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# Ocean acidification interacts with variable light to decrease growth but increase particulate organic nitrogen production in a diatom

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ABSTRACT

Phytoplankton in the upper oceans are exposed to changing light levels due to mixing, diurnal solar cycles and weather conditions. Consequently, effects of ocean acidification are superimposed upon responses to variable light levels. We therefore grew a model diatom *Thalassiosira pseudonana* under either constant or variable light but at the same daily photon dose, with current low (400 μatm, LC) and future high $CO_2$ (1000 μatm, HC) treatments. Variable light, compared with the constant light regime, decreased the growth rate, Chl *a*, Chl *c*, and carotenoid contents under both LC and HC conditions. Cells grown under variable light appeared more tolerant of high light as indicated by higher maximum relative electron transport rate and saturation light. Light variation interacted with high $CO_2$/lowered pH to decrease the carbon fixation rate, but increased particulate organic carbon (POC) and particularly nitrogen (PON) per cell, which drove a decrease in C/N ratio, reflecting changes in the efficiency of energy transfer from photo-chemistry to net biomass production. Our results imply that elevated $pCO_2$ under varying light conditions can lead to less primary productivity but more PON per biomass of the diatom, which might improve the food quality of diatoms and thereby influence biogeochemical nitrogen cycles.

## 1. Introduction

Atmospheric $CO_2$ concentration is rising due to human activity, mainly fossil fuel burning, unprecedently faster than in any previous period of the geological record (Hönisch et al., 2012). The oceans are currently the main sink for anthropogenic $CO_2$, taking up about 26 million tons per day (Gattuso et al., 2010). Absorption of $CO_2$ by oceans lowers the ocean pH and alters seawater chemistry by increasing $H^+$ and $HCO_3^-$ and decreasing $CO_3^{2-}$, termed ocean acidification (OA) (Caldeira and Wickett, 2003). The atmospheric $CO_2$ concentration is expected to reach 800–1000 ppm by the end of this century according to A1Fl scenario of IPCC report (IPCC, 2014), which implies that the oceanic $H^+$ concentration will further increase by 100%–150% while decreasing pH by 0.3–0.4.

Effects of OA on marine organisms and ecosystems have attracted attention of both scientists and societies in part because OA is expected to influence marine ecosystem services (Riebesell and Gattuso, 2015). OA effects on marine phytoplankton have multiple aspects and differ across taxa, regions and across various studies (Gao and Campbell, 2014; Gao et al., 2019). Declining pH can affect intracellular acid-base balance (Pörtner et al., 2010) and nutrient absorption (Shi et al., 2015), reduce algae growth under nutrient limited conditions (Li et al., 2018) or under high levels of sunlight (Gao et al., 2012). pH drops decrease mineralization of diatoms and coccolithophores (see the review by Gao et al. (2019) and literature therein). On the other hand, increased availability of $HCO_3^-$ and dissolved $CO_2$ may benefit photosynthetic carbon fixation, especially in species with weaker carbon concentration mechanisms (CCMs) (Giordano et al., 2005), though intracellular dissolved inorganic carbon concentration can actually decrease under climate change relevant $CO_2$ levels when down-regulation of CCMs outweighs increased passive influx of DIC into cells (Liu et al., 2017).

Responses of diatoms to OA differ under different light conditions (Gao et al., 2012). Since both the quantity and quality of natural solar light changes spatially and temporally, and differently from artificial light often applied in simulation experiments, physiological responses to OA under varying light regimes have been scarcely documented (Zhang et al., 2015; Li et al., 2017). Normally, natural solar radiation follows a sigmoidal diurnal curve which varies across different light dark cycles in different seasons or regions, peaking at mid-day. Phytoplankton cells *in situ* furthermore experience variable light superimposed upon the

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diurnal curve as a result of mixing of the water column and changing weather (Jin et al., 2013a). Responses of phytoplankton to variable light (Van de Poll et al., 2007; Van de Waal et al., 2010; Hoppe et al., 2015; Zheng et al., 2015) include responses to both the average light intensity and the frequency of the variation (Falkowski, 1984; Litchman, 2000).

The measured responses of phytoplankton taxa to OA are inconsistent across studies and even within a taxon, and different experimental setups can bring about differential results (Beardall et al., 2009; Gao et al., 2017). OA effects are not only taxon-specific, but can interact with other co-varying drivers to show synergistic, neutral or antagonistic effects (Riebesell and Gattuso, 2015; Gao et al., 2017; Boyd et al., 2018), with light seen as a key mediator of OA effects (Kranz et al., 2010; Ihnken et al., 2011; Gao et al., 2012; Hoppe et al., 2015; Li et al., 2015, 2017). Variable light regimes could further complicate the responses of phytoplankton to OA as the cells experience low to saturating or inhibitory light levels. The Antarctic diatom *Chaetoceros debilis* indeed showed distinct energy transfer efficiency under interacting influences of OA and variable light conditions (Hoppe et al., 2015). Consequently, responses of diatom growth, photosynthesis and elemental composition to OA obtained from constant light regimes may not translate to responses under variable natural light regimes.

As a major group of phytoplankton, diatoms range widely from coasts to the open ocean and contribute nearly half of marine primary production (Granum et al., 2005; Armbrust, 2009; Finkel et al., 2010). Diatom cell size ranges from a few micrometers to millimeters. Most indoor simulation studies have used a constant light regime to infer OA effects on diatoms, and although constant light is important to infer mechanisms, it may not predict responses to natural conditions. Although there are papers documenting diatom responses under fluctuating light (Hoppe et al., 2015; Li et al., 2017), little is known about the comparative effects of constant and variable light regimes especially at same light dose. Therefore, we hypothesize that OA may exacerbate impacts on diatoms under variable light due to extra energetic costs, and we tested this hypothesis by investigating physiological and biochemical responses of a model diatom to OA under constant *versus* variable light regimes, at the same daily cumulative light dose.

