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

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Abstract

Diel 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. 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), 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). 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).

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). 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): $\Phi_{PSII} = (F_m' - F_t) / F_m' = \Delta F / F_m'$ 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 = $(F_m - F_m') / F_m'$, 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 the onset of light.

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 media were pre-adjusted to their corresponding culture 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). 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).

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. 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 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, 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, 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). 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.

Mean cell sizes were not affected by the fluctuating treatment under either ambient or elevated pCO_2 conditions in *T. weissflogii* (Table 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 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 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 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. 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.

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 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 0.5 h after the onset of light. Elevated pCO_2 decreased Φ_{PSII} by 3%

and 2% for *T. weissflogii* at 6 and 11.5 h after the onset of light, respectively. *T. oceanica* cells under elevated pCO_2 showed 5% and 7% lower Φ_{PSII} compared to those under ambient pCO_2 at 6 and 11.5 h after 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 0.5 h after the onset of light and LCf cells of *T. oceanica* 11.5 h after the onset of light. For steady regimes, elevated pCO_2 showed no detectable effect on NPQ of both species at 0.5 h after the onset of light, while it increased NPQ of *T. weissflogii* by 37.5% and decreased it by 25% relative to values of LCs cells 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 33.3% relative to values of LCs cells at 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} 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 showed a 29% 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.

Both species, regardless of treatment, showed a similar diurnal rhythm of photosynthetic oxygen evolution: oxygen evolution rates reached the highest values 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 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. *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 the onset of light, LCs cells of *T. oceanica* showed 41% higher net oxygen evolution rates per cell than LCf cells. There were no

differences in photosynthetic oxygen evolution rates between HCs and HCf cells 11.5 h after the onset of light.

Elevated pCO_2 increased dark respiration of *T. weissflogii* by 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 60% for cells under the fluctuating regime compared to steady one at ambient pCO_2 level (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. 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 2).

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. In contrast, the oceanic diatom *T. oceanica* was significantly negatively affected by the diurnal variation of carbonate chemistry. The fluctuating regime reduced photosynthetic oxygen evolution rates and enhanced dark respiration 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

289 to increased availability of CO_2 under OA conditions enhanced the growth of diatoms when daytime
290 mean light level was lower than 22-36% of sea surface solar light intensity. However, growth under OA
291 condition decreased when light exceeded 25-42% of incident irradiance (Gao et al. 2012). OA reduced
292 the growth rate of *T. weissflogii* under light and temperature stress, but no effects of OA were detected
293 in the absence of temperature stress (Passow and Laws 2015). Consequently, it appears that effects of
294 OA on phytoplankton species could be region-specific, depending on the local interactions with other
295 abiotic factors.

296 The silicified cell walls of diatoms act as mechanical protection to resist grazers (Hamm et al. 2003),
297 and also have potential roles in photoprotection (Raven and Waite 2004), as well as promotion of
298 catalysis by extracellular carbonic anhydrase (Milligan and Morel 2002). Si:C ratio of both species
299 decreased under the elevated pCO_2 condition, in accordance with results of Tatters et al. (2012) and
300 Mejia et al. (2013). This decreased ratio indicates that the tested species may fix more carbon per silicon
301 assimilated in the OA scenario than under the ambient pCO_2 condition, and so has implications for
302 changes in local and global carbon and silicon budgets. More carbon may be fixed per diatom cell
303 without changes in silicon quota in the OA scenario, and thus the tested species may contribute more to
304 primary production in the ecosystem, especially in Si-limited waters, in the future oceans. However,
305 diatom silicification is under a complex set of controls. For instance, limitation by other nutrients such
306 as, iron (Hutchins and Bruland 1998) and nitrogen (Flynn and Martin-Jézéquel 2000), may act to
307 increase Si quotas and Si:C ratio.

308 Bicarbonate utilization has been suggested to be a general characteristic of marine diatoms, through
309 direct transport or conversion by extracellular carbonic anhydrase (eCA), while the fraction of direct
310 bicarbonate transport and eCA expression varies among species (Martin and Tortell 2008). Pathways
311 that can utilize HCO_3^- and provide CO_2 for Rubisco through C_4 (Reinfelder et al. 2000) or C_3-C_4
312 intermediate photosynthesis (Roberts et al. 2007) have been suggested for *T. weissflogii*. This species
313 takes up both CO_2 and HCO_3^- at a similar rate, and has the ability to adjust uptake rates to cope with a
314 wide range of inorganic carbon supplies (Burkhardt et al. 2001). Moreover, *T. weissflogii* has a
315 markedly higher fraction of direct bicarbonate transport and apparent eCA activity than *T. oceanica*

316 (Martin and Tortell 2008), which may facilitate their inorganic carbon transport and uptake. In this
317 study, *T. oceanica* showed significantly lower oxygen evolution rates in the LCf treatment than in the
318 LCs treatment at 11.5 h after the onset of light, when the highest pH and lowest CO_2 was reached. In
319 contrast, no effects of the fluctuating regime on oxygen evolution rates of *T. weissflogii* were found at
320 this time point. Thus *T. weissflogii* cells were more tolerant of the high pH and low CO_2 period under
321 fluctuating carbonate chemistry than *T. oceanica*.

