



Elevated CO₂ concentrations promote growth and photosynthesis of the brown alga *Saccharina japonica*

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Abstract

Non-photochemical quenching (NPQ) is one of the most important photo-protection mechanisms in brown macroalgae. Global warming and ocean acidification are predicted to impact physiological characteristics of marine algae. However, little is known about the effects of co-occurrence of the elevation of $p\text{CO}_2$ and temperature on photochemical capacity, especially regarding photoprotective mechanisms in brown macroalgae. Here, we studied the separate and combined effects of increases in $p\text{CO}_2$ and temperature on the photochemical characteristics and growth performance in sporophytes of the brown macroalga *Saccharina japonica*. The results showed that the NPQ of *S. japonica* is mainly dependent on the xanthophyll cycle (XC) which appears to be related only to the activation of the enzyme violaxanthin de-epoxidase (VDE), and the transthylakoid proton gradient (ΔpH) could not induce NPQ alone. The elevation of $p\text{CO}_2$ reduced NPQ value of *S. japonica* under high-temperature stress. After 60-day cultivation under the ambient and elevated $p\text{CO}_2$ (400 and 1000 μatm), we further found that the elevation of $p\text{CO}_2$ promoted growth and increased the photosynthetic performance of all three cultivar strains of *S. japonica* that have been traditionally cultured for many years in China.

Keywords Non-photochemical quenching (NPQ) · Ocean acidification · *S. japonica* · Temperature · Transthylakoid proton gradient (ΔpH) · Xanthophyll cycle (XC)

Introduction

Global warming, as well as the ocean acidification derived from anthropogenic CO₂ emissions, is one of the most severe environmental crises in the twenty-first century (Schlüter et al. 2014). The Earth's surface temperature has risen by 0.7 °C during the last century and is expected to increase by an

additional 3 °C by 2100 (Hoegh-Guldberg et al. 2007). As a major global sink for CO₂, oceans have absorbed approximately 30% of anthropogenic CO₂ emissions, which have mitigated CO₂-related global warming (Hall-Spencer et al. 2008). However, CO₂ absorption results in a decrease in oceanic pH and changes in seawater chemistry, with increases in the levels of dissolved carbon dioxide and bicarbonate and a lower degree of carbonate saturation, i.e., ocean acidification (Caldeira and Wickett 2005). The results from a growing number of recent studies conducted in the topics indicated that global warming and ocean acidification interact to affect growth and photophysiology of marine primary producers in varying manners and to varying magnitudes, potentially altering community structure and marine ecosystems (Koch et al. 2013). There is evidence that in the brown macroalga *Macrocystis pyrifera*, the combined increases in temperature and $p\text{CO}_2$ have significantly decreased the germination rates and increased the mortality of kelp spores (Gaitán-Espitia et al. 2014). Olischläger and Wiencke (2013) reported that predicted level of ocean acidification may alleviate the effects of low temperature on the growth and photosynthesis of the red alga *Neosiphonia harveyi*.

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As primary producers, photoautotrophs absorb 30% of the anthropogenically emitted carbon and play a significant role in modulating global climate change (García-Gómez et al. 2014). At the surface of the ocean photosynthetic organisms are often exposed to variations in light intensity. Excitation by an excess of light may cause photoinhibition and the production of reactive oxygen intermediates that are detrimental to plant photosynthetic activity, growth, and productivity (Peers et al. 2009). Increase in the amount of reactive oxygen species (ROS) may cause lipid peroxidation and inactivation of enzymes (Babu et al. 2014). To resist the oxidative stress, plants have evolved a variety of photoprotection mechanisms. Non-photochemical quenching (NPQ) is one of the most important and most rapid regulatory strategies to dissipate excess absorbed light as heat and to prevent photodamage to the reaction center in PSII (Mou et al. 2013). Multiple NPQ components based on various molecular mechanisms have been identified in the plant kingdom. In higher plants, the major component is the transthylakoid proton gradient (ΔpH) dependent quenching (qE), which is modulated by the xanthophyll cycle (XC) and the protein PsbS (Johnson and Ruban 2010). During the XC, the prevailing component, violaxanthin (Vx), is converted to antheraxanthin (Ax) and then to zeaxanthin (Zx) by violaxanthin de-epoxidase (VDE), which is regulated by pH, located in the thylakoid lumen and is inhibited by dithiothreitol (DTT) (Masojidek et al. 2004). Zx synthesized via the XC is essential for thermal dissipation. Similar to higher plants, XC plays a key role in NPQ induction of brown algae (Goss and Lepetit 2015). NPQ induction mainly depends on Zx and it is activated by ΔpH in the thylakoid membrane (Ocampo-Alvarez and García-Mendoza 2013). In addition, the PSII antenna subunit PsbS is essential for qE and no PsbS was found in brown algae (García-Mendoza and Colombo-Pallotta 2007). In contrast to higher plants, qE, a fast NPQ induction mechanism is absent in brown algae (García-Mendoza and Colombo-Pallotta 2007). According to previous study, ΔpH alone could not induce NPQ and appears to be related only to the activation of the VDE in *M. pyrifera* (García-Mendoza and Colombo-Pallotta 2007). Additionally, at the regulatory level, a group of light-harvesting proteins from the LHCSR family is involved in NPQ in green algae, brown algae, and diatoms, but is missing from higher plants (Cao et al. 2013).

In parallel with the diurnal and seasonal variation of light, global climate change has caused additional stresses to phytoplankton in association with the decline in oceanic pH, the warming of seawater, and the thinning of the upper mixing layer as a result of increased stratification (Doney et al. 2012; Gao et al. 2012). In isolation, the elevation of pCO_2 was observed to contribute to a higher capacity for repair of PSII in the diatom *Thalassiosira pseudonana* (McCarthy et al. 2012), a more rapid recovery of PSII from UV damage in *Phaeodactylum tricorutum* (Wu et al. 2014), and a decrease

in UV B-related photochemical inhibition in *Gephyrocapsa oceanica* (Jin et al. 2013). In contrast, NPQ mechanisms of the brown macroalgae *Padina pavonica* and *Cystoseira tamariscifolia* (Korbee et al. 2014) and in the green macroalga *Ulva prolifera* were activated under elevated CO_2 (Liu et al. 2012). Photosynthesis is also known to be highly sensitive to temperature (Mathur et al. 2014). The greenhouse gas-induced increase in temperature has been associated with a three-fold increase in photoinhibition in intertidal benthic microalgal biofilms (Laviale et al. 2014). Furthermore, the combination of increasing pCO_2 with other stresses has been reported to produce antagonistic, synergistic, or neutral effects on physiological performance in various marine organisms (Chen et al. 2014). However, the effects of co-occurrence of the elevation of pCO_2 and temperature on photochemical capacity, especially regarding photoprotective mechanisms, remain unclear.

