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Biogeosciences Discussions

Interactive comment on "Ocean acidification mediates photosynthetic response to UV radiation and temperature increase in the diatom *Phaeodactylum tricornutum*" *by* Y. Li et al.

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Reviewer: However in my opinion there are substantial points related to the effect of CO2 that need to be addressed with more detail. There is also a lack of consistency between the results and conclusions from this study and those published previously from authors belonging to the same group that are vaguely discussed in the manuscript (Wu et al 2010, Gao et al. 2007). For example, in the paper from Wu, Gao and Riebesell (2010) CO2-induced seawater acidification affects physiological performance of the marine diatom Phaeodactylum tricornutum. Biogeosciences 7, the authors conclude (working with the same strain of Phaeodactylum and exactly similar growth conditions)

that in the high CO2 grown cells, the electron transport rate from PSII was photoinhibited to a greater extent at high levels of PAR, while NPQ was reduced compared to low CO2 grown cells. In addition, high CO2 grown cells were downregulated, and growth and photosynthetic carbon fixation rates were enhanced by 5% and 12% respectively compared to cells grown at ambient CO2. As stated above, opposite conclusions are shown in the present manuscript without showing a clear explanation for the differences.

Response: The reviewer had a good point here, we did not discuss the results obtained in these two previous manuscripts.

Consistency: 1) In both studies (Wu et al., 2010 and this work), the growth rate (temperature at 20°C, sub-saturating light levels) was higher in the HC-grown cells as compared to the LC ones.

2) At 20°C, exposures to elevated (over-saturating) PAR, Wu et al. (2010) reported inhibited ETR, and in this study we showed inhibited yield for the HC-grown cells (Fig. 2B).

3) Both studies found no significant changes in Chlα content, and the cells were both grown under sub-saturating PAR levels.

Discrepancy: In Wu et al.'s study, the NPQ for the HC-grown cells was lower than the control when the cells were grown at PAR intensities of 120 μmol m-2s-1 and exposed to actinic light of 840 μmol m-2s-1 within a time frame < 5 min (Wu et al., 2010). In this work, the HC-grown cells showed higher NPQ than the LC-grown ones, with exposures to 290 μmol m-2s-1 (PAR) for a period > 50 min and determined with the actinic light of 300 μmol m-2s-1.

Explanation of the discrepancy: Since CCMs of this diatom becomes down-regulated under elevated CO2 (Burkhardt et al., 2001; Wu et al., 2010, Hopkinson et al., 2011), and levels of light can modulate the efficiency of CCMs (Bartual and Galvez, 2003;

Raven, 2011; Reinfelder, 2011), the cells grown at 70 (present work) and 120 μmol m-2s-1 (Wu et al., 2010) levels would have different levels or CCM-operation efficiency or different levels of energetics, so that the discrepancy might have occurred. In addition, NPQ under solar radiation (long exposures of about 12 h) was remarkably stimulated under elevated CO2 levels of 1000 μatm (Gao et al., 2012).The NPQ measurements were obtained after much longer exposures in this study than in the Wu et al.'s.

We have compared and discussed these at Section 3.2., line 230-231 and Section 4, line 315-335.

Reviewer: The main argument shown by the authors is that the decrease in photoinhibition might be due to UV stimulation of the external carbonic anhydrase (CAe) by UVR. However, it is expected that an acclimation to high CO2 levels similar to that performed for the experimental conditions shown in this paper (> than 20 generations of acclimation to high CO2 levels) would downregulate CA, as also demonstrated in Wu et al. 2010. Under downregulated conditions no activation of CA should be observed, independently of the presence/absence of UVR. Is it possible that despite the long acclimation to high CO2 levels Phaeodactylum cells would not be completely downregulated? It would be interesting to see some results demonstrating the activity of the CAs or other CCMs. Information about growth rates under high/low CO2 conditions would be also helpful. They can give some insights to explain if cells were completely acclimated, and therefore CCMs downregulated.

Response: Yes, It has been suggested in a recent paper using another diatom (Wu et al., 2012) that elevated CO2 level of 800 μatm, compared to the exposures to very high CO2 (5%), may not be enough to switch off the CCM (complete down-regulation). Under moderate levels of UV dose, the diatom Skeletonema costatum showed higher levels of periplasmic proteins, and the presence of UV-A or UV-B appeared to increase the CAe protein (Wu and Gao, Functional Plant Biology, 2009). Although we do not know if the same result would be obtained for other diatoms, such as the species we used here, UV-A stimulated periplasmic proteins or UV-A stimulated carbon fixation

rate (Gao et al., 2007) in cells with non-completely down-regulated CCMs could be responsible for the observed fall of photoinhibition. We agree with the reviewer that further studies on activity of CA and/or CCMs under different CO2 and light +UV levels are needed to examine under what conditions the CCMs can be completely switched off.

Reviewer: Are the subsamples independent replicates? The authors used triplicate samples to measure carbon fixation and photochemical efficiency under the different spectral treatments and temperatures. However it is not clear to me if the subsamples come from only one or several cultures. Which is the volume of the culture/s?

Response: The subsamples are independent replicates (triplicate samples, already added the information in Section 2.1, line 125) and the volume of each culture was 350 ml during the acclimation period.

Reviewer: The authors say that PAM measurements were carried out under 300 umol photons m-2 s-1 PAR for all the treatments, including those with UVA and UVA+UVB. I thought that the Xe-PAM had the capability of exposing samples to PAR and UVR. I understand that samples were measured quite fast after they were collected, however if UVR exposed samples are exposed to PAR we should assume some recovery of photoinhibition caused by UVR. Please clarify why UVA and UVB were not included during the Xe-PAM measurements for the samples coming from the PA and PAB treatments, respectively.

Response: For the XE-PAM, the actinic light was PAR alone, only for the measuring (the pulse) light (very low) was provided with a xenon lamp, which emits UV. Since the measuring light is so low (0.2 μmol m-2s-1), the portion of UV can hardly be effective and measurable. Since the measurement was done within 1 minute, and though the measurement using the PAM was done without UV, the recovery of photoinhibition caused by UV should be very small, and can usually be neglected (see Bouchard et al., Journal of Experimental Marine Biology and Ecology 359, 67–76, 2008; Giordanino

et al., Journal of Photochemistry and Photobiology B: Biology 103, 68–77, 2011).

Reviewer: Which is the reason for growing cultures at low light (70 umol photons m-2 s-1) and expose them for assessment of the photosynthetic characteristics at high light (PAR, 290 umol photons m-2 s-1)?

Response: The reviewer had a point here. It is always better to look at the responses to UV under the same level of PAR as the cells' growth light. We did not perform the experiment in this way, because 1) the diatom grows better under the low light level; 2) during a natural daytime solar cycle, cells experience limiting and then saturating and possibly stressful light levels; therefore, shifting from low-light grown cells to higher light levels reflect some natural conditions (such as cells mixed up from deeper layers), 3) at the PAR level of 70 μmol photons m-2 s-1, the UV level of the solar simulator was too low to represent moderate levels of UV under natural solar radiation.

Reviewer: It would be useful to have absolute rates of damage and repair instead the ratios

Response: We added the data (described in Section 3.2, line 251-258) as suggested by this and another reviewers. The figure was plotted based on the absolute values.

Interactive comment on Biogeosciences Discuss., 9, 7197, 2012.