Marine Pollution Bulletin 163 (2021) 112008

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Ocean acidification interacts with growth light to suppress CO_2 acquisition efficiency and enhance mitochondrial respiration in a coastal diatom

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ARTICLE INFO

Keywords:
Carbonic anhydrase
Growth
Light
Ocean acidification
Thalassiosira weissflogii

ABSTRACT

Diatom responses to ocean acidification have been documented with variable and controversial results. We grew the coastal diatom *Thalassiosira weissflogii* under 410 (LC, pH 8.13) vs 1000 μatm (HC, pH 7.83) pCO_2 and at different levels of light (80, 140, 220 μmol photons m^{-2} s^{-1}), and found that light level alters physiological responses to OA. CO_2 concentrating mechanisms (CCMs) were down-regulated in the HC-grown cells across all the light levels, as reflected by lowered activity of the periplasmic carbonic anhydrase and decreased photosynthetic affinity for CO_2 or dissolved inorganic carbon. The specific growth rate was, however, enhanced significantly by 9.2% only at the limiting low light level. These results indicate that rather than CO_2 "fertilization", the energy saved from down-regulation of CCMs promoted the growth rate of the diatom when light availability is low, in parallel with enhanced respiration under OA to cope with the acidic stress by providing extra energy.

1. Introduction

Diatoms constitute the most abundant group of eukaryotic microalgae with about 100000 species, with new species continuing to be identified (Heydarizadeh et al., 2014; Bork et al., 2015; Beauger et al., 2018). They collectively contribute up to 40% of the marine primary productivity, equivalent to that of all terrestrial rainforests combined (Bowler et al., 2010; Clement et al., 2016; Li et al., 2017). Diatoms are distributed in a broad range of environments, including marine, brackish and fresh waters. They acclimate or adapt to variety of environmental conditions by adjusting their physiological performances (Pasquet et al., 2014).

In general, in seawater availability of dissolved CO_2 is low enough to kinetically limit photosynthesis due to its much lower diffusion rate in water compared with air (Shen et al., 2017). Dissolved CO_2 concentrations in seawater are also usually much lower than that required to half-saturate the Rubisco-catalyzed carboxylation. To adapt to the low CO_2 marine environments, diatoms have developed CO_2 concentration mechanisms (CCMs), to increase both the flux of CO_2 into the cell and the intracellular CO_2 concentrations around Rubisco (Gee and Niyogi, 2017). In phytoplankton, most CCMs facilitate the use of HCO_3^- in photosynthesis via two processes; namely, active uptake of HCO_3^- by anion exchange (AE) and/or HCO_3^- dehydration at the cell surface by external periplasmic carbonic anhydrase (eCA) (Giordano et al., 2005). Physiological and molecular lines of evidence support this role of eCA in diatom CCMs (Clement et al., 2017; Gee and Niyogi, 2017). The eCA activity has been detected in numerous diatom species; increases with decreasing CO_2 availability (Burkhardt et al., 2001; Hopkinson et al., 2011); and is rapidly induced when diatom cells are transferred to low CO_2 medium (Chen and Gao, 2003; Clement et al., 2016).

With increasing anthropogenic CO_2 emissions and its continuous dissolution into oceans, the CO_2 concentration in surface water is predicted to reach 800–1000 μatm by the end of 2100 (Gattuso et al., 2015), resulting in a concomitant decrease of pH, termed ocean acidification (OA) (Tortell et al., 2008; Doney et al., 2009). This raises the question of how effects of increased CO_2 and decreased pH on phytoplankton physiology will relate to projections of future marine primary productivity. There are numerous works showing that elevated CO_2 concentration can stimulate diatom growth and carbon fixation (see the review by Gao and Campbell, 2014 and literature therein). In laboratory studies, the responses of diatoms to OA are highly variable and species-specific (Mackey et al., 2015). For example, the effect of OA on the growth of the diatom *Thalassiosira pseudonana* was sometimes negligible (Yang and Gao, 2012; Wu et al., 2014; Shi et al., 2015), or interacted

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https://doi.org/10.1016/j.marpolbul.2021.112008
Received 16 August 2020; Received in revised form 27 December 2020; Accepted 28 December 2020
Available online 15 January 2021

with growth light (Li and Campbell, 2013). In contrast, growth and photosynthetic C fixation rates in *Phaeodactylum tricornutum* were enhanced by OA (Wu et al., 2010; Hong et al., 2017). Many studies have observed a stimulation, inhibition or neutral effect of OA on growth or primary production in diatom-dominated communities under OA (Hoppe et al., 2015; Gao et al., 2012a; Young et al., 2015, 2016), leading to controversy worth further investigation. An increase in pCO_2 may result in modest savings in the energy expenditure upon carbon fixation (Hopkinson et al., 2011), though short exposures to elevated pCO_2 or lowered pH showed no significant difference in energy use by the CCMs compared to direct use in carbon fixation (Goldman et al., 2017). On the other hand, elevated CO_2 could increase energy requirements under light-stressed condition (Passow and Laws, 2015; Li et al., 2014). The diatom *Proboscia alata* sp. showed increased content of particulate organic carbon when grown under elevated pCO_2 but only at high light levels (Hoogstraten et al., 2012). For the Antarctic diatom *Chaetoceros brevis*, growth remained unaffected by changes in pCO_2 levels irrespective of changing light conditions (Boelen et al., 2011). Nevertheless, under dynamic fluctuating light conditions, elevated pCO_2 decreased primary production of the diatom *C. debilis* (Hoppe et al., 2015).