In coastal ecosystems, macroalgal kelp constructs three-dimensional underwater forests and plays significant ecological role in structuring biodiversity in both cold-temperate and polar coastal regions (Wernberg et al. 2010). Unfortunately, predicted increases in seawater temperatures are forecast to decrease the biodiversity of these cold temperate seaweeds and shift the distributional boundaries northward (Raybaud et al. 2013; Redmond 2013). *Saccharina japonica* is one of the most ecologically and economically important brown macroalgae and is used for food, medicinal, agricultural, and industrial products (Wang et al. 2013). Floating-frame culture using rope rafts has been the most commonly employed cultivation method in the coast of China since the 1950s (Li et al. 2008). During the growing season, from October to June of the following year, this algal frond community forms a dense canopy at the ocean surface, where it is subject to a large diurnal gradient and seasonal variation in light and temperature (Bartsch et al. 2008). Although high capacities for photoacclimation and photoprotection have been reported for another important giant kelp, *M. pyrifera* (García-Mendoza and Colombo-Pallotta 2007), the exact mechanism of photoprotection is not yet known for *S. japonica*. Moreover, responses of this productive macroalga to the isolated and combined effects of ocean acidification and the increasing temperature associated with global climate change are of general concern.

Therefore, in the present study, we first characterized NPQ kinetics in *S. japonica* by using specific chemical inhibitors. Second, we evaluated the effects of temperature and ocean acidification on NPQ in isolation and in combination. To further study the effect of ocean acidification on the growth and photosynthesis in a long-term selection, three strains of *S. japonica* were cultured under two levels of pCO_2 (400 and 1000 μatm) for 60 days. We hypothesized that (1) *S. japonica* shows a photoprotective mechanism similar to that of *M. pyrifera*; (2) although increasing temperature induced a strong NPQ response, the elevation of pCO_2 may alleviate this

response by providing sufficient carbon resources; and (3) increasing $p\text{CO}_2$ would produce a general positive effect on photosynthesis and growth.

Materials and methods

Algal material and culture conditions

Samples of *S. japonica* were collected from Sungo Bay (37°01'–37°09' N, 122°24'–122°35' E) in December 2013, when the mean seawater temperature was 10.32 °C. Within 3 h, the algal samples were transported to the laboratory in a tank of natural seawater. The mean size of the collected samples was 15 cm in length and 3 cm in width. In the laboratory, the intact samples were washed several times with sterile seawater until they were free from visible epiphytes and then pre-cultured in Erlenmeyer flasks supplemented with F/2 medium (Guillard 1975) at 10 ± 1 °C with vigorous air bubbling for 2 days. The lighting conditions were set at 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ supplied by white fluorescent lamps, with a photoperiod of 12 h light/12 h dark.

Chl fluorescence yield and NPQ measurement

In vivo chlorophyll fluorescence measurements were performed at the same temperature (10 ± 2 °C, controlled by an air conditioner) as the natural seawater where the sample was collected by using dual-wavelength pulse amplitude modulated fluorescence monitoring system (Dual-PAM, Heinz Walz, Germany).

Before measurement, the samples were kept in darkness for 20 min and the original fluorescence (F_0) was determined under low measuring light. A saturation light pulse (1287 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 300 ms) was applied to obtain maximum fluorescence (F_m) in the dark-adapted samples. The F_m yield in illuminated samples is denoted as F_m' , and F_t is real-time fluorescence yield. The maximum PSII quantum yield (F_v/F_m) was calculated according to the equation (Hiriart-Baer et al. 2008):

$$F_v/F_m = (F_m - F_0)/F_m \quad (1)$$

The effective PSII quantum yield (YII) was calculated as follows (Genty et al. 1989):

$$YII = (F_m' - F_t)/F_t \quad (2)$$

Relative rate of the photosynthetic electron transport of PSII (rETR) was calculated as (Hiriart-Baer et al. 2008):

$$\text{rETR} = YII \times \text{PAR} \times 0.5 \quad (3)$$

where PAR is the irradiance and 0.5 assumes that half of the light absorbed is distributed to PSII.

NPQ was calculated as (Bilger and Björkman 1990):

$$\text{NPQ} = (F_m - F_m')/F_m' \quad (4)$$

The rapid light response curve (LRC) was also generated under increasing irradiance from 11, 18, 27, 58, 100, 131, 221, 344, 536 to 830 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and fitted by the function (Platt et al. 1980):

$$P = P_s \left(1 - e^{-(\alpha E_d/P_s)} \right) e^{-(\beta E_d/P_s)} \quad (5)$$

where P_s is defined as the maximum potential ETR, α is the slope of the initial of the RLC, and β is the slope of the RLC beyond the onset of saturation, E_d is the downwelling irradiance (400–700 nm) (Ralph and Gademann 2005). The light utilization efficiency (α), maximum electron transport rate of PSII (ETR_{max}), and half saturation irradiance (E_k) were obtained from the LRC (Xu et al. 2015).

To investigate NPQ processes under different inhibitor conditions, actinic light of 1287 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ was used. Minimal irradiance of $> 216 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was necessary to induce NPQ, and irradiances $> 1287 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ did not induce a higher level of NPQ (Fig. S1). Once steady state fluorescence was achieved, saturating pulses were applied every 30 s to measure the F_m under actinic light (F_m').

NPQ kinetics of *S. japonica*

For NPQ kinetics, to minimize the contribution of the saturating light flashes (which are required to determine the NPQ parameter) to NPQ formation, the flashes were separated at 10 s intervals for the first 30 s of induction, 30 s intervals between 30 and 300 s, and 60 s intervals from 300 to 600 s. All measurements were made in triplicate.

The functions of ΔpH and the XC were characterized using the inhibitors ammonium chloride (NH_4Cl) and dithiothreitol (DTT). The inhibitor NH_4Cl is known as the uncoupler of the ΔpH , while DTT was used as a specific inhibitor of VDE (Mou et al. 2013; Zhang et al. 2014). When appropriate, NH_4Cl (8 mmol L^{-1}) or DTT (0.5 mmol L^{-1}) were added at the start of dark incubation to perturb the kinetics of the ΔpH build-up, the XC and NPQ.

