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Differential Photosynthetic Response of a Green Tide Alga *Ulva linza* to Ultraviolet Radiation, Under Short- and Long-term Ocean Acidification Regimes

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ABSTRACT

Both ocean acidification (OA) and solar ultraviolet (UV) radiation can bring about changes in macroalgal physiological performance. However, macroalgal responses to UV radiation when acclimatized to OA under different time scales are rare. Here, we investigate the response of *Ulva linza,* a green tide alga, to UV radiation in the form of photosynthetically active radiation (PAR) or PAB (PAR+UVA+UVB) radiation. Radiation exposures were assessed following long-term (from spore to adult stage, 1 month) and short-term (adult stage, 1 week) OA treatments. Results showed that increased CO_2 decreased the damage rate (*k*) and repair rate (*r*) of thalli grown under short-term OA conditions with PAB treatment, the ratio of *r*:*k* was not altered. Following long-term OA conditions, r was not affected, although k was increased in thalli following PAB treatment, resulting in a reduced ratio of *r*:*k*. The relative level of UV inhibition increased and UV-absorbing compounds decreased when algae were cultured under long-term OA conditions. The recovery rate of thalli was enhanced when grown under long-term OA after UV radiation treatment. These results show that blooming algae may be more sensitive to UV radiation in marine environments, but it can develop effective mechanisms to offset the negative effects, reflecting acclimation to long-term OA conditions.

INTRODUCTION

The stratospheric ozone layer is the principal agent for absorption of ultraviolet radiation in the Earth's atmosphere. Due to anthropogenic destruction and natural degradation, stratospheric ozone concentrations have decreased dramatically over the Arctic, Antarctic and higher latitudes, resulting in the formation of the Montreal protocol in 1987 (1,2). Stratospheric ozone depletion has now stabilized, with both observations and model calculations indicating that replenishment of the Antarctic ozone layer has begun (3). However, at some sites in the northern hemisphere ultraviolet-B (UVB) radiation may continue to increase, due to continuous reduction in aerosol extinction properties (4,5). Depletion of the stratospheric ozone layer is resulting in increased levels of solar UVB (280–320 nm) and ultraviolet-A (UVA, 315–400 nm) radiation exposure (6), modifying the balance between the potentially positive and negative effects of photosynthetically active radiation (PAR), UVA radiation and UVB radiation on living organisms.

Exposure to high levels of UV radiation induces damage in marine primary producers. Among macroalgae, high-intertidal species exhibit high photosynthetic rates, although they are also vulnerable to the harmful effects of increased UV radiation (7). Recent studies have revealed that UV radiation can provoke multiple effects in macroalgae by causing damage to nucleic acids, proteins and lipids (8). In addition, solar UV radiation can disrupt normal DNA base pairing by forming cyclobutane pyrimidine dimers (9), affecting the uptake of nutrients (10), causing inhibition of photosynthesis and reduced the growth rates, significantly damaging photosynthetic pigments (10–14) and altering community structures (15). However, UVA has also been found to stimulate photosynthesis and growth of macroalgae under certain conditions (16,17). UV radiation is not the only stress factor affecting macroalgae, as significant levels of ocean acidification have also been reported in subtidal habitats (18).

Due to the combustion of fossil fuels and other anthropogenic activities, atmospheric CO_2 levels are predicted to increase further to 1000 ppmv by the end of this century (19,20). The increased absorption of atmospheric CO_2 into seawater results in both an increase in surface water temperatures and a decrease in pH by 0.3–0.4 units, inducing ocean acidification (OA) (21,22). Numerous studies have demonstrated that the photosynthetic rates of marine macroalgae are affected by OA (23,24), with short-term OA experiments revealing an immediate response pattern of decreased calcification and growth rates (25–27). Long-term adaptation to OA is a dynamic and complex process. Schlüter *et al.* (28) conducted a long-term OA exposure experiment over 4 years, with 2100 generations of phytoplankton and found that the initial phenotypic and biogeochemical traits were reverted. Few research studies have thoroughly assessed the different stages of the macroalgal life cycle (29) and to our knowledge no studies have been performed to assess adaptation of any macroalgal species to OA (30). In the marine environment, organisms are affected by interacting factors, including OA and UV radiation levels. Furthermore, Gao and Zheng (26) showed

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that OA enhanced UVB radiation sensitivity and reduced photosynthesis in calcified macroalgae.

Marine green algae are mostly distributed in coastal waters, with environmental changes largely related to tidal patterns and human activities. *Ulva linza* is an intertidal green macroalgae that is highly susceptible to fluctuation in physicochemical variables, such as extreme temperatures, high or low salinity levels, desiccation, high CO_2 concentrations and seasonal changes in both solar visible and UV radiation (31–33). Changes in these parameters alter physiological features of *U. linza*, as well as affecting its primary productivity in coastal waters. Elevated CO_2 levels decrease CCM activity and release additional energy as ATP, to help drive repair from UVB damage. To test this hypothesis, the sensitivity of the economically and ecologically important species *U. linza* to short-term (ST) or long-term (LT) OA under PAR and artificial UV radiation (PAB; PAR+UVA+UVB) was investigated, to further our understanding of the processes of acclimation and adaptation of macroalgae to OA and the evolution of green tides.

