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Effects of pH/pCO_2 fluctuation on photosynthesis and fatty acid composition of two marine diatoms, with reference to consequence of coastal acidification

Yu Shang[1], Jingmin Qiu[1], Yuxi Weng[1], Xin Wang[1], Di Zhang[2], Yuwei Zhou[1], Juntian Xu[1,3], Futian Li[1,3]

[1] Jiangsu Key Laboratory of Marine Bioresources and Environment, Jiangsu Ocean University, Lianyungang, 222000, China
[2] School of Ocean, Yantai University, Yantai, 264000, China
[3] Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Jiangsu Ocean University, Lianyungang, 222000, China

Correspondence to: Futian Li (futianli@jou.edu.cn)

Abstract. Coastal waters are impacted by a range of natural and anthropogenic factors, which superimpose on effects of increasing atmospheric CO_2, resulting in dynamically changing seawater carbonate chemistry. Research on influences of dynamic pH/pCO_2 on marine ecosystem is still in its infancy, although effects of ocean acidification have been extensively studied. In the present study, we manipulated the culturing pH/pCO_2 to investigate physiological performance and fatty acid (FA) composition of two coastal diatoms *Skeletonema costatum* and *Thalassiosira weissflogii* in both steady and fluctuating pH/pCO_2 regimes. Generally, seawater acidification and pH variability showed neutral or positive effects on specific growth rate, chlorophyll *a*, and biogenic silica contents of two species. Elevated pCO_2 inhibited net photosynthetic rate by 27% and enhanced mitochondrial respiration rate of *S. costatum* by 36% in the steady pH regime, while these rates were unaltered by elevated pCO_2 in the fluctuating regime. Elevated pCO_2 leaded to 21% lower saturated FA and twofold increase in polyunsaturated FA proportions of *T. weissflogii*. Our results indicate that costal acidification could affect primary production in a different way from ocean acidification. Together with the altered nutritional quality of prey for higher trophic levels, coastal acidification might have far-reaching consequence for marine ecosystem functioning.

1 Introduction

Carbonate chemistry of coastal waters is impacted by biological metabolism, tidal cycles, upwelling, wind forcing, and terrestrial nutrient inputs, in addition to the dissolution of atmospheric CO_2 (Carstensen and Duarte, 2019; Duarte et al., 2013). This results in dynamic changes in carbonate system parameters such as pH and pCO_2 (García-Ibáñez et al., 2024). Thus, short-term pH fluctuations will be superimposed on the downward trend of pH in coastal waters in the context of ocean acidification. Which was supposed to have potential impacts on marine organisms at different trophic levels based on limited researches (Li et al., 2021; Raven et al., 2020; Schaum et al., 2016; Wahl et al., 2018).

Diatoms are usually one of dominant phytoplankton taxa in coastal waters, in which they contribute large proportion of primary production (Tréguer et al., 2018). Coastal diatoms seem to be characterized by high tolerance to dynamic changes in

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abiotic factors (Key et al., 2010; Li et al., 2016; Strzepek and Harrison, 2004), which is supported by their special cell structure or fast acclimation rate and broad ecological niche (Armbrust, 2009). The pH tolerance of diatoms varies among species and some species can adapt to wide range of pH, in which cells show positive growth (Hansen, 2002; Hinga, 2002). Our previous study found that coastal diatom species may benefit from or be tolerant to diurnal pH fluctuation (Li et al., 2016; Shang et al., 2024). This is reasonable given the difference between the diffusion boundary layer (DBL) of cells and bulk seawater amplifies as cell size increases (Flynn et al., 2012). In addition, the carbonate chemistry in coastal waters varies with large amplitude and high frequency (Duarte et al., 2013). Thus, phytoplankton dwelled in coastal waters with turbulent condition should show high tolerance to changes in carbonate chemistry.

Effects of ocean acidification and underlying mechanisms have been extensively studied at different trophic levels on both short- and long-term timescales (Doney et al., 2020; Hancock et al., 2020), yet research on effects of dynamic pH is still in its infancy. Ocean acidification might show kinds of effects on diatoms based on simulated laboratory and field studies, and other environmental drivers could mediate the effects (Gao and Campbell, 2014). These studies are important to reveal the comprehensive consequence of ocean acidification. However, the impacts of fluctuating carbonate chemistry might differ from those of decreased pH and increased CO_2.

