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Ocean acidification and desalination increase the growth and photosynthesis of the diatom *Skeletonema costatum* isolated from the coastal water of the Yellow Sea

Ruijie Wu [a], Yuchen Wu [a], Rui Zhai [a], Kunshan Gao [b], Juntian Xu [a,c,*]

[a] Jiangsu Key Laboratory of Marine Bioresources and Environment/Jiangsu Key Laboratory of Marine Biotechnology, Jiangsu Ocean University, Lianyungang 222005, China
[b] State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China
[c] Key Laboratory of Coastal Salt Marsh Ecosystems and Resources, Ministry of Natural Resources, Jiangsu Ocean University, Lianyungang 222005, China

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ABSTRACT

Global climate changes induce substantial alterations in the marine system, including ocean acidification (OA), desalination and warming of surface seawater. Here, we examined the combined effects of OA and reduced salinity under different temperatures on the growth and photosynthesis of the diatom *Skeletonema costatum*. After having been acclimated to 2 CO_2 concentrations (400 μatm, 1000 μatm) and 2 salinity levels (20 psu, 30 psu) at temperature levels of 10 °C and 20 °C, the diatom showed enhanced growth rate at the lowered salinity and elevated pCO_2 irrespective of the temperature. The OA treatment increased the net photosynthetic rate and biogenic silica (Bsi) contents. Increasing the temperature from 10 to 20 °C raised the net photosynthetic rate by over twofold. The elevated pCO_2 increased the net and gross photosynthetic rates by 20%–40% and by 16%–32%, respectively, with the higher enhancement observed at the higher levels of salinity and temperature. Our results imply that OA and desalination along with warming to the levels tested can enhance *S. costatum*'s competitiveness in coastal phytoplankton communities under influence of future climate changes.

1. Introduction

Anthropogenic CO_2 emissions have been projected to increase atmospheric pCO_2 to about 1000 ppmv by the end of this century (Leung et al., 2022). The oceans are known to continuously take up CO_2, declining pH of seawater and leading to ocean acidification (OA) (Doney et al., 2009). CO_2 in the atmosphere combines with seawater to form H_2CO_3, which then dissociated into HCO_3^- and H^+ to reach equilibrium. However, increase in H^+ concentration leads to a reverse reaction and a decrease in CO_3^{2-} (Chen et al., 2023). Therefore, elevated CO_2 levels result in an increase in the concentrations of HCO_3^- and H^+ in seawater, while the concentration of CO_3^{2-} decreases.

At the same time, progressive global warming is altering the climate. This change also affects the water cycle, leading to modifications in extreme hydrological events and rainfall (Westra et al., 2014). For the oceans, there have been a series of changes in the seawater, including seawater warming, decreased salinity, and shallower mixing layers (Ummenhofer and Meehl, 2017). These changes have altered the amount of CO_2 dissolved in seawater. In coastal areas, the fluctuation in salinity during different seasons can rapidly affect CO_2 fluxes (Woolf et al., 2016), especially when rainfall leads to decreased salinity and increased CO_2 solubility. Therefore, studying the interaction between ocean acidification and various environmental factors is of ecological significance for understanding the impact on diatoms (Qi et al., 2017).

Salinity is a significant regulating factor in the vertical stratification of the ocean, influencing the vertical exchange of carbon and oxygen, among other substances (Du et al., 2019; Sambah et al., 2021). A decrease in salinity often occurs in the upper ocean, especially in nearshore estuarine regions (Liu et al., 2022). This change can alter the osmotic pressure of seawater and affect the osmotic equilibrium of plant cells and the ion flow in electrochemical gradients (Lobban and Harrison, 1994). As important contributors to primary productivity, diatoms respond to changes in salinity by undergoing a series of adaptations. For example, due to the influence of osmotic pressure, diatom cells become

* Corresponding author. Jiangsu Key Laboratory of Marine Bioresources and Environment/ Jiangsu Key Laboratory of Marine Biotechnology, Jiangsu Ocean University, Lianyungang 222005, China.
E-mail address: jtxu@jou.edu.cn (J. Xu).

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smaller under lowered salinity levels (Vidal et al., 2018; Xu et al., 2020).

Diatoms, as the dominant phytoplankton group, contribute about 40% primary production in the oceans, and are primarily dominant in coastal waters (Tréguer et al., 2018). Additionally, they play a crucial role in the marine silicon as well as C cycle. Numerous studies have reported that ocean acidification can either promote diatom growth to some extent (Bautista-Chamizo et al., 2018; Lohbeck et al., 2012; Qu et al., 2017; Wu et al., 2017) or inhibit their growth (Ihnken et al., 2011; Low-DÉCarie et al., 2011; Wu et al., 2010). However, the impact of OA on different species of diatoms varies (Mackey et al., 2015). For example, in communities containing *Skeletonema costatum* and *Nitzschia* spp., acidification only promotes the growth of *S. costatum* (Kim et al., 2006).

Numerous studies have shown that diatoms exhibit adaptive changes in response to various environmental factors such as different seasonal temperatures, ocean acidification, decreased salinity, and changes in light intensity, those studies indicate that growth rate increases with temperature before reaching the optimal growth temperature, but decreases with further temperature increase after reaching the optimal temperature (Thomas et al., 2012), and low salinity environment promotes the growth of phytoplankton (Rai and Rajashekhar, 2014). However, most documented studies have investigated the effects of changes in salinity or pCO_2 on diatoms at a fixed temperature. It is most likely that different levels of temperature can alter the impacts of salinity and/or acidic stress induced by elevated pCO_2.

Skeletonema costatum is considered one of the most abundant and widely distributed diatoms among coastal marine phytoplankton. It is commonly found in coastal and estuarine areas, and becomes dominant in coastal waters during different seasons, leading to large-scale red tides (Li et al., 2011). Therefore, exploring how *S. costatum* responds to climate change drivers is of general importance in predicting its future contributions to marine primary productivity, biogeochemical cycling of carbon, nitrogen, silicon, and other related processes. It has been suggested that effects of OA should be examined under expected combinations of environmental drivers (Gao and Campbell, 2014). Consequently, this study aims to investigate the combined effects of pH, temperature, and salinity on the diatom *S. costatum*. We investigated the combined effects on growth and photosynthetic physiological parameters of the *S. costatum* while growing it under two pCO_2 (400 μatm and 1000 μatm), two levels of salinity (20 psu, 30 psu), and two temperature levels (10 °C, 20 °C). The key results indicate both elevated pCO_2 and reduced salinity enhanced the diatom's growth and photosynthesis at either temperature levels.

