Responses to comments of reviewers

Special comments from associate editor

The issue seems somewhat "philosophical" but I believe reviewer #1 has a good point when he/she suggests a two-way analysis of variance, that would enable you to also test for the combined effects of pH and variability. Further, analysis of variance assumes equal variance in parameters measured for each treatment (has this been tested?).

Response: Thanks for the suggestion. We have changed the statistical analysis to two-way ANOVA. Accordingly, interactions of pCO_2 level and pCO_2 variability on parameters have been stated in the results section. All data used for ANOVA analysis were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene test). This has been added in the revised manuscript (Page 8 line 185-189).

The diurnal pH variation regime used in this study seem very high, could the authors please comment (maybe in the discussion) on how realistic/common this would be for both coastal and oceanic regimes.

Response: The pH variation range used in the present study is realistic in coastal waters. We have added some comments on the variation ranges in coastal and oceanic regimes in the discussion as suggested (Page 17 line 433-437).

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 (Page 5 line 106). Moreover, the second parameter of carbonate system (TA) and other calculated carbonate chemistry parameters have been added in the revised manuscript (Page 6 line 127).

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

Response: Changed (line 20)

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

Response: Changed (line 22)

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". (line 57)

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 pCO_2 conditions in coastal waters (Egleston et al. 2010)." (line 71-80)

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 (line 127). 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 (line 149)

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

Response: Added (line 161)

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: Thanks for the suggestion. We have changed the statistical analysis to two-way ANOVA. Accordingly, interactions of pCO_2 level and pCO_2 variability on parameters have been stated in the results section (line185).

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 (line 106).

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". (line274)

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 (line 291)

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 *p*CO2 condition, and so has implications for changes in local and global silicon budgets". Despite improvable 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 pCO_2 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 340-345)

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

Response: Changed (line 355)

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 CO_2 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 CO_2 period under fluctuating carbonate chemistry than *T. oceanica*." (line 357-365)

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." (line 367-371)

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

Response: Changed (line 393)

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 pCO_2 levels and three temperatures, relative to the results of a short-term experiment conducted on the original collected community (Tatters et al. 2013)." (line 407-410)

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 *p*CO2 that occurred at the end of photoperiod under the LCf condition" in this manuscript.

Response: This sentence has been revised to make it clearer: "Although elevated CO_2 mitigated the negative effects of the fluctuating regime on photosynthetic oxygen evolution rates of *T. oceanica* cells under ambient pCO_2 condition, the effect of the fluctuating regime under elevated pCO_2 tended to be negative, resulting in a decreased growth rate compared to the steady regime." (line 429-433)

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! (line 441-445)

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." (line 449-453)

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." (line 457-460)

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. (line 639)

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. (line 693)

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

Response: Added (line 707 and 714)

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 pCO_2, 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." (line 377-381).

"The differential responses of the tested two species to the fluctuating carbonate chemistry may be partially attributed to the differences in cell size. The differences in carbonate chemistry and pH between the bulk medium and the exterior surface of marine organisms increase as cell size increases (Flynn et al. 2012). 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." (line 397-402).

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. (line 743-746)

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. 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 (line 460). 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 106).

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 O_2 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 O_2 evolution. rETR can be calculated from the Φ_{PSII} data. However, the rETR and O_2 evolution in the present study may not reflect the real relationship between them exactly as the following reasons: 1) while cells for O_2 evolution measurement were concentrated, their concentrations were same as culture for PAM determination. 2) Different light sources were applied for the two measurements: halogen lamp for PAM and LED for oxygen electrode. Moreover, it's better to compare absETR and O_2 evolution. However, we don't have the effective absorption cross section data. Thus the comparison of rETR and O_2 evolution may be not rigorous in the present study.

Photosynthesis and respiration: - please state for how long the O2 rates were measured

Response:We have added the time used to measure oxygen rates (~ 10 min) in the revised manuscript (line 178).

- 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 filtration pressure (< 0.02 MPa) in the revised manuscript (line 174). 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 *p*CO2, 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 (line 127 and Table 1).

Comments of the discussion:

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

Response: Changed. (line 314)

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 375-377).

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 321)

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

the OA scenario than under the ambient pCO_2 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 340-345)

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 CO_2 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 CO_2 period under fluctuating carbonate chemistry than *T. oceanica*." (line 357-365)

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. Unfortunately, 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." (line 375-377). 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 387)

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 412)

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 ± 0.2 to 15.0 ± 0.2 (a higher Revelle factor indicates a lower buffer capacity) when pCO_2 increased from the ambient to the elevated level in the present study." (line 441-443)

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 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." (line 449-453)
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 < 0.01), thus they are not obvious. We have increased the line width of error bar.

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.

List of changes

Abstract

1. Line 20, change “or” to “and”
2. Line 22, add “dynamics of the”
3. Line 27, delete “with higher respiration than cells grown under the corresponding steady regime”
4. Line 29, add “and enhanced dark respiration rates”

Introduction

1. Line 55, add “ocean acidification”
2. Line 57, add “in current and OA scenarios”
3. Line 66, change “Hinga 2002” to “Duarte et al. 2013”
4. Line 71-80, rephrase the sentences to “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 up to 40% compared to the present extent of variation under elevated pCO_2 conditions in coastal waters (Egleston et al. 2010).”

Materials and methods

1. Line 106, add “Triplicate cultures (incubated in 1 L autoclaved Erlenmeyer flasks) were used for each treatment”
2. Line 110, add “cultures were diluted every 24 h at 6 h after the onset of light”
3. Line 127, add “Samples for total alkalinity (TA) measurement were poisoned with a saturated solution of mercuric chloride after filtration. TA was determined by Gran acidimetric titration with a TA analyzer (AS-ALK1+, Apollo SciTech). Certified reference materials obtained from A. G. Dickson at the Scripps Institution of Oceanography were used to assure the accuracy of the TA measurement. TA and pH were applied to CO2SYS software to calculate other carbonate chemistry parameters (Table 1)”
4. Line 133, add “6 h after the onset of light”
5. Line 149, delete “25 mm” , add “1.2 μm pore size”
6. Line 156, change the equation to “$\Phi_{PSII} = (F_m' - F_t) / F_m' = \Delta F / F_m'$”
7. Line 159, change the equation to “$NPQ = (F_m - F_m') / F_m'$”
8. Line 161, add “~ 156 μmol photons m^{-2} s^{-1}”
9. Line 163, change “illumination” to “the onset of light”
10. Line 164, delete “NPQ versus irradiance curves were determined by rapid light curves (RLCs) with 15 s duration for each light level. Although the

values of NPQ derived from RLCs were not as accurate as values from fluorescence induction curves, they provide estimates of the kinetics of NPQ development with increasing light intensity"

11. Line 173, add "6 h after the onset of light"
12. Line 174, add "< 0.02 MPa"
13. Line 178, add "~ 10 min per sample", change "medium" to "media"
14. Line 179, change "medium" to "media", add "of Tris buffered media"
15. Line 185, change statistical analyses to "Data were analyzed by a two-way analysis of variance (ANOVA) with *p*CO2 level and *p*CO2 variability classed as factors in the model, each with two levels (400 ± 15µatm, 1005 ± 40 µatm; and steady, fluctuating *p*CO2, respectively). The interaction of the two factors was also included in the model. All data used for ANOVA analysis were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene test). When p values were under 0.05, the post hoc Duncan test was used to determine the differences between treatments. All data are reported as mean value of triplicate cultures ± standard deviation (SD). "

Results

1. The statements of time points have been unified and they are described with hours after the onset of light now. Moreover, the order of results statement has been changed (always describing the response of *T. weissflogii* before those of *T. oceanica*).
2. Line 197, delete "For clarity, only mean pH values every 1.5 h are shown (Fig. 1)", add "the"
3. Line 198, add "Fig. 1"
4. Line 215, add "A significant interaction between pCO_2 level and pCO_2 variability on growth rate of *T. oceanica* was found"
5. Line 220, change "Table 1" to "Table 2"
6. Line 222, add "resulting in a significant interaction between pCO_2 level and pCO_2 variability"
7. Line 229, change "Table 1" to "Table 2", add "A significant interaction between pCO_2 level and pCO_2 variability on chlorophyll *a* content of *T. oceanica* was found."
8. Line 232, change "Table 1" to "Table 2", add "the"
9. Line 235, add "the"
10. Line 241, add "Significant interactions between pCO_2 level and pCO_2 variability on elemental production rates of *T. oceanica* were found."
11. Line 252, change "Table 2" to "Table 3"
12. Line 274, change "*T. oceanica* cells under the LCf treatment had a 29% decrease of chlorophyll-normalized net oxygen evolution rate relative to the

LCs cells" to "*T. oceanica* cells under the LCf treatment showed a 29% lower chlorophyll-normalized net oxygen evolution rate relative to the LCs cells"

13. Line 276, add "A significant interaction between pCO_2 level and pCO_2 variability on chlorophyll normalized net oxygen evolution rate of *T. oceanica* was found"
14. Line 298, add "net"
15. Line 300, add "the"
16. Line 302, change "Table 1" to "Table 2"
17. Line 305, delete the paragraph

Discussion

1. Line 314, add "for the coastal diatom *T. weissflogii*"
2. Line 315, add "its"
3. Line 316, delete "of the coastal diatom *T. weissflogii*"
4. Line 317, delete "with higher dark respiration under the fluctuating regime than under the steady regime"
5. Line 319,delete "of *T. oceanica*", add "and enhanced dark respiration rates"
6. Line 321, add "which were caused by decreased growth rates"
7. Line 340, rephrase the sentences to "This decreased ratio indicates that the tested species may fix more carbon per silicon assimilated in the OA scenario than under the ambient pCO_2 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"
8. Line 355, change "C_3-C_4 intermediate (Roberts et al. 2007) photosynthesis" to "C_3-C_4 intermediate photosynthesis (Roberts et al. 2007)"
9. Line 358, rephrase the sentences to "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 CO_2 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 CO_2 period under fluctuating carbonate chemistry than *T. oceanica*."
10. Line 366, delete "both" "and OA", change "scenarios" to "scenario"
11. Line 367, rephrase the sentences 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"

12. Line 372, add "under the fluctuating regime in the current scenario"
13. Line 374, change "Beardall and Raven 2012" to "Raven and Beardall, 2005"
14. Line 375, add "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. *T. oceanica* cells showed significantly higher R:P ratios than *T. weissflogii*, especially in the fluctuating regime at ambient pCO_2, 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."
15. Line 387, delete "In contrast", add "the"
16. Line 393, add "can"
17. Line 397, add "The differential responses of the tested two species to the fluctuating carbonate chemistry may be partially attributed to the differences in cell size. The differences in carbonate chemistry and pH between the bulk medium and the exterior surface of marine organisms increase as cell size increases (Flynn et al. 2012). 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."
18. Line 407, change "all of the members of a natural diatom community" to "diatoms of a natural community"
19. Line 412, add "which were caused by decreased growth rates"
20. Line 414, delete the paragraph
21. Line 427, add "the", change "under" to "in the"
22. Line 430, change "Although elevated CO_2 mitigated the limited availability of pCO_2 that occurred at the end of photoperiod under the LCf condition" to "Although elevated CO_2 mitigated the negative effects of the fluctuating regime on photosynthetic oxygen evolution rates of *T. oceanica* cells under ambient pCO_2 condition"
23. Line 433, delete "in our study", add "The diurnal pH variation range (~ 0.5 units) used in the present study is realistic for coastal ecosystems, like upwelling regions (Hofmann et al. 2011), kelp forests (Cornwall et al. 2013), coastal coral reefs (Wang et al. 2014), and tide pools (Morris and Taylor, 1983). In contrast, pH in the open ocean is relatively stable, with a variation range of only ~ 0.024 over a month (Hofmann et al. 2011)."
24. Line 438, delete "~0.5 unites", change "under" to "in the", add "in the present study"
25. Line 441, add "For instance, the Revelle factor increased from 10.6 ± 0.2 to 15.0 ± 0.2 (a higher Revelle factor indicates a lower buffer capacity) when

pCO_2 increased from the ambient to the elevated level in the present study. The increase amplitude of pH variation in coastal water will be more apparent than in oceanic water under an OA scenario, due to high biomass and sufficient nutrients."