Limited studies have focused on how marine phytoplankton perform under fluctuating pH/pCO_2 condition and this hinder our overall understanding of impacts of acidification in coastal regions. To capture more details in fluctuating pH regime, we manipulated the culturing pH/pCO_2 in a stepwise way by adjusting pCO_2 aerated into cultures in the present study, and each step lasts for 24 h. This enables us to investigate cell performance at each pH level in the fluctuating regime, besides the overall responses. We hypothesize that coastal diatoms could tolerate environmental pH fluctuation, given the dynamic carbonate chemistry in coastal waters and the unignorable pH difference between the DBL of cells and bulk seawater.

2 Materials and Methods

2.1 Culture conditions and experiment setup

Two typical diatoms *Skeletonema costatum* (originally isolated from coastal waters of Gaogong Island, Jiangsu Province, China) and *Thalassiosira weissflogii* (CCMA 102, originally isolated from Daya Bay, Guangdong Province, China) were cultured in polycarbonate bottles with 500 ml sterile artificial seawater. Nutrients were added according to F/2 recipe (Guillard and Ryther, 1962) to ensure cells were not limited by nutrients. Triplicate cultures were set for each treatment and they were cultured in one incubator with light intensity of 150 μmol photons m^{-2} s^{-1} and 12:12 h light and dark cycle. Culturing temperature was set at 20 °C, which is in the optimal temperature range for growth of two species. Cultures were diluted every three or four days to make sure cells were in the exponential phase.

To compare effects of pH level and variability on two diatoms, four pH/pCO_2 treatments were set: 1) steady ambient pH/pCO_2 level (LCs); 2) steady future pH/pCO_2 level (HCs); 3) fluctuating ambient pH/pCO_2 level with similar mean values of pH/pCO_2 with those in LCs treatment (LCf); 4) fluctuating future pH/pCO_2 level with similar mean values of pH/pCO_2

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with those in HCs treatment (HCf). LCs and HCs cultures were aerated with ambient air and CO_2-enriched air, respectively. The CO_2-enriched air was achieved by mixing air and CO_2 with a CO_2 Enricher (CE100, Ruihua) and its target pCO_2 level was set as 1000 μatm for HCs cultures. For fluctuating regimes, the pCO_2 of aerating air was adjusted every 24 h in a stepwise way. The pCO_2 was set as follows: 400-280-400-1000-400 μatm for LCf and 1000-400-1000-1750-1000 μatm for HCf treatments, and each step lasted for 24 h. This resulted in pH ranging from 7.85 to 8.35 and from 7.6 to 8.1 under LCf and HCf conditions, respectively (Fig. 1). The aerating rate was controlled at 100 ml min^{-1} by a gas flowmeter, and filter units (SLGPR33RB, Millipore) were used to sterilize the aerating air. Cultures were acclimated to four treatments for at least 10 days (i.e. two pH variation cycles for fluctuating regime) before following parameters were measured.

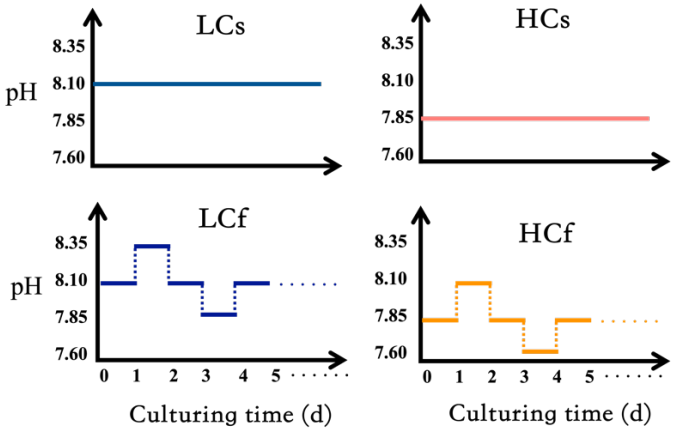

Figure 1. Target culturing pH of steady ambient pH/pCO_2 (LCs), fluctuating ambient pH/pCO_2 (LCf), steady future pH/pCO_2 (HCs), and fluctuating future pH/pCO_2 (HCf) treatments.

2.2 Specific growth rate

Subsamples were collected and fixed with Lugol's solution for cell density measurement. Then samples were counted with a plankton counting chamber (DSJ-01, Xundeng) under an optical microscope (DM500, Leica). Specific growth rate was calculated according to the following equation: μ=ln (N2/N1)/(T2-T1), where N1 and N2 represent cell densities at T1 and T2, respectively.