322 Under the fluctuating regime, *T. oceanica* showed higher respiration rates in the current scenario than
323 under the corresponding steady regime. As with the successful compromise between iron requirements
324 and capacity to acclimate to dynamic light regimes in *T. oceanica* cells (Strzepek and Harrison 2004),
325 this oceanic diatom may also have evolved to acclimate to fluctuating carbonate chemistry in a different
326 way compared with the coastal diatom. The higher respiration rate under the fluctuating regime in the
327 current scenario may imply that this species needs more energy for maintaining its intracellular acid-
328 base balance under dynamic extracellular pH conditions, as dark respiration provides energy for growth
329 and metabolic processes (Raven and Beardall, 2005). Diatoms were shown to exhibit circadian
330 variations in photosynthesis and respiration (Weger et al., 1989; Chen and Gao, 2004). Thus dark
331 respiration might show different pattern at other time points, as photosynthetic oxygen evolution did. *T.*
332 *oceanica* cells showed significantly higher R:P ratios than *T. weissflogii*, especially in the fluctuating
333 regime at ambient pCO_2, and the ratios were within previously reported ranges in diatoms (Geider and
334 Osborne, 1989). The higher R:P ratio indicated greater proportions of photosynthetic fixed carbon and
335 associated energy were used for growth, biosynthesis, and maintaining intracellular homeostasis in the
336 oceanic species. Moreover, the fluctuating regime reduced the production rate of organic matter by *T.*
337 *oceanica* at elevated pCO_2. Depressed biomass build-up has also been found under dynamic light
338 regimes (Wagner et al. 2006; Shatwell et al. 2012; Hoppe et al. 2015). Together with our results, this
339 may imply that organisms that are sensitive to fluctuating abiotic factors maintain intracellular
340 homeostasis under dynamic environments of light or pCO_2 at the expense of reduced biomass
341 production.

342 Either 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 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). 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).

The effect of the fluctuating regime on *T. oceanica* was different 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, 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. 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 same amplitude of pH variation was set 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 in the OA scenario.

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. In contrast, *T. weissflogii* 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), will determine the spatial distribution patterns of species in 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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414 **References**

415 Britton, D., Cornwall, C. E., Revill, A. T., Hurd, C. L., and Johnson, C. R.: Ocean acidification reverses
416 the positive effects of seawater pH fluctuations on growth and photosynthesis of the habitat-forming
417 kelp, *Ecklonia radiata*, Scientific Reports, 6, 26036, doi:10.1038/srep26036, 2016.

418 Brzezinski, M. A., and Nelson, D. M.: The annual silica cycle in the Sargasso Sea near Bermuda, Deep-
419 Sea Res. Part I, 42, 1215-1237, 1995.

420 Burkhardt, S., Zondervan, I., and Riebesell, U.: Effect of CO_2 concentration on C: N: P ratio in marine
421 phytoplankton: A species comparison, Limnol. Oceanogr., 44, 683-690, 1999.

422 Burkhardt, S., Amoroso, G., Riebesell, U., and Sültemeyer, D.: CO_2 and HCO_3^- uptake in marine
423 diatoms acclimated to different CO_2 concentrations, Limnol. Oceanogr., 46, 1378-1391, 2001.

424 Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., Chou, W.-C., Zhai, W.,
425 Hollibaugh, J. T., and Wang, Y.: Acidification of subsurface coastal waters enhanced by
426 eutrophication, Nat. Geosci., 4, 766-770, 2011.

427 Capone, D. G., and Hutchins, D. A.: Microbial biogeochemistry of coastal upwelling regimes in a
428 changing ocean, Nat. Geosci., 6, 711-717, 2013.

429 Chen, X. and Gao, K.: Characterization of diurnal photosynthetic rhythms in the marine diatom
430 *Skeletonema costatum* grown in synchronous culture under ambient and elevated CO_2, Funct. Plant.
431 Biol., 31, 399-404, 2004.

432 Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway,
433 J., and Heimann, M.: Carbon and other biogeochemical cycles, in: Climate change 2013: the physical
434 science basis. Contribution of Working Group I to the Fifth Assessment Report of the
435 Intergovernmental Panel on Climate Change, Cambridge University Press, 465-570, 2014.

436 Comeau, S., Edmunds, P. J., Spindel, N. B., and Carpenter, R. C.: Diel pCO_2 oscillations modulate the
437 response of the coral *Acropora hyacinthus* to ocean acidification, Mar. Ecol. Prog. Ser., 501, 99-111,
438 2014.

439 Cornwall, C. E., Hepburn, C. D., McGraw, C. M., Currie, K. I., Pilditch, C. A., Hunter, K. A., Boyd, P.
440 W., and Hurd, C. L.: Diurnal fluctuations in seawater pH influence the response of a calcifying