2. Material and methods

2.1. Cultures and experimental design

The *Skeletonema costatum* strain used in the experiment was isolated from the coastal waters of Gaogong Island, Lianyungang, Jiangsu Province (119.5 °E, 34.7 °N), China. The seawater salinity in this area varies within a range of 20 psu to 31 psu, and the average temperature of seawater in winter and early spring is about 10 °C, while in summer and early autumn, it is about 20 °C.

This experiment utilized a semi-continuous culture method, in which the filtered coastal seawater was pre-aerated with air of the targeted pCO_2 levels before used for growing *Skeletonema costatum*. The culture medium was diluted with distilled water to obtain the salinity of 20 psu (LS) or used directly (30 psu, HS). The nutrient salts f/2 + Si (Guillard and Ryther, 1962) were added to the seawater after being sterilized. For treatments of different pCO_2, the seawater of different salinity was either bubbled with outdoor air (415 μatm, LC) or with elevated pCO_2 (1000 μatm, HC) passing through a 0.22 μm bacterial filter (Millipore Express) using a CO_2 enricher (HP 1000G-D, Wuhan Ruihua) that mixes pure CO_2 with outdoor air. To examine the effects of pCO_2 and salinity at different temperature levels, the cultures were run at 10 °C (LT) and 20 °C (HT).

Each treatment was run independently in triplicate, with a culture volume of 350 mL (PC bottles) for each. The cultures were carried out under PAR (white LED) of 150 μmol photons m^{-2} s^{-1} (light-dark cycle 12:12 h) in a plant growth chamber (GXZ, Jiangnan, Ningbo), and were diluted every 24 h to have the cell concentration below 20,000 cells mL^{-1} which ensures exponential growth (Gao, 2021).

2.2. Measurement of growth rate and carbonate chemistry

The specific growth rate of the diatoms was determined after they had been grown for 10–15 generations under the combined environmental conditions. Cell counting will be conducted using a 0.1 mL plankton counting chamber under an optical microscope (DM500, Leica, Germany) after the diatom cell were fixed with Lugol's solution. The specific growth rate was calculated as follows:

$$\mu = (\ln N_1 - \ln N_0) / (t_1 - t_0),$$

where N_1 and N_0 represent the cell density at time t_1 and t_0 respectively, ($t_1 - t_0 = 1$ day). The final value used to represent the specific growth rate is the average of the growth rates calculated from the two measurements.

The concentration of DIC was measured after the renewal of the culture medium using a DIC analyzer. The pH changes were determined with a pH meter which was calibrated with standard NBS buffer solution. Subsequently, other parameters of the carbonate system were computed with CO2SYS software based on the known values of DIC, pH, salinity, and nutrients.

2.3. Measurement of photochemical performances

Photochemical performances of PSII were examined by measuring chl *a* fluorescence parameters using a handheld AquaPen-C (AP-C100, Photon Systems Instruments). The rapid light response curves (RLCs) of the diatoms were determined during mid-light period. To determine the RLCs, photosynthetic photon flux density was at 10, 20, 50, 100, 300, 500, and 1000 μmol photons m^{-2} s^{-1}, and each light intensity lasted for 10 s. The rETR rate was calculated as follows according to Wu et al. (2010):

$$\mathrm{rETR} = \mathrm{PAR} \times \mathrm{Y}(II) \times 0.5,$$

where PAR represents Photosynthetically Active Radiation (μmol photons m^{-2} s^{-1}), Y (II) the effective quantum yield of the algal cells with actinic light level similar to that of growth. The value of 0.5 assumes that PSII only absorbs half of the incident light. The Eilers and Peeters formulas are used for nonlinear fitting (Eilers and Peeters, 1988):

$$\mathrm{rETR} = \mathrm{PAR}/\left(\mathrm{a} \times \mathrm{PAR}^2 + \mathrm{b} \times \mathrm{PAR} + \mathrm{c}\right),$$

where a, b and c are parameters, which are calculated according to the following formula:

$$\alpha = 1/\mathrm{c}$$

$$\mathrm{rETR}max = 1/\left(\mathrm{b} + 2\sqrt{ac}\right)$$

$$\mathrm{I_k} = \mathrm{c}/\left(\mathrm{b} + 2\sqrt{ac}\right)$$

Consequently, light utilization efficiency (α) and light saturation point (I_k) were obtained.

2.4. Determination of net photosynthesis and mitochondrial respiration rates

The rates of net photosynthesis and respiration were measured using a Clark-type oxygen electrode (Oxygraph+, Hansatech, UK). The reaction chamber containing the algal suspension was equipped with a constant temperature water bath device (DHX-2005, China) for temperature control. Prior to measurement, the algal suspension was concentrated using a vacuum filtration pump with a pressure below 0.02 MPa to prevent cell rupture. Then, 100 mL of the sample was filtered through a cellulose acetate membrane (New Asia Instrument, Shanghai). Subsequently, the cells were resuspended in 5 mL of pretreated seawater with the same levels of pH, salinity, temperature and light intensity as in the growing conditions, and 2 mL was used for measuring the photosynthetic and respiratory rates, and 1 mL was used for cell counting. Each of the measurements lasted for about 15 min.

The reaction chamber was placed inside an opaque hood to create a dark environment for measurement of dark respiration rate, and a halogen lamp (QVF135, Philips, Netherlands) was used to provide illumination for measuring the net photosynthetic rate.