MATERIALS AND METHODS

Thalli and culture conditions. *Ulva linza* was collected from the intertidal zone of Gaogong island (119.30E, 34.50N), Lianyungang (China) and was transported in ice coolers to the laboratory. Selected thalli were cleaned with filtered natural seawater to remove surface epiphytes. The mature thalli were selected and precultured in aquaculture tanks containing 1 L of aerated and filtered sterile seawater, supplemented with 60 μM NO_3^- and 8 μM PO_4^{3-} . The light intensity was maintained at 300 μmol photons m^{-2} s^{-1} with a 12:12 h light:dark cycle, and the temperature was controlled as 20°C with <0.5°C variation. OA acclimation experiments were performed under short-term and long-term OA conditions. The thalli reaching 1 cm in length were selected and cultured for 1 week under ambient (400 μatm, LC) and elevated (1000 μatm, HC) CO_2 levels, at the same light and temperature conditions as defined above (termed short-term OA). Spores released from the *U. linza* thalli were cultured under both LC and HC conditions, until thalli grew to 1 cm in length, over a period of about 1 month (termed long-term OA). High CO_2 levels were obtained using a CO_2 plant incubator (HP1000G-D, Ruihua Instruments, Wuhan, China). Low CO_2 levels were obtained by pumping in ambient air from outside. The pH levels in the HC and LC cultures were maintained at 7.83 and 8.18, respectively, with variation of <0.04 maintained by constant aeration and renewal of the medium every 2 days. Three triplicate experiments were conducted per treatment.

Estimate of seawater carbonate system. The pH was measured with a pH meter (pH 700, Eutech Instruments, Singapore), and total alkalinity was assessed by titration. Other carbonate system parameters were obtained with CO2SYS software (34), and the equilibrium constants K_1 and K_2 were used for carbonic acid dissociation (35).

UV radiation treatments. Experiments were performed in a dark room with a solar simulator installed, comprising a 1000 W xenon arc lamp and a cooling system (CTP-3000, Japan), which controlled the temperature to be the same as under culture conditions. The thalli were cut into 1 cm segments and cultured under the above-defined conditions for at least 1 h, to minimize the damaging effects of cutting and were then transferred to 30 mL quartz tubes containing 30 mL seawater, which was dark adapted for 15 min and treated with Milli-Q water (as control) or lincomycin (0.5 mg mL^{-1}). Lincomycin was used to inhibit protein synthesis and for determination of the damage rate in the absence of repair. Tubes were then sequentially transferred to a water bath at 1 min intervals. The light levels were maintained at 440 μmol photons m^{-2} s^{-1} for PAR and 41.6 W m^{-2} for UV radiation, which contained 38.96 W m^{-2} UVA and 2.64 W m^{-2} UVB. These UV radiation intensities were selected to reflect the average light intensities experienced during summer and the mid-day intensity in winter at the site. Filters (ZJB280, ZJB400, Mylar-D, Ultraphan, Schott filters, Germany) were used to cover the water bath to shield radiation below either 280 or 400 nm, to create PAR+UVA+UVB (PAB) and PAR treatments, respectively. Temperatures were controlled at 20°C using a cooling system (CTP3000, Eyela). Effective quantum yields were measured at 10 min intervals from 0 to 30 min. After exposure, samples were recovered under low light conditions (30 μmol photons m^{-2} s^{-1}), with effective quantum yields measured at 10, 20, 30, 60 and 120 min. The relative inhibition of effective quantum yield due to UV exposure was calculated according to Eq. (1):

$$\text{Relative UV inhibition}\,(\%) = (P_{\text{PAR}} - P_{\text{PAB}})/P_{\text{PAR}} \times 100 \qquad (1)$$

where P_{PAR} and P_{PAB} are the effective quantum yield under PAR and PAB treatments, respectively. Relative UV inhibition was calculated when P_{PAR} and P_{PAB} were significantly different.

Chlorophyll fluorescence measurements. Samples in these tubes were dark adapted for 15 min and then transferred to the water bath sequentially at 1 min intervals, for light exposure. Chlorophyll fluorescence was determined using a portable fluorometer (AP-C 100, Czech Republic). During the light exposure experiments, samples were removed from the quartz tubes every 10 min for chlorophyll fluorescence measurements, to ensure that each sample was exposed to radiation for the same duration. After three rounds of measurements (30 min), samples without lincomycin were transferred to the pot under low light conditions and maintained at same temperature for recovery, while chlorophyll fluorescence was measured continuously at 10, 20, 30, 60 and 120 min.

Determination of UV-absorbing compounds. About 0.005 g of samples (fresh weight) were mixed with 8 mL absolute methanol (Sangon Biotech, Shanghai, China) at 4°C overnight in the dark, for extraction of UV-absorbing compounds (UVACs). After centrifugation at 5000 ***g*** for 10 min, the supernatants were analyzed using a spectrophotometer (UV-530, Beckman Coulter), with scanning performed in the range of 250–750 nm. The extracted UVACs were obtained according to the method reported by Helbling *et al.* (35). The contents of UVACs were estimated by determining the ratio of UVACs to Chl *a* according to Dunlap *et al.* (36).

