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ORIGINAL PAPER

Effects of increased seawater temperature on UV tolerance of Antarctic marine macroalgae

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Abstract Cold-adapted Antarctic marine macroalgae have different physiological strategies to tolerate the ultraviolet (UV) radiation at low seawater temperatures around 0 °C. The warming of Antarctica's coasts driven by global climate change may alter the physiology such to influence their UV tolerance. This study examined the interactive effects of different seawater temperatures (2 vs. 7 °C) and UV radiation on the physiological performance (primary photochemistry: F_v/F_m, soluble and insoluble phlorotannins, radical scavenging capacity) of seven macroalgae, which are dominant in Antarctic coastal ecosystems. Four brown and three red macroalgae, collected from Fildes Bay (King George Island, South Shetland Islands) in January/February, were exposed to 6 h of UV/temperature stress, followed by a 16-h recovery. The brown macroalgae *Desmarestia menziesii* and *Ascoseira mirabilis* showed the highest UV tolerance at 2 °C, followed by *Desmarestia anceps*, and the rhodophytes *Iridaea cordata*, *Trematocarpus antarcticus*, and *Palmaria decipiens*. *Himantothallus grandifolius* (Phaeophyceae) was sensitive to UV radiation at 2 °C. At 7 °C, UV tolerance was improved in UV-sensitive macroalgae probably due to a more efficient damage repair of the photosynthetic apparatus. Temperature, however, did not modulate UV tolerance in *D. anceps*, indicating an UV-sensitive repair process. Constitutively, high contents of soluble and insoluble phlorotannins and radical scavenging capacities remained unchanged in endemic Desmarestiales. UV induction of soluble phlorotannins along with an increased radical scavenging capacity can be responsible for *A. mirabilis*' high UV tolerance. This study suggests that UV tolerance in macroalgae, which are sensitive to UV radiation at 2 °C, is modulated by temperature. Enhanced UV tolerance at 7 °C can be apparently ascribed to the stimulation of damage repair of the photosynthetic apparatus rather than to an enhanced UV screening or radical scavenging.

Introduction

Antarctic marine macroalgae are strongly adapted to both the low-light conditions and the constantly low seawater temperatures in their subtidal habitats (Gómez et al. 2009; Zacher et al. 2009a). During spring and summer, they are frequently exposed to high solar radiation, including ultraviolet (UV) radiation. Under Antarctic ozone hole events, enhanced UV-B radiation penetrates deeply (≥12 m) into the transparent water column after the break-up of sea ice in October/November, which can harm even macroalgae growing in the lower subtidal (Richter et al. 2008). UV-B radiation alone is not the only stress factor that macroalgae are currently experiencing: significant warming in the Western Antarctic Peninsula region since 1955 was demonstrated for the upper 100 m (Meredith and King 2005). In coastal areas on King George Island (Online Resource 1), seawater temperatures of the upper 30 m (i.e. the habitat of most macroalgae) have increased by 0.32 °C per decade in summer months since 1991 (Schloss et al. 2012). Moreover, summer sea surface temperatures of the Southern

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Ocean were projected to increase by ~1 °C until the end of the 21st century compared to pre-2000 temperatures (Müller et al. 2009). However, because temperature optima of photosynthesis of several Antarctic macroalgae range between 5 and 15 °C (Wiencke et al. 1993), it is possible to argue that they may have the physiological potential to withstand the projected temperature change.

UV radiation can cause adverse effects on biological structures and processes like photosynthesis and gene expression (Bischof et al. 2006; Karsten et al. 2009). However, macroalgae are capable of coping with UV stress by specific metabolic adjustments such as UV shielding and reducing physiological stress. Frequently, UV-exposed macroalgae are generally more UV tolerant than algae from UV-protected habitats due to their high UV acclimation potential. High UV tolerance can be attributed to enhanced antioxidant capacities (e.g. by high antioxidant enzyme activities) to detoxify reactive oxygen species (ROS), fast repair of UV damages, and high UV-screening capabilities (Hoyer et al. 2002; Cruces et al. 2012; Huovinen and Gómez 2013; Rautenberger et al. 2013). Efficient repair of the damaged photosynthetic apparatus by high protein turnover (e.g. D1 protein) is crucial to overcome UV-induced inhibition of photosynthesis (Bouchard et al. 2006). Recent studies suggest that constitutively high levels of phlorotannins account for a remarkable UV tolerance in endemic Antarctic brown macroalgae of the order Desmarestiales at ambient conditions (Huovinen and Gómez 2013). Apart from their UV-absorbing properties, phlorotannins can also participate in ROS scavenging to avoid lethal oxidative damages of membranes, proteins, pigments, and DNA (Gómez and Huovinen 2010; Cruces et al. 2012). Either alone or in combination, these physiological mechanisms protect macroalgae effectively against the harmful effects of UV radiation.

