

MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF CURLY PONDWEED (*POTAMOGETON CRISPUS* L.) TO UV-B RADIATION STRESS

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Abstract. Causes of *Potamogeton crispus* population decline in the late spring and early summer are not fully understood. Given that strong UV-B radiation can penetrate the water column and damage aquatic organisms, it may be a key factor in the *P. crispus* decline. In this study, adult *P. crispus* plants were exposed to different intensities of UV-B radiation for 6 hours every day, the control group was only exposed to UV-A and photosynthetically active radiation. All groups were exposed to UV-A and photosynthetically active radiation at the same intensity. We monitored the morphological changes, and physiological and biochemical indexes. The results showed that plant height, internode length, and leaf area decreased with corresponding increases in radiation intensity. Additionally, photosynthetic pigment content could be improved with low-intensity UV-B radiation (<10.8 kJ/m²), inhibited with continuous radiation, and decreased by high-intensity of UV-B radiation (>10.8 kJ/m²). Catalase activity improved by short-term radiation, but inhibited by long-term radiation. Superoxide dismutase activity increased gradually with longer irradiation time. The malonaldehyde content increased at the beginning but then decreased with continuing radiation. These results suggest that UV-B radiation may contribute to the mass mortality of *P. crispus* in late spring and early summer.

Keywords: *plant height, chlorophyll content, superoxide dismutase activity, malondialdehyde, mass mortality*

Introduction

The significant depletion of the ozone layer over Antarctica, the mid-latitudes and high-latitudes, has raised concerns about the impact of solar ultraviolet-B (UV-B) radiation on marine and freshwater ecosystems. UV-B radiation, which is harmful to plants and human beings (Ulm and Jenkins, 2015), is attenuated rapidly in the water column (Cory et al., 2015). The penetration depth of 10% of surface UV-B radiation is usually less than a few meters, but it can reach several meters in some clear lakes (Williamson, 1995), or even tens of meters in the clearest seas (Williamson, 1995; Buma et al., 2001). The incident depth of 10% of incident UV-B radiation is 7.7 m in Tahoe Lake (America), 10.8 m in Bessvatn (Norway), 12.8 m in Laguna Negra (Chile), and 9 m in the Bellingshausen Sea, even 19.8 m in the Sargasso Sea (Williamson, 1995). Many studies have indicated that increasing UV-B exposure is detrimental to organisms, which causing mutations or even death to aquatic organisms (Buma et al., 2001; Pereira et al., 2014; Al-Aidaros et al., 2015; Häder et al., 2015; Kim et al., 2015; Liu and Wang, 2017; Aksakal and Ciltas, 2018) and leading to a decrease in biomass (Choudhary and Agrawal, 2015). Recent studies have shown that phytoplankton

communities are extremely sensitive to UV radiation (Liu and Wang, 2017), and phytoplankton of the freshwater is more vulnerable to damage from UV radiation than of the marine (Williamson, 1995; Kim et al., 2015). Some studies have shown that UV-B radiation can affect photosynthesis, nitrogen fixation, protein biosynthesis and survive of cyanobacteria, but some protective strategies of Cyanobacteria have been developed to counteract the damaging effects of UV-B radiation (Häder et al., 2015; Mloszewska et al., 2018). UV radiation is harmful to many freshwater zooplankton, acting as a potential driving force for zooplankton community structure in some lakes. The disadvantage effects of ultraviolet radiation on zooplankton include limitations in nutrient uptake, inhibition of photosynthesis, DNA damage, and finally cell death. Earlier studies have suggested that zooplankton move into deep water by vertical migration avoid predation, however, recent researches suggested they maybe escape from surface intense UV radiation (Al-Aidaros et al., 2015; Dumont, 2019). UV-B radiation can produce a series of biological effects on macroalgae at the molecular, cellular, individual and community levels, UV-B radiation can inhibit the growth of several macroalgae, especially for the damage of red algae, brown algae and green algae, by restraining its photosynthesis (Xu et al., 2018). UV-B radiation may affect the physiological and ecological functions of seagrass, and ultimately affect the coastal environment by changing the spatial distribution, species and community functional structure of seagrass (Bischof et al., 2006; Sunny, 2017). Rae et al. (2001) investigated the sensitivity of freshwater macrophytes to UV radiation in New Zealand soon lake, and found that different species of plants have different sensitive degree to UVR and also the different recovery capacity for the damage. Most researches have been focused on the phytoplankton, zooplankton, macroalgae, and seagrass, however, few studies were conducted on the submerged plants, especially freshwater submerged macrophytes.