26. Line 447, add "under fluctuating carbonate chemistry conditions"
27. Line 448, change "under elevated pCO_2 condition" to "in the OA scenario"
28. Line 449, change "poor physiological performance" to "decreased growth and elemental production rates"
29. Line 450, add "in the OA scenario"
30. Line 451, add "Strzepek and Harrison 2004"
31. Line 452, add "where major fluctuations in light and carbonate chemistry will exist, in the future oceans"
32. Line 453, delete "and *T. pseudonana*", change "appear" to "appears"
33. Line 456, add "under OA conditions"
34. Line 459, change " are factors that will help to decide" to "will determine"
35. Line 460, change "" to "both the present and" to "the", add "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."

Acknowledgements

1. Line 466, add "We would like to thank the two anonymous reviewers and Dr. Christine Klaas for their insightful comments on the manuscript."
2. Line 467, add "State Oceanic Administration (SOA, GASI-03-01-02-04)"
3. Line 468, delete "State Oceanic Administration (SOA, GASI-03-01-02-04)"

References

1. Line 476, delete "Beardall, J., and Raven, J. A.: Algal metabolism, in: eLS, John Wiley & Sons, Ltd, doi:10.1002/9780470015902.a0000321.pub2, 2012"
2. Line 492, add the reference "Chen, X. and Gao, K.: Characterization of diurnal photosynthetic rhythms in the marine diatom Skeletonema costatum grown in synchronous culture under ambient and elevated CO2, Funct. Plant. Biol., 31, 399-404, 2004."
3. Line 525, delete "Eberhard, S., Finazzi, G., and Wollman, F.-A.: The dynamics of photosynthesis, Annu. Rev. Genet., 42, 463-515, 2008."
4. Line 533, add "Flynn, K. J., Blackford, J. C., Baird, M. E., Raven, J. A., Clark, D. R., Beardall, J., Brownlee, C., Fabian, H., and Wheeler, G. L.: Changes in

pH at the exterior surface of plankton with ocean acidification, Nature Climate Change, 2, 510-513, 2012."

5. Line 550, add "Geider, R. J., and Osborne, B. A.: Respiration and microalgal growth: a review of the quantitative relationship between dark respiration and growth, New Phytol., 112, 327-341, 1989."
6. Line 557, delete "Goss, R., and Jakob, T.: Regulation and function of xanthophyll cycle-dependent photoprotection in algae, Photosynth. Res., 106, 103-122, 2010."
7. Line 565, delete "Hinga, K. R.: Effects of pH on coastal marine phytoplankton, Mar. Ecol. Prog. Ser., 238, 281-300, 2002."
8. Line 598, delete "Lavaud, J., Rousseau, B., and Etienne, A. L.: General Features of Photoprotection By Energy Dissipation in Planktonic Diatoms (Bacillariophyceae), J. Phycol., 40, 130-137, 2004."
9. Line 616, delete "Müller, P., Li, X.-P., and Niyogi, K. K.: Non-photochemical quenching. A response to excess light energy, Plant Physiol., 125, 1558-1566, 2001."
10. Line 626, add "Morris, S. and Taylor, A. C.: Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools, Estuar. Coast. Shelf S., 17, 339-355, 1983."
11. Line 639, add "Raven, J. A. and Beardall, J.: Respiration in aquatic photolithotrophs, in: Respiration in aquatic ecosystems, edited by: del Giorgio, P. A. and Williams, P.J. le B., Oxford University Press, New York, USA, 36-46, 2005."
12. Line 652, delete "Ruban, A., Lavaud, J., Rousseau, B., Guglielmi, G., Horton, P., and Etienne, A.-L.: The super-excess energy dissipation in diatom algae: comparative analysis with higher plants, Photosynth. Res., 82, 165-175, 2004."
13. Line 689, add "Weger, H. G., Herzig, R., Falkowski, P. G., and Turpin, D. H.: Respiratory losses in the light in a marine diatom: Measurements by short-term mass spectrometry, Limnol. Oceanogr., 34, 1153-1161, 1989."

Figures and tables

1. Line 693, change "pH" to "pH_{NBS}"
2. Line 694, change "squares" to "triangles", add "Here pH values of triplicate cultures in one experimental day are shown"
3. Line 699, change "samples" to "cultures"
4. Line 704, change "samples" to "cultures"
5. Line 707, change "in the middle of photoperiod of" to "determined at 6 h after the onset of light for"
6. Line 711, change "illumination" to "the onset of light", change "samples" to "cultures"
7. Line 714, change "per chlorophyll (a, b) or cell (c, d) in the middle of the photoperiod" to "determined at 6 h after the onset of light"

8. Line 717, change “samples” to “cultures”
9. Line 720, delete figure 6 caption
10. Line 726, add Table 1
11. Line 734, change “Table 1” to “Table 2”
12. Line 736, change “samples” to “cultures”
13. Table 2, add units
14. Line 741, change “Table 2” to “Table 3”, change “illumination” to “the onset of light”
15. Line 742, add “Φ_{PSII} and NPQ were determined under actinic light intensity (~ 156 μmol photons m^{-2} s^{-1}) similar to culture light level after 10 min dark adaptation.”
16. Table 3, add “h”
17. Change figures 1-5, delete figure 6

Physiological responses of coastal and oceanic diatoms to diurnal fluctuations in seawater carbonate chemistry under two CO_2 concentrations

Running head: ocean acidification influences diatoms under fluctuating pH

Futian Li[1], Yaping Wu[1], David A. Hutchins[2], Feixue Fu[2], and Kunshan Gao[1*]

[1]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361102, China

[2] Department of Biological Sciences, University of Southern California, Los Angeles, California, United States of America

[*]Corresponding author e-mail: ksgao@xmu.edu.cn (Kunshan Gao)

Abstract

Diel ~~or~~ and seasonal fluctuations in seawater carbonate chemistry are common in coastal waters, while in the open ocean carbonate chemistry is much less variable. In both of these environments, ongoing ocean acidification is being superimposed on the natural dynamics of the carbonate buffer system to influence the physiology of phytoplankton. Here, we show that a coastal *Thalassiosira weissflogii* isolate and an oceanic diatom, *Thalassiosira oceanica*, respond differentially to diurnal fluctuating carbonate chemistry in current and ocean acidification (OA) scenarios. A fluctuating carbonate chemistry regime showed positive or negligible effects on physiological performance of the coastal species. In contrast, the oceanic species was significantly negatively affected~~, with higher respiration than cells grown under the corresponding steady regime~~. The fluctuating regime reduced photosynthetic oxygen evolution rates and enhanced dark respiration rates of *T. oceanica* under ambient CO_2 concentration, while in the OA scenario, the fluctuating regime depressed its growth rate, chlorophyll *a* content, and elemental production rates. These contrasting physiological performances of coastal and oceanic diatoms indicate that they differ in the ability to cope with dynamic pCO_2. We propose that, in addition to the ability to cope with light, nutrient, and predation pressure, the ability to acclimate to dynamic carbonate chemistry may act as one determinant of the spatial distribution of diatom species. Habitat-relevant diurnal changes in seawater carbonate chemistry can interact with OA to differentially affect diatoms in coastal and pelagic waters.

Key words: diatom, growth, photosynthesis, elemental production rates, fluctuating carbonate chemistry, CO_2

1 Introduction

Anthropogenic emissions of carbon dioxide (CO_2) since the industrial revolution have increased atmospheric pCO_2 levels by 40% (Howes et al. 2015), mainly due to burning of fossil fuels and land use changes (Ciais et al. 2014). The oceans absorb about 30% of the CO_2 emitted by human activities (Sabine et al. 2004), leading to decreases in pH, concentration of carbonate ions, and saturation state of calcium carbonate, along with increases of the concentrations of aqueous CO_2 and bicarbonate (i.e., ocean acidification). The global surface ocean mean pH has already decreased by about 0.1 units since the industrial revolution (Orr et al. 2005; Doney 2010), and a further decrease of 0.3-0.4 units is expected to happen by 2100 under the business as usual scenario (Orr et al. 2005; Gattuso et al. 2015).

For marine organisms, the reduced seawater mean pH caused by ocean acidification (OA) could be detectable on a timescale of years to decades, while striking fluctuations in coastal seawater carbonate chemistry may occur over much shorter timescales in current and OA scenarios. The coastal zone plays a critical role in biogeochemical cycles, and experiences great variability of physical and chemical factors (Drupp et al. 2011). In addition, it is the area most impacted by anthropogenic pressures (Gattuso et al. 1998). Carbonate chemistry in coastal seawater is affected by multiple drivers in addition to atmospheric CO_2 dissolution, such as tidal cycles (Dai et al. 2009; Jiang et al. 2011; Wang et al. 2014), upwelling (Feely et al. 2008; Capone and Hutchins 2013), watershed processes, wind forcing (Drupp et al. 2011), anthropogenic nutrient inputs, aquaculture activities, and changes in ecosystem structure and metabolism (Duarte et al. 2013; Waldbusser and Salisbury 2014). Due to high biomass and sufficient or excess nutrients in coastal waters, biological activities alter pCO_2, resulting in a diel cycle of pH. The diel range of pH variation in some coastal ecosystems can be greater than 1 pH unit (Duarte et al. 2013~~Hinga 2002~~), which corresponds to a 900% change in H^+ concentration.