## 2. Materials and methods

### 2.1. Algae culture

The diatom *Thalassiosira pseudonana* (CCMP 1335) was obtained from Center for Collections of Marine Algae (CCMA) of State Key Laboratory of Marine Environmental Science (Xiamen University). The strain was originally isolated from Moriches Bay in 1958 (Long Island, New York). It was grown in artificial seawater enriched with Aquil Medium (Morel et al., 1979). Cells were cultured semi-continuously under ambient low $pCO_2$ (400 µatm, LC) and elevated high $pCO_2$ (1000 µatm, HC) medium that was pre-bubbled to equilibrium for 12 h with ambient air or $CO_2$-enriched air in a $CO_2$ chamber (HP1000G-D, Ruihua Instrument and Equipment Co. Ltd, China) under 20 °C. The $pCO_2$ in the $CO_2$ chambers was then measured frequently with a portable $CO_2$ meter (M170, Vaisala Oyj). The light dark cycle was set as 12L:12D with 08:00 a.m. to 20:00 p.m. as the photoperiod and 20:00 p.m. to 08:00 a.m. as the nocturnal period. Two light regimes with the same total daily light dose were set. A constant light treatment (abbreviated as "C") was applied at 215 µmol $m^{-2}s^{-1}$ during the photoperiod, close to the mean daytime photon flux in the middle photic zone of the South China Sea (22–36 m depth) (Gao et al., 2012). Alternately a variable light treatment (abbreviated as "V") simulated natural dynamic light conditions, by dividing the photoperiod into 5 stages such that irradiance changed every two or 4 h during the photoperiod, with irradiance of 95 µmol $m^{-2}s^{-1}$ (08:00–10:00), 190 µmol $m^{-2}s^{-1}$ (10:00–12:00), 360 µmol $m^{-2}s^{-1}$ (12:00–16:00), 190 µmol $m^{-2}s^{-1}$ (16:00–18:00) and 95 µmol $m^{-2}s^{-1}$ (18:00–20:00) during each time period (measured with PMA2100, Solar light Co., USA) (Fig. 1). Different layers of neutral density screens were used to achieve the respective light levels described above. The light source was provided by a cool white fluorescent lamp, with an equivalent daily dose of 2.02 MJ $m^{-2}$ ($9.288 \times 10^6$ µmol $m^{-2}$) in the two light regimes. Though variable light regimes have different combinations of average intensity and frequency of period in natural settings, our present study only considers the differential effects of constant vs. variable light within the photoperiod.

Cells were cultured in 1000 ml sealed polycarbonate bottles (Nalgene™, Thermo Scientific) and maintained in exponential phase through dilution with pre-equilibrated LC and HC medium every 2 or 4 days to maintain cell suspension densities ca. $1 \times 10^6$ cells $ml^{-1}$, thereby keeping the carbonate chemistry stable under the two $CO_2$ treatments (Table 1). Cells were grown under the light regimes and $CO_2$ conditions, gently shaken two or three times every day and acclimated for more than 10 generations before being used for sampling and measurement. Each treatment had 3 biological replicate cultures.

### 2.2. Seawater carbonate chemistry

The pH in cultures was measured before each dilution with a pH meter (Mettler Toledo DL15 Titrator, Sweden). The pH meter was calibrated at 3 points with NBS (National Bureau of Standards) buffer solutions (pH 4.01, 7.01 and 10.01, Hanna) every time before measurement. Pilot measures showed that pH remained stable before and after dilution because we pre-equilibrated the diluent media. Known values of pH, $pCO_2$, salinity (35), nutrient concentration ($PO_4^{3-}$: 10 µmol $L^{-1}$, $SiO_4^{2-}$: 100 µmol $L^{-1}$) and temperature (20 °C) were used to derive the total alkalinity (TA), dissolved inorganic carbon (DIC), $HCO_3^-$, $CO_3^{2-}$ and dissolved $CO_2$ (Table 1) using CO2sys_v2.3.xls (Pelletier et al., 2007).

### 2.3. Pigment measurement

Cells grown under different light regimes and $CO_2$ concentrations were sampled at the end of the photoperiod, filtered onto GF/F filter (Whatman, 0.70 µm), extracted with 5 ml methanol (100%) under 4 °C overnight, and centrifuged at 5000 *g* for 10 min. The methanol extract supernatant was then scanned with a spectrophotometer (DU800, Beckman, USA). The chlorophyll *a* (Chl *a*) and Carotenoid were

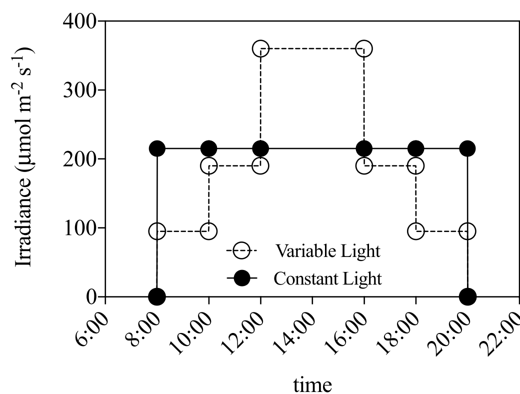


**Fig. 1.** Experimental design for constant and variable light regimes. The light intensity in the constant regime was kept at 215 µmol $m^{-2}s^{-1}$ during the whole photoperiod (08:00–20:00). The variable light regime, which approximated natural light variation during one photoperiod, was cut into 5 stages with light intensity changed manually every two or 4 h using a neutral density net during the photoperiod, with light intensities of 95 µmol $m^{-2}s^{-1}$ (08:00–10:00), 190 µmol $m^{-2}s^{-1}$ (10:00–12:00), 360 µmol $m^{-2}s^{-1}$ (12:00–16:00), 190 µmol $m^{-2}s^{-1}$ (16:00–18:00) and 95 µmol $m^{-2}s^{-1}$ (18:00–20:00) during each time period. The total daily photon dose in the constant and variable light regimes was the same.

**Table 1**
Parameters of seawater carbonate chemistry in constant light under low $CO_2$ (C-LC), constant light under high $CO_2$ (C-HC), variable light under low $CO_2$ (V-LC), variable light under high $CO_2$ (V-HC) before dilution. Known values of pH, $pCO_2$, salinity, nutrients concentration and temperature were used to derive the total alkalinity (TA), dissolved inorganic carbon (DIC), $HCO_3^-$, $CO_3^{2-}$ and $CO_2$* using CO2sys_v2.3.xls. Data are the means ± SD of 3 measurements.

| Treatment | $pH_{Total}$ | $pCO_2$ (μatm) | TA (μmol $kg^{-1}$) | DIC (μmol $kg^{-1}$) | $HCO_3^-$ (μmol $kg^{-1}$) | $CO_3^{2-}$ (μmol $kg^{-1}$) | $CO_2$* (μmol $kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| C-LC | 8.03 ± 0.02 | 399.0 ± 7.0 | 2199.8 ± 53.3 | 1928.6 ± 40.9 | 1733.5 ± 31.5 | 182.2 ± 9.6 | 12.9 ± 0.2 |
| C-HC | 7.70 ± 0.02 | 1002.3 ± 6.7 | 2295.1 ± 72.7 | 2169.9 ± 64.6 | 2037.4 ± 58.5 | 100.2 ± 6.3 | 32.4 ± 0.2 |
| V-LC | 8.03 ± 0.02 | 402.0 ± 4.6 | 2196.6 ± 71.8 | 1927.4 ± 57.9 | 1733.5 ± 46.9 | 180.9 ± 11.2 | 13.0 ± 0.1 |
| V-HC | 7.70 ± 0.02 | 990.0 ± 11.8 | 2267.2 ± 59.7 | 2143.0 ± 52.1 | 2012.0 ± 46.7 | 98.9 ± 5.7 | 32.0 ± 0.4 |

calculated according to Ryckebosch et al. (2011) which was modified from Wellburn (1994). Chlorophyll *c* (Chl *c*) was calculated according to Ritchie (2006).

