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LETTER

Divergent responses of the diatom *Thalassiosira weissflogii* to ocean acidification during light and dark periods

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Scientific Significance Statement

Progressive ocean acidification (OA) due to continuous dissolution of anthropogenic CO_2 into seawater is known to affect diatoms that contribute to approximately 20% of the Earth's primary production. However, impacts of OA on diatoms through a daily cycle remain poorly understood. Our data provide compelling evidence from both physiological and molecular aspects that OA enhances growth of a diatom during the light period by upregulating its photosynthetic CO_2 fixation against the stress of decreased pH, but decreases its apparent specific growth rate during the night period due to the aggravated stress of pH drop from respiratory CO_2 release overlaid with OA. These findings align well with transcriptional imprints, suggesting the essential role of light in modulating the effects of OA on diatoms, with implications for possible seasonal and latitudinal effects of OA given the changing lengths of daytime.

Abstract

Given the limited understanding of discrepancies in responses of diatoms to ocean acidification (OA), we comparatively investigated the physiological and transcriptional performances of a diatom *Thalassiosira weissflogii* acclimated to OA (pH_t drop of 0.35–0.41) between day and night periods. We found that OA enhanced its specific growth rate (up to 10%) in the light period by upregulating light reaction, Calvin cycle and H^+ pumps to cope with the decreased pH. On the other hand, OA reduced its apparent specific growth rate (14%) in the dark period due to additive pH drop caused by OA-enhanced respiratory CO_2 release. In the dark period, the cells could not effectively cope with the decreased pH since H^+ pumps were downregulated. Consequently, OA did not affect cell growth during a 24 h diel cycle. These findings suggest that daytime positive and night negative effects of OA on diatoms could be responsible for differential results observed under different conditions, with implications for possible seasonal and latitudinal effects of OA.

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Dissolution of anthropogenic CO_2 into seawater has caused ocean acidification (OA), which is projected to lower global surface seawater pH by 0.3–0.4 units by the end of this century (IPCC 2021). Ocean acidification has been shown to influence the development, metabolism, and biomass production of many organisms, potentially triggering trophic cascades that affect the productivity and service functions of marine ecosystems (Jin et al. 2015; Sswat et al. 2018; Nissen et al. 2024).

Diatoms, a crucial group within phytoplankton, are widely distributed across coastal and open oceans, from equatorial to polar waters, contributing about 40% to marine primary productivity (Tréguer et al. 2017). OA can enhance the photosynthetic carbon fixation and growth of many diatoms, primarily due to the insufficient amount of CO_2 in seawater relative to its high demand as the substrate for the carboxylation enzyme ribulose-1,5-bisphosphate carboxylase (Rubisco) (Gao et al. 2020). On the other hand, increased CO_2 levels did not significantly affect the photosynthetic carbon fixation of the diatom *Skeletonema costatum* (Chen and Gao 2003) or *Thalassiosira weissflogii* (Goldman et al. 2017), possibly due to their inorganic carbon absorption already being saturated at current CO_2 concentrations. Furthermore, it has been documented that OA impairs algal photosynthesis, potentially as a result of reduced pH (Shi et al. 2019; Gao et al. 2022). Therefore, the impact of OA on phytoplankton largely depends on the balance between the positive effects of increased CO_2 and the negative effects of decreased pH.

Previous studies on the impact of OA on phytoplankton growth have focused on the overall diel responses, without distinguishing the divergent effects of OA between the day and night periods (Gao et al. 2020; Jian et al. 2025). In fact, phytoplankton cells in most regions experience significant diel cycles. Therefore, examining the effects of OA during day and night periods can provide deeper insights into the nuanced effects of OA. Such insights are critical for understanding how micro-level responses to OA translate into broader ecological impacts. Recently, we found that OA promotes the growth of *Phaeodactylum tricornutum* during the day but inhibits it at night (Qu et al. 2021a). However, it remains uncertain whether this phenomenon occurs in other diatom species and what mechanisms underlie these responses. Subsequently, we hypothesize that, during daytime, photosynthetic CO_2 removal (increasing pH of the milieu) acts against the decreased pH associated with OA, thus leading to positive effects of OA; however, during nighttime, the pH drop due to the acidification overlaid with respiratory CO_2 release amplifies the acidification stress, which can disturb intracellular acid–base balance, thereby influencing a range of metabolic activities (Sharbaugh and Laws 2025). *Thalassiosira* is one of the most abundant diatom groups in the oceans based on the data from Tara Oceans expedition (Malviya et al. 2016). Consequently, we chose *Thalassiosira weissflogii* to test our scientific hypothesis proposed above.