When phytoplankton cells are moved up and down within upper mixing layers, they are exposed to continuously changing light conditions due to changing water depths and diurnal solar changes, which alters their photochemical performances (Jin et al., 2013). Excessive or insufficient light levels constrain diatom optimal physiological performance and primary productivity as well as metabolite composition (Barofsky et al., 2009, 2010; Carvalho et al., 2011), though they possess an outstanding capacity to tolerate light fluctuations (Brunet and Lavaud, 2010). Diatom species distributed in coastal waters are frequently exposed changing levels pH due to high biological activities which influence DIC, and may therefore be pre-adapted to tolerate moderate levels of acidic stress (Shi et al., 2009; F. Li et al., 2016). While changing levels of both light and CO_2 can modulate CCMs in diatoms (Chen and Gao, 2003; Raven and Beardall, 2020), physiological performances under influences of OA and changing levels of light need to be explored in the nearshore diatom *Thalassiosira weissflogii* to elucidate contrastingly different results about its responses to ocean acidification (Taucher et al., 2015; W. Li et al., 2016; Zeng et al., 2019). In this work, we hypothesize that different levels of light and CO_2 could interactively modulate the physiological responses of *Thalassiosira weissflogii*, and its growth response may differ from that reported previously when grown under a single constant light level (Wu et al., 2014) or two light levels (Needoba and Harrison, 2004). Our results showed that the OA treatment downregulated the CCMs by lowering activity of periplasmic carbonic anhydrase, suppressed non-photochemical quenching, increased respiration and, in sum, only significantly enhanced the growth in *T. weissflogii* under light-limiting conditions.

2. Materials and methods

2.1. Species and culture condition

Thalassiosira weissflogii (CCMP 1336) was grown in semi-continuous culture using 0.45 μm filtered natural seawater supplemented with Aquil nutrients and vitamins (Morel and Rueter, 1979). We diluted the cultures using pre-CO_2-equilibrated medium every 24 h, and maintained the cell concentrations within a range of 2×10^4 to 6×10^4 cells m^{-1} to ensure stable seawater carbonate chemistry (Table 1). Two levels of CO_2 concentration, 410 μatm (outdoor ambient air, LC) and 1000 μatm (predicted for the end of the century, HC) were achieved in plant growth CO_2 chambers (Ruihua, Wuhan, China) by mixing pure CO_2 with air. The incubation light was provided by white LED light (400–750 nm) in an incubator (Ruihua, Wuhan, China) and was set up to 3 different levels: high (HL, growth-saturating light intensity, 220 μmol photons m^{-2} s^{-1}), medium (ML, medium light intensity, 140 μmol photons m^{-2} s^{-1}) and low light level (LL, 70 μmol photons m^{-2} s^{-1}, growth-limiting light level) (Goldman et al., 2017), by covering bottles with neutral density filters. The light intensities were measured with a Solar Light sensor (PAM2100, USA). Cultures were maintained at 20 °C with a day/night cycle of 12L : 12D. Triplicate cultures (500 ml) were exposed to each light and pCO_2 combination, with a total of 18 independent cultures. All triplicates of the cultures were run in parallel, and each culture was shaken at least 3 times per day.

Table 1
Parameters of seawater carbonate system under the ambient (410 μatm, LC) and elevated (1000 μatm, HC) CO_2 concentration at three different light intensities before dilution in the semi-continuous cultures. Values are means ± SD of triplicate cultures. DIC = dissolved inorganic carbon, TA = total alkalinity. Different superscript letters indicate significant ($p < 0.05$) differences among treatments.

	pH_t	TA (μmol kg^{-1})	DIC (μmol kg^{-1})	HCO_3^- (μmol kg^{-1})	CO_3^{2-} (μmol kg^{-1})	CO_2 (μmol kg^{-1})
LCHL	8.11 ± 0.03[a]	2410.3 ± 11[a]	2126.9 ± 15[a]	1923.3 ± 7[a]	189.9 ± 2[a]	13.7 ± 1[a]
HCHL	7.81 ± 0.02[b]	2393.5 ± 15[a]	2275.3 ± 13[b]	2144.2 ± 12[b]	97.1 ± 3[b]	34.1 ± 1[b]
LCML	8.12 ± 0.02[a]	2413.9 ± 14[a]	2112.2 ± 15[a]	1887.5 ± 17[a]	194.3 ± 6[a]	13.2 ± 2[a]
HCML	7.82 ± 0.03[b]	2401.8 ± 11[a]	2278.2 ± 5[b]	2142.5 ± 11[b]	100.1 ± 3[b]	35.6 ± 2[b]
LCLL	8.13 ± 0.04[a]	2418.7 ± 19[a]	2117.4 ± 9[a]	1910.2 ± 22[a]	191.4 ± 5[a]	15.8 ± 1[a]
HCLL	7.82 ± 0.04[b]	2414.5 ± 13[a]	2279.9 ± 23[b]	2144.3 ± 13[b]	101.2 ± 2[b]	34.4 ± 1[b]

2.2. Seawater carbonate chemistry

To assure the stability of the carbonate system in cultures, pH was measured prior to and after the daily dilution as well as at the middle of the light period using a pH meter (Orion 2 STAR; Thermo Science), which was calibrated with standard NBS buffer (Heiden et al., 2018). Total alkalinity (TA) was determined using the titration method and other parameters of the carbonate system were derived with CO2SYS software (Lewis and Wallace, 1998). The measured pH_{nbs} was converted to pH_t using CO2SYS. All the carbonate chemistry parameters were shown in Table 1.

2.3. Determination of growth rate

The diatom cells under each condition were counted at least three times using a Counter Z2 Particle Count and Size Analyzer (Beckman Coulter Inc., Fullerton, CA, USA), before and after renewal of the medium every 24 h. The specific growth rate (μ, d^{-1}) of *Thalassiosira weissflogii* was determined based on the change in cell counts over 24 h. We grew the diatom *Thalassiosira weissflogii* for about 25 days under different treatments, and the physiological parameters were measured with the cells that had acclimated for at least 10 generations. The specific growth rate (μ, day^{-1}) was calculated using the following equation:

$$\mu\ (d^{-1}) = (\ln N_t - \ln N_{t-1})/\Delta t$$

where N_t and N_{t-1} are the cell counts (cells mL^{-1}) over the time interval of Δt (t_1 and t_{t-1}) respectively.

2.4. Determination of pigment contents

Cell suspensions (100 mL) were filtered onto GF/F filters with low

vacuum pressure (<0.02 MPa) and soaked in methanol over-night at 4 °C (Wellburn, 1994). The extracts were centrifuged at 6000 ×*g* for 10 min to remove debris and glass fibers. The absorption spectra from 400 to 800 nm of the supernatant were measured with a spectrophotometer (DU 800, Beckman, USA). The chlorophyll *a* (Chl *a*) content was determined spectrophotometrically as follows:

$$\text{Chl } a = 16.29 \times (A_{665} - A_{750}) - 8.54 \times (A_{652} - A_{750})$$

where A_{652}, A_{665}, and A_{750} represent absorbances of the methanol extracts at 665, 652, and 750 nm respectively.