### 2.4. Growth rate and cell volume measurements

Growth rate (μ) per day or per hour of each treatment was calculated based on the cell suspension count change according to:

$$\mu = \frac{lnN_t - lnN_0}{t_2 - t_1}$$

where $N_t$ and $N_0$ are cell suspension counts at $t_2$ and $t_1$. Cell counts and cell size were determined with a Z2™ Coulter Counter (Beckman, Buckinghamshire, UK). The growth rate (μ $day^{-1}$) was calculated based on cell density change across a dilution cycle with cell densities measured just after dilution and then again just before the next dilution. The growth rates per hour plotted at 11:00, 14:00, 17:00 and 19:00 were calculated during a photoperiod based on cell density change during 09:00–11:00, 11:00–14:00, 14:00–17:00 and 17:00–19:00.

### 2.5. Photosynthetic performance measurement

Chlorophyll fluorescence variables were determined with a Xe-PAM (Walz, Germany). Rapid light curves (RLCs) were measured at 14:00, 17:00 and 19:00 with actinic irradiance levels set to 0, 156, 226, 337, 533, 800, 1077, 1593 and 2000 μmol $m^{-2}$ $s^{-1}$, with each light level applied for an interval of 10 s, and a saturation pulse of 5000 μmol photons $m^{-2}$ $s^{-1}$ for 0.8 s applied at the end of each light interval. Effective quantum yield ($\Phi_{PSII}$) was measured under culture light at the same time point as RLC, and calculated according to Genty et al. (1990):

$$\Phi_{PSII} = \frac{(F_M^{'} - F_t)}{F_M^{'}}$$

where $F_M$' indicates the light-adapted maximal chlorophyll fluorescence yield, and $F_t$ is the steady fluorescence level during the exposures.

Relative electron transport rate (rETR) was calculated following the equation:

$$rETR = \Phi_{PSII} \times I \times 0.5 \times 0.84$$

where I indicates the actinic light intensity (μmol $m^{-2}$ $s^{-1}$), 0.5 represents the assumption of equal distribution of excitation between PSII and PSI, and 0.84 is an approximation for light interception.

The measured RLCs were fitted with the equation from Eilers and Peeters (1988) to acquire relative maximum electron transport rate ($rETR_{max}$), light use efficiency (α) and saturation light intensity ($I_k$):

$$rETR = \frac{I}{aI^2 + bI + c}$$

where a, b and c are the adjustment parameters, and $rETR_{max}$, α and $I_k$ were calculated as follows:

$$rETR_{max} = \frac{1}{\left[b + 2(ac)^{\frac{1}{2}}\right]}$$

$$\alpha = \frac{1}{c}$$

$$I_k = \frac{rETR_{max}}{\alpha}$$

Note that as the reported irradiance level of actinic light from the built-in monitor on the Xe-PAM (xenon lamp) is about double our comparison measures with a portable light meter (solar light PAM2100) ($Y_{PAM\text{-}actual} = 0.4411{*}X_{PAM\text{-}Built\text{-}in}\text{-}3.0252$), the calculated $I_k$ of different treatments from RLCs were adjusted with the correction factor before reporting.

### 2.6. Particulate organic carbon (POC) and nitrogen (PON) measurements

Cells at the end of the photoperiod were sampled and filtered onto GF/F filters (Whatman, 0.70 μm, pre-combusted at 450 °C for 6 h in muffle furnace), fumed with HCl (12 mol $L^{-1}$) for 12 h to remove the inorganic carbon, and dried in an oven for another 6 h. POC and PON were then measured with a PerkinElmer Series II CHNS/O Analyzer 2400.

### 2.7. POC and PON production calculation

POC and PON production were calculated following (Riebesell et al., 2000; Jin et al., 2013b) according to the following equation:

$$POC\ \ production = \mu(day^{-1}) \times POC(cell^{-1})$$

$$PON\ \ production = \mu(day^{-1}) \times PON(cell^{-1})$$

### 2.8. Carbon fixation rate measurement

Carbon fixation rates were measured throughout the photoperiod at five time points (09:00, 11:00, 14:00, 17:00, 19:00) with the $^{14}C$ isotopic tracer method (Neale et al., 2014). Briefly, the cells grown under different light regimes and $CO_2$ levels were sampled into 20 ml PerkinElmer scintillation vials, injected with 100 μL - 5 μCi ($NaH^{14}CO_3$), and cultured under respective light levels for 1 h. Cells were then immediately filtered onto GF/F filters (Whatman, 0.70 μm) and transferred into 20 ml scintillation vials. Before measurement, filters were fumed with HCl (12 mol $L^{-1}$) for 12 h to remove the inorganic carbon and dried in an oven for another 6 h at 45 °C (Gao et al., 2007). Filters were then transferred combined with 5 ml scintillation cocktail (Tri-Carb 2800 TR, PerkinElmer®), then counted with a liquid scintillation counter (LS 6500, Beckman Coulter, USA). Carbon fixation calculations are detailed elsewhere (Li et al., 2015).

### 2.9. Statistical analysis

Data analysis and plotting were performed using SPSS 19.0 and

GraphPad Prism 7.0. Two - way factorial analyses of variance (Two - way ANOVA) were used to establish interactive effects between light regime (constant or variable) and $CO_2$ concentration (400 μatm or 1000 μatm) on pigments (Chl a, carotenoid, Chl c, carotenoid/Chl a) and elemental composition parameters (POC, PON, POC production, PON production and C/N). Two - way factorial repeated measures analyses of variance (RM-ANOVA) were used to test the effects of light regime and $CO_2$ over time on growth, cell size, fluorescence parameters as well as carbon fixation. Tukey's multiple comparisons test was used to establish significant differences between treatments at a 95% confidence level.