Materials and methods

Experimental design

The diatom *Thalassiosira weissflogii* (CCMP1336) was obtained from the Center for Collection of Marine Bacteria and Phytoplankton of Xiamen University. Before the experiment, diatom cells were dark-cultured for 36 h to synchronize cell division (Qu et al. 2021b). Then the algal cells were cultured directly under two CO_2 concentrations: the ambient atmospheric CO_2 concentration (410 ppmv, AC) and the predicted CO_2 concentration by the end of this century (1000 ppmv, HC) for 15-generation acclimation (~14 d) (Supporting Information Fig. S1). The AC and HC treatments were achieved by aerating ambient outdoor air or a mixture of pure CO_2 with ambient air supplied by a CO_2 enricher (HP100G-D, Ruihua). Each treatment included three independent replicates. To preserve the stability of the seawater carbonate system, the initial cell concentration was set 5×10^3 cells mL^{-1} and the medium was diluted every 24 h to the initial cell concentration. The algal cells were cultured in polycarbonate (PC) bottles at a temperature of 20°C, with a light intensity of 220 μmol photons $m^{-2}\,s^{-1}$, and a light: dark cycle of 12 h : 12 h. The culture medium consisted of filtered (0.45 μm) and sterilized natural seawater, with nutrients enriched according to Aquil medium (Morel et al. 1979). After being acclimated to the CO_2 treatments under the light–dark cycles for 15 generations that follow *Guide to Best Practices in Ocean Acidification Research and Data Reporting* (Riebesell et al. 2011), *T. weissflogii* cells were sampled before the end of the light period (19:30–20:00 h) and the dark period (07:30–08:00 h) to assess physiological and molecular responses. The samples under AC were set as control. No additional "pre-exposure" or "time-zero" control was included, as this study was not designed to examine the effects of culture duration.

To test whether the diel effects of OA persist across irradiance levels, we ran a follow-up experiment. *T. weissflogii* was grown at 35, 70, and 140 μmol photons $m^{-2}\,s^{-1}$ under the same AC and HC conditions described above (Supporting Information Fig. S1). Physiological traits were assessed after cultures had acclimated for at least 15 generations. The light intensity of 220 μmol photons $m^{-2}\,s^{-1}$ used in the main experiment is growth-saturating for *T. weissflogii*, whereas the lower intensities (35, 70, and 140 μmol photons $m^{-2}\,s^{-1}$) are growth-limiting (Qu et al. 2021b). These irradiance levels span a broad range from the light compensation point to the saturation point for this species, representing different light environments encountered across ocean depths where diatoms naturally occur (Gao et al. 2012).

Determination of carbonate system parameters

To determine the carbonate system parameters in the cultures, pH was measured before and after the medium dilution

using a pH meter calibrated with NBS buffer solution. Total alkalinity (TA) was determined by titration (Lewis et al. 1998). Other parameters of the carbonate system were derived using the CO2SYS software based on pH and TA. The measured pH_{NBS} was converted to pH_t with CO2SYS for inter-study comparability.

Measurement of specific growth rate during light and dark periods

The diatom cell numbers were measured using a Counter Z2 Particle Count and Size Analyzer (Beckman Coulter). The specific growth rates (μ) were calculated using the following equation: $\mu = (\ln N_{t2} - \ln N_{t1})/(t_2 - t_1)$, where N_{t2} and N_{t1} represent cell concentrations at times $t2$ and $t1$, respectively, during the light or dark period. Cell numbers were measured 30 min before the onset of the dark (19:30–20:00 h) or light (07:30–08:00 h) periods. Since growth of phytoplankton during night may be negative in terms of biomass loss due to respiration, we expressed it as apparent specific growth rate (μ').

Measurement of photosynthetic oxygen evolution and mitochondrial respiration

Net photosynthetic oxygen evolution and respiratory rates were measured using a Clark-type oxygen electrode (Hansatech). The rates for daytime were measured during the mid-light period, while those for night were measured before the end of dark period (prior to the onset of light at 08:00 h). The diatom cells were collected gently (pump pressures < 0.02 MPa) on PC membranes and resuspended in the fresh AC or HC media before the measurements using a Clark-type electrode (Chlorolab 2, Hansatech) in a reaction chamber placed in a water bath at 20°C. The dark condition was achieved by covering the reaction chamber with a black box. Each measurement lasted for about 5 min.

RNA-seq experimental procedure

Five hundred milliliters of algal culture was filtered onto a 2 μm pore-size PC membrane (Millipore, 47 mm). The membrane with collected cells was immediately transferred to a 2-mL centrifuge tube, and quickly snap-frozen in liquid nitrogen for 5 min before being stored in a −80°C freezer until RNA exaction. Samples underwent RNA extraction, purification, library construction, and were then sequenced using second-generation sequencing (next-generation sequencing) on the Illumina HiSeq platform, with paired-end (Paired, PE) sequencing of these libraries (Jiang et al. 2024).

