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ORIGINAL ARTICLE

Physiological acclimation of *Lessonia spicata* to diurnal changing PAR and UV radiation: differential regulation among down-regulation of photochemistry, ROS scavenging activity and phlorotannins as major photoprotective mechanisms

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Abstract Intertidal macroalgae are constantly subjected to high variations in the quality and quantity of incident irradiance that can eventually generate detrimental effect on the photosynthetic apparatus. The success of these organisms to colonize the stressful coastal habitat is mainly associated with the complexity of their morphological structures and the efficiency of the anti-stress mechanisms to minimize the physiological stress. *Lessonia spicata* (Phaeophyceae), a brown macroalga, that inhabits the intertidal zone in central–southern Chile was studied in regard to their physiological (quantum yield, electron transport rate, pigments) and biochemical (phlorotannins content, antioxidant metabolism, oxidative stress) responses during a daily light cycle under natural solar radiation. Major findings were that F_v/F_m, photosynthetic parameters (ETR_{max}, alpha, E_k) and pigments in *L. spicata* showed an inverse relationship to the diurnal changes in solar radiation. Phlorotannins levels and antioxidant activity showed their highest values in treatment that included UV radiation. There was an increase in SOD and APX in relation at light stress, with a peak in activity between 5.2 and 10.1 W m^{-2} of biologically effective dose. The increase in peroxidative damage was proportional to light dose. These results indicated that different light doses can trigger a series of complementary mechanisms of acclimation in *L. spicata* based on: (i) down-regulation of photochemistry activity and decrease in concentration of photosynthetic pigments; (ii) induction of phenolic compounds with specific UV-screening functions; and (iii) reactive oxygen species (ROS) scavenging activity via complementary repair of the oxidative damage through increased activity of antioxidant enzymes and potentially increased amounts of phenolic compounds.

Keywords Photosynthesis · *Lessonia spicata* · UV radiation · Photoinhibition · Phlorotannins · Antioxidant enzymes

Introduction

Light is the most prominent environmental factors in marine coastal habitats, ranging from limiting levels for macroalgal growth and photosynthesis in the lower subtidal to stressful levels for primary physiological process in the sun-exposed intertidal zone (Hanelt and Figueroa 2012). Since light conditions occurring in the intertidal can frequently and rapidly change throughout the course of a day, macroalgae have developed different photoprotective and acclimation mechanisms to thrive under these unfavorable conditions. Among them, (1) ecological strategies, related either to grow under the canopy or through the colonization of UV-protected areas such as shaded pools and rocky crevices (Bischof et al. 1998; Betancor et al. 2015), (2)

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morphofunctional adaptations related to thallus morphology, thickness or pigmentation (Gómez and Huovinen 2011; Figueroa et al. 2014) and (3) physiological mechanisms associated with rapid adjustments of the photosynthetic machinery (e.g., down-regulation of photosynthesis), synthesis of UV-absorbing compounds and antioxidant responses (Collén and Davison 1999; Cruces et al. 2012). These strategies are strongly related to the specific environmental tolerance thresholds of macroalgae, which finally set the metabolic limits of their vertical distribution along the shore, seasonal growth patterns and abundance (Hanelt et al. 1997; Koch et al. 2016).

Throughout a sunny day, the photosynthetic activity of high light-exposed intertidal macroalgae usually decreases around midday and recovers during the afternoon, showing a strong physiological response to the diurnal changes in solar radiation. For example, when low tides coincide with maximum solar radiation (e.g., during midday in summer), macroalgae that are exposed to high light intensities can eventually generate detrimental effect on the photosynthetic apparatus (Gevaert et al. 2002; Sampath-Wiley et al. 2008). The effect of PAR light and UV radiation on the disruption of the manganese cluster in photosystem II (PSII) might be a beginning of photodamage (Takahashi and Murata 2008; Hou et al. 2013; Hou and Hou 2013). To avoid irreversible damages to the photosynthetic apparatus of sessile intertidal macroalgae, they can activate different efficient photoprotective mechanisms, such as the xanthophyll cycle to prevent photoinhibition of photosynthesis (Krause and Weis 1991; Demmig-Adams and Adams 1992; Hanelt et al. 1994; Nath et al. 2013). Nevertheless, if photoprotection is insufficient, photoinhibition may take place due to structural damages (e.g., of the D1 protein in the PSII reaction center). In the presence of ultraviolet (UV) radiation, the capability of recovering from high light stress can be significantly delayed, depending on the efficiency of the repair of the UV-damaged D1 protein (Häder and Figueroa 1997; Roleda et al. 2006; Takahashi and Badger 2011; Pescheck et al. 2010). High levels of UV-induced reactive oxygen species (ROS) delay the PSII repair cycle by suppressing the repair process of UV-damaged D1 proteins (Takahashi and Badger 2011). Therefore, an effective management of either avoiding enhanced ROS production by photosynthesis or efficient detoxification of ROS is crucial to the survival of macroalgae inhabiting the intertidal zone. Within these mechanisms, which modulate the degree of photoinhibition of photosynthesis, the energy dissipation system and the capacity for damage repair are the most important photoprotective physiological mechanisms (Franklin et al. 2003; Wilhelm and Selmar 2011). However, the physiological bases of these responses to light stress in intertidal macroalgae are not fully understood yet, especially those associated with stress tolerance and resilience to the combination of high PAR and UV radiation (see Bischof et al. 2006; Karsten et al. 2009 and references therein).

