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 will make a substantial revision to address the issues.

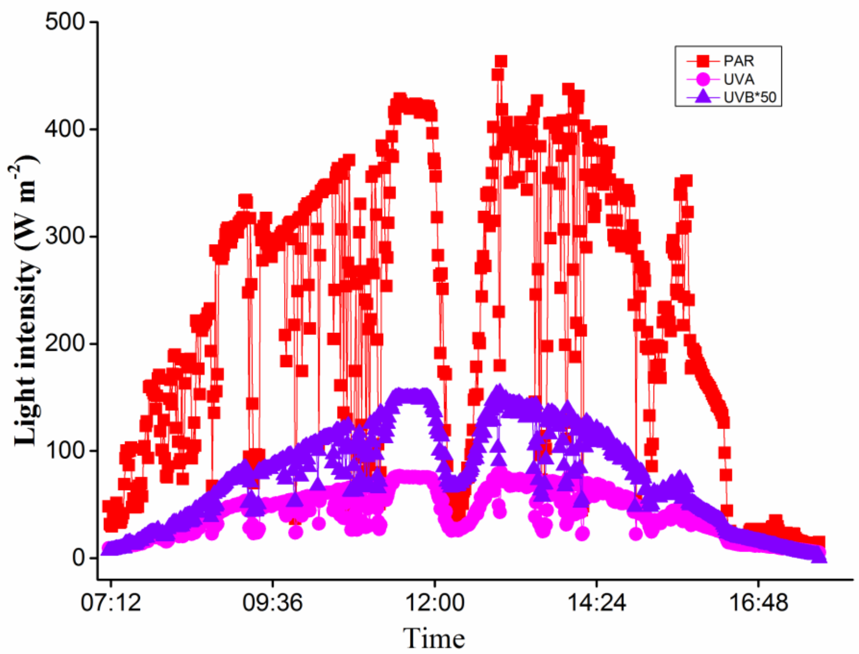
Major Comments:

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 (2017) do cite two review papers (now outdated) as sources for a presumed increase in UV exposure due to mixed layer shoaling and claim that as a result corals would be exposed to higher visible and UV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 will be replaced with more recent and appropriate ones. Somavilla et al. (2017) and more recent reviews supporting warming-enhanced stratification will be cited. It is generally accepted that 1) increasing concentrations of CO_2 and other greenhouse gases plays an important ever-increasing role in determining levels of cloud cover and stratospheric ozone, affecting the amount of UV reaching ocean surface (Williamson et al., 2014); 2) Increasing temperatures enhance stratification and decrease the depth of the upper mixing layer exposing the cells to higher solar radiation and reduce nutrient upward transport from upwelling deeper water (Häder and Gao 2015).

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 in one day in September during one cruise in the South China Sea at 18°N (as shown in the below picture). As pointed out by the reviewer, it is true that the percentage be 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 will reanalyze the data accordingly.

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.

