Interactive comment on "Decreased calcification affects photosynthetic responses of *Emiliania huxleyi* exposed to UV radiation and elevated temperature" *by* K. Xu, K. Gao, V. E. Villafañe, and E. W. Helbling

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***Reviewer**: This paper presents an interesting set of experiments looking at how degree of calcification may affect susceptibility to UV damage (particularly of the photosynthetic apparatus) in a cultured strain of the coccolithophore E. hux. The degree of calcification of the cells was controlling by manipulating Ca2+ in the medium. This work follows on previous work by the same group looking at interactions between UV and calcification in coccolithophores grown at different CO2 levels. The experiments appear to be carefully done and the paper will not require major revisions to be acceptable for final publication. In some cases, all that is missing is appropriate qualifying text. I have listed my biggest general comments here, followed by a number of minor edits and other smaller issues:*

Response: We thank the reviewer for providing several important suggestions for improving our manuscript.

***Reviewer**: I agree that the overall experimental design using high- and low-Ca2+ medium to investigate changes in calcification is "physiologically. . . an effective way to investigate the role of calcification"(p. 860). Ecologically though, other than in the Black Sea (the Cokacar et al. 2001 reference that is given in the text here), I am not familiar with many places where coccolithophore blooms occur at low salinities. Some other references here would bolster the ecological relevance of this methodology- for instance, have coccolithophore blooms been recorded in the Baltic or other estuarine systems?*

Response: The reviewer has a good point. To the best of our knowledge there are no records of coccolithophore blooms in the Baltic Sea at low salinities. However, other blooms had been recorded, as for example *E. huxleyi* blooms in the central Black Sea

at salinity of 18, northeastern Black Sea at salinity of 15-17, and in Sea of Azov at salinity of ~11.

Action taken: Following the reviewer's advice we reworded the sentence that now it reads: "Ecologically, the reduced level of Ca^{2+} may be expected during coccolithophore blooms as observed in previous studies (Pitsyk, 1963; Cokacar et al., 2001; Mikaelyan et al., 2005) in estuarine systems where salinity was less than half the average oceanic value of ~35."

***Reviewer**: Another consideration is that the physiological and geochemical consequences of controlling calcification by lowering seawater [Ca2+] could be different than those of other limiting factors, such as changes in the carbonate buffer system. The authors have published some nice experiments on UV and CO2 interactions in the past (referenced here), but they should still be cautious about extrapolating too freely between these two different ways of limiting calcite production. Perhaps adding some text to the discussion to recognize this would be a good idea.*

Response: The reviewer has a good point and we agree with it.

Action taken: To accommodate the reviewer' suggestion we added the following: "Additionally, and for *E.huxleyi* strain CS-369, both lowering Ca^{2+} concentration and elevated CO_2/decreased pH (Gao et al., 2009) led to a decrease in calcification. However, it is still unknown whether these two different treatments have common mechanisms on regulation of calcification process."

***Reviewer**: These experiments exposed the cells to relatively intense levels of UV-A and UV-B for a very short period of time (2 hours, p. 861, Methods). This shows the responses of heavily- or lightly-calcified cells to a single traumatic UV stress event. How would their responses differ if UV irradiances were less intense, but maintained over much longer time periods (generations)? This type of lower level chronic exposure is certainly also potentially environmentally relevant. Perhaps it would be appropriate to add some consideration of this issue in the discussion section as well.*

Response: The irradiance levels used by us in our experiments were about 18.1, 43.6

and 60% of the maximum values measured in the South China Sea where coccolithophores are abundant (Ho et al., 2010; Li et al., 2011). The reviewer has a good point and it implies whether the cells respond to the irradiance levels or to the dose. Many papers have addressed this issue and in fact, *E. huxleyi* responded to the irradiance and this is why we used for the BWFs, an exposure-response model based on irradiance. The "chronic" and / or "dynamic" (i.e., reversible) effects would depend on the irradiance levels and on the presence of a threshold (of irradiance) above which effects can be observed.

Action taken: Following the reviewer's comments we added sentences to accommodate this point: "The irradiance levels of PAR, UVA and UVB used by us in our experiments were respectively about 18.1, 43.6 and 60% of the maximum values measured in the South China Sea where coccolithophores are abundant (Ho et al., 2010; Li et al., 2011). Previous studies (Guan and Gao, 2010) indicated that *E. huxleyi* response changed with the irradiance and this was the base to use BWFs with an exposure response model based on irradiance (Neale and Kieber, 2000). The "chronic" and/or "dynamic" (i.e., reversible) effects would depend on the irradiance levels and the presence of threshold values above which any effects can be observed (Helbling et al., 1992)."

***Reviewer**: P. 860 line 8-9: this is the wrong reference for the Aquil medium formulation. Instead please use: Price NM et al. (1988/89). Preparation and chemistry of the artificial algal culture mediumAquil. Biological Oceanography 6: 443–461.*

Response: OK.

Action taken: Changed as suggested.

***Reviewer**: P. 860 line 12: Coccolithophore is mis-spelled.*

Response: OK.

Action taken: Changed as suggested.

***Reviewer**: p. 861 line 9: The watts units for irradiance are obsolete and probably not needed, just present the SI units, _mol photons m2 sec 1*

Response: We agree with the reviewer that the units for PAR are normally expressed as µmol photons m^{-2} s^{-1}; however, we disagree in that the units of W m^{-2} are obsolete. This is the way to report UV radiation units, and we included the conversion for PAR to facilitate the comparison between PAR and UVR levels.

