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Response of the red algae *Pyropia yezoensis* grown at different light intensities to CO_2-induced seawater acidification at different life cycle stages

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ABSTRACT

Increasing CO_2 levels in the surface water of oceans are expected to decrease oceanic pH and lead to seawater acidification. The responses of macroalgaea to this acidification of coastal waters have been studied in detail; however, most reports have focused on the adult stage only, while ignoring other life cycle stages. In this study, the economically important seaweed species *Pyropia yezoensis* was cultured under two CO_2 concentrations (ambient CO_2: 400 μatm; elevated CO_2: 1000 μatm) and two light intensities (low light intensity: 80 μmol photons m^{-2} s^{-1}; and high light intensity: 240 μmol photons m^{-2} s^{-1}). The effects on the growth and photosynthetic performance of *P. yezoensis* were explored at different life cycle stages. Relative growth rates were significantly elevated at the conchocelis stage under high light intensity and elevated CO_2 concentration. Moreover, the P_{max} of *P. yezoensis* was also increased under high light intensity. However, this positive effect inversed at the thallus stage. The relative growth rate, relative electron transport rate (rETR), and net photosynthetic rate decreased at the thallus stage in response to high CO_2 concentration. Under low light intensity, elevated CO_2 concentration significantly increased the relative growth rates of conchocelis and thallus stages. These were 269% and 45% higher at elevated CO_2 concentration compared with ambient CO_2 concentrations, respectively. The Chl *a* and phycoerythrin levels were also higher under elevated CO_2 level at the conchocelis stage. However, the rETR for the thallus stage was elevated under low light. This suggests that seawater acidification could positively affect algae at low light conditions (especially at the conchocelis stage). Different growth stages of *P. yezoensis* may respond differently to seawater acidification and changes of light intensity. Thalli growth stage, stocking density, and seawater depth should be considered in different areas to optimize the primary production of macroalgae.

1. Introduction

Seawater acidification is the result of increased oceanic uptake of atmospheric CO_2 [1] and evidence suggests that the oceans absorb approximately 1/3 of the atmospheric CO_2 [2]. This results in a decrease of surface-ocean pH by approximately 0.1 units [3]. Atmospheric CO_2 concentrations are expected to reach 800–1000 ppmv by the end of this century [4]. Consequently, the CO_3^{2-} concentrations will decrease by 56%, while CO_2 and HCO_3^- concentrations will increase by 192% and 14%, respectively. The seawater pH will decrease to 7.65 under high CO_2 levels [4]. In recent years, the responses of coastal algae to the increasing seawater acidification have drawn much attention due to the economic impact of the coastal ecosystem and its importance for coastal primary productivity.

Macroalgae account for about 10% of the total primary productivity of the oceans and they are the main primary producers of coastal regions, thus providing important structural components for organisms in coastal waters. Carbon enrichment can significantly increase the growth of seaweeds. Many studies showed that responses to carbon enrichment depend on physiological mechanisms associated with the utilization of dissolved inorganic carbon (DIC) [5–7]. Most seaweed species can utilize both CO_2 and HCO_3^- from the surrounding seawater as sources of inorganic carbon for photosynthesis [8,9]. As a consequence, seawater acidification could enhance seaweed photosynthesis

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and productivity [10,11]. Atmospheric CO_2 affects the interconversion of HCO_3^- and CO_2 by carbonic anhydrase, which affects the photosynthetic processes of algae [7,10]. Light energy is absorbed by the light-harvesting complex and then transferred to the reaction centers. This process is related to the content of pigments in seaweeds.

Macroalgal growth has also been reported to be negatively affected by seawater acidification [12]. The effect of seawater acidification is modulated by other abiotic factors, such as temperature, nutrients, and light intensity [12–14]. In recent years, the ecological impacts of CO_2 levels on macroalgal growth and the photosynthesis of the adult stages of algae have been studied extensively [15–19]. However, the physiological impacts of the CO_2 concentrations on juvenile stages need to be explored.

Pyropia yezoensis (Ueda) M.S.Hwang & H.G.Choi is one of the economically important seaweed species and is cultivated on a large scale, especially in China, Korea, Japan, and other Asian countries [20]. It has a biphasic life cycle with microscopic, sporophytic filaments (termed the conchocelis phase) and macroscopic, foliose, gametophytic blades (termed the thallus stage). In the natural, the filaments live in the limestone layer of the shells and spend the summer. In the autumn, spores are released to attach to the rocks and grow into fronds. Clam shells and oyster shells are used as substrates to culture filaments to achieve the purpose of artificial seedlings of *P. yezoensis*. However, this method is limited by the insufficient source of shells, and because a large number of shells are processed in seedlings, the procedures are cumbersome, so the free filamentous is cultivated to reform the seedling raising technology [21]. Conchocelis filaments cultured in either seawater or liquid culture medium are called free-living conchocelis, and grow very well at 20–25 °C under 60–100 μmol photons m^{-2} s^{-1}. However, optimal growth of thallus occurs at 10–15 °C under 100–300 μmol photons m^{-2} s^{-1}. Thalli are fixed on ropes and float on the seawater surface, forming a cultivation mesh curtain. They are exposed to different intensities of sunlight depending on the number of their branches and their mass, which can cause them to sink below the surface. Their growth toward larger depths can further influence this. This is an important factor because underwater light intensity decreases with increasing sea depth. Plant photosynthesis has a light compensation point, and algae can grow and develop only under light intensities above the light compensation point [22]. The depth at which the compensation point is located in the seawater is called the compensation depth [23]. Algae can only grow and develop above the compensation depth, which varies with the light intensity (weather, tidal changes, and seawater turbidity) and the seasons.

Both the photosynthetic efficiency and growth rates of *P. yezoensis* are enhanced by elevated CO_2 concentrations [24]. It has also been reported that *P. yezoensis* growth is inhibited by high light intensities [12]. However, little is known about how specific combinations of seawater acidification and light intensity affect *P. yezoensis*. Hence, the present study investigated the physiological response of different life cycle stages of *P. yezoensis* to different CO_2 concentrations and light intensities. The growth and photosynthetic parameters of both free-living conchocelis and thalli were compared and important information for *P. yezoensis* cultivation is reported.

