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ORIGINAL PAPER

Interactive effects of UV radiation and enhanced temperature on photosynthesis, phlorotannin induction and antioxidant activities of two sub-Antarctic brown algae

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Abstract *Lessonia nigrescens* and *Durvillaea antarctica,* two large sub-Antarctic brown algae from the southern Chilean coast, were exposed to solar UV radiation in an outdoor system during a summer day (for 11 h) as well as to artificial UV radiation under controlled laboratory conditions at two temperatures (15 and 20 °C) for 72 h. Chlorophyll *a* fluorescence–based photoinhibition of photosynthesis was measured during the outdoor exposure, while electron transport rates, lipid peroxidation, antioxidant activity and content of phlorotannins were determined at different time intervals during the laboratory exposure. Under natural solar irradiances in summer, both species displayed well-developed dynamic photoinhibition: F_v/F_m values decreased by 70 % at noon coinciding with the levels of PAR >1,500 µmol m^{-2} s^{-1} and UV-B radiation >1 W m^{-2} and recovered substantially in the afternoon. In treatments including UV radiation, recovery in *D. antarctica* started already during the highest irradiances at noon. The results from laboratory exposures revealed that (a) elevated temperature of 20 °C exacerbated the detrimental effects of UV radiation on photochemical parameters (F_v/F_m and ETR); (b) peroxidative damage measured as MDA formation occurred rapidly and was strongly correlated with the decrease in F_v/F_m, especially at elevated temperature of 20 °C; (c) the antioxidant activity and increases in soluble phlorotannins were positively correlated mainly in response to UV radiation; (d) phlorotannins were rapidly induced but strongly impaired at 20 °C. In general, short-term (2–6 h) exposures to enhanced UV radiation and temperature were effective to activate the photochemical and biochemical defenses against oxidative stress, and they continued operative during 72 h, a time span clearly exceeding the tidal or diurnal period. Furthermore, when algae were exposed to dim light and control temperature of 15 °C for 6 h, F_v/F_m increased and lipid peroxidation decreased, indicating consistently that algae retained their ability for recovery. *D. antarctica* was the most sensitive species to elevated temperature for prolonged periods in the laboratory. Although no conclusive evidence for the effect of the buoyancy of fronds was found, the interspecific discrepancies in thermo-sensitivity in the UV responses found in this study are consistent with various ecological and biogeographical differences described for these species.

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Introduction

Marine macroalgae inhabiting the intertidal zone are exposed in a short time span to sharp fluctuations of their physical environment. Solar UV radiation and temperature are among the major factors that determine the physiological performance of seaweeds at intertidal rocky shores. The strength of the impact of these factors depends on the tidal regime and elevation (Huovinen and Gómez 2011) and can also be exacerbated by severe desiccation, hyperosmosis and nutrient depletion, especially during summer (Schonbeck and Norton 1980; Davison and Pearson 1996; Kim et al. 2008). It has been established that exposure to UV-B radiation causes damage to the photosynthetic

apparatus in many species of algae (Dring et al. 1996; Franklin et al. 2003), mainly damage of the thylakoid photochemistry and related processes, which can finally lead to a decrease in oxygen evolution, electron transport, Rubisco activity and finally CO_2 fixation rates (Bischof et al. 2002a; Gómez et al. 2007). On the other hand, low and high temperatures affect also the early reactions of photosynthesis, principally those enzymatic processes in thylakoid membranes, which can diminish the efficiency of photoinhibition (Falk et al. 1990; Öquist et al. 1993). Due to the complex interplay between solar radiation and temperature, the study of the interactive effects of both factors is essential to understand the mechanistic bases of the physiological adaptation of these organisms to the intertidal life (Lotze and Worm 2002; Hoffman et al. 2003; Huovinen et al. 2010). Probably, the most common expression of the physical stress caused by high solar radiation and temperature on photosynthetic organisms is the elevated formation of reactive oxygen species (ROS), and hence, the ability to reduce oxidative stress defines much of the tolerance mechanisms displayed for intertidal macroalgae (Collén and Davison 1999a, b; Aguilera et al. 2002; Bischof et al. 2002b; Choo et al. 2005; Rautenberger and Bischof 2006).

The extent and threshold of the physiological responses depend not only on the duration and magnitude of the environmental stress but also on the timescales of acclimation potential and morpho-functional processes of algae, especially metabolic adjustments and synthesis of anti-stress substances. For example, together with changes in photochemical down-regulation (e.g., dynamic photoinhibition; Gevaert et al. 2002), the synthesis and accumulation of phenolic compounds (phlorotannins) and their antioxidant capacity were found to follow a diurnal course in various brown algae (Connan et al. 2007). In the fucoid *Pelvetia canaliculata*, acclimation to UV radiation based on changes in phenolic compounds and antioxidant capacities has been detected in a time span between 0 and 21 days (Hupel et al. 2011). Recent studies emphasize that bio-optical characteristics associated with ontogeny in *Laminaria* define its UV stress tolerance. In these algae, synthesis and allocation of phenols with putative UV-absorbing properties are modified according to the growth patterns and energy requirements of both sporophytes and early developmental stages (Roleda et al. 2006; Steinhoff et al. 2011).

Lessonia nigrescens (Laminariales) and *Durvillaea antarctica* (Fucales), two large brown algae that coexist and dominate at the infralittoral zone along the South Pacific coast (Santelices et al. 1980; Westermeier et al. 1994), exhibit different physiological capacities to handle environmental stress (Huovinen et al. 2010; Cruces et al. 2012). Apparently, these differences are strongly related with growth patterns and thallus anatomy, which not only can have impact on their population dynamics at a local scale but also can set physiological limits for large-scale biogeographic distribution (Tellier et al. 2009; Huovinen and Gómez 2012). *L. nigrescens* (Fig. 1a) allocates the highest energy resources and biomass to the stipes and holdfast (Westermeier and Gómez 1996), while in *D. antarctica* (Fig. 1b), biomass is mostly allocated to the air-filled lamina (Lawrence 1986). Therefore, a great part of the lamina of *D. antarctica* remains floating at the sea surface, whereas the most compact thallus of *L. nigrescens* can be submerged and completely exposed to air during a tidal cycle (Fig. 1c). Thus, the question rises whether these remarkable differences in morpho-functional organization, which define the intensity and duration of the exposure to solar radiation and temperature in a daily scale, govern the stress tolerance capacities of these species. In the present study we hypothesize that *D. antarctica* can be less affected by the combined action of UV radiation and elevated temperature, and thus, the time span required to activate the anti-stress machinery will be longer and subject to smaller hourly fluctuations compared to the non-floating *L. nigrescens*. The experimental approach was based on outdoor exposure to natural solar radiation to determine the extent of photoinhibition of photosynthesis and on continuous exposures to UV radiation in the laboratory under growth (15 °C) and elevated (20 °C) temperature for 72 h to determine decreases in photochemistry-based photosynthesis, lipid peroxidation, antioxidant activity and content of phlorotannins.

