of different structures. This information will be added.

12) REVIEWER #1: Page 5864, lines 9-10: This statement cannot be made on the basis of Fig. 4 alone. Also Table 1 is not sufficient, because not the complete carbonate chemistry is given. This is crucial. Please provide the complete carbonate chemistry in Table 1. Moreover, Fig. 4 contains error bars; how were they calculated? There is no standard deviation for malformation in Table 1.

REPLY: We will provide the requested data in the revised version so that the reader can follow our conclusions. Error bars in Fig. 4 denote the standard deviation from measured mean malformation of all coccoliths of a treatment. We will add this information to the legend of Fig. 4. Furthermore, we will add standard deviations of malformation to table 1.

13) REVIEWER #1: Page 5864, line 24: Should read ". . .bound to. . ."

REPLY: We thank REVIEWER #1 for spotting that mistake and will change accordingly.

14) REVIEWER #1: Page 5865, line 2: Replace "factors" by e.g. "cellular components".

REPLY: We will change "factors" to "cellular components" as suggested by REVIEWER

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#1.

15) REVIEWER#1: Page 5865, lines 9-10: The explanation would also be feasible without H⁺ easily entering the cytosol. See Langer et al. 2006 for a discussion. The study of Suffrian et al. 2011, however, renders this explanation plausible, not merely feasible. Please make this distinction clear.

REPLY: We changed the sentence to: "This explanation seems plausible since H⁺ is known to easily enter into the cytosol of *E. huxleyi* (Suffrian et al., 2011)."

16) REVIEWER #1: Page 5865, lines 11-13: Agreed. This was actually hypothesised and argued in detail in Langer et al. 2006. Please cite the paper here.

REPLY: We will cite Langer et al. 2006 at this point as suggested by Reviewer #1.

17) REVIEWER #1: Page 5865, lines 20-23: This argument was put forth in Langer et al. 2006 for the first time. Please cite the paper here.

REPLY: We will cite Langer et al. 2006 at this point as suggested by Reviewer #1.

18) REVIEWER #1: Page 5866, line 3: Nor on another *C. leptoporus* strain.

REPLY: We changed the sentence to: "A comparison on the strain level is not possible because there is no such data on different strains of the same species available so far."
"

19) REVIEWER #1: Page 5866, line 17: Hard to judge because not the complete carbonate chemistry is given in Table 1. See above.

REPLY: We will add this information to table 1. (See also REPLY to comment 12)

20) REVIEWER #1: Page 5867, line 6: Replace "co-correlated" by "positively correlated".
REPLY: We will change "co-correlate" to "positively correlate" as suggested by REVIEWER #1.

21) REVIEWER #1: Page 5867, line 19: Why nucleation?

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REPLY: We will remove the term "nucleation" since it might be confusing in the context of coccolith size.

22) REVIEWER #1: Page 5868, lines 5-10: The range in Langer et al. 2006 cannot be narrow and broad at the same time. Please clarify.

REPLY: This paragraph will be changed. See reply to comment 23.

23) REVIEWER #1: Page 5868, lines 10-13: This conclusion is not convincing, because a similar response pattern does not imply a similar coupling. On page 5866, lines 6-8, the authors draw the correct conclusion in a comparably structured argument.

REPLY: REVIEWER #1 addresses our speculation that a similar coupling between CaCO₃ production rates and coccolith weight as the one shown in Fig 6a could also be present in other *E. huxleyi* strains or even other coccolithophore species. Our conclusion is one of the two possibilities. The other possibility is that this coupling varies in between strains and species and there is no overarching similarity. We disagree with REVIEWER #1 that the latter is necessarily the correct conclusion. Langer et al., 2009 have shown that sensitivities of calcification rates to ocean acidification are variable between different *E. huxleyi* strains. However, differences in sensitivities within a certain fCO₂ range do not necessarily result in a fundamentally different response if a broader fCO₂ range is considered. It has been speculated that the general response of presumably all *E. huxleyi* strains to a very broad fCO₂ range is similar (resembling an optimum curve), even though there are strain-specific differences within distinct parts of the entire optimum curve (Bach et al., 2011). We have rephrased this paragraph and will discuss this issue more carefully in a revised version.