During a diurnal cycle, organisms in coastal areas could experience pH values that may be lower than the projected value for the surface ocean in the year 2100 (Hofmann et al. 2011; Hurd et al. 2011; Waldbusser and Salisbury 2014). In contrast, pH in the open ocean is relatively stable, with a variation range of only ~ 0.024 over a month (Hofmann et al. 2011). ~~The~~ Considering the lower buffering capacity ~~will decrease as the increase of dissolved inorganic carbon in both coastal and oceanic~~

~~seawaters~~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~~),)~~ ~~while the variation range of pH in coastal water may be amplified, due to the multiple drivers mentioned above. Diurnal and seasonal variations in pH caused by photosynthesis and respiration could be increased by more than 40% relative to the present extent of variation~~ 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 up to 40% compared to the present extent of variation under elevated pCO_2 conditions in coastal waters (Egleston et al. 2010).

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Responses of fish (Dixon 2014), gastropods (Onitsuka et al. 2014), oysters (Keppel 2015), mussels (Frieder et al. 2014), coral (Dufault et al. 2012; Comeau et al. 2014), canopy-forming kelp (Britton et al. 2016), and coralline algae (Gao et al. 1993; Cornwall et al. 2013; Noisette et al. 2013; Johnson et al. 2014) to diurnally fluctuating pCO_2/pH have been studied recently. Dufault et al. (2012) hypothesized that storage of dissolved inorganic carbon during the night-time high pCO_2 period fueled day-time calcification (and perhaps photosynthesis), resulting in higher calcification and survival rate of coral recruits. Thus, it appears that some marine organisms may benefit from pCO_2 fluctuations. In spite of this body of literature, the responses of marine phytoplankton to fluctuating pH/pCO_2 are still unclear. To our knowledge, only one study has addressed the responses of the marine green alga *Ostreococcus* to fluctuating pCO_2 (Schaum et al. 2016). However, how CO_2 variability affects other major marine phytoplankton groups over either the short- or long-term remains unknown.

Coastal and open ocean species are distinguished by habitat-related differences in cell size, nutrient utilization (Glibert and Ray 1990), photosynthetic architecture (Strzepek and Harrison 2004), and photosynthetic performance (Lavaud et al. 2007; Li et al. 2011; Liu and Qiu 2012). Our study was intended to understand whether coastal and oceanic species also differ in their capacity to respond to fluctuating carbonate chemistry. A coastal *Thalassiosira weissflogii* isolate and an oceanic diatom, *Thalassiosira oceanica*, were used in the present study. We manipulated pCO_2 to mimic diurnally fluctuating carbonate chemistry and hypothesized that coastal diatoms would show better physiological performance under fluctuating carbonate chemistry than oceanic ones, a difference that could

potentially be a key factor influencing the geographical distribution of diatoms.

2 Materials and methods

2.1 Cultures and experimental setup

Thalassiosira weissflogii (CCMP 1336, isolated from coastal Long Island, New York, USA in 1956) and *Thalassiosira oceanica* (CCMP 1005, isolated from the Sargasso Sea in 1958) were incubated in Aquil medium (Sunda et al. 2005~~),~~). Triplicate cultures (incubated in 1 L autoclaved Erlenmeyer flasks) were used for each treatment, illuminated by cool white fluorescent light at an intensity of 115 μmol photons m^{-2} s^{-1}. Cultures were maintained at 20 ^{o}C with a 12 h:12 h light and dark cycle. Cells were maintained at exponential growth phase with maximal concentration < 1.1×10^4 mL^{-1} (*T. weissflogii*) or 3.5×10^4 mL^{-1} (*T. oceanica*) in semi-continuous cultures (cultures were diluted every 24 h at 6 h after the onset of light).

T. weissflogii and *T. oceanica* were acclimated to four treatments: 1) steady carbonate chemistry at ambient pCO_2 level (LCs); 2) diurnally carbonate chemistry fluctuated around ambient pCO_2 level (LCf); 3) steady carbonate chemistry at elevated pCO_2 level (HCs); and 4) diurnally carbonate chemistry fluctuated around elevated pCO_2 level (HCf) for 15 generations before sampling. Steady regimes were bubbled with ambient air (400 ± 15μatm, LCs) or elevated (1005 ± 40 μatm, HCs) pCO_2, which was automatically achieved by mixing air/CO_2 with a CO_2 Enricher (CE100B, RuiHua). The fluctuating regimes were obtained by changing the CO_2 partial pressure every 12 h. Cells were aerated with air of low pCO_2 (i.e., 0 or 557 ± 15 μatm for LCf and HCf, respectively) during the photoperiod; the aeration was changed to high pCO_2 (i.e., 870 ± 19 or 1949 ± 35 μatm for LCf and HCf, respectively) at the beginning of the dark period. Measurements showed that pH gradually increased and decreased, similar to a natural diurnal cycle (see Results). Since pH increased quickly in the first few hours of the photoperiod, the aeration rates were adjusted to make sure the fluctuating regimes reached similar pH values with corresponding steady regimes in the middle of photoperiod and reached target values at the end of photoperiod. The steady regimes were aerated with stable pCO_2 air at the same flow rate as the fluctuating regimes. The pH was measured every 1.5 h by a pH meter (Orion 2 STAR, Thermo

Scientific) calibrated with standard National Bureau of Standards (NBS) buffers. Samples for total alkalinity (TA) measurement were poisoned with a saturated solution of mercuric chloride after filtration. TA was determined by Gran acidimetric titration with a TA analyzer (AS-ALK1+, Apollo SciTech). Certified reference materials obtained from A. G. Dickson at the Scripps Institution of Oceanography were used to assure the accuracy of the TA measurement. TA and pH were applied to CO2SYS software to calculate other carbonate chemistry parameters (Table 1).Subsamples for measurement of physiological parameters were always taken in the middle of the photoperiod (6 h after the onset of light), unless otherwise noted.

2.2 Growth rate and chlorophyll *a* content

Cell concentration and mean cell size were measured by a Coulter Particle Count and Size Analyzer (Z2, Beckman Coulter). Specific growth rate was calculated according the equation: $\mu = (\ln N_1 - \ln N_0) / (t_1 - t_0)$, in which N_1 and N_0 represent cell concentrations at t_1 and t_0. For the chlorophyll *a* content determination, samples were filtered onto GF/F filters (25 mm, Whatman), and extracted overnight at 4 ^{o}C in absolute methanol before centrifugation. The supernatants were analyzed by a UV-VIS Spectrophotometer (DU800, Beckman Coulter) and the chlorophyll *a* content was calculated according to the equation of Ritchie (2006).

2.3 Elemental composition and production rate

Samples for measuring particulate organic carbon (POC) and nitrogen (PON) were filtered onto pre-combusted (450 ^{o}C for 6 h) GF/F filters (25 mm, Whatman). Filters were treated using HCl fumes to remove any inorganic carbon and dried before analysis on a CHNS/O Analyzer (2400SeriesII, PerkinElmer). ~~25 mm p~~Polycarbonate filters (1.2 µm pore size) were used to determine biogenic silica (BSi) by the spectrophotometric method of Brzezinski and Nelson (1995). Production rates of POC, PON, and BSi were calculated by multiplying cellular content by specific growth rate.

2.4 Chlorophyll *a* fluorescence

The photochemical parameters were determined using a Xenon-Pulse Amplitude Modulated fluorometer (Xe-PAM, Walz). Effective photochemical quantum yields were determined according to the equation of Genty et al. (1989): $\Phi_{PSII} = (F_m' - F_t) / F_m' = \Delta F / F_m'$ ~~$\Phi_{PSII} = (F'_m - F_t) / F'_m = \Delta F / F'_m$~~ for light-adapted samples, where F_m' indicates maximum chlorophyll fluorescence of light-adapted samples, and F_t, steady chlorophyll fluorescence of light-adapted samples. Non-photochemical quenching (NPQ) was calculated as: $NPQ = (F_m - F_m') / F_m'$, ~~$NPQ = (F_m - F'_m) / F'_m$,~~ where F_m indicates maximum chlorophyll fluorescence of dark-adapted samples. Φ_{PSII} and NPQ were measured under actinic light intensity (~ 156 µmol photons m^{-2} s^{-1}) similar to culture light level after 10 min dark adaptation. Given the changing carbonate chemistry over a diurnal cycle, Φ_{PSII} and NPQ were determined at three time points: 0.5, 6, and 11.5 h after ~~illumination~~the onset of light. ~~NPQ versus irradiance curves were determined by rapid light curves (RLCs) with 15 s duration for each light level. Although the values of NPQ derived from RLCs were not as accurate as values from fluorescence induction curves, they provide estimates of the kinetics of NPQ development with increasing light intensity.~~

2.5 Photosynthetic oxygen evolution and dark respiration rates

Net photosynthetic oxygen evolution and dark respiration rates were determined using a Clark-type oxygen electrode (Oxygraph, Hansatech) at the experimental temperature. Oxygen evolution rates were measured under 115 µmol photons m^{-2} s^{-1} at the same three time points as mentioned above. Oxygen consumption rates were measured in the middle of photoperiod (6 h after the onset of light), when the steady and fluctuating regimes reached similar pH values. Samples were gently filtered (< 0.02 MPa) onto 47 mm cellulose acetate membranes, and then re-suspended into 20 mmol L^{-1} Tris buffered medium. The re-suspended cells were injected into an oxygen electrode chamber equipped with a magnetic stirrer. Rates of oxygen evolution and consumption were derived from the linear portion of the slope of the oxygen record (~ 10 min per sample). The pH values of Tris buffered ~~medium~~ media were pre-adjusted to their corresponding culture ~~medium~~ media values. That is, pH values of Tris buffered media of the three time points in the LCf treatment were 7.84, 8.14, and 8.35, and those in the HCf

treatment were 7.54, 7.80, and 8.06. Values in the LCs and HCs treatments were set to 8.14 or 7.80 for all three time points, respectively.

2.6 Statistical analyses

Data were analyzed by a two-way analysis of variance (ANOVA) with pCO_2 level and pCO_2 variability classed as factors in the model, each with two levels (400 ± 15µatm, 1005 ± 40 µatm; and steady, fluctuating pCO_2, respectively). The interaction of the two factors was also included in the model. All data used for ANOVA analysis were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene test). ~~Significant differences among treatments were tested using one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$.~~ When ~~necessary~~p values were under 0.05, the post hoc Duncan test was used to determine the differences between treatments. All data are reported as mean value of triplicate ~~samples~~ cultures ± standard deviation (SD).

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3 Results

3.1 Variation of pH in experimental regimes

The variation ranges of pH in the LCf and HCf treatments were 0.52 ± 0.03, and 0.53 ± 0.03, respectively. ~~For clarity, only mean pH values every 1.5 h are shown (Fig. 1).~~ At the beginning of the photoperiod, pH of the LCf regime was 7.84 ± 0.02 (Fig. 1), and then it increased to 8.15 ± 0.03 ~~in the middle of photoperiod~~at 6 h after the onset of light, similar to the value of the LCs regime (8.13 ± 0.02). The pH value of the LCf regime reached 8.35 ± 0.02 at 12 h after the onset of light~~the end of the photoperiod~~, and then decreased to 7.84 ± 0.02. For the HCf regime, pH ranged from 7.54 ± 0.01 to 8.06 ± 0.02, and reached 7.82 ± 0.01 at 6 h after the onset of light~~in the middle of photoperiod~~, similar to the value of the HCs regime (7.79 ± 0.01).

