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Effects of different light conditions on repair of UV-B-induced damage in carpospores of *Chondrus ocellatus* Holm*

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Abstract We evaluated the effects of ultraviolet-B (UV-B) radiation and different light conditions on the repair of UV-B-induced damage in carpospores of *Chondrus ocellatus* Holm (Rhodophyta) in laboratory experiments. Carpospores were treated daily with different doses of UV-B radiation for 48 days, when vertical branches had formed in all treatments; after each daily treatment, the carpospores were subjected to photosynthetically active radiation (PAR), darkness, red light, or blue light during a 2-h repair stage. Carpospore diameters were measured every 4 days. We measured the growth and cellular contents of cyclobutane pyrimidine dimers (CPDs), chlorophyll *a*, phycoerythrin, and UV-B-absorbing mycosporine-like amino acids (MAAs) in carpospores on Day 48. Low doses of UV-B radiation (36 and 72 J/m^2) accelerated the growth of *C. ocellatus*. However, as the amount of UV-B radiation increased, the growth rate decreased and morphological changes occurred. UV-B radiation significant damaged DNA and photosynthetic pigments and induced three kind of MAAs, palythine, asterina-330, and shinorine. PAR conditions were best for repairing UV-B-induced damage. Darkness promoted the activity of the DNA dark-repair mechanism. Red light enhanced phycoerythrin synthesis but inhibited light repair of DNA. Although blue light, increased the activity of DNA photolyase, greatly improving remediation efficiency, the growth and development of *C. ocellatus* carpospores were slower than in other light treatments.

Keyword: *Chondrus ocellatus* Holm; UV-B radiation; blue light; red light; repair

1 INTRODUCTION

During the latter part of the 20th century, the average amount of ultraviolet-B (UV-B) radiation reaching the earth's surface has increased as a result of the depletion of stratospheric ozone (Butler et al., 1999). Over the past decade, stratospheric ozone depletion has slowed and levels have even recovered slightly. However, complete recovery of the ozone layer will be a slow process, and marine organisms will be continuously exposed to high doses of UV-B radiation for some time to come (Hu, 2007). Interest in the effects of UV-B radiation on macroalgae is increasing (Liu et al., 2008).

Macroalgae represent approximately 10% of primary productivity in the ocean (Smith, 1981). They grow in the eulittoral and upper sublittoral zones, where they are exposed to increasing levels of radiation. *Chondrus ocellatus* Holm (Rhodophyta) is a species of red algae that grows abundantly along rocky shores from the middle intertidal into the subtidal zones of Qingdao, Shandong Province, China. It is the dominant species with the greatest biomass in the macrobenthic algae community in the rocky low intertidal zones of Qingdao (Tang et al., 2008). *Chondrus ocellatus* are widely distributed across geographical areas and at different depths.

The effects of UV-B radiation on marine macroalgae include damage to DNA, changes in the DNA repair capacity, and alterations to gene expression, protein structures, photosynthetic pigments, secondary metabolism, morphology, and growth (Frohnmeyer and Staiger, 2003; Xu et al., 2003; He et al., 2004; Bischof et al., 2006; Zhong et

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al., 2009; Pereira et al., 2014; Polo et al., 2014; Simioni et al., 2014). In macroalgae, large molecules can be damaged by high levels of UV-B radiation. Increased UV-B radiation can phototransform DNA, leading to the production of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidinone dimers (Strid et al., 1994), which inhibit DNA replication and transcription (Buma et al., 1995, 2000). Photosynthetic pigments (e.g., chlorophylls, phycobilins, and carotenoids) show varying sensitivities to UV-B radiation; phycobilins are the most sensitive, followed by chlorophylls, and then carotenoids (Teramura, 1983).

Damage to DNA, proteins, and pigments inhibit the normal physiological processes of macroalgae. In addition to impacting photosynthesis and respiration, increased UV-B radiation also damages other physiological process, such as nutrient absorption and zoospore mobility (Renger et al., 1986; Allen et al., 1997; Karsten, 2008). The negative effects of UV-B radiation on these physiological processes can decrease growth rate and even cause death. These effects can change the population size of a given species, ultimately influencing the marine ecosystem (Duffy and Hay, 2000; Sousa and Connall, 1992). Species distributions can also be affected, as algae that were distributed near the surface colonize deeper layers (Wiencke et al., 2006).

Macroalgae have evolved various protection and repair mechanisms to reduce and reverse UV-B-induced damage. The photosynthetic system responds to UV-B radiation via two mechanisms: faster repair of photoinhibition and depressed photoinhibition (Bischof et al., 1999). In green algae, a mechanism exists by which UV damage to the D1 protein in photosystem II can be detected and repaired (Yokthong et al., 2001). Over the long term, a series of antioxidant systems has evolved to scavenge reactive oxygen species (Aguilera et al., 2002b). Algae have also evolved mechanisms to repair UV-B induced damage to DNA, such as light-dependent repair of CPDs in many algal species (Pakker et al., 2000); dark repair also occurs in many plants (Li et al., 2000; He et al., 2006). Some algae also synthesize mycosporine-like amino acids (MAAs), which can absorb UV radiation and emit heat or fluorescence to prevent damage (Dunlap and Shick, 1998; Karsten et al., 1998).

There are few studies which evaluated the effects of UV-B radiation on early development stage and spores. Because the algae in juvenile stages are smaller in size and simple in structures, different kinds of stress can affect the algae more evidently, as shown for various brown algae zoospores and germlings (Wiencke et al., 2000, 2006; Altamirano et al., 2003), unicells of Ulvales (Cordi et al., 2001) and early development of Gigartinales (Roleda et al., 2004). So the effect of UV-B radiation on the microscopic life stages of the algae are essential to recruit. In this study, we evaluated the effects of UV-B radiation on carpospore development, UV-B adaptability, and UV-B screening capacity in the early developmental stages of *C. ocellatus*. We also examined the role of light quality during repair of UV-B-induced damage.

2 MATERIAL AND METHOD

2.1 Algal material

C. ocellatus is abundant species of red alga along the coast of Qingdao, and inhabits the intertidal and upper sublittoral zones of rocky shorelines. It grows from a discoid holdfast and branches four or five times in a dichotomous. *C. ocellatus* with mature carpogonium tissues was collected from rocks in the intertidal zone at Taipingjiao (a public beach) Qingdao, Shandong Province, China, in summer. No specific permissions were required. Experiments using about 200 fronds of gametophytes and were carried out in the laboratory. Immature sporophytes of *C. ocellatus* have the same morphological characters as gametophytes.

2.2 Release of carpospores

Sporangia with carpospores were washed in sterilized seawater and cleaned using a banister brush. The washed gametophytes with carposporophytes were dried in the shade for 4 h at 18°C to promote the spread of spores. Natural seawater was sterilized by filtering through 0.45-μm microporous filtering film and then autoclaving. The three fronds of gametophytes were placed in sterile Petri dishes containing 8 glass slides and 300 mL sterilized seawater. The dishes were kept in the dark until the carpospores attached to the slides (20/3 mm^2), then the attached carpospores were used for UV-B radiation experiments. All cultures were maintained in modified Provasoli culture solution consisting of sterilized seawater enriched with nitrogen (8.24×10^{-4} mol/L $NaNO_3$) and phosphorus (3.26×10^{-5} mol/L $NaH_2PO_4\cdot H_2O$) (Starr and Zeikus, 1993). The cultures were kept at 18±1°C under a 12:12 (L:D) photoperiod of 40 μmol photons/(m^2·s) of photosynthetically active radiation (PAR)

Table 1 UV-B radiation and light treatments during repair periods for carpospores released from *Chondrus ocellatus* blades

Treatment	Dose of UV-B radiation (J/m^2)	PAR group (Control)	Darkness group	Red light group	Blue light group
A	0	0+2 h PAR (control)	0+2 h dark (control)	0+2 h red light (control)	0+2 h blue light (control)
B	36	5 min+ 2 h PAR	5 min+2 h dark	5 min+2 h red light	5 min+2 h blue light
C	72	10 min+2 h PAR	10 min+2 h dark	10 min+2 h red light	10 min+2 h blue light
D	108	15 min+2 h PAR	15 min+2 h dark	15 min+2 h red light	15 min+2 h blue light
E	144	20 min+2 h PAR	20 min+2 h dark	20 min+2 h red light	20 min+2 h blue light
F	180	25 min+2 h PAR	25 min+2 h dark	25 min+2 h red light	25 min+2 h blue light

provided by 40-W daylight fluorescent tubes (Philips, Ningbo, China). Each treatment consisted of five replicates, one dish was one replicate. The culture solution was replaced every 2 d until Day 48, when a vertical branch had formed in each treatment. The carpospores were then assessed for physiological and biochemical indices.