2.5. Determination of pigment

The algal cells were filtered onto a GF/F membrane (25 mm, Whatman, UK) with a pressure below 0.02 MPa. The folded GF/F membrane is placed into a 15 mL centrifuge tube. To each sample, 4 mL of methanol is added, and the tubes were kept in a darkness at 4 °C overnight for extraction. The absorbances at wavelengths of 480, 510, 632, 665, and 750 nm were measured using a UV–visible spectrophotometer (Ultrospect 3300 pro, Amersham Bioscience) after the extracted solution as centrifuged (5000 g) for 5 min at 4 °C with a centrifuge (Biofuge primo R, Thermo, Germany). The concentrations of chlorophyll *a* and carotenoids (pg cell^{-1}) in the samples were calculated based on the dilution factors and cell concentrations according to Ritchie (2006) and Strickland (Strickland and Parsons, 1972).

$$\text{Chl } a\ \left(\mu\text{g mL}^{-1}\right) = 13.2654 \times \left(A_{665} - A_{750}\right) - 2.6839 \times \left(A_{632} - A_{750}\right)$$

$$\text{Carotenoid}\ \left(\mu\text{g mL}^{-1}\right) = 7.6 \times \left(\left(A_{480} - A_{750}\right) - 1.49 \times \left(A_{510} - A_{750}\right)\right)$$

2.6. Determination of biogenic silica content

Biogenic silica (Bsi) of the diatom was determined by collecting the cells polycarbonate membrane (Millipore), followed by drying and analysis according to Brzezinski and Nelson' s method (Brzezinski and Nelson, 1995). After 2–3 h color development, the absorbance was measured at a wavelength of 810 nm using a UV–visible spectrophotometer (Ultrospect 3300 pro, Amersham Bioscience). The Bsi content was calculated based on a standard curve, and the Bsi per cell was obtained based on the sample concentration and filtration volume.

2.7. Date analysis

Statistical analysis was performed using SPSS26 software. All data were presented as mean ± standard deviation (X ± SD). Post-hoc analysis was conducted using Tukey' s test. The experimental data were consistent with a normal distribution ($P > 0.05$) and homogeneity of variance ($P > 0.05$). The differences between LC and HC under the same temperature and salinity levels were analyzed using an independent samples T-test. The differences between different treatment groups under LC or HC were analyzed using one-way ANOVA. The interactive effects of these factors on the specific growth rate, chlorophyll fluorescence parameters, photosynthetic rate, respiration rate, pigment content, and Bsi content of the *S. costatum* strain were analyzed using univariate analysis (Three-way ANOVA). The significance level was set at $P < 0.05$.

3. Result

3.1. Carbonate chemistry

The elevated pCO_2 (HC) led to a significant decline of pH and CO_3^{2-}, along with significant increase of CO_2 and HCO_3^- concentrations. The pH in the cultures treated with LTLS, HTLS, LTHS, and HTHS was respectively lowered by 5.6%, 3.3%, 5.6%, and 3.3% compared to that in the LC culture, with CO_3^{2-} concentrations being correspondingly lowered by 64%, 44%, 63%, and 41%.

The decrease in salinity significantly reduced the levels of DIC, pH, HCO_3^-, and CO_3^{2-}, and the higher temperature led to less decline of pH and CO_3^{2-}, along with lowered DIC concentration.

3.2. Growth rate

The specific growth rates of *Skeletonema costatum* ranged from 0.99 to 2.41 d^{-1} under different treatments.

Salinity, pCO_2, temperature, the interaction between pCO_2 and temperature, the interaction between salinity, pCO_2 and temperature had significant effects on the specific growth rate of *Skeletonema costatum* ($P < 0.05$, Supplementary Table S1). As shown in Fig. 1 The HC significantly promoted the growth by 33%, 31%, 7%, and 15% at LTLS, LTHS, HTLS and HTHS, respectively ($P < 0.05$), compared to the LC, which indicated that HC promoted the growth of *Skeletonema costatum*. The lowered salinity from 30 psu to 20 psu also enhanced the diatoms' s growth by 6%–14%, with the enhancement being more pronounced at high levels of CO_2 and high level of temperature. HT stimulated the growth rate respectively by 110% at LSLC, 68% at LSHC, 101% at HSLC, 75% at HSHC, compared with those in LT ($P < 0.05$, Supplementary Table S7), and with less enhancement observed in HC-grown cells.

When the salinity levels are identical, the interaction of HT and HC promoted the growth of *Skeletonema costatum*, whereas the interaction between LT and LC hindered the growth of *Skeletonema costatum*, respectively ($P < 0.05$, Supplementary Table S8).

Combination of HT, HC and LS (HTLSHC) enhanced the specific

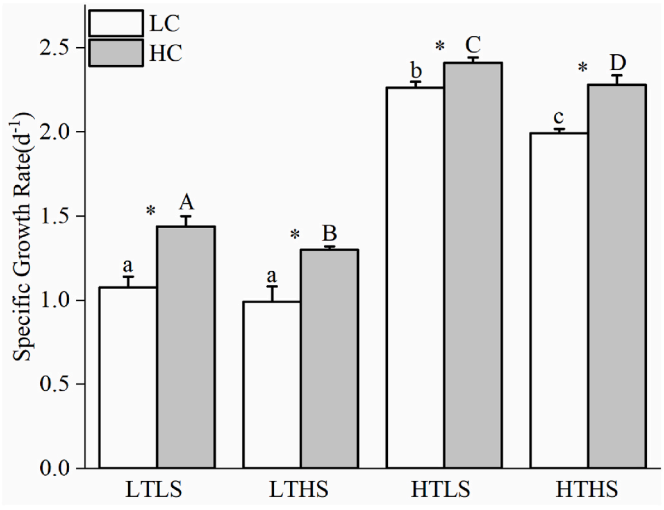

Fig. 1. Specific growth rate of *Skeletonema costatum* grown under different levels of temperature (LT: 10 °C, HT: 20 °C), pCO_2 (LC: 400 μatm, HC: 1000 μatm) and salinity (20 psu, 30 psu) after having acclimated for more than 15 generations. Lower case letters were used to indicate significant differences between different conditions under LC ($P < 0.05$), uppercase letters were used to indicate significant differences between different conditions under HC, and significant differences between LC and HC (*, $P < 0.05$). The error bars indicate the standard deviations (n = 3).

growth rate respectively by 143% compared to LTHTLC ($P < 0.05$, Supplementary Table S6).

3.3. Net photosynthesis and mitochondrial respiration rates

Temperature, pCO_2, the interaction between pCO_2 and temperature, the interaction between salinity and temperature had significant effects on the Net photosynthesis rate and Gross photosynthesis rate of *Skeletonema costatum* ($P < 0.05$, Supplementary Table S2). Temperature, salinity, the interaction between salinity and pCO_2, the interaction between temperature and pCO_2, the interaction between temperature and pCO_2 and salinity had significant effects on the Mitochondrial respiration rate of *Skeletonema costatum* ($P < 0.05$, Supplementary Table S2).