2.5. Measurements of photochemical parameters

To estimate photochemical responses of the cells to different combinations of CO_2 concentration and light intensities, rapid light curves (RLCs), maximum quantum yield (F_v/F_m) and effective quantum yield (F_v'/F_m') were determined using a Multi-Color-PAM (Walz, Germany). Samples were dark-acclimated for 15 min to ensure that all photosystem II (PS II) reaction centers were oxidized and non-photochemical quenching was relaxed. The relative electron transport rate (rETR) was assessed as:

$$\text{rETR} = \text{Y(II)} \times 0.5 \times \text{photon flux density (PFD)}$$

where the yield represents the effective quantum yield of Y(II) ($F_m - F'/F_m'$); the coefficient 0.5 takes into account that roughly 50% of all absorbed quanta reach PSII; PFD is the actinic light intensity (μmol photons m^{-2} s^{-1}). Non-photochemical quenching (NPQ) was calculated using $NPQ = (F_m - F_m')/F_m'$, where F_m and F_m' represent the maximal and effective chlorophyll fluorescence yields in the dark-adapted and light-acclimated cells, respectively. Rapid light curves (RLCs) were measured to establish the relationship between relative electron transport rate (rETR) and light intensity. RLCs were fitted to the following model: $\text{rETR} = \text{PAR} / (a \times \text{PAR}^2 + b \times \text{PAR} + c)$, where PAR was the photon flux density of actinic light (μmol photons m^{-2} s^{-1}), *a*, *b*, and *c* were model parameters. The assay light intensities were increased from 0 to 1723 μmol photons m^{-2} s^{-1} with 12 steps (0, 63, 121, 178, 264, 377, 516, 731, 964, 1214, 1327, 1723 μmol photons m^{-2} s^{-1}) with a duration of 15 s at each step. The light-use efficiency (*a*), light saturation point (I_K), and maximum electron transport rate (ETR_{max}) were calculated from *a*, *b*, and *c* according to the equation in (Eilers and Peeters, 1988).

2.6. Determination of photosynthesis and respiration rates

Net photosynthetic O_2 evolution and dark respiration rates were determined using a Clarke-type electrode (Hansatech, UK). In the middle of the light period, the cells were harvested by filtering them onto mixed cellulose filters (diameter = 25 mm, pore size 8 μm) under gentle vacuum pressure (<0.02 MPa). The packed cells were resuspended in buffered seawater of 20mmol/L Tris, which was pre-equilibrated with the target CO_2 levels, and the pH levels were adjusted to their growth levels (7.83 and 8.13) for the high- and low-CO_2 grown cells, respectively by adding hydrochloric acid or sodium hydroxide. The resuspended cells were injected into an oxygen electrode vessel with a magnetic stirrer held in a water-jacked chamber (for temperature control at 20 °C). The dark respiration and net O_2 evolution rates under the growth light levels were determined by covering the cuvette with black box or by adjusting the distances from the light source (white LED), respectively.

2.7. Determination of photosynthetic response to DIC

We followed Wu et al. (2010) to determine the response of photosynthesis to DIC concentrations using RLC obtained under different DIC concentrations. Briefly, samples were harvested on mixed cellulose filters under gentle vacuum pressure (<0.02 MPa), and re-suspending the cells at 2–4 × 10^4 mL^{-1} in DIC free Tris buffered medium (pH 8.13). The re-suspended diatoms were exposed to light for 15 min under culture conditions to deplete the intracellular inorganic carbon pool. Then sodium bicarbonate solution was injected stepwise to a final DIC concentrations of 8000 μmol L^{-1}. The RLC was measured as mentioned above at each DIC level, and the DIC concentration required for half maximal ETR ($K_{1/2}$) was derived using the Michaelis-Menten formula from the ETR vs DIC curves obtained from the RLCs at various DIC concentrations. $K_{1/2}$ for carbon fixation was taken as the DIC concentration at which half of $rETR_{max}$ was reached. Lower $K_{1/2}$ reflects increased CCMs activity (Li et al., 2018). We obtained $K_{1/2}$ values at growth light levels and at ETR-saturating light levels. Since both types of $K_{1/2}$ values showed the same trend, we used $K_{1/2}$ values determined at growth light levels.

2.8. Measurement of eCA activity

The catalyzing activity of the periplasmic extracellular carbonic anhydrase (eCA) was measured using the cells in their exponential growth phase using an electrometric method (Wilbur and Anderson, 1948) which had been commonly used for evaluation of CA activity (Zeng et al., 2019). The harvested cells about 2 × 10^4 to 4 × 10^4 mL^{-1} were washed and re-suspended in seawater buffered with 20 mmol/L barbiturate at pH 8.3. Then, 5 m of intact cell suspension was incubated in a water-jacketed chamber at 4 °C. The reaction was started by adding 2 m of CO_2-saturated milliQ water to 5 ml of cell buffer that had been kept at 4 °C. The time required for a pH drift from pH 8.3 to 7.3 was recorded. Blanks were performed for each assay by omitting the sample. Enzyme activity was expressed as enzyme units, being calculated from the following equation $EU = 10 \times (T_0/T - 1)$, where T_0 and T represent the times required for the reaction in the absence and presence of the cell samples respectively.

2.9. Statistics

All data are shown as the means ± SD of 3 independent cultures. To test for significant differences among treatments, one-way analyses of variance (ANOVA), with additional normality (Shapiro-Wilk) and post hoc tests were performed ($\alpha = 0.05$). Two-way ANOVA was applied when examining the interactions between CO_2 and light. To test direct effects between two particular treatments standard *t*-tests (level of significance $p < 0.05$) were used. All statistical analyses were carried out with Origin 9.0 and the results presented in Table 2. Different letters in figures and tables indicate statistical differences between treatments based on post-hoc tests.

3. Results

3.1. Carbonate system

The carbonate chemistry parameters were stable, with pH variation less than 0.05 within either the HC or LC treatment (Table 1). The HC treatment did not alter TA, but increased concentrations of DIC, pCO_2 and HCO_3^- by about 2.2%, 59.8% and 10.3%, respectively; and decreased CO_3^{2-} by 48.6% when compared to the LC treatment.