2.3 UV-B treatments

All experimental treatments occurred under laboratory conditions. A rank of four fluorescent UV-B lamps (Q-Lab, Cleveland, OH, USA) provided supplemental UV-B radiation. The UV-B intensity was 7.2 μW/cm^2, as measured with a UV-B type UV radiometer (Beijing Normal University Photoelectric Instrument Factory, Beijing, China). The light was filtered with the cellulose diacetate foil to achieve 0% transmission below 286 nm.

For these experiments, five levels of UV-B radiation (36, 72, 108, 144, and 180 J/m^2) were employed by adjusting the exposure time (5, 10, 15, 20, and 25 min, respectively) (Table 1). The levels of UV-B radiation were setting according to the preliminary analysis. The tetraspores and germlings could not development well as the dose of UV-B radiation higher than 216 J/m^2.The control consisted of carpospores kept under PAR with no UV-B radiation (0 J/m^2). The UV-B radiation treatments were applied each day at 09:00. The UV-B lamps were burned for 100 h before starting the experiment, as recommended by the manufacturer, and were steadied 10 min before the start of each expose to achieve stable irradiance. To test the effects of light quality on repair of UV-B-induced damage, the carpospores were subjected to a 2-h recovery period under PAR, darkness, red light, or blue light after the daily UV-B treatment (Table 1). Red and blue light were supplied at 40 μmol·photons/(m^2·s) using three 40-W Philips fluorescent lamps. After the 2-h recovery period, the carpospores were returned to their culture conditions.

To determine the UV-B radiation effects on the carpospores, the experimental samples (PAR 36–180 J/m^2) were returned to PAR after exposure to UV-B radiation; the control was not exposed to UV-B radiation at all (Table 1, PAR group). To determine the effects of light quality on repair of UV-B-induced damage, experimental groups were subjected to four different light conditions immediately after UV-B exposure. In these experiments, the group kept under PAR for 2 h was the 'normal' light repair group, while the group in darkness could not carry out light repair. These groups were compared with those kept in red or blue light for 2 h.

2.4 Assessment of UV-B-induced damage to *C. ocellatus*

2.4.1 Carpospore diameters

We determined the diameters of carpospores under a fluorescence microscope (CX31; Olympus, Tokyo, Japan) every 4 d from Day 0 until Day 48. The diameters were measured from images using the optical microscopy software package ImagePro Plus ver. 6 (Media Cybernetics, Silver Spring, Maryland, USA). Normal carpospores were approximately disc-shaped. The diameter of each was the average of 50 carpospores diameter in each treatment.

2.4.2 DNA extraction and ELISA of CPDs on Day 48

Total DNA was extracted from 0.1 g carpospores of *C. ocellatus* according to the method of Mayes et al. (2004). To avoid light repair during analysis, the DNA was extracted under dim red light. DNA concentration was determined using a nucleic acid spectrophotometer (Ultrospec 4300 Pro, GE Healthcare, Little Chalfont, UK).

CPDs were quantified by enzyme-linked immunosorbent assay (ELISA) as described by Mori et al. (1991), with a monoclonal antibody, ab10347, supplied commercially (Abcam, Cambridge, UK). Absorbance of the reaction mixture at 490 nm was

measured using a microplate reader (Multiskan MK3, Thermo Fisher Scientific, Massachusetts, USA).

2.4.3 Concentrations of chlorophyll *a* and phycoerythrin on Day 48

Carpospores of *C. ocellatus* (0.1 g) was homogenized in 90% acetone with quartz sand on ice, then kept at 4°C for 24 h before centrifuging at 8 944×*g* at 4°C for 10 min. The absorbance of the supernatant was measured at 647 nm and 664 nm according to the method of Ritchie (2006). The chlorophyll *a* (Chl *a*) content was calculated as:

$$C_a=-1.93\times A_{647}+11.93\times A_{664}, \quad (1)$$

where C_a is the mass ratio of Chl *a* (mg/L) and A_{664} and A_{647} are the optical densities at 664 nm and 647 nm, respectively.

Carpospores of *C. ocellatus* (0.1 g) was washed with distilled water, homogenized and extracted in 0.1 mol/L phosphate buffer (pH 6.5) on ice, then centrifuged at 8 944×*g* at 4°C for 10 min. The concentration of phycoerythrin in the supernatant was determined using the method of Beer and Eshel (1985).

2.4.4 Extraction and analysis of MAAs

0.1 g carpospores of *C. ocellatus* were oven dried at 100°C for 15 min, and MAAs were extracted for 2 h in 25% HPLC-grade methanol (v/v) at 40°C. Then the samples were passed through a 0.22-μm membrane filter. Tryamine hydrochloride (THC) was used as an internal standard in this analysis (Whitehead and Hedges, 2002). THC has a maximum absorption at 280 nm, far lower than that of the MAAs, and a relatively low molecular weight, so it is easily distinguishable from the common MAAs. Prior to liquid chromatograph/mass spectroscopy (LC/MS) analysis, 100 μL of a solution containing 173 mg THC dissolved in 1 mL of MeOH (1 mol/L) was added to 400 μL of MAA standard.

The concentration of MAAs was analyzed with high-performance liquid chromatography (HPLC) (1120 Compact LC; Agilent, Santa Clara, CA, USA) and calculated from the peak area. The mobile phase was 2.5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water, run isocratically at 1.0 mL/min for 15 min. Sample volumes of 10 μL were injected into the Sphereclone C column with precolumn (5 m packing; 250 mm×4 mm I.D.). Peaks were detected via absorbances at 320 nm and 340 nm; samples containing the internal standard were monitored at 280 nm and 320 nm.

MAA types were analyzed using an Agilent 1100 LC/MS system with a diode array detector (DAD) interfaced to a quadrupole mass spectrometer at the First Institute of Oceanography, Qingdao, China. The UV wavelengths monitored were 320 nm and 340 nm, except when the sample contained the internal standard, in which case 280 nm and 320 nm were monitored. We used a C-8 column (250 mm×4.6 mm I.D.) with isocratic elution over a 15-min period. The mobile phase was 65:35 methanol/water acidified to pH 3.8 with acetic acid. The flow rate was 0.8 mL/min. The acquisition methods for LC/MS were based on those of Whitehead and Hedges (2002), and the conditions were optimized to produce positive molecular ions (MH^+) via electrospray ionization. Other LC/MS settings were as follows: gas temperature, 350°C; sheath gas flow, 11 mL/min; scan mode 50–600 amu. Each kind of MAA was determined according to molecular weight from the total ion current. The structural formulae were determined by comparing the examined mass spectrum with the standard mass spectrum and were used to confirm the MAA classes detected in *C. ocellatus*.