The HC treatment significantly promoted the Net photosynthesis rate by 32%, 20%, 40% and promoted the Gross photosynthesis rate by 16%, 26%, 32% at LTHS, HTLS and HTHS, respectively ($P < 0.05$, Supplementary Table S9), compared to LC (Fig. 2A–C). The lowered salinity from 30 psu to 20 psu inhibit the diatom' s mitochondrial respiration by 41%, 29%, and 63% at LTLC, LTHC, and HTLC respectively ($P < 0.05$, Supplementary Table S8), compared to HS (Fig. 2B). Increasing temperature from 10 to 20 °C promoted the Net photosynthesis and Gross photosynthesis more than a twofold.

The combination of HC and HT promoted the Net photosynthesis by 133% (LS) and by 242% (HS), and promoted Gross photosynthesis by 88% (LS) and 42% (HS) ($P < 0.05$, Fig. 2A–C). The combination of HTLSHC promoted the Mitochondrial respiration by 110% compared to HTLSLC, but the combination of HTHSHC inhibited the Mitochondrial respiration compared to HTHSLC ($P < 0.05$, Supplementary Table S6), which indicated that LS alleviated the inhibitory effect of HTHC on Mitochondrial respiration ($P < 0.05$, Fig. 2B).

3.4. Photochemical performances

Under LC the ETR of *S. costatum* tends to rapidly increase and then plateau at LTLS, HTLS, and LTHS (Fig. 3A). Under HC, the ETR of *S. costatum* exhibits a rapid increase followed by a slight decline at HTLS, LTHS, and HTHS (Fig. 3B). The ratio of HC to LC decreases with increasing light intensity in all treatments (Fig. 3C).

The pCO_2 had significant effects on the $rETR_{max}$ and I_k of *Skeletonema costatum*, temperature had significant effects on $rETR_{max}$ and α of *Skeletonema costatum*, and salinity had significant effects on all three indicators above ($P < 0.05$, Supplementary Table S3). The interaction between pCO_2, salinity and temperature had significant effects on the α ($P < 0.05$, Supplementary Table S3).

The HC treatment significantly reduced $rETR_{max}$ by 19% at LTHS and by 12% at HTLS, reduced α value by 14% at LTHS, while decreased I_k by 8% at HTLS compared to the LC ($P < 0.05$, Supplementary Table S9). The lowered salinity from 30 psu to 20 psu significantly increased the diatom' s $rETR_{max}$ by 28%, 26% and 22% at LTHC, HTLC, and HTHC, at the same time it also significantly increased the diatom' s α value by 24% at LTHC ($P < 0.05$, Supplementary Table S8).

The LTHSHC significantly decreased the value of α by 20%, 19%, 27%, 23%, and 22% at LTLSLC, LTLSHC, HTLSLC, HTLSHC, and HTHSHC ($P < 0.05$, Supplementary Table S6), which indicated that the combination of HS, HC, and LT have a detrimental effect on α.

3.5. Pigment content

Temperature, pCO_2, salinity, the interaction between CO_2 and salinity, the interaction between CO_2 and temperature, the interaction between salinity and temperature, and the interaction between CO_2, salinity, and temperature all significantly effects on the Carotenoids content of *S. costatum* ($P < 0.05$, Supplementary Table S4). Except for the interaction between salinity and temperature, all the aforementioned factors had a significant impact on the Chlorophyll *a* content of *S. costatum* ($P < 0.05$, Supplementary Table S4).