Another line of defense against the direct UV exposure in brown macroalgae is their capability of allocating large amounts of UV-absorbing polyphenolic compounds (i.e., phlorotannins) between different parts of their thalli (Pavia et al. 1997; Targett and Arnold 1998). The accumulation of phlorotannins in UV-exposed fronds of brown macroalgae can vary over short periods of time by showing highest contents during low tides and UV stress in summer (Connan et al. 2004; Connan et al. 2007; Rautenberger et al. 2015). In the kelp *Lessonia spicata* (former *Lessonia nigrescens*), the induction of phlorotannins by UV radiation was shown to protect the photosynthetic apparatus and genomic DNA against UV-induced damage (Gómez and Huovinen 2010). However, both soluble and insoluble phlorotannins do not provide a full UV protection, which requires the interplay between different mechanisms of photoacclimation (Gómez and Huovinen 2010; Cruces et al. 2013). Thus, the photosynthetic apparatus has to be protected by other mechanisms that can be more rapidly activated than the accumulation of phlorotannins, e.g., a temporary down-regulation of PSII and the activation of the xanthophyll cycle, which reduces the 'excitation pressure' in PSII (Wilhelm and Selmar 2011; Mou et al. 2013; Yu et al. 2013). However, when high irradiances of UV radiation inhibit the xanthophyll cycle, ROS production can tremendously increase because the excess absorbed excitation energy of PAR is not effectively dissipated (Dring 2005; Bischof et al. 2006; Lesser 2012). The surplus of photosynthetic electrons, which cannot be used by photosynthesis, is transferred to molecular oxygen (i.e., the Mehler reaction or photoreduction of oxygen), generating superoxide anions (O_2^{*-}) and eventually highly hydroxyl radicals (·OH). If high levels of these ROS cannot be efficiently detoxified, for example by antioxidant enzymes such as superoxide dismutase (SOD) and ascorbate peroxidase (APX), photooxidation of pigments and proteins results in bleaching and lethal disintegration of the macroalgal thallus (Bischof et al. 2002a, 2003; Bischof and Rautenberger 2012). In intertidal macroalgae, the potential for enzyme activity to avoid oxidative stress depend on the stress conditions, which vary considerably in the natural habitat (Aguilera et al. 2002; Rautenberger et al. 2013). For example, in the red macroalga *Corallina officinalis*, activities of superoxide dismutase (SOD) and peroxidase (POX) increased significantly under high UV-B stress, while both ascorbate peroxidase (APX) and catalase (CAT) increased their activities only to respond to low irradiances of UV-B radiation (Li et al. 2010). In the Antarctic brown macroalgae *Desmarestia anceps*, the phenotypic plasticity of SOD and the photosynthetic activity seems to be crucial

to withstand the PAR stress in the upper subtidal (5–9 m) (Rautenberger et al. 2013). Therefore, the ability of a macroalgal species to display these photoprotective mechanisms is determined by specific thresholds (Gravot et al. 2010; Wang et al. 2016).

The present study was conceived to examine the interaction between the whole suite of anti-stress mechanisms (i.e., antioxidant enzymes, photochemical down-regulation and phenolic compounds) presently known in the intertidal brown macroalga *L. spicata* (Phaeophyceae) from central to south Chile during the exposure to a diurnal cycle of solar radiation (PAR + UV radiation). In the present study, we tested the prediction if these three different types of photoprotective mechanisms are complementary and vary differentially during the changing light stress periods throughout a day. Therefore, these results will give insights into the different PAR and UV tolerance mechanisms that allow this light-exposed macroalga survive in its harsh rocky intertidal habitat.

Materials and methods

Sampling and acclimation of algae

Thalli from juvenile sporophytes of *Lessonia spicata* (Suhr) Santelices (Laminariales, Phaeophyceae, Ochrophyta) were collected from the rocky intertidal area of Calfuco Beach, Valdivia (39°51′S, 73°23′W), during the austral summer (January 2014). Subsequently, macroalgal thalli were transferred to the Calfuco Coastal Laboratory of Aquatic Resources (Universidad Austral of Chile) where they were cleaned of epibionts and associated animals. Fifteen different individuals were collected and were cut off their blades that were placed into six different containers. Samples were acclimated to 40 µmol photons m^{-2} s^{-1} (PAR; Cool daylight, Phillips, The Netherlands) for 24 h in a 40-L tank with circulating and continuous aerated seawater (salinity 30 PSU) and at a constant temperature of 12 ± 1 °C (corresponding to seawater of the season).

After the pre-acclimation, macroalgae were placed into UV transparent cages ~3 cm underwater to prevent desiccation and heat stress in a 200-L tank with circulating seawater at 30 PSU and constant aeration (Fig. 1). For radiation treatments, two different cutoff filters were used to cover the cages to expose the fronds of *L. spicata* to two radiation conditions: PAR alone (400–700 nm, Ultraphan UV; Digefra GmbH, Munich, Germany) and PAR + UV radiation (280–700 nm, Ultraphan URT; Digefra GmbH, Munich, Germany). The experiment was set to follow of daily course of solar radiation at this location (Fig. 2).

The changes in spectral composition of solar radiation reaching the Earth's surface throughout the experimental day were measured by placing a hyper-spectral radiometer with a cosine-corrected sensor (RAMSES ACC2-UV–Vis; Trios Optical Sensors GmbH, Oldenburg, Germany). Spectroradiometric scans for UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm) were performed every 15 min from 8:00 h in the morning until 20:00 h in the afternoon. The maximum irradiances for each waveband were 2174 µmol photons m^{-2} s^{-1} PAR, 50.3 W m^{-2} UV-A and 2.4 W m^{-2} UV-B at 14:00 h (i.e., solar noon) (Fig. 2). The action spectrum for photoinhibition of photosynthesis considered wavelengths between 280–399 nm was used to calculate biologically effective dose rates (BED), considering that UV radiation is highly photoinhibitory to PSII (Jones and Kok 1966).

Fluorescence measurements

Algal samples were taken from the experimental treatments and incubated for 20 min in the dark and measured for maximum quantum yield of fluorescence of photosystem II (PSII) (i.e., F_v/F_m), which is an indicator of quantum efficiency (Schreiber et al. 1994). The electron transport rate (ETR) was determined through the P–E curves for the relationship between the effective quantum yield (Φ_{PSII}) and the intensity of actinic irradiance (red light diode) as follows:

$$\mathrm{ETR} = \Phi_{\mathrm{PSII}} \times E \times A \times F_{\mathrm{II}} \quad (1)$$

where E is the irradiance of PAR, A the thallus absorptance and F_{II} is the fraction of absorbed quanta directed to PSII (i.e., 0.5), which was estimated by determining the fraction of the chlorophyll *a* associated with PSII and its corresponding light harvesting complex (LHC). The thallus absorptance, A, was determined by placing the algae on top of a cosine-corrected PAR sensor (Licor 192 SB, Li-COR Inc., Lincoln, USA) and calculating the light transmission:

$$A = 1 - T - R \quad (2)$$

where T is the transmittance (light transmitted throughout the sample $= E_t/E_0$), and R is the reflectance (reflected fraction) of the thallus. For defining the ETR versus E curve parameters, a modified nonlinear function of Jassby and Platt (1976) was used:

$$\mathrm{ETR} = \mathrm{ETR}_{\max} \times \tanh(\alpha \times E/\mathrm{ETR}_{\max}) \quad (3)$$

where ETR_{max} is the maximal ETR, tan*h* is the hyperbolic tangent function, α is the initial slope of the ETR–*E* curve, which is an indicator of the efficiency of the electron transport, and E is the incident irradiance. The saturation irradiance for the photosynthetic electron transport (E_k) was calculated as the intercept between α and ETR_{max}.