The effects of temperature and $p\text{CO}_2$ on NPQ kinetics

To identify the isolated and combined effects of temperature and $p\text{CO}_2$ on NPQ, a gradient of three temperatures (5, 10, and 20 °C) was established. Under each temperature, four $p\text{CO}_2$ levels (400, 700, 1000, and 2000 μatm) were applied the pH of culture medium. The experiment was conducted in triplicate with a flask and tubing system similar to that used by Zou and Gao (2009). Culture medium was bubbled either with air ($\sim 400 \mu\text{atm}$) or air/ CO_2 mixed gas in a programmed CO_2

chamber (HP1000G-D, China) to achieve the desired $p\text{CO}_2$ levels and temperature setting. Algal samples were cultured in each condition for 12 h. The pH in each culture medium was measured before and after each transfer using a pH meter (Orion ROSS, Fisher Scientific Instruments) that was calibrated with the National Bureau of Standards (NBS) buffers. Temperature, salinity, and total alkalinity (TA) were also measured periodically (but not at every transfer due to logistical restrictions) throughout the experiment. Other carbonate system parameters were calculated with the CO_2SYS using pH and TA (Table S1). Then, the NPQ kinetics were determined using a Dual-PAM-100 fluorometer at an actinic light intensity of $1287 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. At the end of the experiment, after NPQ determination, algal samples from each treatment were also frozen in liquid nitrogen and preserved for the analysis of pigments involved in the XC.

Isolated effects of ocean acidification on the physiological performance of *S. japonica*

To further study the isolated effects of ocean acidification on the physiological performance of *S. japonica*, mature fronds of three cultivar strains (denoted as BN, Ja, or F) of *S. japonica* were collected from Sungo Bay ($37^\circ 01' - 37^\circ 09' \text{N}$, $122^\circ 24' - 122^\circ 35' \text{E}$) in August 2014. The three cultivar strains are three of the most important local strains with different phenotypic performance and have been traditionally cultured for many years in China. BN showed the highest biomass with a modest ratio of length/width, Ja showed the highest width and modest biomass, and F showed the highest length and minimum width and matured at the last. Juvenile sporophytes were successfully formed by the summer seedling raising method (Tseng 2001) after cultivation for 60 days. During the cultivation period, the three strains were cultured at $10 \pm 1 \text{ }^\circ\text{C}$ and in a pH-adjusted F/2 medium, which was bubbled at one of two different $p\text{CO}_2$ levels (L, $400 \mu\text{atm}$ and H, $1000 \mu\text{atm}$) and exchanged weekly. At the end of experiment, sporophyte size, PSII photosynthetic parameters, and pigment contents were determined.

Xanthophyll determinations

The preserved samples were mechanically disrupted and the pigments were extracted in a tube by adding 3 mL of cold 85% acetone. The extracts were maintained on ice for 1 h, centrifuged at $12,000 \times g$ ($4 \text{ }^\circ\text{C}$) for 5 min, and stored at $-20 \text{ }^\circ\text{C}$ before analysis by high-pressure liquid chromatography (HPLC). Cellular debris was removed by centrifugation and the supernatants were filtered through $0.22\text{-}\mu\text{m}$ syringe filters into amber HPLC vials. The vials were stored on ice in the dark prior to injection. All extraction procedures were performed in a dimly lit room. Concentrations of XC pigments were determined using reversed phase HPLC as described by

Zapata et al. (2000). Pigments were analyzed following Thayer and Bjorkman (1990) with minor modifications using a HP1100 Liquid Chromatograph equipped with a diode array detector (Agilent Technology, USA). An Agilent Technology non-encapped Zorbax ODS column ($4.5 \times 250 \text{ mm}$, 5 mm particle sizes) was used in the separation, preceded by a C18 Adsorbosphere guard column (Alltech Associates, Inc., USA). Pigment quantification was performed using calibration curves calculated from HPLC separations using purified pigment standards obtained from DHI Inc. (Denmark). DPS of the XC pigment pool was calculated as $(Zx + 0.5Ax)/(Vx + Ax + Zx)$ (Casper-Lindley and Björkman 1998); here, ΣXC is the sum of the Vx , Ax , and Zx concentrations. DPS represents the photoprotective state of the XC because the carotenoids Ax and Zx are involved in the dissipation of energy as heat, which confers photoprotection to the photosynthetic apparatus.

Statistical analysis

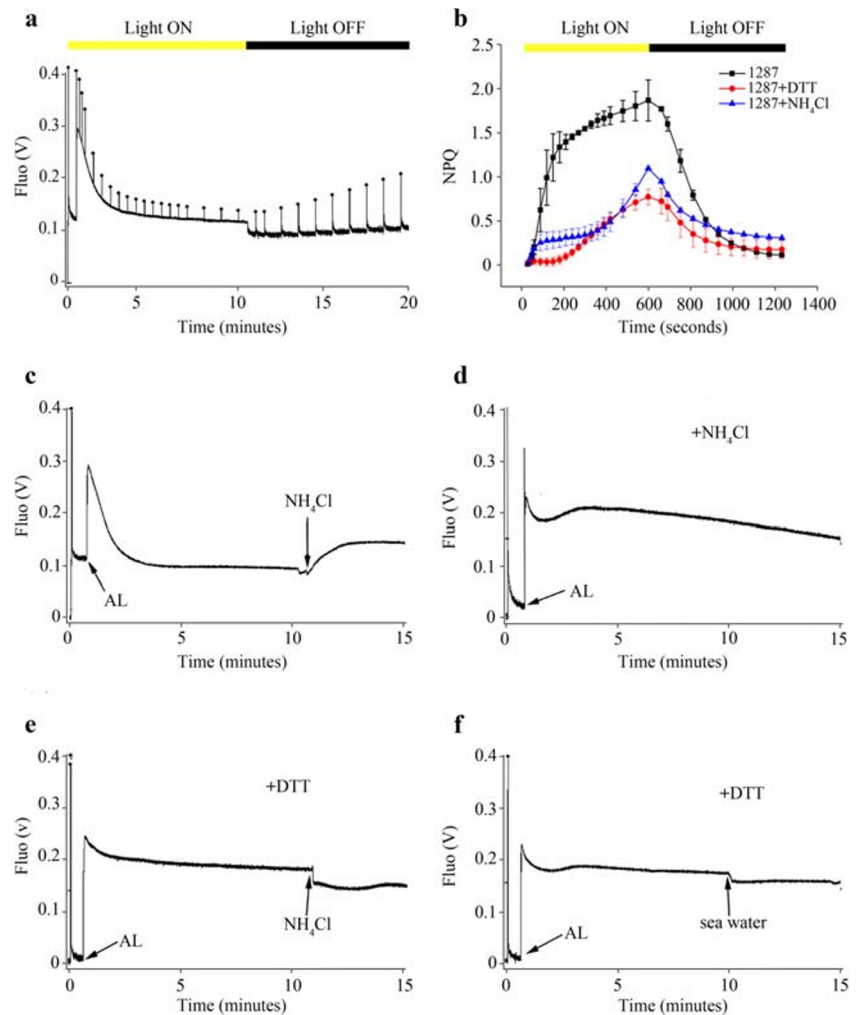
One-way and two-way ANOVAs were employed to analyze the isolated and combined effects, respectively, of $p\text{CO}_2$ and temperature on various physiological parameters using the software SPSS 17.0 (SPSS, USA). Post hoc tests, Tukey's test for two-way ANOVA, and Dunnett's test for multivariate ANOVA were also performed. The assumptions of homogeneity of variance and normality were assessed by examining the scatter plots of the residuals and the normal curves of the residuals, respectively. A significance level of 0.05 was set for all tests.