Potamogeton crispus (Potamogetonaceae) is a submerged herbaceous perennial plant that grows in freshwater lakes, ponds, paddy fields, and rivers worldwide, and produces large quantities of biomass (Wang et al., 2017). The plants usually remain underwater in the early growth stages. Thus, the impact of light on their growth is limited. In the later growth period, when the plant penetrates the water surface after rapid growth in late spring and early summer, intense light begins to inhibit its growth, resulting in the decline of *P. crispus* (Su et al., 2001). But an observation indicated that *P. crispus* grew throughout the year in an artificial lake on an open experimental site where a glass rooftop had been installed. And the plant height of the *P. crispus* was 2-3 times higher than that in the field, but the branches are significantly lower than in the wild. The illumination difference between the inside and outside of the experimental site was small after filtration by the glass, but ultraviolet radiation—especially UV-B—decreased significantly. In general, PAR penetrates a glass greenhouse at an 80% to 85% rate, and UV-A radiation at a 60% to 70% rate; by contrast, UV-B radiation only does so at a 2% to 5% rate. Could this be why *P. crispus* continuously grows in an open experimental site with glass? Jian et al. (2003) found that mass mortality of *P. crispus* is strongly associated with intense light in late spring and early summer. Thus, we have reason to believe that UV-B radiation may contribute to the mass mortality of *P. crispus* in late spring and early summer.

The aim of this study is to understand the effect of UV-B radiation on growth of *P. crispus*, it is helpful to understand the reasons for mass mortality of *P. crispus* in late spring and early summer.

Materials and Methods

Experimental design

The experiment was conducted in an open experimental site where a glass rooftop had been installed (32.11°N, 118.91°E). *P. crispus* plants were collected from the Hongze Lake Nature Reserve on April 15, 2012, in China (Figure 1). These adult plants were transferred into flower pots, of which the upper diameter, base diameter, and the height were 18.0, 12.5, and 15.0 cm, respectively. 15-20 plants were included in each pot. The average plant height was 63 ± 1.8 cm. The roots were fixed with a small amount of clay and gravel in every disk to reduce the effect of sediment. These pots were placed in 200-L plastic buckets, of which the upper diameter, base diameter, and height were $64 \times 52 \times 72$ cm, respectively.



Figure 1. Experimental site (left) and some partial experimental equipment (right)

UV-B lamps made of stainless steel were suspended 120 cm above the plants. The UV-B lamps were manufactured by Nanjing Huaqiang Special Light Source Factory (40 W, peak 313 nm). The treatment intensity was achieved by adjusting the lamp's heights. The UV-B lamps were hung in an east-west direction in order to reduce the influence of the lamps' shade and to ensure that each treatment group obtained equivalent amounts of photosynthetically active radiation (Figure 1). The water temperature was maintained by $23 \pm 2^\circ\text{C}$, the day length was between 13.63 h and 14.18 h, and the sunshine duration was 7.0-11.8 h of sunshine.

The overlying water was tap water with an added nutrient solution. The depth was 70 cm in all treatments.

UV-B intensity setting

UV-B Radiation intensity recorded from April to August in 2004 and 2008 in the Nanjing field were used as the reference values for the experimental intensity (Table 1). In this study, *P. crispus* plants were exposed to UV-B radiation at different intensity ($50 \mu\text{W}/\text{cm}^2$, $100 \mu\text{W}/\text{cm}^2$, $150 \mu\text{W}/\text{cm}^2$, and $200 \mu\text{W}/\text{cm}^2$) for 6 hours (9:00 to 15:00 in local time each day), so that the cumulative daily intensity of UV-B radiation was $10.8 \text{ kJ}/\text{m}^2$, $21.6 \text{ kJ}/\text{m}^2$, $32.4 \text{ kJ}/\text{m}^2$, and $43.2 \text{ kJ}/\text{m}^2$, respectively. The treatment groups were marked as T50, T100, T150, and T200. The control group had a polyester film (125 μm thickness, Shanghai Texiang electrical material Co., Ltd) to filter small amounts of UV-B radiation in the open experimental site with a glass rooftop, so the

control group was only exposed to UV-A and PAR from the solar radiation; the control group was marked as T0. All treatment groups received the same intensity of UV-A and PAR from the solar radiation. The experiment began on May 10, 2012, and the duration of the experimental period was 31 days. UV-B dosimetry was set through the SpectroSense2 (British SKYE company) to connect the SKU 430UV-B sensor (280 - 315 nm). Each group included three replicates. Because the UV-B intensity released from the thick tube was more intense than that released from the thin tube at the same power, T50 and T100 were subjected to the thin UV-B lamps (diameter 14 mm), whereas T150 and T200 were subjected to the thick UV-B lamps (diameter 26 mm). PAR radiation was set in the same condition for all groups, and measured at the beginning and on the 7th day of the experiment (Table 2).