2.3 Chlorophyll *a* and biogenic silica contents

Subsamples were filtered onto GF/F filters (Whatman) for subsequent chlorophyll *a* extraction in 100% methanol at 4 °C. Then they were centrifuged at 5000 g for 10 min before the absorption of supernatant was determined at 632, 665, and 750 nm with a spectrophotometer (Ultrospect 3300 pro, Amersham Bioscience). Biogenic silica (BSi) samples were collected onto polycarbonate membranes (ATTP02500, Millipore). Membranes with cells were digested in NaOH at 95 °C for 45 min, and then HCl was added to terminate extraction. Then ammonium molybdate and mixture of metol-sulfite, oxalic acid,

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sulfuric acid, and MilliQ water were added and let the color develop for 2 h. Then the absorption of the samples was determined at 810 nm to measure the BSi concentration (Brzezinski and Nelson, 1995). Cell concentration, filtration volume, and dilution factor during extraction and measurement were taken into account for calculating chlorophyll *a* and BSi contents.

2.4 Quantum yield of PSII

The AquaPen Chlorophyll Fluorometer (AP-C100, Photon Systems Instruments) was used to measure effective quantum yield of PSII (Φ_{PSII}). Light-adapted samples were used for measurements, which were put at same light and temperature levels as culturing conditions for at least 15 min. For effective quantum yield, blue light was chosen and the saturating pulse was set at 100%. It was calculated as: $\Phi_{PSII} = (F_m' - F_t) / F_m'$, where F_m' and F_t represent the maximum chlorophyll fluorescence of light-adapted samples and the steady state chlorophyll fluorescence, respectively.

2.5 Photosynthetic oxygen evolution and mitochondrial respiration

Subsamples were gently filtered (< 0.02 MPa) onto cellulose acetate membranes and then re-suspended into 20 mmol l^{-1} Tris-buffered medium. The pH values of the Tris-buffered media were pre-adjusted with HCl and NaOH to the corresponding culturing values. Then re-suspended samples were injected into the chamber of a Clark-type oxygen electrode (Oxygraph plus, Hansatech) and the changes in the oxygen level were recorded for at least 10 min for each sample. Light intensity was set as 150 µmol photons m^{-2} s^{-1} for photosynthetic oxygen evolution rate measurement, and a halogen lamp (QVF135, Philips) was used as the light source. For mitochondrial respiration rate measurement, the chamber was covered by aluminum foil to achieve dark condition. Temperature of water jacket of chamber was controlled at 20 °C with a thermostatic water bath (DHX-2005, Xianou). Photosynthetic oxygen evolution and respiration rates were measured at each culturing pH level in fluctuating regimes. Cell density after concentrating was counted as mentioned above to calculate oxygen evolution and consumption rate per cell.

2.6 Fatty acid composition

Cells were collected by gentle filtration (< 0.02 MPa) and centrifugation (5000 rpm, 5 min), and then samples were dried (80 °C, 36 h) and pulverized to fine powders. Then fatty acids (FAs) in samples were converted into fatty acid methyl esters (FAMEs) by chloroform-methanol (V:V=2:1), and their compositions were analyzed with a Shimadzu GC-2010 gas chromatograph-flame ionization detection equipped with a fused silica column (100 m × 0.25 mm × 0.2 µm film thickness, Agilent CP-Sil 88). Standards were used to identify FAMEs by comparing retention times and proportions of FAs were quantified by the percentage of each peak area to total area.

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2.7 Statistical analysis

All data are reported as the mean ± standard deviation (SD). Shapiro-Wilk and Levene tests were used to test the normality and equal variance of data, respectively. One-way analysis of variance (ANOVA) and *post hoc* Tukey test were used to analyze the differences among four treatments.

3 Results

3.1 Specific growth rate, chlorophyll *a* and BSi contents

For simplicity, only pH rather than both pH and pCO_2 was shown in this section. There were no effects of pH level and variability on specific growth rates of both species, although the rate varied among sampling period (Fig. 2). Similarly, effects of pH level or pH variability were not detected for chlorophyll *a* content of *S. costatum* (Fig. 3a). For *T. weissflogii*, no difference between steady and fluctuating regimes was found at each pH level. HCs cells had 44% more chlorophyll *a* content compared to LCs cells, but LCf and HCf cells showed similar content (Fig. 3c). In terms of BSi content, both species had similar content regardless of treatments (Fig. 3b and d).

