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# 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 or 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 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 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.

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**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 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. 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,

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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 (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 buffering capacity will decrease as the increase of dissolved inorganic carbon in both coastal and oceanic seawaters (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 (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

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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), 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 < $1.1 \times 10^4$ $mL^{-1}$ (*T.*

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103 *weissflogii*) or $3.5 \times 10^4$ $mL^{-1}$ (*T. oceanica*) in semi-continuous cultures.

104 *T. weissflogii* and *T. oceanica* were acclimated to four treatments: 1) steady carbonate chemistry at
105 ambient $pCO_2$ level (LCs); 2) diurnally carbonate chemistry fluctuated around ambient $pCO_2$ level
106 (LCf); 3) steady carbonate chemistry at elevated $pCO_2$ level (HCs); and 4) diurnally carbonate
107 chemistry fluctuated around elevated $pCO_2$ level (HCf) for 15 generations before sampling. Steady
108 regimes were bubbled with ambient air (400 ± 15μatm, LCs) or elevated (1005 ± 40 μatm, HCs) $pCO_2$,
109 which was automatically achieved by mixing air/$CO_2$ with a $CO_2$ Enricher (CE100B, RuiHua). The
110 fluctuating regimes were obtained by changing the $CO_2$ partial pressure every 12 h. Cells were aerated
111 with air of low $pCO_2$ (i.e., 0 or 557 ± 15 μatm for LCf and HCf, respectively) during the photoperiod;
112 the aeration was changed to high $pCO_2$ (i.e., 870 ± 19 or 1949 ± 35 μatm for LCf and HCf, respectively)
113 at the beginning of the dark period. Measurements showed that pH gradually increased and decreased,
114 similar to a natural diurnal cycle (see Results). Since pH increased quickly in the first few hours of the
115 photoperiod, the aeration rates were adjusted to make sure the fluctuating regimes reached similar pH
116 values with corresponding steady regimes in the middle of photoperiod and reached target values at the
117 end of photoperiod. The steady regimes were aerated with stable $pCO_2$ air at the same flow rate as the
118 fluctuating regimes. The pH was measured every 1.5 h by a pH meter (Orion 2 STAR, Thermo
119 Scientific) calibrated with standard National Bureau of Standards (NBS) buffers. Subsamples for
120 measurement of physiological parameters were always taken in the middle of the photoperiod, unless
121 otherwise noted.
122

123 **2.2 Growth rate and chlorophyll *a* content**

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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 polycarbonate filters 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

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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. $\Phi_{PSII}$ and NPQ were measured under actinic light intensity similar to culture light level after 10 min dark adaptation. Given the changing carbonate chemistry over a diurnal cycle, $\Phi_{PSII}$ and NPQ were determined at three time points: 0.5, 6, and 11.5 h after illumination. 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, when the steady and fluctuating regimes reached similar pH values. Samples were gently filtered 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. The pH values of Tris buffered medium were pre-adjusted to their corresponding culture medium values. That is, pH values 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

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166 7.80 for all three time points, respectively.

167

168 **2.6 Statistical analyses**

169 Significant differences among treatments were tested using one-way analysis of variance (ANOVA)
170 with a significance level of $p < 0.05$. When necessary, the post hoc Duncan test was used to determine
171 the differences between treatments. All data are reported as mean value of triplicate samples ± standard
172 deviation (SD).