## 3. Results

### 3.1. Growth rate and cell size

Significant interactions between $CO_2$ concentration (400 μatm and 1000 μatm) and light regimes were found for growth rate per day (Table 2). Although the growth rate was not significantly affected by $CO_2$ (Fig. 2A), it was significantly affected by the light regimes (Fig. 2A) (Table 2). Compared to the constant light treatment, under the variable light treatment the growth rates of both LC and HC were significantly decreased by 45% ($P < 0.0001$) and 30% ($P < 0.0001$), respectively (Fig. 2A). According to higher resolution estimates of growth rates ($hour^{-1}$) measured during one photoperiod, growth rate was significantly affected by both time within the photoperiod ($P < 0.0001$) and light regime ($P = 0.001$) individually and interactively ($P < 0.0001$) (Table 3) with all treatments starting at the same low growth rate in the morning, but the constant light treatments sustaining higher growth rates through the mid photoperiod (14:00 to 17:00) ($P < 0.05$) before reconverging with variable light treatments late in the photoperiod (Fig. 2B).

During the photoperiod, light regime and $CO_2$ concentration individually and pair-wise interactively affected the cell size (all $P < 0.05$) (Table 3). Cell size increased with time during the photoperiod under both constant and variable light regimes ($P < 0.0001$) (Fig. 2C). In the cells grown under LC with constant light, the cell size was about 4% larger at both the start ($P = 0.0001$) and the end ($P < 0.0001$) of the photoperiod compared to the variable light regime. However, no significant differences in cell size were detected between constant and variable light under the HC condition (all $P > 0.05$).

### 3.2. Pigments

No significant differences in Chl *a* (Fig. 3A), Carotenoid (Fig. 3B), Chl *c* (Fig. 3C) nor Carotenoid/Chl *a* (Fig. 3D) were found between LC and HC under neither constant nor variable light treatments (all $P > 0.05$). Compared to the constant light treatment Chl *a*, Carotenoid and Chl *c* all decreased under variable light, for both LC and HC (Fig. 3A–C). No significant difference in Carotenoid/Chl *a* was found among the treatments (Fig. 3D). The calculated Chl *a*, Carotenoid and Chl *c* unit to a cell volume base (cell biovolume, $\mu m^{-3}$, calculated based on a sphere) showed similar trends as unit to per cell base (Fig. S1).

**Table 2**
Two - way factorial ANOVA analysis (Two - way ANOVA) of individual and interactive effects of light regime (constant or variable, "L"), $CO_2$ concentration (400 μatm or 1000 μatm, "C") on observed parameters at $P < 0.05$ level.

| | L | | C | | L*C | |
|---|---|---|---|---|---|---|
| | F | P | F | P | F | P |
| Growth ($day^{-1}$) | 316.1 | **<0.0001** | 0.01 | 0.92 | 18.87 | **0.003** |
| Chl *a* | 37.63 | **0** | 1.46 | 0.262 | 0.59 | 0.464 |
| Carotenoid | 58.96 | **<0.0001** | 1.82 | 0.214 | 0.37 | 0.562 |
| Chl *c* | 36.52 | **<0.001** | 0.33 | 0.583 | 0.04 | 0.843 |
| Carotenoid/Chl *a* | 7.46 | **0.026** | 0.04 | 0.854 | 0.09 | 0.773 |
| POC | 26.27 | **<0.001** | 5.01 | 0.056 | 3.28 | 0.108 |
| PON | 48.82 | **<0.001** | 9.72 | **0.014** | 2.63 | 0.144 |
| POC production | 73.11 | **<0.0001** | 26.87 | **<0.001** | 68.87 | **<0.0001** |
| PON production | 8.39 | **0.02** | 40.13 | **<0.001** | 20.86 | **0.002** |
| C/N | 173.5 | **<0.0001** | 32.97 | **<0.001** | 2.28 | 0.169 |

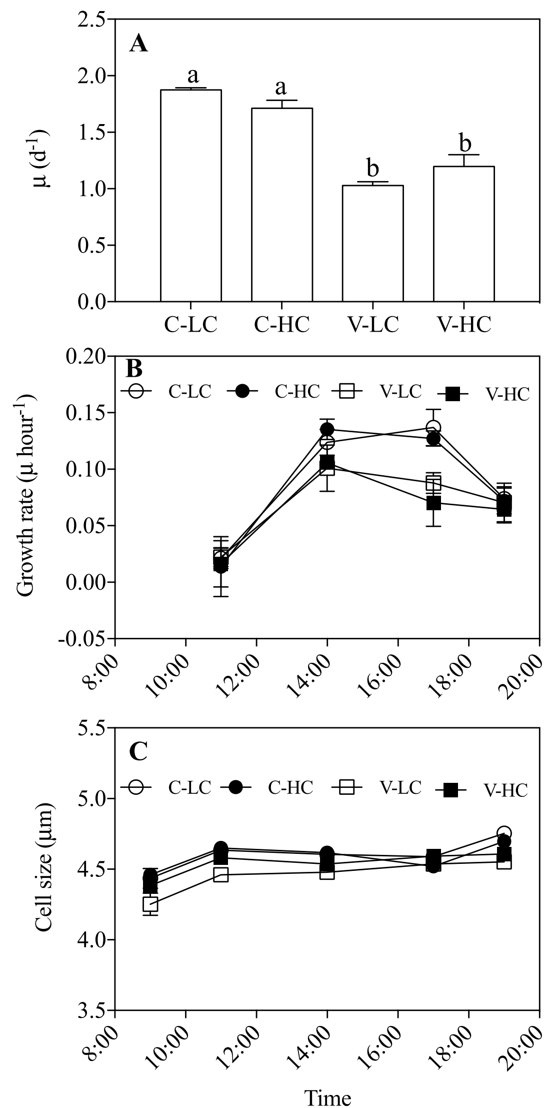


**Fig. 2.** Growth rate ($\mu$ $day^{-1}$) (A), growth rate ($\mu$ $hour^{-1}$) (B) and cell size (C) of *T. pseudonana* cultured in constant light under low $CO_2$ (C-LC), constant light under high $CO_2$ (C-HC), variable light under low $CO_2$ (V-LC), variable light under high $CO_2$ (V-HC) ($n = 3$). The cell counts for growth rate ($\mu$ $hour^{-1}$) and cell size were measured at five time points (09:00, 11:00, 14:00, 17:00 and 19:00) during the photoperiod, and the growth rate ($\mu$ $hour^{-1}$) plotted at 11:00, 14:00, 17:00 and 19:00 were then calculated based on the cell density changes during 09:00–11:00, 11:00–14:00, 14:00–17:00 and 17:00–19:00.