Data analysis. Effective quantum yields were calculated according to Eq. (2) below:

$$\text{Effective quantum yield} = (\text{Fm}' - \text{Ft})/\text{Fm}' \qquad (2)$$

where Fm′ refers to the maximum fluorescence yield and Ft refers to the steady-state fluorescence under actinic light. The rate of UV radiation-induced repair to photosystem II (PSII) (r, min^{-1}) and the damage rate (k, min^{-1}) with lincomycin were calculated according to Eq. (3):

$$\frac{P_t}{P_0} = \frac{r}{k+r} + \frac{k}{k+r}e^{-(k+r)t} \qquad (3)$$

where P_0 and P_t represent the initial effective quantum yield at 0 min and t (min), respectively (37,38). Recovery rates under low light levels were estimated according to Equation (4) below:

$$y = y_0 + c(1 - e^{-\alpha t}) \qquad (4)$$

where y represents the effective quantum yield at time t (min) following low level light treatment, α represents the recovery rate, and y_0 and c are constants (37,38). The relative inhibition of effective quantum yield by UV exposure was calculated according to Eq. (1).Data are shown as mean values ± standard deviation (SD). SPSS 18.0 was used for statistical analysis, with significant differences in the kinetics of photochemical quantum yield among treatments analyzed by repeated measures-ANOVA, while two-way ANOVA or *t*-test analysis was used for all other parameters. A confidence interval of 95% was set for all experiments.

RESULTS

Carbonate system and photochemical quantum yield

Elevated CO_2 concentrations altered carbonate system parameters in seawater (Table 1). Compared with LC, elevated pCO_2 level significantly increased DIC, HCO_3^- and CO_2 by 6.05%, 10.17% and 1.54%, respectively ($P < 0.05$). HC resulted in a significant decrease by 4.40% in pH and 52.28% in CO_3^{2-} concentrations

Table 1. Parameters of the seawater carbonate system under the ambient (LC; 395 μatm) and elevated CO_2 concentrations (HC; 1000 μatm). DIC = dissolved inorganic carbon; TA = total alkalinity. Data are the mean values ± SD (n = 3). Different letters represent significant differences between the CO_2 levels ($P < 0.05$).

Condition	pCO_2 (μatm)	pH	DIC (μmol kg^{-1})	HCO_3^-	CO_3^{2-}	CO_2	TA
LC	417.10 ± 24.28^a	8.18 ± 0.02^a	2077.31 ± 43.76^a	1894.52 ± 44.78^a	168.95 ± 2.16^a	13.84 ± 0.81^a	2315.33 ± 39.11^a
HC	1060.22 ± 45.56^b	7.82 ± 0.01^b	2202.97 ± 54.97^b	2087.16 ± 52.68^b	80.63 ± 1.71^b	35.18 ± 1.51^b	2293.33 ± 54.15^a

($P < 0.05$). Repeated measures-ANOVA analysis showed that UV and CO_2 were the dominant factors affecting the photochemical quantum yield of *U. linza* under short-term conditions (Table S1—Supporting Information and Figure S1A). The photochemical quantum yield was around 0.55 before light exposure, with a rapid decreased observed after PAR and PAB radiation exposure, with a greater reduction observed under PAB than that under PAR conditions (Fig. 1A). No differences were observed between ambient and high CO_2 concentrations under PAB exposure conditions ($P > 0.05$). During the low-light exposure period, UV and CO_2 were the dominant factors affecting the photochemical quantum yield of *U. linza* under short-term conditions (Table S2 and Fig. 1A). At ambient CO_2 levels, the quantum yield recovered to its initial value within 120 or 240 min, when under PAR or PAB treatment conditions, respectively, while with increased CO_2 concentrations cells recovered around 95% of their quantum yield under both PAB and PAR treatment conditions (Fig. 1A). For *U. linza* cultures grown under long-term OA conditions, UV was the dominant factor affecting the photochemical quantum yield (Table S1 and Fig. 1A). A similar trend was observed in *U. linza* cultures grown under both long- and short-term OA conditions (Fig. 1), with quantum yield decreasing gradually when cells were exposed to PAR and PAB radiation, to 0.33 and 0.2, respectively. During the low-light exposure period, both UV and CO_2 were the dominant factors affecting the photochemical quantum yield of *U. linza* under long-term conditions (Table S2 and Fig. 1B). Under elevated CO_2 conditions, the quantum yield of cells recovered to 0.63, after low light conditions and PAB treatment (Fig. 1B).

