

Interactive comment on “Temperature affects the morphology and calcification of *Emiliania huxleyi* strains” by Anaid Rosas-Navarro et al.

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We appreciate the overall positive referee remarks and acknowledge the constructive comments that greatly helped to clarify a number of points and to improve the manuscript.

Below are our detailed responses to the referee's comments, including expected modifications of the manuscript:

COMMENT: I would like to recommend authors to show the reason why the three strains are chosen. This species widely distributed from tropical to sub-polar region. If the strains originated from cooler environment were chosen, the opposite relationships may be shown between production and temperature. The environmental adaptation might be important to include consideration of the result. The other ecological informa-

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tion of them are also valuable if they are available.

REPLY: We agree and have now included the information on strain choice. The new text reads “Clonal cultures of *Emiliania huxleyi* were obtained from the Roscoff Culture Collection. We selected 3 strains of *E. huxleyi*, two from the Japanese coast in the North Pacific Ocean (RCC1710 –synonym of NG1– and RCC1252 –synonym of AC678 and MT0610E–) and a third strain from the same region but of unknown exact origin and strain name, named here IAN01. Strain RCC1710 was collected off Nagasaki at Tsushima straight (Japan) and RCC1252 at Tsugaru straight (Japan), both places are strongly influenced by the Tsushima warm current. Additional information about the strain RCC1252 can be found at: <http://roscoff-culture-collection.org/>.” We selected the strains because they were collected in the tropical North Pacific and we wanted to test different strains from a single area in order to assess the plasticity within strains originating from a particular environmental setting. We also agree that strains from a cooler environment are likely to respond differently to the temperature range tested here, e.g. the optimum will probably be a lower temperature. We now include this point. In the introduction “We selected three strains of *E. huxleyi* from a single area, the Japanese coast in the North Pacific Ocean, in order to assess the plasticity within strains originating from a particular environmental setting” and in the Discussion “All three *E. huxleyi* strains investigated here displayed similar growth rate versus temperature relationships, with an optimum at 20–25°C (Fig. 1a). This is a typical range for many *E. huxleyi* strains (Watabe and Wilbur, 1966; Van Rijssel and Gieskes, 2002; Sorrosa et al., 2005; De Bodt et al., 2010; Langer et al., 2009). We expect that strains isolated e.g. in the Arctic will have a lower temperature optimum, though.” We noticed too late that the strain IAN01 was wrongly labelled at an early stage of the study. Tracing back this label we could do no more than infer that it was isolated in the same area as the other strains, but we could not unambiguously identify its name. That is why we gave it a new name. For our study it is important that IAN01 comes from the same area as the other strains. So it is unfortunate, but not critical, that we do not know its real name.

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COMMENT: P2L30 The authors indicate web site of Roscoff Culture Collection. It was a little bit difficult to find RCC1710 because the distribution was finished (The default setting was "distributed: Yes"). I thought some key information can be summarized in the text or table (e.g. ocean origins). It is also good option to show some details about strains by supplemental information. According to Dr Hagino, RCC 1252 was from Tsugaru straight (Japan) and RCC1710 was captured at off Nagasaki (Japan). Both places are strongly influenced by Tsushima warm current. What is the origin of IAN01? I think it is important facts to keep the reproducibility of the study. Any kind of description would be useful. Can the strain be distributed by request?

REPLY: We now included the information on the strains (see reply to previous comment).

COMMENT: P3L12 The water samples were collected after experimental treatment?

REPLY: Yes, during the harvesting. We have clarified this in the text "During the harvesting, samples for total alkalinity (TA) measurements were sterile-filtered (0.2 μm pore size) and stored in 25 ml borosilicate flasks at 4°C until measurements."

COMMENT: P6L28 Why tube show positive relationship with temperature?

REPLY: We clarified that point "The positive relationship of the mean tube width with temperature reflects the increased coccolith calcite quota at higher temperature. Coccolith mass and coccolith size are positively correlated. Why coccolith mass or size should increase with temperature cannot be decisively answered based on our data."

COMMENT: P7L15/P9L5 Why the malformed percentage are different among the strains?

REPLY: We clarified that point "The fact that the base level of malformations in cultured coccolithophores differs between species and strains (and also varies with time) has been recognized for many years and is now well documented (e.g., Langer and Benner, 2009; Langer et al., 2011, 2013). Also the response of the morphogenetic machinery

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to environmental factors is strain-specific (Langer et al., 2011; Oviedo et al., 2014). We have currently not enough accessory information to formulate a hypothesis why exactly one strain differs from another. That fact that they do indeed differ, however, probably reflects the high genetic diversity in *E. huxleyi*."

- Langer, G. and Benner, I.: Effect of elevated nitrate concentration on calcification in *Emiliania huxleyi*, *Journal of Nannoplankton Research*, 30, 77–80, <http://epic.awi.de/22502/>, 2009.
- Langer, G., Probert, I., Nehrk, G., and Ziveri, P.: The morphological response of *Emiliania huxleyi* to seawater carbonate chemistry changes: an inter-strain comparison, *Journal of Nannoplankton Research*, 32, 29–34, 2011.
- Langer, G., Oetjen, K., and Brenneis, T.: On culture artefacts in coccolith morphology, *Helgoland Marine Research*, 67, 359–369, doi:10.1007/s10152-012-0328-x, <http://link.springer.com/10.1007/s10152-012-0328-x>, 2013.
- Oviedo, A. M., Langer, G. & Ziveri, P.: Effect of phosphorus limitation on coccolith morphology and element ratios in Mediterranean strains of the coccolithophore *Emiliania huxleyi*, *Journal of Experimental Marine Biology and Ecology*, 459, 105–113, 2014.

COMMENT: P11L35 Control commands can be modified.

REPLY: Thanks for noticing the typo, it has been corrected.

COMMENT: P19 Some explanation about empty places might be kindly to readers.

REPLY: We have now added an explanation. "Growth rate and cellular PIC, POC, and TPN content and production of the three strains of *E. huxleyi* at different temperatures. Standard deviation of the triplicates in parentheses. Measured growth rates for extra temperatures from the pre-experiments are included, but PIC, POC and TPN were not measured for these temperatures."