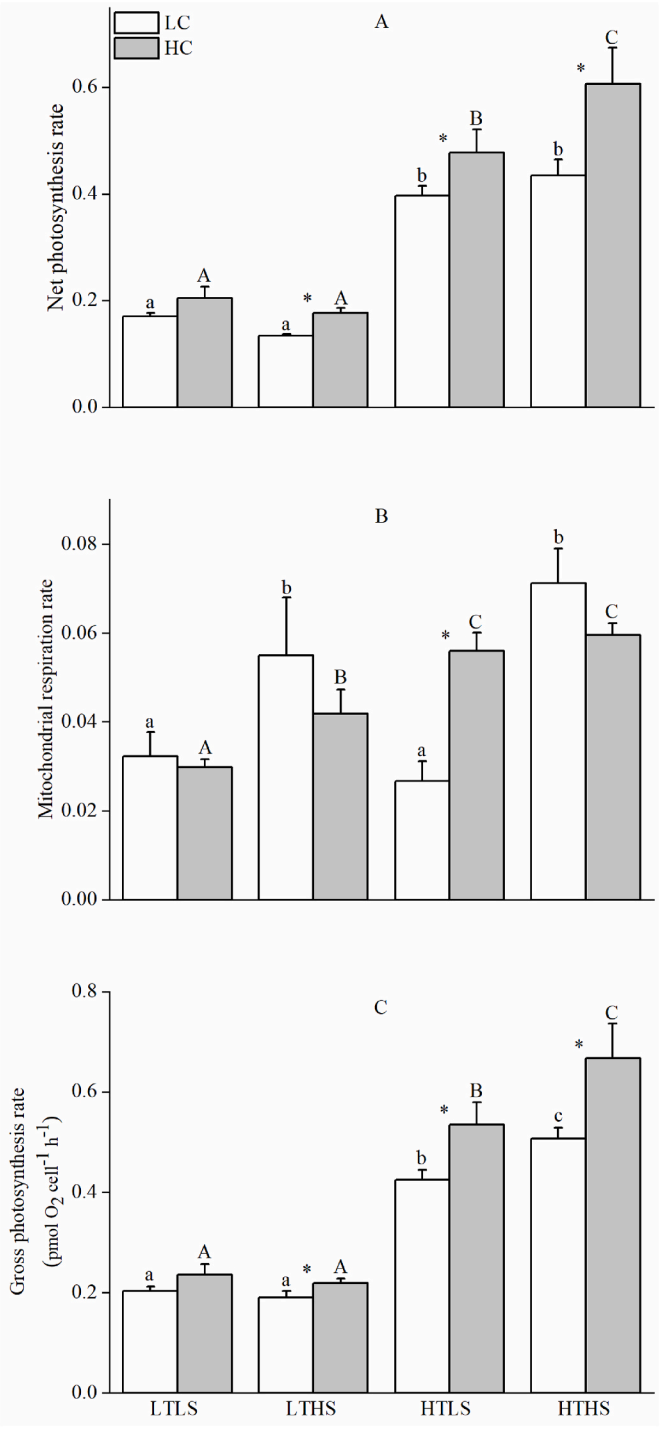

Fig. 2. Net photosynthesis (A), mitochondrial respiration (B) and gross photosynthesis rates (C) of *Skeletonema costatum* (pmol O_2 $cell^{-1}$ h^{-1}) grown under different levels of temperature (LT: 10 °C, HT: 20 °C), pCO_2 (LC: 400 μatm, HC: 1000 μatm) and salinity (20 psu, 30 psu) after having acclimated for more than 15 generations. Different letters and * above the bars indicate significant differences between treatments.

The HC treatment significantly enhanced the intracellular chlorophyll *a* content by 23% at LTHS and by 103% at HTHS (Fig. 4A), and enhanced the diatom' s carotenoid content by 159% at HTHS (Fig. 4B), respectively, compared to the LC ($P < 0.05$, Supplementary Table S9). The lowered salinity from 30 psu to 20 psu reduced the diatom's chlorophyll *a* by 33% at LTHC and by 25% at HTHC ($P < 0.05$, Fig. 4A), and carotenoids reduced by 45% at HTHC ($P < 0.05$, Fig. 4B). Seawater

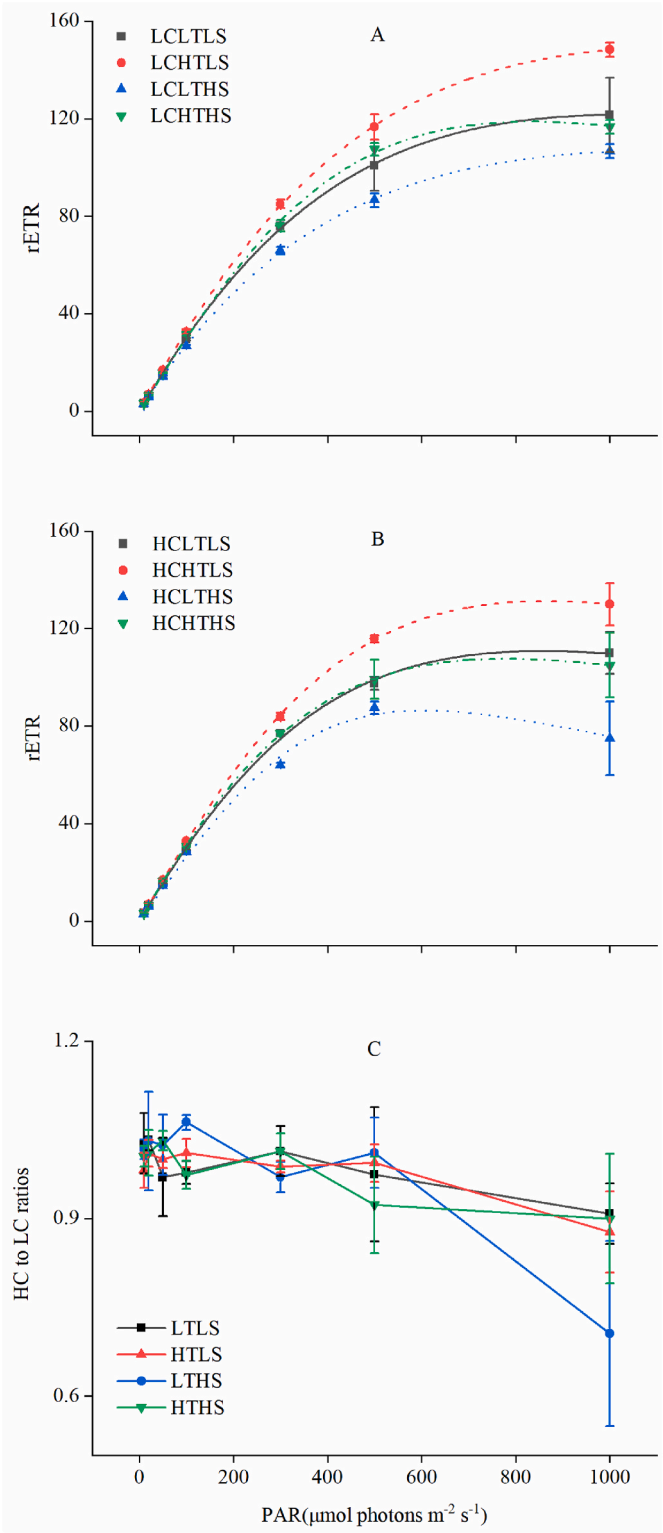

Fig. 3. Rapid light response curves (A, B) and the rate of HC to LC (C) in *Skeletonema costatum* grown under different levels of temperature (LT: 10 °C, HT: 20 °C), pCO_2 (LC: 400 μatm, HC: 1000 μatm) and salinity (20 psu, 30 psu) after having acclimated for more than 15 generations.