2. Materials and methods

2.1. Plant materials

The thalli of *P. yezoensis* (Ueda) M.S.Hwang & H.G.Choi were collected from the farming area of Lianyungang, Jiangsu province, China, at a depth of 20 cm and samples were transported to the laboratory within 3 h. Thalli were cut into segments (0.7 × 0.7 cm) and cultured in 500 ml bottles containing filtered seawater that had been enriched with Von Stosch enrichment (VSE) and essential nutrients, at constant salinity (30 psu). The natural seawater was sourced from the nearby algae cultivation area. The supplemented nutrient content is similar to the concentration of nitrogen and phosphorus that are typically added under *P. yezoensis* field cultivation (N: 7.1 μmol L^{-1} and P: 0.21 μmol L^{-1}). Cultures were continuously aerated and the medium was changed daily. The light intensity was set to 80 μmol photons m^{-2} s^{-1} (light dark cycle 12 h:12 h) at a temperature of 15 °C. Free-living conchocelis were obtained from the Key Laboratory of Marine Biotechnology of Jiangsu Province, Jiangsu Ocean University, China. Conchocelis were crushed into uniform shell sporangia, and were cultured in 500 ml bottles with sterilized seawater that had been enriched with VSE medium and had constant salinity (30 psu). This enriched seawater was replaced each day. The same temperature and light conditions were used for conchocelis and thalli culture.

2.2. Experimental setup

P. yezoensis samples that represent two life cycle stages (free-living conchocelis and thallus) were used in the experiment. After pre-incubation at 80 μmol photons m^{-2} s^{-1} (light dark cycle 12 h:12 h) at a temperature of 15 °C for approximately 2 days, conchocelis and thalli of *P. yezoensis* were cultured under two CO_2 conditions (LC: 400 ppmv; and HC: 1000 ppmv) and light intensities (LL: 80 μmol photons m^{-2} s^{-1}; and HL: 240 μmol photons m^{-2} s^{-1}). Due to the changing climate, the light intensity received by the macroalgae changes [12]. The low light and high light conditions represent the average light intensities of the growth of *P. yezoensis* under these conditions. The 400 and 1000 ppmv pCO_2 represent the current atmospheric CO_2 concentrations and those expected by the end of this century, respectively. The low CO_2 level was obtained by bubbling ambient air and the high CO_2 level was obtained using a CO_2 plant incubator (HP1000G-D, Ruihua Instruments, Wuhan, China) to maintain the stability of the carbonate system. pH levels of 8.18 and 7.83 were maintained in LC and HC cultures, respectively; variations were < 0.05. Each condition was provided in a 500 ml bottle with medium made from natural seawater, that was enriched with VSE at constant salinity (30 psu), and replaced every two days. Three flasks per treatment were evenly and randomly cultivated in three same incubators (GXZ-500C, Ningbo, China) [[5]25]. In this study the design, performance, analysis, and reporting of experimental procedures follow the “Guide to Best Practices for Ocean Acidification Research and Data Reporting [26]”. Thalli were incubated at 15 °C, which simulated the optimum temperature for the growth of *P. yezoensis* in the cultivation areas. The growth rate of thalli was determined every two days, and the photosynthesis, and pigments, chlorophyll fluorescence, and soluble protein content were determined after 1 week.

2.3. Carbonate system parameter determination

The pH of the seawater in the flasks was recorded using a pH meter probe (pH 700, Eutech Instruments, Singapore), calibrated with standard National Bureau of Standards (NBS) buffers (pH = 4.01, 7.00, and 10.01 at 25 °C, Thermo Fisher Scientific Inc., Springfield, USA). The pH meter was re-calibrated after 48 h. pH in the seawater inside flasks was measured after 0, 40, 44, and 48 h of culture. Other carbonate system parameters were indirectly calculated via CO2SYS [27] where the equilibrium constants of K_1 and K_2 were used to calculate the carbon acid dissociation [28]. Total alkalinity (TA) is a measure of the seawater buffer capacity and one of the four parameters of the seawater carbonate system. TA was determined via Gran acidimetric titration on a 25 ml sample with a TA analyzer (AS-ALK1, Apollo SciTech, CO, USA). The TA analyzer was regularly calibrated every two weeks with certified reference materials from the laboratory of Andrew G. Dickson (Scripps Institute of Oceanography, USA) at a precision of 2 μmol kg^{-1} [29].

2.4. Growth measurements

Free-living conchocelis were ground in a shredder for approximately 1 min; then, their growth was determined by measuring the changes in chlorophyll content per unit of volume. Free-living conchocelis were filtered onto GF/F filters (25 mm, Whatman, ANPEL Laboratory Technologies Inc., Shanghai, China), and then extracted in methanol at 4 °C for 24 h. After centrifugation at 5000*g* for 10 min, absorption values of the supernatant were analyzed using an ultraviolet/visible spectrophotometer (Ultrospect 3300 pro, Amersham Bioscience, Uppsala, Sweden). The chlorophyll content was calculated as described by Porra et al. [30]. The growth rate of thalli was determined by measuring changes in area using a digital camera (Canon, EOS 5DSR) and Photoshop CS3 software. The relative growth rate (RGR) was estimated as follows: RGR (% day^{-1}) = Ln (M_t/ M_o) / t × 100, where M_o represents the initial value and M_t represents the value after t days. Sterile seawater (enriched with VSE medium) was renewed after measuring the RGR.

2.5. Photosynthesis determination

The photosynthetic rates of thalli and free-living conchocelis were determined using a Clark-type oxygen electrode (YSI 5300A, USA). The temperature was controlled at a constant 15 °C, using a constant temperature water circulator (DCW-1065, Xianou, China). Thalli were fragmented into circular pieces of about 1 cm, similar to free-living conchocelis, and were then subjected to the above-mentioned culture conditions for 1 h to avoid effects of stress in response to cutting damage [31,32]. Approximately 0.02 g algae segments were transferred into an oxygen electrode chamber, which contained 5 ml natural seawater, enriched with VSE medium, at 15 °C for photosynthesis determination. Similar light penetration was ensured by using quartz tubes, which were made of silicon dioxide and had good light transmission properties. It can be considered that the light intensity inside the quartz tube was the same as the illumination intensity. The measurement was finished within 5 min, during which, the pH did not vary. The decreased oxygen concentration in the seawater was defined as the rate of respiration, and was measured after 2 min of dark adaptation. The same method was used to measure photosynthesis [31,32]. Furthermore, the increased oxygen concentration in seawater was defined as the net photosynthetic rate after an increase of light density. Light density is descried as μmol photons m^{-2} s^{-1} and was adjusted by a double-ended halogen lamp (500 w, 230 v, PHILIPS, Jaingsu, China).

