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# *Interactive comment on* "Physiological responses of coastal and oceanic diatoms to diurnal fluctuations in seawater carbonate chemistry under two $CO_2$ concentrations" *by* Futian Li et al.

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Anonymous Referee #1 General comments This manuscript presents data on the effects of Ocean Acidification on coastal and oceanic diatom species under constant and fluctuating pH regimes. This is a very relevant and timely issue, and the results are very interesting. I am particularly excited about the differences between the coastal and oceanic species investigated here. Before publishing this manuscript, however, the statistics and some parts in the description/discussion of data need to be changed. Unfortunately, I also see two potentially significant problems with this dataset, which hopefully can be resolved by the authors: Firstly, a second parameter of carbonate system is missing to fully constrain carbonate chemistry. Secondly, even though not



clearly mentioned, from the description of the methods and data it sounds like the distinct measurements were conducted from the same incubation bottles (as the authors speak about replicate "samples" but not "incubations" or "replicates"). If this would be true, no statistical analysis or any kind of interpretation would be meaningful to conduct based on this data. I hope this is rather a misunderstanding from my side, because otherwise the authors would have to repeat the experiment with proper replication.

Response: We greatly appreciate the detailed comments and suggestions, which have led to significant improvements of the revised manuscript. We apologize for the vague statement of "replicate samples". Three independent bottles were used in this study. This has been clarified in the "materials and methods" section now. Moreover, the second parameter of carbonate system (TA) and other calculated carbonate chemistry parameters have been added in the revised manuscript.

Specific comments P2 L20: I suggest changing the beginning of the sentence from "Diel or seasonal" to "Diel and seasonal"

Response: Changed

P2 L 22: I suggest changing the sentence from "natural carbonate buffer system" to "natural dynamics in the carbonate buffer system"

Response: Changed

P3 L54-55: Not clear if the statement on "fluctuations in coastal seawater" refers to current or future conditions.

Response: This has been clarified in the revised manuscript: "in current and OA scenarios".

P4 L69-72: The first and second part should be spilt in two separate sentences. Furthermore, something seems to be missing here.

Response: These sentences have been revised as follows: "Considering the lower

buffering capacity in the OA scenario, pH variability would increase in both coastal and oceanic waters (Egleston et al. 2010; Cai et al. 2011; Denman et al. 2011; Wang et al. 2013). The amplitude of pH variation in coastal water will be larger than in oceanic water due to the presence of multiple drivers (Waldbusser and Salisbury 2014). For instance, biological activities could increase variation in pH by up to 40% compared to the present extent of variation under elevated pCO2 conditions in coastal waters (Egleston et al. 2010)."

P6: In the description of the manipulation of and measurements of carbonate chemistry, only pH measurements are mentioned. To constrain carbonate chemistry, however, a second parameter of the carbonate system is critically needed (cf. best practice guide; Riebesell et al 2010). While I understand that it is probably not feasible to measure other parameters as frequently as needed for the fluctuating pH regime, the authors still need to show that they properly controlled carbonate chemistry, e.g. by presenting AT data from the beginning and the end of the experiment.

Response: Total alkalinity data before and after dilution and measuring method were added now. Carbonate chemistry parameters calculated from pH and TA have been shown in Table 1 in the revised manuscript.

P6 L 120-121: The time points of measurements are defined differentially throughout the manuscript. It would be good to have these more consistent. Here for example, also the number of hours after onset of light should be mentioned.

Response: Thanks for the useful suggestion, we have followed.

P7 L 136: Rather than filter size, the pore size seems to be the more relevant information.

Response: Added

P8 L147: How similar was the light? Please be more specific here.

Response: Added



P8 L150-152: Light exposure for 15s is very short, I do not think that NPQ can be robustly estimated under these assay conditions. The authors need to provide evidence for their statement that they really "provide estimates on the kinetics of NPQ development".

Response: We have reconsidered the NPQ data obtained from RLCs, and these data are not closely relevant to the whole story, as pointed out by the referee. Thus, we have deleted these data in the revised manuscript.

P8 L 164-165: Standard errors or deviations of the pH values are missing.

Response: These were pH values of Tris buffered mediums (we have added this information), thus there were no standard deviations.

P9 L169: I do not agree with the way the statistics have been done. From my perspective, you have two independent variables (i.e. LC vs. HC and steady vs. fluctuating) and not one, so the data should have been analyzed using a two-way instead of a one-way ANOVA.

