Response to referee #1

The authors have studied how the effects of ocean acidification, growth temperature and UV exposure interact in terms of growth, photosynthesis, and calcification in the coccolithophor, *Emiliana huxleyi*. The study complements several related studies of how these processes in *E. huxleyi* respond to various combinations of pCO2, growth irradiance and UV Treatments. The novel aspect of this study is the evaluation of these effects at temperatures chosen to bracket this strain's apparent optimum growth temperature at 20°C. The results of this study have the potential to advance our understanding of how this key species responds to the multiple changes occurring in the aquatic environment. To realize this potential, these results need to be better integrated with previous studies. In particular, the present discussion does not do much to show how these results fit into the bigger picture of how *E. huxleyi* responds to ocean acidification and UV. Several speculations are advanced which relate to specifics of this study which I did not considered justified as explained below. Thus, the discussion will require substantial revision to address these issues.

Response: We are grateful for the referee's constructive suggestions on our manuscript. We have studied the comments carefully and made a substantial revision to address the issues.

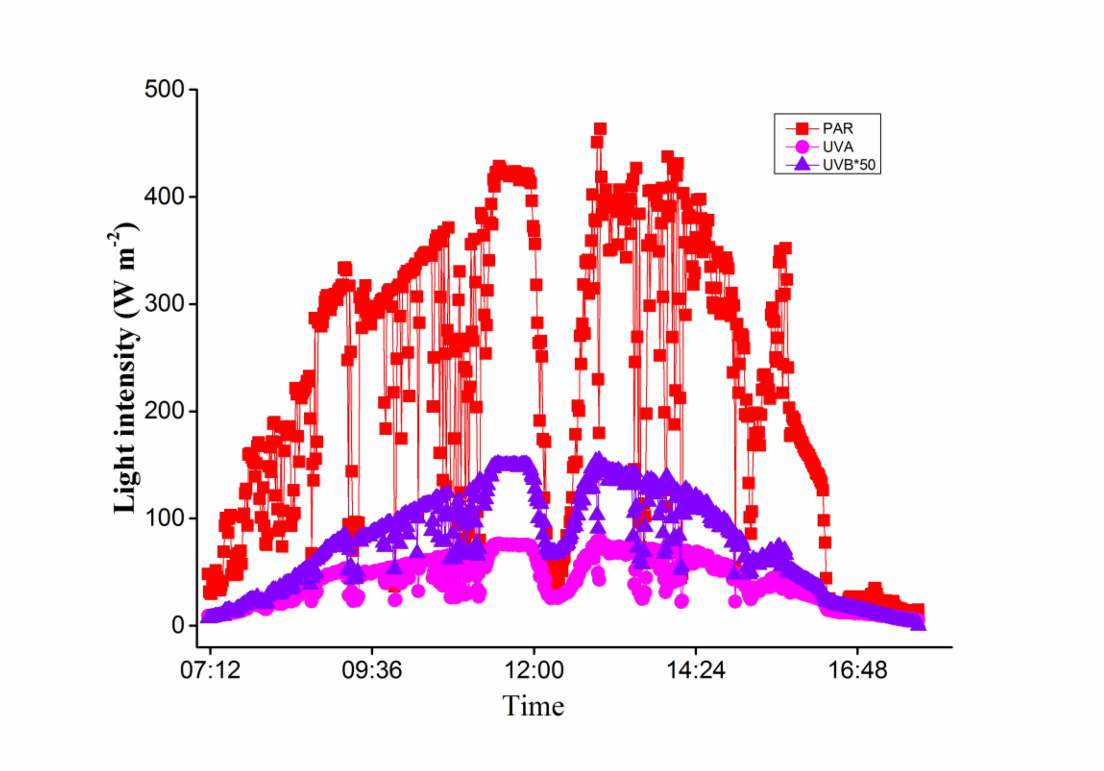
Major Comments:

Line 80 The sentence refers to the possibility that ocean warming will lead to enhanced
stratification and mixed layer shoaling. It cites a reference on UV-temperature interaction in coral reefs (Courtail et al., 2017), that reference presents no direct information on the relationship between ocean warming and mixed layer depth. Courtail et al. (2017) do cite two review papers (now outdated) as sources for a presumed increase in UV exposure due to mixed layer shoaling and claim that as a result corals would be exposed to higher visible and UV radiation. It is difficult to understand this statement given that corals are benthic organisms that do not experience vertical motion. In any case, more recent studies have refuted the assumed relationship between ocean warming and mixed layer shoaling, see Somavilla et al. (2017).