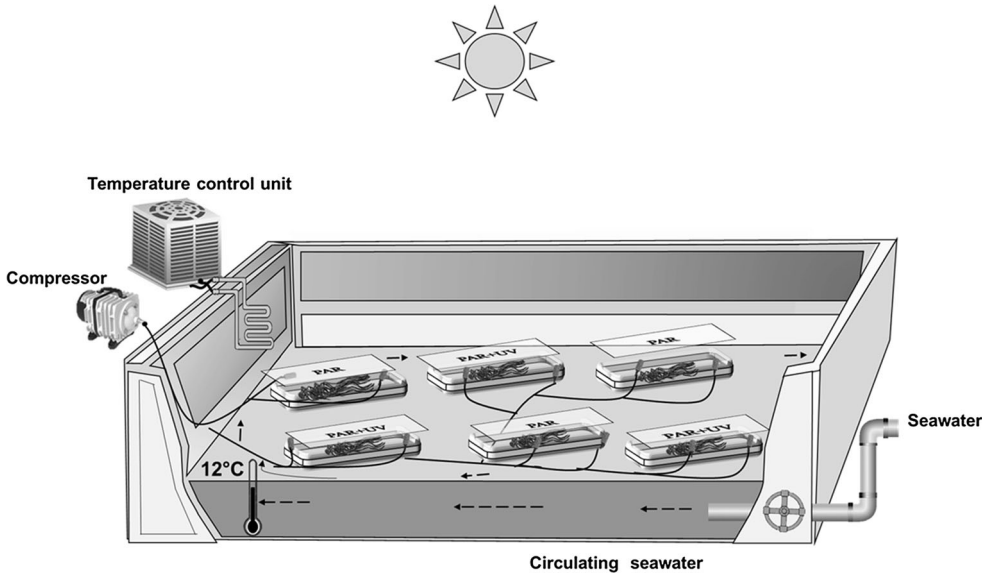

Fig. 1 Schematic drawing of the experimental design for a light cycle with natural light incident

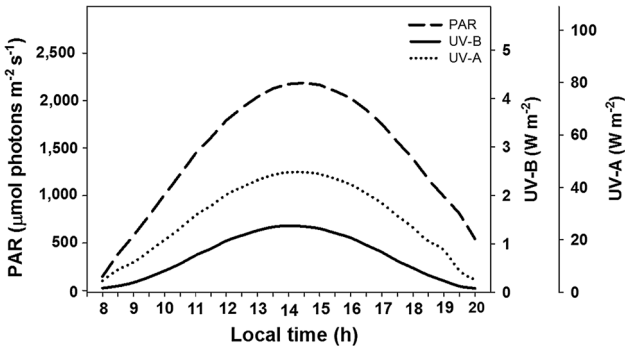

Fig. 2 Irradiances of solar radiation during a course of a day, separated for photosynthetically active (PAR: 400–700 nm), ultraviolet-B (UV-B: 280–315 nm), and ultraviolet-A (UV-A: 315–400 nm) radiation in a summer day in Calfuco Beach, Valdivia (39°51′S, 73°23′W)

Pigment analysis

Chlorophylls (Chl *a*, Chl *c*) and the total carotenoids were determined by extraction form macroalgal thalli with 100 % *N*,*N*-dimethylformamide for 24 h at 4 °C in dark conditions. Subsequently, the absorbance was measured in a spectrophotometer (Scinco, South Korea), and the Chl *a* was calculated according to Inskeep and Bloom (1985). For Chl *c* contents and crude estimations of total carotenoids of the extract, the methodology of Henley and Dunton (1995) was followed.

Quantification of total phenolic compounds

Total phenols were determined using the Folin–Ciocalteu method (Koivikko et al. 2005), following the protocol modified by Cruces et al. (2016). Approximately 75 mg of macroalgal tissue was ground to a fine powder in liquid nitrogen using mortar and pestle. The powder was mixed with 1.5 mL of 70 % (v/v) aqueous acetone. After the acetone extract was centrifuged at 4000 rpm for 10 min at 4 °C, the supernatant was filtered (0.22 µm) to remove impurities. Aliquots of 100 µL of the filtered extract were mixed with 100 µL of ultrapure H_2O (18.2 MΩ cm at 20 °C), 100 µL of 1 N Folin–Ciocalteu reagent (Sigma-Aldrich, USA) and 200 µL of 20 % (w/v) Na_2CO_3. The samples were incubated for 45 min at room temperature in the dark and centrifuged at 5000 rpm for 3 min. Afterward, absorbances were measured photometrically at 730 nm (Multiskan Spectrum microplate reader, Thermo Fisher Scientific Inc., Waltham, USA). The concentration of phlorotannins in the extracts was calculated using a standard curve of phloroglucinol (Sigma-Aldrich, USA). The concentration of total phenolic compounds was expressed as mg g^{-1} fresh weight (FW).

Determination of DPPH-based antioxidant activity

The antioxidant activity of the extracts was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH*) free radical scavenging method described by Brand-Williams et al. (1995), modified for 96-well microplate. DPPH* (150 µM) was prepared freshly in 80 % (v/v) methanol and mixed with 22 µL of the sample extract. The absorbance was measured at 520 nm in a microplate reader using gallic acid as a standard. The antioxidant activity was expressed as mg gallic acid equivalent (GAE) g^{-1} FW.

Lipid peroxidation

The content of malondialdehyde (MDA) equivalents as indicator of oxidative damage in membranes was analyzed

according to Salama and Pearce (1993) with modifications of volumes for 96-well microplate (Cruces et al. 2012). Algal samples of (70 ± 5 mg FW) were ground in liquid nitrogen using mortar and pestle and extracted with 1.5 mL of 0.5 % (w/v) thiobarbituric acid dissolved in 20 % (w/v) trichloroacetic acid. The mixture was incubated in a water bath at 95 °C for 30 min, cooled on ice, and centrifuged at 14,000 rpm for 20 min. Absorbances were read in a 96-well microplates spectrophotometer (Multiskan Spectrum Thermo Scientific, Waltham, MA) at 440, 532 and 600 nm. The amounts of MDA were calculated using the molar wavelength-dependent extinction coefficient of 1.57×10^5 M^{-1} cm^{-1} at 532 nm (Albro et al. 1986). Results are expressed as nmol MDA g^{-1} FW.