3.2 Specific growth rate and mean cell size

Growth rates of *T. weissflogii* were not influenced by diurnally fluctuating carbonate chemistry in either the current or the OA scenario (Fig. 2a). ~~Likewise, there were no differences in growth rates~~

~~between steady and fluctuating regimes for *T. oceanica* under the ambient pCO_2 condition (Fig. 2b). However, fluctuating regime reduced its growth rate by 9% under the elevated pCO_2 condition. OA influenced the growth rate of *T. oceanica*, with rate of HCs cells being 16% lower than LCs cells.~~ No effects of OA on growth rate of *T. weissflogii* were detected. There were no differences in growth rates between the steady and fluctuating regimes for *T. oceanica* under the ambient pCO_2 condition (Fig. 2b). However, the fluctuating regime reduced its growth rate by 9% under the elevated pCO_2 condition. OA influenced the growth rate of *T. oceanica*, with rates of HCs cells being 16% lower than those of LCs cells. A significant interaction between pCO_2 level and pCO_2 variability on growth rate of *T. oceanica* was found. ~~Additionally, growth rates of *T. pseudonana* (CCMP 1335, isolated from Moriches Bay, New York, USA in 1958) were not influenced by the fluctuating regime under both ambient and elevated pCO_2 conditions (data not shown).~~

Mean cell sizes were not affected by the fluctuating treatment under either ambient or elevated pCO_2 conditions in *T. weissflogii* (Table ~~1~~2). *T. oceanica* cells showed minor but significant changes in cell size in the fluctuating treatments. Cells in the LCf treatment cells were 1.2% larger than LCs cells, while HCf cells were 1.4% smaller than cells in the corresponding steady treatments, resulting in a significant interaction between pCO_2 level and pCO_2 variability.

3.3 Chlorophyll *a* content and elemental composition

Chlorophyll *a* contents of *T. weissflogii* in the four treatments were not significantly different. For *T. oceanica*, the fluctuating regime didn't influence chlorophyll *a* content under ambient CO_2 level. However, in the HCf treatment chlorophyll *a* content decreased by 24% compared to the steady regime (Table ~~1~~2). A significant interaction between pCO_2 level and pCO_2 variability on chlorophyll *a* content of *T. oceanica* was found.

POC and PON quotas of both species were elevated in the OA scenario in both the steady and fluctuating regimes, relative to present day pCO_2 levels (Table ~~1~~2). However, no effects of the fluctuating regime on cellular POC and PON contents were detected in either species compared to the steady treatments. The only exception was that POC increased by 9% in the LCf treatment relative to

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the LCs treatment for *T. weissflogii*. Generally, elevated pCO_2 and the fluctuating regime showed no effects on BSi quota of either species, besides a slight decrease in the HCf treatment relative to that of the HCs treatment for *T. weissflogii*.

The fluctuating regime increased the POC production rate of *T. weissflogii* at both ambient and elevated pCO_2 levels, but had no effects on other elemental production rates of this species. By contrast, the fluctuating regime decreased all of the elemental production rates in the OA scenario for *T. oceanica* (Fig. 3). Significant interactions between pCO_2 level and pCO_2 variability on elemental production rates of *T. oceanica* were found. ~~The C:N and Si:C ratios of *T. oceanica* and the Si:C ratio of *T. weissflogii* were lower in the OA scenario, while C:N ratios of *T. weissflogii* were not significant different in the four treatments (Table 1).~~ Slight but significant decreases of the Si:C ratio in the fluctuating regime compared to the steady regime were found at ambient pCO_2 for both species (Table 2). The Si:C ratio of *T. weissflogii* was lower in the OA scenario, and C:N ratios of *T. weissflogii* were not significant different among the four treatments. For *T. oceanica*, cells showed lower C:N and Si:C ratios at elevated pCO_2 relative to cells grown at ambient pCO_2.

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3.4 Chlorophyll *a* fluorescence

The effective photochemical quantum yields of both species varied little at different time points, ranging from 0.54 ± 0.03 to 0.61 ± 0.03 among treatments (Table ~~2~~3). Fluctuating regimes scarcely influenced Φ_{PSII} of either species. The only exception was that Φ_{PSII} of HCf decreased by 8% relative to that of the HCs for *T. weissflogii* at ~~the beginning of the photoperiod~~0.5 h after the onset of light. Elevated pCO_2 decreased Φ_{PSII} by 3% and ~~5~~2% ~~in the middle of the photoperiod~~ for *T. weissflogii* ~~and *T. oceanica*~~ at 6 and 11.5 h after the onset of light, respectively. *T. oceanica* ~~C~~cells under elevated pCO_2 showed ~~2~~5% and 7% lower Φ_{PSII} compared to those under ambient pCO_2 at 6 and 11.5 h after ~~illumination for *T. weissflogii* and *T. oceanica*~~the onset of light, respectively. NPQ under culture light intensity ranged from 0.06 ± 0.01 to 0.23 ± 0.05 at different time points. No detectable effects of the fluctuating regime on NPQ of either species were found, with the exceptions of HCf cells of *T. weissflogii* at ~~the beginning of the photoperiod~~0.5 h after the onset of light and LCf cells of *T. oceanica*

11.5 h after ~~illumination~~the onset of light. For steady regimes, elevated pCO_2 showed no detectable effect on NPQ of both species at ~~the beginning of the photoperiod~~0.5 h after the onset of light, while it increased NPQ of *T. weissflogii* by 37.5% and ~~38.4%~~decreased it by 25% relative to values of LCs cells ~~in the middle of the photoperiod for *T. weissflogii* and *T. oceanica*~~at 6 and 11.5 h after the onset of light, respectively. Values of NPQ of *T. oceanica* HCs cells were enhanced by 38.4% and decreased by ~~25% and~~ 33.3% relative to values of LCs cells at ~~the end of the photoperiod for *T. weissflogii* and *T. oceanica*~~6 and 11.5 h after the onset of light, respectively.

3.5 Photosynthetic oxygen evolution and dark respiration rates

Chlorophyll normalized net oxygen evolution rates of these two species ranged from 0.39 ± 0.07 to 0.55 ± 0.07 μmol O_2 μg chl a^{-1} h^{-1} ~~in the middle of photoperiod~~at 6 h after the onset of light. Neither elevated pCO_2 nor the fluctuating regime showed detectable effects on oxygen evolution rates per chlorophyll of *T. weissflogii* (Fig. 4a), while *T. oceanica* cells under the LCf treatment ~~had~~ showed a 29% ~~decrease of~~lower chlorophyll-normalized net oxygen evolution rate relative to the LCs cells (Fig. 4b). A significant interaction between pCO_2 level and pCO_2 variability on chlorophyll normalized net oxygen evolution rate of *T. oceanica* was found.

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Both species, regardless of treatment, showed a similar diurnal rhythm of photosynthetic oxygen evolution: oxygen evolution rates reached the highest values ~~in the middle of the photoperiod~~at 6 h after the onset of light (Fig. 4c, d). For *T. weissflogii*, effects of fluctuating pCO_2 on net oxygen evolution per cell were only observed ~~in the middle of the photoperiod~~at 6 h after the onset of light for cells at the ambient pCO_2 level, with 7% lower rates in the LCf treatment than in the LCs (Fig. 4c). These effects were more obvious for *T. oceanica* cells ~~at ambient pCO_2 level~~. *T. oceanica* cells in the steady regime under elevated pCO_2 evolved oxygen at 65% higher cell-specific rates than those in the fluctuating regime at 0.5 h after the onset of light (Fig. 4d). At 11.5 h after ~~illumination~~the onset of light, LCs cells of *T. oceanica* showed 41% higher net oxygen evolution rates per cell than LCf cells ~~(Fig. 4d).~~ . There were no differences in photosynthetic oxygen evolution rates between HCs and HCf cells 11.5 h after the onset of light. ~~*T. oceanica* cells in the steady regime under elevated pCO_2 evolved oxygen at 65%~~

~~higher cell-specific rates than those in the fluctuating regime at the beginning of the photoperiod.~~

Elevated pCO_2 increased dark respiration of *T. weissflogii* by ~~59~~57% compared to that at ambient pCO_2 level, while the fluctuating regime had no detectable effect (Fig. 5a). In contrast, dark respiration rates of *T. oceanica* were stimulated by ~~44% and 55~~60% for cells under the fluctuating regime compared to steady one at ambient ~~and elevated~~ pCO_2 level~~s, respectively~~ (Fig. 5b), while no effects of the fluctuating regime at elevated pCO_2 were observed. Dark respiration rates of *T. oceanica* were similar in the steady regimes of ambient and elevated pCO_2 levels. ~~When dark respiration rates were normalized per cell, they generally showed the same patterns as chlorophyll normalized rates, with different amplitudes of variation (Fig. 5c, d). The exception was that no effects of fluctuating regime on dark respiration per cell of *T. oceanica* were found in the OA scenario.~~ The respiration to net photosynthesis (R:P) ratios for *T. weissflogii* under elevated pCO_2 was higher than at ambient pCO_2 by 73%, while no effects of the fluctuating regime were detected. R:P ratios for *T. oceanica* cells was higher by 104% in the fluctuating regime than for cells in the corresponding steady regime at ambient pCO_2 level (Table ~~1~~2).

~~**3.6 NPQ versus irradiance curves**~~

~~The development kineties of NPQ with increasing light intensity differed in the two diatoms (Fig. 6). No effects of fluctuating regimes on NPQ were found in either species, so for clarity, only steady regimes are shown. *T. weissflogii* had higher NPQ values than *T. oceanica* above a light intensity of 330 μmol photons m^{-2} s^{-1}, and its maximal extent of NPQ under the highest light of RLCs was 6-7 times higher than that of *T. oceanica*.~~

4 Discussion

Both species were influenced by elevated pCO_2 in several ways, while they responded differentially to fluctuating regime. In general, for the coastal diatom *T. weissflogii*, the fluctuating pCO_2 regime showed either positive (POC cellular quota and production rate) or no obvious effects on its

physiological performance ~~of the coastal diatom *T. weissflogii*~~. In contrast, the oceanic diatom *T. oceanica* was significantly negatively affected by the diurnal variation of carbonate chemistry~~, with higher dark respiration under the fluctuating regime than under the steady regime~~. The fluctuating regime reduced photosynthetic oxygen evolution rates ~~of *T. oceanica*~~and enhanced dark respiration ~~under~~rates under ambient pCO_2 concentration, while in the OA scenario, the fluctuating regime depressed its growth rate, chlorophyll *a* content, and elemental production rates (which were caused by decreased growth rates).