De novo transcriptome analysis

Raw sequencing data were filtered to remove reads with adapters, lengths less than 50 bp, and average sequence quality below Q20. The resulting high-quality sequences were assembled de novo to obtain transcript sequences, which were then clustered to select the longest transcripts as Unigenes. These Unigenes were then used for subsequent GO, KEGG, eggNOG, and other annotations. The filtered sequences were aligned to Unigene set to determine the read counts for each Unigene, providing a foundation for further expression difference analysis and enrichment analysis. The absolute value of $\log_2$ fold change ≥ 1 and false discovery rate ≤ 0.001 were used to assess the significance of the differential expression genes (DEGs) across various groups (Jiang et al. 2024). Further cluster analysis, functional enrichment analysis and KEGG pathway analysis were carried out with the "phyper" package in R software.

Statistical analysis

Results in this study were expressed as means of three biologically independent replicates ± standard deviation. All data were analyzed by the software SPSS v.26. Data from every treatment was confirmed to a normal distribution (Shapiro–Wilk, $p > 0.05$), and the variances were equal (Levene's test, $p > 0.05$). Two-way ANOVA was conducted to assess the effects of CO_2 and day/night periods on specific growth rate, respiration rate, photosynthetic rate, seawater pH and CO_2 concentration. Least significant difference was conducted for post hoc investigation. A confidence interval of 95% was set for all tests. Effect sizes were evaluated using partial eta squared (η_p^2). The thresholds for small, medium, and large effect sizes in partial eta squared are 0.01, 0.06, and 0.14, respectively (Richardson 2011). Pearson correlation of multiple variables was conducted to test the reliability of three biological replicates in gene expression (Supporting Information Fig. S2).

Results

Thalassiosira weissflogii could grow in both light and dark periods in terms of increase of cell numbers (Fig. 1). CO_2 interacted with the diel cycle on its specific growth rate ($F_{(1,8)} = 23.051$, $p = 0.001$,$\eta_p^2 = 0.742$). The HC enhanced the specific growth rate by 10% in the light period but reduced it during the night period by 14% compared to AC. Therefore, the gross effect of HC on the specific growth rate during a 24 h diel cycle was neutral (Fig. 1, inset).

Both CO_2 ($F_{(1,8)} = 18.141$, $p = 0.003$, $\eta_p^2 = 0.694$) and day vs. night periods ($F_{(1,8)} = 90.397$, $p < 0.001$, $\eta_p^2 = 0.919$) affected the respiration rates of *T. weissflogii* (Fig. 2A). Compared to AC, HC increased the respiration rate by 10% in the light period and by 14% in the dark period in the cells grown under 220 μmol photons m^{-2} s^{-1}, indicating a stronger impact of HC in dark period. Respiration rates in light period were higher than those in dark period regardless of CO_2 concentration (Fig. 2). CO_2 ($F_{(1,8)} = 20.583$, $p = 0.002$, $\eta_p^2 = 0.720$) and day vs. night periods ($F_{(1,8)} = 24.499$, $p = 0.001$, $\eta_p^2 = 0.754$) also imposed significant effects on the photosynthetic rates of *T. weissflogii* (Fig. 2B). The HC enhanced both gross and net photosynthetic rates by a similar extent (~10%).

In terms of transcriptomic response, in the light period, HC led to 4074 upregulated DEGs and 2871 downregulated DEGs compared to AC (Fig. 3). In contrast, HC resulted in

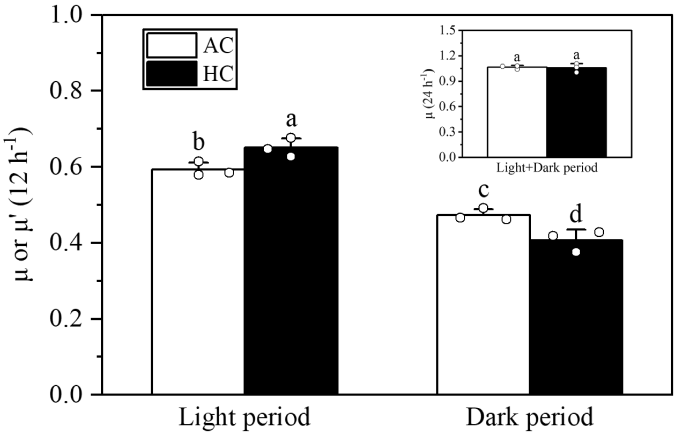

Fig. 1. Daytime specific growth rate (μ) and night apparent specific growth rate (μ') of *Thalassiosira weissflogii* grown under ambient (AC) and high CO_2 (HC) levels. Inset: specific growth rate expressed as per day over the 24 h diel cycle. The error bars indicate the standard deviations ($n = 3$). The white dots stand for the values of three biological repetitions. Different letters indicate that there is a significant difference among treatment groups ($p < 0.05$).

similar numbers of upregulated DEGs (1908) and downregulated DEGs (1907) in the dark period. These results suggest different transcriptional response patterns of *T. weissflogii* to HC when grown in the light and dark periods. Compared to the effect of high CO_2, light had a much larger effect on gene expression of *T. weissflogii*, resulting in 20,470 upregulated DEGs and 13,390 downregulated DEGs under AC and 21,042 upregulated DEGs and 10,239 downregulated DEGs under HC. When considering the combined effects of CO_2 and light, the number of DEGs was lower than the sum of those induced by CO_2 or light alone and even fewer than those caused by light individually, suggesting an interactive effect of CO_2 and light on the gene expression of *T. weissflogii*.