Results

The general NPQ feature of *S. japonica*

Multiple intensities of actinic light were applied to measure NPQ kinetics (Fig. S1). Minimal irradiance of $> 216 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was necessary to induce NPQ, and irradiances $> 1287 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ did not induce a higher level of NPQ. Therefore, an actinic light of $1287 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ was established as the final applied irradiance to saturate photosynthesis and induce maximal NPQ. At the actinic light intensity of $1287 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, the basic characteristics of NPQ of *S. japonica* were further studied by Chl *a* fluorescence quenching (Fig. 1). In *S. japonica*, NPQ was rapidly induced when the light switched on and exhibited reversibility in darkness (Fig. 1a). To further study the role of ΔpH and XC in NPQ, the various concentrations of uncoupling agents NH_4Cl or DTT were added at the start of the dark incubation (Fig. S2). NPQ decreased significantly with an increase in the concentration of NH_4Cl or DTT. The final concentrations of NH_4Cl and DTT determined to be the

Fig. 1 **a** Chlorophyll *a* fluorescence quenching in *S. japonica*. **b** Effects of NH_4Cl or DTT on NPQ kinetics. Before measuring NPQ values, samples were pre-incubated in the dark for 20 min with NH_4Cl (8 mmol L^{-1}) or DTT (0.5 mmol L^{-1}). **c** Effect of NH_4Cl on fluorescence quenching in *S. japonica* over 15 min of exposure to actinic light (up arrow), with NH_4Cl added after 10 min. **d** Effect of NH_4Cl on fluorescence quenching in *S. japonica* over 15 min of exposure to actinic light (up arrow), with NH_4Cl added before light exposure. **e** Chlorophyll *a* fluorescence quenching with DTT and NH_4Cl added after light exposure for 10 min. **f** Chlorophyll *a* fluorescence quenching with DTT and seawater added after light exposure for 10 min. The values are represented as means \pm SD, $n = 3$



minimum concentrations required to achieve complete inhibition were 8 mmol L^{-1} and 0.5 mmol L^{-1} , respectively.

The NPQ values of DTT-treated and control samples were determined within 10 min of illumination at $1287 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Fig. 1b). The mean NPQ value in the control samples was approximately 1.87. In contrast to control samples, there was an obvious decrease in NPQ (0.7) after the addition of 0.5 mmol L^{-1} DTT, indicating that the de-epoxidation of XC pigments is required for NPQ induction in *S. japonica*. NPQ also decreased significantly with the addition of 8 mmol L^{-1} NH_4Cl at the start of the dark incubation (Fig. 1b). Chl *a* fluorescence quenching was also monitored under 15 min of high light exposure, by adding NH_4Cl during the process (Fig. 1c) or before light exposure (Fig. 1d). Both assays induced a relaxation of fluorescence quenching, which appears to be correlated with the ΔpH induced by NPQ. However, the samples incubated with DTT before light exposure did not exhibit the slow relaxation of NPQ after the addition of NH_4Cl (Fig. 1e), although a slight increase may be induced by seawater (Fig. 1f). These results indicated that the

slow reversibility of NPQ only occurred in the presence of de-epoxidated XC pigments and that the role of this gradient appears to be related only to the activity of the XC.

The levels of the pigments involved in the XC under the treatment conditions evaluated were determined by HPLC (Table 1). The high light condition induced higher levels of Zx/Chls and DPS, indicating that the high light was effective in inducing Zx synthesis and increasing the de-epoxidation index of the XC. When samples were treated with inhibitors, the Zx/Chls and DPS of the HL- NH_4Cl samples decreased from 0.509 ± 0.042 to 0.295 ± 0.004 and from 0.080 ± 0.043 to 0.024 ± 0.001 , respectively ($p < 0.05$), which indicates that the ΔpH may affect the activation of the XC. DTT-treated samples induced substantially lower levels of Zx/Chls and DPS, demonstrating that 0.5 mmol L^{-1} DTT can almost entirely block the conversion of Vx into Ax and Zx under high light conditions. Similarly, the correlation analysis further indicates that NPQ showed a strong positive correlation with Zx/Chls ($R^2 = 0.802$) (Fig. 2a), and NPQ also showed a strong positive correlation with DPS ($R^2 = 0.788$) (Fig. 2b).

Table 1 Pigment contents involved in Z_x in dark-adapted samples of *S. japonica* collected from various experimental conditions. The values are represented as means \pm SD, $n = 3$. Different lowercase letters indicate significant differences among the treatments at $p < 0.05$

Treatment	Chl <i>c</i> /Chl <i>a</i>	Vx/Chls	Ax/Chls	Zx/Chls	Σ XC/Chls	DPS
Control	0.144 \pm 0.002b	0.097 \pm 0.004b	0.023 \pm 0.002b	0.016 \pm 0.004b	0.136 \pm 0.010b	0.204 \pm 0.021c
High light	0.166 \pm 0.016a	0.075 \pm 0.018b	0.138 \pm 0.007a	0.080 \pm 0.043a	0.293 \pm 0.054a	0.509 \pm 0.042a
HL-NH ₄ Cl	0.157 \pm 0.005a	0.152 \pm 0.004a	0.135 \pm 0.015a	0.024 \pm 0.001b	0.311 \pm 0.018a	0.295 \pm 0.004b
HL-DTT	0.152 \pm 0.028a	0.100 \pm 0.049b	0.015 \pm 0.002b	0.006 \pm 0.004b	0.121 \pm 0.043b	0.112 \pm 0.084d