Response: It is true that the cited literature was inappropriate. This literature has been replaced with more recent and appropriate one in line 81 (Capotondi et al. 2012, Journal of Geophysical Research: Oceans). It is generally accepted that increasing concentrations of CO_2 and other greenhouse gases play an important ever-increasing role in determining levels of cloud cover and stratospheric ozone , thus affecting the amount of UV reaching the ocean surface (Williamson et al., 2014, Nature Climate Change). As the reviewer notes, virtually all previous work has suggested increasing temperatures enhance stratification and decrease the depth of the upper mixed layer, exposing the cells to higher solar radiation and reduced nutrient upward transport from deeper layers (Häder and Gao 2015, Frontiers in Environmental Science). We have rewritten this part on lines 80-88 to reflect both this accepted viewpoint and the newer suggestion of Somavilla et al. 2017 (line 82) that this is not true.

Line 189 “ratios … were about 30% higher”
What is the source for the UV:PAR relationship? The percentage looks about right for comparison of the solar-simulator output to incident, midday, near-solstice irradiance in the subtropics but the UV:PAR ratios for incident radiation will be lower than that averaged over the day and over seasons (as I assume was done to estimate the PAR mean light level). Also as a fraction of incident, mean UV in the mixed layer will be less than mean PAR in the mixed layer since UV is attenuated more strongly than PAR.

Response: The UV:PAR relationship comes from the mean light level on one sunny day in September during one cruise in the South China Sea at 18^{o}N (as shown below). As pointed out by the reviewer, it is true that the percentage is lower than that averaged over the seasons and that mean UV in the mixed layer is less than mean PAR. Logistically and technically, we were not able to manipulate different ratios of UV to PAR.



Line 231 “two sample paired t-tests”
If these are comparisons between specific treatments included in the ANOVA analysis, post-hoc multiple comparisons should be applied to test for the significant effects.

Response: Yes, post-hoc multiple comparisons should be applied after the ANOVA analysis, we have corrected the statement in line 235 and reanalyzed the relevant data using this method.