### 3.3. Photosynthetic performance

Rapid light curves measured at the middle of the photoperiod (14:00) showed highest values of $rETR_{max}$, α and $I_k$ compared to late photoperiod (17:00) and the end of the photoperiod (19:00) (Fig. 4 A-C, Fig. 5A–C). At the mid-photoperiod (14:00), no significant difference in α and $\Phi_{PSII}$ among treatments was found (all $P > 0.05$) (Fig. 5 B, D). Under the variable light regime, $rETR_{max}$ and $I_k$ increased when compared to the constant light regime (Fig. 5C). There was no significant difference in $rETR_{max}$ and $I_k$ between LC and HC under the same light regime (Fig. 5A, C).

In the later photoperiod (17:00) $rETR_{max}$ in variable light compared to the constant light regime was increased in LC but not in HC ((Fig. 5A).

**Table 3**
Two - way factorial repeated measures analyses of variance (RM-ANOVA) of light regime (constant or variable, "L") and $CO_2$ concentration (400 μatm or 1000 μatm, "C") over time during photoperiod ("T") on growth, cell size, fluorescence parameters as well as carbon fixation at P < 0.05 level.

| Effect | L | | C | | L*C | | T | | T*L | | T*C | | T*L*C | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | P | F | P | F | P | F | P | F | P | F | P | F | P |
| Growth ($h^{-1}$) | 24.53 | **0.001** | 0.74 | 0.414 | 0.30 | 0.596 | 119.85 | **<0.0001** | 9.20 | **<0.0001** | 1.23 | 0.319 | 0.05 | 0.984 |
| Cell size | 43.52 | **<0.001** | 5.53 | **0.047** | 10.86 | **0.011** | 125.40 | **<0.0001** | 12.23 | **<0.0001** | 4.77 | **0.004** | 0.75 | 0.564 |
| rETR | 36.40 | **<0.001** | 8.26 | **0.021** | 0.74 | 0.415 | 2907.94 | **<0.0001** | 122.63 | **<0.0001** | 9.63 | **0.010** | 0.38 | 0.696 |
| α | 0.50 | 0.498 | 1.68 | 0.231 | 0.01 | 0.924 | 17.37 | **<0.0001** | 1.31 | 0.298 | 1.32 | 0.295 | 0.36 | 0.705 |
| $I_k$ | 56.68 | **<0.0001** | 2.63 | 0.144 | 1.46 | 0.262 | 324.52 | **<0.0001** | 20.14 | **<0.0001** | 10.89 | **0.001** | 3.27 | 0.064 |
| $\Phi_{PSII}$ | 58.93 | **<0.0001** | 0.03 | 0.876 | 3.72 | 0.090 | 2.15 | 0.187 | 4.50 | 0.055 | 0.35 | 0.714 | 3.02 | 0.113 |
| Carbon fixation | 131.61 | **<0.0001** | 13.73 | **0.006** | 1.22 | 0.302 | 39.12 | **<0.0001** | 6.59 | **0.001** | 4.36 | **0.006** | 2.86 | **0.039** |

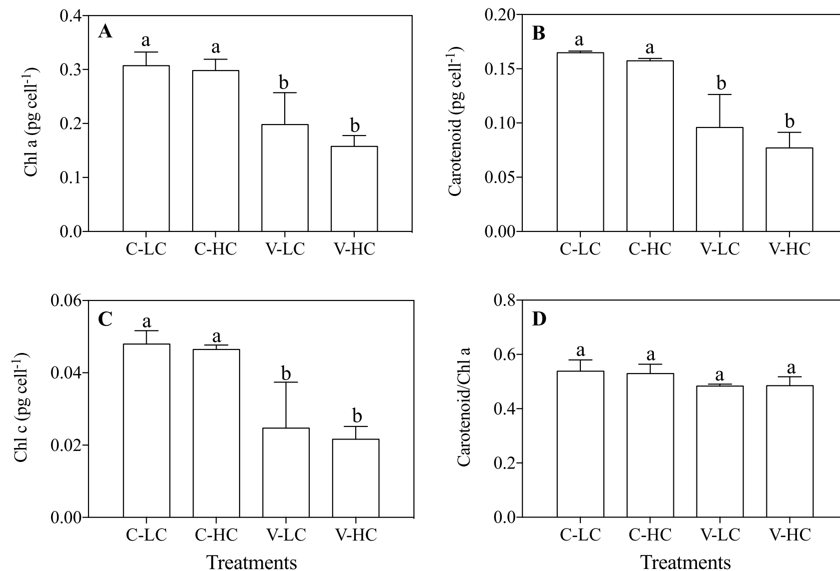


**Fig. 3.** Chl *a* (A), Carotenoid (B), Chl *c* (C) contents (units per cell) and the ratio of Carotenoid to Chl *a* (Carotenoid/Chl *a*) (D) of *T. pseudonana* cultured in constant light under low $CO_2$ (C-LC), constant light under high $CO_2$ (C-HC), variable light under low $CO_2$ (V-LC), variable light under high $CO_2$ (V-HC) (n = 3). Cells grown under different light regimes and $CO_2$ concentrations were sampled at the end of the photoperiod for pigment measurements.

Under variable light, HC decreased $rETR_{max}$ in comparison with LC ((Fig. 5A). No significant difference in α among treatments was found (all P > 0.05) (Fig. 5B). The $I_k$ of LC and HC in variable light was increased compared to cells grown in constant light (Fig. 5C). No significant difference of $I_k$ between LC and HC in the constant light regime was found (all P > 0.05); however, in the variable light regime, $I_k$ decreased in HC when compared to the LC grown cells. There was no significant difference in $\Phi_{PSII}$ between LC and HC treatments in either light regime (all P > 0.05); the $\Phi_{PSII}$ of LC and HC in variable light was increased (P = 0.005) compared to the constant light grown cells (Fig. 5D).

At the end of the photoperiod (19:00), no significant differences in $rETR_{max}$ (Fig. 5A), α (Fig. 5B), nor $I_k$ (Fig. 5C) were detected between the constant and variable light regimes in both LC and HC conditions. There was also no significant difference in $rETR_{max}$, α, $I_k$ and $\Phi_{PSII}$ between LC and HC treatments under constant or variable light regimes (Fig. 5). The $\Phi_{PSII}$ of LC and HC in variable light were higher than the cells grown in constant light (Fig. 5D). Two-factorial repeated measures analyses of variance (RM-ANOVA) indicated that light regime and $CO_2$ concentration individually and/or synergistically with time of photoperiod affected the fluorescence based photosynthetic performances (detailed in Table 3).

### 3.4. POC, PON, POC production, PON production and carbon fixation rate

There was no significant difference in POC per cell between LC and HC under either constant or variable light (Fig. 6A). There was also no significant difference in POC quota between constant and variable light in the LC condition, however, under the HC treatment, variable light increased the POC relative to the constant light treatment (Fig. 6A). Two-way ANOVA indicated a significant effect of light regime on cellular POC content (Table 2).