3.2. Growth rate

The specific growth rates of *T. weissflogii* were stable under each treatment after ten generations of acclimation to the treatments (Suppl. Fig. S1). The growth rates of *T. weissflogii* were influenced by the light intensities and CO_2 concentration individually and interactively. Within the tested range of light levels, the higher the light intensity, the faster the specific growth rate (Fig. 1A), showing a linear relationship with a daily light use efficiency which was about 0.02–0.03. The HC treatment

Table 2
Summary of Two-way ANOVA analyses for interactive effects of pCO_2 and light intensities on growth, photosynthesis, pigments, eCA activity, fluorescence parameters in *Thalassiosira weissflogii*. The symbol '*' indicates the interactions between factors, df = degrees of freedom, F = F value, P = probability, the significant differences level was set at $p < 0.05$.

Response variable	Factor variables	df	Mean square	F	P
μ	pCO_2	1	0.002	4.241	0.062
μ	Light	2	0.264	674.795	<0.01
μ	pCO_2*Light	2	0.003	6.855	0.010
Net photosynthesis rate/Chl *a*	pCO_2	1	<0.01	0.100	0.757
Net photosynthesis rete/Chl *a*	Light	2	0.351	252.981	<0.01
Net photosynthesis rate/Chl *a*	pCO_2*Light	2	0.003	2.020	0.175
Respiration rate/Chl *a*	pCO_2	1	<0.01	12.434	0.004
Respiration rate/Chl *a*	Light	2	0.002	230.709	<0.01
Respiration rate/Chl *a*	pCO_2*Light	2	<0.01	0.166	0.849
Daytime C fixation rate/Chl *a*	pCO_2	1	0.078	0.404	0.537
Daytime C fixation rate/Chl *a*	Light	2	42.459	219.057	<0.01
Daytime C fixation rate/Chl *a*	pCO_2*Light	2	0.386	1.990	0.179
eCA activity	pCO_2	1	0.514	88.878	<0.01
eCA activity	Light	2	3.957	684.780	<0.01
eCA activity	pCO_2*Light	2	0.106	18.376	<0.01
$K_{1/2}$(A)	pCO_2	1	0.001	9.673	0.021
$K_{1/2}$(A)	Light	2	0.014	53.972	<0.01
$K_{1/2}$(A)	pCO_2*Light	2	<0.01	0.820	0.484
$k_{1/2}$(B)	pCO_2	1	0.003	34.743	0.001
$k_{1/2}$(B)	Light	2	0.013	136.060	<0.01
$k_{1/2}$(B)	pCO_2*Light	2	<0.01	0.557	0.600
Chl *a*	pCO_2	1	0.109	17.690	0.001
Chl *a*	Light	2	1.48E+00	<0.01	<0.01
Chl *a*	pCO_2*Light	2	0.023	<0.01	0.055
NPQ	pCO_2	1	0.006	9.989	0.008
NPQ	Light	2	0.079	121.413	<0.01
NPQ	pCO_2*Light	2	<0.01	0.457	0.644

did not bring about a significant difference in the growth under HL when compared to the LC treatment at the equivalent light levels. However, HC promoted the growth rate of *T. weissflogii* by about 9.2% under the LL condition. In addition, the cell size increased with decreasing light intensity, but did not show significant difference between the HC and LC treatments (Fig. 1B).

3.3. Pigment contents

Cellular chlorophyll contents increased in the HC-grown cells, but the contents of carotenoids were lower in HC- than in LC-grown cells under low light condition (Fig. 2). Light intensities and CO_2 concentrations had no interactive effects on the contents of Chl *a* (Table 2). The contents of Chl *a* increased with decreasing light levels, with carotenoids being almost unaffected except for the low light-grown cells (Fig. 2b), resulting in a decline of Car to Chl *a* ratio as light availability decrease. In terms of CO_2 effects, the HC-grown cells showed a significantly lower Car to Chl *a* ratio but only under the LL level (Fig. 2c).

3.4. Photochemical responses

CO_2 concentrations and light intensities had no interactive effects on $rETR_{max}$ (Table S1). CO_2 concentration did not give rise to significant effects on ETR_{max} and I_k irrespective of the light intensities. Even though the cells grown under different levels of light and CO_2 concentration showed contrasting differences in $rETR_{max}$ and I_k, light-use efficiency (α) was not significantly altered under any of the combinations. The dark-adapted maximum PSII quantum yield (F_v/F_m) of LC-grown cells was not affected by the growth light condition, but the HC-grown cells

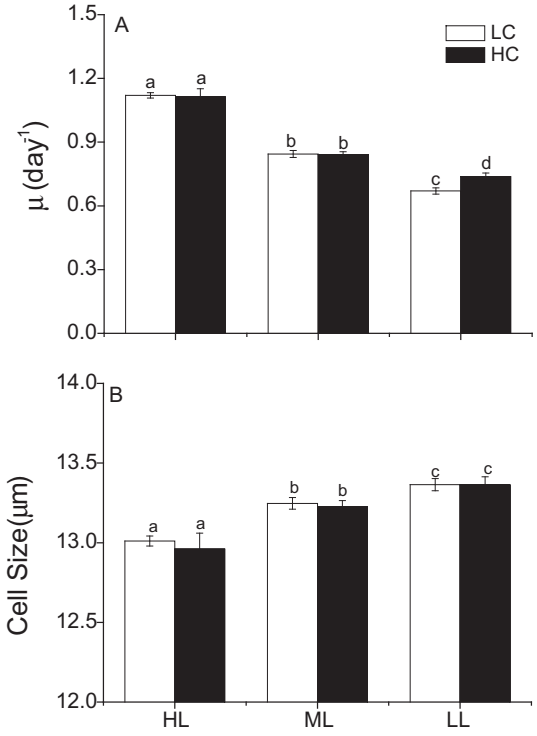

Fig. 1. The relative growth rate (μ, d^{-1}) (A) and cell size (B) of *T. weissflogii* cells grown under different levels of light intensities and CO_2 concentration. Values are the means ± SD of triplicate cultures. Different superscript letters indicate significant ($p < 0.05$) differences among treatments.

markedly increased F_v/F_m regardless of the light treatments (Fig. 3A). Light intensities and CO_2 concentration had no interactive effects on rETR (Table 2), though rETR decreased with decreasing light intensities. In the HL-grown cells, rETR was higher in than ML- or LL-grown cells (Fig. 3B). On the other hand, higher growth light levels resulted in higher values of NPQ, which was moderated by HC (Fig. 3C).