A photosynthesis-irradiation curve (P-E curve) was also obtained during 30 min of different irradiances (100, 200, 300, 400, 500, and 600 μmol photons m^{-2} s^{-1}) and durations of light irradiation.

The maximum net photosynthetic rate (P_{max}) and photosynthetic efficiency (α) were calculated by curve fitting to Eq. (1):

$$P = P_{max} \times \tanh(\alpha \times E/P_{max}) + R_d, \quad (1)$$

where P represents the gross photosynthetic rate and R_d represents the respiration rate.

2.6. Measurement of chlorophyll fluorescence

The chlorophyll concentration is one of the most important indicators for the growth of algae. This concentration was measured using a pulse modulation fluorometer (Water-PAM, Walz, Germany). Actinic light measurements were consistent with the light intensity. Thalli were cultured without light for 15 min, a saturating pulse of light (5000 μmol photons m^{-2} s^{-1}; 0.8 s) was provided, and Fv/Fm was measured. The relative electron transport rate (rETR) was calculated as follows [33]:

$$rETR\ (\mu mol\ e^{-1}\ m^{-2}\ s^{-1}) = DF/Fm' \times 0.15 \times EPFD \quad (2)$$

where DF/Fm′ represents the effective photosynthetic quantum yield of PSII in response to light irradiation, which is a reflection of the original light energy capture efficiency of an open PSII reaction center [34]. EPFD represents the photosynthetically active photon flux density. The coefficient of 0.15 represents the value of FII, which is the fraction of DF/Fm′ directed to PSII including its light harvesting complexes (LHCs) [34]. The rapid light curves were determined under nine different light conditions. The light curve fitted the following equation [35]:

$$rETR = E/(aE^2 + bE + c) \quad (3)$$

where E represents the light intensity and a, b, and c represent adjustment parameters.

The light saturation parameter of light-response (E_k), the photosynthetic efficiency (α), and the maximum relative electron transfer rate ($rETR_{max}$) were calculated as follows:

$$E_k = (c/a)^{1/2} \quad (4)$$

$$\alpha = E/c \quad (5)$$

$$rETR_{max} = E/(b + 2(ac)^{1/2}) \quad (6)$$

2.7. Measurement of soluble protein content

For thalli, approximately 0.02 g of samples from each culture condition were ground in a mortar with 0.1 M phosphate buffer (pH 6.8) and then centrifuged for 10 min at 5000*g*. The supernatant was used to measure the soluble protein content according to the Bradford (1976) assay, using bovine serum albumin as standard [36]. Since the amount of free-living conchocelis is relatively small, 20 ml of algae was concentrated and then ground as mentioned above to measure the soluble protein content.

2.8. Photosynthetic pigments

Chlorophyll *a* concentration was estimated according to Wellburn [37]. For thalli, approximately 0.02 g of samples were extracted in 5 ml of anhydrous methanol in the dark for 24 h at 4 °C. The absorbances were measured at 666 nm and 652 nm, using an ultraviolet/visible (UV/vis) spectrophotometer (Ultrospect 3300 pro, Amersham Bioscience, Sweden). The chlorophyll *a* and b contents (Chl *a*) were calculated according to Porra et al. [[30]]. Approximately 0.08 g of sample was ground with 0.01 g quartz sand in suitable 0.1 M phosphate buffer (pH 6.8). 0.1 M phosphate buffer (pH 6.8) was added to 8 ml for further extraction. The mixture was then centrifuged at 5000*g* for 15 min. The supernatants were used to determine the phycoerythrin (PE) and phycocyanin (PC) concentrations at 455, 564, 592, 618, and 645 nm according to Beer and Eshel [[38]. Since the amount of free-living conchocelis is relatively small, 20 ml of algae was concentrated and extracted as mentioned above for the measurement of photosynthetic pigments.

2.9. Data analysis

Data are described as replicate means ± standard deviations (SD). Each treatment condition used three replicates. Experimental data were analyzed using Origin 7.0 software. Three-way ANOVA was used to analyze the effects of stage, CO_2 and light intensity on carbonate system parameters, RGR, P_{max}, α, E_k, R_d, E_c, ETR_{max}, and Fv/Fm. Two-way ANOVA was used to analyze the effects of CO_2 and light intensity on protein, Chl *a*, PE, and PC of free-living conchocelis and thallus stage of *P. yezoensis* at high light or low light under ambient CO_2 or elevated CO_2. Student's *t*-test was used to compare the difference of protein, Chl *a*, PE, PC at high light or low light conditions under either ambient CO_2 or elevated CO_2 levels at same stage of *P. yezoensis*. And Student's *t*-test was also used to compared carbonate system parameters, RGR, P_{max}, α, E_k, R_d, E_c, ETR_{max}, and Fv/Fm under same conditions at free-living conchocelis and thallus stage of *P. yezoensis* when stage, CO_2 and light

Table 1
Parameters of the seawater carbonate system under high light (HL) or low light (LL) conditions under either ambient CO_2 (LC) or elevated CO_2 (HC) levels of free-living conchocelis and thallus of *P. yezoensis*. DIC = dissolved inorganic carbon, TA = total alkalinity.