Materials and methods

Sampling and algal material

Juvenile fronds of *Lessonia nigrescens* and *Durvillaea antarctica* were collected in austral summer (December) from intertidal rocky shores of Playa Rosada, coast of Valdivia, southern Chile (39°51′S, 73°23′W), and transferred to the Marine Laboratory of Calfuco, located in the vicinity. The algae were cleaned of visible epiphytes and acclimated for 24 h in tanks with circulating seawater, continuous aeration, salinity of 30 psu, at a temperature corresponding to natural surface water temperature in the sampling site (15 ± 1 °C) and under dim light (40 µmol m^{-2} s^{-1}, Daylight, TL Phillips, the Netherlands).

Experimental design

Outdoor exposure

The daily course of photosynthesis measured as maximum quantum yield of fluorescence (F_v/F_m) (see below) was

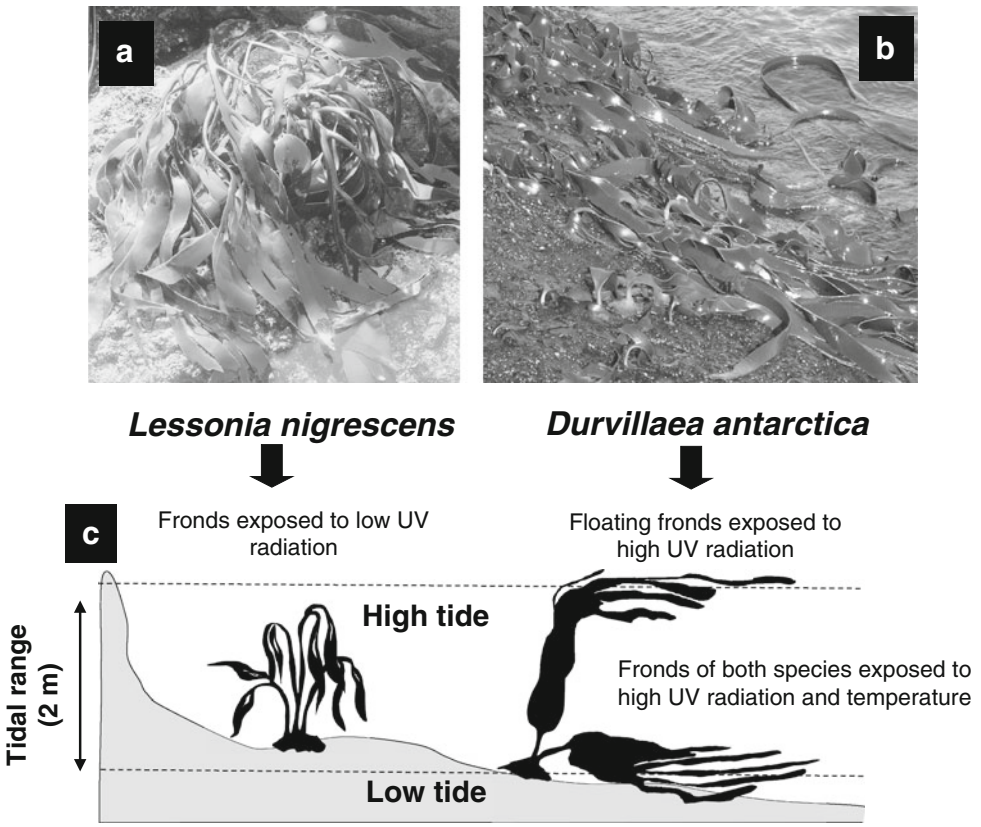

Fig. 1 The exposure of *Lessonia nigrescens* (**a**) and *Durvillaea antarctica* (**b**) to elevated solar UV radiation and temperature in the intertidal habitats in the coast of southern Chile. The "kelps" are the dominant organisms at these sites but show different population dynamics as well as morpho-functional organization, mainly a different biomass allocation between lamina and the attachment structures (stipes and holdfast). Two environmental scenarios during high and low tides are also illustrated (**c**)

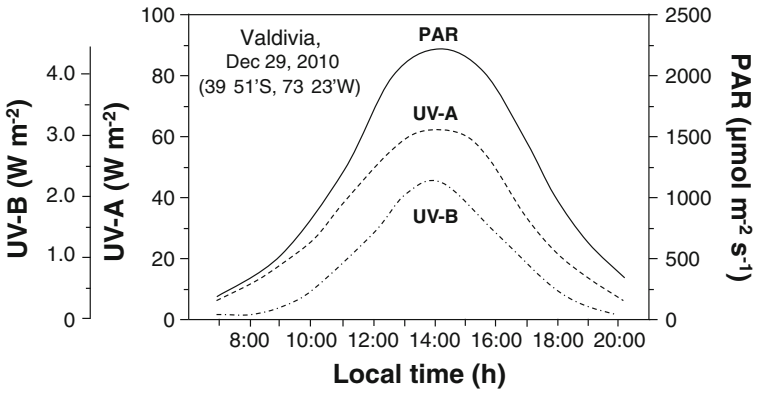

Fig. 2 Daily course of solar radiation measured in the coast of Valdivia during an outdoor exposure of algae

determined at 1-h intervals from 9:00 to 20:00 h on December 29, 2010. Pieces of fronds (12) from three individuals of each species were separated in two plastic cages covered by two cutoff foils, Ultraphan 295 and 395 nm (Digefra, Munich, Germany), resulting in UV + PAR and only PAR conditions, respectively. Although the UV-B range begins at 280 nm, the use of the Ultraphan foil cutting off at 295 nm has proved to be suitable for this type of studies as the energy between 280 and 295 nm reaching the earth surface is negligible. The whole system was submerged in a large 200-l flat tank with circulating seawater and vigorous air bubbling such that thalli were floating along a water column of 10 cm (Fig. 2a). The constant seawater flux kept an average temperature between 14 and 16 °C. Only during some hours at noon, temperature in the tank increased by 4 °C, which is the range that algae experience during low tide at this latitude in summer. Instantaneous irradiances of UV-B (295–315 nm), UV-A (315–400 nm) and PAR (400–700 nm) incident to the water surface of the tank were determined during the study period using a hyperspectral radiometer RAMSES-ACC2-UV–vis (Trios Optical Sensors, Oldenburg, Germany). Maximal values of UV-B and UV-A during noon were close to 2.1 and 64 W m^{-2}, respectively. PAR values

peaked, 2,250 μmol m^{-2} s^{-1} (Fig. 2). The attenuation of these levels of solar radiation in the tank was negligible.