Table 1. UV-B radiation intensity ($\mu W \cdot cm^{-2}$) from April to August in 2004 and 2008 in the Nanjing, China

Year	April	May	June	July	August
2004	170.24	172.91	239.80	156.17	147.66
2008	131.20	178.82	109.51	177.50	139.50

Table 2. Light intensity ($W \cdot m^{-2}$) received at the surface of the experimental water

Time	T0	T50	T100	T150	T200	Random error
10 May, 2012	181.6	167.7	167.4	164.3	178.6	7.7
17 May, 2012	47.0	47.6	45.1	44.4	45.7	1.3

The ability of UV-B to penetrate the water should be considered. Taking T150 as an example, the UV-B intensity at the surface water was 62.4% of the air intensity, but the intensity was reduced to 54.0%, 30.5%, 10.1%, 3.5%, 1.4%, and 0.7% at depths of 10, 20, 30, 40, 50, and 60 cm, respectively. Irradiation time was controlled by a digital timer. UV-B radiation can attenuate exponentially in the water, and the attenuation model is in accordance with the following formula: $E_d = E_0 e^{-K \cdot d}$, where E_0 is the incident UV-B intensity, K is the attenuation coefficient, and d is the depth of the water (Bernhard et al., 2018; Overmans and Agustí, 2020), K is 0.0868 in this experiment. According to calculation, T150 and T200 could penetrate to the underwater depth of 90 cm, while T100 and T50 could penetrate to the underwater depth of 80cm. However, the experimental water was only 70cm deep, so UV-B radiation could penetrate to the bottom all four treatments.

Determination of monitoring indexes

Plant height: The lengths of 15-20 plants in the three parallel treatment groups were measured from the border to the top of the main plant stem using a stainless steel ruler, and the average length was calculated.

Internode length: The 10-15th internode length from the top was also measured in the three parallel treatment groups, 15-20 internode lengths were measured and the average value was calculated each time.

Leaf area: The first three to five fully expanded leaves from the top to the bottom of the plants were selected to measure the leaf area. The length of the main leaf's veins was measured using a ruler, and the leaf width was measured at the leaf's widest point.

20-30 leaves were measured in each treatment and the average value was calculated at every sampling time. The leaf area of *P. crispus* was calculated by the following equation (1) (Wang et al., 2016):

$$\text{leaf area} = 0.87 \times \text{leaf length} \times \text{leaf width} - 0.21 \quad (\text{Eq.1})$$

The plant height, internode length and leaf area were measured at 9 o'clock at the beginning of the experiment, and at days 16, 24, and 31, respectively.

Chlorophyll concentrations were measured by extracting fresh leaf tissue with 80% acetone and centrifuging the sample at 3000 g for 5 mins. The absorbance of the extract was determined at 663, 645, and 440 nm. Chlorophyll concentrations were calculated using the published formulae (Strain and Svec, 1966).

Catalase activity (CAT) was determined by measuring the decrease in absorbance of the reaction mixture at 240 nm (Havir and McHale, 1987). The activity was assayed for 1 min in a reaction solution composed of 2.9 mL potassium phosphate buffer 50 mmol (2.85 mL, pH 7.0), H₂O₂ 12.5 mmol (50 µL), and 100 µL of crude extract.

Superoxide dismutase (SOD) activity was determined by measuring the ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT) according to the method of Beauchamp and Fridovich (1971). One enzyme unit of SOD activity was defined as the amount of enzyme that inhibits 50% NBT reduction measured at 560 nm. The blue formazan produced by NBT photoreduction was measured as an increase in absorbance at 560 nm.

Lipid peroxidation of leaves was determined using a thiobarbituric acid (TBA) test by measuring the malondialdehyde (MDA) level (Heath and Packer, 1968). The extinction coefficient was determined at 600 nm, 532 nm, and 450 nm (De Vos et al., 1989).

The physiological and biochemical parameters were determined at the beginning of the experiment, and at days 16, 23, and 31, respectively, 150-180 leaves were sampled in each treatment at every sampling time.

