Biogeosciences Discuss.,
doi:10.5194/bg-2016-281-AC2, 2016

Biogeosciences Discussions
Open Access
EGU

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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Received and published: 3 October 2016

Anonymous Referee #2 The manuscript "Physiological responses of coastal and oceanic diatoms diurnal fluctuations in seawater carbonate chemistry under two CO2 concentrations" by Li/Gao et al presents very interesting and novel findings on the CO2 response of T. weissflogii and T. oceanica. I'm very pleased that the authors aim to mimic the natural environmental conditions focusing on diurnal fluctuations in CO2 availability (or pH) coastal species can experience. Based on their findings the authors discuss the niche distribution and their adaptation potential to certain habitats of different species. The study is well designed and the paper is well written. There are, however, a few shortcomings that should be revised before the study can be published.

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I also advise the authors to tone down some of their findings and try to generalize less.

Response: We would like to thank the referee for the recognition of the value of our work and the valuable and constructive comments. We have followed the referee's suggestion of generalizing less, for example, a sentence "However, phytoplankton are known to exhibit species-specific response to environmental factors (including OA, fluctuating carbonate chemistry etc.), thus more studies on the responses of phytoplankton at the species and community levels are needed to predict such broad biogeographic trends." has been added at the end of discussion. Moreover, given the species-specific responses, we have revised "diatoms" to "the tested species" in some discussion. Detailed responses to each of the comments are listed below.

Comments on the methods: - I could not find much information on the statistical replication in the MS text.

Response: This information is now added in the materials and methods section (Line 104-105).

Photophysiology: - the 15s light acclimation applied seems to be very short. Can the authors cite studies using this short time in RLCs for determining NPQ? Did the authors also obtain rETR rates? These data should be available in the dataset given by the PAM and could give additional information on the physiological performance of the cells. For example one could compare rETR with O2 evolution rates.

Response: We agree with the referee that 15s is too short. We have reconsidered the NPQ data obtained from RLCs, and these data are not closely relevant to the whole story. Thus, we have deleted these data in the revised manuscript. It's useful to compare rETR and O2 evolution. rETR can be calculated from the ΦPSII data. However, the rETR and O2 evolution in the present study may not reflect the real relationship between them exactly as the following reasons: 1) while cells for O2 evolution measurement were concentrated, their concentrations were same as culture for PAM determination. 2) Different light sources were applied for the two measurements: halogen

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lamp for PAM and LED for oxygen electrode. Moreover, it's better to compare absETR and O2 evolution. However, we don't have the effective absorption cross section data. Thus the comparison of rETR and O2 evolution may be not rigorous in the present study.

Photosynthesis and respiration: - please state for how long the O2 rates were measured - why did the authors decide to measure respiration only in the middle of the day while they measured O2 evolution/PS three times a day?

Response:The reason we only measured respiration of one time point is the limited volume of culture (1L) used in the present study. Because cells need to be concentrated for measurements of photosynthesis and respiration, the culture volume is not enough for measuring the two parameters at three time points.

- Did the authors check ifthe cells where physiologically OK after filtering (e.g measuring Fv/Fm prior and post filtering)? This is critical information to obtain reliable data.

Response: We have added the time used to measure oxygen rates ($\sim$ 10 min) and filtration pressure ($<$ 0.02 MPa) in the revised manuscript. Cells were checked by microscope, and no damage cell was found. To measure Fv/Fm is a fast and effective way, thanks for the suggestion!

Carbonate chemistry: When one conducts CO2 experiments it is usually preferred to measure more than the pH to constrain the carbonate chemistry. Although I agree with the authors that the difference and shifts in pH are caused by modulating the pCO2, it would be necessary to measure at least one other parameter such as total alkalinity or DIC to fully characterize the carbonate chemistry. The additional information would also help in the discussion regarding buffer capacities. Please add the missing information if available.

Response: Total alkalinity data and other calculated carbonate chemistry parameters have been added in the revised manuscript.

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Comments of the discussion: Line 272/273: please bring T. w. to the front of the sentence.

Response: Changed.

Line 275: the authors state that T.o. has a higher dark respiration. I don't understand why the authors did not measure a dark-respiration in the morning and evening when they also measured photosynthesis. The intregration of the data could show a different scenario. What is the reason these data where not acquired or shown?

Response: As the reason mentioned above, we only measured respiration in the middle of photoperiod. There is no doubt that circadian variations should be considered when discussing the results. We have added a part of discussion about the possibility that physiological performance may differ at different time point: "Diatoms were shown to exhibit circadian variations in photosynthesis and respiration (Weger et al., 1989; Chen and Gao, 2004). Thus dark respiration might show different pattern at other time points, as photosynthetic oxygen evolution did."