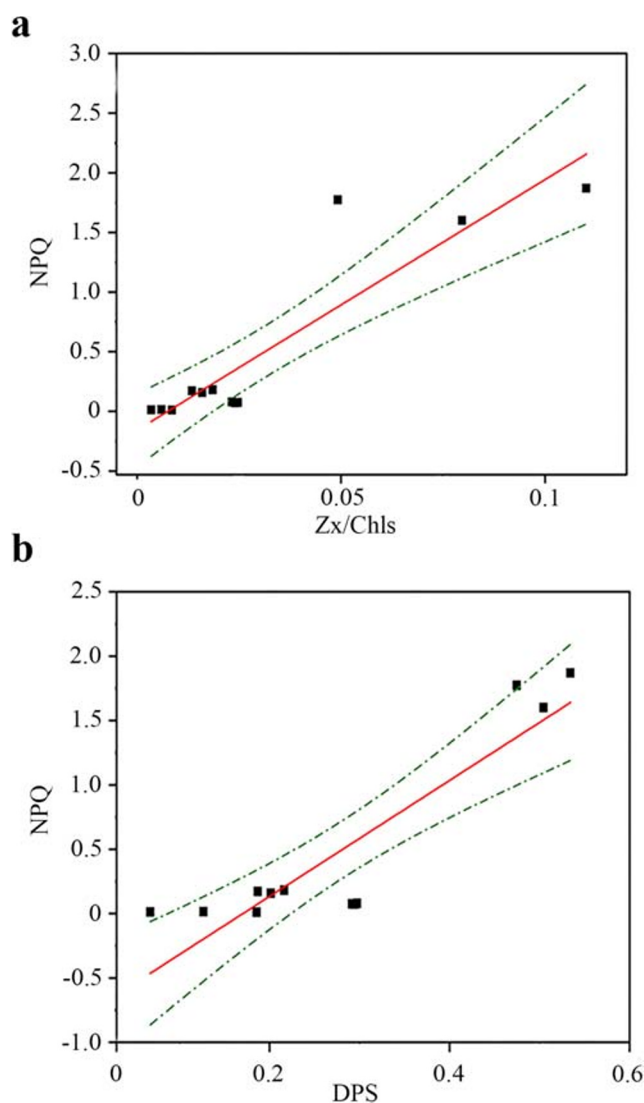
The isolated and combined effects of $p\text{CO}_2$ and temperature on NPQ in young sporophyte of *S. japonica*

Statistical analysis showed that there were significant effects of temperature and $p\text{CO}_2$ on NPQ kinetics across 1200 s of *S. japonica* (Fig. 3, Table S2). Although NPQ increased with the increasing temperature from 5 to 10 °C and 20 °C (Table S3), the elevation of $p\text{CO}_2$ reduced value of *S. japonica* (Table S4). Analysis of pigments involved in Z_x indicated that Z_x/Chls and DPS showed trends similar to those of the various treatments; high temperature increased these parameters, and the elevation of $p\text{CO}_2$ reduced them, except for the variation in Z_x/Chls at the lowest temperature of 5 °C (Table 2). Consistently, correlation analysis demonstrates that the correlation coefficient R^2 (0.280) between Z_x/Chls and NPQ was substantially lower than that between DPS and NPQ (0.735) (Fig. 4).

The physiological performances of various strains of *S. japonica* under ambient and elevated $p\text{CO}_2$ are shown in Fig. 5. Although there were significant differences in blades size among the three tested strains, the elevation of $p\text{CO}_2$ dramatically promoted growth in all three strains ($p < 0.001$) (Fig. 5a). In terms of the PSII photosynthetic parameters, the F_v/F_m , YII (Fig. 5b) and ETRII (Fig. 5c) values were increased significantly under the elevated $p\text{CO}_2$ treatment for all three strains. From the LRCs, other parameters including α , ETR_{max} , and E_k were calculated (Table 3). Statistical analysis revealed significant differences in ETR_{max} ($p < 0.001$), E_k ($p < 0.001$), and α ($p = 0.036$) among the three strains. The elevation of $p\text{CO}_2$ treatment resulted in a significant decrease in α ($p < 0.001$), but marked increases in ETR_{max} ($p < 0.001$) and E_k ($p < 0.001$). Additionally, although one-way ANOVA indicated that the effects of $p\text{CO}_2$ on the levels of the pigments Chl *a*, Chl *c*, and carotenoids were not significant, all of these levels were, on average, greater under the $p\text{CO}_2$ of 1000 μatm (Fig. 5d).

Discussion

According to the results, we speculated that the NPQ mechanism in brown macroalga *S. japonica* is mainly associated

**Fig. 2** Relationship between NPQ and Z_x (a) or DPS (b) in *S. japonica*. The dashed line represents $\pm 95\%$ confidence intervals

with the XC and that a change in the ΔpH alone could not induce NPQ (Fig. 1). The elevation of pCO_2 reduced NPQ values of *S. japonica*, which could save energy spent in thermal dissipation and invest more energy to promote growth and photosynthesis (Figs. 3 and 5). Although the basic principle of NPQ, the dissipation excessive light energy as heat is identical

across photoautotrophs, the regulation mechanisms vary significantly in organisms (Zhang et al. 2014). In green algae, NPQ is mainly controlled by the ΔpH and further depends on the activation of the XC to varying extents depending on taxon or lineage (Lunch et al. 2013; Mou et al. 2013). For diatoms and brown algae, the ΔpH is not sufficient to induce NPQ, which has been mainly associated with the formation of diatoxanthin (Dtx) and Zx (Cao et al. 2013). In this study, the NPQ kinetics in *S. japonica* was examined by the addition of the chemical uncouplers NH_4Cl and DTT (Fig. 1).

The significant decrease in NPQ with the addition of NH_4Cl indicates that the disruption of ΔpH may induce a reduction in NPQ (Fig. 1 b, c and d). An even more highly significant decline in NPQ was also induced by the addition of DTT (Fig. 1b). Chlorophyll *a* fluorescence quenching analysis further demonstrates that the addition of NH_4Cl to DTT-pretreated samples did not relax NPQ (Fig. 1e). The slight decline in fluorescence quenching may be attributable to the addition of seawater (Fig. 1f). Therefore, ΔpH was not the factor inducing NPQ. Further pigment analysis indicated that