173

174 **3 Results**

175 **3.1 Variation of pH in experimental regimes**

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

183

184 **3.2 Specific growth rate and mean cell size**

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

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187 between steady and fluctuating regimes for *T. oceanica* under the ambient $pCO_2$ condition (Fig. 2b).
188 However, fluctuating regime reduced its growth rate by 9% under the elevated $pCO_2$ condition. OA
189 influenced the growth rate of *T. oceanica*, with rate of HCs cells being 16% lower than LCs cells. No
190 effects of OA on growth rate of *T. weissflogii* were detected. Additionally, growth rates of *T.*
191 *pseudonana* (CCMP 1335, isolated from Moriches Bay, New York, USA in 1958) were not influenced
192 by the fluctuating regime under both ambient and elevated $pCO_2$ conditions (data not shown).
193 Mean cell sizes were not affected by the fluctuating treatment under either ambient or elevated $pCO_2$
194 conditions in *T. weissflogii* (Table 1). *T. oceanica* cells showed minor but significant changes in cell size
195 in the fluctuating treatments. Cells in the LCf treatment cells were 1.2% larger than LCs cells, while
196 HCf cells were 1.4% smaller than cells in the corresponding steady treatments.
197
198 **3.3 Chlorophyll *a* content and elemental composition**
199 Chlorophyll *a* contents of *T. weissflogii* in the four treatments were not significantly different. For *T.*
200 *oceanica*, the fluctuating regime didn't influence chlorophyll *a* content under ambient $CO_2$ level.
201 However, in the HCf treatment chlorophyll *a* content decreased by 24% compared to the steady regime
202 (Table 1).
203 POC and PON quotas of both species were elevated in the OA scenario in both the steady and
204 fluctuating regimes, relative to present day $pCO_2$ levels (Table 1). However, no effects of fluctuating
205 regime on cellular POC and PON contents were detected in either species compared to the steady
206 treatments. The only exception was that POC increased by 9% in the LCf treatment relative to the LCs
207 treatment for *T. weissflogii*. Generally, elevated $pCO_2$ and fluctuating regime showed no effects on BSi

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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). 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.

### 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). Fluctuating regimes scarcely influenced $\Phi_{PSII}$ of either species. The only exception was that $\Phi_{PSII}$ of HCf decreased by 8% relative to that of the HCs for *T. weissflogii* at the beginning of the photoperiod. Elevated $pCO_2$ decreased $\Phi_{PSII}$ by 3% and 5% in the middle of the photoperiod for *T. weissflogii* and *T. oceanica*, respectively. Cells under elevated $pCO_2$ showed 2% and 7% lower $\Phi_{PSII}$ compared to those under ambient $pCO_2$ 11.5 h after illumination for *T. weissflogii* and *T. oceanica*, 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 fluctuating regime on NPQ of either species were found, with exceptions of HCf cells of *T. weissflogii* at the beginning of the photoperiod and LCf cells of *T. oceanica* 11.5 h after illumination. For steady regimes, elevated $pCO_2$

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showed no detectable effect on NPQ of both species at the beginning of the photoperiod, while it increased NPQ by 37.5% and 38.4% relative to values of LCs cells in the middle of the photoperiod for *T. weissflogii* and *T. oceanica*, respectively. Values of NPQ of HCs cells were decreased by 25% and 33.3% relative to values of LCs cells at the end of the photoperiod for *T. weissflogii* and *T. oceanica*, 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. Neither elevated $pCO_2$ nor 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 a 29% decrease of chlorophyll-normalized net oxygen evolution rate relative to the LCs cells (Fig. 4b).

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 (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 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. At 11.5 h after illumination, LCs cells of *T. oceanica* showed 41% higher net oxygen evolution rates per cell than LCf cells (Fig. 4d). *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.

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Elevated $pCO_2$ increased dark respiration of *T. weissflogii* by 59% compared to that at ambient $pCO_2$ level, while fluctuating regime had no detectable effect (Fig. 5a). In contrast, dark respiration rates of *T. oceanica* were stimulated by 44% and 55% for cells under the fluctuating regime compared to steady one at ambient and elevated $pCO_2$ levels, respectively (Fig. 5b), while no effects of elevated $pCO_2$ were observed. 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 photosynthesis (R:P) ratios for *T. weissflogii* under elevated $pCO_2$ was higher than at ambient $pCO_2$ by 73%, while no effects of 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).

### 3.6 NPQ versus irradiance curves

The development kinetics 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

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Both species were influenced by elevated $pCO_2$ in several ways, while they responded differentially to fluctuating regime. In general, the fluctuating $pCO_2$ regime showed either positive (POC cellular quota and production rate) or no obvious effects on 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* under ambient $pCO_2$ concentration, while in the OA scenario, the fluctuating regime depressed its growth rate, chlorophyll *a* content, and elemental production 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.