3.5. Photosynthetic and respiratory activities

The respiration rate, net photosynthetic rate and daily net primary production (daytime O_2 evolution) of *T. weissflogii* all showed a decreasing trend with the decrease of growth light levels under both CO_2 concentrations (Fig. 4). Such physiological traits were similar whether expressed per cell or per Chl *a*. However, the HC treatment significantly enhanced the respiration rate per Chl *a* or per cell by about 14.5% under HL or 17.0% under LL condition (Fig. 4A). Light intensities and CO_2 concentration had no interactive effects on the chlorophyll-normalized net photosynthetic rate, and no significant differences in the photosynthetic rates was detected between the LC and HC-grown cells irrespective of the growth light intensities. The daily photosynthetic carbon fixation (integrated daytime production minus nighttime respiratory loss) showed the same trend as net photosynthetic rate (Fig. 4C).

3.6. Enzyme activity

Light intensities and CO_2 concentration had an interactive effect on eCA activity (Fig. 5A). The eCA activity increased with increasing growth light levels. Under the LC condition, the eCA activity in the HL-grown was higher by about 52.4% than ML- and LL-grown ones, respectively. The HC-treatment resulted in a higher $K_{1/2}$, reflecting the decreased affinity of DIC or CO_2 for photosynthesis and a down-regulation of CCMs in the diatoms (Fig. 6). While the $K_{1/2}$ increased with decreasing growth light levels and elevating CO_2 concentration,

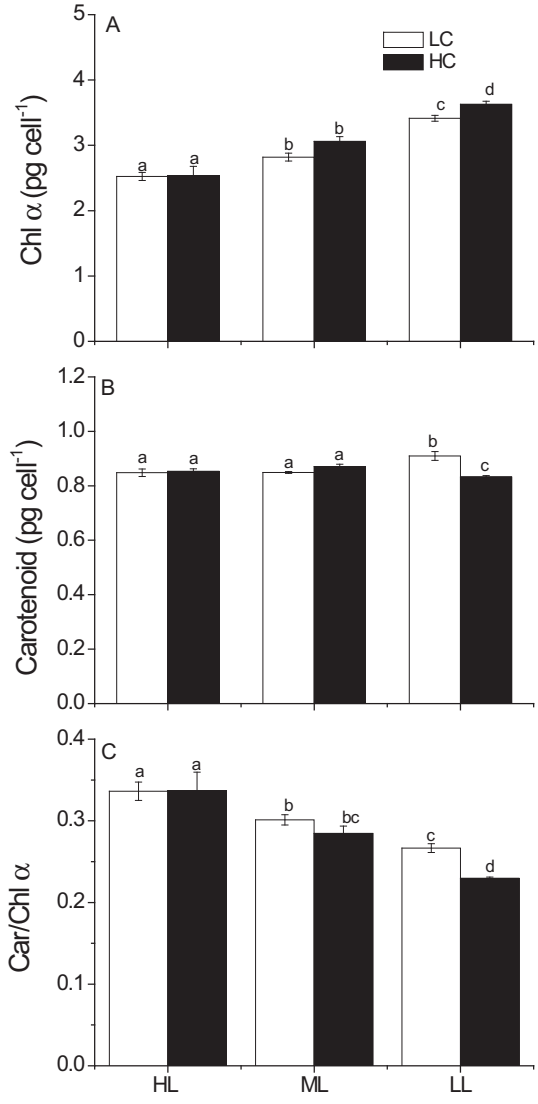

Fig. 2. The contents of Chl *a* (A), Car (B) and the mass:mass ratios of Car/Chl *a* (C) of *T. weissflogii* grown at different light intensities and two CO_2 concentration levels. Values are means ± SD of triplicate cultures. Different superscript letters indicate significant ($p < 0.05$) differences among treatments.

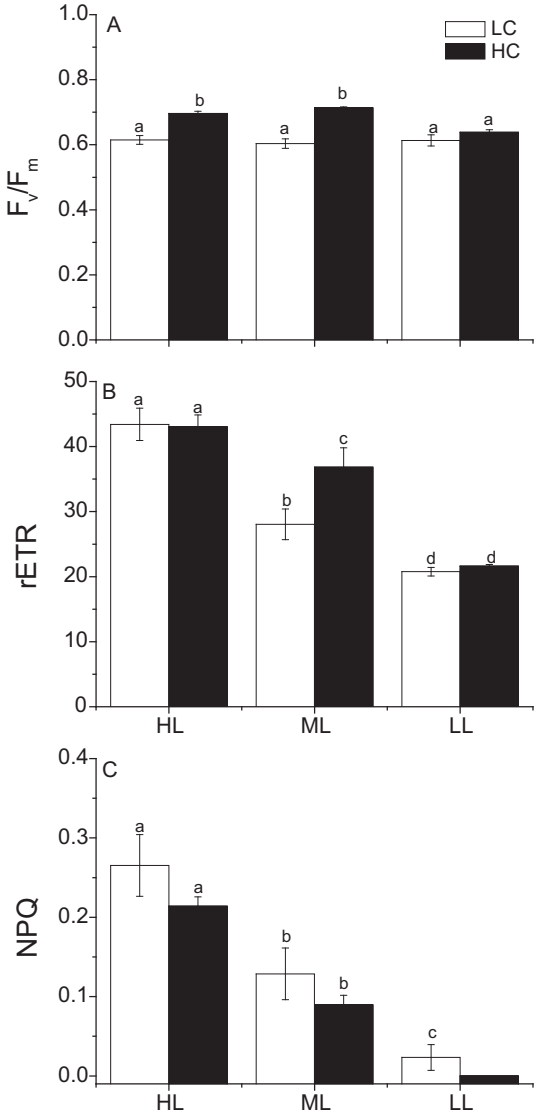

Fig. 3. The dark-adapted maximum PSII quantum yield (F_v/F_m, A) and the relative electron transfer rates (rETR, B) and non-photochemical quenching (NPQ, C) of *T. weissflogii* grown under different growth light intensities and two CO_2 concentration levels. Values are means ± SD of triplicate cultures. Different superscript letters indicate significant ($p < 0.05$) differences among treatments.