In the light period, HC downregulated the expression of genes related to CCMs (*CA* and *PEPCK*) by 1.45–1.67 $\log_2$ folds (Fig. 4). On the other hand, HC significantly upregulated the expression of genes related to light harvesting (*LhcA1*) and Calvin cycle (*PGK*) by 1.15 $\log_2$ folds and 2.46 $\log_2$ folds, respectively. Meanwhile, HC significantly upregulated genes related to fatty acid degradation (*ACSL* and *HADH*), glycolysis (*ALDO*, *PGK*, *PGAM*, *ENO*, and *PK*) and TCA cycle (*DLAT*, *CS*, *DLD*, *FUM*, and *MDH*) by 1.25–1.60 $\log_2$ folds, 1.10–3.91 $\log_2$ folds and 1.23–6.45 $\log_2$ folds, respectively, indicating increased energy supply that can support rapid growth. Meanwhile, HC also upregulated genes related to cell membrane proton pump (*PMA*) and V-type proton pump (*Subunit C*, *Subunit F*, and *Proteolipid subunit*) by 1.24 $\log_2$ folds and 1.24–1.91 $\log_2$ folds, respectively.

In the dark period, HC did not significantly affect the expression of genes related to CCMs (*CA*, *PEPC*, and *PEPCK*) while significantly downregulated genes related to Calvin cycle (*Rubisco* and *TKT*) by 1.23 $\log_2$ folds (Fig. 5). The HC downregulated genes related to fatty acid degradation (*ACSL*, *ACADM*, and *ECH*), glycolysis (*PGAM*) and TCA cycle (*DLD*, *OGDH*, and *MDH*) by 1.10–1.80 $\log_2$ folds, 1.39 $\log_2$ folds and 1.09–1.46 $\log_2$ folds, respectively, but upregulated genes related to Cytochrome c oxidase (*COX1*, *COX2*, *COX3*, *COX11*, and *COX15*) by 1.20–4.11 $\log_2$ folds. The HC also upregulated genes related to plasma membrane H^+-ATPase (*PMA*) by 1.04 $\log_2$ folds but did not significantly affect genes related to V-type proton pump. These results demonstrate that the transcriptomic responses of *T. weissflogii* to OA were notably distinct between the light and dark periods: OA promoted the expression of genes associated with Calvin cycle, fatty acid degradation, glycolysis, and TCA cycle in the light period, whereas it suppressed these pathways in the dark period.

The stimulative effect of OA during the daytime and its inhibitory effect at night at the light density of 220 μmol photons $m^{-2}\,s^{-1}$ was similarly observed in a supplementary

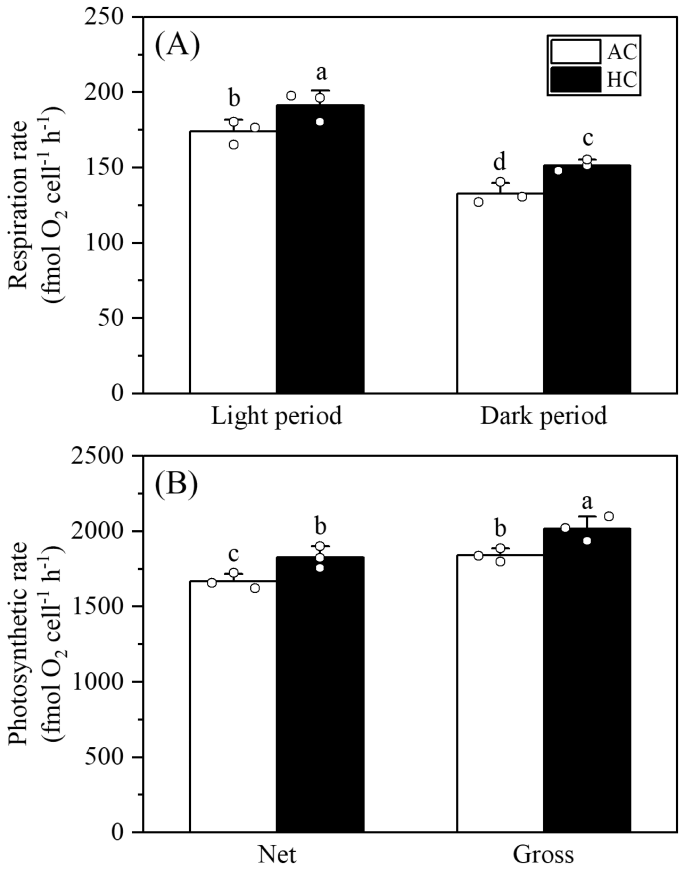

Fig. 2. Respiration (**A**) and photosynthetic (**B**) rates of *T. weissflogii* grown under ambient (AC) and high CO_2 (HC) conditions. Net and Gross represent net and gross photosynthetic rates, respectively. The error bars indicate the standard deviations ($n = 3$). The white dots stand for the values of three biological repetitions. Different letters above the columns indicate significant difference among treatment groups ($p < 0.05$).