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292 The silicified cell walls of diatoms act as mechanical protection to resist grazers (Hamm et al. 2003),
293 and also have potential roles in photoprotection (Raven and Waite 2004), as well as promotion of
294 catalysis by extracellular carbonic anhydrase (Milligan and Morel 2002). Si:C ratio of both species
295 decreased under the elevated $pCO_2$ condition, in accordance with results of Tatters et al. (2012) and
296 Mejia et al. (2013). This decreased ratio indicates that diatoms may have reduced silicon requirements
297 per carbon fixed under an OA scenario than under the ambient $pCO_2$ condition, and so has implications
298 for changes in local and global carbon and silicon budgets. In Si-replete regions, a lower ratio may
299 reduce the ballasting function of silica in carbon export by diatoms (Mejia et al. 2013). In Si-limited
300 waters, a consequence may be that the proportion of diatoms in phytoplankton communities may
301 increase due to reduced Si requirements (Mejia et al. 2013). However, diatom silicification is under a
302 complex set of controls. For instance, limitation by other nutrients such as, iron (Hutchins and Bruland
303 1998) and nitrogen (Flynn and Martin-Jézéquel 2000), may act to increase Si quotas and Si:C ratio.
304 Bicarbonate utilization has been suggested to be a general characteristic of marine diatoms, through
305 direct transport or conversion by extracellular carbonic anhydrase (eCA), while the fraction of direct
306 bicarbonate transport and eCA expression varies among species (Martin and Tortell 2008). Pathways
307 that can utilize $HCO_3^-$ and provide $CO_2$ for Rubisco through $C_4$ (Reinfelder et al. 2000) or $C_3$-$C_4$
308 intermediate (Roberts et al. 2007) photosynthesis have been suggested for *T. weissflogii*. This species
309 takes up both $CO_2$ and $HCO_3^-$ at a similar rate, and has the ability to adjust uptake rates to cope with a
310 wide range of inorganic carbon supplies (Burkhardt et al. 2001). Moreover, *T. weissflogii* has a
311 markedly higher fraction of direct bicarbonate transport and apparent eCA activity than *T. oceanica*
312 (Martin and Tortell 2008). In this study, *T. oceanica* showed significantly lower oxygen evolution rates

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313 in the LCf treatment than in the LCs treatment before the end of the photoperiod, when the highest pH
314 and lowest $CO_2$ was reached. In contrast, no effects of fluctuating regime on oxygen evolution rates of
315 *T. weissflogii* were found at this time point. As shown here, cells of *T. weissflogii* benefited from their
316 inorganic carbon transport and uptake characteristics and were more tolerant of the high pH and low
317 $CO_2$ period under fluctuating carbonate chemistry than *T. oceanica*.

318 Under the fluctuating regime, *T. oceanica* showed higher respiration rates in both the current and OA
319 scenarios than under the corresponding steady regime. Just as *T. oceanica* makes a successful
320 compromise between iron requirements and capacity to acclimate to dynamic light regimes (Strzepek
321 and Harrison 2004), this oceanic diatom may also sacrifice its ability to acclimate to fluctuating
322 carbonate chemistry, since this is a characteristic of coastal rather than oceanic habitats. The higher
323 respiration rate may imply that this species needs more energy for maintaining its intracellular acid-base
324 balance under dynamic extracellular pH conditions, as dark respiration provides energy for growth and
325 metabolic processes (Beardall and Raven 2012). Moreover, the fluctuating regime reduced the
326 production rate of organic matter by *T. oceanica* at elevated $pCO_2$. Depressed biomass build-up has also
327 been found under dynamic light regimes (Wagner et al. 2006; Shatwell et al. 2012; Hoppe et al. 2015).
328 Together with our results, this may imply that organisms that are sensitive to fluctuating abiotic factors
329 maintain intracellular homeostasis under dynamic environments of light or $pCO_2$ at the expense of
330 reduced biomass production.

331 In contrast, either positive (POC production rate) or no obvious effects of fluctuating regime on
332 biomass production were found in the coastal species *T. weissflogii*. Coastal calcifying organisms have
333 shown the ability to achieve homeostasis within critical tissues to facilitate calcification under dynamic

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334 pH/$pCO_2$ condition, and this was suggested to be associated with diurnal and seasonal pH fluctuations
335 in coastal waters (Hendriks et al. 2015). Thus, some organisms could take advantage of the fluctuating
336 carbonate system regime to mitigate the negative effects of ocean acidification on physiological
337 performance. For instance, growth and calcification of corals benefit from oscillatory $pCO_2$ (Dufault et
338 al. 2012; Comeau et al. 2014). Organisms like *T. weissflogii* whose physiological performance were
339 enhanced or unaltered under dynamic carbonate chemistry conditions thus could be at a distinct
340 advantage in competing with species that showed negative responses to this condition (such as *T.*
341 *oceanica* in the present study).