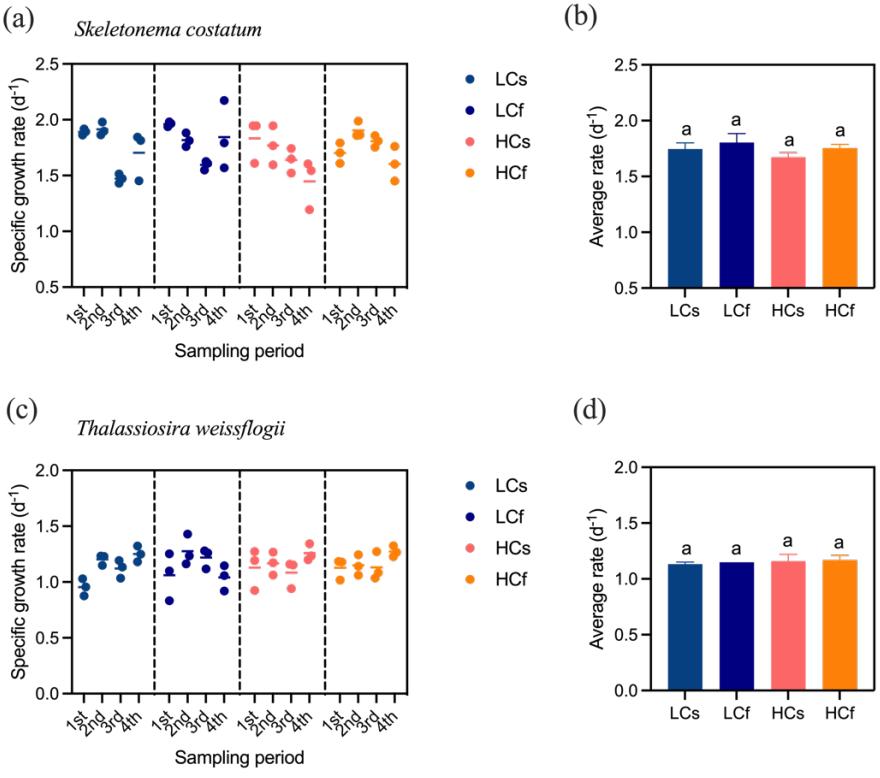

Figure 2. Specific growth rate of *Skeletonema costatum* and *Thalassiosira weissflogii* cells grown under steady ambient pH/pCO_2 (LCs), fluctuating ambient pH/pCO_2 (LCf), steady future pH/pCO_2 (HCs), and fluctuating future pH/pCO_2 (HCf).

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Scatter plots show the specific growth rate of all replicates in each sampling period and the short line indicates the mean value of three replicates. Four sampling periods include all pH levels in the fluctuating regime and average rates are calculated from the means of triplicate cultures across four sampling periods. The different letters indicate significant ($p < 0.05$) differences among treatments.

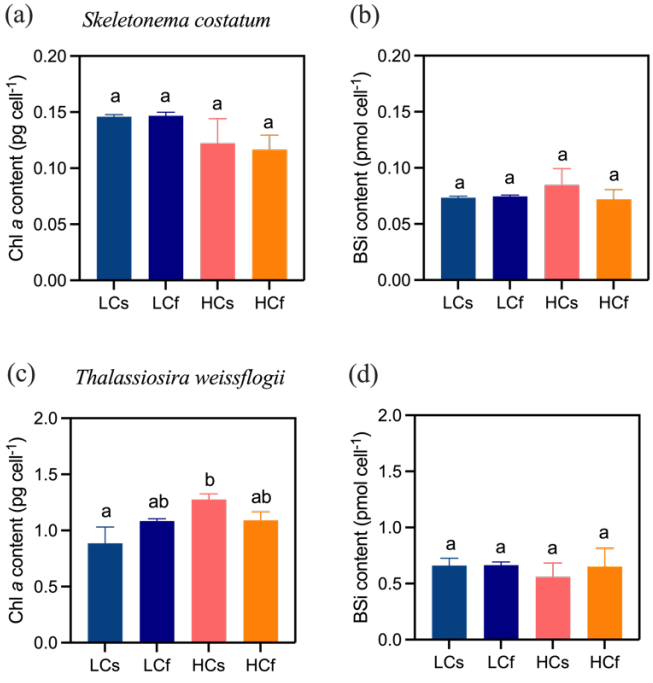

Figure 3. Chlorophyll *a* and biogenic silica contents of *Skeletonema costatum* and *Thalassiosira weissflogii* cells grown under steady ambient pH/pCO_2 (LCs), fluctuating ambient pH/pCO_2 (LCf), steady future pH/pCO_2 (HCs), and fluctuating future pH/pCO_2 (HCf). Values are the means ± SD of triplicate cultures. The different letters indicate significant ($p < 0.05$) differences among treatments.