light intensities and CO_2 concentration had no interactive effects on $K_{1/2}$ (Table S2). The $K_{1/2}$ values increased significantly in HL and HC-grown cells either based on the ETR obtained at their growth light (Fig. 5B) or saturating light levels (Fig. S2), indicating that the downregulation of CCMs can be detected under either ETR-saturating or limiting light levels. Nevertheless, the HC treatment significantly enhanced the rETR$_{max}$ by about 17% in LL level. The efficiency of carbon acquisition, expressed as the ratio of rETR$_{max}$ to $K_{1/2}$ for CO_2 decreased under HC, but not under LL. The parameters obtained from the growth light or from saturating light intensity had no significant difference.

4. Discussion

Our results showed that OA treatment did not result in significant effects on growth rate in *T. weissflogii* when grown under HL (220 μmol photons m^{-2} s^{-1}) or ML (140 μmol photons m^{-2} s^{-1}). Under the LL (80 μmol photons m^{-2} s^{-1}) conditions, OA promoted the growth rate of *T. weissflogii* (Fig. 1), differing from other studies in which OA had non or negative effects on the growth rate of *T. weissflogii* (Seebah et al., 2014; Passow and Laws, 2015; Taucher et al., 2015). There have been 16 published works on *T. weissflogii* growth responses to OA (Table 3), with stimulative, neutral and negative effects documented under different conditions. In this work, we demonstrated that OA lowered periplasmic carbonic anhydrase (eCA) activity, down-regulated the CCMs, and increased mitochondrial respiration, with enhanced growth rate only at light-limiting conditions (Fig. 7). This was consistent with other diatom species that OA only stimulated their growth under limiting light levels (Gao et al., 2012a). The enhanced growth under insufficient light supply was attributed to saved energy due to down-regulation of CCMs (Gao et al., 2012b). In the present study, extra energy freed up from down-regulated operation of CCMs should be responsible for the enhanced growth rate in *T. weissflogii* under low light, and it was not "CO_2 fertilization" since no enhancement of growth was observed under high light when more CO_2 was demanded for carboxylation (Fig. 7). It was most likely that down-regulated CCMs could have reduced intracellular DIC concentration in *T. weissflogii* as in another diatom *Phaeodactylum tricornutum* (Liu et al., 2017), that is, increased CO_2 availability outside the cells reduces its intracellular concentration. On the other hand, increased respiration under OA indicates that the cells required extra

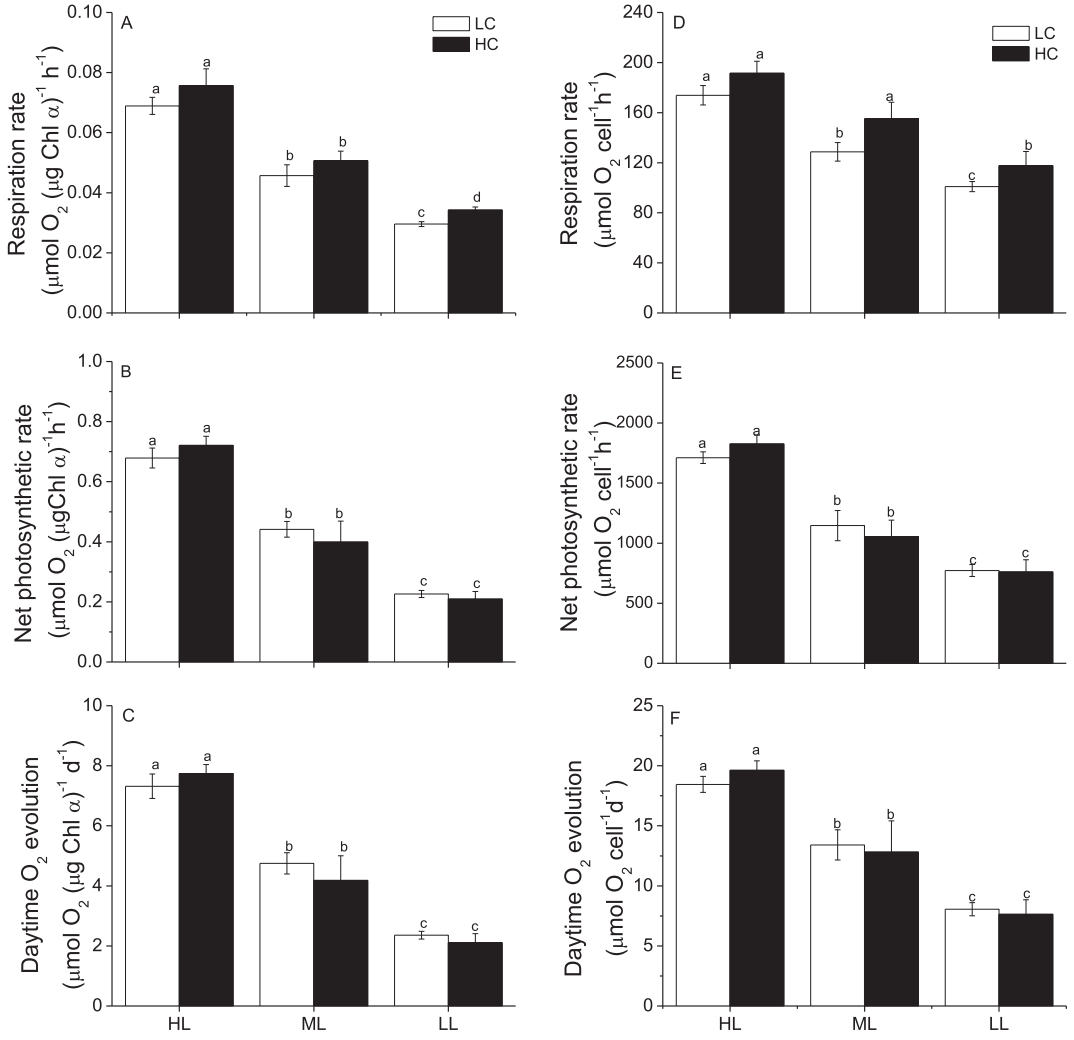

Fig. 4. The chlorophyll-normalized dark respiration rates (A), net photosynthetic rate per cell (B) and daytime O_2 evolution (C) of *T. weissflogii*, and cell-normalized dark respiration rates (D), net photosynthetic rate (E) and daytime O_2 evolution (F) of *T. weissflogii* at different light intensities and CO_2 concentration levels. Values are means ± SD of triplicate cultures. Different superscript letters indicate significant ($p < 0.05$) differences among treatments.