Response: In our opinion, two-way ANOVA is used when the experiment has one measurement variable and two nominal variables (often called "factors" or "main effects"), like CO2 and temperature. In the present study, four treatments used (LCs, LCf, HCs, and HCf) are all about seawater carbonate chemistry (one environmental factor), just like different levels of pCO2, thus we think one-way ANOVA is suitable for this study.

P8 L171-172: The authors state that all data is reported as "mean value of triplicate samples". Does this mean that there was no true replication in the experiments, and samples were taken from the same incubation bottles? This needs to be clarified. If the latter is the case, statistical analysis is not possible, as this would mean n=1.

Response: We apologize for the vague statement of "replicate samples". Three independent bottles were used for one treatment in this study. This has been clarified in the "material and methods" section now.

P9 L177: I would still prefer to see the error bars.

Response: Added

P11 L 210-216: I find the structure of the results section partially confusing (especially in this section). I would try to structure it more clearly, e.g. by always describing the responses of T. weissflogii before those of T. oceanica.

Response: Thanks for the useful suggestion! We have followed and changed the order of statement.

P12 L 239: Can cells “have a decrease” in something? Consider revising.

Response: Revised as follows: “T. oceanica cells under the LCf treatment showed a 29% lower chlorophyll-normalized net oxygen evolution rate relative to the LCs cells”.

P12 L241-249: I find this section also quite confusing, also because the time points are sometimes described with hours and sometimes descriptive (e.g. middle of photoperiod).

Response: The statements of time points have been unified and they are described with hours after the onset of light now.

P13 L 251: I think this should read “while the fluctuating regime had no detectable effect”.

Response: Changed

P13 L 263-258: Given the limited usefulness of these super short RLCs, do you really need this data for your argumentation?

Response: We have deleted this part of results.

P15 L 296-298: The authors state that “diatoms may have reduced silicon requirements per carbon fixed under an OA scenario than under ambient pCO2 condition, and so has implications for changes in local and global silicon budgets”. Despite im-



provable grammar in this sentence, I find the use of the term “silicon requirements” in this context rather misleading because BSi per cell is only affected by OA in one out of four situations and the change in BSi:POC ratio is rather driven by changes in POC quota (Figure 3).

Response: We have revised the statement, now it reads as follows: “This decreased ratio indicates that the tested species may fix more carbon per silicon assimilated in the OA scenario than under the ambient pCO2 condition, and so has implications for changes in local and global carbon and silicon budgets. More carbon may be fixed per diatom cell without changes in silicon quota in the OA scenario, and thus the tested species may contribute more to primary production in the ecosystem, especially in Si-limited waters, in the future oceans.”

P15 L308: Consider changing “C3-C4 intermediate (Roberts et al. 2007) photosynthesis” to “C3-C4 intermediate photosynthesis (Roberts et al. 2007)”.

Response: Changed

P16 L314: Details on CCM characteristics were not “shown here”, but rather hypothesized.

Response: Thanks for pointing this out. These sentences have been revised as follows: “Moreover, T. weissflogii has a markedly higher fraction of direct bicarbonate transport and apparent eCA activity than T. oceanica (Martin and Tortell 2008), which may facilitate their inorganic carbon transport and uptake. In this study, T. oceanica showed significantly lower oxygen evolution rates in the LCf treatment than in the LCs treatment at 11.5 h after the onset of light, when the highest pH and lowest CO2 was reached. In contrast, no effects of the fluctuating regime on oxygen evolution rates of T. weissflogii were found at this time point. Thus T. weissflogii cells were more tolerant of the high pH and low CO2 period under fluctuating carbonate chemistry than T. oceanica.”

P16 L319-322: I do not like the use of the word “sacrifice” in this context. This sounds like an active decision by the algae, rather than a process where evolution is acting upon an organism.

Response: We have revised this sentence to “As with the successful compromise between iron requirements and capacity to acclimate to dynamic light regimes in T. oceanica cells (Strzepek and Harrison 2004), this oceanic diatom may also have evolved to acclimate to fluctuating carbonate chemistry in a different way compared with the coastal diatom.”

P17 L337: Consider changing from “calcification of corals benefit” to “calcification of corals can benefit”.