Statistical analysis

Data from three replicates of all treatments were subjected to analysis of variance using SPSS 16.0. All data were presented as the mean ± SE. Comparisons between treatments were performed using two-way ANOVA, and Tukey's post-test was performed to determine the significant differences between different treatment groups on the same sampling day, different sampling date for the same treatment group. The significance of treatment effects was determined at the 0.05 probability level, the level of probability was set at 95% confidence interval ($p < 0.05$).

Results

Growth status

The leaves near the water surface in the groups from T100 to T200 were bleached from 7 d to 31 d, whereas the leaves growing underwater remained healthy. The harmful effects in all treatment groups increased with increasing radiation intensity. Some plants from the T150 and T200 groups died, but the roots of some of these plants were still linked to the stem at 16 d. At 22 d, the leaves in all treatment began to shrink, T0, T50,

and T100 also showed a declining trend at 22 d, two new branches appeared in T0, and T150 and T200 had no intact plants, with more broken branches and stem fractures in the roots, and they were beginning to float on the water (Table 3). At 31 d, there were 18 well-developed specimens in T0 in the three parallel groups, including 6 new branches, and some branches were broken from the original plants, whereas T50, T100 only had 9, 7 intact plants and more broken branches, the broken branches of T150 and T200 have been decomposed. During the whole experiment, the plant height, internode length, and leaf area in T50-T200 decreased continuously with corresponding increases in UV-B radiation intensity.

Table 3. Number of intact specimens in the three parallel groups

Treatment	0d	16d	22d	31d
T0	50	50	38	18
T50	52	45	16	9
T100	50	37	17	7
T150	58	22	0	/
T200	58	13	0	/

Morphological indexes

The plant heights of T100, T150, and T200 reduced after 16 days of UV-B radiation, but the heights of T0 and T50 plants increased slightly by 2.1% and 0.9%, respectively, from the beginning of the experiment (Table 4), whereas the heights of T100, T150, and T200 remained relatively consistent, decreased by 1.6%, 8.3%, and 2.0% from the beginning, respectively ($p>0.5$). The heights of T50, T100, T150, and T200 decreased from the beginning by 12.5%, 11.0%, 22.6%, and 24.1%, respectively at 24 d. T0 increased continuously after from 24 d to 31 d. Although all treatment groups had a significant difference at 24 d compared with the values at the beginning of the experiment ($p<0.05$), the heights of T50-T200 decreased more significantly as the radiation intensity increased. After 24 days of UV-B radiation, the heights of T50, T100, T150, and T200 were lower than that of T0 by 21.4%, 22.9%, 28.2%, and 34.1%, respectively. The plants in T150 and T200 died at 31 d. Although T0-T100 still grew, the growth rates decreased from those at the beginning of the experiment. This decrease became more significant from 24 d to 31 d ($p<0.05$).

The internode lengths of T50, T100, T150, and T200 differed significantly ($p<0.05$) after days 16 of UV-B radiation compared with the beginning (Table 4), and were lower than that of T0 by 59.5%, 71.7%, 77.4%, and 71.6%, respectively. At 24 d, the internode lengths of all groups decreased, but the differences were not significant between the treatments ($p>0.05$). The average internode length of T50 was 3.8% greater than T0, T100 was lower than T0 by 8.0%, but the internode lengths of T150 and T200 were significantly lower than T0 (38.7% and 36.3%, respectively) at 24 d. The internode lengths of T0, T50, and T100 at 31 d differed significantly from those at the beginning of the experiment ($p<0.05$), but the differences among those treatments were much less.

The leaf area of T0 at 16 d had increased by 13.8% (Table 4), and thereafter decreased continuously. The leaf areas for T50-T200 decreased significantly ($p<0.05$) from the beginning to the day 24, and were 48.7%, 51.9%, 57.3%, and 58.1% lower than those at the beginning, respectively. The leaf areas of T0, T50, and T100 decreased

significantly ($p < 0.05$) at 31 d compared with those at the beginning, accounting for 63.7%, 70.4%, and 70.5% decreases.