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

352 Diatoms have an efficient dissipation of excess excitation energy through NPQ (Goss and Jakob
353 2010), which can be three to five times larger than that of higher plants (Ruban et al. 2004). NPQ
354 processes can be initiated in seconds to minutes (Müller et al. 2001; Eberhard et al. 2008), and so are

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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 fluctuating regime on *T. oceanica* was different under 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 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 same amplitude of pH variation (~0.5 units) was set under current and elevated $pCO_2$ scenarios. 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). 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* would be amplified under elevated

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376 $pCO_2$ condition.

377 Given the poor physiological performance of *T. oceanica* under fluctuating seawater carbonate
378 chemistry, and its limited ability to dissipate excess excitation energy through NPQ under high light,
379 this species is unlikely to be able to acclimate to coastal habitats. In contrast, *T. weissflogii* and *T.*
380 *pseudonana* appear to be insensitive to, even benefit from, fluctuating carbonate chemistry. This striking
381 contrast of physiological traits in coastal and oceanic diatoms suggests that the ability to cope with
382 fluctuating carbonate chemistry may play a role in influencing the geographic distributions of species. It
383 is possible that this ability, together with the abilities to cope with nutrient (Irwin et al. 2006), light
384 (Lavaud et al. 2007; Lavaud and Lepetit 2013; Laviale et al. 2015), and predation pressure (Irigoien et
385 al. 2005), are factors that will help to decide the spatial distribution patterns of species in both the
386 present and future oceans.

387

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598 **Figure captions**

599 Figure 1. Measured pH variation over a diel cycle in the four experimental treatments (LCs, closed
600 squares; LCf, open squares; HCs, closed circles; HCf, open circles).

601

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

605

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

610

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

617

618 Figure 5. Dark respiration rates per chlorophyll (a, b) or cell (c, d) in the middle of the photoperiod for

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619 *T. weissflogii* (a, c) and *T. oceanica* (b, d) under steady (closed bars) and fluctuating (open bars) regimes
620 of ambient (LC) and elevated (HC) $pCO_2$ levels. Values are means ± SD of triplicate samples. The
621 different letters indicate significant ($p < 0.05$) differences among treatments.

622

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

628

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Table 1. 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. 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 | 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 | 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 | 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. Effective photochemical quantum yields ($\Phi_{PSII}$) and non-photochemical quenching (NPQ) determined 0.5, 6, and 11.5 h after illumination 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. The different letters indicate significant ($p < 0.05$) differences among treatments.

| | *T. weissflogii* | | | | *T. oceanica* | | | |
|---|---|---|---|---|---|---|---|---|
| Time | LCs | LCf | HCs | HCf | LCs | LCf | HCs | HCf |
| $\Phi_{PSII}$ | | | | | | | | |
| 0.5 | 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 | 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 | 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 | 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 | 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 | 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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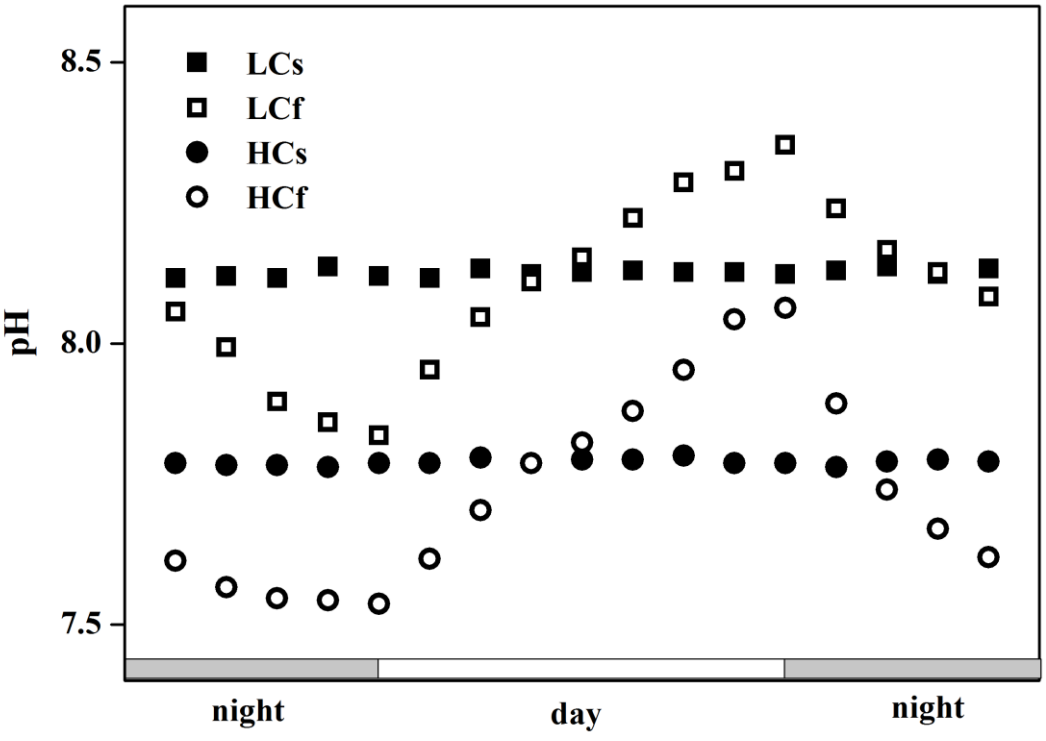