Activities of antioxidant enzymes

Antioxidant enzyme activities were analyzed according to a modified protocol of Aguilera et al. (2002). Samples (0.5 ± 0.05 g FW) were homogenized in 3 mL of 50 mM phosphate buffer (pH 7.0, 20 °C) containing 1 mM EDTA, 1 % (w/v) polyvinylpolypyrrolidone (PVPP) and complete protease inhibitor cocktail (Sigma-Aldrich, USA). After the crude extract was centrifuged (12,000 rpm, 10 min, 4 °C), the supernatant was aliquoted (200 µL) and stored at −80 °C until further measurement of activities of antioxidant enzymes. All steps were carried out on ice. Superoxide dismutase (SOD; EC. 1.15.1.1) activities were indirectly analyzed from 50 µL of the crude extract using the commercial WST-SOD Assay Kit (Dojindo Molecular Technologies Inc., Rockville, MD, USA) in which superoxide anions generated by xanthine oxidase reduced the 2-(4-iodophenyl)-3-(4-nitrophenyl)-5(2,4-disulfo-phenyl)-2H-tetrazolium monosodium salt (WST-1) to produce a water-soluble WST-1 formazan dye. This product was photometrically measured at 450 nm (Multiskan Varioskan; Thermo Fisher Scientific Inc., Waltham, USA). Several dilutions were used to calculate SOD activities, which resulted in a 50 % inhibition (IC_{50}) of the reduction rate of blanks (i.e., without samples or SOD). One unit of SOD is defined as the amount of enzyme in the reaction mixture that inhibits the reduction reaction of WST-1 with O_2^{*-} by 50 %. Catalase (CAT; EC 1.11.1.6) activities were analyzed by adding 50 µL of the crude extract to 200 µL of a 50 mM potassium phosphate buffer (pH 7.0, 20 °C). The reaction started by adding 150 µL of 15 % (v/v) hydrogen peroxide (H_2O_2) to the mixture, and the decrease in absorptance at 240 nm was measured at 20 °C over 5 min. CAT activities were calculated by subtracting the non-enzymatic reaction and using the extinction coefficient for H_2O_2 of 0.0398 mM^{-1} cm^{-1} at 240 nm (Rao et al. 1996). Ascorbate peroxidase (APX; EC 1.11.1.11) was extracted with the identical protocol as used for both SOD and CAT with the modification that 0.5 mM of reduced ascorbate was added to the extraction buffer (see above) to ensure the stability of APX (Chen and Asada 1989). APX activities were assessed according to Chen and Asada (1989), and the decrease in absorptance was recorded photometrically at 290 nm over 10 min at 20 °C after 50 µL of the crude extract was added to 200 µL of 50 mM phosphate buffer (pH 7.0, 20 °C) containing 0.1 mM of H_2O_2 and 0.5 mM ascorbate. APX activities were calculated by subtracting the non-enzymatic reaction and using an extinction coefficient for ascorbate of 2.8 mM^{-1} cm^{-1} (Nakano and Asada 1981). Activities of all antioxidant enzymes were expressed as U mg^{-1} protein.

Total soluble proteins of crude extracts were determined using a commercial protein assay (Sigma-Aldrich, USA), based on the Bradford method (Bradford 1976). Protein contents were analyzed photometrically at 595 nm, and concentrations were calculated compared with a standard curve of bovine serum albumin (BSA) (Winkler Ltda, Santiago, Chile).

Statistical analysis

Means and standard deviations were calculated from 15 independent replicates of individual blades per treatment ($n = 15$). ETR parameters were determined using 3–4 replicates. Data were compared using a two-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc analysis of means when differences were detected. Time of exposure and UV treatments were considered as the main factors. Arcsine transformations were used to meet the ANOVA requirements. Homogeneity of variances and normal distribution were examined using the Levene and Shapiro–Wilk W tests, respectively. To determine the correlation between DPPH-based antioxidant activity and phlorotannins, Pearson's test was performed. Statistical significance was set to $P < 0.05$.

Results

Changes in photosynthetic activities during diurnal light stress

There are significant effects on almost all photosynthetic parameters in response to the changing PAR and UV radiation regimes throughout the day. The maximum PSII quantum yield (F_v/F_m) showed an inverse relationship with the levels of solar radiation (Fig. 3). At the beginning of the experiment at 8:00 h, F_v/F_m was highest (0.74 ± 0.04) but decreased with increasing radiation stress: The PAR + UV radiation treatment caused a more pronounced

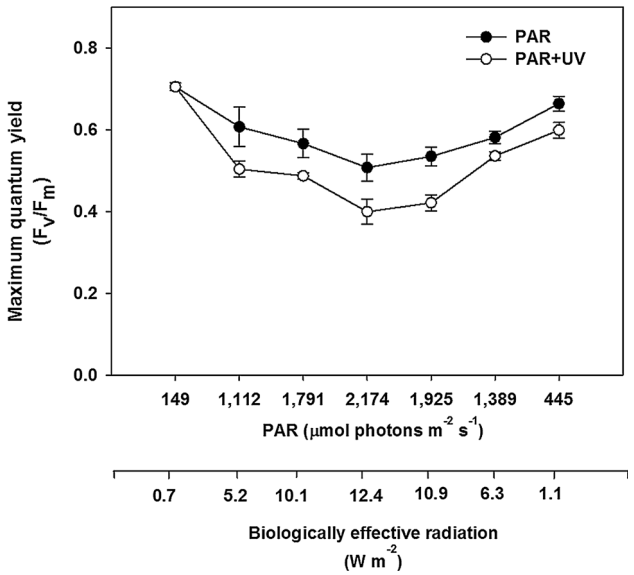

Fig. 3 Variation of maximal quantum yield (F_v/F_m) of *Lessonia spicata* during a diurnal light stress under natural solar radiation (see Fig. 2). Data are mean ± standard deviation; $n = 15$. We use the action spectrum for photoinhibition of photosynthesis (Jones and Kok 1966) to calculate biologically effective dose rates