Rising seawater temperatures can affect macroalgal UV tolerance significantly, depending on whether the changes are within the species-specific optimum temperature range of growth and/or photosynthesis (Fredersdorf et al. 2009). Thus, the question rises whether a scenario of enhanced temperature modifies the UV stress tolerance of seaweeds. Evidences from two Antarctic and sub-Antarctic eurytherm *Ulva* species indicate that photosynthetic UV tolerance was higher at 10 than at 0 °C, which is in accordance with their optimum growth temperatures between 5 and 15 °C (Rautenberger and Bischof 2006). Temperature changes that lead the physiology of macroalgae to their thermal limits exacerbates the detrimental UV effects, e.g. by down-regulation of repair and photoprotection and increased ROS production (Gómez et al. 2001; Fredersdorf et al. 2009; Steinhoff et al. 2011; Heinrich et al. 2012; Cruces et al. 2013). In the Arctic/cold-temperate brown macroalga *Saccharina latissima*, which shows a physiological optimum at ~7 °C, UV damage of the photosynthetic D1 protein was less severe at 12 than at 2 °C, demonstrating both the benefits and negative effects of temperature shifts (Heinrich et al. 2015). The UV-induced activation of the mitogen-activated protein kinases (MAPK) pathways, which constitute the central core of a complex signalling network to respond to environmental stress by expression of genes involved in UV protection, was found to be higher activated in the cold-adapted endemic Arctic *Laminaria solidungula* when its surrounding seawater temperature was 5 °C above that of its natural habitat (i.e. 2 °C), the lower subtidal. In the shallow water species *S. latissima*, which is regularly exposed to 5–7 °C in summer (Hanelt et al. 2001; Brey 2009), low temperatures around 2 °C resulted in an increased stress signalling due to higher activities of MAPK pathways (Parages et al. 2013). By such short-term laboratory stress experiments, the key mechanisms of regulation and acclimation underlying long-term trends can be examined to understand the potential ecological impact on larger scales such as on communities and ecosystems. However, multifactorial experiments studying the interaction between these two factors in field-grown Antarctic macroalgae are still very limited, although they are sensitive to changes in their environment (Eggert 2012).

The present study examined the interactive effects of temperature and UV radiation on the physiology of Antarctic brown and red macroalgae in order to gain insights into the actual or potential impact of ocean warming on cold-adapted organisms. We address the question if Antarctic macroalgae still have a high potential of UV tolerance under rising temperatures and, if so, how this is achieved. We hypothesise that a short-term increase in seawater temperature has a beneficial effect on continuously running metabolic processes rather than on inductions to compensate for negative UV effects. Therefore, we presume that a moderately rising temperature of 5 °C has a positive effect on the repair of the UV-damaged photosynthetic apparatus due to the permanently high turnover of the D1 protein, which can be reflected by a rapid recovery of the F_v/F_m. In contrast, induction of UV-absorbing compounds and antioxidant defence strategies, which require longer times, may only play a minor role.

Materials and methods

Adult thalli of four brown [*Desmarestia anceps* Montagne, *Desmarestia menziesii* J.Agardh, *Himantothallus grandifolius* (A.Gepp & E.S.Gepp) Zinova, and *Ascoseira mirabilis* Skottsberg] and three red macroalgae [*Iridaea cordata* (Turner) Bory de Saint-Vincent, *Trematocarpus antarcticus* (Hariot) Fredericq, Moe & Ramirez, and *Palmaria decipiens* (Reinsch) R.W.Ricker] were collected randomly