Laboratory exposures

The stressful effects of UV radiation and temperature were assessed by exposing algae for 72 h without dark regime in a tank system similar as in the outdoor incubation (Fig. 3a). Solar radiation was replaced by a combination of UV (Q-Panel-313 and 340; Q-Panel Co., Cleveland, OH) and PAR (Daylight, Philips) fluorescent lamps whose irradiance levels for the distinct wavelengths were set at 1.9 W m^{-2} for UV-B, 11.1 W m^{-2} for UV-A and 70 μmol m^{-2} s^{-1} for PAR (Fig. 3b). Although the UV-B level is within the range recorded in southern Chile in summer (see Fig. 2), PAR and UV-A levels were significantly lower than the field conditions, due mainly to the difficulty of simulating natural PAR/UV ratios under artificial conditions. However, the setup and levels of UV radiation used in our experiments are suitable to induce effects on various physiological processes of algae (e.g., inhibition of photosynthesis, Fig. 3b). Temperature in the tank was regulated using a heating unit, so that algae were exposed to temperatures of 15 ± 1 °C (water temperature during summer) and 20 ± 1 °C (elevated temperature, 4–5 °C above the upper summer range for the latitude). Four treatments were used: (1) 15 °C and PAR, (2) 15 °C and PAR + UV, (3) 20 °C and PAR and (4) 20 °C and PAR + UV. Measurements of photochemical reactions were made at 0, 2, 4, 6, 12, 24, 48 and 72 h. In each case, 6–10 individual thallus pieces from each species were measured. From this material, six pieces from each species were frozen in liquid nitrogen and stored at −80 °C until further biochemical analyses. After 72 h of exposure, the remaining algae were returned to the original culture conditions (15 °C, 40 μmol m^{-2} s^{-1}) for a 6-h recovery.

Photochemical reactions

Maximum quantum yield (F_v/F_m) of in vivo chlorophyll *a* fluorescence of photosystem II (PSII) of algae adapted to darkness was measured using a PAM-2000 fluorometer (Walz, Germany). The electron transport rate (ETR) was estimated through P–I curves. Frond pieces of 1 cm in diameter were put in a dark chamber and irradiated with increasing intensities of PAR (up to 500 μmol photon m^{-2} s^{-1}) provided by the PAM device (Schreiber et al. 1994). ETR was determined relating effective quantum yield (Φ_{PSII}) and the intensity of the actinic irradiance as follows:

$$ETR = \Phi_{PSII} \times E \times A \times 0.5$$

where E is the incident irradiance of PAR and A the thallus absorptance. The factor 0.5 comes from the assumption that two quanta are required for the transport of one electron in two photosystems. Considering the changes in

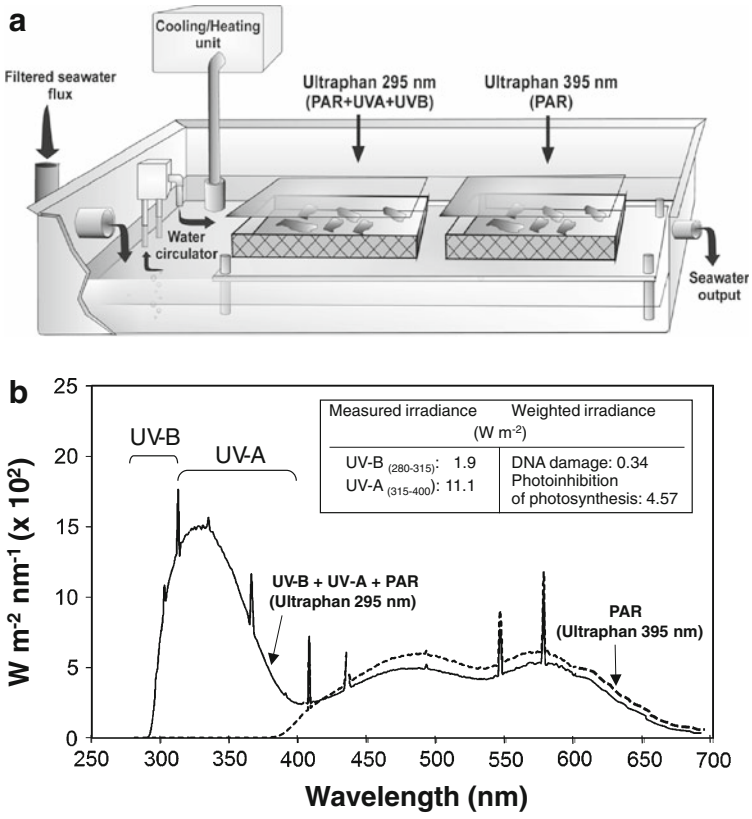

Fig. 3 Schematic diagram of experimental system used for incubation of algae (**a**). In the laboratory incubations under artificial lamps, the water temperature was set using a heating/cooling unit. In outdoor experiments, the constant flux of filtered seawater maintained the temperature 15 ± 3 °C; **b** spectral emission of the combination of UV (Q-panel) and PAR (DL Philips) lamps and weighted irradiances according to DNA damage (Setlow 1974) and photoinhibition of photosynthesis (Jones and Kok 1966) biological weighting functions

absorption characteristics and pigment arrangement of algae from different divisions, it has been suggested that the fraction of chlorophyll associated with PSII can vary (Grzymski et al. 1997). Absorptance was determined by placing the algae on a cosine-corrected PAR sensor (Licor 192 SB, Lincoln, USA), and calculating the light transmission as:

$$A = 1 - E_t E_o^{-1}$$

where E_t is the irradiance below the alga (transmitted light) and E_o the incident irradiance. The ETR parameters were estimated through a modified nonlinear function of Jassby and Platt (1976):

$$\mathrm{ETR} = \mathrm{ETR}_{max} \times \tan h(\alpha \times E/\mathrm{ETR}_{max})$$

where ETR_{max} is the maximal ETR at saturating irradiance, tanh the hyperbolic tangent function, α the initial slope of the P–I curve which is an indicator of the efficiency of the electron transport and E the incident irradiance.