OA depressed the growth of *T. oceanica*, consistent with results of a previous study (King et al. 2015), which showed a similar decrease (19%) to the present study (16%). No detectable effects of OA on growth of *T. weissflogii* were found, as reported by previous studies (Burkhardt et al. 1999; Shi et al. 2009; Reinfelder 2012; King et al. 2015; Passow and Laws 2015; Taucher et al. 2015). However, the growth responses of diatoms have also been shown to be affected by interactions between OA and other abiotic factors. For instance, the energy saved from active inorganic carbon acquisition mechanisms due to increased availability of CO_2 under OA conditions enhanced the growth of diatoms when daytime mean light level was lower than 22-36% of sea surface solar light intensity. However, growth under OA condition decreased when light exceeded 25-42% of incident irradiance (Gao et al. 2012). OA reduced the growth rate of *T. weissflogii* under light and temperature stress, but no effects of OA were detected in the absence of temperature stress (Passow and Laws 2015). Consequently, it appears that effects of OA on phytoplankton species could be region-specific, depending on the local interactions with other abiotic factors.

The silicified cell walls of diatoms act as mechanical protection to resist grazers (Hamm et al. 2003), and also have potential roles in photoprotection (Raven and Waite 2004), as well as promotion of catalysis by extracellular carbonic anhydrase (Milligan and Morel 2002). Si:C ratio of both species decreased under the elevated pCO_2 condition, in accordance with results of Tatters et al. (2012) and Mejia et al. (2013). This decreased ratio indicates that ~~diatoms~~ the tested species may fix more carbon per silicon assimilated ~~have reduced silicon requirements per carbon fixed under an~~ in the OA scenario than under the ambient pCO_2 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~~In Si-replete regions, a lower ratio may reduce the ballasting function of silica in carbon export by diatoms (Mejia et al. 2013). In Si-limited waters, a consequence may be that the proportion of diatoms in phytoplankton communities may increase due to reduced Si requirements (Mejia et al. 2013)~~. However, diatom silicification is under a complex set of controls. For instance, limitation by other nutrients such as, iron (Hutchins and Bruland 1998) and nitrogen (Flynn and Martin-Jézéquel 2000), may act to increase Si quotas and Si:C ratio.

Bicarbonate utilization has been suggested to be a general characteristic of marine diatoms, through direct transport or conversion by extracellular carbonic anhydrase (eCA), while the fraction of direct bicarbonate transport and eCA expression varies among species (Martin and Tortell 2008). Pathways that can utilize HCO_3^- and provide CO_2 for Rubisco through C_4 (Reinfelder et al. 2000) or C_3-C_4 intermediate photosynthesis (Roberts et al. 2007)~~ photosynthesis~~ have been suggested for *T. weissflogii*. This species takes up both CO_2 and HCO_3^- at a similar rate, and has the ability to adjust uptake rates to cope with a wide range of inorganic carbon supplies (Burkhardt et al. 2001). 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 ~~before the end of the photoperiod~~at 11.5 h after the onset of light, when the highest pH and lowest CO_2 was reached. In contrast, no effects of the fluctuating regime on oxygen evolution rates of *T. weissflogii* were found at this time point. ~~As shown here, cells of *T. weissflogii* benefited from their inorganic carbon transport and uptake characteristics and~~Thus *T. weissflogii* cells were more tolerant of the high pH and low CO_2 period under fluctuating carbonate chemistry than *T. oceanica*.

Under the fluctuating regime, *T. oceanica* showed higher respiration rates in ~~both~~ the current ~~and OA~~ scenario~~s~~ than under the corresponding steady regime. ~~Just as *T. oceanica* makes a~~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 ~~sacrifice~~

its ability to acclimate to fluctuating carbonate chemistry in a different way compared with the coastal diatom, since this is a characteristic of coastal rather than oceanic habitats. The higher respiration rate under the fluctuating regime in the current scenario may imply that this species needs more energy for maintaining its intracellular acid-base balance under dynamic extracellular pH conditions, as dark respiration provides energy for growth and metabolic processes (Raven and Beardall, 2005)(Beardall and Raven 2012). 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. *T. oceanica* cells showed significantly higher R:P ratios than *T. weissflogii*, especially in the fluctuating regime at ambient pCO_2, 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. Moreover, the fluctuating regime reduced the production rate of organic matter by *T. oceanica* at elevated pCO_2. Depressed biomass build-up has also been found under dynamic light regimes (Wagner et al. 2006; Shatwell et al. 2012; Hoppe et al. 2015). Together with our results, this may imply that organisms that are sensitive to fluctuating abiotic factors maintain intracellular homeostasis under dynamic environments of light or pCO_2 at the expense of reduced biomass production.

In contrast, eEither positive (POC production rate) or no obvious effects of the fluctuating regime on biomass production were found in the coastal species *T. weissflogii*. Coastal calcifying organisms have shown the ability to achieve homeostasis within critical tissues to facilitate calcification under dynamic pH/pCO_2 condition, and this was suggested to be associated with diurnal and seasonal pH fluctuations in coastal waters (Hendriks et al. 2015). Thus, some organisms could take advantage of the fluctuating carbonate system regime to mitigate the negative effects of ocean acidification on physiological performance. For instance, growth and calcification of corals can benefit from oscillatory pCO_2 (Dufault et al. 2012; Comeau et al. 2014). Organisms like *T. weissflogii* whose physiological performance were enhanced or unaltered under dynamic carbonate chemistry conditions thus could be at a distinct advantage in competing with species that showed negative responses to this condition (such

as *T. oceanica* in the present study). The differential responses of the tested two species to the fluctuating carbonate chemistry may be partially attributed to the differences in cell size. The differences in carbonate chemistry and pH between the bulk medium and the exterior surface of marine organisms increase as cell size increases (Flynn et al. 2012). 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.

Schaum et al. (2016) found that short-term plastic responses to high pCO_2 disappeared in a green microalgae after extended experimental evolution at high pCO_2, particularly in fluctuating pCO_2 treatments. Whether a similar phenomenon may be operative in other algal groups such as diatoms following exposures to high, fluctuating pCO_2 that are longer than those we employed, is currently unknown. However, it is notable that growth rates and competitive abilities of ~~all~~ diatoms of ~~the members of~~ a natural ~~diatom~~ community showed little change following one year of conditioning at two pCO_2 levels and three temperatures, relative to the results of a short-term experiment conducted on the original collected community (Tatters et al. 2013). Regardless of the responses of cell physiology to different timescales of changes in pCO_2 concentrations, it is a significant observation that the fluctuating regime reduced the production rate of organic matter in *T. oceanica* at elevated pCO_2 (which were caused by decreased growth rates).

~~Diatoms have an efficient dissipation of excess excitation energy through NPQ (Goss and Jakob 2010), which can be three to five times larger than that of higher plants (Ruban et al. 2004). NPQ processes can be initiated in seconds to minutes (Müller et al. 2001; Eberhard et al. 2008), and so are the first lines of defense for cells to respond to light stress (Lavaud et al. 2004; Lavaud et al. 2007). Strzepek and Harrison (2004) found *T. weissflogii* had higher NPQ values than *T. oceanica* under both low and high light conditions. Similarly, *T. weissflogii* showed 6–7 times higher NPQ than *T. oceanica* at high light in this study. This difference may reflect their contrasting habitats, since species from fluctuating light environments need a greater and more flexible capacity for photoprotection than those from relatively stable light environments (Lavaud et al. 2007). In addition to greater and more flexible capacity to dissipate excess excitation energy of coastal species, they were less sensitive to UV stress~~

~~than offshore ones. Inhibition of phytoplankton primary production induced by UV-A increases from coastal to offshore waters (Li et al. 2011). Thus, NPQ capacity and UV sensitivity could be a major factor influencing geographic distribution patterns of phytoplankton (Laviale et al. 2015).~~

The effect of the fluctuating regime on *T. oceanica* was different ~~under~~ in the current and OA scenarios. Under elevated rather than current pCO_2 condition, fluctuating carbonate chemistry decreased pigment content and the production rate of organic matter. Although elevated CO_2 mitigated the negative effects of the fluctuating regime on photosynthetic oxygen evolution rates of *T. oceanica* cells under ambient pCO_2 condition~~limited availability of pCO_2 that occurred at the end of photoperiod under the LCf condition~~, the effect of the fluctuating regime under elevated pCO_2 tended to be negative, resulting in a decreased growth rate compared to the steady regime. ~~In our study,~~ The diurnal pH variation range (~ 0.5 units) used in the present study is realistic for coastal ecosystems, like upwelling regions (Hofmann et al. 2011), kelp forests (Cornwall et al. 2013), coastal coral reefs (Wang et al. 2014), and tide pools (Morris and Taylor, 1983). In contrast, pH in the open ocean is relatively stable, with a variation range of only ~ 0.024 over a month (Hofmann et al. 2011). ~~the~~The same amplitude of pH variation ~~(~ 0.5 units)~~ was set ~~under~~ in the current and elevated pCO_2 scenarios in the present study. Buffering capacity will decrease as the increase of dissolved inorganic carbon in both coastal and oceanic seawater under projected elevated pCO_2 conditions (Egleston et al. 2010; Cai et al. 2011; Denman et al. 2011; Wang et al. 2013). For instance, the Revelle factor increased from 10.6 ± 0.2 to 15.0 ± 0.2 (a higher Revelle factor indicates a lower buffer capacity) when pCO_2 increased from the ambient to the elevated level in the present study. The increase amplitude of pH variation in coastal water will be more apparent than in oceanic water under an OA scenario, due to high biomass and sufficient nutrients. With a larger diurnal pH variation range in the future ocean, *T. oceanica* would be affected more than observed in the present study. Thus, based on our results, the competitive disadvantage for organisms like *T. oceanica* under fluctuating carbonate chemistry conditions would be amplified ~~under elevated pCO_2 condition~~in the OA scenario.

Given the decreased growth and elemental production rates ~~poor physiological performance~~ 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. In contrast, *T. weissflogii* ~~and *T. pseudonana*~~ appears to be insensitive to, even benefit from, fluctuating carbonate chemistry. This striking contrast of physiological traits in coastal and oceanic diatoms suggests that the ability to cope with fluctuating carbonate chemistry may play a role in influencing the geographic distributions of species under OA conditions. 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), ~~), are factors that will help to decide~~will determine the spatial distribution patterns of species in ~~both the present and~~the future oceans. 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.

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Figure captions

Figure 1. Measured pH_{NBS} variation over a diel cycle in the four experimental treatments (LCs, closed ~~squares~~triangles; LCf, open ~~squares~~triangles; HCs, closed circles; HCf, open circles). Here pH values of triplicate cultures in one experimental day are shown.

Figure 2. Specific growth rates of *T. weissflogii* (a) and *T. oceanica* (b) under steady (closed bars) and fluctuating (open bars) regimes of ambient (LC) and elevated (HC) pCO_2 levels. Values are means ± SD of triplicate ~~samples~~cultures. The different letters indicate significant ($p < 0.05$) differences among treatments.