by SCUBA diving from the mid subtidal (7–10 m) at Fildes Bay on King George Island, South Shetland Islands (62.216°S, 58.960°W) in January and February 2014 (Online Resource 1). The macroalgal material was collected such that the biological replicates in the experiment derived from different individuals of similar size and age of a species. After the fronds were transported in seawater-filled dark boxes to the laboratory at the Antarctic research station 'Base Profesor Julio Escudero', they were carefully cleaned and washed with filtered (0.45 µm) seawater. Apical pieces of *D. anceps* and *D. menziesii* (four to five fronds per individual and species, 0.2 m in length) or algal discs of all other macroalgae (5–20 discs per individual and species, 0.18 m in diameter) were maintained at 18 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR: 400–700 nm) and 2 ± 1 °C. Samples were kept separately in 1-L plastic vessels with filtered seawater (salinity 33.8 ± 0.2, conductivity 52.9 ± 0.2 mS cm^{-1}, pH 8.29 ± 0.04, $n = 3$; Multi 350i, WTW GmbH, Weilheim, Germany), which was continuously aerated and changed daily. Seawater was used for both macroalgal cultures, and the experiment was acquired from 10 m water depth at Fildes Bay, ~1.5 km off the Uruguayan Antarctic Base 'Artigas'.

Experimental design

Samples taken from macroalgal cultures (three apical pieces or discs per individual and species) were exposed in cell culture plates (50 mL per well; TrueLine, USA) to 18 µmol photons m^{-2} s^{-1} PAR at 2 ± 1 °C for 24 h prior to the experiment. For the interactive bi-factorial experiment (i.e. UV radiation × temperature), specimens used as controls were kept under PAR alone at 2 ± 1 °C, whereas others were transferred either to PAR + UV at 2 ± 1 °C, PAR alone at 7 ± 1 °C, or PAR + UV at 7 ± 1 °C for 6 h. For radiation treatments, two cut-off filters were used to cover culture plates: PAR (≥395 nm: Ultraphan URUV; Digefra GmbH, Munich, Germany) and PAR + UV (≥295 nm: Ultraphan URT; Digefra GmbH, Munich, Germany). Experimental irradiances were 18 µmol photons m^{-2} s^{-1} PAR which was additionally supplemented with 1.51 W m^{-2} UV-A (315–400 nm) and 0.26 W m^{-2} UV-B (295–315 nm) in the PAR + UV treatment (Huovinen and Gómez 2013). By keeping irradiance of PAR low, effects of UV radiation on macroalgal physiology will be unmasked by PAR. Right after the experimental treatments, all specimens were shifted back to low PAR alone (18 µmol photons m^{-2} s^{-1}) in a 16 h recovery phase at 2 ± 1 °C. This experimental setup was designed as 'repeated measures' or 'within-subjects design' with three observations (F_v/F_m) on each experimental unit consisting of three apical pieces or discs from one individual of each species. Between-subjects and biological replicates in the experiment derived from different individuals of similar size and age of a species.

Samples for biochemical analysis of phlorotannins and total antioxidant activities were only taken after 6 h of the UV/temperature treatments and frozen in liquid nitrogen. Frozen samples were transported to the laboratory in Valdivia, Chile, and stored at −80 °C until analysis.

Chlorophyll *a* fluorescence

Maximum PSII-quantum yield or F_v/F_m was determined using the Junior-PAM chlorophyll *a* fluorometer (Walz GmbH, Effeltrich, Germany) at 2 ± 1 °C. After macroalgal samples were exposed to far red light for 5 s (30 µmol photons m^{-2} s^{-1}) and subsequently incubated in the dark for 5 min, a saturation pulse (0.6 s; 3,000 µmol photons m^{-2} s^{-1}; 1.0 mm distance between the light guide and sample) was applied. Afterwards, basal (F_0) and maximum (F_m) fluorescence of dark-adapted samples were measured to calculate F_v/F_m, the ratio of variable ($F_v = F_m - F_0$) to maximum fluorescence (Schreiber et al. 1995).