2.5 Data analysis

Each treatment consisted of five replicates. We used one-way analysis of variance and generalized linear model-univariate analysis to test the significance of differences. We used SPSS 13.0 for statistical analyses (IBM, Chicago, IL, USA). Levene's test was used to assess homogeneity of variance. When the *P* value of homogeneity of variance was greater than 0.05, the least significant difference (LSD) test was used for multiple comparisons, otherwise Dunnett's T3 was used ($P<0.05$).

3 RESULT

The diameters of carpospores subjected to different UV-B-radiation and light-repair conditions during the experiment are shown in Fig.1. The diameters differed after Day 8 in the PAR group (Fig.1a). Carpospore diameters increased under 0 and 36 J/m^2 UV-B radiation, but at higher UV-B radiation levels (72–180 J/m^2), carpospore growth was strongly inhibited. The diameters of carpospores exposed to 36 J/m^2 UV-B radiation were significantly greater than those in other treatments, including the control. On Day 48, the average diameter across the six UV-B treatments in the PAR was greater than the averages in the groups

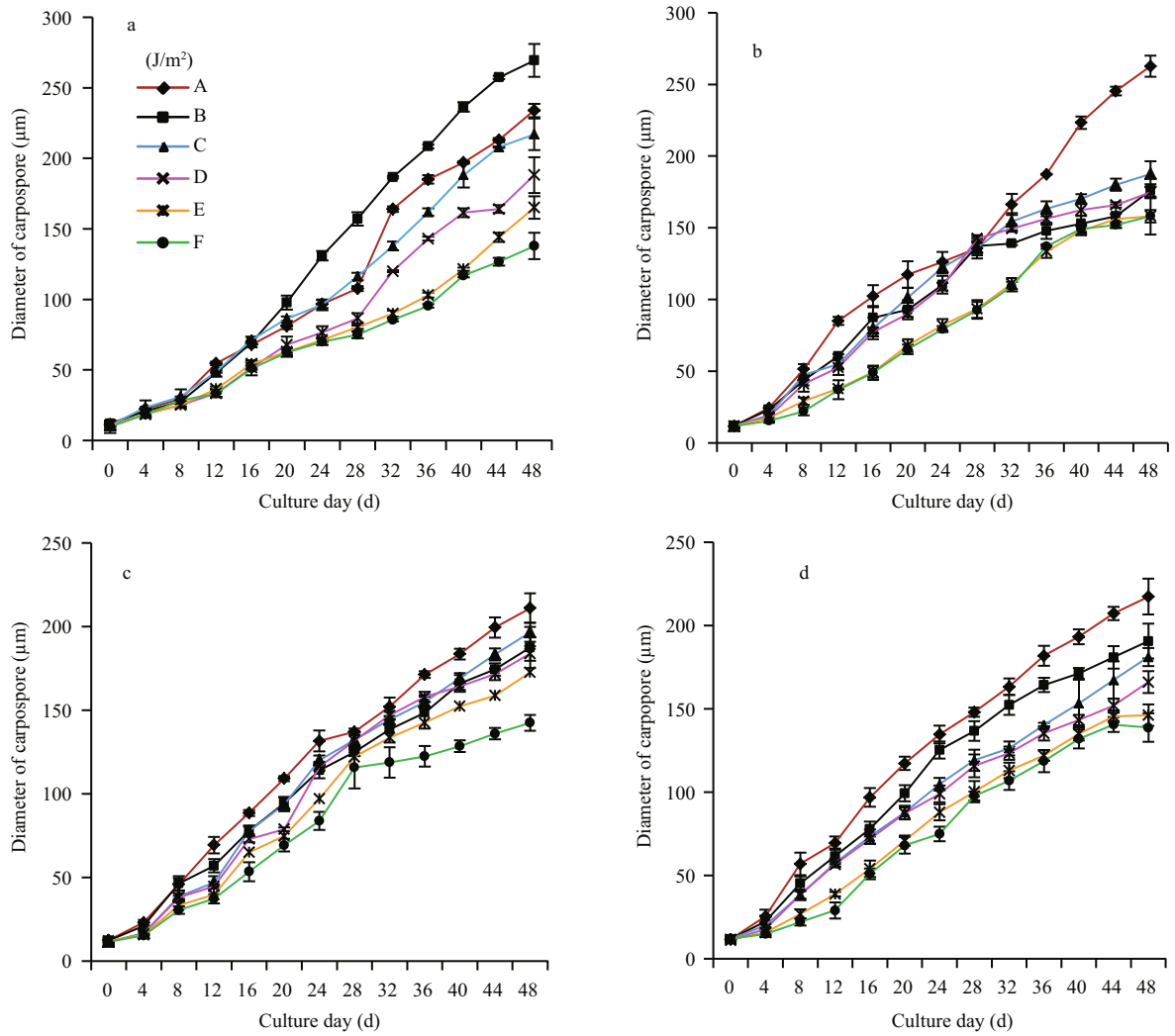

Fig.1 Variation in carpospore diameters under different UV-B radiation and light conditions

Diameters of *Chondrus ocellatus* carpospores kept under photosynthetically active radiation (a), in the dark (b), and under red (c) and blue (d) light after exposure to different doses of UV-B radiation are shown. Diameters were measured every 4 d from Day 0 to Day 48. Letters (A–F) refer to the UV-B radiation doses (0–180 J/m^2) in Table 1. Data are mean values±SD (n=5).

kept in darkness, blue light, or red light for the 2-h repair period (P<0.001). The lowest average diameter was in the blue light group (P<0.001). In the dark (Fig.1b), red-light (Fig.1c) and blue-light (Fig.1d) groups, the carpospore diameters differed on Day 4. In the dark group, the diameters of carpospores that were not subjected to UV-B radiation were the same as those in the PAR group and significantly greater than those in the red and blue groups on Day 48 (P<0.001). Carpospores subjected to 36 J/m^2 UV-B radiation had slightly smaller diameters than those in the control and 72 J/m^2 UV-B radiation treatments. At higher UV-B doses, carpospore diameter was significantly smaller than in the control. Carpospore diameter in the red light group (Fig.1c) showed the same trend as in the darkness group. In the blue light group (Fig.1d), at higher UV-B radiation levels, carpospore diameter decreased significantly.

Inverted fluorescence microscope images of carpospores on Day 48 after treatment with different UV-B radiation levels are shown in Fig.2. Normal carpospores were approximately disc-shaped, with a smooth edge and dark color (Fig.2a). Carpospores exposed to 36 J/m^2 UV-B radiation (Fig.2b) grew more rapidly than those in the control, but had rougher edges. When UV-B radiation levels exceeded 72 J/m^2 (Fig.2c, d), carpospore growth was inhibited and their diameters were smaller than those in the control and low-dose (36 J/m^2) UV-B treatment. In addition, carpospores subjected to UV-B levels greater than 72 J/m^2 had indistinct edges, irregular shapes, incompactly-arranged cells, and a light color. The

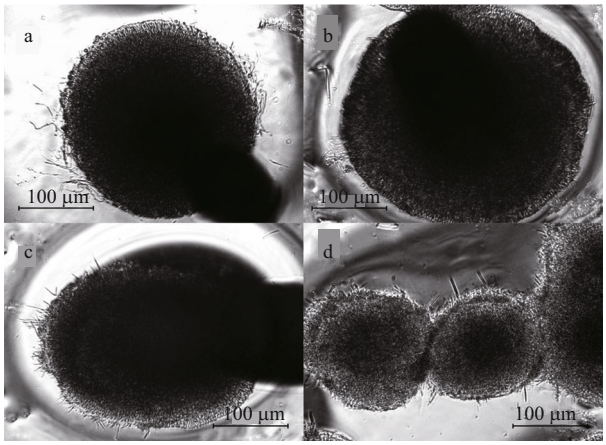

Fig.2 Carpospores in UV-B radiation plus PAR treatments on Day 48

Chondrus ocellatus carpospores exposed to photosynthetically active radiation (PAR) after normal culture without UV-B radiation (a) and after UV-B radiation treatment with 36 J/(m^2·d) UV-B (b), 72 J/(m^2·d) UV-B (c), and 180 J/(m^2·d) UV-B (d) were observed under an inverted microscope. Scale bar: 100 μm.