Table 4. Morphological change of *P. crispus* affected by UV-B radiation

Morphological indexes	Treatment	0d	16d	24d	31d
Plant height/cm	T0	64.1±2.3Aa	65.4±1.2Aa	70.6±1.7Ba	55.3±4.2Ca
	T50	63.4±3.4Aa	64.0±1.4Aa	55.5±3.4Bb	54.3±3.7Ba
	T100	61.2±3.2Aa	60.2±1.9Ab	54.4±1.6Bb	53.3±2.0Ba
	T150	65.4±3.2Aa	60.0±1.9Bb	50.7±3.1Cb	
	T200	61.3±2.2Aa	60.0±1.9Ab	46.5±1.5Bc	
Internode length/cm	T0	3.1±0.4Aa	3.5±0.6Ba	1.1±0.1Ca	0.6±0.1Da
	T50	3.1±0.6Aa	1.4±0.3Bb	1.1±0.1Ba	0.6±0.0Ca
	T100	3.4±0.1Aa	1.0±0.2Bb	1.0±0.2Ba	0.6±0.1Ca
	T150	3.5±0.6Aa	0.8±0.2Bc	0.7±0.1Bb	
	T200	3.3±0.4Aa	1.0±0.2Bb	0.7±0.1Cb	
Leaf area/cm ²	T0	1.5±0.2Aa	1.7±0.2Aa	0.8±0.1Ba	0.6±0.0Ca
	T50	1.5±0.1Aa	1.1±0.1Bb	0.8±0.1Ca	0.5±0.1Db
	T100	1.6±0.1Aa	1.0±0.2Bb	0.8±0.2Ba	0.5±0.0Cb
	T150	1.6±0.1Aa	0.8±0.1Bc	0.8±0.1Ba	
	T200	1.6±0.1Aa	0.6±0.1Bc	0.7±0.1Ba	

Lowercase letters indicate statistical differences between different treatment groups on the same sampling day, capital letters indicate statistical differences between different sampling date for the same treatment group, the equal letters indicate no differences and different letters indicate significant differences. The same situation was applied to the following monitoring indicators and parameters in the tables below.

Chlorophyll content

From 16 d to 31 d, chlorophyll a (Chla) and chlorophyll b (Chlb) concentrations in all treatments were significantly different ($p < 0.05$) from the values at the beginning of the experiment (Table 5), Chla and Chlb concentrations of the T0 and T50 treatments increased, however, the concentrations of T50-T200 decreased with increasing radiation intensity. At 23 d, the values were significantly different from those at the beginning, whereas T0 increased continually, T50 decreased slightly, Chla and Chlb concentrations of T100-T200 increased from the values at 16d, but they still decreased with increasing radiation intensity. Chla and Chlb concentrations of T0-T100 decreased with increasing radiation intensity at 31 d.

Table 5. Chlorophyll content of *P. crispus* affected by UV-B radiation

Chlorophyll content	Treatment	0d	16d	23d	31d
Chlorophyll a content(mg·L ⁻¹)	T0	2.18±0.17Aa	2.32±0.14Aa	4.20±0.15Ba	4.70±0.17Ca
	T50	2.24±0.13Aa	3.75±0.11Bb	3.23±0.08Cb	4.26±0.12Db
	T100	2.18±0.15Aa	1.34±0.13Bc	2.51±0.10Cc	3.05±0.12Dc
	T150	2.00±0.16Aa	1.16±0.06Bc	2.33±0.09Cc	
	T200	2.08±0.13Aa	1.14±0.09Bc	2.00±0.12Ad	
Chlorophyll b content(mg·L ⁻¹)	T0	0.77±0.09Aa	0.95±0.07Aa	1.63±0.07Ba	1.71±0.04Ba
	T50	0.81±0.06Aa	1.53±0.06Bb	1.37±0.07Cb	1.75±0.08Da
	T100	0.82±0.09Aa	0.51±0.07Bc	1.05±0.07Cc	1.23±0.07Db
	T150	0.79±0.08Aa	0.54±0.07Bc	0.99±0.08Cc	
	T200	0.76±0.06Aa	0.46±0.08Bc	0.84±0.07Cd	

CAT activity

CAT activities of T0 and T50 were higher at the beginning of the experiment (Table 6). At 16 d, T50-T200 groups increased with increasing radiation intensity, and had a significant difference compared with the beginning ($p < 0.05$); the CAT activity of T200 was 143.2% greater than that of T0, CAT activities of T50 and T100 had no significant difference than the beginning ($p > 0.05$). While the activities of T150 and T200 were 31.3% and 383.3% higher than the beginning and higher than T0 at 23 d. At 31 d, the CAT activities of T0-T100 still had no significant difference compare with the beginning ($p > 0.05$), and increased with the increasing intensity.