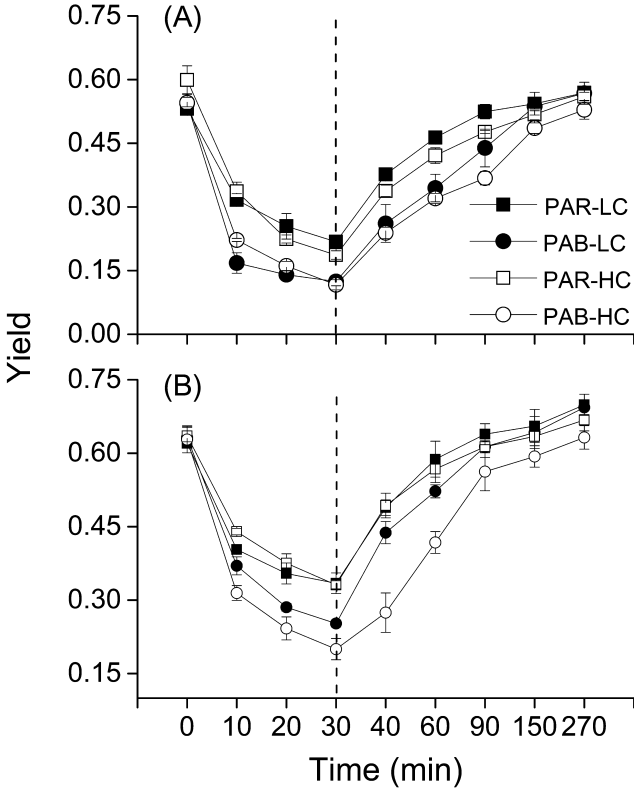

Figure 1. The quantum yields of *U. linza* grown under different CO_2 concentrations (395 and 1000 μatm) in short-term (A) and long-term (B) cultivation for 30 min under PAR or PAB radiation, with subsequent recovery under low light levels for 240 min. Vertical lines represent SD, n = 3.

The repair rate and damage rate of photosystem II

Under PAR exposure conditions, two-way ANOVA showed that CO_2 and time had no interactive effect, with CO_2 being the dominant factor affecting *U. linza* repair rates (r) (Table S3 and Fig. 2A). The repair rates (r) for photosystem II significantly decreased from 0.07 ± 0.02% (LC) to 0.02 ± 0.01% (HC) following short-term exposure, with a similar trend found following long-term exposure, with increased CO_2 causing repair rates (r) to decrease to 64.98% (Fig. 2A; $P < 0.05$). CO_2 levels and exposure duration had the main interactive effect, with each factor having a dominant effect on *U. linza* repair rates (r) (Table S3 and Fig. 2B). The damage rate (k) was 0.21 ± 0.03% (LC) and 0.07 ± 0.01% (HC), respectively, under short-term cultivation, while thalli grown under long-term exposure conditions reduced the damage rate (k) to 0.06 ± 0.00% (LC) and 0.05 ± 0.01% (HC), respectively (Fig. 2B). The difference between LC and HC was significant following both short- and long-term OA. Exposure duration and CO_2 levels did not have a dominant interactive effect on the ratios of repair to damage (r/k) in *U. linza,* while exposure duration did have a dominant effect (Table S3 and Fig. 2B). Although the ratio of repair to damage (r/k) showed no significant changes under short- or long-term OA acclimation periods ($P > 0.05$), it decreased by 18.11% with long-term increased CO_2 exposure.

When thalli were exposed to PAB, exposure duration and CO_2 levels had an interactive effect, with CO_2 having a dominant effect on *U. linza* repair rates (r; Table S4 and Fig. 3A; $P < 0.05$). Repair rates (r) decreased from 0.06 ± 0.001% to 0.03 ± 0.01% following exposure to short-term increased CO_2 concentrations (Fig. 3A). Following long-term cultivation, elevated CO_2 concentrations reduced repair rates (r) by 5.12%, although no significant difference was observed between ambient and increased CO_2 concentrations ($P > 0.05$). Exposure duration and CO_2 levels had a dominant interactive effect, with both factors having dominant effects on the damage rate (k; Table S4 and Fig. 3B; $P < 0.05$). Following increased CO_2 levels, the damage rates (k) were 0.19 ± 0.04% (LC) and 0.11 ± 0.01% (HC) under short-term conditions, while long-term cultivation reduced them to 0.07 ± 0.01% (LC) and 0.09 ± 0.01% (HC), respectively (Fig. 3B). In contrast, long-term exposure to