3.2 Quantum yield of PSII

Lower pH enhanced effective quantum yield of PSII of *S. costatum* by 9% and 13% compared to ambient pH condition for steady and fluctuating regimes, respectively (Fig. 4a). No difference between steady and fluctuating regimes was found at each pH level. Similarly, no difference between steady and fluctuating regimes was found for *T. weissflogii*, and effects of decreased pH were only observed in the fluctuating regime, with 10% higher effective quantum yield of PSII in HCf cells than LCf ones (Fig. 4b).

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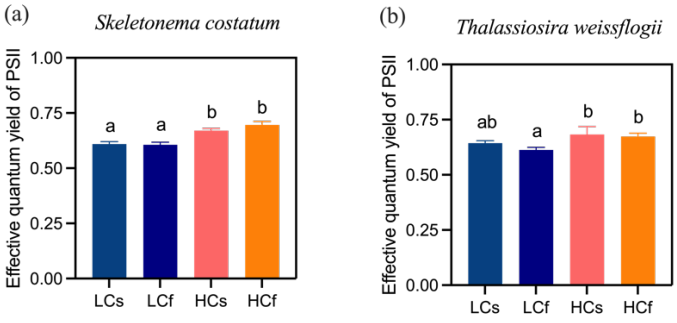

Figure 4. Effective quantum yield of PSII of *Skeletonema costatum* and *Thalassiosira weissflogii* cells grown under steady ambient pH/pCO_2 (LCs), fluctuating ambient pH/pCO_2 (LCf), steady future pH/pCO_2 (HCs), and fluctuating future pH/pCO_2 (HCf). Values are the means ± SD of triplicate cultures. The different letters indicate significant ($p < 0.05$) differences among treatments.

3.3 Photosynthetic oxygen evolution and mitochondrial respiration

In the steady regime, seawater acidification inhibited photosynthetic oxygen evolution rate of *S. costatum* by 27%, while no effects of elevated CO_2 were observed in the fluctuating regime (Fig. 5a and b). Although photosynthetic rate changed with pH in the fluctuating regime, the average rates of cells cultured under LCf and HCf conditions were similar with the rates under corresponding steady conditions. For *T. weissflogii*, its photosynthetic rate was generally insensitive to changes in mean level of pH or pH variability (Fig. 5e and f).

Seawater acidification enhanced mitochondrial respiration rate of *S. costatum* by 36% in the steady regime, while there was no difference in the rate between LCf and HCf conditions (Fig. 5c and d). *S. costatum* cells cultured under LCf condition showed increased mitochondrial respiration rate with increasing pH levels in the regime, while the rates of HCf cells were similar among three pH levels. For *T. weissflogii*, its mitochondrial respiration rate varied at different pH levels in the fluctuating regime, but the average rates of LCf and HCf conditions were similar with the rates under corresponding steady conditions, and no effects of seawater acidification were found (Fig. 5g and h).

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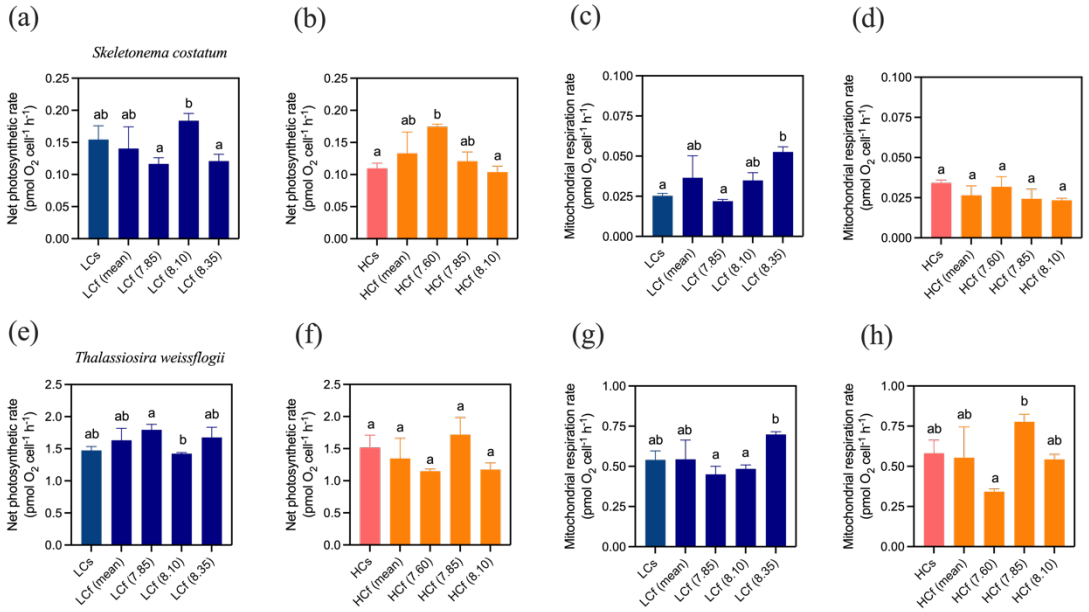