Table 6. CAT activity, SOD activity and malonaldehyde content of *P. crispus* by UV-B radiation

Physiological and biochemical parameters	Treatment	0d	16d	23d	31d
CAT activity/(U·g ⁻¹ FW·min ⁻¹)	T0	4.4±0.4Aa	3.2±0.5Ba	4.1±0.4Aa	3.8±0.4Aa
	T50	4.5±0.4Aa	4.9±0.4Ab	4.9±0.3Ab	4.6±0.4Ab
	T100	4.6±0.4Aa	4.9±0.2Ab	4.9±0.3Ab	4.9±0.5Ab
	T150	4.6±0.2Aa	6.1±0.5Bc	6.0±0.5Bc	
	T200	4.1±0.2Aa	7.8±0.5Bd	19.7±1.7Cd	
SOD activity/(U·g ⁻¹ FW)	T0	25.4±1.4Aa	18.8±1.8Ba	53.6±2.2Ca	109.1±8.5Da
	T50	24.3±1.6Aa	21.9±2.0Aa	56.3±2.5Ba	86.0±1.2Cb
	T100	24.6±1.3Aa	32.4±2.0Bb	59.6±1.7Cb	65.0±1.3Dc
	T150	26.3±1.2Aa	76.9±4.4Bc	82.5±3.4Bc	
	T200	23.5±1.2Aa	78.7±1.5Bc	71.4±2.3Cc	
MDA content/(n mol·g ⁻¹ FW)	T0	30.9±2.1Aa	26.9±2.0Aa	24.4±1.4Ba	25.1±2.1Ba
	T50	29.6±2.9Aa	29.9±1.9Aa	28.9±1.1Ab	20.2±1.5Bb
	T100	30.9±1.1Aa	35.1±1.1Bb	23.1±1.0Ca	18.3±0.5Db
	T150	30.9±2.2Aa	50.7±3.3Bc	21.8±1.2Ca	
	T200	30.9±2.1Aa	43.1±2.1Bd	20.5±0.8Cc	

SOD activity

SOD activity of each group produced a significant difference from the beginning ($p < 0.01$) (Table 6) after 16 days of radiation, the activity gradually increased in T50 to T150, and all treatments were higher than that of T0. The activities of T0, T50 were slightly lower than what was measured at the experiment's onset. Each treatment's SOD activity significantly increased at 23 d ($p < 0.01$) compared with the beginning, and T150 maintained the highest value among all treatments, and the activity of T150 and T200 had increased by 213.3%, 204.0% compared to the beginning, the activities in T0 to T150 continually increased. At 31 d, T0 to T100 had increased significantly compared with their respective values at 23 d ($p < 0.05$), but they maintained a downward trend during the experimental period. The SOD activity of T0 reached the highest value of all groups, increasing by 329.5% compared to its initial value.

MDA content

At 16 d, the MDA concentrations of T0 and T50 remained basically unchanged, but the MDA concentrations in T150 and T200 increased significantly (63.9% and 39.3%, respectively) with the beginning ($p > 0.05$) (Table 6). At 23 d, the concentrations in all

treatment groups decreased, but there were no significant differences compared with the beginning ($p>0.05$); The concentrations of T100, T150, and T200 decreased significantly from the experiment's onset ($p<0.05$), accounting for 25.2%, 29.5%, and 33.8% decreases, respectively, and the concentrations of T100, T150, and T200 were also lower than those of T0 and T50. At 31 d, the MDA concentrations in T0 to T100 were lower than those at the beginning, and decreased with the increasing intensity.

Discussion

Although ultraviolet radiation can be attenuated by the water column, 10% of incident UV-B radiation is still present at substantial depths: from several to dozens of meters. In the middle and lower reaches of the Yangtze river area in China, the maximum depth of UV-B penetration is approximately 2.25 m, the harmful ultraviolet radiation can reach the lake bed in some lakes, threatening aquatic organisms (Zhang et al., 2005). The UV-B radiation may not only affect the creatures in the water, but also damage the benthos (Puthumana et al., 2017).

