UV-susceptibility of photosynthesis of adult sporophytes of four brown Antarctic macroalgae (Phaeophyceae)

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Introduction

The Antarctic ozone hole is reported to develop annually in Austral spring since the late 1970s and results in increasing irradiances of solar UV-B radiation on the earth's surface and in the aquatic environment (Farman *et al.* 1985, Häder *et al.* 1998). Exposure of organisms to enhanced UV-B radiation results in multiple damage, e.g. dimerisation of DNA molecules, formation of reactive oxygen species, inhibition of photosynthesis and growth (Roleda 2006, Lesser 2006, Rautenberger & Bischof 2006, Mansilla *et al.* 2006). Due to a lower solar zenith angle over Antarctica, the irradiance of ultraviolet radiation is usually lower in comparison to the tropics. Therefore, Antarctic macroalgae are generally considered to be adapted to these low UV irradiances and may thus exhibit a higher UV-susceptibility than temperate or tropical macroalgae. In this study we aimed to evaluate the susceptibility of photosynthetic efficiency of four abundant field-grown macroalgae from Antarctica, exposed to artificially increased irradiances of UV-radiation.

Material and methods

In January and February 2005, individuals of four brown macroalgal (Phaeophyceae) species, *Adenocystis utricularis* (collected from the eulittoral), *Ascoseira mirabilis* (collected 1.50 m below low tide level), and *Desmarestia menziesii* (collected 1 m below low tide level; see also Klöser *et al.* 1996) were collected at "Peñon Uno" (Potter Cove, King George Island). Individuals of *Desmarestia anceps* (sublittoral) were obtained by SCUBA diving between 5 to 6 meters depth at "Peñon de Pesca". Upon collection, algal material was immediately covered with black plastic bags and transferred to a climate chamber set to a temperature of 2 °C near the Dallmann Laboratory, Jubany Base. Until experimental exposure, algae were maintained at 15 μmol photons m^{-2} s^{-1} PAR. From these cultures the experimental material was distributed into Petri dishes and exposed to 15 μmol photons m^{-2} s^{-1} of PAR (L58W, Osram, Germany). After 12 hours of pre-acclimation in PAR alone, this radiation regime was supplemented by 9.5 W m^{-2} UV-A (UV-A-340, Q-Panel Lab Products, USA) and 0.87 W m^{-2} UV-B (TL20W/12RS, Philips, The Netherlands), measured by an UV/VIS spectroradiometer (Ramses ACC, TriOS GmbH, Germany). The petri dishes were covered by different cut-off filters in order to generate three light/UV

conditions: PAR alone (λ≥400 nm, Ultraphan URUV, Digefra, Germany), PAR+UV-A (λ≥320 nm, Folanorm SF-AS, Folex, Germany) or PAR+UV-A+UV-B (λ≥295 nm, Ultraphan URT, Digefra, Germany). Under these conditions, the specimens were exposed for four hours and were subsequently transferred to dim PAR (15 μmol photons m^{-2} s^{-1}), to observe recovery from UV-exposure after 24 or 28 hours. Photosynthetic activity was measured as optimum quantum yield of photosystem II ($Fv/Fm=(Fm-F_0)/Fm$) according to Schreiber *et al.* (1994) using a portable PAM-2100 chlorophyll fluorometer (Walz, Germany). Variable chlorophyll fluorescence is a well suited and rapid technique to detect UV-induced stress in algae (Clendennen *et al.* 1996, Hanelt *et al.* 1997, Bischof *et al.* 1998a,b). Before measurements were performed, samples were exposed for 5 minutes to darkness. The protocol of Fv/Fm measurements in brown macroalgae followed the procedure described by Hanelt (1998). Measurements of photosynthetic activity were performed after 4 hours of exposure to PAR alone and UV radiation and after 4 and 24 under recovery conditions. For controls (non-photoinhibited cultures), Fv/Fm ratios for *A. mirabilis* (0.691±0.035), *A. utricularis* (0.755±0.006), *D. menziesii* (0.780±0.007) and *D. anceps* (0.753±0.004) were determined and remained constant during the whole experimental time. The means of the respective Fv/Fm values of controls were normalized to 100% photosynthetic efficiency and all the following readings were calculated as a percentage of these. Statistical analyses were performed using JMP IN 5.1 (SAS Institute Inc., USA) after arcsin-transformation. One-way analysis of variance (ANOVA) with repeated measurements and a subsequent post-hoc test according to Tukey's HSD was conducted in order to identify significant differences between treatments. A level of probability of $p \leq 0.05$ was applied.