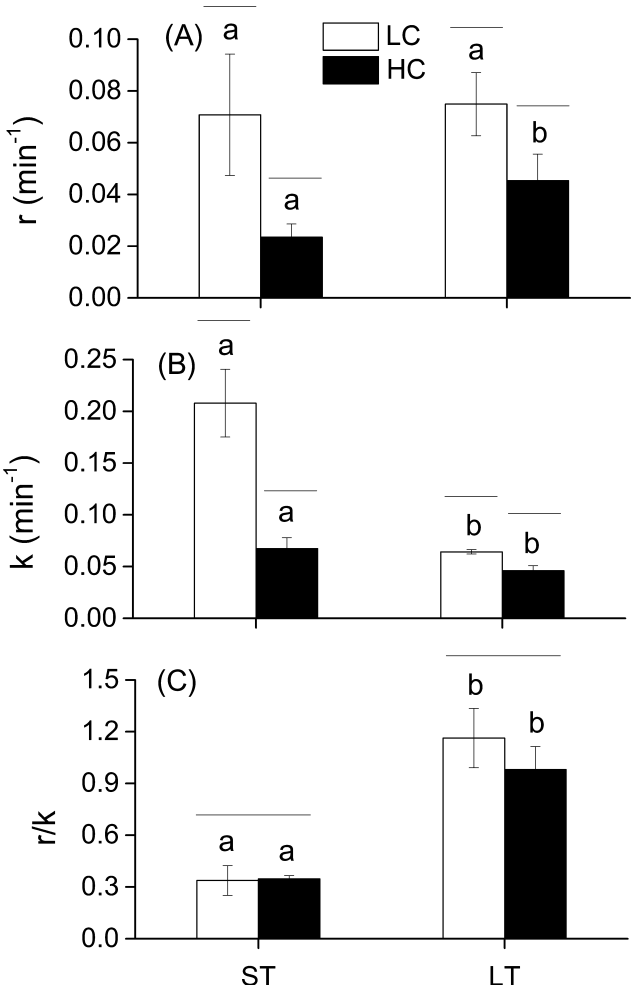

Figure 2. The repair rate (A) and damage rate (B) of photosystem II in *U. linza* during PAR exposure under different CO_2 concentrations (395 and 1000 μatm), following short- or long-term cultivation and the ratio of repair to damage rate (C). Horizontal lines represent the significant differences ($P < 0.05$) between ambient and elevated CO_2 levels, under the same culture times. Different letters represent the significant differences ($P < 0.05$) between short- or long-term cultivation, at the same CO_2 concentration conditions.

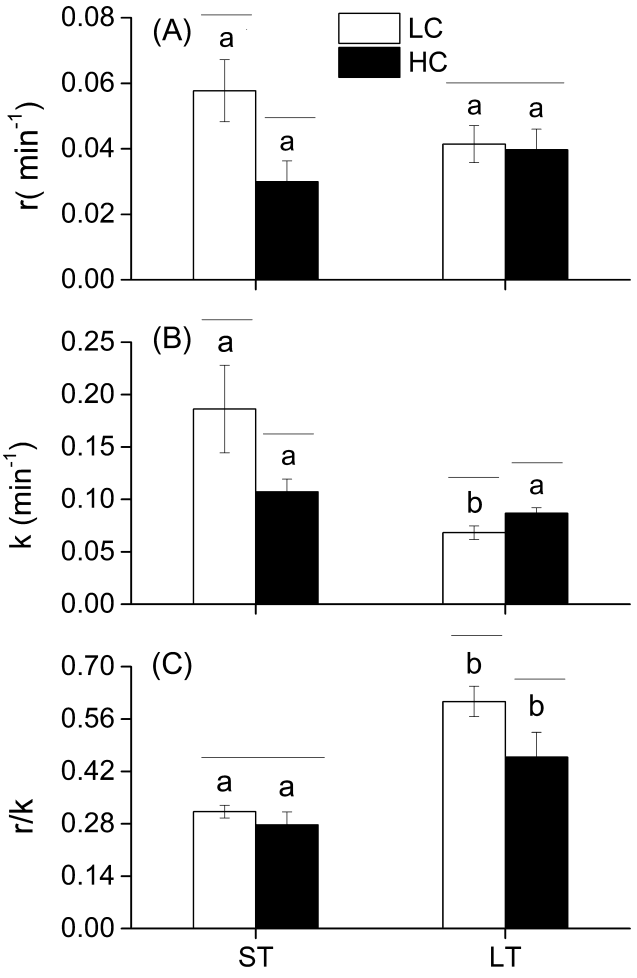

Figure 3. The repair rate (A) and damage rate (B) of *U. linza* photosystem II, during PAB exposure to different CO_2 concentrations (395 and 1000 μatm) under short- or long-term cultivation times, and the ratio of repair to damage rate (C). Horizontal lines represent significant differences ($P < 0.05$) between ambient and elevated CO_2 levels, at the same culture time. Different letters represent significant differences ($P < 0.05$) between short-term and long-term cultivation times, under the same CO_2 concentration conditions.

elevated CO_2 levels caused the damage rate (k) to significantly increase to $0.09 \pm 0.01\%$, as compared with ambient levels ($0.07 \pm 0.01\%$; $P < 0.05$). Both time and CO_2 had a dominant effect on the ratios of repair to damage (r/k; $P < 0.05$), while no interactive effect was observed between these two factors (Table S3 and Fig. 3C; $P > 0.05$). The ratios of repair to damage (r/k) in *U. linza* grown under short-term exposure conditions were $0.31 \pm 0.02\%$ (LC) and $0.278 \pm 0.03\%$ (HC), respectively, increasing to $0.61 \pm 0.04\%$ (LC) and $0.46 \pm 0.07\%$ (HC) in *U. linza* grown under long-term exposure conditions. Elevated CO_2 concentrations significantly reduced the ratio of repair to damage (r/k) by 33.33%, following long-term cultivation ($P < 0.05$).

The relative inhibition induced by UV radiation on the photosystem II

Figure 4 shows the relative inhibition induced following UV radiation exposure. CO_2 and duration of exposure had no effect on the relative degree of UV inhibition and no interactive effect was observed between these two factors (Table S5 and Fig. 4). Long-term exposure conditions decreased the relative degree of UV inhibition to 24.58%, as compared to short-term exposure conditions (38.27%). The difference between short- and long-term exposure conditions were not significant at ambient CO_2 concentrations ($P > 0.05$). In contrast, long-term OA exposure increased the relative degree of UV inhibition by 5.59%, as compared with short-term OA exposure, although no significant differences were observed between them ($P > 0.05$). Under long-term exposure conditions, elevated CO_2 concentrations significantly increased the relative degree of UV inhibition by 37.91% ($P < 0.05$).