UV-B radiation can attenuate exponentially in the water, and the attenuation model is in accordance with the following formula: $E_d = E_0 e^{-K \cdot d}$, where E_0 is the incident UV-B intensity, K is the attenuation coefficient, and d is the depth of the water (Bernhard et al., 2018; Overmans and Agustí, 2020). Based on the above theory, the higher the incident UV-B radiation, the greater the UV-B radiation that can penetrate into the water in this experiment. UV-B radiation will permeate deep into the water column and increase the damage to *P. crispus*. *P. crispus* plants were collected from the Hongze Lake Nature Reserve before the start of the experiment, the plants submerged in deeper water, the ultraviolet radiation in the sunlight was not strong in winter and spring. When the experiment began, the plants grew rapidly to the surface and were exposed to UV-B radiation. The top leaves of the plants are usually injured more severely because of continuous radiation from the top and then died, so the plant height decreased. Because plant biomass is mainly concentrated on the top of the plant, the injuries at the top of the plants due to the increasing radiation intensity will rapidly affect the physiological functions of the plant, and limit plant growth. This may be the reason why all plants of T150 and T200 declined and then decayed after 24 days of continuous UV-B radiation.

Chlorophyll concentration is closely related to photosynthesis in plants and can reflect light-energy utilization by chloroplasts (Huang et al., 2013). Enhanced UV-B radiation can decrease the chlorophyll concentrations of plants (Ma et al., 2016; Zhang et al., 2017). However, the sensitivity of chlorophyll to UV-B radiation can be quite different among different species, and the decline in chlorophyll concentration may be due to inhibition of chlorophyll biosynthesis (Gao et al., 2019), enhancement of chlorophyll photodegradation (Petrović et al., 2017), or a combination of both. However, low intensity UV-B radiation can make pigment molecules of LHCII (light-harvesting pigment protein complex II) absorb more energy and transfer more energy to the PSII core to stimulate photosynthesis (Teramura et al., 1991). It has been reported that T50 treatment for a short period increases chlorophyll concentration, but high-intensity radiation results in a strong bleaching effect on leaf pigment cells (Wang et al., 2010). Some pigment proteins can absorb UV-B energy, and they produce specific photochemical reactions that decrease chlorophyll concentration (Castenholz and Garcia-Pichel, 2014). The top leaves of T100 to T200 plants were damaged when they approached the water surface, and chlorophyll concentration decreased significantly

under intense UV-B radiation. In particular, when the radiation intensity surpassed 21.6 kJ/m², acute injury occurred, and the top leaves were strongly bleached (Wang et al., 2010). Chlorophyll concentration decreased gradually with increasing radiation intensity. At 23 d, the biomass of the top of the plants declined when the radiation intensity was greater than 21.6 kJ/m², which resulted in decreased plant height because new-growth leaves were used in the measurements, so the chlorophyll concentration of T150 and T200 showed an increasing trend compared with 16 d. At 23 d, because T50 received low-intensity radiation, the chlorophyll concentration was only slightly lower than that of the control group; by contrast, the chlorophyll concentration in the other groups decreased substantially with increasing radiation intensity. The leaves were farther from the radiation source and received less radiation at 31 d. Therefore, injury to the plants was reduced, and the concentration of the chlorophyll increased compared with the aforementioned cases.

UV-B radiation can induce generation of reactive oxygen species (ROS) (Yokawa et al., 2016), and increased ROS causes lipid peroxidation and protein oxidation (Pospíšil and Yamamoto, 2017). These ROS are highly reactive because they can interact with a number of cellular molecules and metabolites, leading to a number of destructive processes that cause cellular damage (Choudhury et al., 2017). Plants contain antioxidant metabolites, enzymes and nonenzymes to a variable extent, which have the ability to detoxify ROS (Abid et al., 2018). CAT and SOD can play key roles in eliminating superoxide (O₂⁻) and hydrogen peroxide (H₂O₂) (Chen et al., 2016). CAT are important protective enzymes that remove H₂O₂ and decompose H₂O₂ into O₂ and H₂O, protecting plants from the toxicity associated with H₂O₂. SOD is an enzyme capable of superoxide anion radical scavenging, and it can convert superoxide radicals to molecular oxygen and hydrogen peroxide (Perry et al., 2010). CAT and SOD activities in *P. crispus* increase under adverse conditions, such as heavy metal stress (Hu et al., 2007; Xu et al., 2010) and high nutrients (Zhang et al., 2009). If the stress exceeds a certain threshold, the enzyme activity decreases. UV-B radiation can produce the same effect on the antioxidant enzyme system of *P. crispus*. In this experiment, reactive oxygen concentrations increased with increasing radiation intensity in the initial radiation stage, and the antioxidant enzyme system was enhanced for scavenging reactive oxygen species. SOD activity also increased with increasing radiation intensity because it could decompose the superoxide anion into hydrogen peroxide and oxygen. However, the decomposition products could aggravate the H₂O₂ concentration in the leaves. In this study, CAT activities increased more than SOD activity, and they reached the highest levels at the early stage of radiation treatment; by contrast, SOD activity continued to grow with radiation. In general, the activities of all kinds of antioxidant enzymes rose with increasing radiation intensity, and this might have been inhibited by UV-B radiation. Furthermore, damage to enzymatic activities in the plants was irreversible under intense radiation conditions, which ultimately caused the plants to die. After 16 days of radiation, the plants apparently entered an emergency reaction period, and the activities of CAT reached their highest levels for the entire monitoring period. The plants' resistance was more obvious with increasing radiation intensity. SOD activity gradually increased with increasing radiation time and intensity. Because the T200 group received the highest radiation intensity, the damage was the most obvious in these plants. The SOD activity showed a decreasing trend, likely because the radiation intensity exceeded its tolerance threshold. Physiological function was reduced by increasing irradiation time, and reactive oxygen species increased. Therefore, SOD