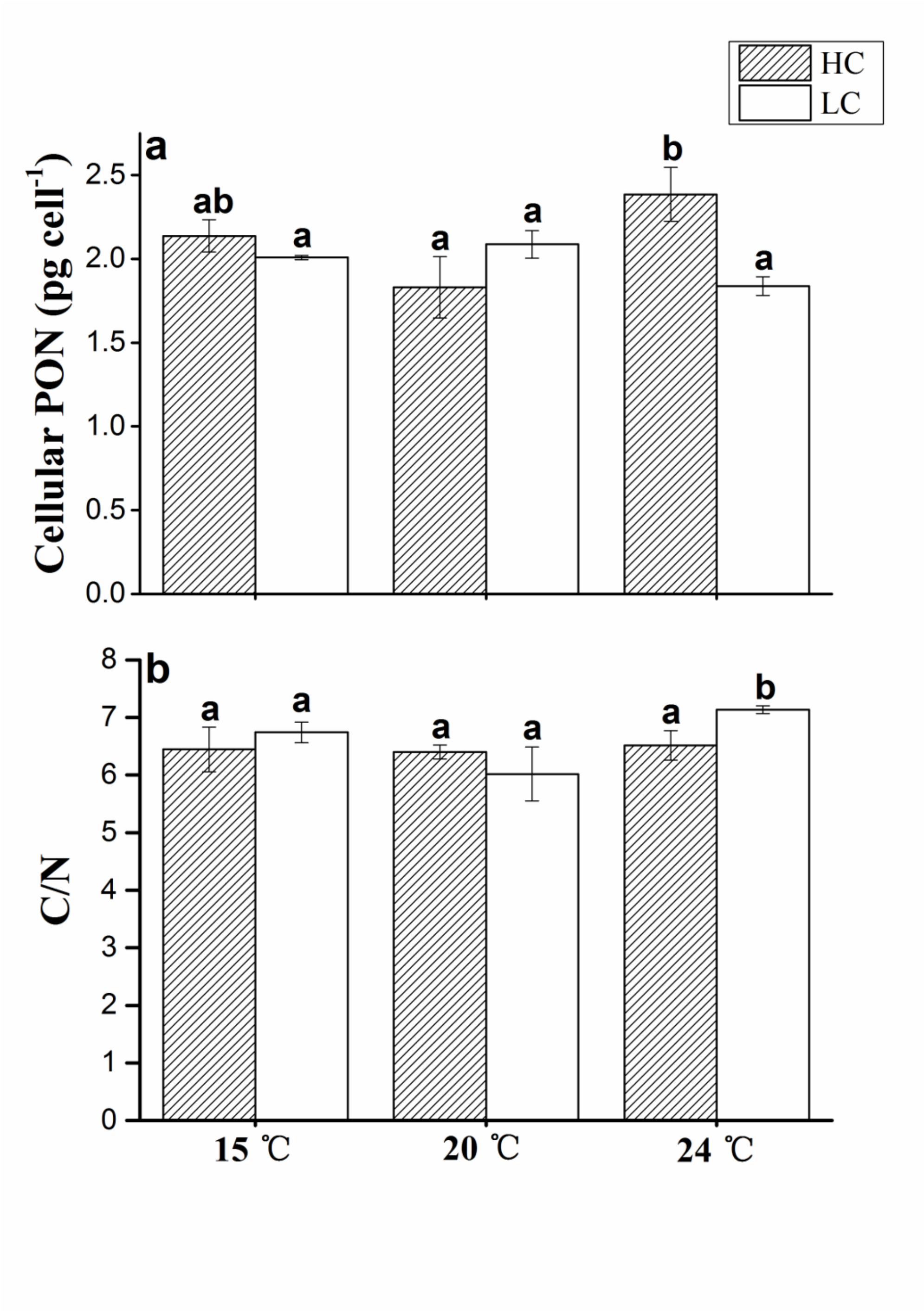
Lines 292-361 (Sections 3.5 and 3.6)
These two sections read as if they are two different data sets whereas in reality it is just the same data viewed in two different ways. There is substantial redundancy between the two sections and it should be condensed into one section. For example, lines 303 – 305 state that photosynthesis was reduced by 33.4% in HC cells and 19.9% in LC at 20°C, essentially the same result is presented in lines 334-335 in the next section. If differences between treatments (e.g. PA and PAB) are not significant, then the inhibition percentage should not be considered significantly different from zero.
It makes sense to discuss the Cal/Pho ratio either as being either increased or decreased in a treatment vs the ratio in the control. On the other hand, the terms “inhibition” and “stimulation” are appropriate for the rates themselves but not the ratio of rates. From that standpoint, it seems that everything that needs to be said about treatment effects of Cal/Pho are covered in lines 322-330, and lines 355-361 can be dropped.

Response: These are very helpful suggestions. We have condensed and rewritten Sections 3.5 and 3.6 as the referee suggested in lines 304-344. Then, we have deleted panel g, h and i in figure 6 (C/P inhibition) and dropped lines 355-361 in the original manuscript. Other panels in figure 5 and 6 are also adjusted accordingly.