441 macroalga to ocean acidification, P. Roy. Soc. Lond. B Bio., 280, 20132201,
442 doi:10.1098/rspb.2013.2201, 2013.
443 Dai, M., Lu, Z., Zhai, W., Chen, B., Cao, Z., Zhou, K., Cai, W. J., and Chenc, C. T. A.: Diurnal
444 variations of surface seawater pCO_2 in contrasting coastal environments, Limnol. Oceanogr., 54,
445 735-745, 2009.
446 Denman, K., Christian, J. R., Steiner, N., Pörtner, H.-O., and Nojiri, Y.: Potential impacts of future
447 ocean acidification on marine ecosystems and fisheries: current knowledge and recommendations for
448 future research, ICES J. Mar. Sci., 68, 1019-1029, 2011.
449 Dixon, R. L.: Behavioral responses of common juvenile estuarine fishes to diel-cycling hypoxia and
450 corresponding pH fluctuations: a comparative approach, Ph.D. thesis, University of Delaware, USA,
451 2014.
452 Doney, S. C.: The growing human footprint on coastal and open-ocean biogeochemistry, Science, 328,
453 1512-1516, 2010.
454 Drupp, P., De Carlo, E. H., Mackenzie, F. T., Bienfang, P., and Sabine, C. L.: Nutrient inputs,
455 phytoplankton response, and CO_2 variations in a semi-enclosed subtropical embayment, Kaneohe
456 Bay, Hawaii, Aquat. Geochem., 17, 473-498, 2011.
457 Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., Carstensen, J.,
458 Trotter, J. A., and McCulloch, M.: Is ocean acidification an open-ocean syndrome? Understanding
459 anthropogenic impacts on seawater pH, Estuar. Coast., 36, 221-236, 2013.
460 Dufault, A. M., Cumbo, V. R., Fan, T.-Y., and Edmunds, P. J.: Effects of diurnally oscillating pCO_2 on
461 the calcification and survival of coral recruits, P. Roy. Soc. B Biol. Sci., 279, 2951-2958, 2012.
462 Egleston, E. S., Sabine, C. L., and Morel, F. M.: Revelle revisited: Buffer factors that quantify the
463 response of ocean chemistry to changes in DIC and alkalinity, Global Biogeochem. Cy., 24, 2010.
464 Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., and Hales, B.: Evidence for upwelling
465 of corrosive" acidified" water onto the continental shelf, Science, 320, 1490-1492, 2008.
466 Flynn, K. J., and Martin-Jézéquel, V.: Modelling Si–N-limited growth of diatoms, J. Plankton Res., 22,
467 447-472, 2000.

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.

Frieder, C. A., Gonzalez, J. P., Bockmon, E. E., Navarro, M. O., and Levin, L. A.: Can variable pH and low oxygen moderate ocean acidification outcomes for mussel larvae?, Global Change Biol., 20, 754-764, 2014.

Gao, K., Aruga, Y., Asada, K., Ishihara, T., Akano, T., and Kiyohara, M.: Calcification in the articulated coralline alga *Corallina pilulifera*, with special reference to the effect of elevated CO_2 concentration, Mar. Biol., 117, 129-132, 1993.

Gao, K., Xu, J., Gao, G., Li, Y., Hutchins, D. A., Huang, B., Wang, L., Zheng, Y., Jin, P., and Cai, X.: Rising CO_2 and increased light exposure synergistically reduce marine primary productivity, Nature Climate Change, 2, 519-523, 2012.

Gattuso, J.-P., Frankignoulle, M., and Wollast, R.: Carbon and carbonate metabolism in coastal aquatic ecosystems, Annu. Rev. Ecol. Syst., 405-434, 1998.

Gattuso, J.-P., Magnan, A., Billé, R., Cheung, W., Howes, E., Joos, F., Allemand, D., Bopp, L., Cooley, S., and Eakin, C.: Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios, Science, 349, aac4722, doi:10.1126/science.aac4722, 2015.

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.

Genty, B., Briantais, J.-M., and Baker, N. R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence, BBA Gen. Subjects, 990, 87-92, 1989.

Glibert, P., and Ray, R.: Different patterns of growth and nitrogen uptake in two clones of marine *Synechococcus* spp, Mar. Biol., 107, 273-280, 1990.

Hamm, C. E., Merkel, R., Springer, O., Jurkojc, P., Maier, C., Prechtel, K., and Smetacek, V.: Architecture and material properties of diatom shells provide effective mechanical protection, Nature, 421, 841-843, 2003.

Hendriks, I. E., Duarte, C. M., Olsen, Y. S., Steckbauer, A., Ramajo, L., Moore, T. S., Trotter, J. A., and McCulloch, M.: Biological mechanisms supporting adaptation to ocean acidification in coastal ecosystems, Estuar. Coast. Shelf S., 152, A1-A8, doi:10.1016/j.ecss.2014.07.019, 2015.

Hofmann, G. E., Smith, J. E., Johnson, K. S., Send, U., Levin, L. A., Micheli, F., Paytan, A., Price, N. N., Peterson, B., and Takeshita, Y.: High-frequency dynamics of ocean pH: a multi-ecosystem comparison, PloS One, 6, e28983, doi: 10.1371/journal.pone.0028983, 2011.

Hoppe, C. J., Holtz, L. M., Trimborn, S., and Rost, B.: Ocean acidification decreases the light-use efficiency in an Antarctic diatom under dynamic but not constant light, New Phytol., 207 (1), 159-171, 2015.

Howes, E. L., Joos, F., Eakin, M., and Gattuso, J.: An updated synthesis of the observed and projected impacts of climate change on the chemical, physical and biological processes in the oceans, Frontiers in Marine Science, 2, 36, doi:10.3389/fmars.2015.00036, 2015.