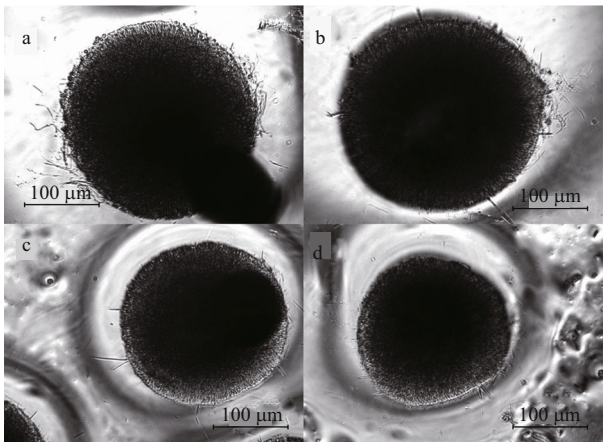

Fig.3 Carpospores exposed to PAR, dark, red light, and blue light after non-UV-B treatment

Chondrus ocellatus carpospores were observed under an inverted microscope on Day 48 after a non-UV-B radiation treatment (0 J/(m^2·d) UV-B) followed by exposure to photosynthetically active radiation (PAR) (a), dark (b), red light (c), and blue light (d). Scale bar: 100 μm.

inverted fluorescence microscope images clearly show that in the control, carpospores grew better in the PAR (Fig.3a) and darkness (Fig.3b) treatments than in the other two groups (Fig.3c, d). When the UV-B radiation level was 180 J/m^2 (Fig.4), UV-B radiation adversely affected carpospores in all four light treatments, as described above. Carpospores in the blue light group also showed signs of damage; they had hyaline vesicles at the edges and were very light in color.

The absorbance of CPDs in *C. ocellatus* changed with UV-B radiation level. As shown in Fig.5, UV-B significantly affected DNA and accumulation of

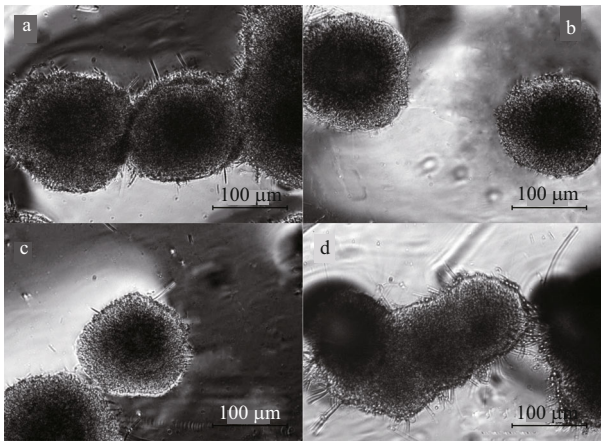

Fig.4 Carpospores exposed to PAR, dark, red light, and blue light after UV-B treatment

Chondrus ocellatus carpospores were observed under an inverted microscope on Day 48 after a 180 J/(m^2·d) UV-B radiation treatment followed by exposure to photosynthetically active radiation (PAR) (a), dark (b), red light (c), and blue light (d). Scale bar: 100 μm.

CPDs in the PAR group (P<0.001; Fig.5a). As the UV-B radiation level increased from 0 J/m^2 to 72 J/m^2, the CPD absorbance increased, peaking at a value of 1.0, then decreased after exposure to 108 J/m^2 UV-B radiation. At 144 J/m^2 UV-B, the absorbance of CPDs (0.35) was significantly lower. The control groups also formed some CPDs. In the group kept in darkness (Fig.5b), CPD absorbance increased with the level of UV-B radiation (P<0.001). In the red-light group (Fig.5c) (P<0.001), the absorbance of CPDs increased as the level of UV-B radiation increased to 72 J/m^2, where the peak value of 1.76 was seen; at 108 J/m^2 UV-B radiation, CPD content decreased (0.64 absorbance) then increased. In the blue-light group (Fig.5d), CPD absorbance did not differ significantly among the 36, 108, 144, and 180 J/m^2 UV-B treatments (P=0.384). The average absorbance values in the blue-light groups (0.5791) were lower than those in the PAR, darkness, and red-light groups. Carpospores in the red-light group had the highest CPD contents.

As shown in Fig.6, UV-B radiation significantly damaged some pigments in *C. ocellatus* in the PAR group (P<0.001) (Fig.6a). The low level of UV-B radiation (36 J/m^2) increased Chl-*a* synthesis, and Chl-*a* content rose to 2.29 mg/L. As the level of UV-B radiation increased further, the Chl-*a* content decreased significantly. In the group kept in darkness (Fig.6b), the Chl-*a* content increased with the UV-B radiation level from 36 to 108 J/m^2, peaking at 1.05 mg/L, then decreasing at higher UV-B levels (P<0.001). The Chl-*a* content in the red-light (P<0.001; Fig.6c) and blue-light (P<0.001; Fig.6d)