		pH	pCO_2 (μatm)	DIC	HCO_3^- (μmol kg^{-1})	CO_2	TA
Free-living conchocelis	HL-LC	8.15 ± 0.01	538.51 ± 2.62	2513.92 ± 23.64	2299.86 ± 19.44	17.77 ± 0.09	2774.42 ± 28.82
	LL-LC	8.19 ± 0.01	706.85 ± 14.23	3613.89 ± 118.99	3285.32 ± 103.67	23.33 ± 0.47	3982.98 ± 135.01
	HL-HC	7.85 ± 0.01	1268.08 ± 77.83	2827.99 ± 190.46	2673.49 ± 179.61	41.85 ± 2.57	3124.01 ± 230.70
	LL-HC	7.77 ± 0.01	1642.28 ± 117.73	3034.68 ± 226.28	2879.58 ± 214.72	54.20 ± 3.89	3124.01 ± 230.70
Thallus	HL-LC	8.33 ± 0.01	400.92 ± 7.11	3067.51 ± 39.59	2692.74 ± 29.54	13.23 ± 0.23	3542.16 ± 51.91
	LL-LC	8.35 ± 0.01	468.56 ± 19.36	3378.39 ± 169.20	2983.31 ± 146.54	15.46 ± 0.64	3851.72 ± 191.39
	HL-HC	7.97 ± 0.03	1134.07 ± 105.12	3435.96 ± 27.27	3215.55 ± 36.39	37.43 ± 3.0.47	3642.30 ± 8.12
	LL-HC	7.98 ± 0.04	1257.90 ± 26.19	3444.85 ± 59.73	3236.87 ± 55.97	41.51 ± 0.86	3626.19 ± 62.38

intensity exerted a significant interactive. Repeated measures ANOVA (RM-ANOVA) was used to analyze pH of the seawater after 0, 15, 19, 23, and 48 h of culture. A 95% confidence interval was set for all tests.

3. Results

3.1. Carbonate system parameters

The results of three-way ANOVA indicated that stage, CO_2, and light intensity exerted a significant interactive effect on pH, DIC, HCO_3^-, and TA ($p < .05$). Under LL, the increased pCO_2 concentration decreased the DIC, TA, and HCO_3^- levels by 579.21, 858.97, and 405.74 μmol kg^{-1}, respectively. While increased CO_2 increased DIC, TA, and HCO_3^- by 314.01, 349.59, and 373.63 μmol kg^{-1} compared with LC at free-living conchocelis stage under HL (Table 1). The pH were decreased 5.13% and 3.68% at HC than at LC under LL and HL condition, respectively. For thallus, under LL, the DIC, HCO_3^-, and CO_2 levels increased at HC by 66.46, 253.56, and 26.05 μmol kg^{-1}, respectively, compared with LC (Table 1). While, they were increased by 368.45, 522.81, and 24.20 μmol kg^{-1} compared with LC, respectively, under HL. At HC the pH were decreased 0.37 and 0.36 compared with LC, respectively, under LL and HL conditions. TA was decreased from 3851.72 ± 191.39 (LC) to 3626.19 ± 62.38 (HC) under LL condition. However, it was increased from 3542.16 ± 51.91(LC) to 3642.30 ± 8.12 (HC) under HL condition. Moreover, the pH value inside flasks did not change significantly during culture ($p > .05$) (48 h; see Table 2).

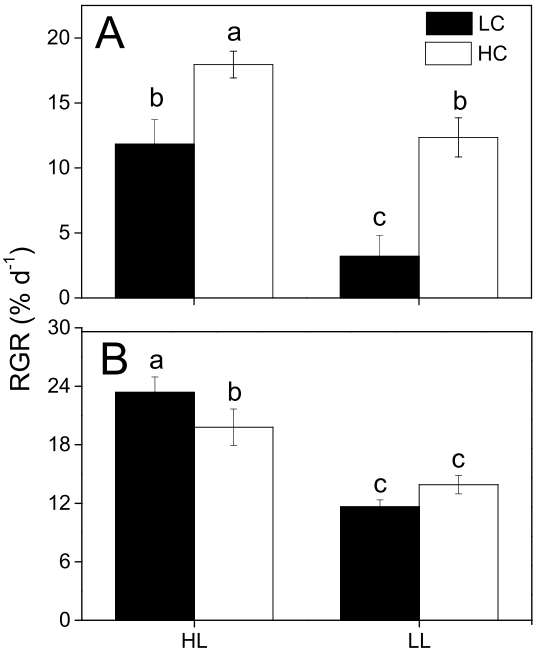

Fig. 1. Relative growth rate (RGR) of free-living conchocelis (A) and thallus (B) of *P. yezoensis* under high light (HL) or low light (LL) under ambient CO_2 (LC) or elevated CO_2 (HC). Significant differences are indicated by different letters. Values represent the mean ± SD (n = 3).

3.2. Growth

Free-living *P. yezoensis* conchocelis and thallus grew steadily during the culture periods. The three-way ANOVA indicated that stage, CO_2, and light intensity exerted no significant interactive effect on RGR ($p > .05$) (Table S2). For free-living conchocelis stage, RGR was significantly higher under elevated CO_2 levels than that under normal CO_2 levels for both light intensities ($p < .05$) (Fig. 1A). Furthermore, RGR increased from 11.83 ± 1.88% to 17.95 ± 1.02% and from 3.21 ± 1.61% to 12.35 ± 1.51% at HL and LL conditions, respectively. However, the RGR of free-living conchocelis was lower at LL conditions compared with HL conditions ($p < .05$), with increases from 3.21 ± 1.61% (LC) to 11.83 ± 1.88% (HC) and 12.35 ± 1.51% (LC) to 17.95 ± 1.02% (HC) under ambient air and elevated CO_2 levels, respectively. For thallus of *P. yezoensis*, under HL, RGR was lower at HC than at LC, while, under LL, RGR was higher at HC than at LC (Fig. 1B). Student's *t*-test showed that the RGR was the highest at LC under HL for free-living conchocelis and thallus stage of *P. yezoensis* ($p < .05$).

Table 2
pH of the seawater after 0, 15, 19, 23, and 48 h of culture under high light (HL) or low light (LL) conditions under either ambient CO_2 (LC) or elevated CO_2 (HC) levels of free-living conchocelis and thallus of *P. yezoensis*.

		15(h)	19(h)	23(h)	48(h)
Free-living conchocelis	HL-LC	8.17 ± 0.00^a	8.15 ± 0.01^a	8.16 ± 0.01^a	8.15 ± 0.01^a
	LL-LC	8.21 ± 0.01^a	8.19 ± 0.01^a	8.17 ± 0.00^a	8.19 ± 0.01^a
	HL-HC	7.84 ± 0.01^a	7.85 ± 0.01^a	7.84 ± 0.01^a	7.85 ± 0.01^a
	LL-HC	7.75 ± 0.01^a	7.77 ± 0.01^a	7.75 ± 0.01^a	7.77 ± 0.01^a
Thallus	HL-LC	8.22 ± 0.03^a	8.31 ± 0.04^a	8.37 ± 0.05^a	8.33 ± 0.01^a
	LL-LC	8.18 ± 0.02^a	8.24 ± 0.01^a	8.33 ± 0.01^a	8.35 ± 0.01^a
	HL-HC	7.81 ± 0.01^a	7.96 ± 0.02^a	7.98 ± 0.04^a	7.97 ± 0.03^a
	LL-HC	7.80 ± 0.01^a	7.93 ± 0.01^a	7.92 ± 0.01^a	7.98 ± 0.04^a

Different letters represent significant differences ($p < 0.05$) after analyzed by RM-ANOVA.