Hurd, C. L., Cornwall, C. E., Currie, K., Hepburn, C. D., McGraw, C. M., Hunter, K. A., and Boyd, P. W.: Metabolically induced pH fluctuations by some coastal calcifiers exceed projected 22nd century ocean acidification: a mechanism for differential susceptibility?, Global Change Biol., 17, 3254-3262, 2011.

Hutchins, D. A., and Bruland, K. W.: Iron-limited diatom growth and Si: N uptake ratios in a coastal upwelling regime, Nature, 393, 561-564, 1998.

Irigoien, X., Flynn, K., and Harris, R.: Phytoplankton blooms: a 'loophole'in microzooplankton grazing impact?, J. Plankton Res., 27, 313-321, 2005.

Irwin, A. J., Finkel, Z. V., Schofield, O. M. E., and Falkowski, P. G.: Scaling-up from nutrient physiology to the size-structure of phytoplankton communities, J. Plankton Res., 28, 459-471, 2006.

Jiang, Z.-P., Huang Jr, -. C., Dai, M., Kao, S. J., Hydes, D. J., Chou, W.-C., and Jan, S.: Short-term dynamics of oxygen and carbon in productive nearshore shallow seawater systems off Taiwan: Observations and modeling, Limnol. Oceanogr., 56, 1832-1849, 2011.

Johnson, M. D., Moriarty, V. W., and Carpenter, R. C.: Acclimatization of the crustose coralline alga *Porolithon onkodes* to variable pCO_2, PloS One, 9, e87678, doi:10.1371/journal.pone.0087678,

2014.

Keppel, A. G., Breitburg, D. L., Wikfors, G. H., Burrell, R. B., and Clark, V. M.: Effects of co-varying diel-cycling hypoxia and pH on disease susceptibility in the eastern oyster, *Crassostrea virginica*, Mar. Ecol. Prog. Ser., 538, 169-183, 2015.

King, A. L., Jenkins, B. D., Wallace, J. R., Liu, Y., Wikfors, G. H., Milke, L. M., and Meseck, S. L.: Effects of CO_2 on growth rate, C: N: P, and fatty acid composition of seven marine phytoplankton species, Mar. Ecol. Prog. Ser., 537, 59-69, 2015.

Lavaud, J., Strzepek, R. F., and Kroth, P. G.: Photoprotection capacity differs among diatoms: Possible consequences on the spatial distribution of diatoms related to fluctuations in the underwater light climate, Limnol. Oceanogr., 52, 1188-1194, 2007.

Lavaud, J., and Lepetit, B.: An explanation for the inter-species variability of the photoprotective non-photochemical chlorophyll fluorescence quenching in diatoms, BBA Bioenergetics, 1827, 294-302, 2013.

Laviale, M., Barnett, A., Ezequiel, J., Lepetit, B., Frankenbach, S., Méléder, V., Serôdio, J., and Lavaud, J.: Response of intertidal benthic microalgal biofilms to a coupled light–temperature stress: evidence for latitudinal adaptation along the Atlantic coast of Southern Europe, Environ. Microbiol., 17 (10), 3662-3677, 2015.

Li, G., Gao, K., and Gao, G.: Differential impacts of solar UV radiation on photosynthetic carbon fixation from the coastal to offshore surface waters in the South China Sea, Photochem. Photobiol., 87, 329-334, 2011.

Liu, S.-W., and Qiu, B.-S.: Different responses of photosynthesis and flow cytometric signals to iron limitation and nitrogen source in coastal and oceanic *Synechococcus* strains (Cyanophyceae), Mar. Biol., 159, 519-532, 2012.

Martin, C. L., and Tortell, P. D.: Bicarbonate transport and extracellular carbonic anhydrase in marine diatoms, Physiol. Plantarum, 133, 106-116, 2008.

Mejia, L. M., Isensee, K., Méndez-Vicente, A., Pisonero, J., Shimizu, N., González, C., Monteleone, B., and Stoll, H.: B content and Si/C ratios from cultured diatoms (*Thalassiosira pseudonana* and

549 *Thalassiosira weissflogii*): Relationship to seawater pH and diatom carbon acquisition, Geochim.
550 Cosmochim. Ac., 123, 322-337, 2013.
551 Milligan, A. J., and Morel, F. M.: A proton buffering role for silica in diatoms, Science, 297, 1848-1850,
552 2002.
553 Morris, S. and Taylor, A. C.: Diurnal and seasonal variation in physico-chemical conditions within
554 intertidal rock pools, Estuar. Coast. Shelf S., 17, 339-355, 1983.
555 Noisette, F., Egilsdottir, H., Davoult, D., and Martin, S.: Physiological responses of three temperate
556 coralline algae from contrasting habitats to near-future ocean acidification, J. Exp. Mar. Biol. Ecol.,
557 448, 179-187, 2013.
558 Onitsuka, T., Kimura, R., Ono, T., Takami, H., and Nojiri, Y.: Effects of ocean acidification on the early
559 developmental stages of the horned turban, *Turbo cornutus*, Mar. Biol., 161, 1127-1138, 2014.
560 Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N.,
561 Ishida, A., and Joos, F.: Anthropogenic ocean acidification over the twenty-first century and its
562 impact on calcifying organisms, Nature, 437, 681-686, 2005.
563 Passow, U., and Laws, E. A.: Ocean acidification as one of multiple stressors: growth response of
564 *Thalassiosira weissflogii* (diatom) under temperature and light stress, Mar. Ecol. Prog. Ser., 541, 75-
565 90, 2015.
566 Raven, J. A. and Beardall, J.: Respiration in aquatic photolithotrophs, in: Respiration in aquatic
567 ecosystems, edited by: del Giorgio, P. A. and Williams, P.J. le B., Oxford University Press, New
568 York, USA, 36-46, 2005.
569 Raven, J., and Waite, A.: The evolution of silicification in diatoms: inescapable sinking and sinking as
570 escape?, New Phytol., 162, 45-61, 2004.
571 Reinfelder, J. R., Kraepiel, A. M., and Morel, F. M.: Unicellular C_4 photosynthesis in a marine diatom,
572 Nature, 407, 996-999, 2000.
573 Reinfelder, J. R.: Carbon dioxide regulation of nitrogen and phosphorus in four species of marine
574 phytoplankton, Mar. Ecol. Prog. Ser., 466, 57-67, 2012.
575 Ritchie, R. J.: Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and