Figure 1

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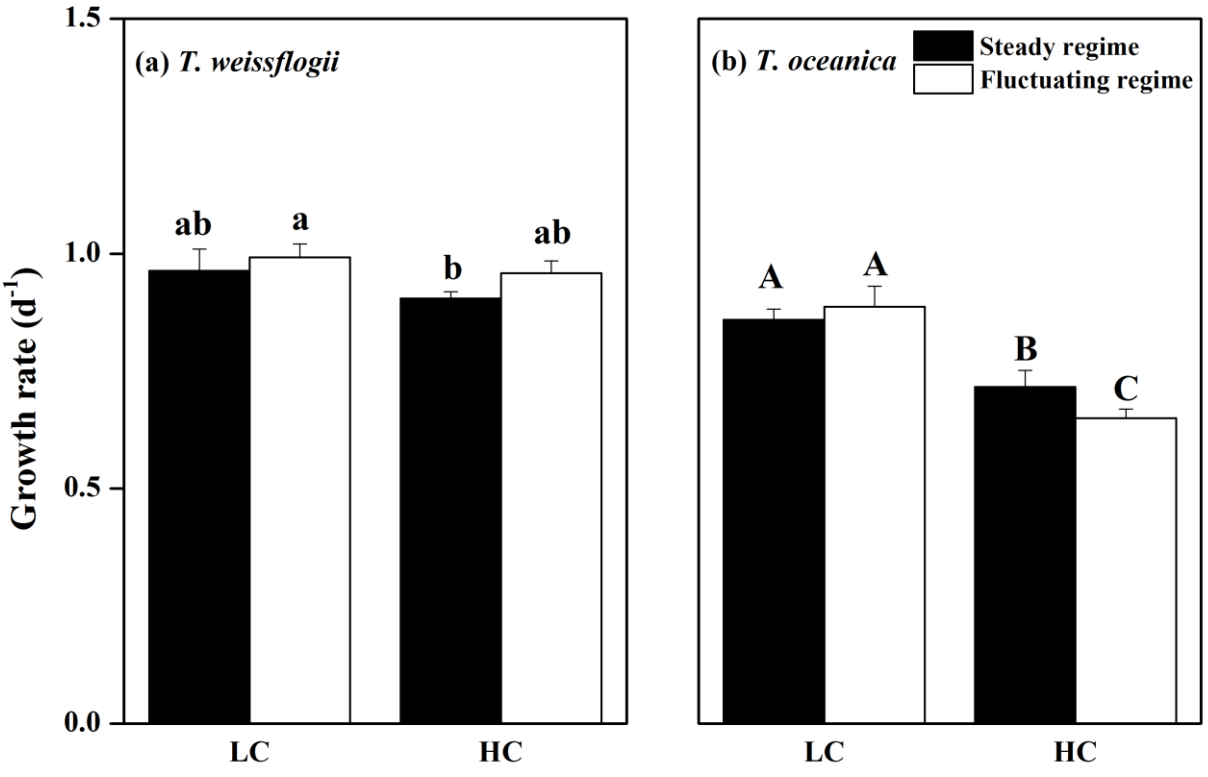