Response: Changed

P17 L 346-349: I don’t think the authors can claim that “all of the members” of a natural diatom community” have been investigated in this species (e.g. cf. Schaum et al. 2012 for intraspecific plasticity).

Response: This sentence has been revised to “However, it is notable that growth rates and competitive abilities of diatoms of a natural community showed little change following one year of conditioning at two pCO2 levels and three temperatures, relative to the results of a short-term experiment conducted on the original collected community (Tatters et al. 2013).”

P18 L360-364: UV comes in as a bit of a surprise here and I am not convinced it really feeds into the argumentation/story of this manuscript.

Response: We have reconsidered the relevance of this section of discussion and NPQ data obtained from RLCs to the whole story. These paragraph seems to be a little redundant in the discussion, thus we have deleted it.

P18 L367-268: I do not find data that would show that “elevated CO2 mitigated the limited availability of pCO2 that occurred at the end of photoperiod under the LCf con-



dition” in this manuscript.

Response: This sentence has been revised to make it clearer: “Although elevated CO2 mitigated the negative effects of the fluctuating regime on photosynthetic oxygen evolution rates of T. oceanica cells under ambient pCO2 condition, the effect of the fluctuating regime under elevated pCO2 tended to be negative, resulting in a decreased growth rate compared to the steady regime.”

P18 L371-372: It may be worth mentioning that this is even more so in coastal compared to oceanic environments.

Response: Added. Thanks for the suggestion!

P19 L377: I don’t think the responses really classify as “poor physiological performance”.

Response: This sentence has been revised: “Given the decreased growth and elemental production rates of T. oceanica under fluctuating seawater carbonate chemistry in the OA scenario, and its limited ability to dissipate excess excitation energy through NPQ under high light (Strzepek and Harrison 2004), this species is unlikely to be able to acclimate to coastal habitats, where major fluctuations in light and carbonate chemistry will exist, in the future oceans.”

P19 L385: I don’t understand the last part of this sentence, what is meant by “factors that will help to decide the spatial distribution patterns of species”?

Response: Now it reads as “It is possible that this ability, together with the abilities to cope with nutrient (Irwin et al. 2006), light (Lavaud et al. 2007; Lavaud and Lepetit 2013; Laviale et al. 2015), and predation pressure (Irigoien et al. 2005), will determine the spatial distribution patterns of species in the future oceans.”

P20 L397: Something went wrong with this citation.

Response: We have changed the reference to another one, since detailed citation

information was missed for the former reference.

P30 L 599-600: The used pH scale and error estimates are missing. Furthermore, it should be mentioned if this is 1) an average over all days, or an example and 2) averaged over the biological replicates (I assume were used) or just one bottle.

Response: The scale and error bars are now added, and we also clarify that these data show an example of the experimental days and are the mean values of triplicate cultures.

P30 L611 and L618: For consistency, I would also mention the number auf ours after start of the photoperiod in these captions.

Response: Added

P32 Table 1: The differences in cell size between both species are an interesting aspect that should be discussed in terms of their implications for surface:volume ratios, carbon acquisition and pH homeostasis. Similarly, also the R:P ratio is an interesting parameter (e.g. the significantly higher ratio in T. oceanica under LCf), that is currently not discussed in the manuscript. Furthermore, units of ratios are missing.

Response: Thanks for the suggestion. Units of ratios are added now. We have added some discussion about the cell size difference between this two species and the R:P ratio as follows: "T. oceanica cells showed significantly higher R:P ratios than T. weissflogii, especially in the fluctuating regime at ambient pCO2, and the ratios were within previously reported ranges in diatoms (Geider and Osborne, 1989). The higher R:P ratio indicated greater proportions of photosynthetic fixed carbon and associated energy were used for growth, biosynthesis, and maintaining intracellular homeostasis in the oceanic species."

"The differential responses of the tested two species to the fluctuating carbonate chemistry may be partially attributed to the differences in cell size. Flynn et al. (2012) showed that the differences in carbonate chemistry and pH between the bulk medium and the



exterior surface of marine organisms increase as cell size increase. Thus the larger species, T. weissflogii theoretically possesses higher adaptability to cope with the varied carbonate chemistry and pH, as they are frequently encountered in the natural coastal waters and their exterior surfaces."

P33 Table 2: The irradiance level used for these measurements should be mentioned in the caption. For clarity, I would furthermore call the time point really "time point rather than "time" and add a "h" after the number of hours.

Response: Changed and added.

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