Analysis of phlorotannins in brown macroalgae

Soluble and insoluble fractions of phlorotannins in brown macroalgae were determined after Koivikko et al. (2005), modified by Gómez and Huovinen (2010). Frozen samples (−80 °C) were dried in silica gel and ground to a fine powder under liquid nitrogen using mortar and pestle. The powder (5–15 mg) was mixed with 70 % acetone (1 mL 15 mg^{-1}) and shaken (200 rpm) overnight at 4 °C. After centrifugation (10,000×*g*, 6 min, 4 °C), the supernatant was reduced by 30 % on a shaker (200 rpm) at room temperature (RT). To 100 µL of the extract, 100 µL of ultrapure H_2O (18.2 MΩ cm at 20 °C) and 100 µL of 1 N Folin-Ciocalteu (Winkler Ltda., Santiago, Chile) were added. After 5 min of incubation, 200 µL of 20 % $NaCO_3$ was added. This mixture was incubated at RT in the dark for 60 min and afterwards centrifuged (2,500×g, 3 min, 4 °C). The soluble fraction of phlorotannins dissolved in the supernatant was measured photometrically at 730 nm (Multiskan Spectrum plate reader, Thermo Scientific, Waltham, Massachusetts, USA). The extraction procedure was repeated until all soluble phlorotannins were extracted from the samples. Afterwards, the cell wall-bound, insoluble fraction of phlorotannins were analysed from the same samples. Air-dried pellets of specimens were washed (15 min) several times with 1 mL of different solvents at RT: 100 % methanol (1×), ultrapure H_2O (2×), 100 % methanol (3×), 100 % acetone (2×) and 100 % diethylether (2×). Solvents were carefully removed from the pellets after centrifugation (8,000×*g*, 3 min, RT). Subsequently, pellets were dried at 60 °C for 1 h and incubated in 800 µL of preheated (60 °C)

1 M NaOH for 17 h at 25 °C. Afterwards, samples were vortex mixed for 1 min and centrifuged (8,000×*g*, 5 min, RT). To neutralise 100 µL of the supernatant, 10 µL of 85 % H_3PO_4 was added and vortex mixed. From these samples, total phenol contents were analysed photometrically using H_2O–Folin–Ciocalteu–$NaCO_3$ method as described above. A standard curve of known concentrations of phloroglucinol (Sigma-Aldrich Chemie GmbH, Steinheim, Germany; 5–100 µg mL^{-1}) was used to calculate the content of phlorotannins and normalised to macroalgal dry weight (DW): mg g^{-1} DW.

Total antioxidant activity in brown and red macroalgae

Total cellular antioxidant activity was determined using the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) assay of Fukumoto and Mazza (2000) that was adjusted for macroalgae (Cruces et al. 2012, 2013). Dried macroalgal specimens (silica gel) were ground to a fine powder and extracted in 70 % acetone overnight (17 h) at 4 °C in the dark. One millilitre of 150 µM DPPH (Sigma-Aldrich Chemie GmbH, Steinheim, Germany; prepared freshly in 80 % methanol) was added to 100 µL of either undiluted (red macroalgae) or 1:10–1:80 diluted (brown macroalgae) extracts and incubated in the dark (at RT) until radical scavenging was completed after either five (brown macroalgae) or 6 h (red macroalgae). Optimal incubation time was tested in preliminary test runs. Changes in optical absorption of the DPPH radicals were measured photometrically at 520 nm (Varioskan Flash plate reader, Thermo Scientific, Waltham, Massachusetts, USA). An inhibition curve of known concentrations (0–0.1 mg mL^{-1}) of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) dissolved in 80 % methanol was used to calculate total radical scavenging capacity of macroalgal specimens. Radical scavenging capacity was expressed as Trolox equivalent (TE) and normalised to macroalgal dry weight (mg TE g^{-1} DW).

Statistical analysis

Arithmetic means and standard deviations (SDs) were calculated from four independent replicates per treatment ($n = 4$). Normal distribution of raw data and their residuals were tested by the Shapiro–Wilk *W* test. Two-way analyses of variance with repeated measures (RM-ANOVA) were conducted to identify statistically significant differences of means of F_v/F_m between and within UV/temperature treatments (two levels of each between-subjects factor called 'light' and 'temperature') under consideration of Mauchly's sphericity test (three levels of the within-subjects factor called 'time': initial, treatment, and recovery; $\alpha = 0.05$). A Greenhouse-Geisser correction was applied when sphericity was violated ($\varepsilon < 0.75$). Post hoc contrast analyses were performed to identify the effects of UV radiation and temperature between different 'time' levels. A two-way ANOVA with Tukey's honest significance difference (HSD) post hoc test (heteroscedasticity assumed: Levene test) was performed to compare between means of phlorotannins and total radical scavenging activity in macroalgae after 6 h of UV/temperature treatments. A 5 % significance level ($P = 0.05$) was applied in all statistical tests. All statistical analyses were performed using the software package JMP 11.0 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Photosynthetic stress tolerance