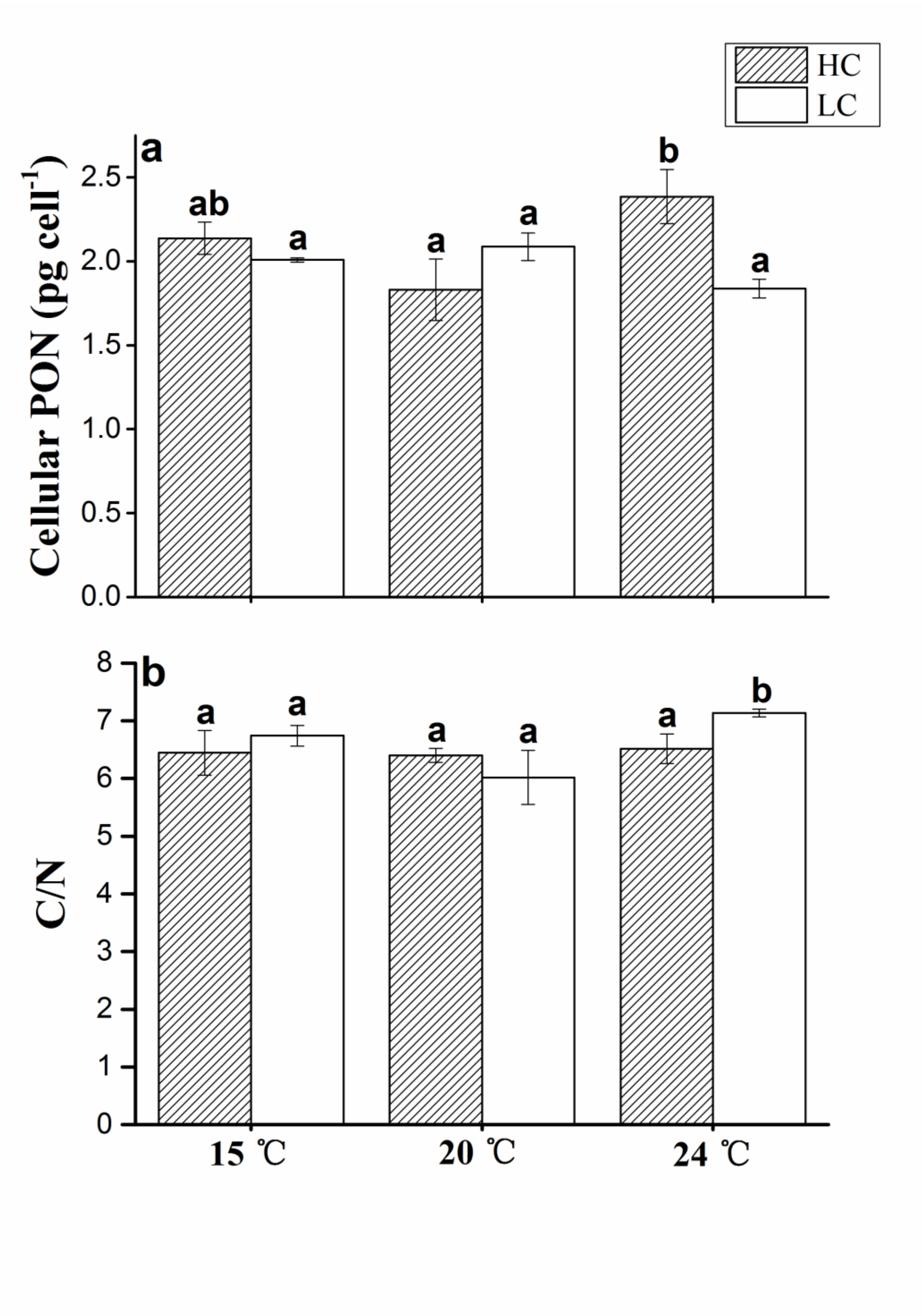
Response: These are very helpful suggestions. We will condense Sections 3.5 and 3.6 as the referee suggested. Then, We will delete panel g, h and i in figure 6 (C/P inhibition) and drop lines 355-361 in the manuscript.

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 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 will cite the literatures raised by the reviewer and revise the discussion.

Yes, it is true we are not sure exactly what kind of UVACs were, so better to avoid usage of MAAs. Yes, in Xing et al., no UVAC were accumulated during PAR alone exposure and the UVAC. We will revise this part of 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*, the light intensity they used was similar to us. Gao et al. (2009) reported that UVA inhibited calcification in *E.huxleyi* CS-369, and the light intensity used by them was over twice as high as the one we used. We speculate that the different levels of UVA could be responsible for these discrepancies, given that UVA enhances photosynthesis during cloudy days but inhibit it on Sunny days (Gao et al. 2007 Plant Physiology). Level or dose dependence of Calcification on UVA should also be considered, though not well documented yet. Further studies are needed to examine relationship of *E.huxleyi*'s calcification with varying levels of UV radiations.

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 and UVA-related stimulation. Nevertheless, presence of UVA could still stimulate calcification by enhancing bicarbonate utilization, as found in another alga (Xu and Gao 2010). Well, this is simply a postulation, we will try to shorten and revise the discussion.

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 will revise the wording. What we mean here is that ignorance of the presence of UVR, like that in previous studies that examined the combined effects of ocean acidification and temperature on *E.huxleyi* under PAR alone, can result in more reduction of calcification, compared to our results with UVR.

Minor Comments

Line 180 elemental samples

Better – "samples for elemental analysis"

We will revise according to the referee's suggestion

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, will be corrected.

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

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, will corrected, sorry for the typo.

Line 212ff Methods formulas state that Inh was calculated as a percentage, but figure (6) contains only fractions
We will correct the scale of Y axes in figure (6).

Line 496 Misspelling – Author is Banaszak
We will correct 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 will correct it.

There are a number of other typos in the reference list
We will check carefully and correct all of them.

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.