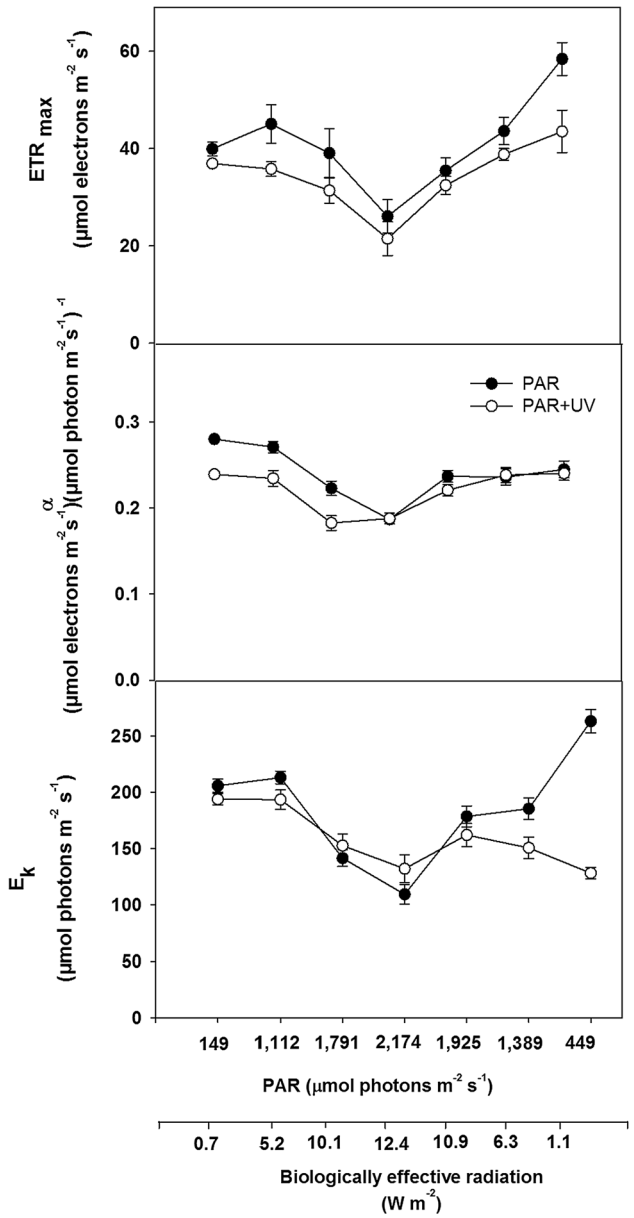

Fig. 4 Maximum electron transport rate (ETR_{max}), electron transport efficiency (α) and light saturation points of photosynthesis (E_k) of *Lessonia spicata* exposed to natural irradiances of photosynthetically active radiation (PAR: 400–700 nm) and ultraviolet (UV: 280–400 nm) radiation during a diurnal light stress under natural solar radiation. Values are mean ± standard deviation; $n = 3$–4. We use the action spectrum for photoinhibition of photosynthesis (Jones and Kok 1966) to calculate biologically effective dose rates

decline in F_v/F_m than PAR alone. At 14:00 h, when solar radiation was highest (2174 µmol photons m^{-2} s^{-1} and 12.4 W m^{-2} of BED), F_v/F_m decreased at its lowest values. The F_v/F_m was reduced by 29 % under high PAR stress, while it decreased by 45 % under exposure to PAR + UV radiation. Afterward, F_v/F_m started to recover from radiation stress by gradually reaching initial F_v/F_m values, while the recovery of F_v/F_m in UV-treated samples remained incomplete.

Similar to F_v/F_m, the photosynthetic parameters ETR_{max}, alpha and E_k also showed an inverse relationship to the diurnal changes in solar radiation (Fig. 4). The photosynthetic electron transport capacity (ETR_{max}) decreased by ~50 % under both radiation conditions similarly compared at the beginning of the experiment (38 µmol electrons m^{-2} s^{-1}) but recovered completely in the late afternoon. The electron transport efficiency (α) in *L. spicata* showed a decline in values in the solar noon (~30 %) achieving a recovery in both exposures. The saturation irradiance electron transport (E_k) in *L. spicata* was close to 200 µmol photons m^{-2} s^{-1}. It was observed that in both exposures *L. spicata* decreased in average by 40 % ($P < 0.001$) in the solar noon in relation to its initial values (Fig. 4).

Diurnal changes in pigments

Photosynthetic pigments were strongly affected by the two changing radiation conditions throughout the day, showing a reverse relationship in their contents with the irradiances of PAR and UV radiation (Table 1). While the decrease by 30 % in Chl *c* was induced by maximum irradiances of PAR, the additional exposure to UV radiation resulted in a stronger decline (−47 % at 14:00 h) and incomplete recovery in Chl *a* values. In general, the total contents of carotenoids were sensitive to UV radiation showing a delay in the recovery.

Table 1 Chlorophylls *a*, *c* and carotenoids levels in *Lessonia spicata* exposed to PAR versus UV radiation

Local time (h)	Chl *a* (mg g^{-1} FW)		Chl *c* (mg g^{-1} FW)		Carotenoids (mg g^{-1} FW)	
	PAR	PAR + UV	PAR	PAR + UV	PAR	PAR + UV
8	0.74 ± 0.04^{Aa}	0.71 ± 0.01^{Ac}	0.08 ± 0.01^{Aa}	0.08 ± 0.004^{Aa}	0.28 ± 0.001^{Aa}	0.27 ± 0.005^{Aa}
10	0.72 ± 0.05^{Aa}	0.62 ± 0.08^{Aabc}	0.07 ± 0.005^{Aa}	0.07 ± 0.001^{Aab}	0.25 ± 0.006^{Ab}	0.24 ± 0.001^{Bb}
12	0.64 ± 0.02^{Ab}	0.61 ± 0.02^{Ab}	0.07 ± 0.012^{Ab}	0.06 ± 0.012^{Ab}	0.28 ± 0.005^{Aa}	0.17 ± 0.007^{Bd}
14	0.52 ± 0.07^{Ac}	0.38 ± 0.02^{Bd}	0.05 ± 0.004^{Ab}	0.05 ± 0.007^{Ac}	0.15 ± 0.004^{Ad}	0.14 ± 0.006^{Ac}
16	0.57 ± 0.01^{Ac}	0.41 ± 0.01^{Bd}	0.06 ± 0.005^{Aa}	0.05 ± 0.003^{Bb}	0.19 ± 0.01^{Ac}	0.19 ± 0.022^{Acd}
18	0.62 ± 0.05^{Aab}	0.51 ± 0.03^{Bc}	0.08 ± 0.002^{Aa}	0.06 ± 0.003^{Ab}	0.25 ± 0.002^{Ab}	0.20 ± 0.004^{Bc}
20	0.63 ± 0.02^{Ab}	0.56 ± 0.03^{Bc}	0.07 ± 0.004^{Aa}	0.07 ± 0.018^{Ab}	0.28 ± 0.005^{Aa}	0.21 ± 0.003^{Bc}

Different letters indicates statistical differences between treatments and times according to ANOVA and Tukey's HSD post hoc test. Values are mean ± standard deviation; $n = 15$. Different capital letters indicate significant differences ($P \leq 0.05$) between treatments of light (PAR and PAR + UV) at the same local time. Different lowercase letters indicate significant differences ($P \leq 0.05$) between exposure times for the same similar light treatment (PAR or PAR + UV)

Responses of phenolic compounds during the light cycle

Contents of total soluble phenolic compounds showed a strong variation in response to the changing radiation conditions during the day (Fig. 5). Phenols increased gradually from 0.8 to 3.3 mg g^{-1} FW after six hours of exposure to high PAR (solar noon) and decreased to the initial values within four hours ($P < 0.001$). A similar pattern but with an even greater induction of these compounds (4.2 mg g^{-1} at 12.4 W m^{-2} of BED) could be detected under UV stress at all times. It is noteworthy that the soluble phenolic compounds decreased rapidly between 16:00 and 18:00 h in both radiation treatments (Fig. 5).