In *D. anceps*, F_v/F_m decreased slightly under exposure to UV radiation by a similar degree between 7 and 10 % at both 2 and 7 °C, respectively ($P < 0.05$) but it was indistinguishable from initial values after the 16 h recovery period in dim PAR (i.e. complete recovery from the previous experimental treatment) (Fig. 1a, Online Resource 2 Table S1). In contrast, no UV or temperature-induced changes in F_v/F_m were measured in *D. menziesii* across all treatments (Fig. 1b, Online Resource 2 Table S2). In *H. grandifolius*, F_v/F_m decreased significantly by 42 % ($P < 0.05$) under the UV treatment at 2 °C but recovered completely in dim PAR afterwards. A negative UV effect was not present either at increased temperature alone (i.e. PAR alone at 7 °C) or in combination with UV radiation (Fig. 1c, Online Resource 2 Table S3). In *A. mirabilis*, there were no statistical differences in F_v/F_m after both 6 h of UV/temperature treatments and 16 h of recovery in dim PAR ($P > 0.05$) (Fig. 1d, Online Resource 2 Table S4).

In all three red macroalgae studied, UV radiation caused a marked decrease in F_v/F_m at ambient seawater temperature (2 °C). At 7 °C; however, this negative UV effect was either less pronounced or absent (Fig. 2). In *I. cordata*, *T. antarcticus,* and *P. decipiens*, F_v/F_m decreased by 15, 30, and 18 % ($P < 0.05$) from their initial values at 2 °C, respectively, but recovered completely after 16 h in dim PAR. In *I. cordata*, the UV-induced decline in F_v/F_m by 5 % at 7 °C was less pronounced at 2 °C (Fig. 2a, Online Resource 2 Table S5). When *T. antarcticus* was incubated at 7 °C, there were no changes in F_v/F_m, neither as an effect of the increased seawater temperature at PAR alone nor in combination with the exposure to UV radiation (Fig. 2b, Online Resource 2 Table S6). In *P. decipiens*, an 18 % decrease in F_v/F_m was measured under the PAR + UV treatment at 2 °C which was still present (7 % decrease in F_v/F_m) but less pronounced at 7 °C (Fig. 2c, Online Resource 2 Table S7). In both cases, F_v/F_m was similar to the initial F_v/F_m values after 16 h of