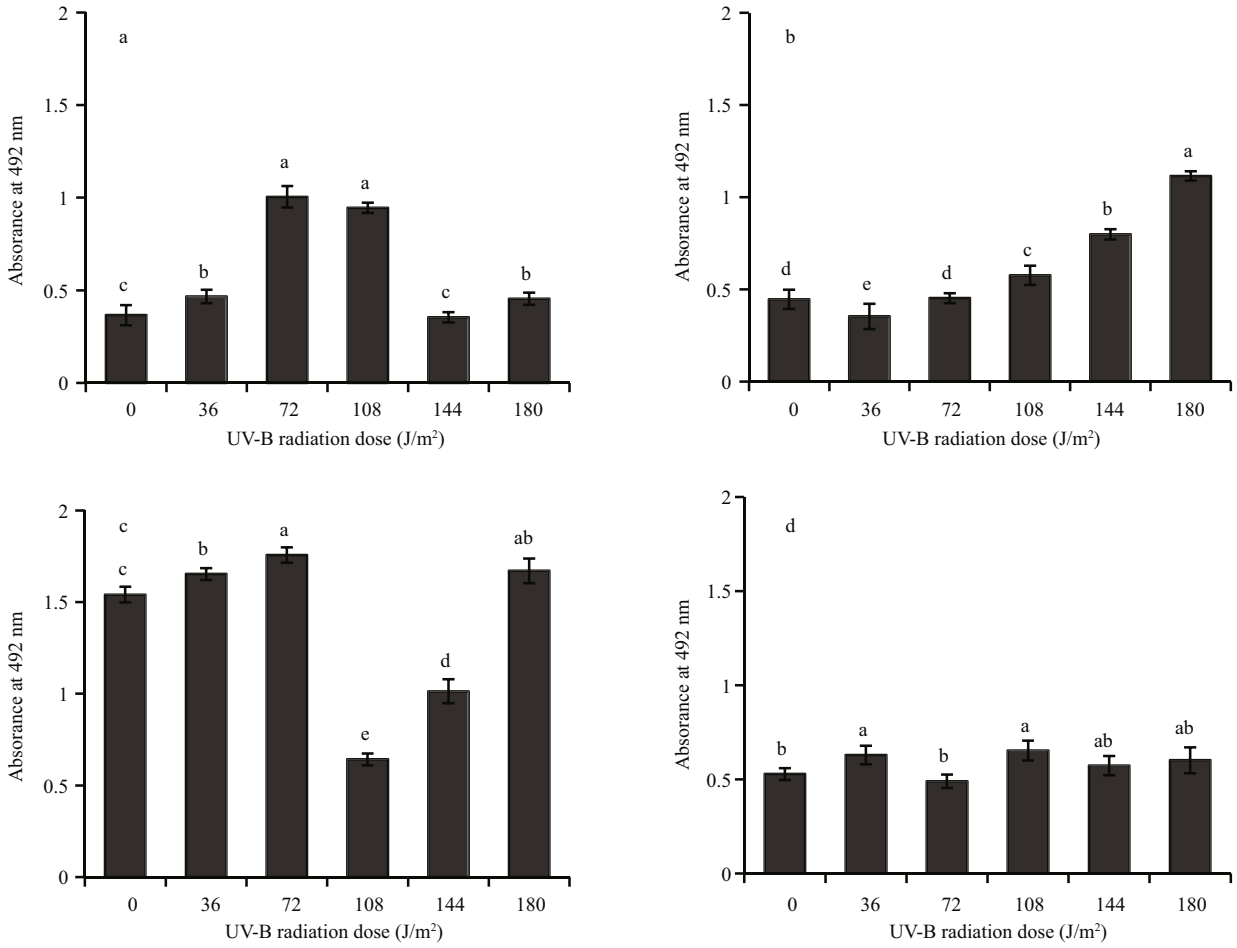

Fig.5 CPD concentrations in carpospores under different UV-B radiation and light conditions

Concentrations of cyclobutane pyrimidine dimers (CPDs) in *Chondrus ocellatus* carpospores were determined on Day 48 after different doses of UV-B radiation followed by photosynthetically active radiation (a), dark (b), red light (c), and blue light (d). Data are mean values±SD (n=5). Different letters above columns indicate significantly different mean values (LSD test, P<0.05).

group showed the same trends as observed in the PAR group, with peaks of 1.35 and 1.36 mg/L, respectively.

There were significant changes in the phycoerythrin contents in response to UV-B radiation, as shown in Fig.7. The trends in phycoerythrin contents were the same as those observed for Chl-*a* contents in the PAR (P<0.001; Fig.7a) and blue-light (P<0.001; Fig.7d) groups. A low level of UV-B radiation (36 J/m^2) resulted in increased phycoerythrin synthesis, and the phycoerythrin content increased to 0.02 and 0.012 mg/L, respectively, in the two groups; at higher UV-B levels, the phycoerythrin content decreased significantly. The phycoerythrin contents decreased significantly with increasing levels of UV-B radiation in the darkness (P<0.001; Fig.7b) and red-light (P<0.001; Fig.7c) groups. The average phycoerythrin content was lowest in the blue-light group.

MAAs were analyzed by LC/MS (Fig.8). The carpospores of *C. ocellatus* contained three kinds of MAAs: palythine (245.112 9), asterina-330 (289.138 1), and shinorine (333.129 2). These MAAs were quantified by HPLC. We observed the same retention times of compounds extracted from carpospores subjected to different treatments in the four groups, implying that their chemical compositions were identical in the different samples. The MAA contents in the carpospores from the four groups are shown in Fig.9. The MAA contents of the PAR group increased (0.115 mg/g), decreased (0.018 mg/g), and then increased again with increasing doses of UV-B radiation (P<0.001; Fig.9a). A similar trend was observed in the groups kept in darkness (P<0.001; Fig.9b) and red light (P<0.001; Fig.9c); the MAA contents peaked in response to 36 J/m^2 UV-B radiation at 0.087 and 0.139 mg/g, respectively, but decreased at higher UV-B levels. In the red-light group, the MAA content of carpospores subjected to 36 J/m^2 UV-B was significantly greater than that in the PAR,

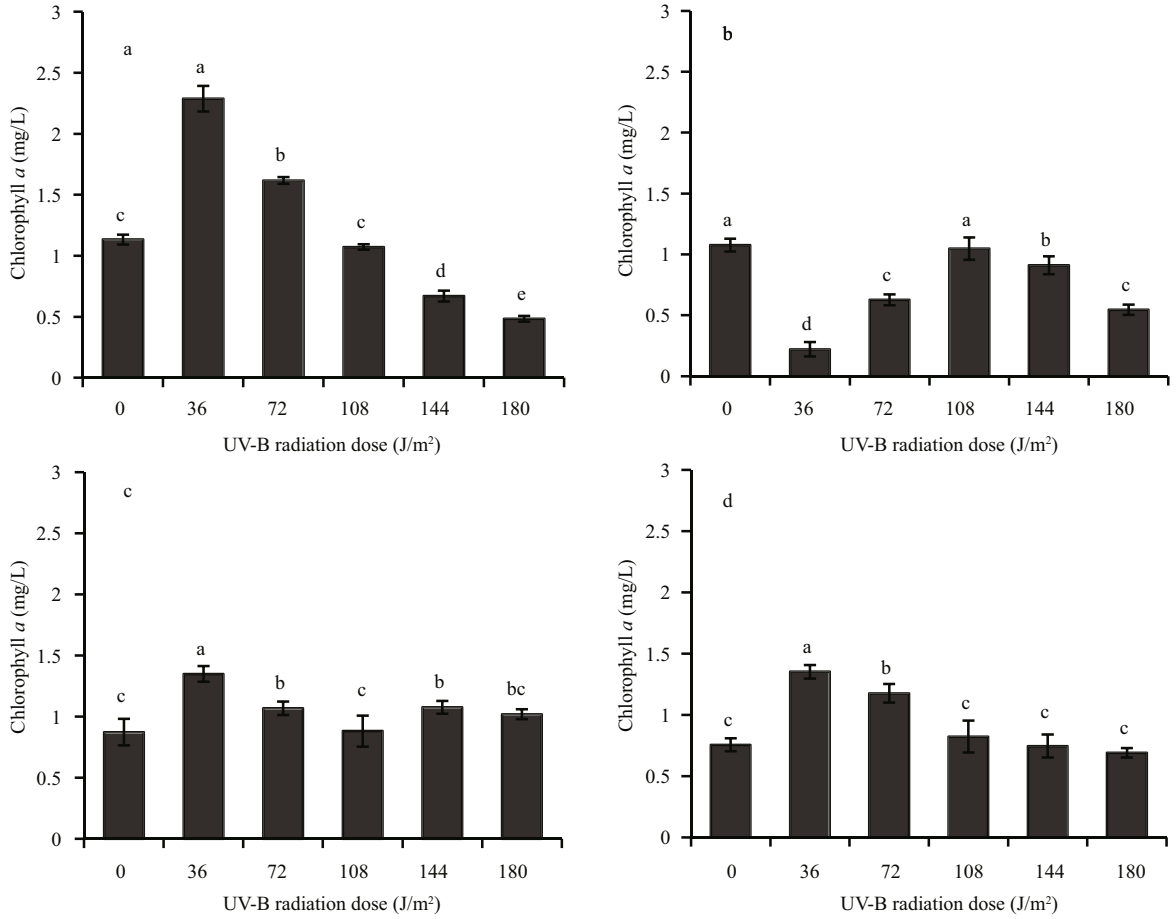

Fig.6 Chl-*a* concentrations in carpospores under different UV-B radiation and light conditions

Concentrations of Chl-*a* in *Chondrus ocellatus* carpospores were determined on Day 48 after different doses of UV-B radiation followed by photosynthetically active radiation (a), dark (b), red light (c), and blue light (d). Data are mean values±SD (n=5). Different letters above columns indicate significantly-different mean values (LSD test, P<0.05).

darkness, or blue-light groups (P<0.001). In the blue-light group (P<0.001; Fig.9d), MAA contents increased with UV-B radiation level from 0 to 72 J/m^2 (0.148 mg/g), but decreased at 108 J/m^2 UV-B to the lowest value of 0.28 mg/g. The highest average MAA content was in the blue-light group (P<0.001).