576 ethanol solvents, Photosynth. Res., 89, 27-41, 2006.
577 Roberts, K., Granum, E., Leegood, R. C., and Raven, J. A.: Carbon acquisition by diatoms, Photosynth.
578 Res., 93, 79-88, 2007.
579 Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., Wong, C.,
580 Wallace, D. W., and Tilbrook, B.: The oceanic sink for anthropogenic CO_2, Science, 305, 367-371,
581 2004.
582 Schaum, C.-E., Rost, B., and Collins, S.: Environmental stability affects phenotypic evolution in a
583 globally distributed marine picoplankton, ISME J., 10, 75-84, 2015.
584 Shatwell, T., Nicklisch, A., and Köhler, J.: Temperature and photoperiod effects on phytoplankton
585 growing under simulated mixed layer light fluctuations, Limnol. Oceanogr., 57, 541-553, 2012.
586 Shi, D., Xu, Y., and Morel, F.: Effects of the pH/pCO_2 control method on medium chemistry and
587 phytoplankton growth, Biogeosciences, 6, 1199-1207, 2009.
588 Strzepek, R. F., and Harrison, P. J.: Photosynthetic architecture differs in coastal and oceanic diatoms,
589 Nature, 431, 689-692, 2004.
590 Sunda, W. G., Price, N. M., and Morel, F. M.: Trace metal ion buffers and their use in culture studies, in:
591 Algal culturing techniques, Elsevier Academic Press, 35-63, 2005.
592 Tatters, A. O., Fu, F.-X., and Hutchins, D. A.: High CO_2 and silicate limitation synergistically increase
593 the toxicity of *Pseudo-nitzschia fraudulenta*, PloS One, 7, e32116,
594 doi:10.1371/journal.pone.0032116, 2012.
595 Tatters, A. O., Roleda, M. Y., Schnetzer, A., Fu, F., Hurd, C. L., Boyd, P. W., Caron, D. A., Lie, A. A.,
596 Hoffmann, L. J., and Hutchins, D. A.: Short-and long-term conditioning of a temperate marine
597 diatom community to acidification and warming, Philos. T. Roy. Soc. B, 368, 20120437,
598 doi:10.1098/rstb.2012.0437, 2013.
599 Taucher, J., Jones, J., James, A., Brzezinski, M. A., Carlson, C. A., Riebesell, U., and Passow, U.:
600 Combined effects of CO_2 and temperature on carbon uptake and partitioning by the marine diatoms
601 *Thalassiosira weissflogii* and *Dactyliosolen fragilissimus*, Limnol. Oceanogr., 60, 901-919, 2015.
602 Wagner, H., Jakob, T., and Wilhelm, C.: Balancing the energy flow from captured light to biomass under

603 fluctuating light conditions, New Phytol., 169, 95-108, 2006.
604 Waldbusser, G. G., and Salisbury, J. E.: Ocean acidification in the coastal zone from an organism's
605 perspective: multiple system parameters, frequency domains, and habitats, Annual Review of Marine
606 Science, 6, 221-247, 2014.
607 Wang, G., Jing, W., Wang, S., Xu, Y., Wang, Z., Zhang, Z., Li, Q., and Dai, M.: Coastal acidification
608 induced by tidal-driven submarine groundwater discharge in a coastal coral reef system, Environ.
609 Sci. Technol., 48, 13069-13075, 2014.
610 Wang, Z. A., Wanninkhof, R., Cai, W.-J., Byrne, R. H., Hu, X., Peng, T.-H., and Huang, W.-J.: The
611 marine inorganic carbon system along the Gulf of Mexico and Atlantic coasts of the United States:
612 Insights from a transregional coastal carbon study, Limnol. Oceanogr., 58, 325-342, 2013.
613 Weger, H. G., Herzig, R., Falkowski, P. G., and Turpin, D. H.: Respiratory losses in the light in a marine
614 diatom: Measurements by short-term mass spectrometry, Limnol. Oceanogr., 34, 1153-1161, 1989.
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Figure captions

Figure 1. Measured pH_{NBS} variation over a diel cycle in the four experimental treatments (LCs, closed triangles; LCf, open 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 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 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 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 the onset of light. Values are means ± SD of triplicate 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 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. Values are means ± SD of triplicate cultures. The different letters indicate significant ($p < 0.05$) differences among treatments.