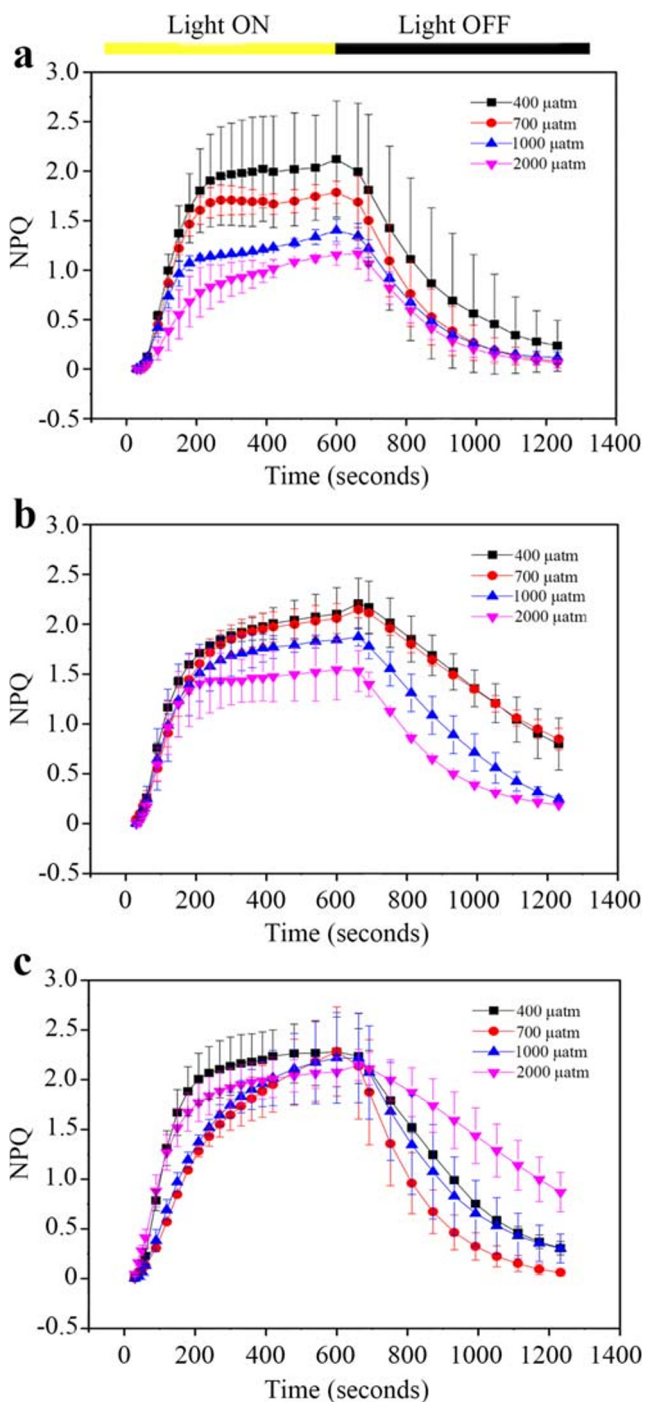


Fig. 3 The effect of increasing pCO_2 on NPQ in *S. japonica* at cultivation temperatures of 5 °C (a), 10 °C (b), and 20 °C (c). The values are represented as means \pm SD, $n = 3$

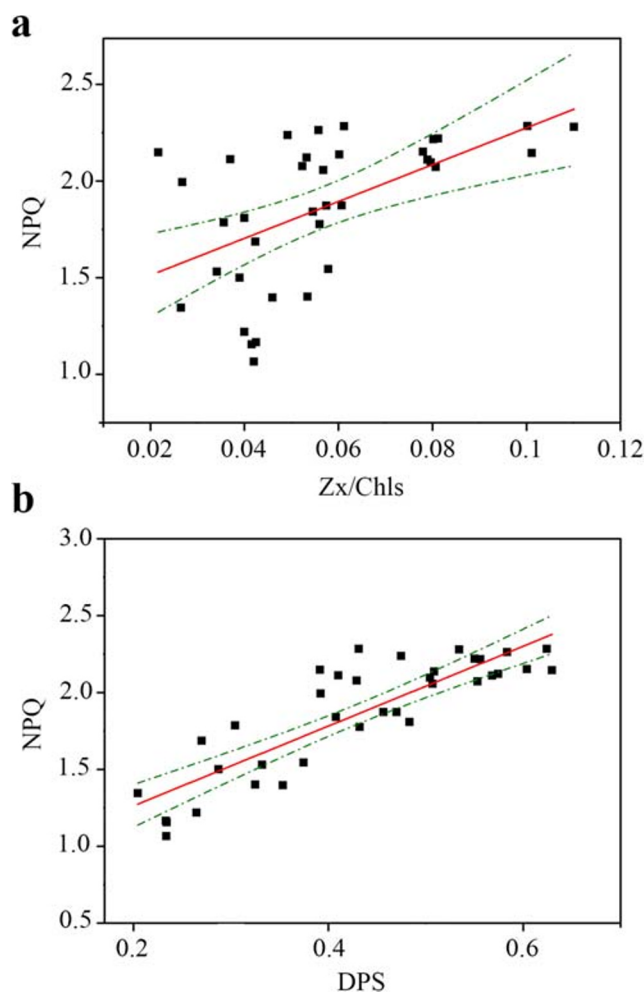


Fig. 4 Relationship between NPQ and Zx (a) or DPS (b) in *S. japonica*. The dashed line represents \pm 95% confidence intervals

Table 2 Combined effects of temperature and $p\text{CO}_2$ on levels of pigments involved in Zx in *S. japonica*. The values are represented as means \pm SD, $n = 3$. The different lowercase letters indicate significant differences among the treatments at $p < 0.05$

