

***Interactive comment on “CO<sub>2</sub>-induced seawater acidification affects physiological performance of the marine diatom *Phaeodactylum tricornutum*” by Y. Wu et al.***

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Response to Referee #1 The ms addresses the timely topic of effects of increased CO<sub>2</sub> concentrations in seawater on a diatom. The ms is well organized and the discussion is generally supported by the experimental results. The ms would benefit from more details in the material and methods section, and a less speculative discussion. Response: We have detailed the M&M, and revised discussion accordingly.

Specific remarks: line 2, p. 3856: is there also “non CO<sub>2</sub> induced ocean acidification”? Response: Ocean acidification has been proved to be induced by atmospheric CO<sub>2</sub> increase, though other acidic gases, such as SO<sub>2</sub> or NO<sub>2</sub>, may also contribute.

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20 generations acclimation: with the reported growth rates, that would be about 10 days. Is that really sufficient for acclimation to the changed CO<sub>2</sub> conditions? Response: 20 generations was considered enough for phytoplankton cells to acclimate the changed CO<sub>2</sub>, since 5-10 generations was recommended in “Guide to best practices for ocean acidification research and data reporting” (digital version at <http://www.epoca-project.eu/index.php/guide-to-best-practices-for-ocean-acidification-research-and-data-reporting.html>).

The authors use K<sub>1/2</sub>, better would be to use the generally accepted K<sub>m</sub> or K<sub>s</sub>: half saturation value for uptake or growth. And why indicate (line 10, p. 3856) as “photosynthetic” affinity, it is simply affinity. In general the ms has a rather high “cliff-hanger” contents: some parameters are stimulated, some reduced, and the result..., could be different (a balance, line 18 p. 3855). So, what will it be ? Also it is confusing that on the one hand it is indicated that growth (net or gross ?) increased, but that the balance could be positive or negative: : .. If growth (net ?) is stimulated, than obviously the balance is positive (stimulating). So, in other words, is it not clear that productivity will increase? Response: We have replaced K<sub>1/2</sub> with K<sub>m</sub>, and reworded related sentences (line 10, p3856). Though both positive and negative effects were observed in present study, the stimulated growth indicated that the net effects of OA could be positive under low light condition. But for the effects on oceanic primary productivity, it is hard to conclude, since physiological responses to OA depend on light levels, OA stimulates the photo inhibition under high light conditions and enhances respiration. We believe this is the way to explain in view of the drastic changes in solar radiation in natural seawaters. .

Lines 15, p 3856, “Increasing... to : : : : :respiration” (line 18), is a repetition of the previous sentences. Delete or make shorter. Response: Deleted as suggested

P 3862 line 19: use half saturation constant (K<sub>m</sub>) rather not affinity Response: reworded as suggested.

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Why report CO<sub>2</sub> in Pa? And not in atm or mol.kg? Response: Pascal (Pa) is a SI (international system) unit, and acceptable in peer reviewed journal, such as Limnology and Oceanography. At 20 oC and normal pressure (1 atm), 1 Pa CO<sub>2</sub> equals to 9.87 ppmv or  $\mu\text{atm}$ , while mol kg<sup>-1</sup> is a unit represents concentration, such as CO<sub>2</sub> in seawater (see the 7th column of table 1).

Why was CCM not measured? Response: "CCMs are polyphyletic, involving active transports of HCO<sub>3</sub><sup>-</sup>, CO<sub>2</sub> and/or H<sup>+</sup>, or an energized biochemical mechanism as in C4 and CAM plants" (Raven et al., 2008, Phil. Trans. R. Soc. B), thus it is complicated to determine CCM directly. Key physiological parameters are widely used to reflect the activity of CCM, such as Km (Xu and Gao 2009 functional Plant Biol., Aizawa and Miyachi 1986 FEMS MicroBiol Lett.) and intracellular DIC pool (Tortell et al., 2000, Limnol. Oceanogr.)

p.3858 line 9:"automatic system for DIC measurements", specify. Response: Specified.

NBS standards are used for pH measurements. This will give an certain offset in the calculation of CO<sub>2</sub> speciation. How much? It seems that the nutrients were added as nominal additions. Was nutrient draw down measured during the experiments? If so, please specify. If not, how did the authors correct for changes in nutrient concentrations (needed in CO<sub>2</sub> speciation calculations) ? Nutrient draw down will affect alkalinity. And it is likely that nutrients were removed: 20000-30000/ml cells are capable of doing that. Please provide details on this. Response: Since the CO<sub>2</sub>SYS software allows several pH scale input (pHfree, pHtotal, pHNBS, pHseawater), the calculation of speciation of carbonate system will offset automatically when using pHNBS. The referee is correct that nutrients drawdown (mainly P and Si) will affect alkalinity. We did not measure that, but, we recalculated the carbonate system according to theoretic daily nutrients drawdown (47.1, 5.1, 6 and 49.5, 5.4, 6.3  $\mu\text{mol L}^{-1} \text{d}^{-1}$  for N, P and Si under LC and HC conditions, respectively), which was calculated from daily integrated carbon fixation based on the reported ratios of N, P to C (Burkhardt et al, 1999, Limnol. and Oceanogr.) and Silica content per biovolume (Conley and Kilham 1989, Limnol. and

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Oceanogr.). Table 1 was revised and detailed information was added in M&M.

The discussion is sometimes highly speculative, for example p 3865 lines 1- 12 is full of: "estimates", roughly's", "would allow", "would lead", "would increase" : : : etc. Delete or be more specific. Response: this paragraph has been revised.

The authors refer to Riebesell paper (and others) (p 3865, line 6) to indicate that negative effects on calcifying *E. huxleyi* under increased CO<sub>2</sub> conditions (contradiction the results with *P. tricornutum*), but leave out for example the Iglesias Rodrigues reference, supporting the present findings of stimulation of growth/productivity of *E. huxleyi* under high CO<sub>2</sub> conditions. Response: as suggested, we have cited the paper by Iglesias-Rodriguez et al. and reworded the sentence.

The scale of Fig 1 is inappropriate, better give a smaller range, allowing better insight in the differences. Response: as suggested, we have rescaled Fig. 1.

Fig 2: n (number of analyses) is indicated as 3- 12. Be more specific, what was the exact number for every average? Response: we have specified n.

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