24) REVIEWER #1: Page 5868, line 17: There are also published data (Langer, G. , Gussone, N. , Nehrke, G. , Riebesell, U. , Eisenhauer, A. and Thoms, S. (2007) Calcium isotope fractionation during coccolith formation in *Emiliania huxleyi*: Independence of growth and calcification rate, *Geochemistry, Geophysics, Geosystems*, 8, Q05007).

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Please cite the paper.

REPLY: We will cite the paper in a revised version of the manuscript.

25) REVIEWER #1: Page 5869, line 9: The term “ecophysiologicaly” is not ideal, because it usually means “physiologicaly”. What about “ecologicaly” or “community”?

REPLY: We will change "ecophysiologicaly" to "ecologicaly" as suggested by REVIEWER #1.

—————REPLIES TO COMMENTS MADE BY REVIEWER #2—————

1) REVIEWER #2: Page 5851, Lines 5-7; Raffi et al. (2006) reported the first appearance of *Emiliana huxleyi* at 291 ka, and crossover of *G. caribbeanica* and *E. huxleyi* (onset of *E. huxleyi* acme) at 82-63 ka (Raffi, I. et al., 2006. A review of calcareous nannofossil astrobiochronology encompassing the past 25 million years. *Quaternary Science Reviews*, 25: 3113-3137).

REPLY: We will add the dates approximated by Raffi et al., (2006) to the revised version of the manuscript.

2)REVIEWER #2: Page 5851, Lines 5-7; As authors said, currently it is usually considered that *E. huxleyi* evolved from *G. oceanica* since *E. huxleyi* and *G. oceanica* are identical in SSU rDNA sequences, and *G. oceanica* have longer fossil record than *E. huxleyi*. However, it is important to keep in mind that there is a possibility that *E. huxleyi* separated from other members of *Gephyrocapsa* (e.g., *G. mullerae*, *G. ericsonii*, *G. ornate*). Currently there is no genetic data for other members of *Gephyrocapsa* , and it is difficult to discuss about direct ancestor of *E. huxleyi*.

REPLY: We will replace '*Gephyrocapsa oceanica*' by '*Gephyrocapsa spec.*' as suggested by REVIEWER #2.

3) REVIEWER #2: Page 5852, Line 10; Please provide information of origin (sampling locality, date) and morphotype of the strain PML B92/11.

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REPLY: *E. huxleyi* strain PML B92/11 is morphotype A and was isolated in 1992 at the field station of the University of Bergen (Raunefjorden ; 60°18' N, 05°15'E). This information will be added to a revised version of the manuscript.

4) REVIEWER #2: Page 5852, Line 14:Please provide information of temperature of origin of the strain B92/11. Since the lines 13-14 of page 5870 says 'It is likely that coccolithophores are adapted to the mean temperature of their natural habitat (Buitenhuis et al., 2008)', information of temperature of origin of the strain B92/11 would be useful for interpretation of the results of this experiments. Mean temperature data can be obtained from World Ocean Atlas 2005/2009 website by NOAA for free.

REPLY: We will change the sentence to: 'It is likely that coccolithophores are adapted to the temperature regime of their natural habitat (Buitenhuis et al., 2008)'. The mean surface temperature during a *E. huxleyi* bloom at the site of isolation ranges from 10-12°C (compare e.g. Schulz et al., 2008).

5) REVIEWER #2: Page 5857; How many coccoliths per sample were measured under the SEM?

REPLY: On average, 82 coccoliths per sample were investigated for DSA, DSL and DSW and 36 for CAL and CAW. We will add this information to the revised version of the manuscript as suggested by REVIEWER #1 and REVIEWER #2.