4 DISCUSSION

4.1 Increased UV-B negatively affected carpospores of *C. ocellatus*

In some rhodophytes, UV-B radiation negatively affected growth, photosynthetic pigments, and photosynthetic parameters and caused some ultraviolet-absorbing/screening substances to accumulate (Eswaran and Rao, 2001; Hoyer et al., 2001; Eswaran et al., 2002; Schmidt et al., 2009; Schmidt et al., 2010a, b, c; Schmidt et al., 2012a, b). Our results show that UV-B significantly stressed *C. ocellatus* carpospores. The low dose of UV-B radiation promoted growth and development of *C. ocellatus*; carpospore diameters were significantly higher in this treatment than in the other treatments (including the control) in the PAR group on Day 48. The low UV-B dose may have promoted the activities of some protection and repair mechanisms in the cells, so the algae could acclimatize better to the conditions. According to Liu et al. (2008), a low-dose UV-B promoted zoospore germination and the formation of male and female gametophytes. Our results showed that when the UV-B radiation exceeded 72 J/m^2, carpospore growth decreased significantly. In addition, morphological changes induced by UV-B radiation were very significant; at high UV-B levels, the discoidal body of *C. ocellatus* had indistinct edges, irregular shapes, incompactly-arranged cells, and a light color. According to Scariot et al. (2013), UV-B radiation induced morphological changes in tetrasporelings, such as altered length-to-width ratio, twisted thalli, loss of pigmentation, and differentiation

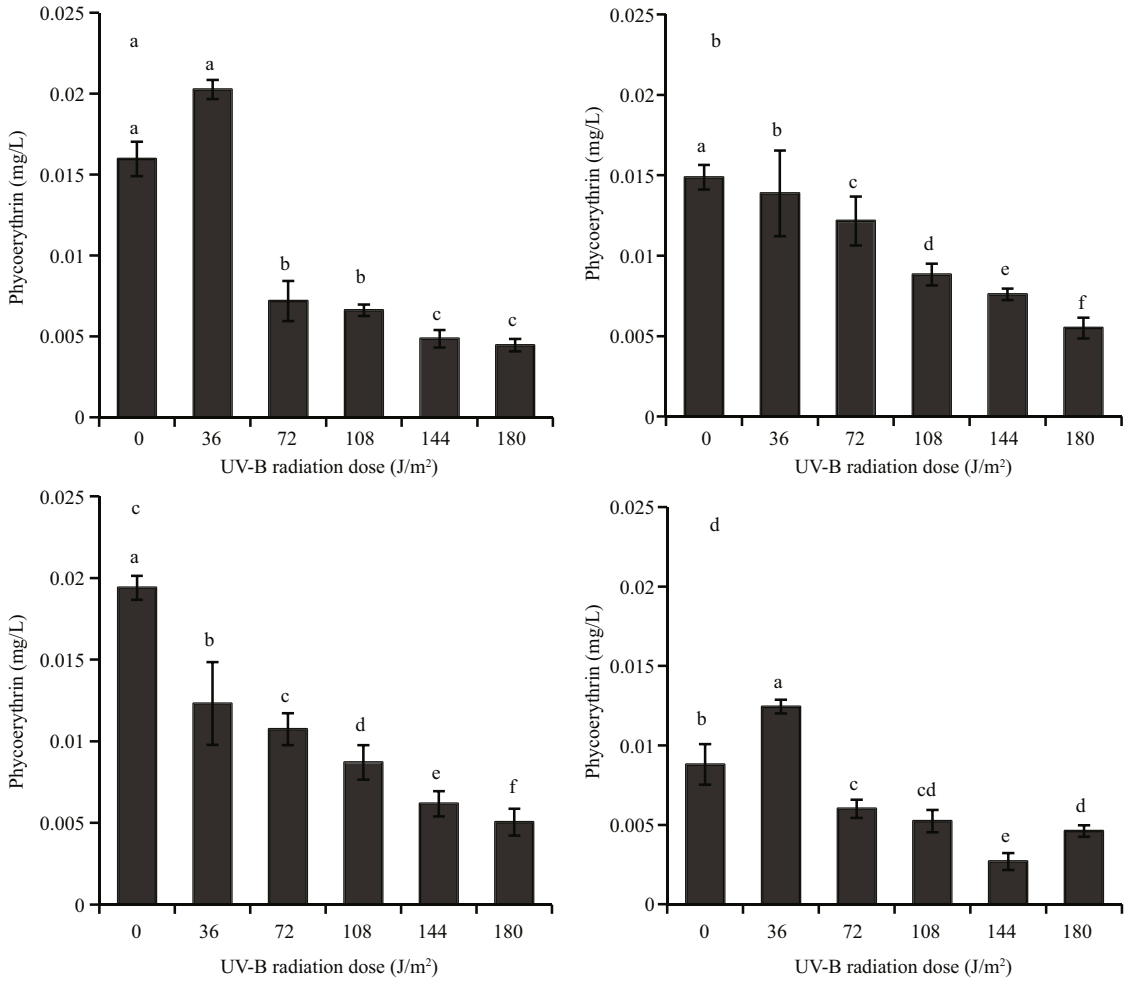

Fig.7 Phycoerythrin concentrations in carpospores under different UV-B radiation and light conditions

Concentrations of phycoerythrin in *Chondrus ocellatus* carpospores were determined on Day 48 after different doses of UV-B radiation followed by photosynthetically active radiation (a), dark (b), red light (c), and blue light (d). Data are mean values±SD (n=5). Different letters above columns indicate significantly different mean values (LSD test, P<0.05).

of more than one apical cell. As well as in young gametophytes of *Gelidium floridanum*, the ultrastructure also changed by UV-B radiation (Simion et al., 2014). These changes may be due to the negative effects of UV-B radiation, including bleaching of photosynthetic pigments, damage to photosystem II, and the formation of CPDs and reactive oxygen species. These changes inhibit physiological and biochemical reactions, ultimately delaying the development of the carpospores.