Temperature	$p\text{CO}_2$ (μatm)	Chl <i>c</i> /Chl <i>a</i>	Vx/Chls	Ax/Chls	Zx/Chls	$\Sigma\text{XC/Chls}$	DPS
5 °C	400	0.178 \pm 0.066bc	0.045 \pm 0.019f	0.063 \pm 0.001c	0.040 \pm 0.019b	0.148 \pm 0.000c	0.484 \pm 0.129bc
5 °C	700	0.199 \pm 0.011bc	0.141 \pm 0.000c	0.062 \pm 0.044c	0.039 \pm 0.005b	0.242 \pm 0.040b	0.289 \pm 0.024de
5 °C	1000	0.241 \pm 0.039ab	0.172 \pm 0.037b	0.065 \pm 0.001c	0.040 \pm 0.019b	0.278 \pm 0.019ab	0.263 \pm 0.085e
5 °C	2000	0.244 \pm 0.045a	0.214 \pm 0.029a	0.067 \pm 0.028c	0.042 \pm 0.001b	0.322 \pm 0.057a	0.233 \pm 0.001e
10 °C	400	0.166 \pm 0.016c	0.075 \pm 0.018ef	0.138 \pm 0.007a	0.080 \pm 0.043a	0.293 \pm 0.054ab	0.509 \pm 0.042b
10 °C	700	0.199 \pm 0.011bc	0.079 \pm 0.032e	0.098 \pm 0.048b	0.061 \pm 0.001ab	0.237 \pm 0.081b	0.463 \pm 0.055bc
10 °C	1000	0.207 \pm 0.006b	0.089 \pm 0.018e	0.090 \pm 0.016bc	0.056 \pm 0.002ab	0.235 \pm 0.032b	0.430 \pm 0.034c
10 °C	2000	0.242 \pm 0.041ab	0.114 \pm 0.010d	0.074 \pm 0.001bc	0.046 \pm 0.017b	0.234 \pm 0.026b	0.355 \pm 0.030d
20 °C	400	0.093 \pm 0.023d	0.028 \pm 0.009g	0.129 \pm 0.003a	0.078 \pm 0.031a	0.235 \pm 0.044b	0.606 \pm 0.029a
20 °C	700	0.135 \pm 0.005c	0.043 \pm 0.015f	0.132 \pm 0.001a	0.079 \pm 0.031a	0.253 \pm 0.016b	0.570 \pm 0.087ab
20 °C	1000	0.143 \pm 0.001c	0.053 \pm 0.002f	0.130 \pm 0.006a	0.081 \pm 0.001a	0.264 \pm 0.009b	0.552 \pm 0.004ab
20 °C	2000	0.150 \pm 0.002c	0.065 \pm 0.019ef	0.059 \pm 0.006c	0.037 \pm 0.022b	0.161 \pm 0.035c	0.411 \pm 0.027cd

a high light intensity induces significant increases $\Sigma\text{XC/Chls}$ and DPS; in the presence of DTT, the $\Sigma\text{XC/Chls}$ and DPS decreased significantly from 0.293 ± 0.054 and 0.509 ± 0.042 to 0.121 ± 0.043 and 0.112 ± 0.084 , respectively (Table 1). In particular, the Zx/Chls ratio decreased to a barely detectable level. This indicates that the NPQ induction in *S. japonica* closely followed the synthesis of Zx in XC (Fig. 2a) and that ΔpH alone could not induce NPQ, which appears to be solely related to the activation of the VDE, similar to the

NPQ regulation mechanism of the giant kelp *M. pyrifera* (García-Mendoza and Colombo-Pallotta 2007).

Marine algae experience diurnal and annual changes in irradiance and temperature, which may vary from limitation to levels potentially inhibiting photosynthesis. Moreover, desynchronized variations in light and temperature may lead to photoinhibition occurring at a relatively low light saturation (Vonshak et al. 2001). Although photosynthesis is known to be highly sensitive to temperature, information on the effects

Fig. 5 The isolated effect of increasing $p\text{CO}_2$ on growth (a), the PSII photosynthetic parameters of F_v/F_m , YII (b), and ETRII (c), and the pigment contents (d) of *S. japonica* cultured under either a low $p\text{CO}_2$ of 400 μatm (L) or a high $p\text{CO}_2$ of 1000 μatm (H) for 60 days. The values are represented as means \pm SD, $n = 3$

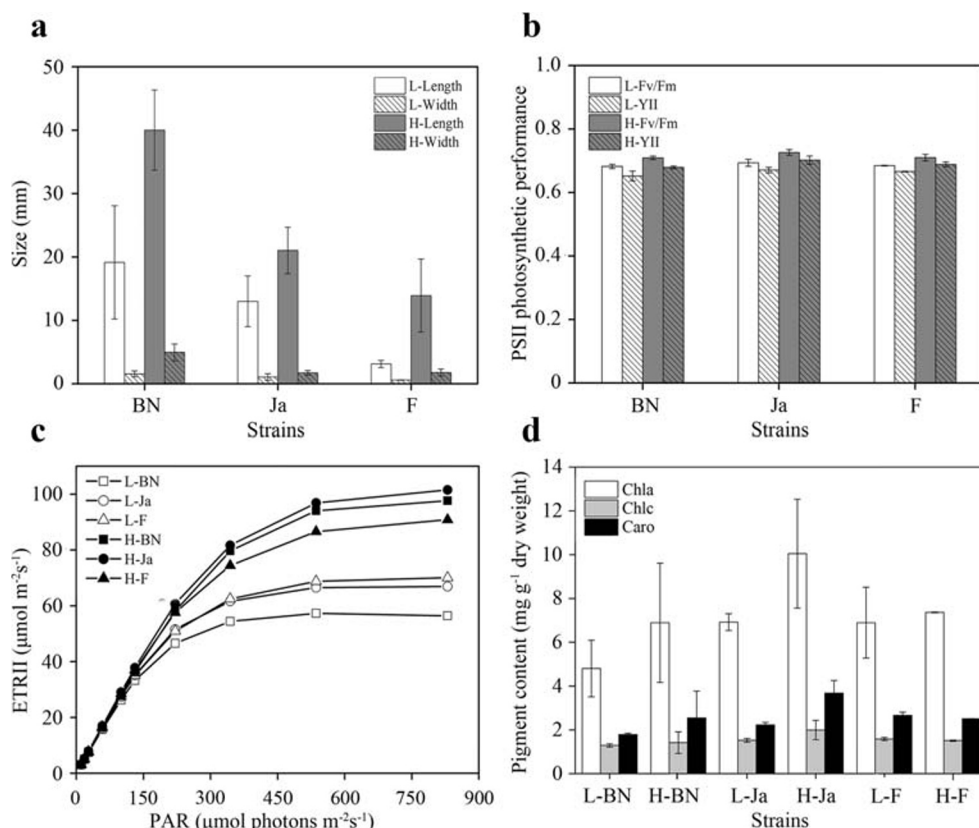


Table 3 The photosystem II light-response curve parameters of *S. japonica* cultured under either a low $p\text{CO}_2$ of 400 μatm (L) or a high $p\text{CO}_2$ of 1000 μatm (H) for 60 days. α , light utilization efficiency;

ETR_{max} , maximum electron transport rate of PSII; E_k , half saturation irradiance. The values are represented as means \pm SD, $n = 3$