160 Figure 5. Net photosynthetic rate and mitochondrial respiration rate of *Skeletonema costatum* and *Thalassiosira weissflogii* cells grown under steady ambient pH/pCO_2 (LCs), fluctuating ambient pH/pCO_2 (LCf), steady future pH/pCO_2 (HCs), and fluctuating future pH/pCO_2 (HCf). Values are the means ± SD of triplicate cultures. The different letters indicate significant ($p < 0.05$) differences among treatments. In the fluctuating regime, net photosynthetic rate at each pH level was measured.

3.4 Fatty acid composition

165 The proportion of saturated FA (SFA) of *S. costatum* slightly decreased at lower pH, without significant changes in monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) proportions (Fig. 6a). Effects of pH fluctuation were only observed for PUFA of *S. costatum* at ambient pH level, with 14% lower proportion under LCf condition. FA compositions of *T. weissflogii* were markedly altered by seawater acidification rather than pH fluctuation, with 21% lower SFA and twofold increase in PUFA compared with ambient pH level (Fig. 6b). The decrease in SFA proportion of *T. weissflogii* was
170 contributed by all main SFA except C17:0, which showed higher proportion under seawater acidification conditions (Fig. 7). Enhanced PUFA was mainly resulted from higher eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) under seawater acidification conditions regardless of steady or fluctuating regimes.

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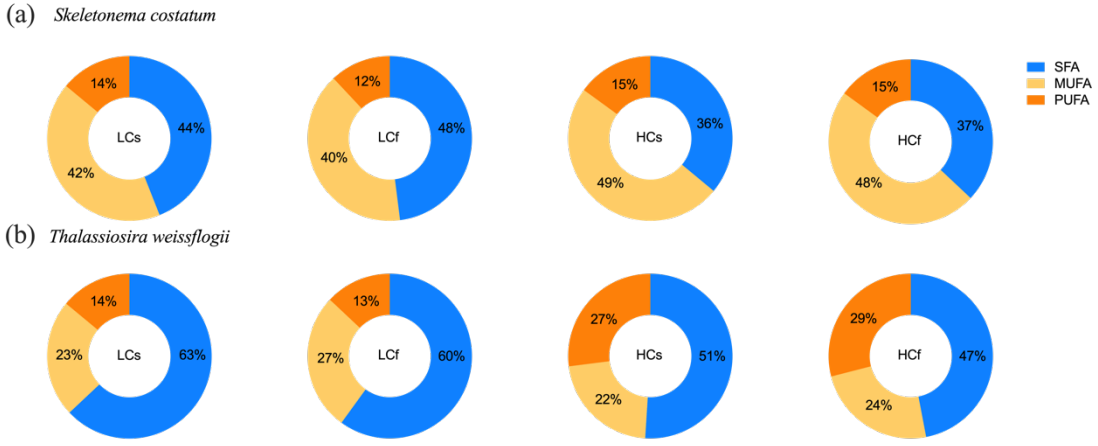

Figure 6. Saturated FA (SFA), monounsaturated FA (MUFA), and polyunsaturated FA (PUFA) proportions of *Skeletonema costatum* and *Thalassiosira weissflogii* cells grown under steady ambient pH/pCO_2 (LCs), fluctuating ambient pH/pCO_2 (LCf), steady future pH/pCO_2 (HCs), and fluctuating future pH/pCO_2 (HCf). Values are the means of triplicate cultures.