There was no significant difference in PON per cell between LC and HC in the constant light regime (Fig. 6B). However, the HC treatment increased PON in the variable light regime ((Fig. 6B). Compared to the constant light regime, cells grown under variable light showed increased PON under both LC and HC (Fig. 6B). Both light regime and $CO_2$ concentration significantly affect the cellular PON content (Table 2).

In the variable light regime, daily POC production in the HC grown cells increased compared to the LC condition (Fig. 6C). In the LC condition, the daily POC production of variable light grown cells decreased compared to the constant light regime, however, there was no significant difference in daily POC production between constant and variable light regimes in the HC condition (Fig. 6C). Light regime and $CO_2$ concentration individually and interactively affect the daily POC production (all P < 0.05) (Table 2).

In the variable light regime, cells grown under HC showed an

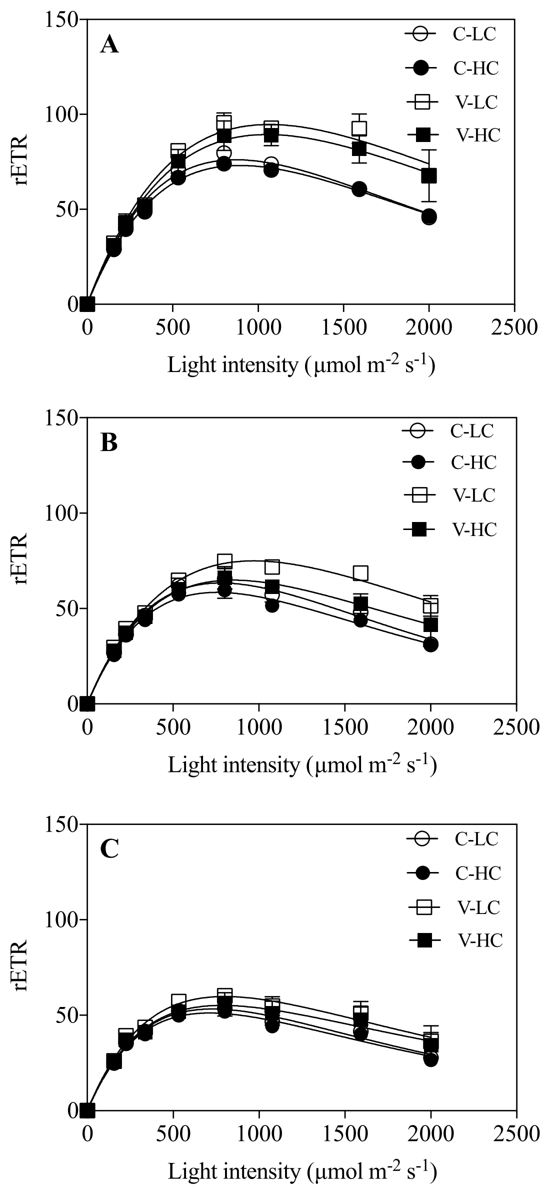


**Fig. 4.** Rapid light curves of *T. pseudonana* cultured in constant light under low $CO_2$ (C-LC), constant light under high $CO_2$ (C-HC), variable light under low $CO_2$ (V-LC), variable light under high $CO_2$ (V-HC) measured at mid-photoperiod at 14:00 (A), late-photoperiod at 17:00 (B), and the end of the photoperiod at 19:00 (C) (n = 3).

increase in daily PON production compared to the LC treatment (Fig. 6D). There was no significant difference in daily PON production between the two light regimes in the LC treatment; however daily PON production was increased in variable light under the HC condition ($P = 0.003$) (Fig. 6D). Light regime and $CO_2$ concentration individually and interactively affected daily PON production (Table 2).

Under constant light, the C/N decreased in HC grown cells, however, no difference was found in the variable light regime under LC nor HC treatments (Fig. 6E). Compared to the constant light regime, the C/N of cells grown under the variable light regime was decreased in both LC and HC (Fig. 6E). Both light regime and $CO_2$ concentration significantly affect the C/N (Table 2).

The carbon fixation rate increased from the start of the photoperiod (09:00) to the mid photoperiod (14:00) in both light regimes and $CO_2$ treatments, but then decreased in the variable light regime later in the photoperiod (Fig. 6F). Cells grown in variable light always showed a lower carbon fixation rate in both LC and HC compared to those grown in constant light. Two-factorial repeated measures analyses of variance (RM-ANOVA) indicated that light regime, time during photoperiod, and $CO_2$ concentration significantly affect carbon fixation rate (Table 3). There was also an interactive effect of time during photoperiod with both light regime and $CO_2$ concentration (Table 3). The calculated POC, PON, POC production, PON production and carbon fixation rate unit in a cell volume base ($\mu m^{-3}$) (Fig. S2) showed similar trends as unit in per cell base (Fig. 6).

## 4. Discussion

We found that OA and variable light interacted to alter C & N stoichiometry and diurnal photosynthesis in the model diatom species *Thalassiosira pseudonana*. Our variable light regime significantly decreased growth and pigmentation, but enhanced tolerance of cells to high light through increased $rETR_{max}$ and $I_k$, though this increase under variable light was moderated under the HC condition. Light variation interacted with OA to decreased carbon fixation rate, but promoted the synthesis of particulate organic nitrogen (PON), and enhanced daily PON production. Increased PON synthesis and thus decreased C/N may improve the diatom cell quality as a food source for high level predators, though at the expense of decreased growth and production. Interactive effects of OA and variable light on the particulate organic matters of diatom also may have significant implications on the biogeochemical cycles of C and N through vertical export.

Effects of OA on marine phytoplankton under a constant light regime have been shown in numerous studies (Kranz et al., 2010; Wu et al., 2010; Passow and Laws, 2015; Shi et al., 2015; Valenzuela et al., 2018). Under fluctuating sunlight, OA enhances growth under low but inhibits it under high levels of daily mean PAR (Gao et al., 2012). While these interactive effects of OA and light regimes or intensities on phytoplankton are modulated by nutrient availability and other drivers (see the review by Gao et al. (2019) and literature therein), the effect of variable light on growth rate can be species-specific and is strongly related to both the average irradiance level and variation period (Litchman, 2000; Zheng et al., 2015). Variable light decreased the growth rate of both LC and HC grown cells in the present study, consistent with earlier studies (Marshall et al., 2000; Van de Poll et al., 2007; Boelen et al., 2011; Hoppe et al., 2015). This may reflect lowered efficiency of energy transfer from biochemistry to biomass production (Hoppe et al., 2015), as dynamic light can result in an increased metabolic cost from photo-protection and/or acclimation and increased PSII protein turnover rate (Behrenfeld et al., 1998; Marshall et al., 2000; Hoppe et al., 2015).