Results and Discussion

In all specimens of tested brown macroalgae, photosynthetic efficiencies (Fv/Fm) were affected by the incident ultraviolet radiation. In all species, 4 hours of exposure to either UV-A or UV-B radiation led to a decrease of the optimum quantum yield of PS II (Fig. 1). In *A. mirabilis* exposed under PAR+UV-A, Fv/Fm did not decrease significantly from that of its control (PAR alone) and consequently there was no recovery. Thus, photochemistry of the photosynthetic process was apparently not inhibited by UV-A radiation. Furthermore, the reduction of optimum PS II-quantum yield by only 10% after 4 hours of PAR+UV-A+UV-B exposure and subsequent complete recovery within 4 hours after removal of UV-B radiation might suggest a down-regulation of photosynthesis as a possible strategy of protection against ultraviolet radiation like evidenced for PAR exposure (Osmond 1994, Franklin & Forster 1997). Another effective UV-protection in macroalgae might be based on thallus morphology: thicker algae show less sensitivity to UV radiation than filamentous species (Halldal 1964, Franklin & Forster 1997). *A. mirabilis* consists of optically dark-pigmented leathery fronds (Wiencke & Clayton 2002), and, thus incident UV radiation might be reflected, attenuated or absorbed by the thallus itself to elongate the optical path (Caldwell *et al.* 1983). Furthermore, UV absorbing

compounds like pholorotannins may provide cellular protection (Pavia *et al.* 1997), but at present it is not known whether *A. mirabilis* contains UV absorbing components in sufficient quantities.

In the other three species, both PAR + UV-A and PAR+ UV-A + UV-B radiation also caused photoinhibition and, thus, a significantly stronger decrease of Fv/Fm by 6 to 13% and 16 to 21%, respectively. In specimens of *Adenocystis utricularis*, which were collected in the eulittoral, Fv/Fm exhibited a reduction by 9 and 16% after 4 hours of exposure to PAR + UV-A and to PAR + UV-A + UV-B, respectively. Subsequently to these exposures, this species recovered almost completely within 4 hours, as indicated by an increasing Fv/Fm (Fig. 1). Again, such a fast recovery from UV-A and UV-B radiation suggests that photosynthesis in *A. utricularis* might also rather down-regulated (rapidly-reversible) than damaged as suspected in *A. mirabilis*. Hanelt *et al.* (1994) also observed similar kinetics of recovery in field-grown *A. utricularis,* but exposed to natural solar radiation. The authors concluded that PAR-induced photoinhibition was rather a dynamic, regulatory process than photodamage of photosystem II. Thus, this species might be able to acclimate to strong white light (Hanelt *et al.* 1994) and as well as to UV-A and UV-B radiation, as demonstrated in this study.

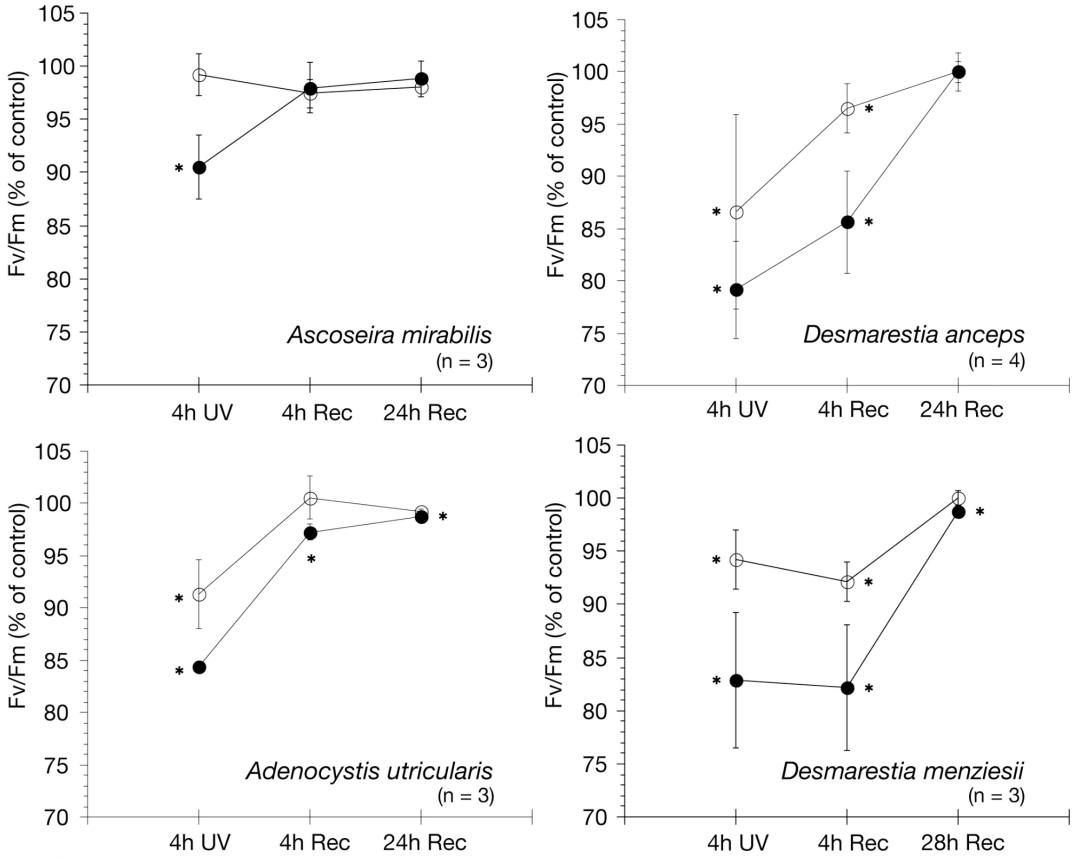

Fig. 1: Impacts of a 4 hours exposure to artificial PAR + UV-A (open circles) and PAR + UV-A + UV-B (closed circles) radiation on optimum quantum yield of PS II (Fv/Fm) of adult sporophytes of four brown macroalgae *Ascoseira mirabilis*, *Adenocystis utricularis*, *Desmarestia anceps* and *Desmarestia menziesii* and their recovery after 4 and 24 hours in dim PAR light. Note that *D. menziesii* was measured after 28 hours of recovery. Error bars represent the coefficients of variation. Asterisks represent significant differences from control values (=100%).