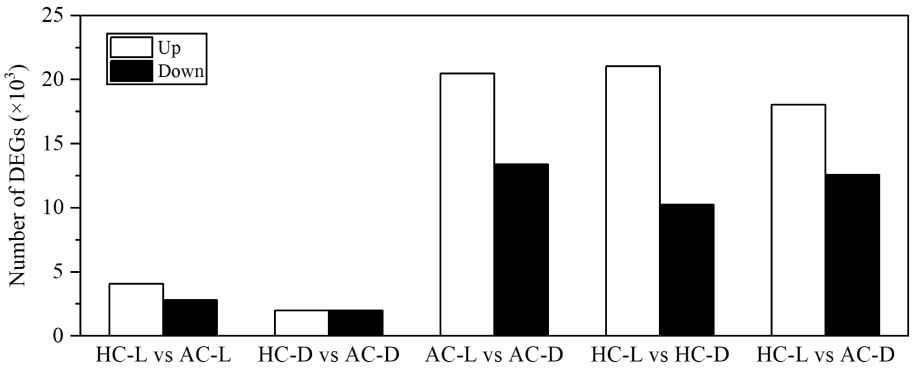

Fig. 3. Number of DEGs in *T. weissflogii* grown under ambient (AC) and high CO_2 (HC) conditions in light (L) and dark (D) periods. The values are means of three biological replicates.

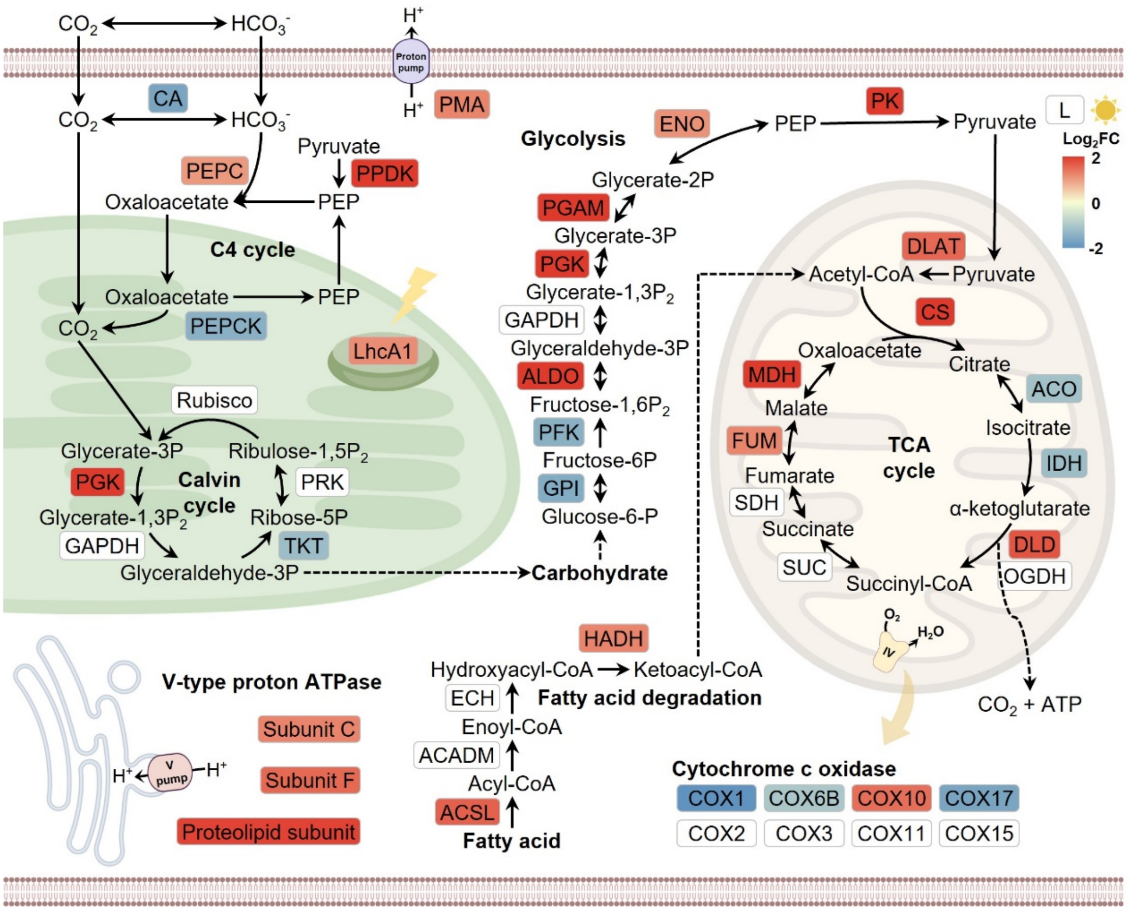

Fig. 4. Transcriptomic responses of *T. weissflogii* to ocean acidification in the light (L) period. The red and blue colors in a certain symbol represent significantly up and downregulated genes, respectively ($p < 0.05$ and $|\log_2 FC| > 1$). Solid lines indicate direct reactions, while dashed lines represent multi-step reactions. Full names of differentially expressed genes can be found in Supporting Information Tables S1 and S2.