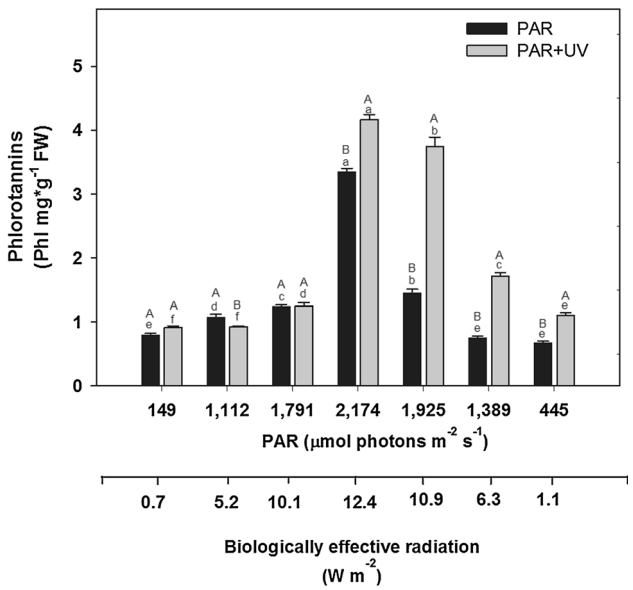

Fig. 5 Contents of total soluble phlorotannins in *Lessonia spicata* exposed to changing irradiances of photosynthetically active (PAR: 400–700 nm) and ultraviolet (UV: 280–400 nm) radiation during a diurnal light stress on a summer day (see Fig. 2). Different capitol and small case letters denote statistically significant differences between different treatments and times exposure according. Values are mean ± standard deviation; $n = 15$. We use the action spectrum for photoinhibition of photosynthesis (Jones and Kok 1966) to calculate biologically effective dose rates

Lipid peroxidation and antioxidant capacities

Lipid peroxidation measured as MDA contents varied in relation to the changing radiation conditions during the day, while antioxidant capacities did not show such a clear pattern (Table 1). MDA contents increased with increasing irradiances of PAR until 14:00 h and decreased afterward. Under exposure to PAR + UV radiation, the radiation-dependent pattern of lipid peroxidation was similar but with statistically significant MDA contents from 12:00 until 18:00 h.

The antioxidant capacity showed response associated with the changing irradiances of solar radiation (Table 2). The antioxidant capacity increased by twofold during the maximum radiation (14 h) under both radiation treatments of PAR and PAR + UV.

Antioxidant enzymatic activities

The activities of the two antioxidant enzymes SOD and APX showed a similar pattern in which they were stimulated within the first two (10:00 h) to four (12:00 h) hours under radiation stress. Afterward, activities of both enzymes decreased gradually until they reached initial values at the end of the experiment (Figs. 6 and 7). CAT activities, however, showed their maximum in the later afternoon between 18:00 and 20:00 h. The activities of these three antioxidant enzymes were not considerably affected by UV stress, even though slight changes were detected (Fig. 8).

Table 2 Lipid peroxidation levels measured as formation of malondialdehyde (MDA) and radical scavenging activity in *Lessonia spicata* exposed to PAR versus UV radiation

Local time (h)	MDA (nmol g^{-1} FW)		Antioxidant activity (mg GAE g^{-1} FW)	
	PAR	PAR + UV	PAR	PAR + UV
8	19.35 ± 1.46^{Ac}	21.47 ± 1.53^{Ad}	8.99 ± 1.49^{Abc}	7.89 ± 1.34^{Ac}
10	26.50 ± 2.02^{Aa}	27.08 ± 2.26^{Ac}	9.69 ± 1.95^{Ab}	11.03 ± 0.66^{Ab}
12	26.36 ± 2.81^{Bab}	34.96 ± 0.81^{Aa}	10.90 ± 1.54^{Ab}	12.17 ± 1.88^{Ab}
14	29.47 ± 1.45^{Bd}	37.36 ± 3.83^{Aa}	16.21 ± 2.10^{Aa}	17.08 ± 1.69^{Aa}
16	22.51 ± 1.09^{Bb}	31.70 ± 1.38^{Ab}	15.79 ± 1.07^{Aa}	16.37 ± 1.24^{Aa}
18	17.37 ± 1.62^{Bcd}	26.44 ± 1.74^{Ac}	6.89 ± 1.42^{Bc}	12.91 ± 1.69^{Aab}
20	18.53 ± 1.30^{Ac}	20.33 ± 2.86^{Ad}	5.77 ± 1.80^{Ac}	9.05 ± 1.61^{Ac}

Different letters indicates statistical differences between treatments and times according to ANOVA and Tukey's HSD post hoc test. Values are mean ± standard deviation; $n = 15$. Different capital letters indicate significant differences ($P \leq 0.05$) between treatments of light (PAR and PAR + UV) at the same local time. Different lowercase letters indicate significant differences ($P \leq 0.05$) between exposure times for the same similar light treatment (PAR or PAR + UV)

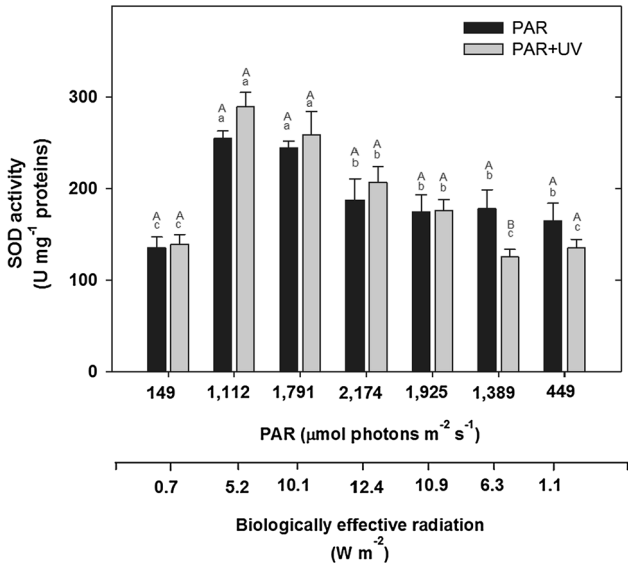

Fig. 6 Total activity of superoxide dismutase (SOD) in *Lessonia spicata* exposed to a diurnal light stress during the course of a summer day. Data are mean ± standard deviation; $n = 15$. Different capital letters indicate significant differences ($P \leq 0.05$) between treatments of light (PAR and PAR + UV) at the same local time. Different lowercase letters indicate significant differences ($P \leq 0.05$) between exposure times for the same similar light treatment (PAR or PAR + UV). We use the action spectrum for photoinhibition of photosynthesis (Jones and Kok 1966) to calculate biologically effective dose rates