Lipid peroxidation

Oxidative damage in membranes was measured as the content of malondialdehyde (MDA) equivalents according to Salama and Pearce (1993) with modifications of volumes for 96-well microplate (Cruces et al. 2012). Algal samples of approximately 50–70 mg fresh weight were ground in liquid nitrogen and extracted with 1.5 mL of 0.5 % w/v thiobarbituric acid 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. Absorbance was read at 440, 532 and 600 nm. Amounts of MDA were calculated using an extinction coefficient of 157 M^{-1} cm^{-1}.

Antioxidant activity

The radical scavenging activity of fronds was tested using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method of Brand-Williams et al. (1995) as modified by Fukumoto and Mazza (2000) for 96-well microplate. DPPH* (150 µM) was prepared in 80 % methanol and mixed with 22 µl of algal extract. The absorbance was measured at 520 nm using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as standard. The antiradical activity was defined as µmol Trolox equivalent on a dry weight basis.

Phlorotannins

Phlorotannins were determined using the Folin–Ciocalteu method (Koivikko et al. 2005) with modification of volumes for 96-well microplate (Cruces et al. 2012). For the determination of the soluble fraction in the cytosol, 10 mg of frozen algal material was homogenized with liquid nitrogen in a mortar. After adding 1 ml acetone (70 %), the extracts were kept shaking overnight at 4 °C. After centrifugation (4,000 rpm, 10 min), 50 µl of supernatant was mixed with 250 µl of dH_2O, 200 µl of 20 % $NaCO_3$ and 100 µl of 2 N Folin–Ciocalteu reagent. The samples were incubated for 45 min (at room temperature in darkness) and centrifuged (5,000 rpm, 3 min) and the absorbance read at 730 nm in Multiskan Spectrum spectroradiometer (Thermo Fisher Scientific Inc., Waltham, MA).

The content of insoluble phlorotannins (the cell wall-bound fraction) was quantified according to the methodologies described by Strack et al. (1989) and modified by Koivikko et al. (2005). The alkaline treatment was repeated four times, and the aliquots from each treatment were analyzed separately. The precipitated fraction was successively extracted using a series of solvents: methanol, H_2O, methanol, acetone and diethylether. After drying for 1 h at 60 °C, the insoluble residue was dissolved in 800 µl of 1 M aqueous NaOH and stirred for 2.5 h. Samples were centrifuged (3,000 rpm, 5 min), and 100-µl aliquots were neutralized with 10 µl of H_3PO. The absorbance was read at 730 nm.

The content of phlorotannins in the extracts of soluble and cell wall-bound phlorotannins was determined using phloroglucinol (SIGMA) as a standard. Based on calibration curves, the phlorotannin contents were expressed in dry weight units.

Statistical analyses

Data were compared using two-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc analysis of means when differences were detected. Time of exposure and treatment were considered as the main factors. ANOVA assumptions (homogeneity of variances and normal distribution) were examined using the Levene and Shapiro–Wilk W tests, respectively. Proportions and percentage data were arcsine transformed. Correlation analysis was performed to determine the correlation between physiological variables.

Results

The daily course of F_v/F_m under natural solar radiation indicated similar responses in both species. In general, a well-developed dynamic photoinhibition of F_v/F_m was observed, which started when PAR was >1,000 µmol m^{-2} s^{-1} and persisted for several hours until a recovery occurred in the afternoon (Fig. 4). Under full solar radiation (PAR + UV), the pattern of the daily course of F_v/F_m

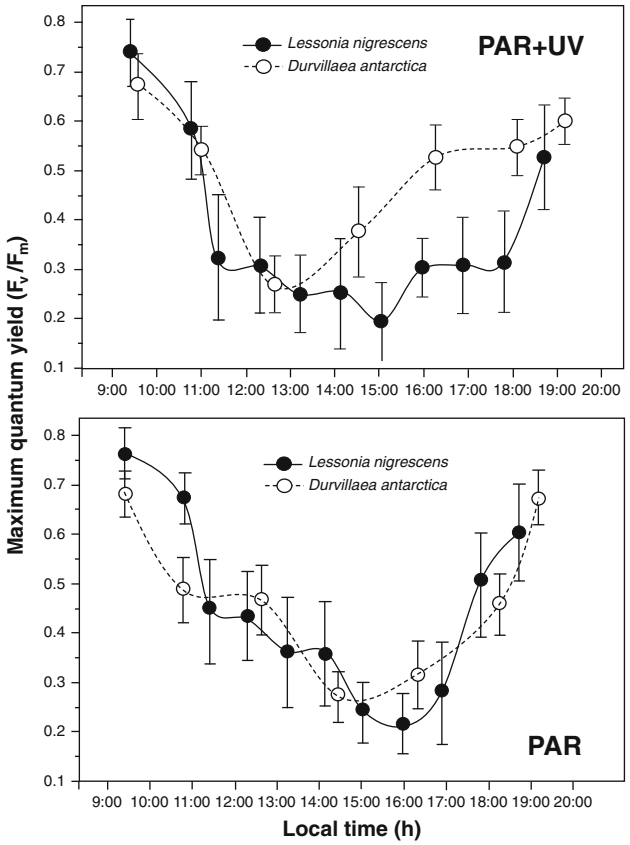

Fig. 4 Daily course of maximal quantum yield (F_v/F_m) of *L. nigrescens* and *D. antarctica* measured at hourly intervals under natural solar radiation in an outdoor tank. Results of exposure to full solar spectra and PAR alone are shown. Irradiances of UV-B, UV-A and PAR at different time intervals for the entire period are indicated in Fig. 2. Values of F_v/F_m are mean ± S.E., $n = 6$–8

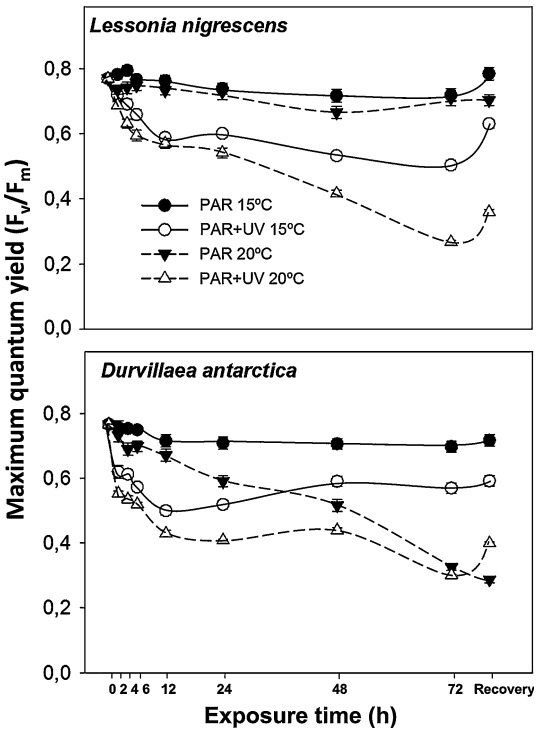

Fig. 5 Effect of UV radiation and temperature on the maximal quantum yield of fluorescence (F_v/F_m) of two kelps at different exposure times during 72 h and recovery for 6 h. Values are mean ± S.E., $n = 10$