Action taken: No action was taken and we kept the sentence unchanged.

***Reviewer**:* p. 864*: Which carotenoids (or xanthophylls) from E. hux would you expect to be included in these measurements using a classic spectrophotometric method from Strickland and Parsons? I assume they include 19-hexanoyloxyfucoxanthin, and what else?*

Response: Diadinoxanthin and diatoxanthin should be included in carotenoids xanthophylls.

Action taken: No action was taken and we kept the sentence unchanged.

***Reviewer**: p. 864, line 15 and Fig 1a: This SEM appears to show a lysed or ruptured cell- is there a better picture of an intact cell without coccoliths availablefrom this treatment?*

Response: The SEM samples were treated according to Trimborn et al. (2007), and we observed that naked cells collapse after treatment.

Action taken: No action was taken and we kept the Figure unchanged.

***Reviewer**: Fig 3 and p. 865 of the text: The decreased inhibition in HCa compared to LCa treatments is not very obvious for either UVA or UVB alone, it is most noticeable in the UVR graph. Even here, the differences in inhibition are relatively small. The same is true for the declining (Fig 3a) and increasing (3b) trends with time, they may be statistically significant, but they are not very big. It would be good to mention this in the text here.*

Response: The reviewer has a point in this and we agree with it.

Action taken: We improved the sentence that now reads: "In general, the *Y* inhibition increased with increasing exposure time to UVR wavebands. HCa had significantly but slightly lower inhibition ($p<0.05$) than LCa treatments at 40 min and 80 min, but there were no significant differences between the two Ca^{2+} concentrations after 2 h. However, inhibition of *Y* due to UVA (Fig. 3A) decreased slightly but significantly with time ($p<0.001$), whereas that due to UVB slightly increased ($p<0.001$)."

***Reviewer**: P. 865, lines 26-29, and figure 4: The difference in NPQ values for HCa and LCa cells is not only "more evident" early in the incubation, it actually seems to disappear almost completely by 2 hours (Fig 4c). A more careful description of the data trends is needed here.*

Response: We agree with the reviewer.

Action taken: This part was reworded and the following sentence was added: "After 2 h, there was no significant difference between the two Ca^{2+} concentrations at 20 ^{o}C ($p>0.05$, Fig. 4C)."

***Reviewer**: p. 866, line 18: Define C/P ratio for readers here. You mean Calcification to Photosynthesis, but it could be read as Carbon to Phosphorus.*

Response: We agree with the reviewer's comment.

Action taken: We reworded the sentence that now reads: "The calcification to photosynthesis ratio (*C*/*P*) of…"

***Reviewer**: p. 867 and Fig 7: Since there were no significant treatment-related trends in the BWFs, this graph is not very useful to the paper. It could be left out and this could be stated briefly in words.*

Response: We agree with the reviewer in that there were no significant treatment-related trends in the BWFs. However, not only significant differences but the shape and weighting values of the BWFs are important for prediction of the potential impact of different levels of UVR. In fact, this was one of the questions raised by this reviewer.

Action taken: No action was taken as we think it is important to have the BWFs shown as a Figure.

Reviewer: *p. 868, line 27: This reference appears to have a typo. Is "Adams III" a correct surname?*

Response: The reviewer is right, the surname is Adams, without III. The correct citation for the author is Adams, W. W., III

Action taken: Changed.

Reviewer*: p. 869, line 22: "lose", not "loose"*

Action taken: Changed.

Reviewer*: p. 869, line 27 to p. 870: This sentence is long and awkward and should be re-written.*

Response: We agree with the reviewer.

Action taken: The sentence was changed and now reads: "Therefore, the LCa-grown cells might not need energy as much as the HCa-grown cells do to calcify due to reduced availability of Ca^{2+}. Then photosynthetic activity were down-regulated, as reflected by the lower *Y* and carbon fixation rates."

Reviewer*: p. 870, line 26: How were enzymes like phosphatases "affected" by low Ca in Shaked et al? Some elaboration is probably needed here.*

Response: We were quoting a published work by Shaked et al., 2006, where the authors showed that phosphatase activity decreased with reduced Ca^{2+} concentrations.

Action taken: We reworded the text and now reads: "Additionally, phosphatase activity in *E. huxleyi* decreased with reduced Ca^{2+} concentrations as shown in a previous study (Shaked et al., 2006)."

***Reviewer**:* p. *870-871: The authors need to be careful about drawing string parallels between these experiments with coccolithophorecultures, and calcification by corals on the Great Barrier Reef. This may be over-intepreting your results a bit.*

Response: We think that the reviewer has a very good point here.

Action taken: We deleted the sentence quoting the work of McNeil et al. 2004, and De'ath et al. 2009.

***Reviewer**: p. 871, line 12: This is an excellent point, yes E hux is very cosmopolitan species and is tremendously diverse genetically and morphologically, and various strains and species will differ greatly in their responses to temperature. The authors may want to speculate whether this could also be the case for responses to UV radiation.*

Response: Although many studies focused on mechanisms of calcification and photosynthesis of coccolithophores, very few considered the effects of UV radiation.

Action taken: Following the reviewer's advice we slightly modified the sentence to accommodate the suggestion. The following sentence was added: "Many studies focused on the effects of ocean acidification on coccolithophore (Riebesell et al., 2000; Iglesias-Rodriguez et al., 2008; Feng et al., 2008; De Bodt et al., 2010), but few considered the role of UVR in natural conditions (Gao et al., 2009)."