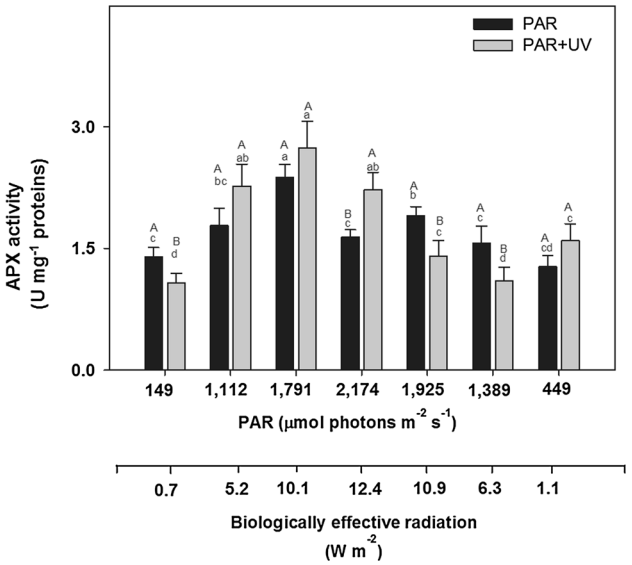

Fig. 7 Total activity of ascorbate peroxidase (APX) in *Lessonia spicata* exposed to a diurnal light stress during the course of a summer day. Values are mean ± standard deviation; $n = 15$. Different capital letters indicate significant differences ($P \leq 0.05$) between treatments of light (PAR and PAR + UV) at the same local time. Different lowercase letters indicate significant differences ($P \leq 0.05$) between exposure times for the same similar light treatment (PAR or PAR + UV). We use the action spectrum for photoinhibition of photosynthesis (Jones and Kok 1966) to calculate biologically effective dose rates

Discussion

Photoinhibition of photosynthesis and increased phenolic compounds a common and synchronized strategy to withstand light stress

The results of the present study show that the intertidal brown macroalga *Lessonia spicata* exhibits differential photoprotective responses and capacities of acclimation to the changing radiation regimes of PAR and UV radiation throughout a day. Although photochemical down-regulation as a photoprotective mechanism is well expressed, this process alone does not seem to provide full protection against high irradiances of PAR and UV radiation at solar noon. This study reveals that *L. spicata* employs a combination of both UV-screening phlorotannins and certain major antioxidant enzymes to respond to these unfavorable light conditions.

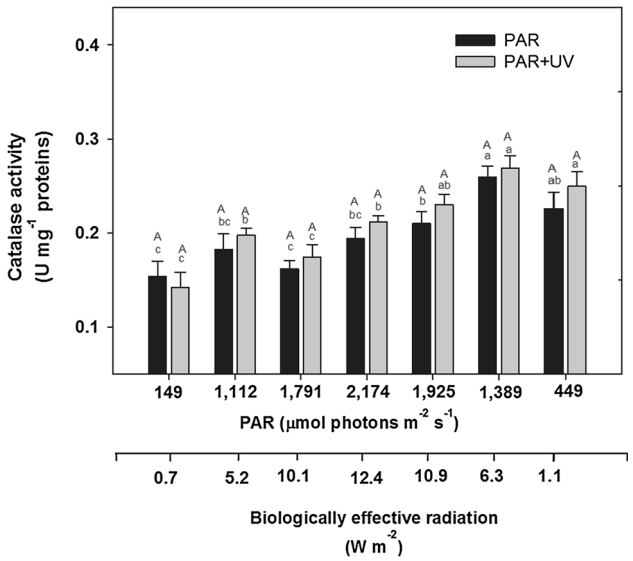

Fig. 8 Total activity of catalase in *Lessonia spicata* exposed to a diurnal light stress during the course of a summer day. Values are mean ± standard deviation; $n = 15$. Different capital letters indicate significant differences ($P \leq 0.05$) between treatments of light (PAR and PAR + UV) at the same local time. Different lowercase letters indicate significant differences ($P \leq 0.05$) between exposure times for the same similar light treatment (PAR or PAR + UV). We use the action spectrum for photoinhibition of photosynthesis (Jones and Kok 1966) to calculate biologically effective dose rates

In general, the gradual decline in photosynthetic parameters (F_v/F_m, E_k, ETR_{max} and α) toward solar noon and their subsequent recovery in late afternoon can be related to the capacities of photoacclimation of *L. spicata*. Changes in the functionality of the photosynthetic apparatus allow *L. spicata* to dissipate excess light energy harmlessly to prevent oxidative stress (Adams et al. 2006). Under both light treatments, this macroalga was able to regulate the flow of absorbed photons through its efficient mechanism of photoinhibition, allowing an adequate balance between the photoinactivation of PSII and its repair (Takahashi and Badger 2011). It was demonstrate that approximately one-third of photodamage to PSII is associated with UV radiation primarily affecting the repair of the D1 protein (Shelly et al. 2003; Takahashi and Badger 2011). Likewise, the photophysiological response studied in *L. spicata* can also be associated with the complexity of their morphological structures and the utilization efficiency of absorbed light energy as a way to minimize the physiological stress by high irradiances (Roleda et al. 2006; Figueroa et al. 2014). The massive thallus of *L. spicata*, which consists of different cell layers (i.e., medulla, cortex and meristoderm), has a lower content of chlorophyll-per-weight unit compared to other intertidal macroalgae but shows a high light absorptance (94 %) (Gómez et al. 2004; Gómez and Huovinen 2011). It has been demonstrated that morphofunctional patterns are related to carbon fixation processes acting as a compensatory mechanism to respond to enhanced solar radiation, especially macroalgae with an efficient light-independent carbon fixation (LICF) (Gómez and Huovinen 2012). Because the potential effect of UV radiation in the decreased of the activity of the carbon-fixing enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RubisCO), the LICF emerges as a complementary pathway involved in the strategy to minimize the carbon losses in glycolysis of translocated carbohydrates via carboxylating enzymes (Kremer 1984; Allen et al. 1997; Mackerness et al. 1999; Bischof et al. 2002b). For example, in *L. spicata* under doses of UV exposure close to the used in this work, F_v/F_m decreased to 30 %, and however, the photosynthetic C-fixation rates as LICF rates increased to 45 % (Gómez et al. 2007).