was similar to that under PAR alone in *L. nigrescens*, but in *D. antarctica* the recovery started already during the period where irradiances were at their highest. In contrast, the inhibition of F_v/F_m in *L. nigrescens* continued for longer time compared to *D. antarctica*. In both species, final rates of recovery in the afternoon were close to 90 % compared to the morning values in algae exposed to PAR, while in those kept under full solar radiation, recovery was slightly lower (Fig. 4).

During the laboratory exposure, F_v/F_m in *L. nigrescens* remained relatively constant during 72 h under low PAR conditions in both studied temperatures (15 and 20 °C) (Fig. 5). Instead, in *D. antarctica* elevated temperature (20 °C) caused decrease in F_v/F_m with time under low PAR conditions, especially after 48-h exposure. Under UV exposure the studied species exhibited a decrease in F_v/F_m, the impact being more pronounced at elevated temperature and with time ($p < 0.01$; two-way ANOVA; Tukey's test; Table 1). In general, *L. nigrescens* showed a recovery, which was better in treatment including UV radiation. In *D. antarctica*, recovery was observed at 20 °C and UV, but not under PAR alone (Fig. 5).

The initial ETR_{max} values were 36.6 µmol e^- m^{-2} s^{-1} (*L. nigrescens*) and 43.5 µmol e^- m^{-2} s^{-1} (*D. antarctica*) (Fig. 6). ETR_{max} was affected by both UV radiation and temperature in both species, the impact being the most pronounced under simultaneous UV exposure and elevated temperature (Table 1). *D. antarctica* showed the strongest reduction in ETR_{max} close to 89 % (Fig. 6). In general, time of exposure and treatment as well as their interactions affected significantly the ETR_{max} ($p < 0.01$; two-way ANOVA; Tukey's HSD test; Table 1). Recovery between 10 and 15 % was observed in *L. nigrescens* in all treatments, but only in *D. antarctica,* a slight recovery was observed only under 15 °C + UV and 20 °C + PAR (Fig. 6).

UV radiation increased the levels of lipid peroxidation as compared to PAR treatment, which was also associated with the time of incubation (<0.01; Table 1 and Fig. 7). Elevated temperature (20 °C) caused stronger increase in lipid peroxidation than UV exposure at 15 °C. The strongest increase was seen under UV exposure at elevated temperature, with the highest levels measured in *D. antarctica* at 72 h of exposure (~110 nmol MDA g^{-1} FW). Antioxidant activity increased only under UV exposure at 15 °C, showing

Table 1 Summary of two-factorial ANOVA for each species with UV (A) and time (B) treatment as factors

Dependent variable	Factor	*Lessonia nigrescens*				*Durvillaea antarctica*			
		d.f.	MS	*F*	*p* value	*d.f.*	MS	*F*	*p* value
F_v/F_m	*A*	3	0.6	379.0	<0.001	3	0.5	440.1	<0.001
	B	8	0.1	85.9	<0.001	8	0.2	160.3	<0.001
	A × *B*	24	0.03	19.9	<0.001	24	0.05	34.5	<0.001
	Error	180	0.002			180	0.001		
ETR_{max}	*A*	3	516.8	109.7	<0.001	3	2,103.2	316.9	<0.001
	B	8	201.1	42.7	<0.001	8	452.4	68.1	<0.001
	A × *B*	24	25.8	5.5	<0.001	24	103.3	15.5	<0.001
	Error	72	4.7			72	6.6		
Phlorotannin content	*A*	3	64.1	497.4	<0.001	3	675.8	719.6	<0.001
	B	8	13.7	106.7	<0.001	8	94.0	100.1	<0.001
	A × *B*	24	12.4	96.3	<0.001	24	40.5	43.2	<0.001
	Error	167	0.1			169	0.9		
Lipid peroxidation	*A*	3	4,927.2	933.0	<0.001	3	19,547.0	1,009.0	<0.001
	B	8	1,131.3	214.2	<0.001	8	6,191.4	319.6	<0.001
	A × *B*	24	222.1	42.0	<0.001	24	1,614.3	83.32	<0.001
	Error	175	5.2			169	19.3		
Antioxidant activity	*A*	3	26,966.5	931.5	<0.001	3	23,296.6	168.9	<0.001
	B	8	5,136.1	177.4	<0.001	8	4,103.1	29.7	<0.001
	A × *B*	24	3,132.5	108.2	<0.001	24	1,472.1	10.6	<0.001
	Error	170	28.9			171	137.8		

The main effects and their interactions are indicated

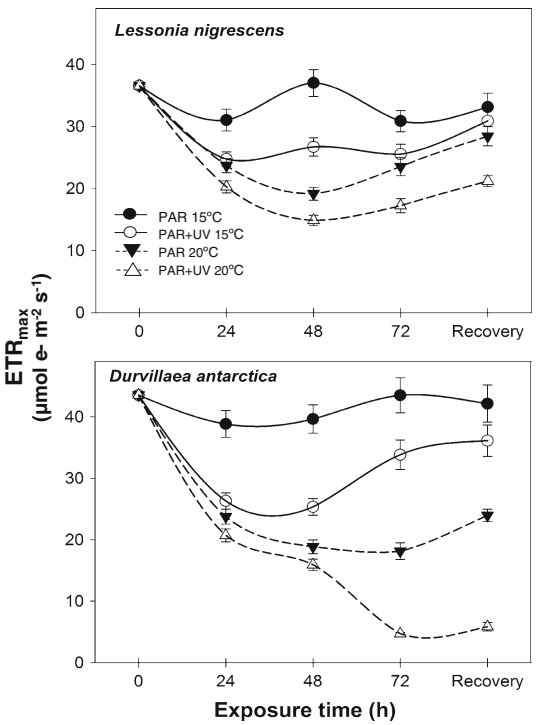

Fig. 6 Effect of UV radiation and temperature on the maximal electron transport rate (ETR_{max}) calculated from P–I curves at different exposure times during 72 h and recovery for 6 h. Data are mean ± S.E.; $n = 3$–4