energy during night to cope with the acidic stress. In addition, enhanced respiration could theoretically lead to an increased growth rate as mitochondrial respiration provides energy for cell biosynthesis and maintenance (Hoppe et al., 2015; F. Li et al., 2016; Li et al., 2018). Even though the OA treatment did not result in any difference in the daily net primary productivity (Fig. 4C, F), across growth lights, the enhanced growth in the HC-/LL-grown cells could also be attributed to lowered energy transfer loss in addition to the saved energy from down-regulated CCMs operation.

The none enhancement of growth under HL and ML levels by OA (Fig. 1) could be attributed to that the energy freed up from CCMs-down-regulation was relatively small compared to the relatively sufficient energy supply under the higher light levels when photosynthesis-driven energy supply was relatively high (Goldman et al., 2017). The diatom *Chaetoceros* sp. also showed little or no growth response to OA, with apparent light-dependent differences (Boelen et al., 2011; Ihnken et al., 2011). *Thalassiosira pseudonana* showed an increased metabolic burden to maintain the photosynthetic system under OA and high light condition, which could counter the saving energy from down-regulation of the CCMs (Li et al., 2014). In the present work, the rate of net photosynthesis of LL-grown cells was lower by about 55% of than that at HL level, so the energetic cost for maintaining a stable physiological performance becomes a big fraction in the cells growing under the low light level, thus the saved energy from down-regulated CCMs aided to raise the growth.

As the enzyme Ribulose-1,5-bisphosphate carboxylase–oxygenase (RubisCO) catalyzes both carboxylation and oxygenation reaction. Since intracellular CO_2 concentration becomes lower in a diatom grown under elevated CO_2 concentrations compared to the cells grown under lower CO_2 levels (Liu et al., 2017), the ratio of CO_2 to O_2 surrounding Rubisco was supposed to decline. Therefore, OA induced down-regulation of CCMs could result in enhanced of photorespiration (Gao et al., 2012a; (Xu and Gao, 2012). In the present work, we did not measure photorespiration, the non-enhanced growth of *T. weissflogii* could be partially due to increased photorespiratory carbon loss. Since high light resulted in a high photorespiration, down-regulation of CCMs by OA could further exacerbate it, therefore, excessive light levels might reduce the growth rate of diatoms (Gao et al., 2012b). In this study, the high light was only at a growth-saturating level, which was not high enough to cause photoinhibition.

In our study, the F_v/F_m and effective quantum yield of diatom were generally higher especially under LL level, indicating that diatoms were in a good physiological state. Similar to other studies, the $rETR_{max}$ and I_k were lower under lower growth light intensities (Ralph and Gademann, 2005). Under HL condition, OA had no influence on relative electron transfer rate and light harvesting efficiency (α), indicating that OA had insignificant effects on electron transfer efficiency for biomass conversion (Ihnken et al., 2011). The energy saved from the down-regulation of CCMs activity may be used by the LL-grown cells to better deal with

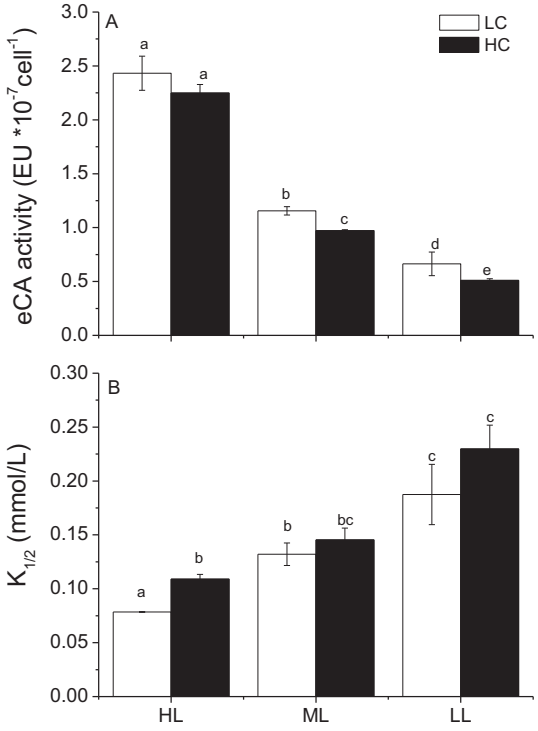

Fig. 5. The extracellular carbonic anhydrase activity (eCA, A) and $K_{1/2}$ (DIC concentration required for half maximal ETR) under growth light intensity (B) of *T. weissflogii* at different light intensities and CO_2 concentration levels. Values are means ± SD of triplicate cultures. Different superscript letters indicate significant ($p < 0.05$) differences among treatments.

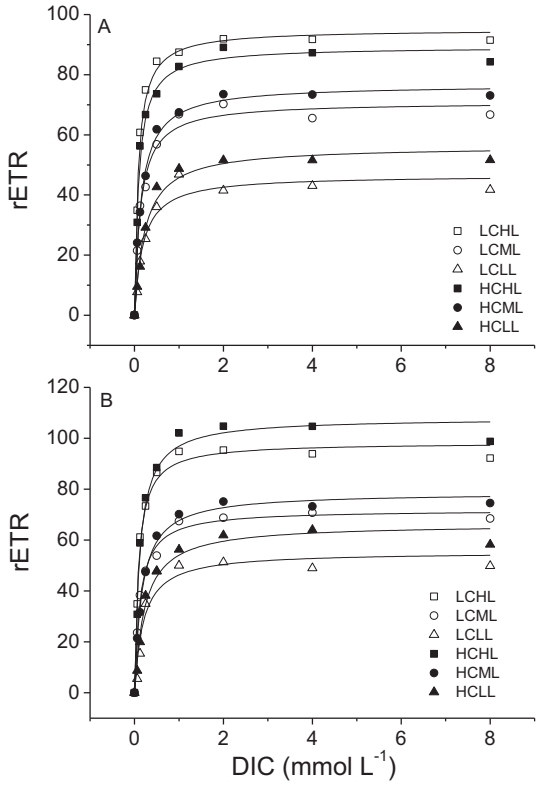

Fig. 6. rETR under their growth light intensities (A) and under their saturated light intensity (B) as a function of DIC concentration for the cells grown at the different light and CO_2 concentration levels.