experiment in which *T. weissflogii* was cultured under three different light intensities of 35, 70, and 140 μmol photons $m^{-2}\,s^{-1}$ (Supporting Information Fig. S3A, B). When the opposing effects of HC during light and dark periods were integrated to obtain daily growth rates, HC treatment stimulated the specific growth rate at the light intensities of 35 and 70 μmol photons $m^{-2}\,s^{-1}$, but did not impose a significant effect at the light intensity of 140 μmol photons $m^{-2}\,s^{-1}$

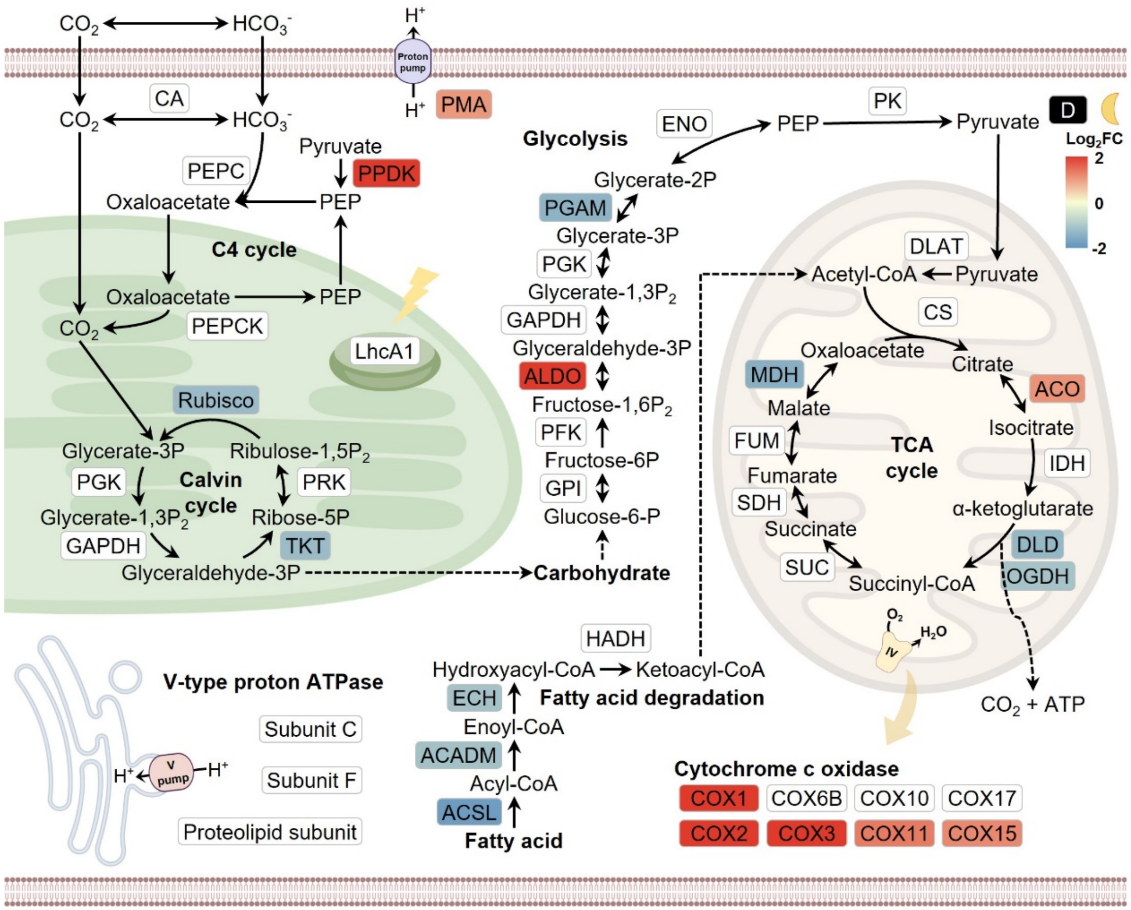

Fig. 5. Transcriptomic responses of *T. weissflogii* to ocean acidification in dark (D) period. The red and blue colors in a certain symbol represent significantly up and downregulated genes, respectively ($p < 0.05$ and $|\log_2 FC| > 1$). Solid lines indicate direct reactions, while dashed lines represent multistep reactions. Full names of differentially expressed genes can be found in Supporting Information Tables S1 and S2.

