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RESEARCH NOTE

Early life stages of the South Pacific kelps *Lessonia nigrescens* and *Lessonia trabeculata* (Laminariales, Phaeophyceae) show recovery capacity following exposure to UV radiation

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This study examined the recovery capacity of early life stages of the South Pacific kelps *Lessonia nigrescens* and *Lessonia trabeculata* after exposure to artificial ultraviolet radiation (UV-$B_{280\text{–}315\ \mathrm{nm}}$, UV-$A_{315\text{–}400\ \mathrm{nm}}$). Spores (3 h postrelease) were exposed to different UV doses applied as daily pulses (0.5, 1, 2, 3, 4 h) during 6 days. Afterwards cultures were kept under PAR condition and fertility of gametophytes and formation of sporophytes measured up to day 45 (for *L. nigrescens*) and 65 (for *L. trabeculata*). The irradiation with UV-B radiation for 6 days strongly impaired spore germination: at biologically effective UV doses ($\mathrm{BED_{Caldwell}} > 2\ \mathrm{kJ\ m^{-2}}$; pulses > 2 h per day), complete mortality of the spores was observed. In contrast, spores irradiated with UV-A retained a germination capacity close to 80 and 70% under high doses (75 $\mathrm{kJ\ m^{-2}}$; 4 h per day). The surviving spores from both treatments exhibited a recovery during their further development as the fertility of gametophytes and formation of sporophytes increased with age of culture. In the intertidal *L. nigrescens*, fertility of gametophytes from spores irradiated with UV-B during 0.5 and 1 h per day was between 70 and 100% after 45 days. Treatments with UV-A did not affect markedly the fertility and production of sporophytes, as values were always between 80 and 100% after 45 days. In the subtidal *L. trabeculata*, recovery of cultures irradiated with UV-B and UV-A were lower, in particular the formation of sporophytes, as compared to *L. nigrescens*. Two major findings are outlined: (1) exposure of spores to UV-B radiation affects the further development of the life cycle, and (2) the surviving spores retained their viability and were able to complete the life history.

KEY WORDS: Germination, Kelp, *Lessonia*, Microscopic stages, Recovery, Spores, UV radiation

INTRODUCTION

Reproductive phases (spores and early developmental stages) of macroalgae are more sensitive to UV radiation than their adult stages. Due to their small size, low pigmentation and a narrow path cross section, these early life cycle stages are strongly impaired by UV wavelengths, which reach easily target molecules such as DNA, with consequences for their viability, motility, germination capacity, nuclear division and translocation (Huovinen *et al.* 2000; Wiencke *et al.* 2000; Altamirano *et al.* 2003a, 2003b; Roleda *et al.* 2005; Véliz *et al.* 2006). For example, most kelps are fertile the year-round but preferentially reproduce during winter and spring, when light conditions do not cause photoinhibition of early stages (Lüning 1980; Wiencke 1990; Tala *et al.* 2004; Roleda *et al.* 2005).

Although the ability for acclimation to high light conditions increases with ontogenic development of the thallus (Dring *et al.* 1996; Hanelt *et al.* 1997; Roleda *et al.* 2004), free-living microscopic stages of macroalgae are exposed to extremely changing light conditions in terms of intensity and dose in the water column. This "planktonic behaviour" is associated with rapid physiological adjustments in order to recover from the light stress and to acclimate (Franklin & Forster 1997). Thus, it is interesting to address questions related to the potential UV acclimation at spore levels, as apparently much of the mechanisms involved in the recovery after solar radiation stress described in macroalgae and phytoplankton (Bischof *et al.* 1998; Falkowski & LaRoche 1991; Falkowski & Raven 1997) might be exhibited by zoospores of kelps (Swanson & Druehl 2003; Roleda *et al.* 2005, 2006). This has ecological implications, as the fate of early stages has consequences for the maintenance of the adult population (Reed 1990; Wiencke *et al.* 2006).

The aim of this study was to examine the recovery capacity of early developmental stages of two dominant South Pacific kelps, *Lessonia nigrescens* Bory (intertidal) and *Lessonia trabeculata* Villouta & Santelices (subtidal). Spores were

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initially irradiated with different UV doses applied as daily pulses during 6 days and the subsequent recovery of gametophytes and young sporophytes under conditions deprived of UV monitored during 45 and 65 days.