6) REVIEWER #2: Page 5858, Line 28; I think your method is also useful for *E. huxleyi* var. *corona*.

REPLY: We will add this information to the revised version of the manuscript.

7) REVIEWER #2: Page 5861, Line 22-23; I think Fig 5 needs to be referred for this sentence.

REPLY: We will refer to Fig. 5 in this sentence as suggested by REVIEWER #2.

8) REVIEWER #2: Page 5862, Lines 2-4; Please refer the paper that showed DIC and

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fCO₂ range in the last 270 (or 290) kyr if available.

REPLY: The given numbers on DIC and seawater fCO₂ represent the approximated range *E. huxleyi* might have experienced during glacial times and possibly will experience in the future due to ocean acidification (Sarmiento and Gruber, 2006).

REFERENCES:

Bach, L. T., Riebesell, U., and Schulz, K. G.: Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliana huxleyi*, *Limnol. Oceanogr.*, 56, 2040–2050, 2011.

Brand, L. E.: Genetic variability and spatial patterns of genetic differentiation in the reproductive rates of the marine coccolithophores *Emiliana huxleyi* and *Gephyrocapsa oceanica*, *Limnol. Oceanogr.*, 27, 236–245, 1982.

Buitenhuis, E. T., Pangerc, T., Franklin, D. J., Le Quéré, C., and Malin, G.: Growth rates of six coccolithophorid strains as a function of temperature, *Limnol. Oceanogr.*, 53, 1181–1185, 2008.

Dickson, A. G.: An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data, *Deep-Sea Res.*, 28, 609–623, 1981.

Iglesias-Rodriguez, M. D., Schofield, O. M., Batley, J., Medlin, L. K., and Hayes, P. K.: Intraspecific genetic diversity in the marine coccolithophore *Emiliana huxleyi* (Prymnesiophyceae): the use of microsatellite analysis in marine phytoplankton population studies, *J. Phycol.*, 42, 526–536, 2006.

Kester, D., Duedall, I. W., Connors, D. N., and Pytkowicz, R. M.: Preparation of artificial seawater, *Limnol. Oceanogr.*, 1, 176–179, 1967.

Langer, G., Geisen, M., Baumann, K.-H., Kl Áías, J., Riebesell, U., Thoms, S., and Young, J. R.: Species-specific responses of calcifying algae to chang-

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ing seawater carbonate chemistry, *Geochem. Geophys. Geosy.*, 7, Q09006, doi:10.1029/2005GC001227, 2006.

Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of *Emiliana huxleyi* to changing seawater carbonate chemistry, *Biogeosciences*, 6, 2637–2646, doi:10.5194/bg-6-2637-2009, 2009.

Raffi, I., Backman, J., Fornaciari, E., Pälike, H., Rio, D., Lourens, L., Hilgen, F.: A review of calcareous nannofossil astrobiochronology encompassing the past 25 million years. *Quaternary Sci. Rev.*, 25, 3113–3137, 2006.

Sarmiento, J. L., Gruber, N. (Eds.): *Ocean biogeochemical dynamics*, Cambridge University Press, Cambridge, United States of America, 2006.

Schulz, K. G., Riebesell, U., Bellerby, R. G. J., Biswas, H., Meyerhöfer, M., Müller, M. N., Egge, J. K., Nejtgaard, J. C., Neill, C., Wohlers, J., and Zöllner, E.: Build-up and decline of organic matter during PeECE III, *Biogeosciences*, 5, 707–718, 2008.

Suffrian, K., Schulz, K. G., Gutowska, M., Riebesell, U., and Bleich, M.: Cellular pH measurements in *Emiliana huxleyi* reveal pronounced membrane proton permeability, *New Phytol.*, 190, 595–608, 2011.

Young, J. R. and Westbroek, P.: Genotypic variation in the coccolithophorid species *Emiliana huxleyi*, *Mar. Micropaleontol.*, 18, 5–23, 1991.

Interactive comment on *Biogeosciences Discuss.*, 9, 5849, 2012.

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