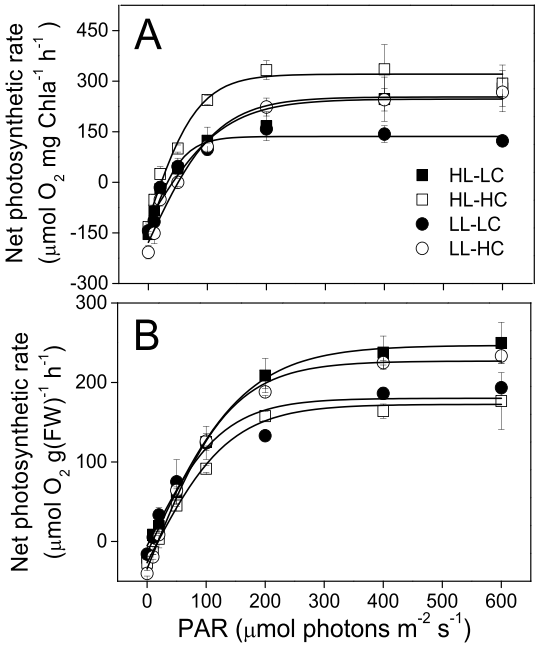

Fig. 2. Net photosynthetic rate and respiration rate of free-living conchocelis (A) and thallus (B) of *P. yezoensis* under high light (HL) or low light (LL) under ambient CO_2 (LC) or elevated CO_2 (HC). Values represent the mean ± SD (n = 3).

3.3. Photosynthesis and respiration rate

The results of three-way ANOVA indicated that stage, CO_2, and light intensity exerted a significant interactive effect on α, and Ec ($p < .05$) (Table S2). For free-living conchocelis, α significantly increased from 3.1 ± 1.3 to 5.3 ± 0.9 at HL under LC and HC conditions, respectively ($p < .05$). While, no significant difference was found at both HC and LC under LL conditions ($p > .05$). Under HL, E_c declined from 41 ± 12 to 21 ± 3 at LC and HC condition, respectively ($p < .05$). However, E_c was 56.67% higher at HC than at LC under the LL condition ($p < .05$). For thallus stage, no significant difference was found between LC and HC at HL or LL for α ($p > .05$). E_c significant increased from 9 ± 1 to 18 ± 1 at HL under LC and HC conditions, respectively ($p < .05$), while it was significant increased from 5 ± 2 to 18 ± 1 at LL under LC and HC conditions, respectively ($p < .05$). The P_{max}, and R_d of free-living conchocelis significantly increased compared with thallus by Student's *t*-test regardless the light intensity and CO_2 concentrations (Fig. 2, Table 3). For free-living conchocelis, under the LL condition, the P_{max} significantly increased at the HC level compared with samples cultured at LC ($p < .05$). With an increase of 20.13%, R_d was higher at HC compared with LC under LL conditions. No significant difference was observed for E_k under HL at HC and LC, while, it was significantly increased from 58 ± 10 to 116 ± 22 at LC and HC conditions under LL conditions, respectively ($p < .05$) (Table 3). For thallus stage, under the LL condition, the P_{max} significantly increased at the HC condition compared with that at LC ($p < .05$) (Table 3), while, opposite trend was found under HL condition for P_{max}. It was significantly declined 22.98% at HC than at LC condition ($p < .05$). With an increase of 76.47% and 46%, respectively, R_d was significantly higher at HC compared with LC under LL and HL condition ($p < .05$). The similar trends were observed in E_c of thallus. No significant difference was found between LC and HC at HL or LL for E_k ($p > .05$).

3.4. Chlorophyll fluorescence parameters

The results of three-way ANOVA indicated that stage, CO_2, and light intensity had a significant interactive effect on $rETR_{max}$ and α ($p < .05$) (Table S4). For free-living conchocelis, under LL conditions, the $rETR_{max}$ significantly increased to 43.57 ± 2.52 at HC condition compared with LC ($p < .05$) (Table 4). While, it was significant decreased to 33.33 ± 2.42 (HC) from 56.69 ± 1.39 (LC) under HL condition ($p < .05$) (Table 4). Furthermore, under HL conditions, the α of free-living conchocelis was 33.33% lower at HC compared with conchocelis cultured at LC conditions ($p < .05$). However, it was 66.66% higher at HC than that cultured at LC conditions under LL ($p < .05$). Compared with free-living conchocelis, thallus had higher $rETR_{max}$ for thallus, the $rETR_{max}$ significantly increased under LC than at HC condition at LL and HL ($p < .05$) (Table 4). The change of α was not statistically significant ($p > .05$) (Fig. 3, Table 4). Student's *t*-test showed that $rETR_{max}$ was increased by 78.77 at free-living conchocelis compared with that at thallus stage at LC under LL condition. No significant difference was found between LC and HC at HL or LL for E_k at free-living conchocelis stage ($p > .05$). For thallus, E_k was 15.73% lower at HC than that at LC under HL conditions ($p < .05$), and the change of α was not statistically significant ($p > .05$) (Fig. 3, Table 4).

Three-way ANOVA indicated that stage, CO_2, and light intensity exerted no significant interactive effect on Fv/Fm ($p > .05$) (Table S5). Under LL conditions, a slight increase was observed when free-living conchocelis were cultured under HC conditions. While, it was lower under HL and HC condition than under LC condition. For thallus, it was significant higher 7.41% under LL condition than HL condition at LC ($p < .05$) (Fig. 4B).