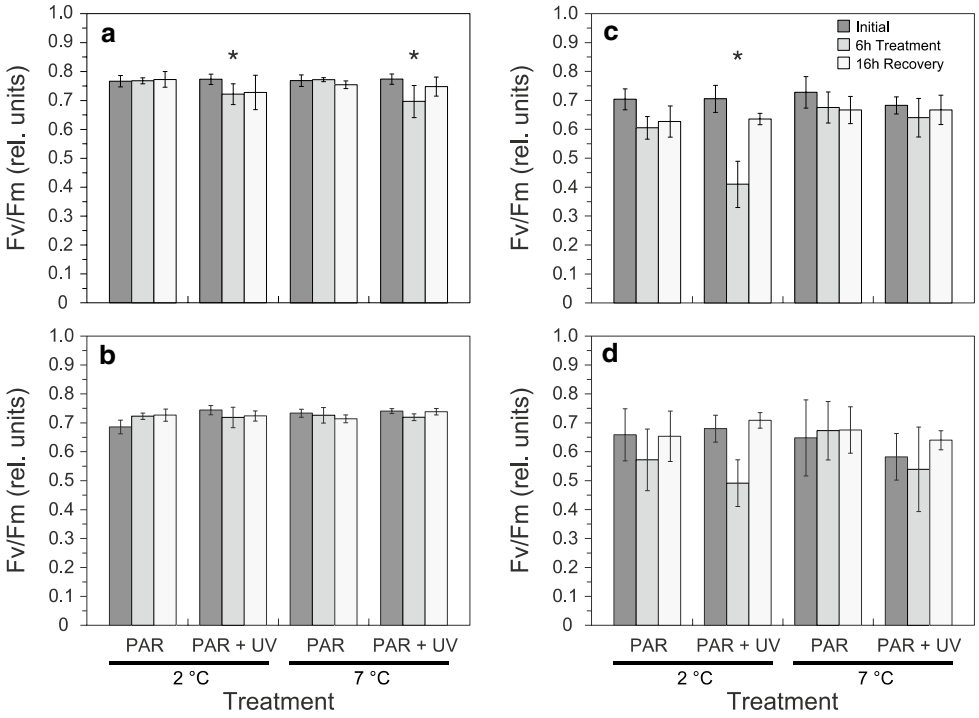

Fig. 1 Changes in F_v/F_m of the brown macroalgae, **a** *D. anceps*, **b** *D. menziesii*, **c** *H. grandifolius*, and **d** *A. mirabilis* before, after 6 h of UV/temperature treatments and 16 h of recovery in dim white light. Data are means ($n = 4$) and *error bars* represent SDs. *Asterisks* denote statistical differences ($P < 0.05$)

recovery in dim PAR ($P > 0.05$), indicating a full recovery. In all three red macroalgal species, there was no effect of the increased seawater temperature (i.e. PAR alone at 7 °C).

Soluble and insoluble phlorotannins in brown macroalgae

Contents of both the soluble and insoluble fractions of phlorotannins in *D. anceps*, *D. menziesii,* and *H. grandifolius* were high, especially in *H. grandifolius* (maximal values close to 100 mg g^{-1} DW) and remained unchanged due to the 6 h exposure to UV radiation at both seawater temperatures (Fig. 4a–c). In these three species of Desmarestiales, the contents of insoluble phlorotannins were lower than those of soluble phlorotannins, ranging between 18 and 40 mg g^{-1} DW, i.e. 25 and 40 % of the total phlorotannin content. In *A. mirabilis,* contents of soluble phlorotannins increased slightly under the PAR + UV treatment at both 2 and 7 °C (2-way ANOVA, $F(1,1) = 6.79$, $P = 0.023$). Contents of soluble phlorotannins were 8.2 and 13.4 % higher than under the PAR alone at 2 and 7 °C, respectively (Fig. 3d). In contrast to Desmarestiales, the content of the insoluble fraction was twice (67 %) as high as the content of the soluble (33 %) fraction but did not change in response to the UV/temperature treatments.

Antioxidant activity in brown and red macroalgae

The total radical scavenging capacity varied considerably between the different macroalgal species studied. It is striking that total radical scavenging capacity of brown macroalgae was between 50 and 190× higher than the average total radical scavenging capacity of the three red macroalgae tested. In brown and red macroalgae except *A. mirabilis*, radical scavenging capacity did not change due to the exposure to UV radiation at both temperatures and under PAR alone at 7 °C (Fig. 5). A significant increase in radical scavenging capacity was measured under the PAR + UV treatment at both seawater temperatures in *A. mirabilis* (2-way ANOVA, $F(1,1) = 12.84$, $P = 0.004$; Fig. 4c).

Discussion

The results of the present study show species-specific effects of increased seawater temperature on UV tolerance of dominant brown and red Antarctic macroalgae. At low temperature, both *D. menziesii* and *A. mirabilis* showed the highest UV tolerance, followed by *D. anceps* along with the three red macroalgae. *H. grandifolius* was less tolerant to UV radiation at ambient conditions due to its strong decline in F_v/F_m at 2 °C. UV-induced damages to PSII disappeared in *H. grandifolius* and the rhodophytes completely at 7 °C, which made them as tolerant to UV stress as *D. menziesii* and *A. mirabilis*.