MATERIAL AND METHODS

The sampling location, cultivation technique, light source and evaluation of the microscopic development of early stages were described by Véliz *et al.* (2006). In the present study, spores (3 h postrelease) were irradiated with PAR+UV-A+UV-B and PAR+UV-A using a combination of $\text{UV-B}_{(280\text{–}315\ \text{nm})}$, $\text{UV-A}_{(315\text{–}400\ \text{nm})}$ (Q-Panel, Co., Cleveland, OH, USA) and $\text{PAR}_{(400\text{–}700\ \text{nm})}$ (photosynthetic active radiation; Philips, Amsterdam, The Netherlands) lamps and cutoff foils (Ultraphan 295; Digefra GmbH, Munich, Germany) and Folex 320 (Folex GmbH, Dreieich, Germany). During 6 days, pulses of 0.5, 1, 2, 3 and 4 h per day were applied in order to set doses between 4.2 and 34.3 kJ m^{-2} for UV-B and 9.3 and 74.4 kJ m^{-2} for UV-A. These biologically effective doses (0.6–5.0 kJ m^{-2}) (BEDs; generalized plant damage; Caldwell 1971) are in the range that measured in clear skies during the austral summer (Huovinen *et al.* 2006). After exposure to UV radiation, the spores were returned to standard conditions (15 ± 1°C, 12:12 light:dark photoperiod and 60 μmol photons $\text{m}^{-2}\ \text{s}^{-1}$ of PAR) where recovery was evaluated after 7, 15, 25 and 45 days (or 65 days in the case of *L. trabeculata*). Petri dishes with spores exposed only to PAR were used as controls.

A two-way analysis of variance was applied to the data set for each species, with radiation treatment (PAR+UV-A+UV-B, PAR+UV-A and PAR controls) as the first factor and time exposure (0.5, 1, 2, 3 and 4 h) as the second factor. The null hypothesis was rejected to 95% of confidence. The normality (Kolmorov–Smirnov test) and homoscedasticity (Barlett test) of the percentage values was obtained using square root arcsine transformation. A *post hoc* Dunnett test was applied for comparisons with the control (Zar 1999).

GERMINATION CAPACITY OF SPORES

The exposures to PAR+UV-A+UV-B during the first week of culture caused a significant ($P < 0.05$) decrease in the spore germination of *L. nigrescens* (10–30%) and *L. trabeculata* (30–50%), which was observed in a dose-responsive manner (Fig. 1). Complete mortality of spores was observed in PAR+UV-A+UV-B treatment in both species at exposures > 2 h, equivalent to $\text{BED}_{\text{Caldwell-300}} >$ 2 kJ m^{-2}. Although a significant ($P < 0.05$) decrease in the spore germination was also observed under UV-A radiation, nonlethal effects were detected along the whole range of unweighted doses applied to the cultures (50% decreases by doses close 50 kJ m^{-2}) (Fig. 1).