3.5. Soluble protein

For free-living conchocelis two-way ANOVA showed that light intensity and CO_2 had no significant interactive effect ($p > .05$), while both light intensity and CO_2 had main effects on the soluble protein content ($p < .05$) (Table S6). The soluble protein content decreased from 10.17 ± 0.70 (LC) to 8.13 ± 0.39 (HC) under the HL condition, and no significant difference was found between HC and LC under the LL condition ($p > .05$) (Fig. 5A). Two-way ANOVA showed that light intensity and CO_2 had a significant interactive effect on soluble protein

Table 3
Maximum net photosynthetic rate (P_{max}), light use efficiency (α), light saturation parameters (E_k), respiration rate (R_d), and light compensation point (Ec) of free-living conchocelis and thallus of *P. yezoensis* under high light (HL) or low light (LL) under ambient CO_2 (LC) or elevated CO_2 (HC). Significant differences are indicated by different letters (Student's *t*-test). Values represent the mean ± SD (n = 3).

		P_{max}	α	E_k	R_d	E_c
Free-living conchocelis	HL-LC	372 ± 73^{ab}	3.1 ± 1.3^{c}	144 ± 90^{ab}	115 ± 22^{b}	41 ± 12^{abc}
	HL-HC	433 ± 51^{a}	5.3 ± 0.9^{a}	85 ± 22^{b}	111 ± 13^{b}	21 ± 3^{c}
	LL-LC	279 ± 26^{b}	4.9 ± 1.2^{ab}	58 ± 10^{b}	143 ± 12^{a}	30 ± 5^{b}
	LL-HC	432 ± 33^{a}	3.8 ± 0.6^{bc}	116 ± 22^{a}	179 ± 28^{a}	47 ± 2^{a}
Thallus	HL-LC	261 ± 24^{a}	1.6 ± 0.1^{b}	163 ± 2^{a}	14 ± 1^{c}	9 ± 1^{b}
	HL-HC	200 ± 15^{b}	1.4 ± 0.2^{b}	142 ± 34^{ab}	26 ± 5^{b}	18 ± 1^{a}
	LL-LC	187 ± 6^{b}	1.7 ± 0.6^{ab}	122 ± 48^{ab}	8 ± 1^{d}	5 ± 2^{c}
	LL-HC	261 ± 8^{a}	1.9 ± 0.1^{a}	139 ± 5^{b}	34 ± 3^{a}	18 ± 1^{a}

Table 4
Maximal electron transport rate ($rETR_{max}$), light use efficiency (α), and light saturation parameters (E_k) of free-living conchocelis and thallus of *P. yezoensis* under either high light (HL) or low light (LL) under ambient CO_2 (LC) or elevated CO_2 (HC). Significant differences are indicated by different letters (Student's *t*-test). Values represent the mean ± SD (n = 3).

		$rETR_{max}$	α	E_k
Free-living conchocelis	HL-LC	56.69 ± 1.39^{a}	0.06 ± 0.00^{a}	936.76 ± 44.75^{a}
	HL-HC	33.33 ± 2.42^{c}	0.04 ± 0.00^{b}	779.59 ± 136.95^{a}
	LL-LC	24.58 ± 0.72^{d}	0.03 ± 0.00^{b}	753.13 ± 63.45^{a}
	LL-HC	43.57 ± 2.52^{b}	0.05 ± 0.00^{a}	793.98 ± 50.95^{a}
Thallus	HL-LC	88.63 ± 5.46^{ab}	0.06 ± 0.00^{a}	1513.12 ± 167.45^{ab}
	HL-HC	74.70 ± 5.31^{b}	0.06 ± 0.01^{a}	1275.31 ± 224.63^{b}
	LL-LC	103.35 ± 8.22^{a}	0.06 ± 0.00^{a}	1697.63 ± 231.32^{a}
	LL-HC	84.29 ± 6.10^{b}	0.06 ± 0.01^{a}	1430.49 ± 243.99^{b}

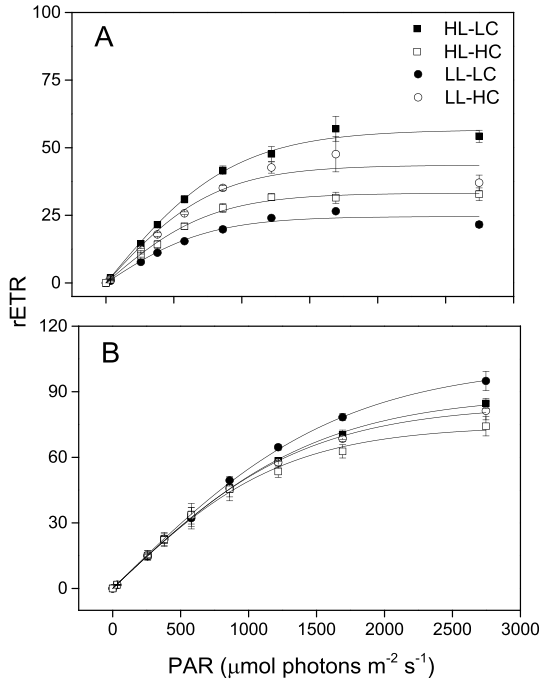

Fig. 3. Relative electron transport rate (rETR) of free-living conchocelis (A) and thallus (B) of *P. yezoensis* under high light (HL) or low light (LL) under ambient CO_2 (LC) or elevated CO_2 (HC). Values represent the mean ± SD (n = 3).

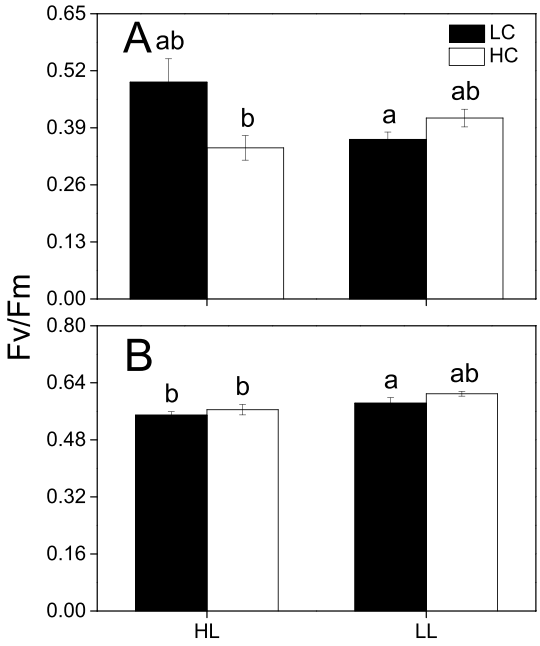

Fig. 4. The maximal quantum yield (Fv/Fm) of free-living conchocelis (A) and thallus (B) of *P. yezoensis* under either high light (HL) or low light (LL) under ambient CO_2 (LC) or elevated CO_2 (HC). Significant differences are indicated by different letters. Values represent the mean ± SD (n = 3).

content for thallus stage ($p < .05$) (Table S6). The soluble protein content was 35.67% higher at HC than that at LC under LL conditions ($p < .05$). While, no significant difference was found both at LC and HC condition under HL ($p > .05$) (Fig. 5B).