644 Table 1. Carbonate chemistry parameters of culture media before and after dilution under steady and fluctuating regimes of ambient (LC) and elevated

645 (HC) pCO_2 levels. Values are means ± SD of triplicate cultures. The different letters indicate significant ($p < 0.05$) differences among treatments.

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	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 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 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}

Table 3. Effective photochemical quantum yields (Φ_{PSII}) and non-photochemical quenching (NPQ) determined 0.5, 6, and 11.5 h after 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 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}

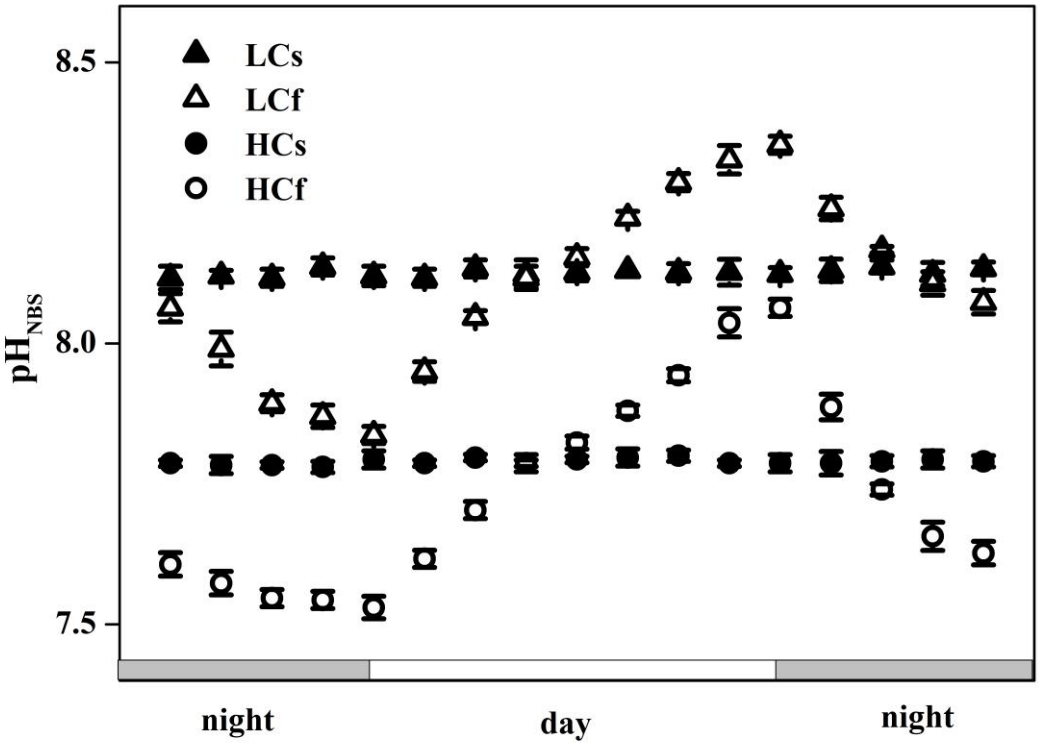
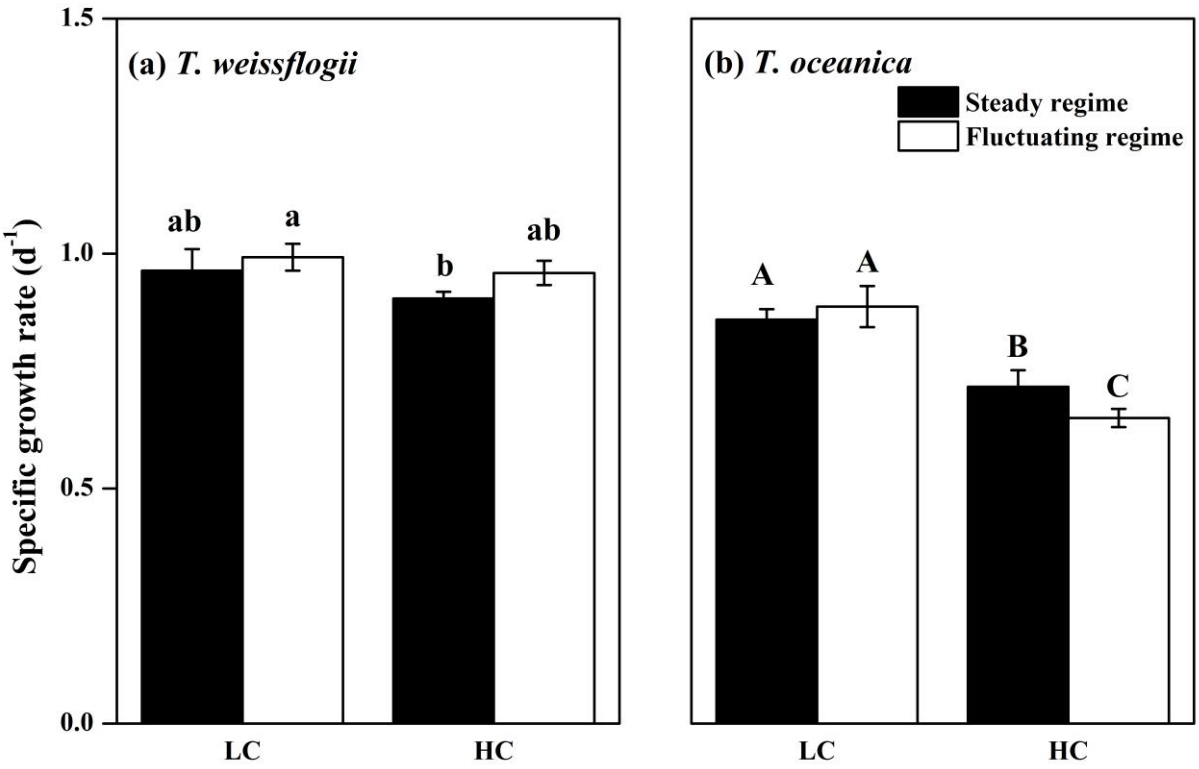