Figure 2

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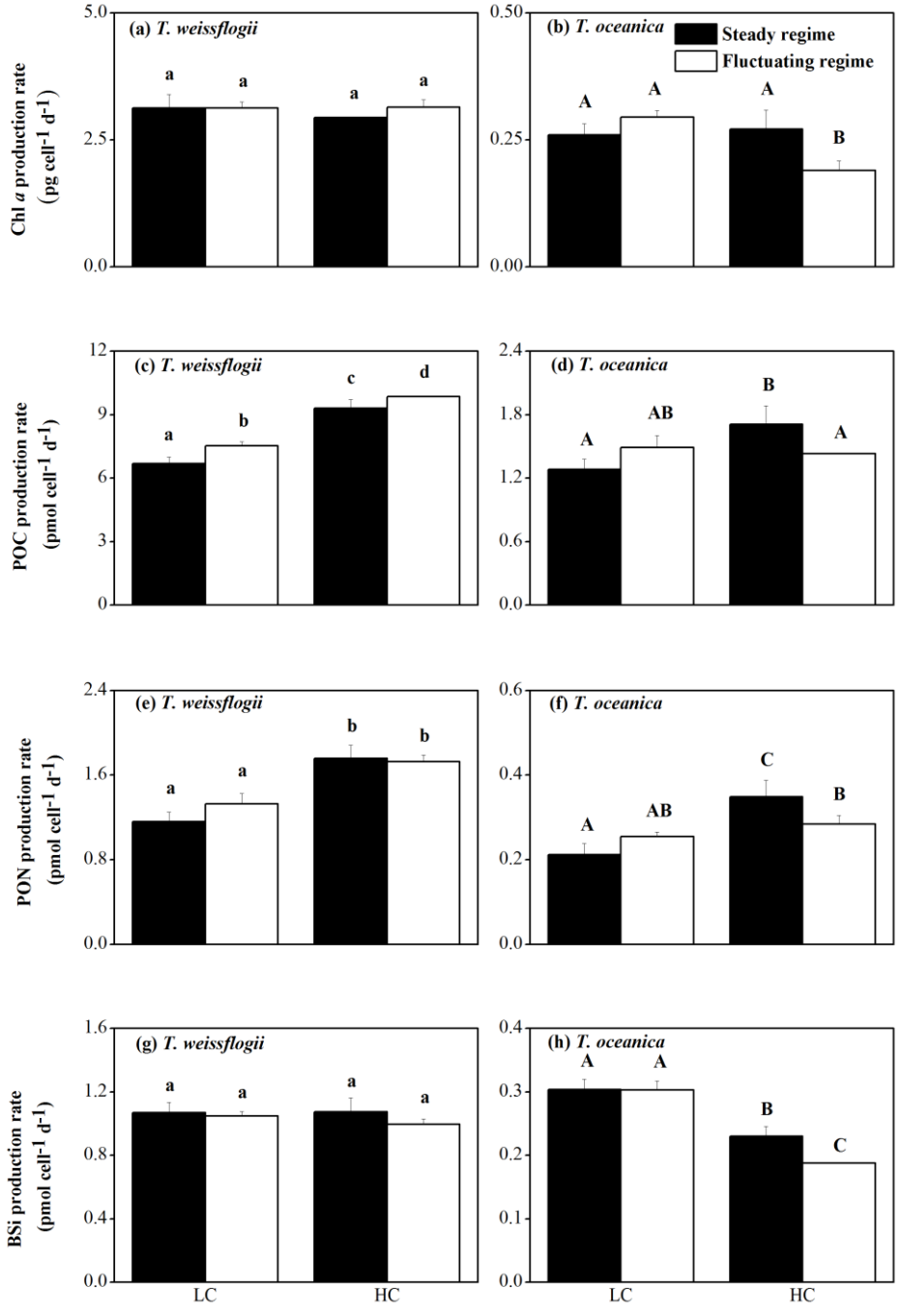