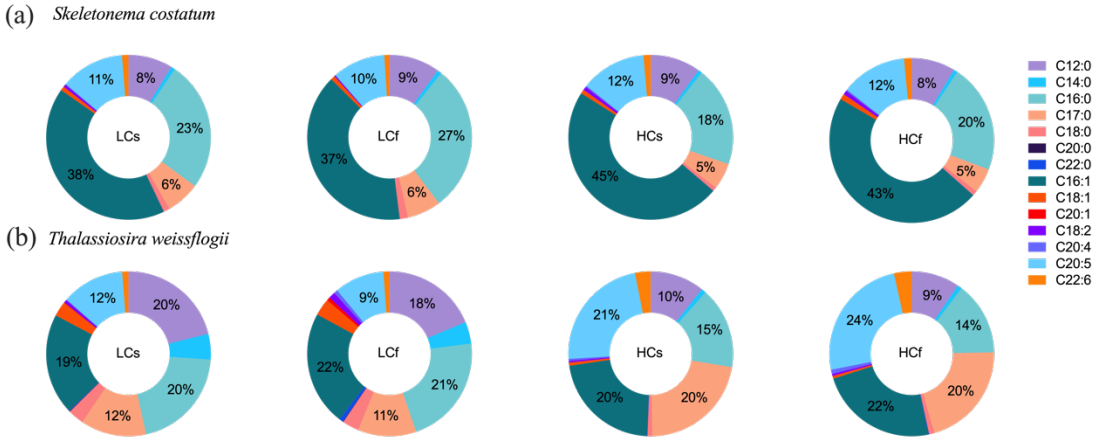

Figure 7. FA composition of *Skeletonema costatum* and *Thalassiosira weissflogii* cells grown under steady ambient pH/pCO_2 (LCs), fluctuating ambient pH/pCO_2 (LCf), steady future pH/pCO_2 (HCs), and fluctuating future pH/pCO_2 (HCf). Values are the means of triplicate cultures.

4 Discussion

4.1 Effects of pH changes on photosynthesis of two diatoms

The amplitude of pH changes in coastal regions could be greater than 1 unit within 24 hours (Duarte et al., 2013), which outdistanced the expected 0.3 units drop of pH by the end of this century (Gattuso et al., 2015). The high frequency of pH changes in coastal waters might potentially impact the acclimation and adaptation of phytoplankton to ocean acidification

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(Schaum et al., 2016). There have been limited researches investigating effects of short-term dynamic changes in pH on phytoplankton, in which neutral or positive effects were usually observed (Li et al., 2016; Li et al., 2021). In these studies, the holistic response of phytoplankton to fluctuating pH was investigated, but the specific response to different pH levels in the fluctuating regime was usually missing. However, the high pH/low CO_2 period might show negative effects when the duration of treatment extends from several to 24 hours.

The diffusion boundary layer (DBL) exists at the interface of cells, in which the microenvironment is different from the bulk seawater. For example, pH in DBL could increase substantially when photosynthesis happens inside the cell, and larger cells could experience more significant changes in pH within DBL (Chrachri et al., 2018). Thus, just like the condition in coastal waters, stable seawater carbonate chemistry is not realistic in DBL of large cells. For this reason, it seems reasonable to expect neutral or positive effects of pH fluctuation on coastal phytoplankton. Indeed, our previous comparative-study provide experimental evidence for this hypothesis. We found the diurnal pH fluctuation depressed the growth and photosynthesis of oceanic diatom under either ambient or elevated CO_2 conditions, whilst coastal diatom *T. weissflogii* was insensitive to pH fluctuation, even enhanced production rate of particulate organic carbon was observed in fluctuating regime (Li et al., 2016). In line with this, *T. weissflogii* was found to be tolerant to decreased pH/increased pCO_2, while seawater alkalization significantly inhibited growth when medium pH was greater than 8.44 (Li et al., 2019).

In our previous study, we manipulated seawater carbonate chemistry with a high frequency of pH change, and a whole period of pH fluctuation was set as 24 hours (Li et al., 2016). Here, the period was set as 5 days, and we found the net photosynthetic rate of *T. weissflogii* was unaltered regardless of the pH levels at which cells were cultured in the fluctuating regime. For *S. costatum*, the photosynthetic rate varied in a way dependent on the mean pH of the fluctuating regime. The maximum photosynthetic rate of *S. costatum* was observed at pH 8.1 for LCf cultures, and the rate decreased with increasing pH from 7.6 to 8.1 for HCf cultures. In spite of this, there were no difference in the average photosynthetic rate between steady and fluctuating regimes at ambient or elevated CO_2 levels. Similarly, the mitochondrial respiration rates were similar when comparing the average rate of cells grown under fluctuating condition and corresponding steady condition, although the rate was more sensitive to pH changes than photosynthetic rate. Based on the results of photosynthesis in the present study, effects of seawater acidification could be overestimated in some regions if the prediction doesn't take pH/pCO_2 variability into account. If the steady regime was used to simulate coastal acidification, inhibited primary production could be observed for some species. However, no effects were found when the coastal acidification was simulated more realistically in a fluctuating regime.