Along with the adjustments of the photosynthetic apparatus, some intertidal brown macroalgae can additionally protect their photosynthetic machinery by synthesizing and accumulating polyphenols under high PAR and UV radiation stress (Flores-Molina et al. 2016; Gómez et al. 2016). In addition, studies have shown the key role of shorter wavelengths in the induction of polyphenols, which include photoregulatory processes and cumulative radiation effects (Connan et al. 2007; Gómez and Huovinen 2010). Although phlorotannin levels in *L. spicata* increased significantly under PAR, the highest concentrations were observed in treatment that included UV radiation. Such a response has been associated with a putative UV-absorbing role of phlorotannins because of their absorption in the UV-B waveband (Swanson and Druehl 2002; Henry and Van Alstyne 2004). Therefore, their high concentrations, which can reach up to 3 % of the dry weight of *L. spicata* (Cruces et al. 2016), their absorption range in the UV spectrum and their induction by UV radiation, suggest an important UV-screening function of the soluble phlorotannins (Pavia et al. 1997). This direct relationship between high doses of solar radiation and concentrations of phlorotannins in *L. spicata* presents a seasonal pattern of photoprotection due to the increase in up to 10-fold of BED at midlatitudes (39°S) during the summer in relation to the winter season (Huovinen et al. 2006; Gómez and Huovinen 2010). In winter, the induction of phlorotannins during UV exposure is much lowest or practically null since these compounds are used especially for active growth via processes of biomass formation (Gómez et al. 2016; Tala et al. 2016). It must be mentioned that we used the model for photoinhibition of photosynthesis in isolated chloroplasts of higher plants (Jones and Kok 1966) to set the harmful irradiances of processes related to decreases in photochemical processes (e.g., F_v/F_m). Therefore, the comparison of radiant exposure between different taxa with their inherent morphological and physiological features (high plants vs macroalgae) can lead to misinterpretations of the

results (see Cullen et al. 1992; Cullen and Neale 1996). Clearly, few functions are available for photosynthesis in macroalgae (Miller et al. 2009) and also, for other metabolic functions, such as antioxidant enzymatic activity, peroxidation.

Antioxidant capacity: phenolic compounds versus antioxidant enzymes

A high amount of PAR and UV-related damage is caused indirectly through the production of free radicals and other reactive chemical species in chloroplasts and mitochondria and, therefore, requires efficient ROS scavenging mechanisms (Agati et al. 2013). The positive correlation between the concentration of phlorotannins and the total cellular antioxidant capacity in *L. spicata* suggests that soluble phlorotannins may act as efficient ROS scavengers (Cruces et al. 2012, 2013, 2016). In this regard, the reduction in oxidative stress associated with polyphenols includes electron donation ability, deprotonation equilibrium, stability of radicals and chemical hardness (Cheng et al. 2002). These bioactive properties are due to the neighboring hydroxyl groups, allowing them to act as reducing agents, hydrogen donors and singlet oxygen (Lopes et al. 1999; Michalak 2010; Cruces et al. 2016). It has been demonstrated that the accumulation of both superoxide radicals and H_2O_2 as a consequence of increasing methyl jasmonate levels can increase phlorotannins biosynthesis in brown macroalgae (Küpper et al. 2009; Chowdhury et al. 2015). However, the transduction pathways regulating the synthesis of phenols in macroalgae by action of solar radiation are still poorly understood (Meslet-Cladière et al. 2013). The generation of intracellular ROS may also be part of a signaling cascade that allows organisms to perceive environmental stress (Mackerness 2000; Suzuki et al. 2012). Overall, the induction of phenolic compounds between different species could be associated with species-specific adjustments of the primary and secondary metabolism to acclimate and potentially to adapt to the changing solar radiation in the intertidal habitat.

The increase in oxidative damage (e.g., lipid peroxidation) in *L. spicata* was proportional to the dose and could be closely linked to differential regulation of key antioxidant mechanisms, which includes the activity of SOD, APX and CAT. During low levels of oxidative stress (e.g., high tide is related to low light stress), antioxidative responses are finely modulated, while that detoxification during ROS excess takes place during high light stress (e.g., during low tide) (Gill and Tuteja 2010; Foyer and Noctor 2016). *Lessonia spicata* showed an increase in SOD and APX activities mediated by light stress, with a peak in antioxidant activity between 5.2 and 10.1 W m^{-2} of biologically effective dose, when photoinhibition reaches percentages between 14 and 30 %. The different relationship between light and enzyme activity is generally determined by thresholds of enzymatic action, which is species-specific, as well as by other stressors in their natural habitat (Aguilera et al. 2002). Some studies have shown that the SOD activity, as a first line of defense against photosynthetically produced ROS, is related to an increased formation of superoxide anions and the solar radiation threshold (Alscher et al. 2002; Sung et al. 2009). For example, it has been observed that when the UV doses increased over 2.5 W m^{-2}, the detoxification capacity of superoxide anion decreased in *Ulva fasciata* (Shiu and Lee 2005). For APX, it has been mentioned that its synthesis and activity would increase with high light reaching very high affinities at low concentrations of H_2O_2 (at micromolar and submicromolar range) (Cakmak and Marschner 1992; Mittler and Poulos 2007). In contrast to CAT whose low affinity with H_2O_2 would induce its activity only in a milieu characterized by high concentrations of H_2O_2 (millimolar range) (Nicholls et al. 2001), offsetting in some degree the lower activity of APX during phases of decreasing light intensity (Pnueli et al. 2003; Vandenabeele et al. 2004). Thus, the balance between SOD and APX-CAT in synchrony with different mitigation mechanisms seems to be crucial to reach the steady state of superoxide radical and H_2O_2, minimizing cell damage in intertidal macroalgae (Mittler 2002; Suzuki et al. 2012).

Conclusions

Lessonia spicata is a brown macroalgal species that inhabits the rocky intertidal of midlatitudes of central to southern Chile (29–41°S) where it is regularly and frequently exposed to high irradiances of PAR in combination with UV radiation during low tides (Tellier et al. 2009; González et al. 2012). Their success to colonize this coastal habitat can be related to intrinsic morphological, physiological and ecological adaptations in virtue of its massive thallus conferring high resistance to physical stress. These fundamental structural features together with their position on the shore define the intensity and duration of exposure to solar radiation that finally set their physiological responses. Additionally, the mechanisms of photoacclimation allow *L. spicata* to response efficiently to the light stress in its habitat (i.e., high PAR + UV radiation). In particular, stress tolerance under increasing PAR levels toward noon was mediated by dynamic photoinhibition and the complementary action of an antioxidant system conformed by phenolic compounds and ROS scavenging enzymes. Both types of antioxidant mechanisms are dependent on BED: At low solar irradiances, enzyme activity prevailed while when at highest irradiances ROS

scavenging relied on phenolic compounds (i.e., phlorotannins). Our results indicated that the influence of UV radiation on biological process of *L. spicata* is dependent on the exposure time, on those short-term responses are mediated by down-regulation of the photochemical machinery, and the increase in the synthesis of antioxidant enzymes, while long-term responses are mediated primarily by an increase in the induction of soluble phlorotannins.

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