3.6. Photosynthetic pigments

For free-living conchocelis, light intensity and CO_2 had an interactive effect on Chl *a* and PE ($p < .05$) (Fig. 6A,C) (Table S7). The contents of both Chl *a* and PE significantly increased under HC compared with LC under LL conditions ($p < .05$) (Fig. 6A, C), while no significant differences were observed between HC and LC under HL conditions ($p > .05$) (Fig. 6E). For thallus, light intensity and CO_2 also had an interactive effect on Chl *a* and PE ($p < .05$) (Fig. 6B,D) (Table S7). The content of Chl *a* was 23.06% lower at LC than at HC under HL condition (Fig. 6B), while, it was 29.97% higher at LC than that at HC under LL condition. The PE contents were significantly higher (by 27.87% and 44.97%) at HC compared with the LC condition under HL and LL ($p < .05$) (Fig. 6D). No significant difference was found between HC and LC under LL and HL conditions on PC ($p > .05$) (Fig. 6F).

4. Discussion

Changes of chlorophyll content and photosynthetic parameters of algae are important indices with which the level of algal absorption and the conversion of light energy can be determined, and this indicates the tolerance to light intensity [39] [40]. A high light intensity provides more energy for the photosynthesis of algae and for growth. Due to environmental climate change, coupled with their life in the intertidal zone, the light intensity the macroalgae are exposed to also changes [31]. Seawater acidification exerts an important impact on primary producers of marine ecosystems, especially on macroalgal species, such as *P. yezoensis* [31]. In the present study, differences in carbonate system parameters between light treatments and between conchelis and thallus treatments were minor in comparison to differences between CO_2 treatments suggesting that CO_2 had a greater impact on the seawater carbonate system. And in our experiment, elevated CO_2 enhanced conchocelis growth regardless of light intensity, while thallus growth was reduced at low light. The decrease in growth could be due to mechanisms of photoinhibition [41]. Conchocelis may be more resistant to photodamage due to their increased carbon fixation capability, although the PSII photochemical activity was affected by HL conditions. The net photosynthetic rate were significantly enhanced for *P. yezoensis* at the conchocelis stage and in a controlled seawater carbonate system

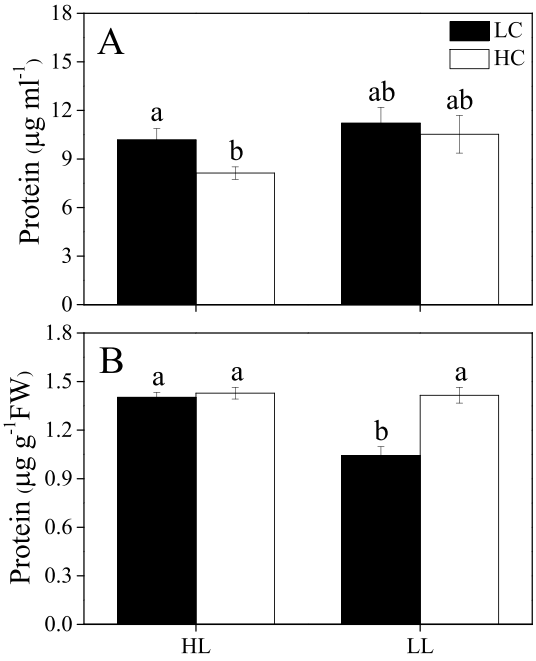

Fig. 5. Soluble protein contents of free-living conchocelis (A) and thallus (B) of *P. yezoensis* under either high light (HL) or low light (LL) under ambient CO_2 (LC) or elevated CO_2 (HC). Significant differences are indicated by different letters. Values represent the mean ± SD (n = 3).

under high light stress at HC provided further evidence to support this (Fig. 1A) [42]. Both the soluble protein content and light compensation point decreased. The significant increase of the light use efficiency under HC compared with LC under HL also corroborated this hypothesis, which suggests that elevated CO_2 levels can alleviate the negative effects of high light intensity on conchocelis. The opposite impact was observed at the thallus stage. Under HL conditions, a significant decrease was found in the RGR and photosynthetic rate of thallus under HC conditions, which is possibly because the elevated CO_2 concentration significantly decreased NifH levels compared with low light under HL conditions. Pigment contents including Chl *a*, PE, and PC were significantly increased at HC compared with LC under HL conditions to capture more light energy and to compensate for the reduced light energy efficiency and electron transfer rate. Similar results were also reported for *Ulva lactuca* by Liu et al. [43] and Olischläger et al. [44], respectively. Simultaneously, the O_2-based photosynthetic efficiency was higher than ETR-based photosynthetic efficiency in all conditions because non-photochemical quenching could occur in PS II reaction centers [45]. Other quenchings such as energy dependent quenching and thermal energy dissipation also could alter the relationship between photochemical and fluorescence yields [46]. And the light saturation parameters based on rETR was also about 7–10 times higher than that based on photosynthetic, especially when there is less utilization of inorganic carbon, which indicated that excessive electron flow was not used for an increase in gross O_2 uptake, the Mehlerascorbate-peroxidase reaction, and the photosynthetic carbon oxidation enhanced at high irradiance or low inorganic carbon [47,48]. The difference in results observed for both stages could be due to the different response mechanism of thallus to light intensity.