Line 395ff Explanations of higher resistance to UV in 24°C HC. Here the authors argue that increased cellular nitrogen in this treatment was responsible for the lower sensitivity to UVA via enhanced defense/repair memchanisms. I question the rationale here, because the enhanced PON is accompanied by larger cell volume and more POC, so there is no indication that the C:N ratio has changed. An increase in the content of nitrogen intensive defense mechanisms, relative to other components of the photosynthetic apparatus, sufficient to change sensitivity to UV would be expected to lower the C:N ratio, compare (for example) with results of Lesser et al. (1994)
Estimating from the figures for example, I get these POC: PON (mass:mass) ratios
15° HC POC:PON = 11.9/2.1=5.67 (6.6 molar)
24°C HC 13/2.25 =5.78 (6.7 molar)
These are not significantly different. Apart from this, I question whether mycosporine amino acid (MAA) accumulation could be involved in the enhanced resistance, particularly since no measurements of absorbance or pigments were made. Xing et al found that UV absorbing compounds (UVAC) were accumulated when cells were grown in the presence of UV, but no UVAC were accumulated during PAR only exposure as used in this study. Moreover, Xing et al never definitively identified the UVAC as MAAs. Other studies of *E. huxleyi* have found only low or trace quantities of MAAs

Response: We realized the inappropriate explanation of lower sensitivity to UVA in the HC treatment at 24 ˚C. We just focused on changes in PON and neglected the simultaneous changes in POC content and the POC/PON ratio. We have read the results of Lesser and others, and agree with the reviewer that lower C:N ratio is responsible for the decreased sensitivity to UV. Therefore, we added POC/PON as panel b in figure 3 as shown below. As indicated in it, in HC treatment at 24 ˚C, though cellular POC was significantly increased, PON had a larger increment than POC. As a result, POC/PON ratio was significantly decreased, and this could be the explanation for the lower sensitivity to UVA in HC treatment. We have cited the literature raised by the reviewer and revised the discussion in lines 386-398.

Yes, it is true we are not sure exactly what kind of UVACs were accumulated, so it is better to avoid usage of MAAs. And Xing et al. found no UVAC were accumulated during PAR alone exposure and the UVAC were not identified as MAAs in their UV treatment. We have deleted the ambiguous discussion.



Lines 422 – Better explanation needed for the discrepancy between different studies – how do differences in growth irradiance and treatment exposure explain why UVA sometimes stimulates vs inhibits calcification rate?

In the existing studies that examined effects of UV radiation on *E. huxleyi* calcification, Xu et al. (2015) found that moderate levels of UVA increase particulate inorganic production in *E.huxleyi*, and the irradiance intensity they used was similar to ours. Gao et al. (2009) reported that UVA inhibited calcification in *E. huxleyi* CS-369, and the UV intensity used was over twice as high as the one we used. We speculate that different levels of UVA could be responsible for these discrepancies, given that low to moderate levels of UVA enhances photosynthesis but high levels of it inhibit it (Gao et al. 2007 Plant Physiology). Stimulation of calcification by moderate levels of UVA could be expected, considering the experimental evidence that presence of UV stimulated PIC production in *E. huxleyi* when grown under reduced levels of solar radiation (Guan and Gao 2010, Environ Experimental Botany). The underlying mechanism needs to be further studied.

Lines 431ff Speculation on reason for UVA-stimulation
Here the authors invoke UVA-dependent bicarbonate uptake as a mechanism to account for the enhanced calcification under the PA treatment. The arguments advanced are not justified at the irradiance levels used in the experiment. At the PAR levels used in the exposure, both photosynthesis and calcification rate are irradiance saturated (cf. Jin et al. 2017). So the additional photon energy coming from UVA is unlikely to increase calcification. Likewise, if irradiance effects are saturated, differences in coccolith layer thickness are unlikely to have an effect mediated by light transmission.

Response: It is true that both photosynthesis and calcification rate were irradiance saturated. From this point of view, it is hard to think of UVA-related stimulation. Nevertheless, presence of UVA could still stimulate calcification by enhancing bicarbonate utilization, as found in another alga (Xu and Gao 2010, Photochemistry and Photobiology B: Biology). We agree that this is speculative though, and so we have shortened and revised the discussion in line 400-411.

Line 468 This implies the presence of UVR could compensate…
Don’t understand the reasoning here, episodic exposure to UV as used in the experiment is already occurring in natural assemblages and will continue in the future, so what is the potential for UVR effects to compensate for future increases in temperature and acidification?