(Supporting Information Fig. S3C), which is similar to that of 220 μmol photons m^{-2} s^{-1} (Fig. 1). For the diatom cells grown under lower light intensities (35, 70, and 140 μmol photons m^{-2} s^{-1}), HC similarly increased respiration rates during both light and dark periods (Supporting Information Fig. S4A, B), relative to the high light intensity. The HC also stimulated net photosynthetic rates at the light intensities of 35 and 70 μmol photons m^{-2} s^{-1} but did not significantly affect it at 140 μmol photons m^{-2} s^{-1} PAR (Supporting Information Fig. S4C).

Discussion

Almost every organism on the planet experiences a diel cycle due to the Earth's rotation. Numerous algal metabolic activities have been shown to exhibit periodical oscillation in response to the 24 h light and dark cycles. In terms of cell division, some species have the peaks of increased cell numbers in the dark period while some species have that during the light period; still, there are species that maintain nearly consistent division rates throughout the light and dark cycle (Nelson and Brand 1979; Qu et al. 2021a). While phytoplankton lose biomass during night, most of them increase their cell numbers (per volume of water) due to cell division, exhibiting apparent growth. Our results demonstrated that the diatom *T. weissflogii* grows faster under the simulated OA conditions during daytime but slower during night in terms of increased cell numbers in contrast to the control (AC); such day vs. night inverse responses to OA are supported by the expression levels of numerous genes related to photosynthesis, CCMs and other key metabolisms. Such divergent effects of OA under light vs. night periods could explain the neutral effects of OA on the growth of *T. weissflogii* in our previous work (Qu et al. 2021b). This finding may also be used to explain insignificant effects of OA on growth of other diatoms, whose responses to OA have not been distinguished between day and night (Gao and Campbell 2014; Ahmad et al. 2024). It can be expected that OA can exert more positive effects on diatoms in the areas or seasons with longer daytime.

The positive influence of OA on the growth of *T. weissflogii* in the light can likely be ascribed to the increased rate of

photosynthesis or CO_2 removal from the medium, mitigating the lowered pH effects induced by OA (Supporting Information Fig. S5). Based on the transcriptional data, the HC-downregulated genes of *CA* and *PEPCK* are associated with CCMs, indicating that OA modulates both biophysical and biochemical aspects of this mechanism. Previous studies show that *T. weissflogii* operates efficient CCMs including bicarbonate transporters and carbonic anhydrases (Roberts et al. 1997; Hopkinson et al. 2011). Elevated levels of CO_2 can usually downregulate algal and cyanobacterial CCMs since more CO_2 supply through passive diffusion signifies a diminished necessity for them to actively concentrate CO_2 (Hopkinson et al. 2011; Raven and Beardall 2014). On the other hand, HC-upregulated genes of *PGK* are related to Calvin cycle, indicating that the energy conserved from downregulated CCMs may be redirected to fuel the Calvin cycle, thereby accelerating CO_2 fixation and growth of diatoms (Hopkinson et al. 2011; Gao and Campbell 2014). Meanwhile, HC upregulated fatty acid degradation, glycolysis and TCA cycle, which can supply energy for other substance syntheses and metabolic activities, ultimately facilitating growth. OA usually has dual effects, generated by increased CO_2 and decreased pH, respectively (Supporting Information Fig. S5). While increased CO_2 availability commonly demonstrates positive effects on algal growth, decreased pH can lead to negative effects by disturbing the acid–base balance between extracellular and intracellular environments (Shi et al. 2019; Gao et al. 2022). Compared to the dark period, the stress resulting from decreased pH was less intense during the light period due to photosynthesis-driven CO_2 removal (H^+ decline). This mechanism is supported by our experimental measurements that demonstrate pH values in the light period were significantly higher than those in the dark period for both AC and HC conditions (Supporting Information Fig. S5). Furthermore, the plasma membrane H^+ pump was upregulated to maintain the cross-membrane H^+ gradient and normal metabolic activities under OA (Fig. 4). In parallel, V-type H^+ pump was also upregulated to maintain the pH neutrality of the cytoplasmic matrix and the pH acidity within organelles (Schumacher 2014). In short, the *T. weissflogii* cells that had been acclimated to OA coped efficiently with the decreased pH in light period by upregulating the genes involved in H^+ pumps as well as photosynthesis.