Temperature effects

The unaffected stress and photophysiology of the cold-adapted Antarctic macroalgae under UV exclusion at 7 °C

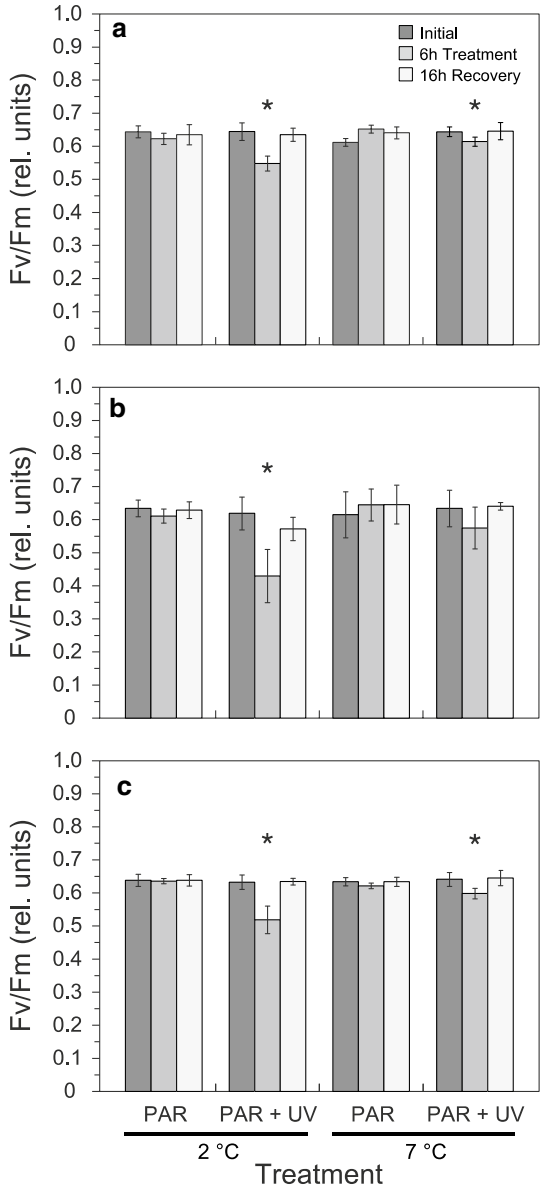

Fig. 2 Changes in F_v/F_m of the red macroalgae, **a** *I. cordata*, **b** *T. antarcticus*, and **c** *P. decipiens* before, after 6 h of UV/temperature treatments and 16 h of recovery in dim white light. Data are means ($n = 4$) and *error bars* represent SDs. *Asterisks* denote statistical differences ($P < 0.05$)

demonstrates that these algae are physiologically capable of tolerating higher temperatures over short periods of time. Temperatures optima for photosynthesis (i.e. 5–10(15) °C), which range well above current Antarctic seawater temperatures (Wiencke et al. 1993), are in accordance with the unchanged photosynthesis, measured as F_v/F_m, at 7 °C in this study. However, increased seawater temperature (3.5 °C) in combination with ocean acidification conditions, which have been projected for Antarctic waters, appears to affect photosynthesis of *D. menziesii* such that higher light intensities are required to saturate the photosynthetic electron transport (Schoenrock et al. 2015). Additionally, it should be emphasised that, although the algae were collected between 7 and 10 m depth, at least three out of seven species tested (i.e. *A. mirabilis*, *I. cordata,* and *P. decipiens*) grow in shallow waters where seawater temperatures can vary. Extreme fluctuations in temperature occur, for example, in intertidal rock pools on King George Island (up to 14 °C in summer). Therefore, a physiological potential to acclimate to higher temperatures can be part of a 'safety mechanism' that allows Antarctic macroalgae not only to withstand short-term thermal stress but also to maintain their physiological performance and primary production across a wide depth distribution range along the shore.

Interactive effects: temperature as a factor that affects macroalgal UV tolerance