The positive and negative effects of OA interacting with variable light can depend upon the balance between energy savings through down-regulated CCMs versus energy costs associated with acidic stress and variable light levels (Giordano et al., 2005; Gao et al., 2007, 2019; Li et al., 2019). Numerous studies have shown that the OA effects can be modulated by light quality and quantity (Ihnken et al., 2011; Gao et al., 2012; Jin et al., 2017; Li et al., 2017; Wu et al., 2017) or changing light regimes during mixing (Jin et al., 2013a). Low light limits energy supply, and therefore, energy saved from downregulated CCMs can favor the growth and metabolic activities (Gao et al., 2012; Li et al., 2017). In contrast, high or excessive light levels may induce photo-inhibition as a result of photo damage on the key photosynthetic apparatus, especially the D1 protein (Jansen et al., 1999; Crawfurd et al., 2011). The counteracting repair processes are costly, therefore, the energy saved from downregulation of CCMs can actually exacerbate the photoinhibition since CCM paths may dissipate excess energy (see the review by Gao et al. (2019) and literature therein). In addition, disturbance to intracellular acid-base balance under OA may induce an increased energy cost through increased respiration (Raven and Crawfurd, 2012), which was observed in diatoms and phytoplankton assemblages (Wu et al., 2010; Jin et al., 2015). In the present study, OA did not show significant effects on the growth rate under constant and variable light regimes with

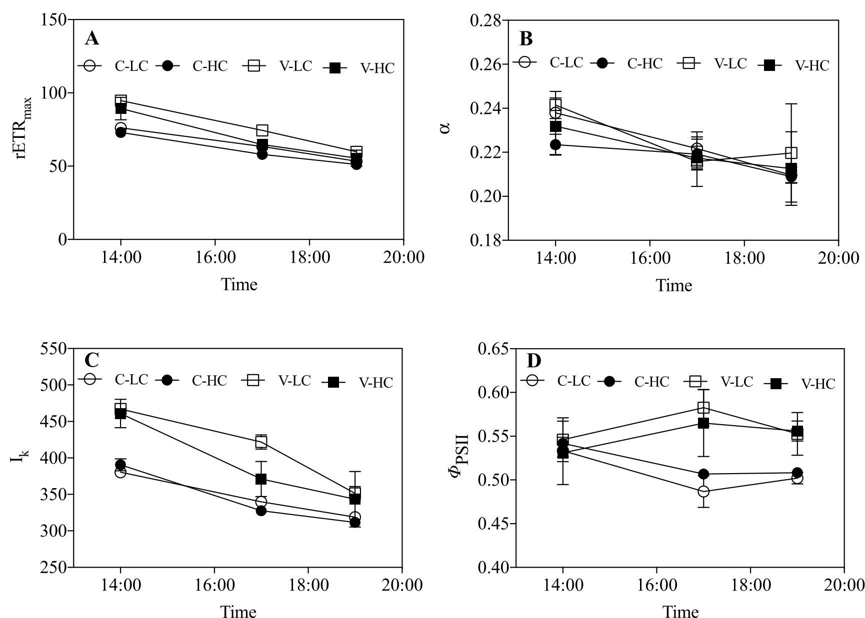


**Fig. 5.** The fitted rETR$_{max}$ (A), α (B) and $I_k$ (C) from the rapid light curves of Fig. 4, and the effective quantum yield ($\Phi_{PSII}$) at the culture light level (D) of *T. pseudonana* cultured in constant light under low $CO_2$ (C-LC), constant light under high $CO_2$ (C-HC), variable light under low $CO_2$ (V-LC), variable light under high $CO_2$ (V-HC) measured at mid-photoperiod at 14:00 late-photoperiod at 17:00, and the end of the photoperiod at 19:00 (n = 3).

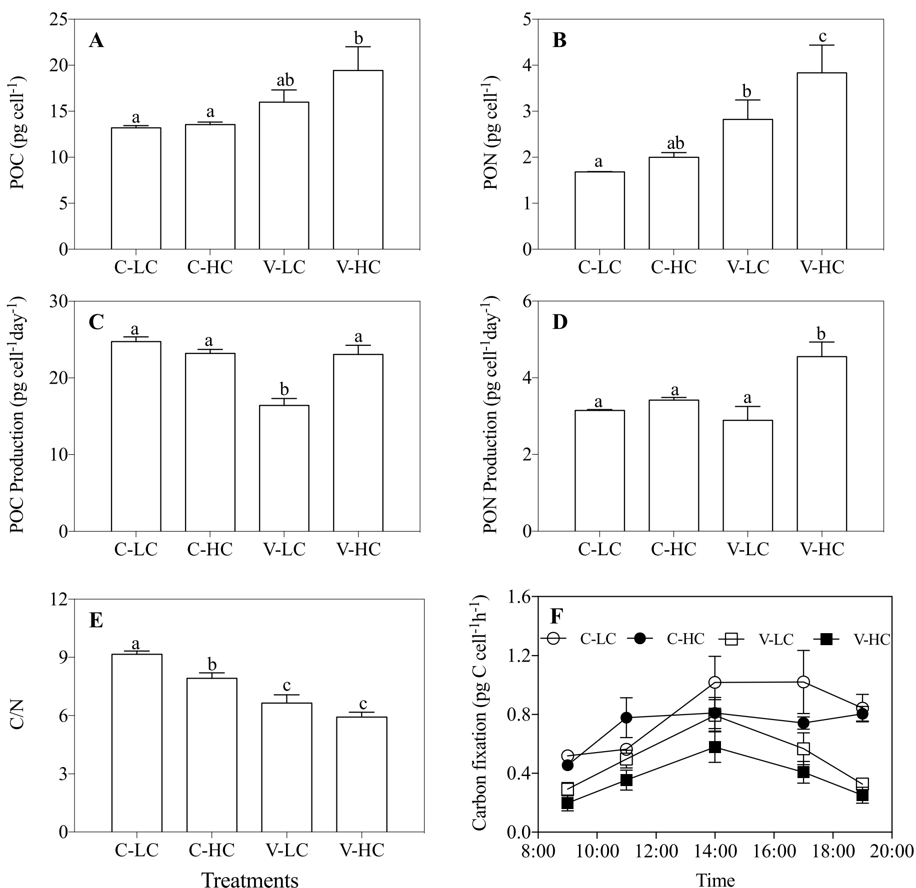


**Fig. 6.** Particulate organic carbon (POC, per cell) (A), particulate organic nitrogen (PON, per cell) (B), POC production (C), PON production (D), C/N (E) and carbon fixation rate (F) of *T. pseudonana* cultured in constant light under low $CO_2$ (C-LC), constant light under high $CO_2$ (C-HC), variable light under low $CO_2$ (V-LC), variable light under high $CO_2$ (V-HC). Cells grown under different light regimes and $CO_2$ concentrations were collected at the end of photoperiod for POC and PON measurements. (n = 3).