In contrast, *Desmarestia anceps* collected in the sublittoral between 5 and 6 meters depth was a more sensitive species than *A. mirabilis* obtained from 1.5 meters depth with respect to exposure to both, UV-A and UV-B radiation, and recovery from UV-B radiation (Fig. 1). Fv/Fm in *D. anceps* was reduced by 13% and 21% due to PAR + UV-A and PAR + UV-A + UV-B exposure, respectively. Similar results were also obtained after exposure of *Desmarestia menziesii* to PAR + UV-A (6% of control) and PAR + UV-A + UV-B (17% of control) radiation for 4 hours (Fig. 1). The depression of photosynthetic efficiency was not statistically different between both species. Fv/Fm after 4 hours of recovery from PAR + UV-A + UV-B exposure was not statistically different between both *Desmarestia* species and significantly lower than in *A. mirabilis* and *A. utricularis*. A complete recovery was only measured 24 and 28 hours after of PAR + UV-A and PAR + UV-A + UV-B-exposure. Thus, UV-exposure caused a strongly delayed recovery in both *Desmarestia* species. Hence, such a delayed recovery may reflect a slowly reversible photoinhibition indicating that proteins in photosystem II might be damaged (Osmond 1994) due to incident ultraviolet radiation. Although phlorotannins were found in both *Desmarestia* species (Fairhead *et al.* 2005), Fairhead *et al.* (2006) could not provide any evidence for phlorotannins as UV-screens recently.

In various studies on macroalgae, Fv/Fm was a more sensitive parameter for UV-induced stress to photosynthesis rather than the maximum electron transport rate ($ETR_{max.}$) because light capture by the antennae system might be more affected than photosynthetic reaction centers which may still remain active (Bischof *et al.* 1999, Hanelt *et al.* 1997). Furthermore, besides the degree of inhibition of photosynthetic efficiency, the velocity of recovery in dim PAR after removal of UV radiation can be regarded as an even more significant parameter for evaluation of UV-susceptibility (Bischof *et al.* 1999, Hanelt *et al.* 1997). Many studies comparing the UV-susceptibility in macroalgae from different shore levels revealed lower sensitivity of intertidal species and a higher UV-sensitivity with increasing growth depth (reviewed by Franklin & Forster 1997, Bischof *et al.* 1998a,b). This general pattern could not be confirmed by this study on field-grown macroalgae because neither exposure to UV radiation nor recovery from ultraviolet radiation has revealed an UV-susceptibility in relation to depth distribution. Recapitulatory, *A. mirabilis* from 1.5 meters water depth seems to be a less sensitive species with respect to UV-A and UV-B radiation than *A. utricularis* occurring in the eulittoral. Rapidly reversible UV-induced photoinhibition is applied by *A. mirabilis* and *A. utricularis* to protect them from enhanced UV radiation. A similar pattern of reduction of photosynthetic efficiency and a delayed recovery due to UV-exposure was measured in both species of *Desmarestia*: *D. menziesii* obtained from 1 meter below tide level and *D. anceps* collected between 5 and 6 meters in the sublittoral. The similarity of results from these closely related species (Peters *et al*. 1997) might be due to similar morphological characteristics (Wiencke et al.1995, 1996) of lateral branches and probably by a comparable physiological potential of acclimation. Therefore, probably both *Desmarestia* species could occupy the same zone of the phytal and could exist up to the same depths considering UV-susceptibility. Delépine (1966) and DeLaca & Lipps (1976) reported that both species form mixed stands at their southern distributional limit (Melchior Island) whereas *D.*

anceps mainly occurs in the central sublittoral due to lower turbulence resistance and feeding preference by the fish *Notothenia neglecta* for *D. menziesii* growing in the lower sublittoral at King George Island, the center of their geographical distribution (Klöser *et al.* 1996).

Hence, in this study, the decrease of Fv/Fm and the velocity of recovery in dim PAR after UV-exposure were highly species-specific and not ruled by tidal distribution.

In most previous studies on UV-susceptibility of Antarctic macroalgae, laboratory-grown material raised from stock cultures was used (e.g. Bischof *et al.* 1998b), while the present study used field-grown macroalgal material. In contrast to laboratory-grown algal material, which should be used to study mechanisms of adaptation, field-grown material is normally exposed to a broad spectrum of environmental factors and, thus, it is suitable for investigating mechanisms of acclimation.

In summary, these results support the importance of UV-B radiation in structuring seaweed communities from Antarctica.

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