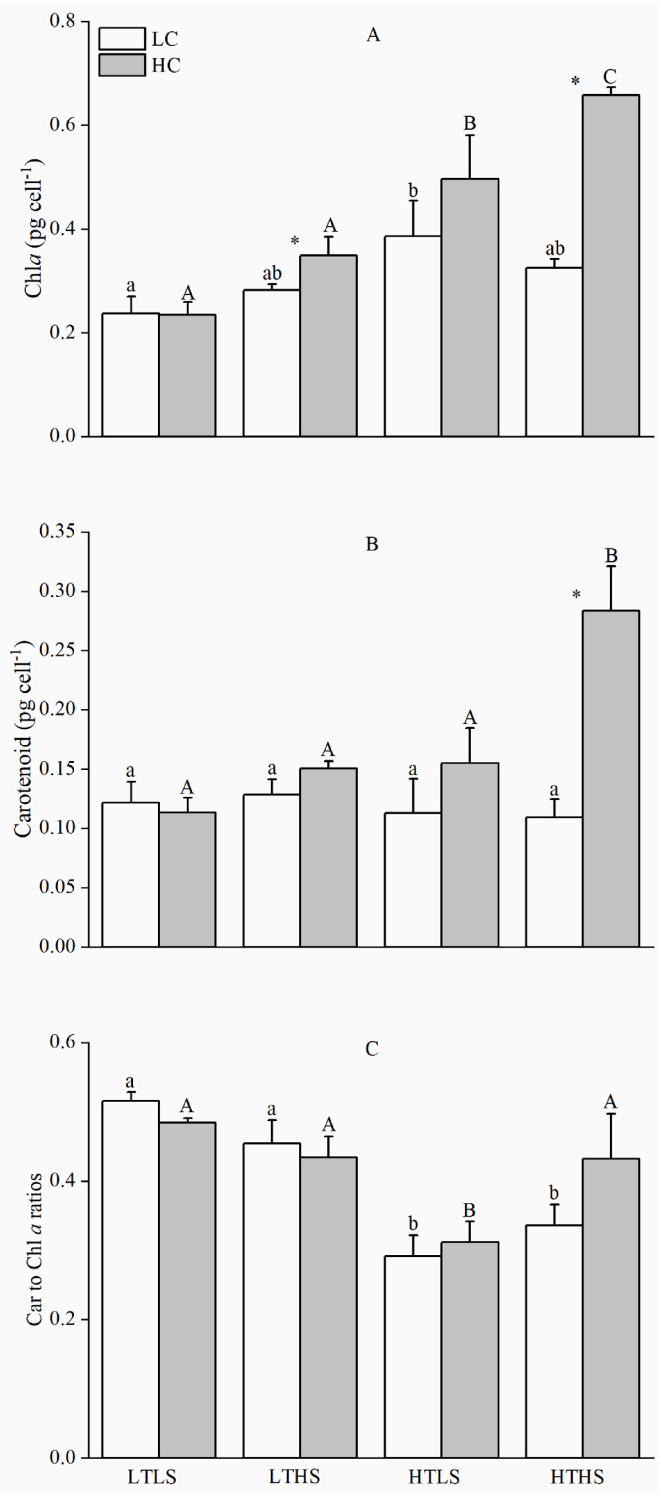

Fig. 4. Contents of chlorophyll *a* (A), carotenoids (B) and the ratios of Car. to Chl *a* (C) of *Skeletonema costatum* grown under different levels of temperature (LT: 10 °C, HT: 20 °C), pCO_2 (LC: 400 μatm, HC: 1000 μatm) and salinity (20 psu, 30 psu) after having acclimated for more than 15 generations. Different letters and * above the bars indicated significant difference between the treatments.

desalination has different effects on Car./Chl *a* under different temperature or CO_2 conditions. At lower temperatures, seawater desalination increased the Car./Chl *a*, while at higher temperatures, seawater desalination decreased the Car./Chl *a* (Fig. 4C).

The combination of HC and LS decreased the chlorophyll *a* of *S. costatum* by 1–33% at 10 °C and decreased the carotenoid content by 1.4–4.7% at 10 °C. Combination of HC, higher temperature and higher salinity (HTHSHC) significantly higher increase the chlorophyll *a* and carotenoid content compared to other levels ($P < 0.05$, Supplementary

Table S6).

3.6. Biogenic silica contents

Temperature, pCO_2, the interaction between CO_2 and temperature, the interaction between CO_2, salinity, and temperature had significant effects on the Bsi content of *S. costatum* ($P < 0.05$, Supplementary Table S5).

The HC treatment significantly promoted the biogenic silica content of *S. costatum* by 118.78% at LTLS and by 38.74% at HTHS compared to LC ($P < 0.05$, Fig. 5A). The LT treatment significantly promoted the biogenic silica content of *S. costatum* by 80% at HSHC compared to HT ($P < 0.05$, Fig. 5B). At 30 psu, HC has little effect on *S. costatum*' s Bsi content. However, at low temperature and desalination condition, HC significantly promoted the Bsi content (Fig. 5B). The combination of HC and desalination promoted the BSi content of *S. costatum* by 44–119% at 10 °C (Fig. 5A).

4. Discussion

Reduction of salinity usually leads to higher levels of pCO_2 and lowered levels of DIC (Li et al., 2011). While we could not accurately distinguish the different impacts of lowered salinity and elevated pCO_2 on carbonate chemistry (Table 1), our results clearly demonstrated that either elevated pCO_2 or reduced salinity enhanced the growth and photosynthesis of *S. costatum*, and it is most likely that increased pCO_2 under lowered salinity is responsible for the observed enhancement.

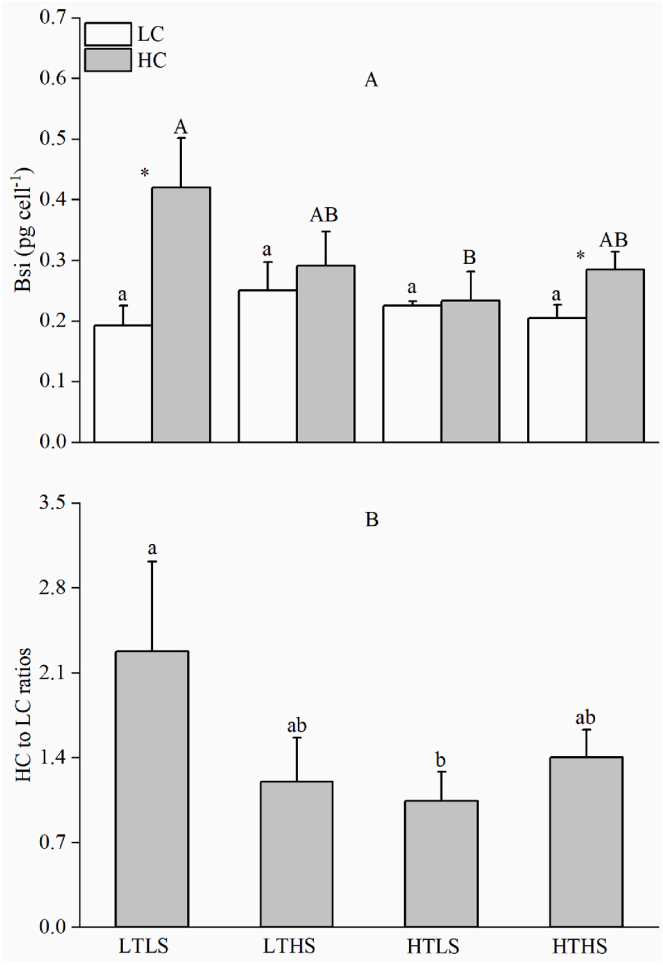

Fig. 5. Biogenic silica contents (A) and the rate of HC to LC (B) of *Skeletonema costatum* grown under different of pCO_2, temperatures and salinity after having acclimated for more than 15 generations, all abbreviations are the same as in Fig. 1. Different letters above the bars indicated significant difference between treatments.