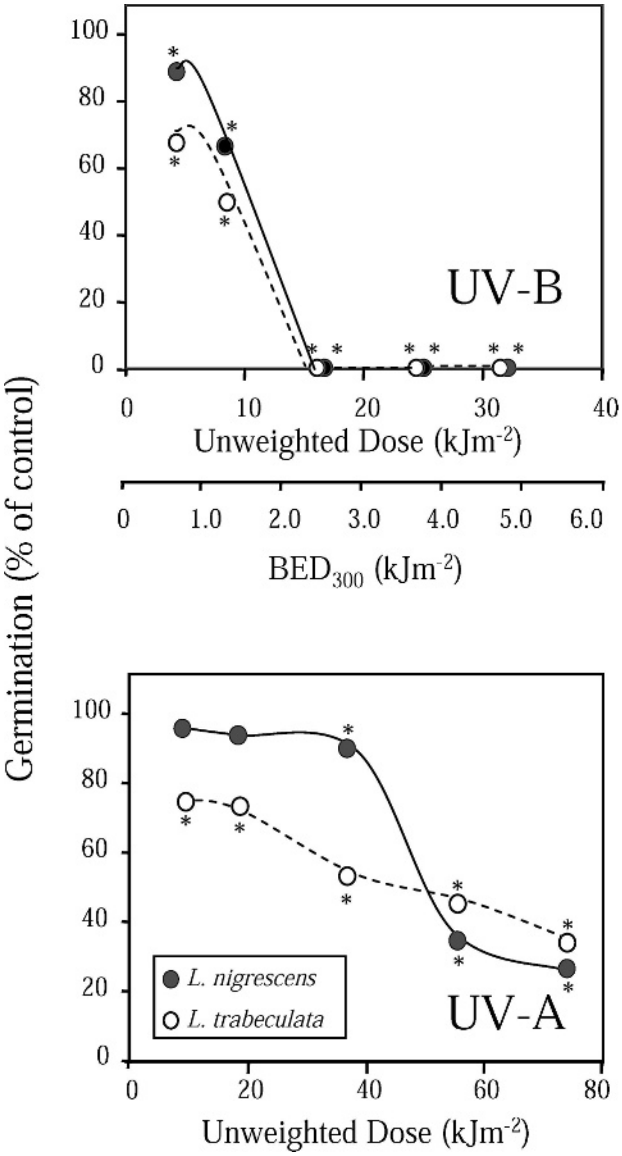

Fig. 1. Germination of spores of *Lessonia nigrescens* and *Lessonia trabeculata* at different doses of UV-B and UV-A radiation applied as daily pulses. Biologically effective dose (BED) of UV-B was calculated using a weighting function for generalized plant damage (Caldwell 1971). Data are means ± s (n = 3). Asterisks denote significant differences from the control (PAR) for each species (from data not normalized to the control; Dunnett test, $P < 0.05$).

Spore mortality by UV-B wavelengths has been related, among others, to DNA damage generated by the formation of photoproducts (e.g. T<>T dimers) (Mitchell & Karentz 1993; Huovinen *et al.* 2000; Wiencke *et al.* 2000; Roleda *et al.* 2005). Moreover, lower viability can be exacerbated by enhanced energy requirements for repair mechanisms, and by photoinhibition of photosynthesis (Wiencke *et al.* 2000; Roleda *et al.* 2005). Our results indicated that germination abruptly decayed when BED increased between 1.2 and 2 kJ m^{-2}, suggesting that spores do not show acclimation over these levels, even with a recovery period of several hours after each UV pulse. In the case of UV-A, only 50–70% of the spores remained alive and with a potential for germination. Although spores of kelps can become particularly sensitive to UV-A radiation (which is associated with oxidative cellular stress and the accumulation of

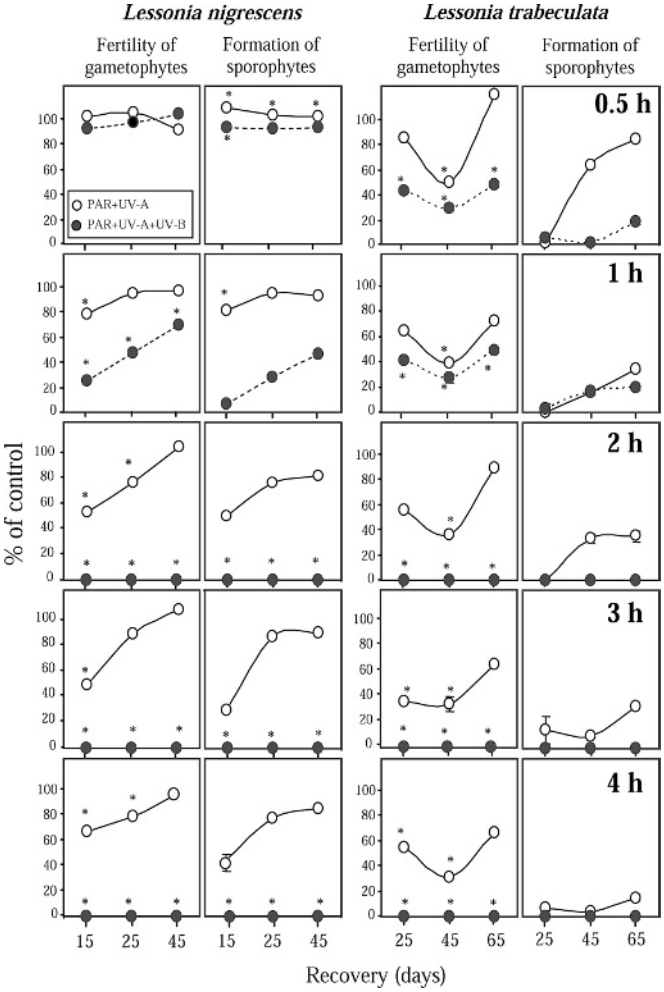

Fig. 2. Effects of different UV doses applied as daily pulses during the first 6 days of development of the spores on fertility of gametophytes and sporophytes production in *Lessonia nigrescens* and *Lessonia trabeculata* measured during recovery under PAR condition. Data are means ± *s* ($n = 3$). Asterisks denote significant differences from the control (PAR) for each species (from data not normalized to the control; Dunnett test, $P < 0.05$).

harmful free radicals), as has been observed in the endemic high Arctic *Laminaria solidungula* J. Agardh (Wiencke *et al.* 2004), the results in *Lessonia* emphasize the impact of UV-B radiation expressed as a lysis of the entire cell. In this sense, daily doses of UV-A equivalent to summer days at sea surface (Huovinen *et al.* 2006) could not be regarded as detrimental for spore survival, as it can also act as a trigger of, for example, photoliase-mediated photoreactivation processes of the DNA molecule (Häder & Sinha 2005).