Figure 3. Production rates of chlorophyll *a* (a, b), POC (c, d), PON (e, f), and BSi (g, h) of *T. weissflogii* (a, c, e, g) and *T. oceanica* (b, d, f, h) under steady (closed bars) and fluctuating (open bars) regimes of ambient (LC) and elevated (HC) pCO_2 levels. Values are means ± SD of triplicate ~~samples~~cultures. The different letters indicate significant ($p < 0.05$) differences among treatments.

Figure 4. Chlorophyll-normalized net oxygen evolution rates determined at 6 h after the onset of light ~~in the middle of photoperiod of~~ for *T. weissflogii* (a) and *T. oceanica* (b) under steady (closed bars) and fluctuating (open bars) regimes of ambient (LC) and elevated (HC) pCO_2 levels. Oxygen evolution rates per cell of *T. weissflogii* (c) and *T. oceanica* (d) of the four treatments determined 0.5, 6, and 11.5 h after ~~illumination~~the onset of light. Values are means ± SD of triplicate ~~samples~~cultures. The different letters indicate significant ($p < 0.05$) differences among treatments.

Figure 5. Dark respiration rates determined at 6 h after the onset of light ~~per chlorophyll (a, b) or cell (c, d) in the middle of the photoperiod~~ for *T. weissflogii* (a~~, c~~) and *T. oceanica* (b~~, d~~) under steady (closed bars) and fluctuating (open bars) regimes of ambient (LC) and elevated (HC) pCO_2 levels. Values are means ± SD of triplicate ~~samples~~cultures. The different letters indicate significant ($p < 0.05$) differences

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among treatments.

~~Figure 6. Non-photochemical quenching (NPQ) versus irradiance curves of *T. weissflogii* (solid lines) and *T. oceanica* (dashed lines) measured at ambient (squares) and elevated (circles) pCO_2 levels. Values are means ± SD of triplicate samples. The maximum light intensities in RLCs were set as 1593 µmol photons m^{-2} s^{-1} for *T. oceanica* of ambient pCO_2 level and 2130 µmol photons m^{-2} s^{-1} for the remaining measurements.~~

Table 1. Carbonate chemistry parameters of culture media before and after dilution under steady and fluctuating regimes of ambient (LC) and elevated (HC) pCO_2 levels. Values are means ± SD of triplicate cultures. The different letters indicate significant ($p < 0.05$) differences among treatments.

	pH_{NBS}	TA ($\mu mol\ kg^{-1}$)	DIC ($\mu mol\ kg^{-1}$)	HCO_3^- ($\mu mol\ kg^{-1}$)	CO_3^{2-} ($\mu mol\ kg^{-1}$)	CO_2 ($\mu mol\ kg^{-1}$)
After dilution						
LCs	8.12 ± 0.03^a	2397 ± 7^a	2132 ± 20^a	1929 ± 27^a	188 ± 8^a	16 ± 1^a
LCf	8.13 ± 0.01^a	2398 ± 2^a	2128 ± 6^a	1922 ± 10^a	191 ± 4^a	15 ± 1^a
HCs	7.80 ± 0.02^b	2392 ± 5^a	2279 ± 10^b	2144 ± 12^b	98 ± 3^b	37 ± 1^b
HCf	7.80 ± 0.02^b	2406 ± 13^a	2288 ± 19^b	2152 ± 20^b	100 ± 3^b	36 ± 2^b
Before dilution						
LCs	8.13 ± 0.02^a	2399 ± 2^a	2133 ± 7^a	1929 ± 12^a	189 ± 5^a	15 ± 1^a
LCf	8.14 ± 0.02^a	2388 ± 19^a	2116 ± 21^a	1910 ± 22^a	191 ± 5^a	15 ± 1^a
HCs	7.79 ± 0.02^b	2401 ± 6^a	2287 ± 7^b	2153 ± 8^b	98 ± 4^b	37 ± 2^b
HCf	7.82 ± 0.01^b	2408 ± 9^a	2283 ± 12^b	2144 ± 13^b	104 ± 2^b	34 ± 1^b

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Table ~~1~~2. Cell size, respiration to photosynthesis ratio (R:P), cellular quotas of chlorophyll, particulate organic carbon (POC), particulate organic nitrogen (PON), and biogenic silica (BSi) and elemental ratios of *T. weissflogii* and *T. oceanica* under steady and fluctuating regimes of ambient (LC) and elevated (HC) pCO_2 levels. Values are means ± SD of triplicate ~~samples~~cultures. The different letters indicate significant ($p < 0.05$) differences among treatments.

	T. weissflogii				*T. oceanica*			
	LCs	LCf	HCs	HCf	LCs	LCf	HCs	HCf
Cell size (μm)	12.17±0.05^{a}	12.17±0.05^{a}	12.20±0.04^{a}	12.18±0.04^{a}	5.58±0.01^{A}	5.65±0.03^{B}	5.71±0.02^{C}	5.63±0.02^{B}
Cellular quotas								
Chl *a* (pg cell^{-1})	3.24±0.14^{a}	3.15±0.05^{a}	3.25±0.05^{a}	3.27±0.07^{a}	0.30±0.03^{A}	0.33±0.02^{AB}	0.38±0.06^{B}	0.29±0.02^{A}
POC (pmol cell^{-1})	6.94±0.36^{a}	7.59±0.23^{b}	10.28±0.29^{c}	10.28±0.28^{c}	1.49±0.12^{A}	1.68±0.20^{A}	2.38±0.17^{B}	2.20±0.07^{B}
PON (pmol cell^{-1})	1.21±0.14^{a}	1.34±0.12^{a}	1.94±0.11^{b}	1.80±0.06^{b}	0.25±0.03^{A}	0.29±0.01^{A}	0.49±0.03^{B}	0.44±0.03^{B}
BSi (pmol cell^{-1})	1.11±0.01^{ab}	1.06±0.04^{a}	1.19±0.10^{b}	1.04±0.04^{a}	0.35±0.03^{A}	0.34±0.03^{A}	0.32±0.02^{AB}	0.29±0.01^{B}
Ratios								
C:N (pmol:pmol)	5.78±0.40^{a}	5.68±0.32^{a}	5.30±0.20^{a}	5.72±0.24^{a}	6.10±0.60^{A}	5.87±0.70^{AB}	4.90±0.16^{B}	5.05±0.36^{B}
Si:C (pmol:pmol)	0.16±0.01^{a}	0.14±0.01^{b}	0.12±0.01^{c}	0.10±0.01^{c}	0.24±0.02^{A}	0.20±0.02^{B}	0.14±0.01^{C}	0.13±0.01^{C}
R:P (fmol cell^{-1} h^{-1}: fmol cell^{-1} h^{-1})	0.08±0.01^{a}	0.08±0.02^{a}	0.13±0.03^{b}	0.14±0.03^{b}	0.27±0.07^{A}	0.55±0.12^{B}	0.29±0.06^{A}	0.39±0.04^{A}

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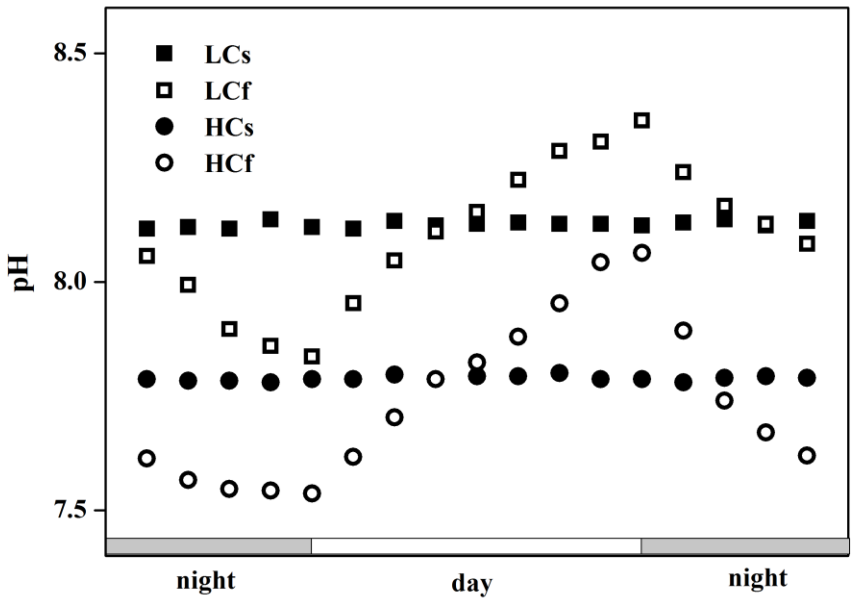
Table ~~2~~3. Effective photochemical quantum yields (Φ_{PSII}) and non-photochemical quenching (NPQ) determined 0.5, 6, and 11.5 h after ~~illumination~~ the onset of light of *T. weissflogii* and *T. oceanica* under steady and fluctuating regimes of ambient (LC) and elevated (HC) pCO_2 levels. Φ_{PSII} and NPQ were determined under actinic light intensity (~ 156 µmol photons m^{-2} s^{-1}) similar to culture light level after 10 min dark adaptation. Values are means ± SD of triplicate ~~samples~~cultures. The different letters indicate significant ($p < 0.05$) differences among treatments.

	T. weissflogii				*T. oceanica*			
Time point	LCs	LCf	HCs	HCf	LCs	LCf	HCs	HCf
Φ_{PSII}								
0.5 h	0.61±0.01^{a}	0.61±0.01^{a}	0.59±0.01^{a}	0.54±0.03^{b}	0.57±0.03^{A}	0.58±0.01^{A}	0.59±0.06^{A}	0.61±0.03^{A}
6 h	0.60±0.01^{a}	0.60±0.01^{a}	0.58±0.01^{b}	0.59±0.01^{b}	0.57±0.01^{A}	0.57±0.01^{A}	0.54±0.01^{B}	0.56±0.01^{AB}
11.5 h	0.58±0.01^{a}	0.58±0.01^{a}	0.57±0.01^{b}	0.57±0.01^{ab}	0.61±0.03^{A}	0.60±0.03^{AB}	0.57±0.01^{B}	0.57±0.01^{AB}
NPQ								
0.5 h	0.13±0.02^{a}	0.13±0.01^{a}	0.13±0.01^{a}	0.23±0.05^{b}	0.10±0.02^{A}	0.06±0.02^{A}	0.08±0.05^{A}	0.12±0.09^{A}
6 h	0.08±0.03^{a}	0.08±0.02^{ab}	0.11±0.01^{b}	0.09±0.01^{ab}	0.13±0.02^{A}	0.14±0.01^{A}	0.18±0.02^{B}	0.19±0.01^{B}
11.5 h	0.08±0.01^{a}	0.07±0.01^{a}	0.06±0.01^{b}	0.07±0.01^{ab}	0.09±0.02^{A}	0.06±0.01^{B}	0.06±0.01^{B}	0.07±0.01^{AB}