In this study, UV-B radiation induced CPD formation in the DNA, and we observed the patterns of repair under different light conditions. DNA is a main target molecule of UV-B radiation. In rhodophytes, UV-B-induced damage can be remedied via light repair (photoreactivation), dark repair (nucleotide excision repair), and recombination repair (Britt, 1999). Here, when the UV-B radiation level increased to 144 J/m^2, the CPD content decreased significantly. Tetraspores may have several repair mechanisms that are induced by serious DNA damage. In some bacteria have some repair mechanisms in response to UVR-induced damage. These mechanisms are usually classified into dark repair and photoreactivation. All mechanisms are inducible as part of the SOS regulon, and the induction is dependent on the level of DNA damage.Some bacteria can repair UVR-induced damage via dark repair and photoreactivation mechanisms that are induced as part of the SOS regulon upon DNA damage (Zenoff et al., 2006). These SOS repair mechanisms may also exist in the tetraspores of *C. ocellatus*. If so, they could have repaired the CPDs that accumulated in *C. ocellatus* cells, decreasing the CPD content. As the UV-B radiation increased further, the rate of CPD formation would have exceeded that of DNA repair,

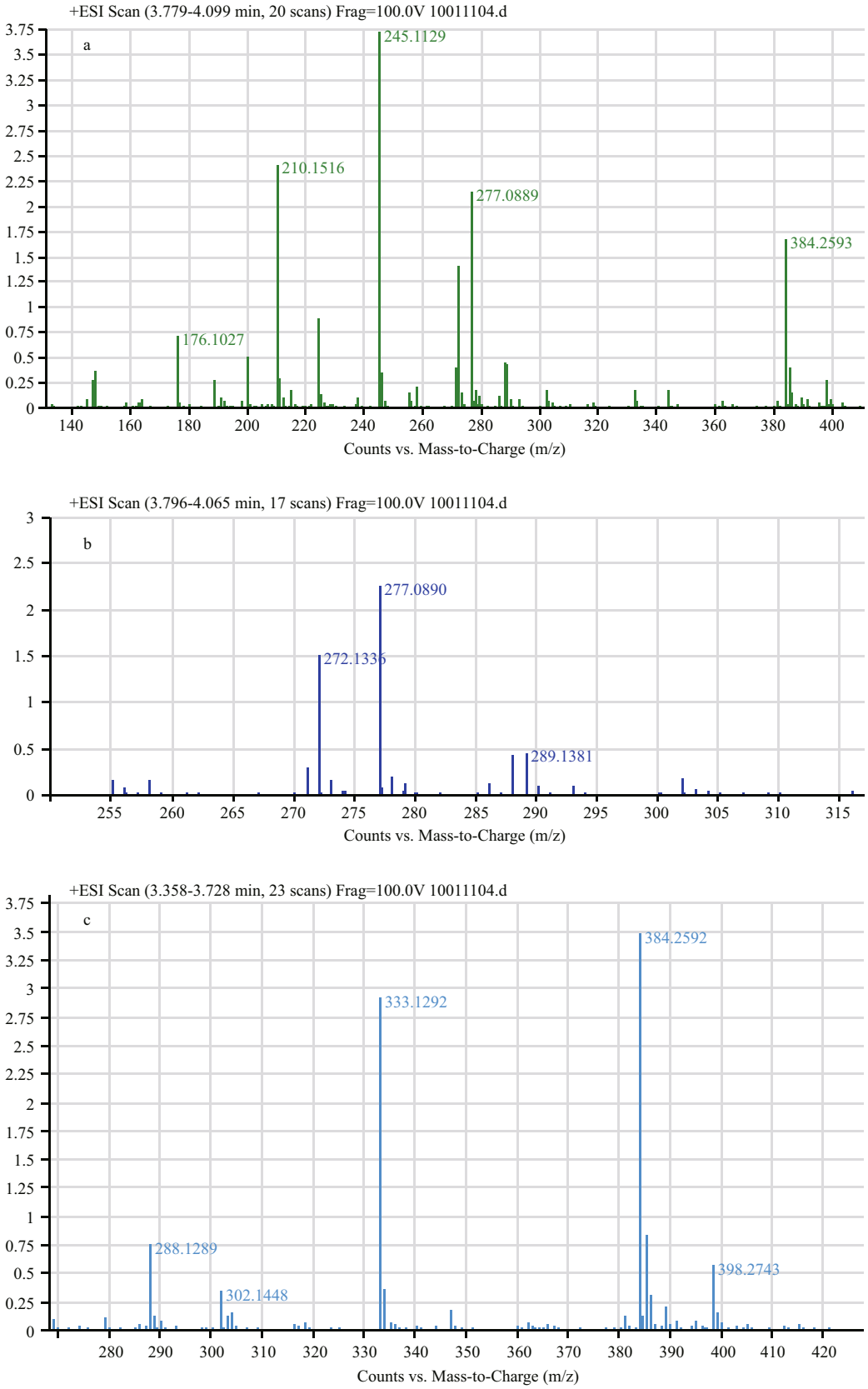

Fig.8 Extracted ion current of MAAs in carpospores of *Chondrus ocellatus*

Extracted ion currents of mycosporine-like amino acids (MAAs; a. palythine (245.112 9); b. asterina-330 (289.138 1); c. shinorine (333.129 2)) in carpospores of *C. ocellatus* are shown. Molecular weights were determined by LC/MS.

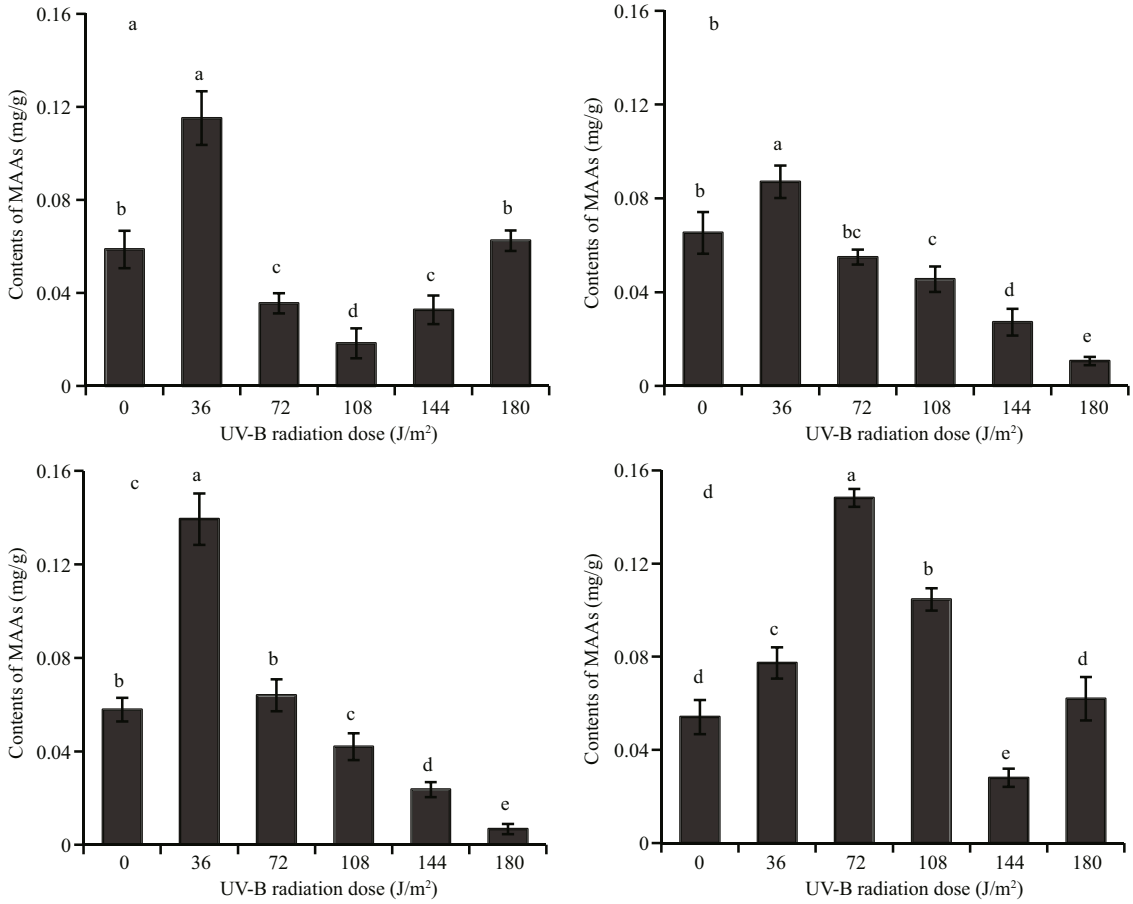

Fig.9 MAA concentrations in carpospores under different UV-B radiation and light conditions

Mycosporine-like amino acid (MAA) concentrations in *Chondrus ocellatus* carpospores were determined on Day 48 after different doses of UV-B radiation followed by photosynthetically active radiation (a), dark (b), red light (c), and blue light (d). Data are mean values±SD (n=5). Different letters above columns indicate significantly-different mean values (LSD test, P<0.05).

and/or the DNA photolyase would be damaged by UV-B radiation, explaining the increases in CPDs under high levels of UV-B radiation.