Figure 1



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673 Figure 2

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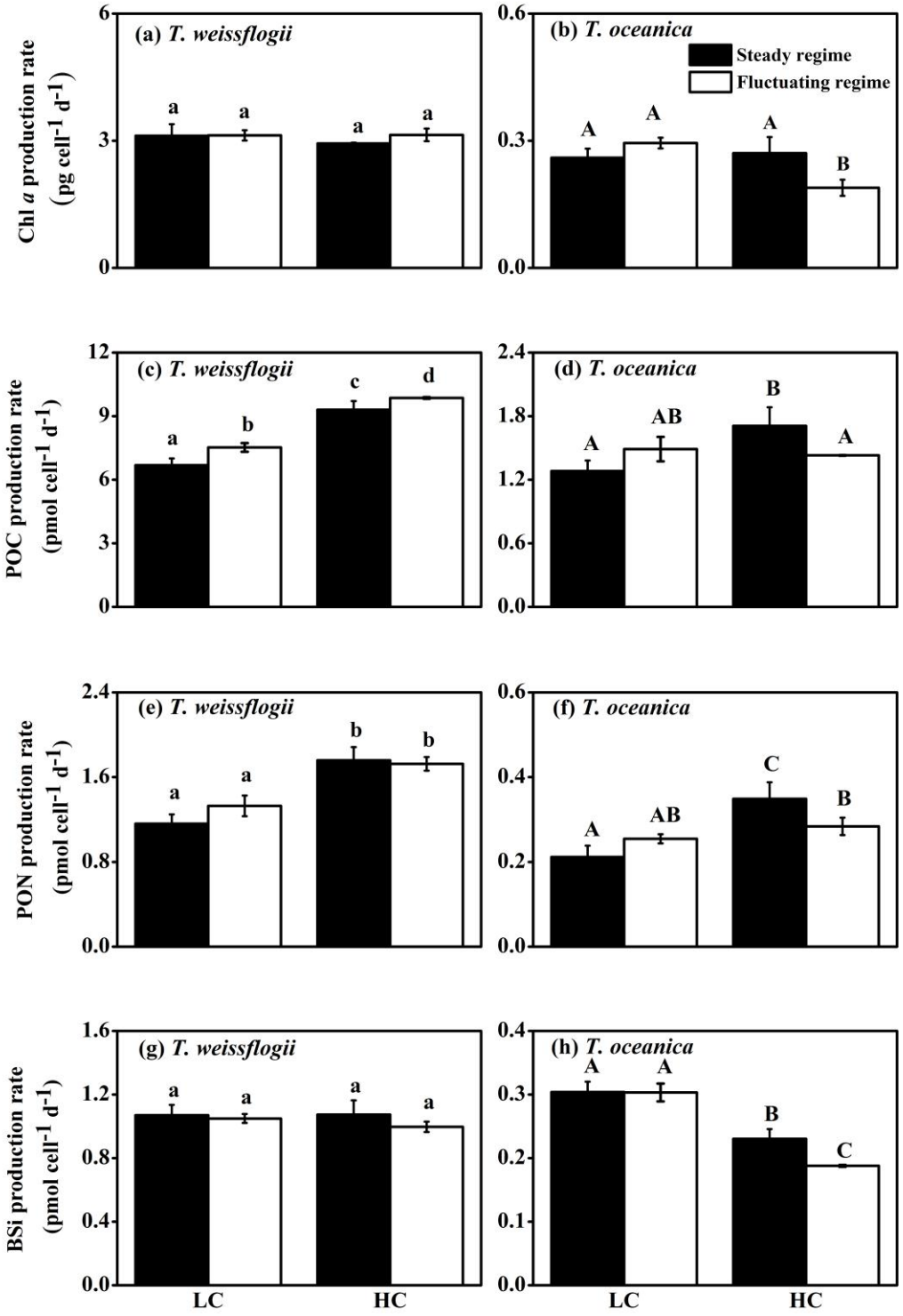
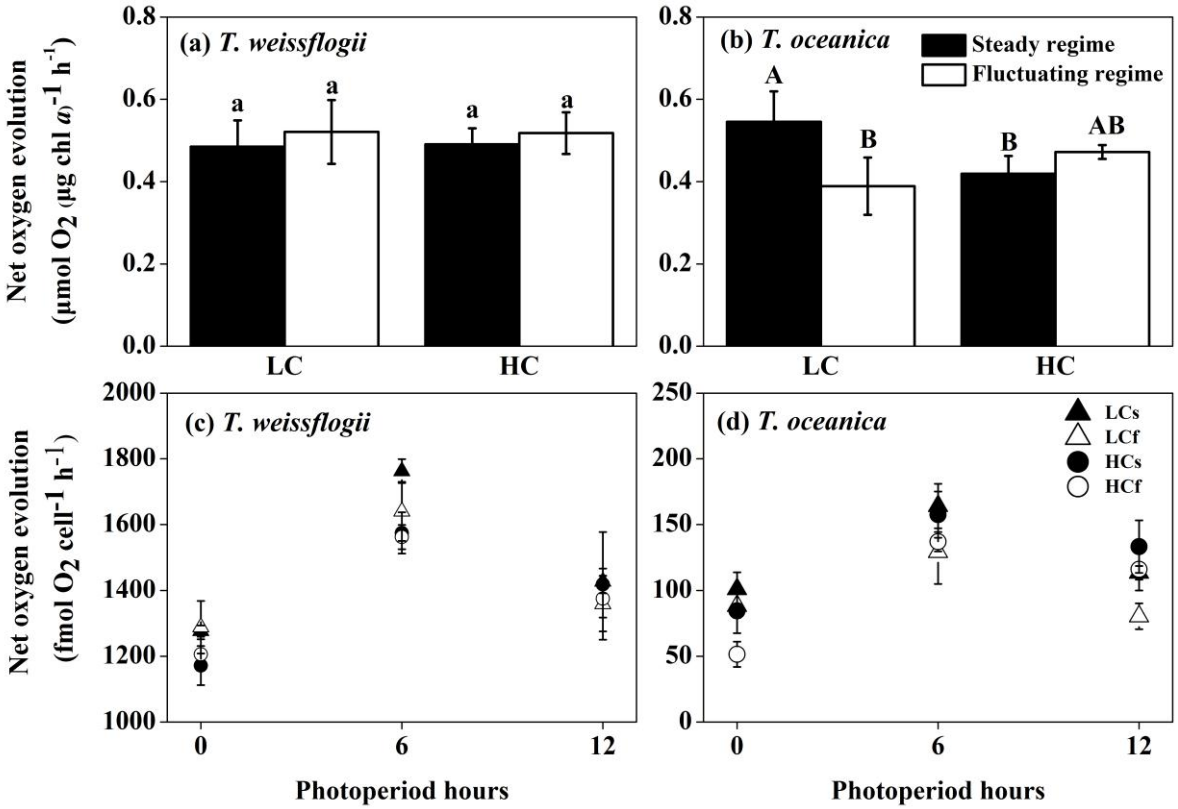