4.2 Fatty acid composition was altered by pH mean level

Omega-3 long-chain essential fatty acids are integral to key functions in aquatic and terrestrial organisms, which are directly or indirectly contributed by phytoplankton in marine ecosystem (Hixson and Arts, 2016). Among these fatty acids, DHA and EPA are well known due to their benefits in enhancing nutritional quality of marine primary consumers (Kainz et al., 2004), especially herbivorous copepods and rotifers which are prey for secondary consumers such as fishes and

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crustaceans. The only way of obtaining essential FAs for marine animals is through their diet, as they cannot synthesize them *de novo* (Brett and Müller-Navarra, 1997). Diatoms are the main producers of EPA in marine ecosystem, which play a critical role in growth, development, and reproduction of marine consumers (Budge et al., 2014). EPA is the major PUFA in both species tested here, with its proportion accounting for more than 10% in total FA under all conditions. DHA is the second most abundant PUFA in two diatoms, although its proportion is much lower than EPA.

Kinds effects of ocean acidification on FAs have been documented in previous studies. In terms of FA composition, different phytoplankton strains of one species appeared to respond to increased CO_2 in a varied way. For instance, the EPA and DHA fractions of total FA in a highly CO_2-tolerant strain of *T. weissflogii* were lower when cells were cultured under 5%, 10%, and 20% CO_2, compared with control air condition (Ishida et al., 2000). While EPA and DHA or total PUFA proportions of *T. weissflogii* (CCMP2599) were not altered when pCO_2 levels increased from 320 to 690 and 2900 ppm (King et al., 2015).

In the present study, we also found species-specific responses of FA proportion to seawater acidification. PUFA and MUFA of *S. costatum* were unaltered by increased pCO_2, whilst PUFA of *T. weissflogii* increased substantially at elevated CO_2 level. The increased proportion of PUFA was mainly attributable to higher EPA and DHA proportions. Given the varied responses among species, the effects of ocean acidification on FA composition of phytoplankton community would be mediated by community structure. In a mixed phytoplankton assemblage including *T. weissflogii*, seawater acidification was conducive to the accumulation of unsaturated FAs, and this change could be transferred to higher trophic level through marine food web (Wang et al., 2017). However, for copepods fed a high-pCO_2 *Thalassiosira pseudonana* diet, a decrease in both copepod somatic growth and egg production was found, which was caused by lower PUFA in their diet (Rossoll et al., 2012). Thus, seawater acidification might have far-reaching consequences for marine ecosystem functioning through altering intracellular macromolecules in primary producers.

5 Conclusions

Although temperature, light intensity, and nutrient limitation usually have prominent influences on photosynthetic performance and nutritional quality of primary producers, the regulating effects of seawater acidification should not be ignored, especially given the cascading effects throughout marine food webs. In the present study, the growth and most parameters of two typical coastal diatom were impacted by neither decreased pH nor pH fluctuation, indicating their tolerance to pH changes. Nevertheless, fatty acid composition of *T. weissflogii* was altered by seawater acidification, with PUFA proportion increased twofold compared to ambient CO_2 condition. Although the deterioration of nutritional quality (Rossoll et al., 2012) and lower production of PUFA (Hixson and Arts, 2016) were projected in the more warmed and acidified ocean, our results suggest that seawater acidification might increase EPA and DHA production with unaltered growth and photosynthesis in *T. weissflogii*-dominant regions. Furthermore, taking dynamic carbonate chemistry into account would help investigate and predict consequences of coastal acidification more properly.

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Data availability. Data presented in this study have been deposited in the Zenodo repository: https://doi.org/10.5281/zenodo.13142180 (Shang et al., 2024).

Author contributions. YS, JQ: methodology, formal Analysis, investigation, writing-original draft; YW, XW, YZ: investigation; JX, DZ: conceptualization, writing-review & editing, supervision; FL: conceptualization, methodology, validation, investigation, writing-review & editing, funding acquisition.

Competing interests. The authors declare that they have no conflict of interest.

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