Recovery rate constants

Under low light conditions, UV and CO_2 had dominant effects on the rate of recovery (r_D), although no interactive effect was observed between these two factors under short-term exposure conditions (Table S6 and Fig. 5A). High CO_2 concentrations

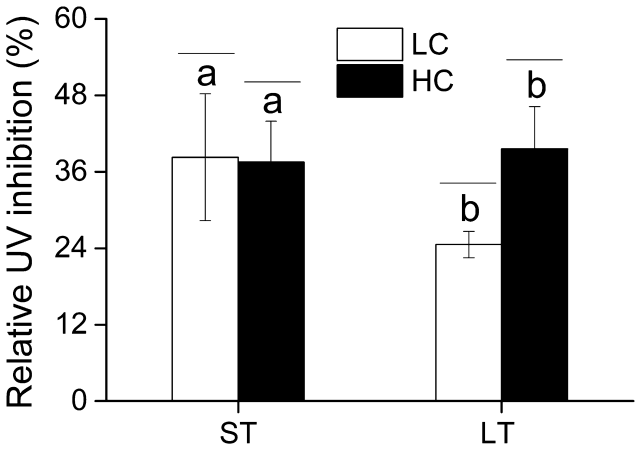

Figure 4. The relative inhibition induced by UV radiation on the photosystem II of *U. linza* during PAR or PAB exposure, under different CO_2 concentrations (395 and 1000 μatm) with short- or long-term cultivation. Error bars indicate the standard deviation (n = 3). Horizontal lines represent significant differences ($P < 0.05$) between ambient and elevated CO_2 levels, at the same culture time. Different letters represent significant differences ($P < 0.05$) between short- and long-term cultivation, under the same CO_2 concentration conditions.

decreased the rate of recovery (r_D) by 52.38% following PAR exposure and by 33.33% following PAB exposure, under short-term exposure conditions (Fig. 5A). There were no interactive effects observed between UV and CO_2 levels, with neither of these factors having an observable effect on the rate of recovery (r_D) under long-term exposure conditions (Table S6 and Fig. 5B). Under long-term increased CO_2 levels, the rate of recovery decreased by 18.52% following PAR exposure, while following PAB exposure, rates of recovery (r_D) increased significantly by 25% ($P < 0.05$) (Fig. 5B), suggesting that the damage induced by UV radiation could be effectively repaired.

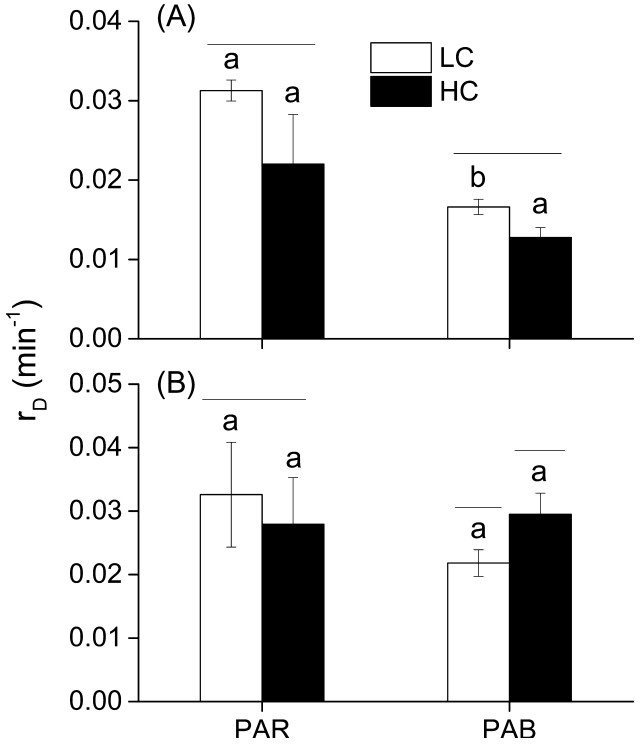

Figure 5. The recovery rate constants (r_D) under short-term (A) and long-term (B) cultivation periods with PAR or PAB exposure and low light levels, at different CO_2 concentrations (395 and 1000 μatm). Horizontal lines represent significant differences ($P < 0.05$) between ambient and elevated CO_2 levels, under the same light irradiation conditions. Different letters represent significant differences ($P < 0.05$) between PAR and PAB exposures, under the same CO_2 concentration conditions.

UV-absorbing compounds

The UV spectra of methanol extracts of *U. linza* showed similar pattern. Relatively higher levels of absorption were observed at 430 and 480 nm, following both short- and long-term OA exposure (Fig. 6A), with no significant differences between them observed ($P > 0.05$). Exposure duration and CO_2 level had no interactive effects on the concentration of UVACs, while exposure duration alone was found to have a dominant effect (Table S7 and Fig. 6B). Under short-term exposure conditions, no significant differences in UVACs were observed between ambient or high CO_2 concentrations ($P > 0.05$) (Fig. 6B). Conversely, increased CO_2 concentrations significantly increased the concentration of UVACs under long-term exposure conditions ($P < 0.05$).