Figure 3

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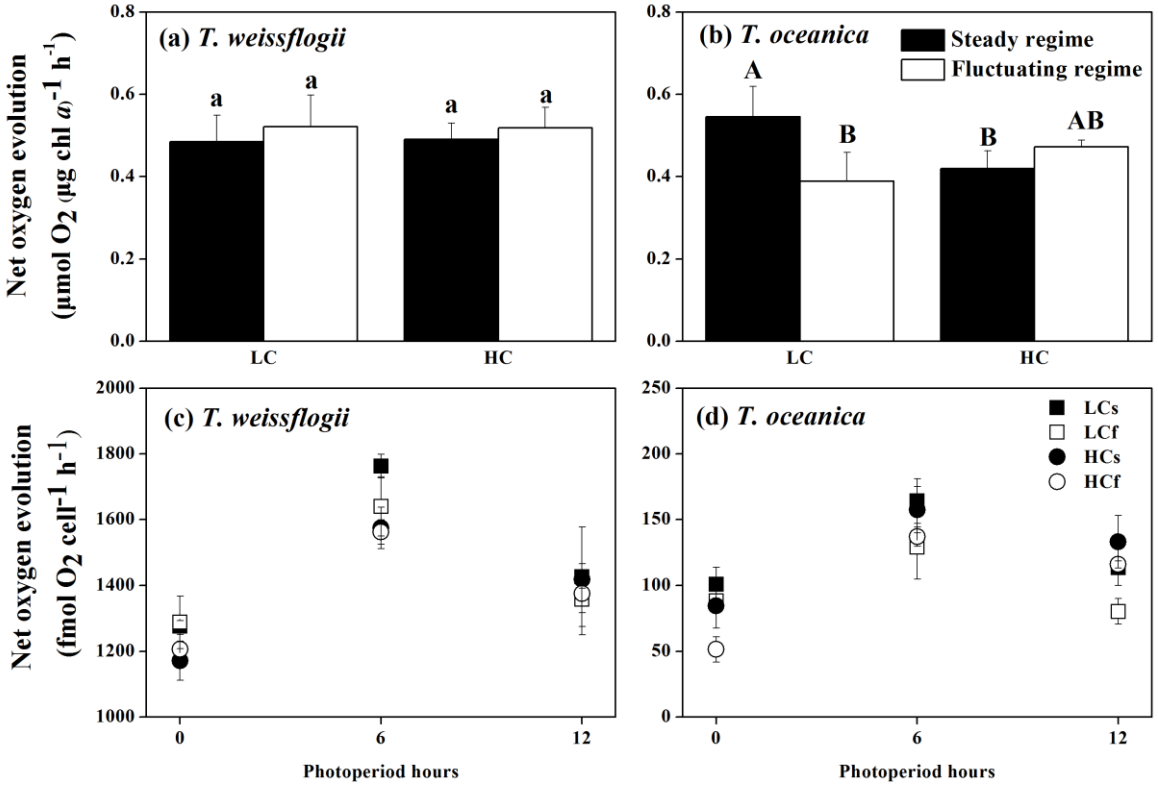


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653 Figure 4

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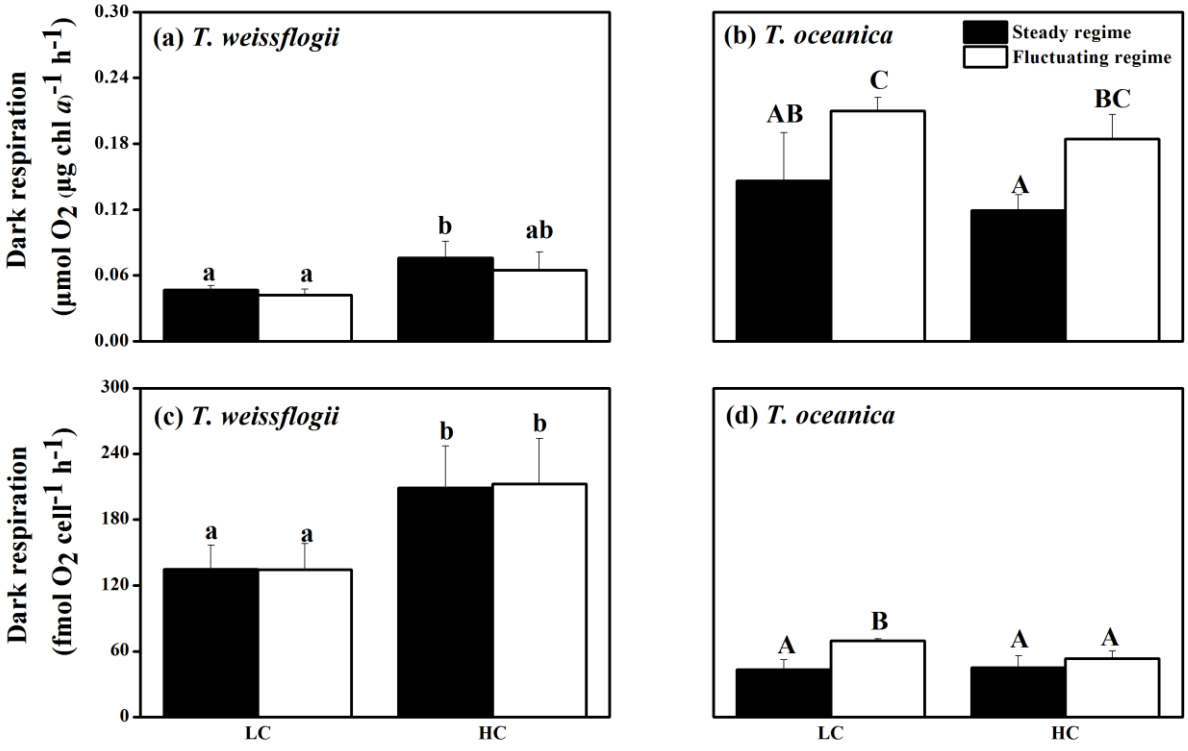