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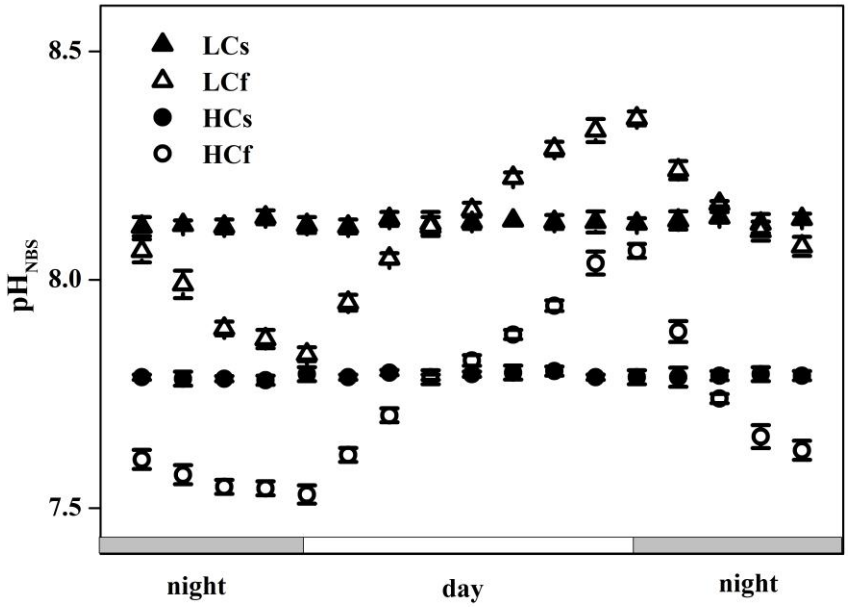

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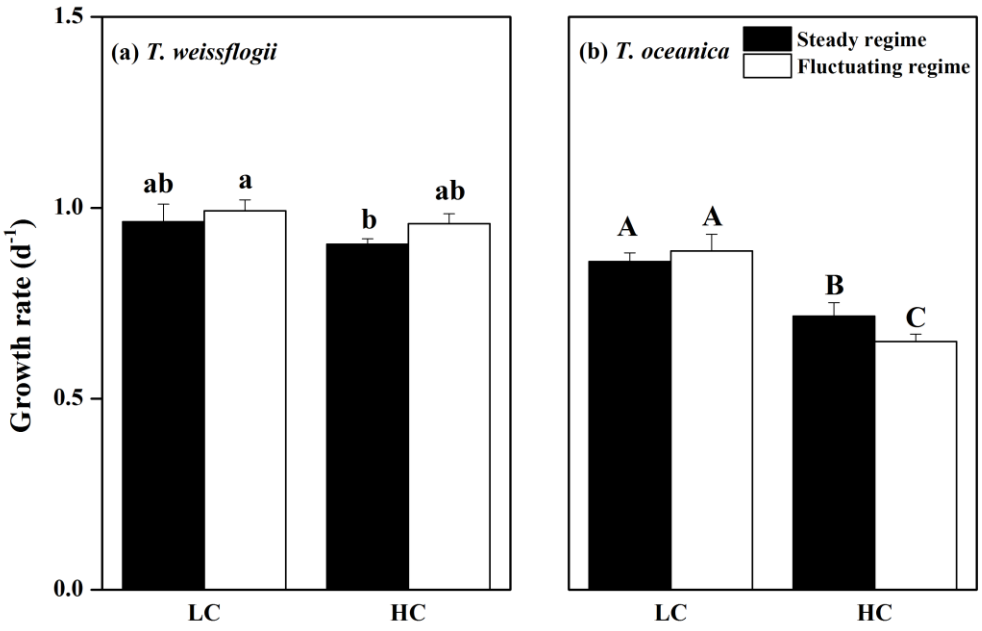

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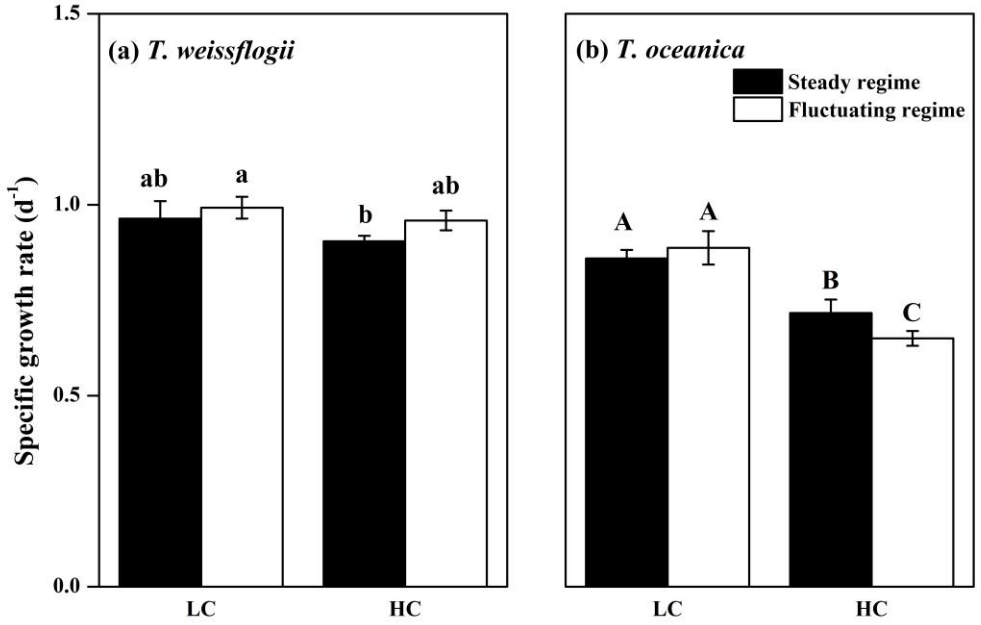

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758 Figure 2

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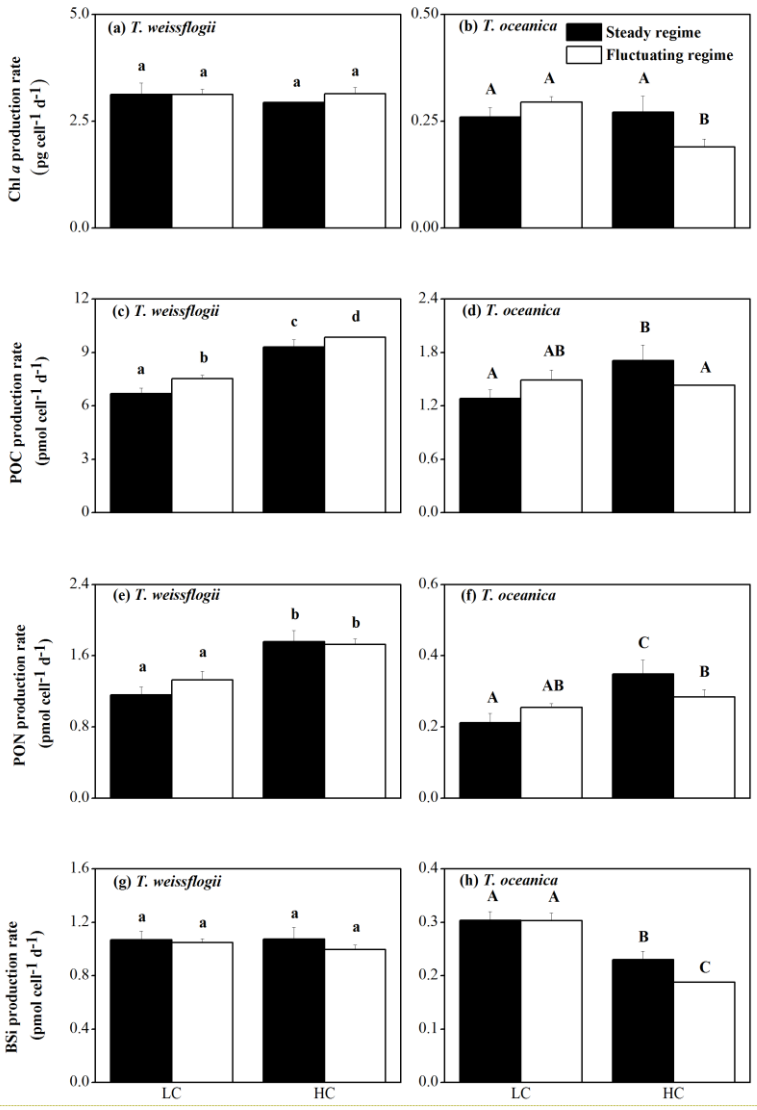

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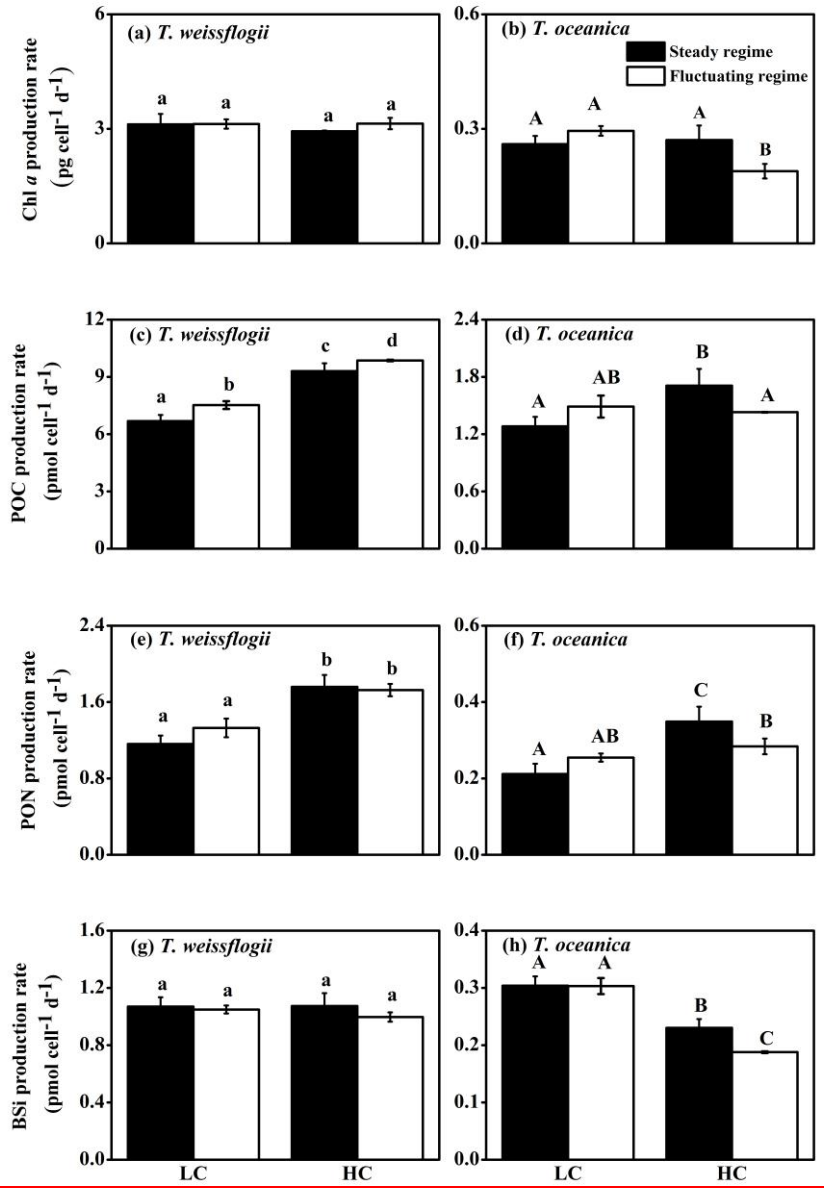