DISCUSSION

Thalli grown under long-term OA exposure conditions showed higher sensitivity to UV, as compared with those grown under

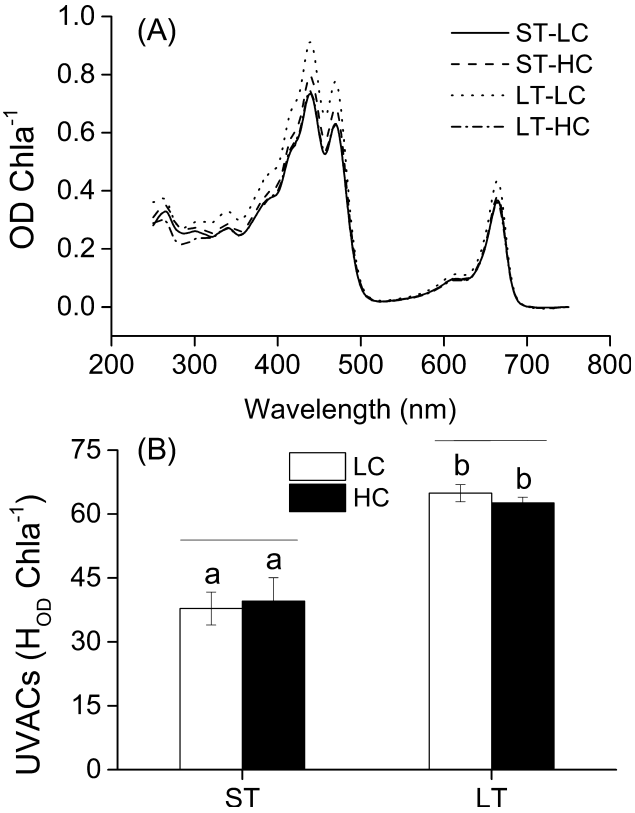

Figure 6. The absorption spectrum of methanol extracts of *U. linza* chlorophyll (A) and UV-absorbing compounds (B), under short- and long-term cultivation periods at both ambient and elevated CO_2 levels. Horizontal lines represent significant differences ($P < 0.05$) between ambient and elevated CO_2 levels, with the same culture time. Different letters represent significant differences ($P < 0.05$) between short-term and long-term cultivation periods, under the same CO_2 concentration conditions.

short-term OA exposure conditions. This effect can be attributed to the decrease in *r*:*k* in algae cultivated under long-term OA conditions (Fig. 3). Long-term OA exposure had no effect on the repair constant (*r*), but significantly enhanced the rate constant for damage by UV radiation (*k*), represented by the damage to photosynthetic performance. Previous research has shown that UV radiation can cause severe damage to nucleic acids, proteins, photosynthesis and reproduction (15,39–41). Photosystem II is a main photochemical reaction site for photosynthesis and is susceptible to light quality and intensity. High PAR irradiance and UV radiation can result in obvious destruction of this pigment–protein complex, containing a heterodimer of D1 and D2 polypeptides (42,43). Simultaneously, high PAR irradiance and UV radiation can increase the rate of repair and induce protective mechanisms which enhance UV tolerance (44). It has also been shown that after 20 days, elevated CO_2 levels can increase DIC in the culture media, photosynthesis and the growth of thalli (45). Xu *et al.* (46) found that after culturing under 1000 μatm CO_2 for 2 months, OA regulated the photochemical and photorespiratory pathways of *U. prolifera*. Increased non-photochemical quenching (NPQ) of cells was observed following UV radiation, in the diatom *Phaeodactylum tricornutum* grown under increased CO_2 levels for more than 20 generations, as well as a minor decrease in damage to PS II (47). Chen and Gao (48) reported that after nine generations of *Phaeocystis globosa* cultivation under high CO_2 concentrations, even though increased UVB-related photoinhibition of growth and NPQ was observed, the chlorophyll *a* (Chl *a*) content and dark respiration rate increased, resulting in an increase in the cellular energy demand due to the increased level of stress. Xu *et al.* (49) found that ephemeral species of the genus *Ulva* could increase their photosynthesis and growth rates under elevated CO_2 conditions, while longer-living perennial species showed smaller responses. In order to protect themselves from the damage caused by UV radiation, macroalgae can utilize escape, repair and barrier mechanisms to protect them during the process of adaptation and evolution. Franklin *et al.* (50) found that diverse strategies to resist UV damage were developed in cyanobacteria, including mechanisms such as gliding motility which allows escape from diurnal high UV intensities. In addition, UVACs such as scytonemin, carotenoids and mycosporine-like amino acids have been reported to accumulate as a response to UV radiation (51–54). UVACs play an important role in the protection of macroalgae against UV damage (50). For *U. linza*, the concentration of UVACs were significantly reduced under short-term OA exposure regimes (Fig. 6), resulting in more damage to photosystem II by UVB radiation. Studies have shown that after calcifying phytoplankter *Emiliania huxleyi* was exposed to UV radiation for 30 days, both calcification and photosynthesis were significantly inhibited. In addition, these rates were further inhibited with increasing acidification, indicating that the effect of a high-CO_2 and low-pH ocean enhanced the negative effects of UVR on *E. huxleyi,* the main pelagic calcifying species (54).