Previous studies have examined the individual effects of ocean acidification (Dong et al., 2020; Kim et al., 2006; Li et al., 2021) and decreased salinity (Ebrahimi and Salarzadeh, 2016; Qasim et al., 1972; Zang et al., 2022) on the growth rate and photosynthetic performance of diatoms. Their findings suggest that the effects vary under different conditions combined with ocean acidification, Overall, ocean acidification has a beneficial impact on the growth not only in diatoms (Dong et al., 2020; Kim et al., 2006; Li et al., 2021), but also in other groups of algae such as *Gracilariopsis lemaneiformis* (Chen et al., 2023). On the contrary, different results have been observed in some large algae species (Zhou et al., 2022). It is worth noting that a decrease in salinity does have a certain degree of inhibitory effect on the growth of diatoms, this is contrary to our experimental results. However, interestingly, for certain types of algae, such as *Emiliania huxleyi*, a decrease in salinity is actually considered a beneficial condition for their growth (Xu et al., 2020), which is consistent with our experimental results.

There is still a lack of comprehensive research on how these factors interact and influence *Skeletonema costatum* under varying seasonal temperatures. In this study, we investigated the effects of ocean acidification and seawater desalination on *Skeletonema costatum* in the seasonal variation of surface water temperatures ranging from 10 °C to 20 °C along the coast of the Yellow Sea. The aim was to understand the impact of environmental factor changes on physiological indicators of *S. costatum* and explore how it responds to these environmental changes, and whether it increases the likelihood of harmful algal blooms (HABs). Our study results indicate that, when considered as individual factors, most indicators of the diatom *Skeletonema costatum* increase at 20 °C compared to 10 °C. The HC treatment positively impacts the growth rate and photosynthesis of *Skeletonema costatum*, but negatively affects the potential maximum relative electron transport rate and the ability to tolerate strong light. Additionally, the growth of *Skeletonema costatum* is enhanced when the salinity decreases from 30 psu to 20 psu, but the pigment content concurrently decreases.

Previous studies have shown that diatoms, such as *S. costatum*, have the ability to actively uptake CO_2 and HCO_3^-. They are capable of utilizing both CO_2 and HCO_3^- for photosynthesis (Korb et al., 1997). In this process, intracellular carbonic anhydrase (CA) catalyzes the conversion of HCO_3^- to CO_2, thereby enhancing the photosynthetic carbon fixation efficiency of diatom cells and their adaptation to stressful conditions (Chen and Gao, 2004a,b). Our study has found that ocean acidification alters the marine carbonate system, resulting in increased concentrations of dissolved inorganic carbon (DIC), CO_2, and HCO_3^-, this indicates that there is more CO_2, and HCO_3^- available for *S. costatum* to utilize in order to increase photosynthesis and promote growth in HC environment (Table 1). Existing studies have also indicated that the HC treatment increases the availability of CO_2 and HCO_3^- in seawater, which promotes the carbon fixation of cells to some extent (Kottmeier et al., 2016; Riebesell and Tortell, 2011; Wu et al., 2010).

For phytoplankton, the carbon concentration mechanism (CCM) is regulated by HCO_3^- in the culture medium (Matsuda et al., 2002). An increase in DIC may result in down-regulation of the CCM to save energy (Li et al., 2014). In this experiment, the increase in pigment content of *S. costatum* under HC conditions may be attributed to the energy saved through CCM down-regulation (Figs. 1 and 2A).

Although *S. costatum* can adapt to a wide range of salinities, its optimal growth salinity varies with changes in the marine environment. It is one of the most common red tide organisms in coastal waters worldwide. Lower salinities may alter ion concentrations, electrochemical gradients, and osmotic pressure both inside and outside the cells of *S. costatum*. These changes require additional energy to meet the passive or active transport of CO_2 and nutrients (Xu et al., 2020), and potentially altering the adaptation mechanisms and nutrient uptake capabilities of algal cells. Previous studies have shown that algal cell

Table 1
Parameters of seawater carbonate system in the cultures of *Skeletonema costatum* prior to dilution, which was executed every 24 h. The pH and DIC were directly measured, and other parameters were derived using the CO2SYS.

		DIC (μM)	pH_{NBS}	TA (μM)	CO_2 (μM)	HCO_3^- (μM)	CO_3^{2-} (μM)
LC	LTLS	1717.96 ± 27.59^{b}	8.22 ± 0.02^{bc}	1863.45 ± 2.69^{c}	15.15 ± 0.68^{a}	1618.39 ± 11.58^{b}	85.55 ± 3.30^{c}
	HTLS	1494.06 ± 9.53^{c}	8.20 ± 0.02^{c}	1667.72 ± 15.31^{d}	11.04 ± 0.34^{b}	1383.62 ± 5.94^{c}	99.40 ± 3.94^{b}
	LTHS	2187.50 ± 18.87^{a}	8.29 ± 0.02^{a}	2448.63 ± 23.73^{a}	14.04 ± 0.51^{a}	2010.22 ± 16.63^{a}	163.24 ± 5.86^{a}
	HTHS	1793.69 ± 47.65^{b}	8.26 ± 0.01^{ab}	2081.09 ± 48.94^{b}	9.95 ± 0.40^{b}	1613.39 ± 44.67^{b}	170.35 ± 2.70^{a}
HC	LTLS	1760.28 ± 3.47^{B}	7.76 ± 0.01^{A}*	1795.29 ± 5.50^{C}*	45.60 ± 0.96^{A}*	1683.81 ± 3.63^{B}*	30.87 ± 0.78^{C}*
	HTLS	1524.81 ± 130.94^{C}	7.93 ± 0.01^{C}*	1622.19 ± 133.48^{C}	21.68 ± 1.99^{B}*	1447.74 ± 124.45^{C}	55.39 ± 4.71^{B}*
	LTHS	2262.96 ± 73.33^{A}	7.83 ± 0.02^{B}*	2345.28 ± 73.04^{A}	43.49 ± 2.33^{A}*	2158.69 ± 70.05^{A}*	60.77 ± 2.60^{B}*
	HTHS	1881.03 ± 33.46^{B}	7.99 ± 0.01^{D}*	2050.08 ± 36.22^{B}	20.21 ± 0.29^{B}*	1760.93 ± 30.74^{B}*	99.88 ± 2.73^{A}*