Parameters	L-BN	L-F	L-Ja	H-BN	H-F	H-Ja
α	0.322 \pm 0.014	0.324 \pm 0.001	0.327 \pm 0.005	0.294 \pm 0.012	0.316 \pm 0.002	0.310 \pm 0.006
ETR_{max}	58.867 \pm 7.651	70.967 \pm 1.201	71.200 \pm 7.318	98.700 \pm 7.400	88.267 \pm 9.318	102.467 \pm 13.381
E_k	183.200 \pm 24.520	218.733 \pm 3.408	210.167 \pm 14.087	336.9 \pm 36.846	278.933 \pm 28.689	330.633 \pm 38.000

of changing temperature on associated photoprotective mechanisms is rather scarce. A few studies have reported that high temperature may increase the de-epoxidation rate in the XC, the repair rate of PSII and RUBISCO activity, leading to a reduction of photoinhibition in the diatom *T. pseudonana* (Cabrerizo et al. 2014; Halac et al. 2014). Low temperature has also been identified to play a synergistic role with excess illumination, limiting electron transport and carbon fixation rates, thereby inducing photoinhibition even under relatively weak light conditions (Gerotto et al. 2011; Halac et al. 2014). Thus, a suboptimal physiological temperature, either high or low, may result in photodamage to photosynthetic organisms (Fig. 3). Similar to our results, previous studies also showed that NPQ increased with temperature in the kelp *Alaria esculenta* (Roleda. 2009). In parallel, there were, in general, increases in the Z_x/Chls , $\Sigma\text{XC}/\text{Chls}$, and DPS involved in the Z_x under high temperature (Table 2).

The ongoing CO_2 emission-induced ocean acidification has been reported to result in various physiological effects on phytoplankton, indicating complex responses among various species and numerous interactions with other environmental factors (Chen et al. 2014). Even within the brown macroalgae, there is inconsistency among the findings on the effects of ocean acidification on species. Swanson and Fox

(2007) reported that CO_2 enrichment promoted growth in the emergent kelp species *Nereocystis luetkeana* but inhibited growth in the submergent *Saccharina latissima*. Species-specific responses of photosynthesis to changes in seawater carbonate chemistry have also been identified within brown algae (Xu and Gao 2012). In the present study, the predicted $p\text{CO}_2$ -induced ocean acidification promoted the growth and photosynthesis of the sporophytes of *S. japonica*, one of the most typical giant kelps in China, during long-term cultivation (Fig. 5). The positive effect of ocean acidification may be attributable to the increased concentration of inorganic carbon resources. As the main form of inorganic carbon for photosynthesis, HCO_3^- can be acquired by algal cells either via active transport or via a carbon-concentrating mechanism (CCM). For the kelp *Laminaria digitata*, an increase in carbon resources increased the DIC uptake, but decreased the activity of external carbonic anhydrase (exCA), which is involved in CCM (Klenell et al. 2004). The energy savings from the down-regulation of CCM may benefit growth and photosynthesis. However, except for the positive effect of excess carbon availability, the elevation of $p\text{CO}_2$ also induces a lower value of α in *S. japonica* sporophytes (Table 3), suggesting that the projected level of ocean acidification may reduce its light use efficiency, which contradicts the findings of Li et al. (2014) for the diatom *P. tricornutum*.

Considering the effect of ocean acidification on NPQ, the elevation of $p\text{CO}_2$ has been demonstrated to increase the susceptibility to photoinhibition of several temperate phytoplankton species (Trimborn et al. 2014). In contrast, the projected increase in ocean acidification was found to alleviate UV inhibition in the diatom *P. tricornutum* and the coccolithophore *Gephyrocapsa oceanica* (Jin et al. 2013, Wu et al. 2014). Unfortunately, no data were available regarding the effect of ocean acidification on NPQ within the scope of brown macroalgae. Our present study thus presents the first evidence that CO_2 -induced seawater acidification reduces the NPQ response of *S. japonica* to high light and alleviates the effect of high temperature on NPQ. Consistent with the NPQ results, the DPS of the XC decreased significantly in response to the increase in $p\text{CO}_2$ from 400 to 2000 μatm (Table 2). Although the underlying mechanism remains unknown, the decrease in NPQ may have been attributed to the increased level of PAR at which light becomes excessive for electron transport rate, as evidenced by the higher ETR_{max} and

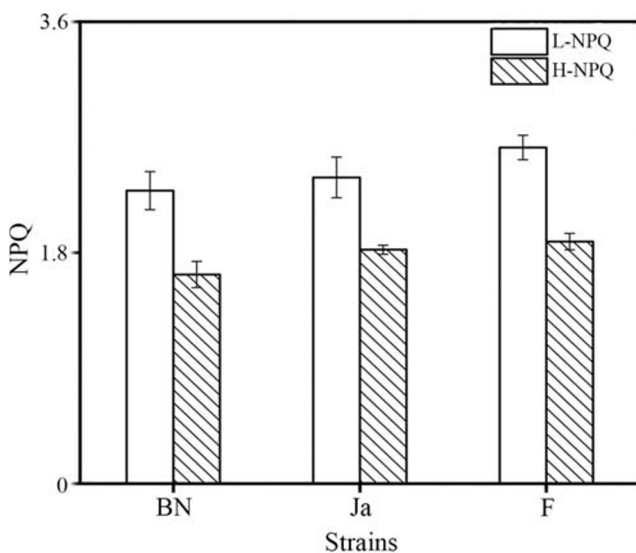


Fig. 6 The NPQ of *S. japonica* under two levels of $p\text{CO}_2$ (L, 400 μatm and H, 1000 μatm). The values are represented as means \pm SD, $n = 3$

E_k under the elevation of $p\text{CO}_2$ (Table 3). In addition, the correlation analysis indicates that there are poor relationships between Z_x/Chls and NPQ ($R^2 = 0.280$), and there are strong positive between DPS and NPQ ($R^2 = 0.735$) (Fig. 4). According to the results of Ocampo-Alvarez and García-Mendoza (2013), DPS instead of absolute concentration of Z_x play a key role during NPQ induction in *M. pyrifera* (Fig. 6). Moreover, we speculate that elevate $p\text{CO}_2$ reduced the activity of Z_x epoxidase enzyme and inhibited the conversion from Z_x to V_x at lower (5 °C) and higher (20 °C) temperatures and the underlying mechanisms should be further investigated.

In conclusion, with progressive climate change, the enhanced stratification in association with global warming and the increased acidification resulting from ongoing CO_2 emissions will expose marine primary producers to a more complex marine environment and will enhance light stress for photoautotrophs living in the upper mixing layer. We speculated that the photoprotective mechanism of NPQ to high light is mainly associated with the XC in the giant kelp *S. japonica*. CO_2 -induced ocean acidification and high temperature exert antagonistic effects on NPQ. In isolation, the projected level of ocean acidification promoted growth and photosynthesis by *S. japonica* sporophytes, but decreased the NPQ activity under high light conditions. Thus, the predicted ocean acidification may alleviate the effect of high temperature on giant kelp, conserving energy for growth and photosynthesis.

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