Ulva linza grown under long-term OA conditions can limit these negative effects by enhancing the recovery ability under low light conditions. The photochemical efficiency recovery rate (r_D) significantly enhanced with increased CO_2 levels after PAB exposure under low light conditions, while the recovery rate (r_D) showed notable hysteresis at low CO_2 concentrations. These results may relate to the preferential degradation and resynthesis of the D1 and D2 protein subunits (55). The damaged D1 protein can be replaced by *de novo* protein synthesis, inserting the new D1 protein into PSII (55,56). Moreover, damage to the D1 protein has been demonstrated to be directly proportional to light intensity (57,58) and the D1 protein turnover rate is 50- to 100-fold faster than other chloroplast-related proteins (59). This high turnover has been associated with *in vivo* photoinhibition, which has been represented as an imbalance between the degradation and synthesis of D1 proteins (60). Previous studies have found that adversity stress factors can cause photoinhibition, such as UVA radiation, high PAR irradiance, low or high temperatures, acidic and alkali environments (61–63). Macroalgae also develop some strategies to cope with this stress. Crawley *et al.* (64) reported that elevated CO_2 concentrations can increase photo-acclimation to sub-saturating light intensities, enhance dark respiration per cell and xanthophyll de-epoxidation, as mechanisms to reduce damage. Long-term OA can enhance the turnover of D1/D2 proteins in this green tidal alga under low light conditions, which may involve strategies such as increasing the activities of enzymes for synthesis of D1 or D2 proteins or other mechanisms.

The exposure of the green tide alga *U. linza* to UV radiation under short-term OA condition treatments utilized adult thalli and the exposure lasted for 1 week. These results show that CO_2 simultaneously decreased the damage rate (*k*) and repair rate (*r*) of thalli grown under short-term OA conditions with PAB treatment, resulting in no significant difference in the effect of UV radiation. However, long-term OA exposures were performed from the spore germination stage to adult thalli stages and lasted about one month. OA has been reported to have different effects on thalli during different life cycle stages. Roleda *et al.* (65) found that increased CO_2 levels caused a significant decreased in germination rates in the giant kelp, *Macrocystis pyrifera* (Laminariales), with no significant difference in the rate of male to female gametophyte ratio. Under pH 7.86 and 7.61 conditions, the gametophytes were 32% and 10% larger, respectively, as compared to control (pH 8.19), suggesting that pH was a primary environmental modulator of sex (65). Increased CO_2 concentrations in artificial cultures reduced *Corallinaceae* spore production and growth, causing an increase in the mortality of germination disks (66). Olischläger *et al.* (67) reported that future CO_2 concentrations could cause a slight enhancement in photosynthetic performance and a significant increase in growth of *U. lactuca.* It is of note that a large number of studies have shown that different species and different life stages of the same species present with varying responses to OA. Gao *et al.* (32) found that higher CO_2 concentrations increased germination rates and weight, with no significant differences in the growth rate of *Ulva* spores cultivated under low and high CO_2 levels. It was found that high CO_2 concentrations significantly increased the reproductive rate and lipid content of *Ulva* spores and decreased the protein content of adult *Ulva* plants. This may be due to *Ulva* spores being more sensitive to OA, as well as accumulation of the influence of OA throughout cultivation to the adult stage (32). Long-term OA exposure decreased the growth rate of *U. linza* (68) and the red coralline algae *Gracila*ria *tenuistipitata* (69), as well as decreasing calcification, respiration rate and altering intracellular acid–base stability (26). Overall, this results in a decline in the ability of thalli to resist UV radiation and improves the corresponding ability to repair.

CONCLUSION

Future CO_2-induced OA conditions are likely to mediate the physiological performance of green tide algae. In the natural environment, although *U. linza* in surface marine waters suffers from significant negative effects at mid-day due to UV radiation levels, in the evening the damage to thalli can be effectively repaired, suggesting that algae have a strong ability to adapt to changes in their environment. Simultaneously, OA can provide a more adequate carbon source, promoting the growth of algae and therefore encouraging large-scale green tide bloom events.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Table S1. Repeated Measures-ANOVA for the effects of UV and CO_2 on initial photochemical quantum yield of *U. linza* under short and long-term OA.

Table S2. Repeated Measures-ANOVA for the effects of UV and CO_2 on initial photochemical quantum yield of *U. linza* under dim light at short and long-term OA.

Table S3. Two-way analysis of variance for the effects of time and CO_2 on repair rates (r), damage rate (k) and the ratio of repair to damage (r/k) of *U. linza* at short and long-term OA under PAR radiation.

Table S4. Two-way analysis of variance for the effects of time and CO_2 on repair rates (r), damage rate (k) and the ratio of repair to damage (r/k) of *U. linza* at short and long-term OA under PAB radiation.

Table S5. Two-way analysis of variance for the effects of time and CO_2 on relative UV inhibition of *U. linza* at short and long-term OA.

Table S6. Two-way analysis of variance for the effects of UV and CO_2 on rate for recovery (r_D) of *U. linza* at short and long-term OA. UV*CO_2 means the interactive effect of UV and CO_2.

Table S7. Two-way analysis of variance for the effects of time and CO_2 on UV-absorbing compounds (UVACs) of *U. linza* at short and long-term OA.

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