Response: We have revised the wording in lines 427-430. What we mean here is that ignoring presence of UVR, as in previous studies that examined the combined effects of ocean acidification and temperature on *E. huxleyi* under PAR alone, may underestimate calcification.

Minor Comments
Line 180 elemental samples
Better – “samples for elemental analysis”

We have revised according to the referee’s suggestion at line 184 in the revised version.

Line 181: …90 mL quartz tubes (volume 100 mL) Are the numbers are reversed? Obviously, 100 mL can’t fit in a 90 mL tube

They were reversed, and have been corrected at line 185 now.

Line 183 – How many tubes were incubated in each treatment?

Three replicate tubes incubated in each treatment (line 187).

Line 186 “PAM2100”
The model number is incorrect, I expect what was meant was PMA2100, which is a logger that can be used with several types of sensors, so specify which sensors were used for each channel.

It is PMA2100, we have corrected this in line 190, sorry for the typo.

Line 212ff Methods formulas state that Inh was calculated as a percentage, but figure (6) contains only fractions

We have corrected the scales of the Y axes.

Line 496 Misspelling – Author is Banaszak

We have corrected it.

Line 651 “Response of growth and photosynthesis of Emiliania huxleyi to visible and UV irradiances under different light regimes.”Citation is incorrect year 2015, vol 91:343-9

We have corrected it.

There are a number of other typos in the reference list

We have checked carefully and corrected them.

Response to referee #2

This MS presents a comprehensive study on the effects of multi-stressors (ocean acidification, warming and UV radiation) on biochemical characteristics and the photosynthetic activity of the coccolithophorid Emiliania huxleyi. Due to the complexity of the experimental setup and the numerous analyses performed, the results presented are original and they will certainly be of interest to a large readership. The different sections of the MS are overall well written. The many results are presented in clear manner in tables and figures; this latter point clearly represents a challenge of this MS. The discussion could, however, be more concise. In its present form, it contains quite a lot of repetition of the results and it is not easy to follow the order of the points that are discussed. My suggestion is to focus on the discussion of a few key findings, certainly including the effect of UV radiation.

Response: We would like to thank the reviewer for their constructive comments. We have followed these suggestions and made considerable efforts to revise and simplify our discussion, focusing on key findings and making it more concise. Please refer to the revised discussion section. We deleted some speculations such as the role of MAAs in protecting *E.huxleyi* against UV radiation (lines 379-399), shortened the explanation for UVA-stimulated calcification (lines 400-411). We also added some words to integrate our results with previous studies (lines 420-440).

References:

Xu, J. and Gao, K.: Use of UV-A Energy for Photosynthesis in the Red Macroalga *Gracilaria lemaneiformis*, Photochem. Photobiol., 86, 580-585, 2010

Xu, K. and Gao, K.: Solar UV Irradiances Modulate Effects of Ocean Acidification on the Coccolithophorid *Emiliania huxleyi*, Photochem. Photobiol., 91, 92-101, 2015.

Gao, K., Ruan, Z., Villafan, V. E., Gattuso, J.-P., and Helbling, E. W.: Ocean acidification exacerbates the effect of UV radiation on the calcifying phytoplankter *Emiliania huxleyi*, Limnol. Oceanogr., 54, 1855-1862, 2009.

Williamson, C. E., Zepp, R. G., Lucas, R. M., Madronich, S., Austin, A. T., Ballaré, C. L., Norval, M., Sulzberger, B., Bais, A. F., McKenzie, R. L., Robinson, S. A., Häder, D.-P., Paul, N. D., and Bornman, J. F.: Solar ultraviolet radiation in a changing climate, Nature climate change, 4, 434-441, 2014.

Häder, D.-P., Gao, K. S.: Interaction of anthropogenic stress factors on marine phytoplankton, Frontiers in Environmental Science, 3, 2015.

Gao, K. S., Wu, Y. P., et al.: Solar UV radiation drives CO_2 fixation in marine phytoplankton: A double-edged sword, 144, 2007.