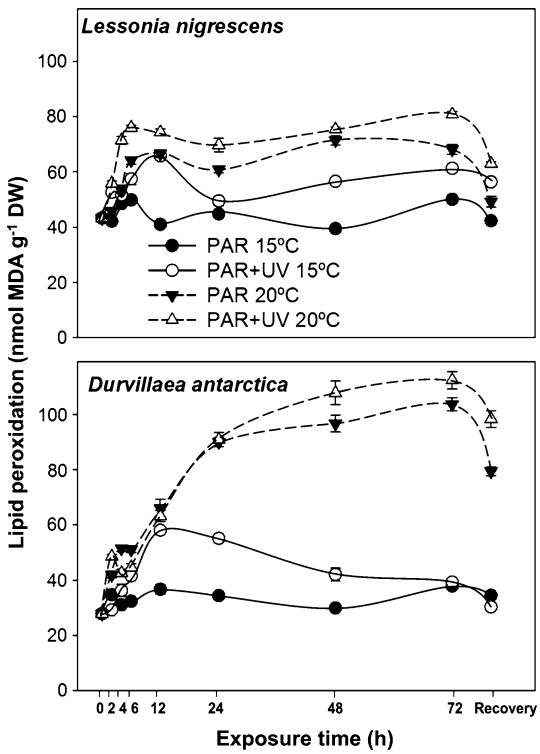

Fig. 7 Effect of UV radiation and temperature on lipid peroxidation measured as formation of malondialdehyde (MDA) of two kelps at different exposure times during 72 h and recovery for 6 h. Data are mean ± S.E.; $n = 6$

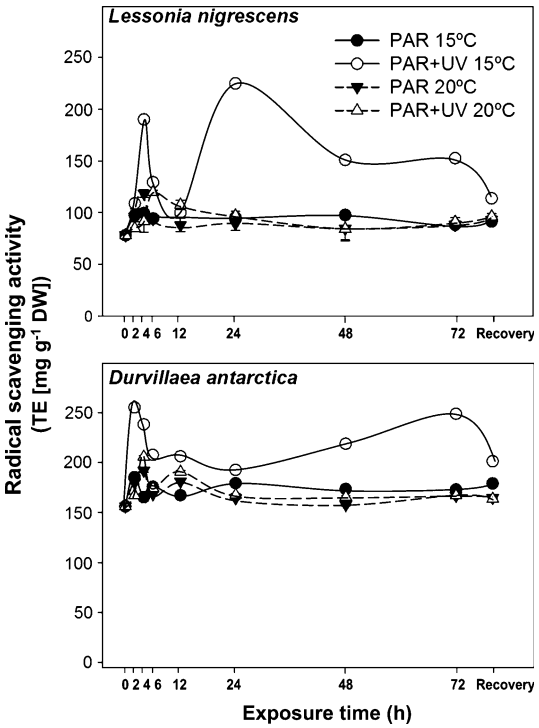

Fig. 8 Effect of UV radiation and temperature on the radical scavenging activity (% of the initial value) of two kelps at different exposure times during 72 h and recovery for 6 h. Data are mean ± S.E.; $n = 6$

maximum first at short term (few hours) and later after 24 in *L. nigrescens* and 72 h in *D. antarctica* ($p < 0.01$; Table 1 and Fig. 8).

The initial concentrations of soluble phlorotannins in the two species ranged between 3.9 mg g^{-1} DW in *L. nigrescens* and 10.8 mg g^{-1} DW in *D. antarctica* (Fig. 9). Similarly, insoluble phlorotannins varied from 2.3 to 4.8 mg g^{-1} DW in *L. nigrescens* and *D. antarctica*, respectively. In general, induction of soluble phlorotannins in *L. nigrescens* was observed under UV exposure and during the first hours, with higher induction at 15 °C as compared to 20 °C. In *D. antarctica,* values fluctuated strongly with the highest contents in soluble phlorotannins reaching maxima of 25 mg g^{-1} DW after 2- and 4-h exposure. After 48 h, values close to 20 mg g^{-1} DW were registered ($p < 0.05$ ANOVA, Tukey's HSD, Table 1). Insoluble phlorotannins were less affected by UV and elevated temperature treatments; however, values measured in *D. antarctica* incubated at 20 °C considerably increased after 48 h ($p < 0.05$; Fig. 9).

Soluble phlorotannins were highly correlated with the antioxidant activity of extracts in the studied species. In contrast, lipid peroxidation was negatively correlated with the F_v/F_m values (Table 2). Although hourly variation in insoluble phlorotannins was not important, some significant and positive correlation was observed with lipid peroxidation.

Discussion

Photosynthetic responses

The outdoor exposures to natural solar radiation revealed a well-developed dynamic photoinhibition in both species, supporting the idea that efficient photochemical adjustment in response to natural solar radiation triggered by high PAR at noon (>2,000 µmol m^{-2} s^{-1}) is an important mechanism to protect the photosynthetic apparatus. In the case of *D. antarctica*, a remarkable capacity to recover even at high full solar radiation at noon was detected. During this time period, temperatures were some degrees higher, which could have induced a more efficient and faster recovery of photochemistry (measured as F_v/F_m) in this species. High temperatures in the range of ecological limits, that is, those at which algae can be exposed in the field (Davison 1991), have been claimed to be less detrimental to photochemistry than low temperatures. In fact, repair and photoprotective processes during short-term light-limited photosynthesis, including turnover of D1 proteins, synthesis of photosynthetic pigments and UV-screening substances, can be stimulated with increasing temperatures (Wünschmann and Brand 1992). In contrast, short-term exposures to low temperatures can exacerbate the sensitivity to high light due to an inhibition of the energy transfer from the light-harvesting complexes to the photosystems (Schofield et al. 1998), thus reducing the capacity for recovery (Gómez et al. 2001). Alternatively, the daily course of F_v/F_m described for *D. antarctica* under full solar radiation could be related to a beneficial effect of UV radiation on recovery of photochemistry, which has been reported for other seaweeds subjected to high irradiances (Flores-Moya et al. 1999; Xu and Gao 2010). In all, the interspecific differences in photochemical reactions probably respond to the history of exposure to high UV radiation and temperature in the field; however, data are not conclusive to support the idea that, for example, buoyancy of fronds govern such patterns.