Figure 5

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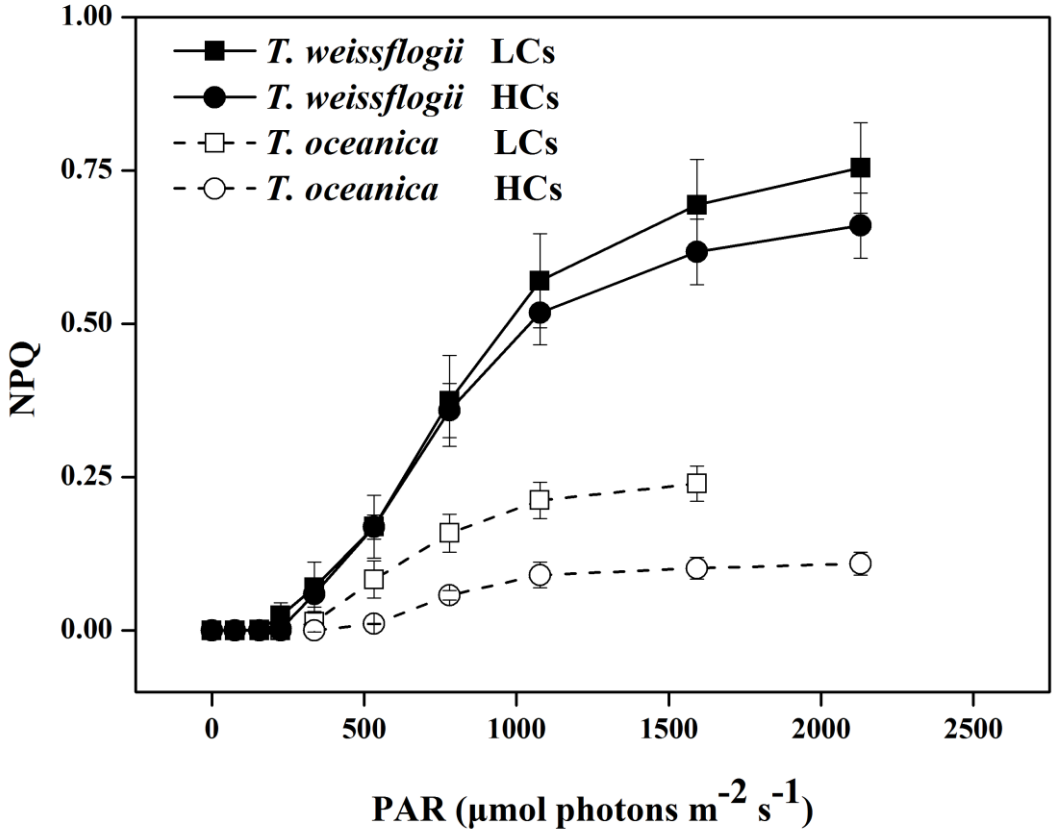


Figure 6