Under LL conditions, the growth significantly exceeded that at HC compared with LC for both conchocelis and thallus stages. This might be due to the energy saved by the down-regulation of CCM operation [49] as well as the N_2 fixation, which is enhanced at limited light levels [42]. Further research also showed that high levels of CO_2 significantly increase the growth rates of *P. yezoensis* due to the higher resulting rates of nutrient uptake [50]. And in the sea water carbonate system under high CO_2 concentration the pH, concentration of HCO_3-, and total alkalinity were declined, and the CO_2 was increased compared with that under ambient CO_2 level, suggested that the conversion process of HCO_3^- to OH^- and CO_2 during photosynthesis occurs much faster than the release of H^+ from HCO_3^-. Hence, an increase of the CO_2 concentration in the ocean can enhance the photosynthesis and growth of algae [51]. A similar result was reported by Xu et al. [31]. In the present study, under LL conditions, growth was higher under HC conditions in free-living conchocelis. This was likely related to CCMs: in

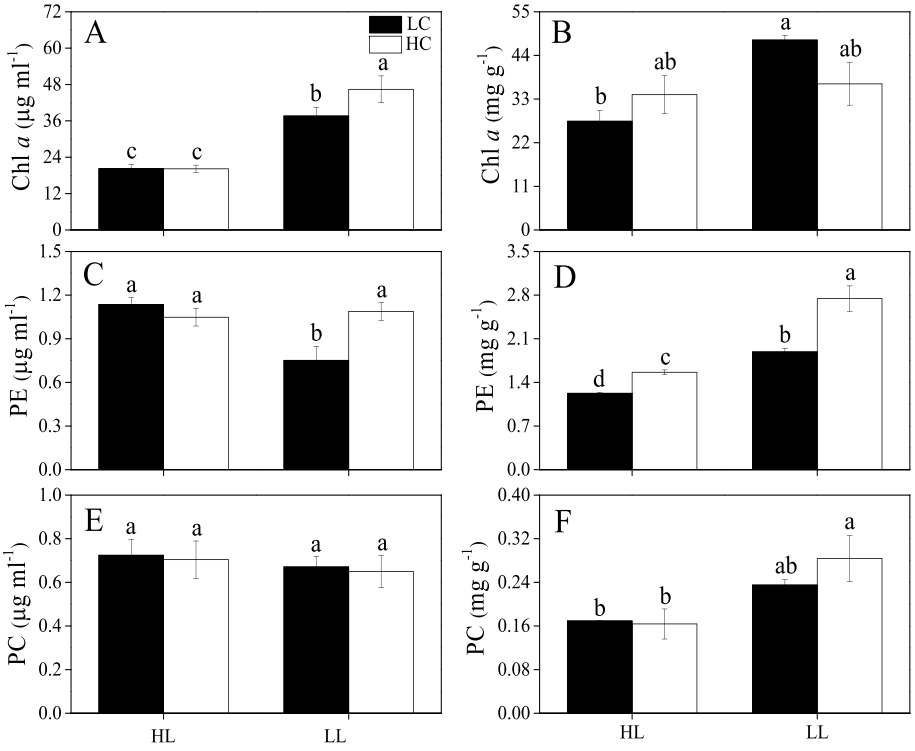

Fig. 6. Chlorophyll *a* (Chl *a*) (A and B), phycoerythrin (PE) (C and D), and phycocyanin (PC) levels (E and F) of free-living conchocelis (A, C, E) and thallus (B, D, F) of *P. yezoensis* under either high light (HL) or low light (LL) under ambient CO_2 (LC) or elevated CO_2 (HC). Significant differences are indicated by different letters. Values represent the mean ± SD (n = 3).

response to higher CO_2 levels, less energy is required to drive CCM, which led to enhanced growth under light-limiting conditions [28]. However, $rETR_{max}$, the quantum yield of PSII (Fv/Fm), Chl *a*, and PE increased. These results indicate that macroalgae utilize multiple mechanisms to adapt to changes of light intensity [52]. It is also possible that part of the energy was absorbed by other processes such as non-photochemical quenching and heat dissipation. The increased respiration rate also supports this hypothesis. For thallus of *P. yezoensis*, the RGR increased at HC under LL. This result is consistent with the growth of free-living conchocelis. However, the rETR for thallus was clearly elevated at LC under LL conditions, suggesting that the electron transport rate was enhanced at these conditions. It is also likely that part of the generated energy was absorbed by the electron transport process, thus inhibiting the growth of thallus under LC and LL conditions. The observed decrease of net photosynthetic rate and soluble protein content also confirmed this result.

The different stages in the algal life cycle have adapted to different light intensities due to the differences in seasonal effects, cloud thickness, water mixing, stratification, transparency, photoperiod, and the water depths they occupy. Seawater acidification can exert a positive effect on the growth of *P. yezoensis* at the free-living conchocelis stage, particularly for LL. In the natural environment, the free-living conchocelis penetrate into the shell and grow in the subtidal zone. Thus, they are produced at low irradiance compared to haploid thalli. This indicates that future CO_2-enriched seawater can significantly stimulate the growth of *P. yezoensis* conchocelis. On the other hand, ocean acidification has a minor stimulating effect on growth of *P. yezoensis* thalli at LL and even reduces it at HL. These results suggest that different stages of *P. yezoensis* may show different responses to seawater acidification and different light intensities. In sea-farming areas, the different growth stages of algae, their stocking density, and a depth-dependent strategy should be considered in different areas to optimize the primary production stages of macroalgae.

5. Conclusion

In the natural environment, increases in CO_2-inducing conditions can mediate the physiological performance of *P. yezoensis*. The RGR of free-living conchocelis significantly increased under elevated CO_2 conditions with intense light, while thallus showed a significant decrease. Conchocelis and thalli had significantly enhanced RGR under low light and elevated CO_2 concentrations compared with ambient CO_2 concentrations. This study provides important information on the physiological performance of different life stages of *P. yezoensis* in response to different CO_2 and light intensity treatments. The presented results offer important insight toward optimizing the primary production of *P. yezoensis* under the constraints of global climate change.

CRediT authorship contribution statement

Jing Ma:Investigation, Formal analysis, Writing - original draft.**Tianpeng Xu**:Investigation.**Menglin Bao**:Investigation. **Huimin Zhou**:Investigation.**Tianzhi Zhang**:Investigation.**Zhenzhen Li**:Investigation.**Guang Gao**:Formal analysis, Writing - original draft.**Xinshu Li**:Methodology.**Juntian Xu**:Methodology, Formal analysis, Writing - original draft.

Declaration of competing interest

The authors declare no conflict of interest.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2020.101950.

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