The results from the laboratory incubations indicated that the exposure to combined UV radiation and elevated temperature for 72 h in the laboratory was stressful and led to a decline in F_v/F_m and ETR parameters in the studied species, most markedly in treatments at 20 °C. Three main findings can be outlined: (a) Photochemical kinetics (F_v/F_m and ETR_{max}) in *D. antarctica* were much more affected by high temperature than in *L. nigrescens*; (b) photosynthesis of *L. nigrescens* was highly responsive to UV treatments under the two temperatures, confirming previous studies in this species (Gómez et al. 2005, 2007); and (c) the adjustments of the photochemistry machinery of algae are activated in the lapse of few hours when solar radiation is maximal, but apparently, these mechanisms remain operational for timescales of hours and days.

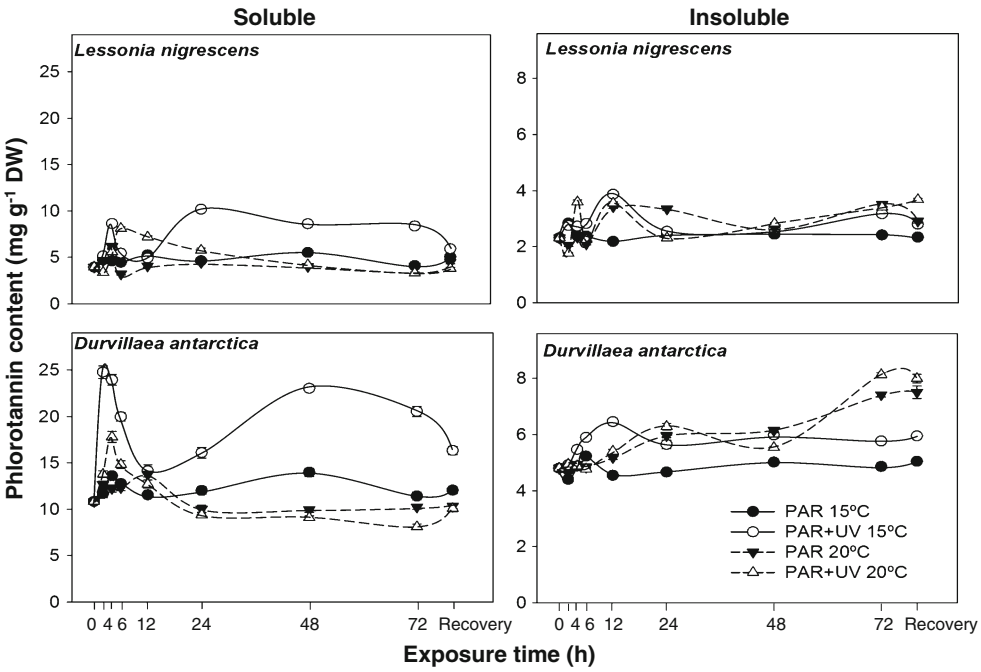

Fig. 9 Effect of UV radiation and temperature on the concentration of soluble and insoluble phlorotannins of two kelps at different exposure times during 72 h and recovery for 6 h. Data are mean ± S.E.; $n = 6$

Table 2 Results from the correlation analysis between physiological variables using Pearson coefficients are indicated

Species	*Lessonia nigrescens*				*Durvillaea antarctica*			
Physiological parameter	Sol Phl	Ins Phl	Lipid peroxidation	Antioxidant activity	Sol Phl	Ins Phl	Lipid peroxidation	Antioxidant activity
F_v/F_m	−0.12	−0.45*	−0.66*	−0.12	0.23	−0.83*	−0.78*	0.13
Sol phlorotannins		−0.04	−0.07	0.88*		−0.25	−0.59*	0.90*
Ins phlorotannins			0.50*	−0.06			0.74*	−0.16
Lipid peroxidation				−0.16				−0.48*

Statistical significance was set to $p < 0.05$ (*)

Similar to a previous study using shorter exposure time (3 h) (Cruces et al. 2012), impairment of photosynthesis in the studied species was exacerbated at 20 °C. However, after 48 h, photochemistry acclimated and after 72 h, a tendency of recovery in the F_v/F_m was evident. The well-developed dynamic photoinhibition under natural solar radiation clearly indicated that both species use this type of mechanism as an efficient primary strategy to cope with light stress in the field. In the laboratory, responses revealed also a remarkable UV tolerance of their photochemical machinery. Thus, it is surprising that these algae, which were submitted to high UV radiation (and eventually elevated temperature) continuously for 72 h, were able to show, at least in the short lapse of 6 h, a capacity for recovery. Similarly, temperature of 20 °C, although affected photosynthesis, did not cause critical decreases in F_v/F_m, reinforcing the idea that an ability of the photosynthetic machinery to withstand extreme changes in solar irradiance is functional to cope with other environmental stressors (e.g., temperature) (Davison 1991).

Peroxidation, antioxidant activity and the role of phlorotannins

MDA formation (an indicator of lipid peroxidation) increased in response to temperature of 20 °C in both species. However, there were temporal differences between the species: In *L. nigrescens,* peroxidation strongly increased during the first 24, but then the values were relatively constant along the exposure time. In contrast, MDA in *D. antarctica* increased substantially at 20 °C (with and without UV) during the entire experimental period. These results were consistent with (a) the marked decreases in photochemical processes occurring in the thylakoids (F_v/F_m and ETR) at 20 °C; and (b) a significant negative correlation between lipid peroxidation and antioxidant activity, supporting the idea that metabolism in this species is sensitive to temperatures above the growth temperature in southern Chile (see below). On the other hand, Cruces et al. (2012) indicated that in the lapse of 3 h, lipid peroxidation in these kelps was low as a result of an

efficient short-term antioxidant activity. The present study confirmed this tendency in the first hours (2–6 h). However, it revealed that peroxidation increased considerably after 12 h, suggesting that prolonged stress can affect membrane processes irreversibly.

In the present study, UV radiation caused less MDA production compared to elevated temperature, which confirms the previous studies carried out in temperate species of *Ulva* (Bischof et al. 2002b, 2006). In this species, which normally is exposed to high solar radiation in the upper littoral, the formation of oxidative stress products not always is triggered by high doses of UV-B radiation, but by mid- or low doses (Shiu and Lee 2005) or PAR (Rautenberger and Bischof 2006; Bischof et al. 2002b). In general, the high positive correlation between increased lipid peroxidation (probably increased ROS) and decreases in fluorescence found in the studied algae could be associated with the damage to cell membranes, reflected in changes in fluidity, and lipid photooxidation, which consequently can cause thylakoid disorganization and eventually decreased photosynthesis (Rijstenbil et al. 2000; Demmig-Adams et al. 2008). For example, in red algae, exposure to UV radiation for 8–72 h causes different ultrastructural damage such as formation of "inside-out" vesicles in the thylakoid membrane or detachment of the chloroplast envelope, which can be reversible in some species (Poppe et al. 2003). Additionally, high energy quanta of the UV band affects other chromophores, for example, aromatic residues and protein bonds, which in turn results in conformational changes of key functional molecules (Pattison et al. 2012). Interestingly, the highest ROS scavenging activity was observed in UV treatments at 15 °C, which extended for various days, confirming that antioxidative responses are effectively activated by UV radiation, especially in *L. nigrescens*.