On the other hand, the stress of decreased pH, which was caused by OA and the respiration-induced pH drop in dark period, led to less cell division or slower apparent growth rate (Fig. 1 and Supporting Information Fig. S5). In addition, V-type H^+ pump was not upregulated by HC in dark period, and plasma membrane H^+ pump was not upregulated as highly as that in light period (Fig. 5). Consequently, the aggravated pH drop resulted in more energy expenditure and carbon loss, that must be responsible for the lower apparent specific growth rate (Fig. 1). On the other hand, the diatom's CCMs appeared not to be downregulated by HC in dark period, indicating that OA-modulated regulation of CCMs is light dependent. The mitochondrial respiration rates were lower in dark period compared to light period regardless of the pCO_2 treatments, aligning with previous study on *T. weissflogii* by Goldman et al. (2017). In the present study, OA enhanced mitochondrial respiration rates during both phases (Fig. 2A), which is consistent with previous findings about *Phaeodactylum tricornutum* (Qu et al. 2021a). Sharbaugh and Laws (2025) also found OA increased the mitochondrial respiration of *Synechococcus* sp. Collectively, these findings suggest that the diel effects of OA on phytoplankton may be conserved. It is likely that the absence of light energy supply at night along with respiratory carbon loss leads to shortage of energy demanded for the operation of the periplasmic H^+ pumps (Schumacher 2014). Therefore, it appears that *T. weissflogii* coped less efficiently with the decreased pH induced by OA in dark period (Supporting Information Fig. S5), leading to less energy supply for its cell division as reflected by the apparent specific growth rate (Fig. 1). Depending on whether the positive effects during the daytime outweigh the negative effects at nighttime, the overall impacts of OA on diatom growth can be promotional, inhibitory, or neutral. This mechanism can account partially for the divergent effects of OA on diatom growth reported in previous studies (Gao and Campbell 2014; Somma et al. 2025).

Our results imply that diatoms distributed at different latitudes or seasons (with different daytime lengths) may show positive, negative or neutral responses to OA, due to the divergent effects of OA during day and night. Seasonal and latitudinal variations in daylight duration may modulate the balance between OA's positive (light-period) and negative (dark-period) effects on diatom growth. In temperate and high-latitude regions, summer is characterized by extended photoperiods (e.g., 16–20 h at 50°N/S), amplifying the window for OA-enhanced photosynthesis and growth and potentially boosting diatom productivity. Conversely, winter in these regions features short photoperiods (e.g., 4–8 h) and prolonged darkness shifts the balance toward OA's dark-period inhibition, reducing apparent growth rates and possibly diminishing diatom standing stocks. Such seasonal shift could alter the timing and magnitude of diatom blooms, which are foundational to regional food webs and carbon cycles (Tréguer et al. 2017). Enhanced diatom growth during summer in high latitudes could increase carbon export to the deep ocean, while reduced winter growth may decrease off-season carbon cycling. In addition, combined with the results of the supplementary experiment OA promotes the growth of *T. weissflogii* under lower light intensities, but exerts a neutral effect under higher light intensities. This phenomenon could be attributed to the attenuated growth-promoting effect of OA during the light period under higher light conditions. Under high light conditions, the energy conserved via CCM downregulation by OA can promote photorespiration and photodamages to photosystems (Gao et al. 2012; Li and Campbell 2013), therefore, leading to less C assimilation. Consequently, it is likely that OA

may have no significant impact on diatom productivity in some regions of the surface oceans, but may exhibit a growth-promoting effect with increasing water depth. These shifts could have feedbacks on atmospheric CO_2 levels, though the magnitude of this feedback requires further modeling to integrate diel, seasonal, and latitudinal and water depth dynamics (Boyd and Van Mooy 2025).

Conclusions

The present study distinctly reveals the contrasting response of *T. weissflogii* to OA when grown in light and dark periods. OA can stimulate growth of *T. weissflogii* in light period but reduce cell growth in dark period, ultimately resulting in a seemingly neutral effect over a complete diel cycle. In light period, the cells downregulate CCMs and upregulate light reaction and Calvin cycle, which contributes to the increased growth. In addition, the H^+ pumps are also upregulated to deal with the pH disturbance caused by OA. In contrast, during the dark period, the H^+ pumps do not function as efficiently as they do in light period, and the Calvin cycle is downregulated, leading to the decreased growth. Our findings strongly indicate that light plays a pivotal role in enabling the ecologically important diatom *T. weissflogii* to respond to OA. For this specific phytoplankton species, individuals in summer with longer light periods may benefit from OA, while those in winter characterized by longer dark periods may experience more adverse effects of OA. Furthermore, the diel effects of OA on growth of *T. weissflogii* are robust across irradiance conditions. However, whether these findings can be extrapolated to other phytoplankton taxa necessitates further experimental verification in the future.

Author Contributions

Kunshan Gao conceived and designed the experiments. Liming Qu and Jingke Ge performed the experiments. Guang Gao, Liming Qu, and Jingke Ge analyzed the data. Guang Gao wrote the manuscript. Kunshan Gao revised the manuscript. All authors reviewed the manuscript.

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Conflicts of Interest

None declared.

Data Availability Statement

The physiological data presented here are publicly available from Mendeley Data repository at https://doi.org/10.17632/cjwppwd3m5.1, and the transcriptomic sequencing data are publicly available in the Genome Sequence Archive under accession numbers CRA031331 (accessible at https://ngdc.cncb.ac.cn/gsa).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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