675

676 Figure 3



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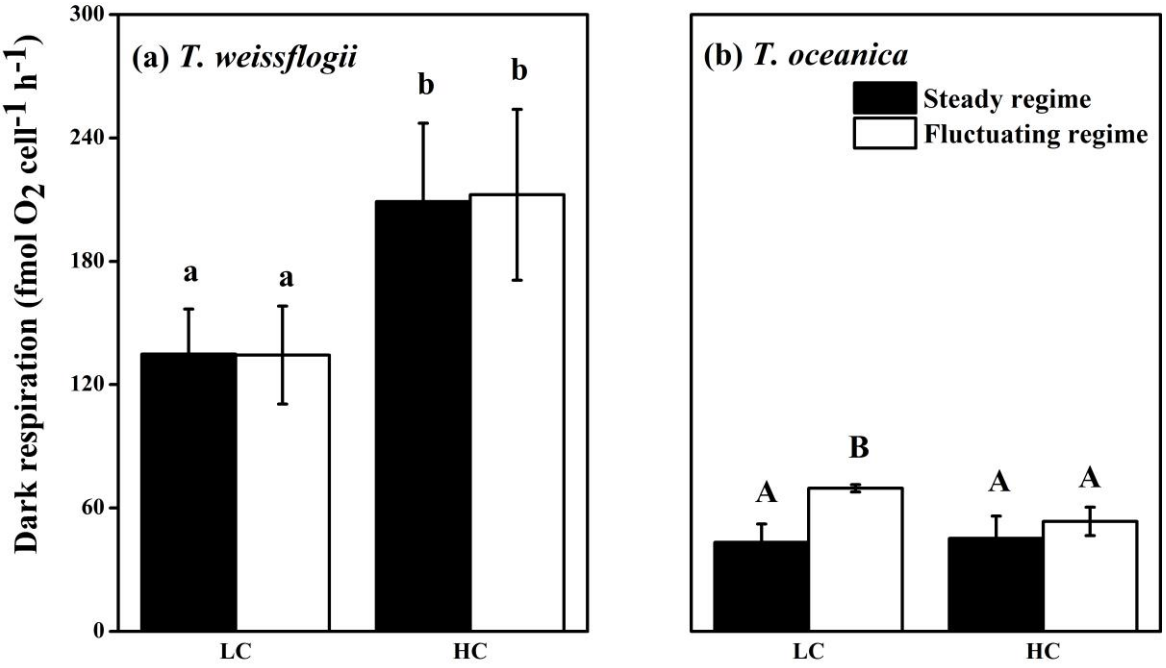
678 Figure 4



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680 Figure 5

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