The maximal contents of phlorotannins in the two studied species varied from 10 mg g^{-1} DW in *L. nigrescens* to 26 mg g^{-1} DW in *D. antarctica* and were determined in the UV treatment at 15 °C. The high contents of phlorotannins measured in *D. antarctica* appear to be constitutive, and in general, they were always higher than in *L. nigrescens*. These levels and their variation along the study period were highly correlated with the antioxidant activity of algal extracts. Based on this evidence, we suggest that ROS scavenging measured in the present study in brown algae is mostly due to phlorotannins than antioxidant activity due to enzymes (e.g., catalases, gluthation reductase and superoxide dismutase) as has been reported previously (Aguilera et al. 2002; Collén and Davison 1999b). High levels of phlorotannins have been correlated with enhanced ROS scavenging activity in intertidal kelps exposed to high UV doses and metals, suggesting that these compounds represent primary metabolic anti-stress agents (Huovinen et al. 2010; Cruces et al. 2012). In the kelp *Laminaria digitata*, high tolerance of sporogenesis and low DNA damage of reproductive cells were related with high concentrations of phenols in the paraphysis (Gruber et al. 2011). In *Saccharina latissima*, this higher allocation of phenolics in soral tissues was correlated with an enhanced antioxidant capacity compared to vegetative regions (Holzinger et al. 2011). At a cellular level, the action mechanism of interaction between phlorotannins and ROS is unclear, but it has been suggested that, at least in vitro, phlorotannins act as an electron donor in stabilization of free radicals (Ahn et al. 2007). On the other hand, phenols may be desulfated and excreted in the form of free molecules in the cytosol (Abdala-Díaz 2001), a process that would allow their action as antioxidant (Connan et al. 2007). Elevated temperature of 20 °C in the present study impaired the synthesis of phlorotannins considerably, which was directly associated with increased lipid peroxidation and low F_v/F_m. These findings could point to an effect of elevated temperature on Golgi–ER membrane complex, the most probable site of biogenesis of phlorotannins (Schoenwaelder and Clayton 2000). Thus, in some cold-adapted species, such as the sub-Antarctic *D. antarctica*, temperatures above the upper limit recorded in summer in southern Chile could impair irreversibly phlorotannin metabolism and hence harm this antioxidative mechanism. Apparently, this will depend on the duration of the thermal stress, as the antioxidant activity mediated by phlorotannins is efficient during short-term (3 h) exposures to temperatures close to higher than 20 °C (Cruces et al. 2012). The question whether ROS scavenging via enzymes can act complementarily when the non-enzymatic mechanism is inhibited remains open, but evidence from *Scytosiphon lomentaria* growing in copper-impacted sites indicates that both systems can act concerted to control the levels of cellular ROS (Contreras et al. 2005). However, it must be emphasized that antioxidant enzymes, in general, are much more thermolabile than other molecules with ROS scavenging potential (Choo et al. 2004).

The insoluble phlorotannins varied less compared to the soluble fraction, which was an important confirmation that insoluble cell wall-bound phlorotannins have a structural function and thus their changes can be associated with cell formation processes rather than rapid changes in response to environmental stress (e.g., UV radiation) (Koivikko et al. 2005). The uniform distribution of insoluble phlorotannins in the cell suggests that they act as a physical barrier for UV wavelengths protecting the whole suite of intracellular components, including nuclear DNA and the photosynthetic machinery (Gómez and Huovinen 2010). Although the putative UV photoprotection of insoluble cell wall-bound phlorotannins may be regarded as a secondary function derived from a peripheral localization causing a physical

interception of UV photons, a functional role of these compounds cannot be ruled out. For example, it is known that the cell wall-bound phenols in terrestrial plants, such as ferulic esters, can act as strong antioxidants (Nara et al. 2006) and in algae, phenols have been involved in wound sealing and healing (Lüder and Clayton 2004; Halm et al. 2011). In the case of *D. antarctica*, the decreased content of soluble phlorotannins in treatments at 20 °C was associated with an relative increase in the concentration of insoluble phlorotannins, which were positively correlated with lipid peroxidation. Thus, it might be reasonable to argue that relative increases in this cell wall-bound fraction of phlorotannins, due to their closeness to the cell membranes, represent any type of damage repair caused by the action of UV radiation and temperature stress in the cortex.

Ecological remarks

No clear evidence for a differential response to stress by UV radiation and elevated temperature was found in the two studied species, suggesting that the type of frond is not a major factor explaining the stress tolerance mechanisms of these species. Although we recognize the limitations of mechanistic laboratory responses as proxies to explain ecological and biogeographical processes, our results can be useful to delineate some ecophysiological characteristics underlining field patterns. In this sense the differences and similarities in sensitivity of photochemical reactions, antioxidant capacity and phlorotannin synthesis observed in these species could be related to the distribution ranges in the Chilean coast as well as to the morpho-functional patterns. For example, short-term responses to withstand, for example, low tide at noon, were similar in both algae, but after prolonged stress, the most eurythermal species, *D. antarctica*, became physiologically limited at 20 °C. This higher sensitivity of *D. antarctica* to elevated temperature could be related to its narrow range of distribution along the Chilean coast (between 29° and 55°S), compared to *L. nigrescens*, which extends its distribution to latitudes close to 16°S (Peters and Breeman 1993; Tellier et al. 2009; Huovinen and Gómez 2012).

The strong intertidal character of both species could explain some of the found similarities. It has been recognized that morpho-functional features that allow *L. nigrescens* and *D. antarctica* to persist in the wave-battered infralittoral zone, for example, large and thicker thalli, appear to be important to withstand or at least minimize the impact of enhanced solar radiation (Gómez et al. 2005; Gómez and Huovinen 2011). However, they also display efficient capacity for ROS scavenging and rapid metabolic adjustments to withstand UV and temperature stress, which can finally be essential to guarantee survival. In all, the amplification of the optimal temperature for photoprotection above the growth temperature could be advantageous for sub-Antarctic species, not only to cope with physical stress in the intertidal zone, but also to expand in their distributional range.

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