activities at 23 d were decreased. The plants died at 31 d due to excessive radiation; the activities of CAT and SOD in the other groups were gradually reduced. Thus, the active oxygen produced in plants increased with UV-B radiation, whereas the scavenging activity of the plants decreased, resulting in a gradual increase in CAT activity. Additionally, because the top leaves of the plants were gradually dying, the plant heights decreased, so the received radiation intensity of the plants declined. The leaves used for analysis were new-growth leaves, which may also explain why the antioxidase activity was lower than in the initial stages of radiation.

Under a ray of light or under the action of free radicals, one hydrogen molecule breaks from the lipid molecules (LH) and forms a lipid free radical (L·). The L can then react with oxygen to form peroxy radicals (LOO), which can attack other lipid molecules, seize the hydrogen atom, and produce new free radicals and lipid hydroperoxide (LOOH) (Ayala et al., 2014). This reaction repeats and results in continuous consumption of lipid molecules and the generation of a number of lipid peroxides (Ayala et al., 2014). In studies of terrestrial plants, UV-B radiation increased the levels of lipid peroxidation products (MDA) significantly (Singh et al., 2014; Gęgotek et al., 2017; Chen et al., 2019), and it changed the membrane fatty acid composition, decreased the unsaturation index, and eventually injured the plants. In the present study, *P. crispus* plants produced a large amount of peroxides under intense radiation during the initial stage. As a result, leaf MDA concentration reached its maximum at 16 d. Concurrently, mass production was reduced, possibly because of continuous consumption of lipid peroxides. Membrane permeability was also increased with long-term radiation, causing gradual bleaching or death of the plants' top leaves and leading to decreased plant height. When middle leaves replaced the top leaves and became the new top leaves, the actual radiation intensity received was reduced, and the MDA concentrations in the leaves were lowered. Because the new top leaves received less radiation due to the decreased plant height, the release of peroxides in the top leaves was also tempered; this explains why the MDA concentrations of the T50-T200 groups decreased at 23 d and why the T0-T150 groups showed a decreasing trend at 31 d. This variation may suggest that oxidation products were gradually reduced because the lipid molecules were continuously oxidated.

Conclusion

UV-B radiation can accelerate plants' decline when the exposure intensity exceeds 32.4 kJ/m², the plants that received this level of radiation declined and died within 31 days, whereas a few plants still grew when the exposure intensity less than 21.6 kJ/m². UV-B radiation reduced the plant heights, and also shortened internode length and shrunk leaf area. Chla and Chlb contents all decreased under UV-B radiation when the UV-B radiation intensity surpassed 10.8 kJ/m², and the inhibition effects were further elevated as the radiation intensity increased. CAT activities were improved under radiation for a short time and gradually increased with increasing radiation intensity. However, CAT activities were inhibited with continuing exposure to radiation. SOD activity increased gradually with prolonged irradiation time and increased radiation intensity. MDA content was improved at the beginning of the UV-B radiation, and then gradually increased with increasing radiation intensity, but decreased with prolonged exposure. These results indicate that UV-B radiation may be an important factor leading to mass mortality of *P. crispus* in late spring and early summer.

Accordingly, we deduce that the sharp enhancement of ultraviolet radiation on the land surface in the late spring and early summer leads to mass mortality of the wild *P. crispus* population. However, further research is necessary to study whether UV-B radiation acts with other environmental factors to affect physiological activity and contribute to the decline of *P. crispus*.

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