Line 278: the elemental production rates are depressed due to the reduced growth rate not due to the change in elemental composition – please make this more clear here.

Response: we have added a statement here.

Line 296: Why do the authors state that both species have lower Si requirements under OA? According to table 1, T.w. increases Si per cell in the HCs acclimation while the HCf acclimation is similar to the LC acclimations. T.o. only decreases Si per cell in the HCf acclimation. I understand that the Si:C ratio decreases! Ratios can be interpreted differently and this is sometimes confusing – but when you state that the Si requirement decreases I would normalize it on a cellular basis. Additionally – the term Si requirement seems to be improbably chosen in this context.

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

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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."

Line 315: I'm confused by the statement that T.w. benefit from the C acquisition pathways "as shown" in this study. I do not find any data on either HCO3- of CO2 usage or CA activities, inhibitor studies on eCA or transport. Please be clearer. I assume that the authors mean that the general characteristics of Ci uptake measured by others can explain some of the findings of this study.

Response: These sentences have been revised to make it clearer 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."

Line 318 following: again – I really would have preferred to see diurnal measurements on respiration. I have one more concern here – respiration does not depend on the Chl a concentration but rather on the activity of mitochondria. As such, normalization based on per cell or per C might have been more appropriate for this study. Additional – regarding the O2 evolution shown in Fig. 4 – please indicate if the integrated O2 evolution differs between the treatments.

Response: We agree with the referee that respiration does not depend on the chl a, thus we deleted the chl a normalized respiration in the revised manuscript. Unfortu-

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nately, respiration was only measured in the middle of photoperiod. We understand the referee's concern and have added some discussion about the possibility that physiological performance may differ at different time point: "Diatoms were shown to exhibit circadian variations in photosynthesis and respiration (Weger et al., 1989; Chen and Gao, 2004). Thus dark respiration might show different pattern at other time points, as photosynthetic oxygen evolution did." Since dark respiration is not the same as respiration in the light, we didn't use dark respiration to infer the gross photosynthetic oxygen evolution.

Line 331: why does this sentence start with "in contrast"? I see no reason for this here.

Response: Deleted.

Line 349-351: Please indicate that the mentioned changes are based on the decrease in growth rate rather than a decrease in the elemental composition of the cells. The production rates (growth rate times elemental composition) are only affected due the change in growth and this should be highlighted.

Response: We have added a statement here.

Line 371: It would come handy here to have a well described carbonate system (2-3 measured parameters) to support this discussion on lower buffer capacity in an OA ocean.

Response: We have calculated the Revelle factor in the current and OA scenarios to reflect the buffer capacity here: "For instance, the Revelle factor increased from 10.6 $\pm$ 0.2 to 15.0 $\pm$ 0.2 (a higher Revelle factor indicates a lower buffer capacity) when pCO2 increased from the ambient to the elevated level in the present study."

Line 377: Please rephrase "poor physiological performance". In my understanding T.o. has similar or higher Chl a content, similar POC/cell, similar PON per cell, similar BSi per cell, growth is similar (LC), POC and PON production rate is higher (LC), quantum yield is high (LC and HC), O2 evolution is similar (HC): : :. Overall – the cells do pretty

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well (physiologically) under fluctuating pH. I agree with the authors that there are some pathways and responses in which the fluctuating cells do not do well – but this does not give them a general poor performance. The NPQ response is also questionable since I'm not sure that a 15s light acclimation can give reliable data on NPQ. Where does T. pseudonana come from in the discussion here?

Response: This sentence has been revised to: "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." We have deleted the T. pseudonana information and NPQ data obtained from RLCs.

Additional comments: Line 158: add a "the" to photoperiod

Response: We unify the expression of time points as hours after the onset of light.

Fig. 1: Add error bars to the graph.

Response: Added.

Fig. 2: why are the different letters (statistics) lower case for T.w. and upper case for T.o.? This is true for all figures. The growth rate should also state "μ"

Response: Because only four treatments of one species were compared. If we use lower case for all eight bars, it may mislead readers. The word "specific" was added in front of "growth rate" in the revised manuscript.

Fig. 3: I miss the error bar in d) and h) HCf. Although the error is small – it should be visible!

Response: The error bars in d) and h) is very small (SD ËĆ 0.01), thus they are not obvious. We have increased the line width of error bar.

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Fig. 4: The font size should be a little bigger for the x/y axis numbers x axis and descriptions. Please be aware that the super and sub script is messed up

Response: Changed.

Fig 5: same comment as for Fig. 4

Response: Changed.

Fig. 6: The data for T.o. below 400 _E are not visible. Please change the size of the squares/circles that all data are visible.

Response: Fig. 6 has been deleted now.

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