Note: LC (400 μatm) and HC (1000 μatm CO_2) represent the CO_2 levels of the culture medium before inoculation of the diatom; LT: 10 °C, HT: 20 °C: 20 or 30: salinity. The data in the table are means ± SD (n = 3). Lower case letters indicate the significant difference ($P < 0.05$) between groups treated with different salinity and temperature under LC condition; uppercase letters indicate the significant difference ($P < 0.05$) between levels of different salinity and temperature under HC condition; * indicates a significant difference between the LC and HC treatments groups under the same temperature and salinity conditions ($P < 0.05$).

Table 2
The fitted values of $rETR_{max}$, α, and I_k for *S. costatum* grown under different pCO_2, temperatures and salinity, which were derived from Fig. 3.

		$rETR_{max}$	α	I_k
LT	LSLC	125.97 ± 16.45^{a}	0.33 ± 0.03^{a}	387.97 ± 58.20^{a}
	LSHC	111.89 ± 4.93^{a}	0.32 ± 0.02^{a}	346.45 ± 7.80^{a}
	HSLC	107.29 ± 2.96^{ab}	0.30 ± 0.01^{ab}	354.07 ± 17.07^{a}
	HSHC	87.24 ± 6.73^{b}	0.26 ± 0.02^{b}	334.83 ± 5.98^{a}
HT	LSLC	150.52 ± 3.02^{A}	0.36 ± 0.01^{A}	423.68 ± 9.55^{A}
	LSHC	131.75 ± 5.15^{B}*	0.34 ± 0.01^{A}	$389.12 \pm 4.46A^{B}$*
	HSLC	119.09 ± 1.20^{BC}*	0.31 ± 0.02^{A}	383.08 ± 19.37^{AB}
	HSHC	108.13 ± 9.26^{D}	0.33 ± 0.02^{A}*	326.61 ± 40.10^{B}

The $rETR_{max}$ indicates relative maximal electron transport rate, α - the light use efficiency, I_k (μmol photons m^{-2} s^{-1}) is the light intensity for the saturated ETR. Lower case letters indicate the significant difference ($P < 0.05$) between groups treated with different salinity and CO_2 under LT condition; uppercase letters indicate the significant difference ($P < 0.05$) between levels of different salinity and CO_2 under HT condition; * indicates a significant difference ($P < 0.05$). The data in the table are means ± SD (n = 3).

growth is typically promoted under low salinity conditions (Xu et al., 2020), which is consistent with our findings (Fig. 1). Although a decrease in salinity leads to a reduction in pigment content, an increase in $rETR_{max}$ and α provides support for growth (Table 2). Under low salinity conditions, diatoms experience an increase in the rate of nutrient uptake, including silicates, during rapid growth. Therefore, as salinity decreases, the rate of silicate absorption increases, resulting in an increase in the cellular content of Bsi (Fig. 5).

Temperature is an important factor that affects algal growth. Current research suggests that ocean warming has a greater impact on diatoms than ocean acidification, making it the primary driving force for diatoms to adapt to oceanic changes (Zhong et al., 2021). As the temperature rises, the growth rate and net photosynthetic rate of diatoms increase (Boyd et al., 2016; Kranz et al., 2015). Our experimental findings align with previous research results, the increase in temperature promoted the growth rate and photosynthetic rate of *S. costatum*, furthermore, it seems that temperature plays a dominant role. (Figs. 1 and 2A).

Interestingly, reduced salinity significantly attenuated the enhancing effect of HC on pigment content, as well as net photosynthetic rate and $rETR_{max}$. However, the impact on I_k was found to be contrary, and this trend was not reflected in the growth rate. Since reduction of salinity increases partial pressure of pCO_2 along with decreased DIC, the down-regulation of CCMs in the diatom was modulated which led to down-regulated levels of pigmentation and electron transport.

In our experiment, *S. costatum* was cultured individually in adapted media. However, in natural environments, there is competition among different algal species, as well as interactions between the environment and species. For instance, in the nearshore estuarine waters of Lianyungang (118° 24′ 03″–119° 54′ 51″ E, 33° 58′ 55″ ~ 35° 08′ 30″ N), China, such as the Qiangwei River estuary, Guan River estuary, and Lin Hong River estuary, the salinity can drop as low as 20‰ or even lower. In winter or during rainy seasons, the environmental changes, including temperature and salinity, become even more unpredictable, Therefore, to better understand the mechanisms underlying the effects of ocean acidification and decreasing salinity on *S. costatum*, further investigation is needed to explore the impact of interspecies competition on *S. costatum* in natural environments.

5. Conclusion

We explored the potential impacts of ocean acidification and increased precipitation on coastal waters under different seasonal temperatures, and found that ocean acidification and seawater desalination promoted the growth of *S. costatum* under the simulated conditions. Enhanced photosynthesis and associated increase of energy supply mechanistically led to the resilience to the acidic stress under the elevated CO_2 and/or reduced salinity and enhanced growth of the diatom. Synergistic effects of OA and the desalination with increased temperature within the range of 10–20 °C implies that *S. costatum* in the coastal waters of the Yellow Sea can benefit from the ocean global changes over most seasons.

CRediT authorship contribution statement

Ruijie Wu: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Yuchen Wu:** Validation, Investigation. **Rui Zhai:** Validation, Investigation. **Kunshan Gao:** Writing – review & editing, Resources. **Juntian Xu:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition, Formal analysis.

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.

Data availability

Data will be made available on request.

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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.2024.106450.

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