FURTHER DEVELOPMENT OF GAMETOPHYTES AND SPOROPHYTES

Spores that survived the UV irradiation were able to germinate and thus allowed the further development of the life cycle. The recovery capacity was expressed as increases in fertility of gametophyte and sporophyte production in relation to the duration of the UV exposure and recovery time (Fig. 2). Clearly, the recovery was lower in cultures previously exposed to PAR+UV-A+UV-B as compared to PAR+UV-A (Dunnett test, $P < 0.05$). In general, final recovery in the fertility of gametophytes and formation of sporophytes were higher in *L. nigrescens* than in *L. trabeculata*, particularly after the UV-B treatments. For example, when spores were exposed to UV radiation during 1 h per day, fertility and formation of sporophytes measured at day 45 were close to 70 and 50% of control *L. nigrescens* and close to 55 and 20% for *L. trabeculata*, respectively. With increasing duration of UV pulses (2–4 h per day), recovery of fertility of gametophytes and formation of sporophytes was always lower in *L. trabeculata* than *L. nigrescens* (Fig. 2). These results confirm that depth distribution of parental sporophytes is an important factor explaining the differential sensitivity of brown algal zoospores to UV radiation, which has been reported in several brown algal species (Hanelt *et al.* 1997; Bischof *et al.* 1998; Wiencke *et al.* 2000, 2004; Altamirano *et al.* 2003a; Roleda *et al.* 2004, 2005).

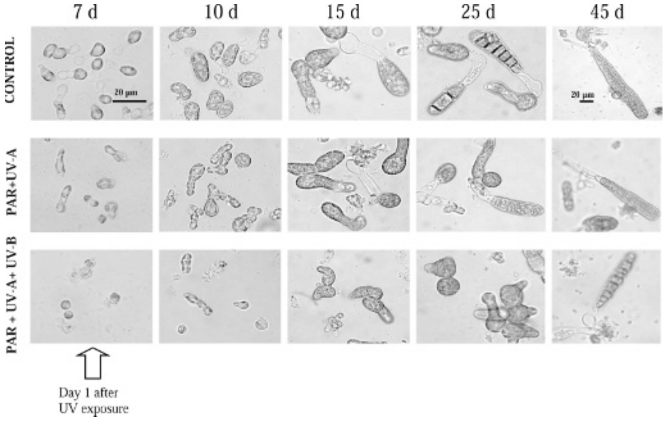

Fig. 3. Development of early stages of *Lessonia nigrescens* after exposure of spores to different UV radiation conditions for 6 days. Photographs represent gametophytes and embryonic sporophytes at different time intervals during a 45-day recovery period. Scale bar = 20 µm.

The lower germination due to the UV treatments did not result in significant effects on the sexual differentiation of survived gametophytes when the cultures were returned to standard conditions: 15 days after the UV exposure, the mean proportion of female gametophytes in the control was 42 ± 0.1% for *L. nigrescens* and 53 ± 0.1% for *L. trabeculata*. On the other hand, the formation of sporophytes was the most UV- sensitive variable in both species. Although effects on the development were observed in both UV treatments, due to the ability for recovery after UV-A exposure, gametophytes and embryonic sporophytes appear similar as controls at day 45, while cultures irradiated with UV-B showed clear signals of damage (Fig. 3).

Overall, our results indicate that the nonlethal UV damage of spores can be "transferred" to the further development stages, including gametophyte fertility and subsequent sporophyte formation. Nevertheless, a recovery capacity was observed through the time of development, allowing the spores that survived to complete their life cycle until sporophyte formation (Fig. 3). Taking into account the recent evidence on recovery capacity of early stages of kelps (Roleda *et al.* 2004, 2005, 2006), the increases in fertility of gametophyte and sporophyte formation in absence of UV stress suggest a possible strategy related to the transition from the pelagic to the benthic life form: benthic habitats might be regarded as less UV stressful as compared to the changing solar environment along the water column. In fact, settled spores are exposed to low light levels (PAR and UV) as compared to motile zoospores (Lüning 1980; Roleda *et al.*, 2006), and, additionally, the parental canopy can attenuate the impact of incident solar radiation. This is more relevant in intertidal habitats. It remains still open whether the nonlethal effects of UV radiation acting early in the life cycle stages (free zoospores) imply altered morphogenic development or decreases in survival chances of recruits in the natural habitat.

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