Table 3
Documented specific growth rates (μ) of *T. weissflogii* grown under different light, temperature and CO_2 conditions. T indicates temperature, "N" no significant change under OA, "U" Unknown the result, "+" OA increase the growth rate, "−" OA decrease the growth rate.

Light (μmol photons m^{-2} s^{-1})	Photoperiod	T (°C)	OA (μ)	CCMP	Reference
35, 65	14L:10D	15, 20	−, N	1053	Passow and Laws, 2015
50	14L:0D	25	N	Isolated Japan	(Ishida et al., 2000)
50	14L:10D	15, 20	N	1336	Seebah et al., 2014
50, 120	12L:12D	17	N	1010	Larsen et al., 2015
100	14L:10D	15, 20	N	1053	Taucher et al., 2015
100	12L:12D	18, 24	N	102	Gao et al., 2018
115	12L:12D	20	N	1336	F. Li et al., 2016
120	12L:12D	20	U	102	Zeng et al., 2019
150	12L:12D	20	N	102	(Li et al., 2019)
15, 140	12L:12D	18	U	1336	Needoba and Harrison, 2004
20, 120	12L:12D 2L:22D	5–15	U	1336	Walter et al., 2015
200	12L:12D	18	N	1336	Reinfelder, 2011
230	12L:12D	20	+	102	Gao et al., 2018
235	12L:12D	20, 25	U	UN	Helbling et al., 2011
350	12L:12D	20	+	1336	Wu et al., 2014
Solar radiation	12L:12D	25–27	N	102	W. Li et al., 2016
80	12L:12D	20	+	1336	This paper
140	12L:12D	20	N	1336	This paper
220	12L:12D	20	N	1336	This paper

acidic stress associated with the pH decline in comparison with the HL cells (Liu et al., 2017). Higher respiration rates at low light had previously been observed under OA and it was attributed to the increased contribution of light-dependent respiration pathways, which could generate ATP without depleting the cell's carbon pool (Fisher and Halsey, 2016). Therefore, compared to the large requirement for CO_2 by the HL-grown cells with high photosynthetic rate, the cells grown under LL required less CO_2 and benefited from the down-regulation of CCMs in order to cope with the acidic stress of OA.

Thalassiosira weissflogii and other diatoms distributed in nearshore waters experience episodical and diel changes in carbonate chemistry due to tidal changes, biological production and wind-driven mixing. Therefore, they are exposed to changing levels of light and CO_2/pH at different timings or weather conditions under the ongoing global ocean acidification. On the basis of the present results, coastal diatoms including *T. weissflogii* may benefit from increased CO_2 availability during twilight periods or under cloudy weather conditions and endure acidic stress of OA even under fluctuating or fast changing light conditions. On the other hand, fast mixing can frequently expose the cells to high and low light conditions, modulating OA effects on its physiological performances as in other phytoplankter (Jin et al., 2013) The balance of positive and negative effects of OA on diatom depend on physical and chemical conditions in different waters, will eventually lead to "winner" or "loser", therefore, influencing diatom community structure and related biogeochemical processes.

CRediT authorship contribution statement

Liming Qu: Conceptualization, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Douglas A. Campbell:** Formal analysis, Writing – review & editing. **Kunshan Gao:** Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing.

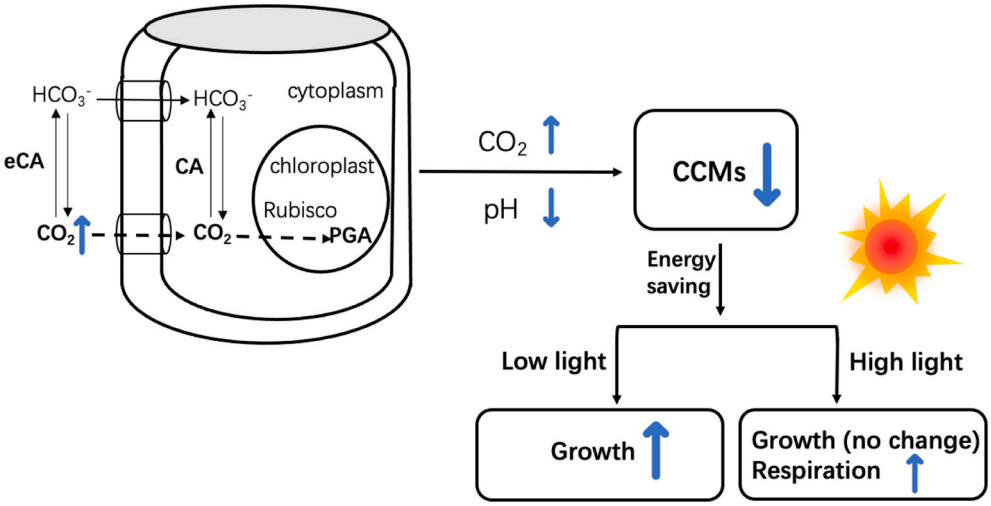

Fig. 7. Illustration about the effects of ocean acidification (OA) on the diatom *T. weissflogii*: while CCMs were equally down-regulated under low and high light levels, its growth was only stimulated by OA under growth-limiting light conditions, indicating that it is energy saving from down-regulation of CCMs that promotes the growth under low light, and there was no "CO_2 fertilization" effect under high light.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The study was supported by National Natural Science Foundation of China (41720104005, 41721005). The authors are grateful to the laboratory engineers Xianglan Zeng, Wenyan Zhao and to the Ph.D students, Xiaowen Jiang, Jiazhen Sun and He Li, for their logistical and technical supports.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112008.

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