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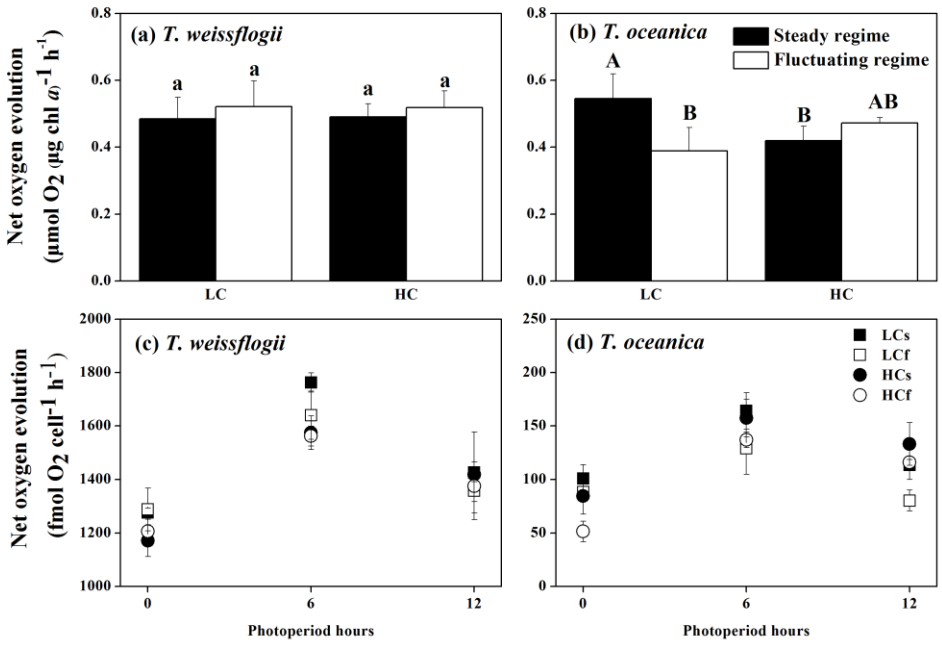

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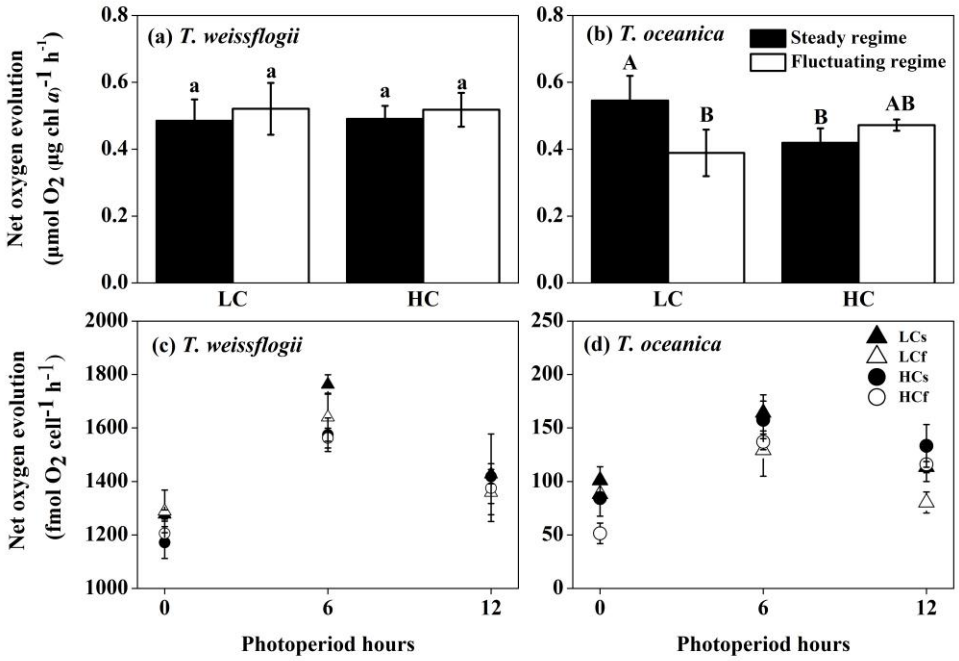
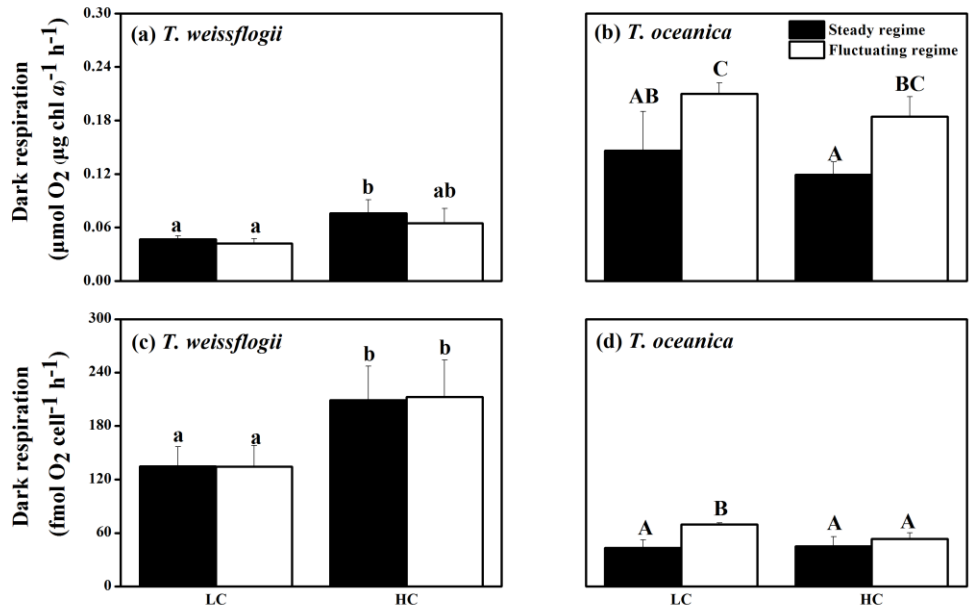

(a) T. weissflogii
(b) T. oceanica
Steady regime
Fluctuating regime
Net oxygen evolution (μmol O2 (μg chl a)-1 h-1)
LC
HC
(c) T. weissflogii
(d) T. oceanica
LCs
LCf
HCs
HCf
Net oxygen evolution (fmol O2 cell-1 h-1)
Photoperiod hours

Figure 4



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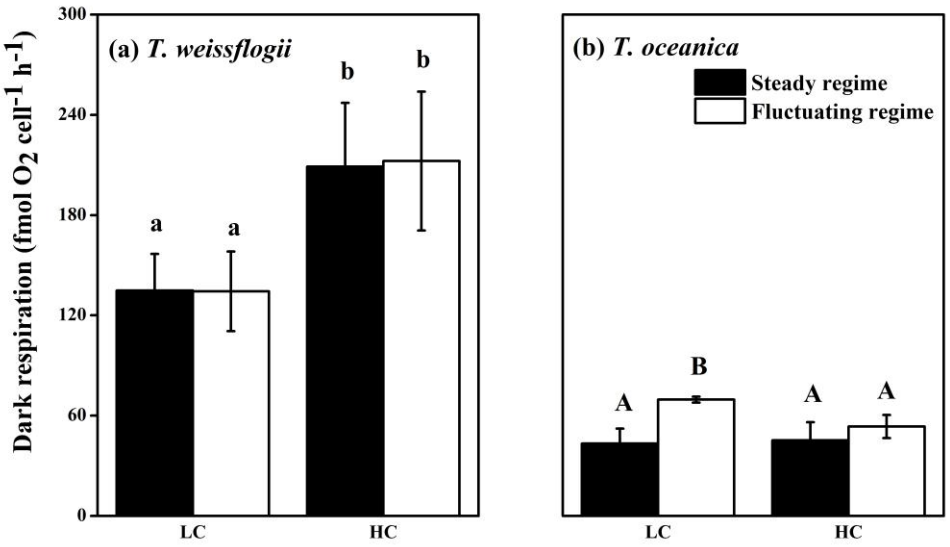
(a) T. weissflogii
(b) T. oceanica
Steady regime
Fluctuating regime
Dark respiration (fmol O2 cell-1 h-1)
300
240
180
120
60
0
a
a
b
b
A
B
A
A
LC
HC
LC
HC

Figure 5

带格式的: 字体: (默认) Times New Roman, 12 磅

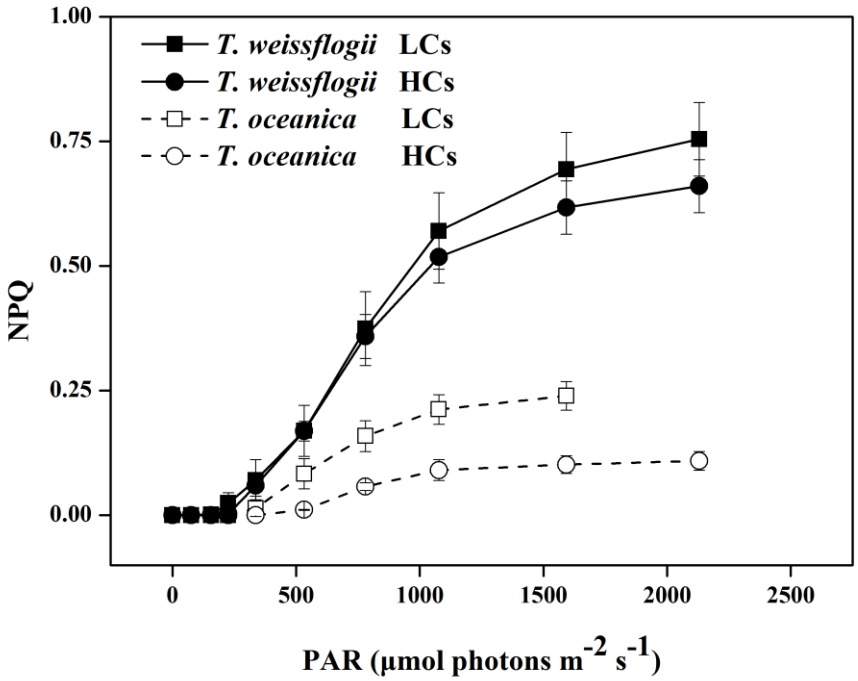

Figure 6