same daily dose of 2.02 MJ $m^{-2}$ (9.288 × $10^6$ μmol $m^{-2}$) and the same daytime average light intensity of 215 μmol $m^{-2}s^{-1}$. This is consistent with the results reported in an Antarctic diatom at an average light intensity of 90 μmol $m^{-2}s^{-1}$ (Hoppe et al., 2015). The light intensity in the present work may not be sufficiently high to induce photoinhibition in *T. pseudonana* (Li and Campbell, 2013); however, energy expenditure used in acid-base balance modulation may be increased under OA conditions, as increased carbon loss due to enhanced respiration and/or

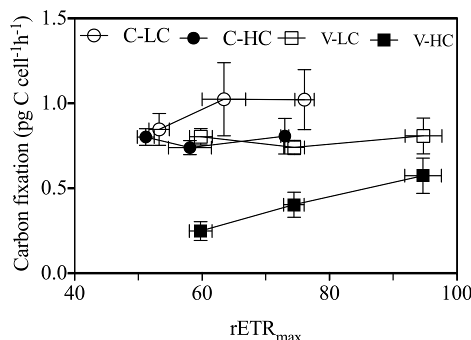


**Fig. 7.** Carbon fixation rate as a function of $rETR_{max}$ of *T. pseudonana* cultured in constant light under low $CO_2$ (C-LC), constant light under high $CO_2$ (C-HC), variable light under low $CO_2$ (V-LC), variable light under high $CO_2$ (V-HC) (n = 3). The carbon fixation rates and $rETR_{max}$ were taken from mid-photoperiod at 14:00, late-photoperiod at 17:00, and the end of the photoperiod at 19:00 from Figs. 5 and 6.

photorespiration may lead to less or no stimulation of growth under OA (Wu et al., 2010; Gao et al., 2012; Li et al., 2012, 2017).

High $CO_2$ did not affect the contents of Chl *a*, Chl *c* and carotenoid, in line with former studies (Wu et al., 2010; Li et al., 2012; Hennon et al., 2017). The variable light regime decreased pigmentation, perhaps because the exposure to high light (up to 360 μmol $m^{-2}s^{-1}$) during the photoperiod (Fig. 3A–C) led to downregulation of antenna pigments, a strategy for cells to acclimate to high light (Gordillo et al., 2003). The unchanged ratio of Carotenoid to Chl *a* (Fig. 3D) and the increased ability to tolerate high light (higher $I_k$) and higher $rETR_{max}$ combine to show significant photosynthetic acclimation under variable light such that changes in electron transport preempt any increase in photoprotective carotenoids relative to light capturing chlorophylls. The operation of both electron transport and light protection under dynamic light may benefit from other electron sinks such as the water-water cycle, the photorespiratory pathway or cyclic electron transport (Asada, 1999; Ort and Baker, 2002; Wagner et al., 2006). Consistent with an increased diversion of electrons, variable light significantly decreased the overall carbon fixation in both LC and HC conditions, which indicates that under dynamic light, the coupling from photo-chemistry to net biomass production decreased (Wagner et al., 2006; Ihnken et al., 2011; Jin et al., 2013a). Lowered carbon fixation rate with similar or even higher $rETR_{max}$ of HC and variable light grown cells indicate a lower conversion efficiency of electron transport rate compared with other treatments (Fig. 7). Maximization of $rETR_{max}$, $I_k$, α and carbon fixation in the mid-photoperiod implies that the effects of variable light on photosynthetic performances are dependent upon time of day.

Elemental stoichiometry is critical ecologically in the marine food web through predator-prey interactions and thereby influences marine biogeochemistry. The Redfield ratio of C/N has significant implications for carbon and nitrogen exports in the water column. POC and PON contents in phytoplankton vary widely under environmental changes relevant to climate change (Finkel et al., 2010; Van de Waal et al., 2010; Li et al., 2012). Increased (Riebesell et al., 2007; Bellerby et al., 2008) or un-changed C/N (Li et al., 2012) have been documented under OA, which will interact with expected nutrient limitations upon the surface ocean as a result of stratification under global warming (Van de Waal et al., 2010). Therefore, the C/N ratio of phytoplankton is generally expected to be enhanced in the future open ocean. However, this may not be a universal pattern as co-varying drivers change spatially and temporally (Finkel et al., 2010). In the present study variable light increased the POC and PON quota in the diatom, while under HC the PON production increased even though the growth rate was lowered (Figs. 1 and 6). The variable light regime decreased the POC production due to a lower growth rate but OA counteracted this decrease in POC production. Enhanced daily PON production and decreased C/N under OA with variable light may provide higher-quality food on a cell basis, as lower C/N normally indicates better nutritional value (Giani, 1991; Iglesias-Rodriguez et al., 2008; Li et al., 2012; Mei et al., 2005). The decreased C/N under variable light is, however, at the expense of lowered production (growth) (Fig. 2A). In addition, an increase of PON production per biomass may have significant implications on the vertical export of POC and PON, especially when considering trophic grazing-selection pressure on differentially nutritious food supplies. It is also worth noting that the wide distribution of *T. pseudonana* spans from pelagic to coastal waters (Alverson et al., 2011). The present results were acquired based on nutrient replete cultures, and response patterns may be altered in different habitats with different nutrient availabilities (Van de Waal et al., 2010).

## 5. Conclusion

The effects of OA are different under variable light than under constant light conditions. Ocean acidification interacts with variable light to affect the photo-physiological performance and ultimately decrease primary production of the model diatom *T. pseudonana*. While effects of OA are most likely modulated by co-varied drivers including temperature, nutrients, solar UV radiation and deoxygenation (see the review by Gao et al. (2019), our finding that interaction of variable light and OA enhance particular organic nitrogen quota and its production rate improves our understanding of diatom responses to the multiple drivers of ocean climate change.

## Declaration of competing interest

The authors declare that they have no conflicts of interest.

## CRediT authorship contribution statement

**Wei Li:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Tifeng Wang:** Formal analysis, Writing - original draft, Methodology, Investigation. **Douglas A. Campbell:** Formal analysis, Writing - original draft, Writing - review & editing. **Kunshan Gao:** Formal analysis, Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2020.104965.

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