UV-B radiation also decreased the concentrations of phycoerythrin and Chl-*a* in *C. ocellatus*. Phycoerythrin, which absorbs at 498–565 nm, is the most important light-harvesting protein in photosystem II in red algae (Sui and Zhang, 1998; Munier et al., 2014), and also plays important roles in many physiological and biochemical reactions. In some red algae, a high dose of UV-B radiation leads to a sharp decrease in phycoerythrin content (Aguilera et al., 1999; Schmidt et al., 2010a, b, c; Schmidt et al., 2012a, b). Recently, Xu and Gao (2009) reported that phycoerythrin contents decreased significantly in response to UV-B radiation in *Gracilaria lemaneiformis*. In our experiments, the phycoerythrin contents in carpospores were significantly lower at high UV-B doses (>36 J/m^2). Under these conditions, there were also significant decreases in Chl-*a* content, consistent with previous reports on the effects of UV-B radiation on Chl-*a* content (Sinha and Häder, 1998; Eswaran et al., 2002).

MAAs, which comprise a cyclohexenone or -hexenimine core conjugated with the nitrogen moiety of an amino acid, are strong antioxidants (Shick and Dunlap, 2002). These compounds also function as important UV-absorbing sunscreens in red algae (Karsten and Wiencke, 1999). Total MAA levels in *Palmaria palmata* were greater in samples from shallow waters (1.5 m depth) than in those from deeper waters (3 m). Furthermore, MAA contents in *P. palmata* and *Devaleraea ramentacea* were lower in samples collected in spring or when covered with ice than in samples collected in summer, when the UV-B radiation level was higher (Aguilera et al., 2002a). In this experiment, the MAA contents increased rapidly when the alga was exposed to a low dose of UV-B radiation (36 J/m^2). We speculated that there is a threshold of UV radiation for MAA synthesis. As the UV-B radiation level increased, MAA synthesis was promoted, and the cellular

contents increased significantly. As UV-B levels rose, MAA contents significantly decreased, suggesting that MAAs are consumed as UV-B protectants or as antioxidants more rapidly than they can be synthesized under these conditions. In red algae, MAAs had important antioxidant activity and provided some protection against UV-B radiation (Dunlap and Yamamoto, 1995). In cyanobacteria, MAA contents increased significantly with UV-B dose (Rajeshwar et al., 2003; Rastogi and Incharoensakdi, 2014).

In general, UV-B radiation negatively affected early development of *C. ocellatus* carpospores, even though their growth was promoted at lower doses. The results prove that low-dose UV-B radiation promotes MAA synthesis, but at higher doses, UV-B radiation inhibited carpospore growth, induced CPDs, bleached pigments, and consumed MAAs.

4.2 Effects of light quality on repair of UV-B-induced damage

PAR, the band of solar radiation from 400 nm to 700 nm, is used for photosynthesis. Our results showed that carpospore diameters in the PAR treatment were significantly higher than those in other three groups. We hypothesize that the carpospores kept in the dark after exposure to UV-B radiation were unable to repair UV-B-induced damage, which red light inhibited some repair processes, and that blue light enhanced the effects of UV-B radiation. The diameters of carpospores differed after Day 8 in the PAR group and after Day 4 in the three other groups; thus, the damage induced by UV-B radiation appeared later in the PAR group, indicating that PAR plays an important role in repair. In other studies, PAR promoted adaptive mechanisms of plants exposed to UV-B radiation (Teramura, 1980; Cen and Bornman, 1990). Pradhan et al. (2006) reported that the degree of damage to the photosynthetic apparatus of wheat leaves subjected to UV-B treatment was more severe than that in a UVB+PAR treatment. In this study, we observed a decrease in CPD content during DNA repair in the PAR treatment. These results indicated that light repair was active in carpospores. Britt (1995) indicated that UV-B damage to DNA was mainly repaired by photoreactivation.

In the groups kept in darkness after exposure to UV-B radiation, CPD absorbance decreased in the treatment exposed to low UV-B doses (36–108 J/m^2). We hypothesize the existence of a repair mechanism other than photoreactivation in carpospores, because without PAR, the light-dependent repair process could not operate. Dark (nucleotide excision) repair is thought to operate in carpospores. It may be inhibited under PAR when the DNA damage is mild, while in darkness, it is activated to repair the DNA damaged by UV-B radiation. He et al. (2006) studied the effects of UV-B radiation on CPD contents in mung bean and observed that dark repair occurred to DNA, but that photoreactivation did not in darkness. Furthermore, the results of the present study suggest that, as the level of UV-B radiation increased, CPD formed more rapidly, exceeding the rate of DNA repair and accumulating in carpospores. The variation of Chl-*a* was different from that in other groups. So the dark condition may aggravated the damage of UV-B radiation on Chl-*a*. The content of Chl-*a* in 36 J/m^2 treatment decreased significantly.

Our experimental results showed that CPD contents were higher in the red-light group than in the other groups. The light-repair process may not be able to operate under red light, allowing a large number of CPDs to accumulate in the carpospores. Some study indicated that dim red light could inhibit DNA photorepair (Freeman et al., 1989). With the CPDs accumulated to a dose in the algae, there may some another repair mechanism activated, so the CPDs contents decreased. The phycoerythrin content of the group subjected to no UV-B followed by red light was higher than that in the groups kept in PAR, darkness, or blue lightgroups not subjected to UVB . In other words, during carpospore culture, red light could promote phycoerythrin synthesis. Similarly, our results indicated that red light also promoted MAAs synthesis.

In the blue-light group, the carpospores had small diameters on Day 48 of the experiment, suggesting a synergism between blue light and UV-B radiation that exacerbates damage. The blue light dose may not activate light-dependent repair mechanisms except DNA photolyase after UV-B exposure. Other studies have reported on the wavelengths of light that different plant photoreceptors absorb. Because only trace amounts of blue light were absorbed by photoreceptors, *Arabidopsis* growth was inhibited under this color of light (Shao, 2001). Blue light has been reported to promote the activity of DNA photolyase (Britt, 1995; Zhong et al., 2009). In another study, the lethal and mutagenic effects of CPDs induced by far-UV (200–300 nm) were reversed by irradiation with near-UV/blue-light (300–500 nm); this photoreactivation was catalyzed by photolyase, a blue-light-activated DNA repair enzyme (Thompson

and Sancar, 2002). Our results showed that CPD contents were lower in the blue-light group than in other groups. Blue light also decreased the sensitivity of MAAs synthesis to UV-B radiation. In the blue-light group, the highest MAA content occurred in carpospores exposed to 72 J/m^2 UV-B radiation, while in the PAR, darkness, and red-light groups, the highest MAA contents were in carpospores exposed to 36 J/m^2 UV-B radiation.

In general, PAR was best for repairing of UV-B-induced damage. The activity of DNA dark repair was promoted in darkness and inhibited by PAR. Red light promoted phycoerythrin synthesis but inhibited DNA light repair, allowing a large number of CPDs to accumulate. Blue light promoted the activity of DNA photolyase, greatly improving remediation efficiency, but carpospore growth was inhibited.

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