Interactions between UV radiation and seawater temperature on F_v/F_m were previously reported for eurytherm polar and cold-temperate macroalgae. The negative UV effects on F_v/F_m, which were present at low temperatures, could be counterbalanced when the temperature rise was within the species-specific thermal tolerance range (Van de Poll et al. 2002; Rautenberger and Bischof 2006; Fredersdorf et al. 2009). By the present study, such interaction was shown for the kelp-like *H. grandifolius* and the red macroalgae *I. cordata*, *T. antarcticus,* and *P. decipiens*. This effect seems to be related to the higher temperature optima of photosynthesis rather than to the low temperature tolerance range of macroalgal growth (Wiencke and tom Dieck 1989; Wiencke et al. 1993). The marked loss in PSII function measured as the decline in F_v/F_m under UV stress at 2 °C can be ascribed to the damaging effects of UV radiation on PSII reaction centres, mainly by the degradation of the weakly linked D1 protein. At polar seawater temperatures, the biosynthetic D1 repair can be slowed down due to temperature-sensitive enzymatic steps of this process, which enhances the rate of photodamage to PSII (Hanelt 1998). At 7 °C, however, the PSII repair cycle was able to compensate for the UV-induced PSII damage probably by higher D1 turnover rates. This result shows that an increased temperature mitigates the damaging effects of UV radiation to PSII in these four macroalgae, which improves their UV tolerance significantly.

In *D. anceps*, the same degree of UV-induced damage to PSII at both seawater temperatures suggests that temperature did not affect the PSII repair cycle, although the metabolic activity presumably increased at 7 °C (Wiencke et al. 1993). A possible explanation is that the PSII repair cycle is particularly sensitive to UV radiation in *D. anceps*. This assumption can be supported by a recent study in which the recovery of F_v/F_m in dim PAR after

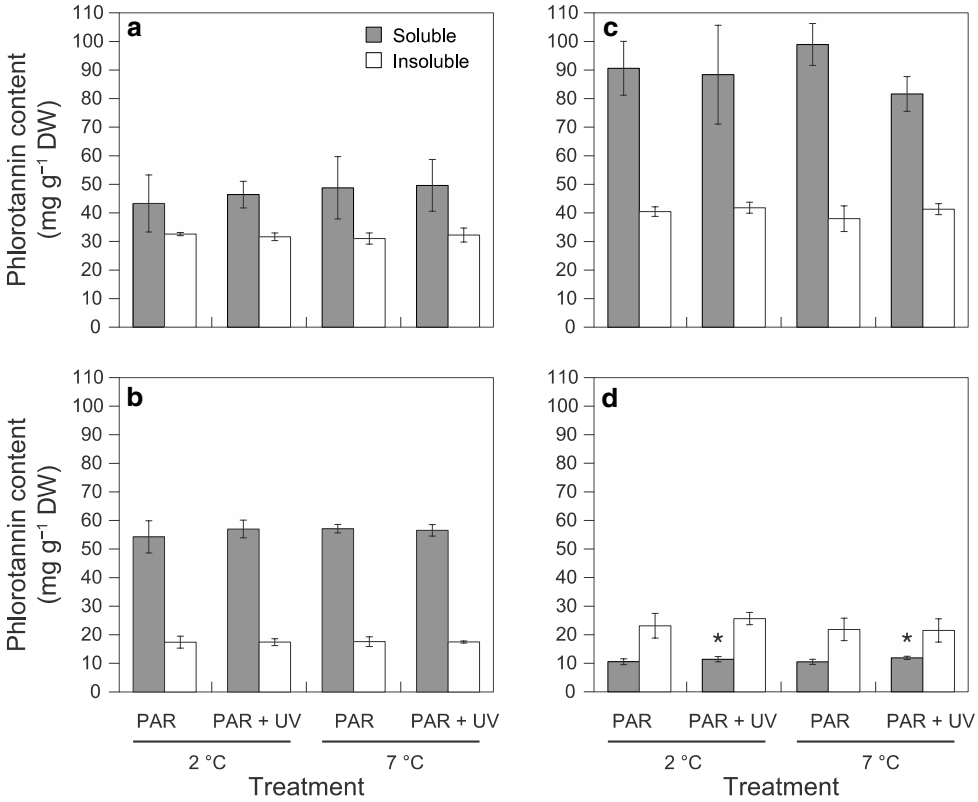

Fig. 3 Contents of both the soluble (*grey columns*) and the insoluble (*white columns*) fractions of phlorotannins in the brown macroalgae, **a** *D. anceps*, **b** *D. menziesii*, **c** *H. grandifolius*, and **d** *A. mirabilis* after 6 h of UV/temperature treatments. Data are means ($n = 4$) and *error bars* represent SDs. *Asterisks* represent statistically significant differences of means between treatments ($P < 0.05$). DW–macroalgal dry weight

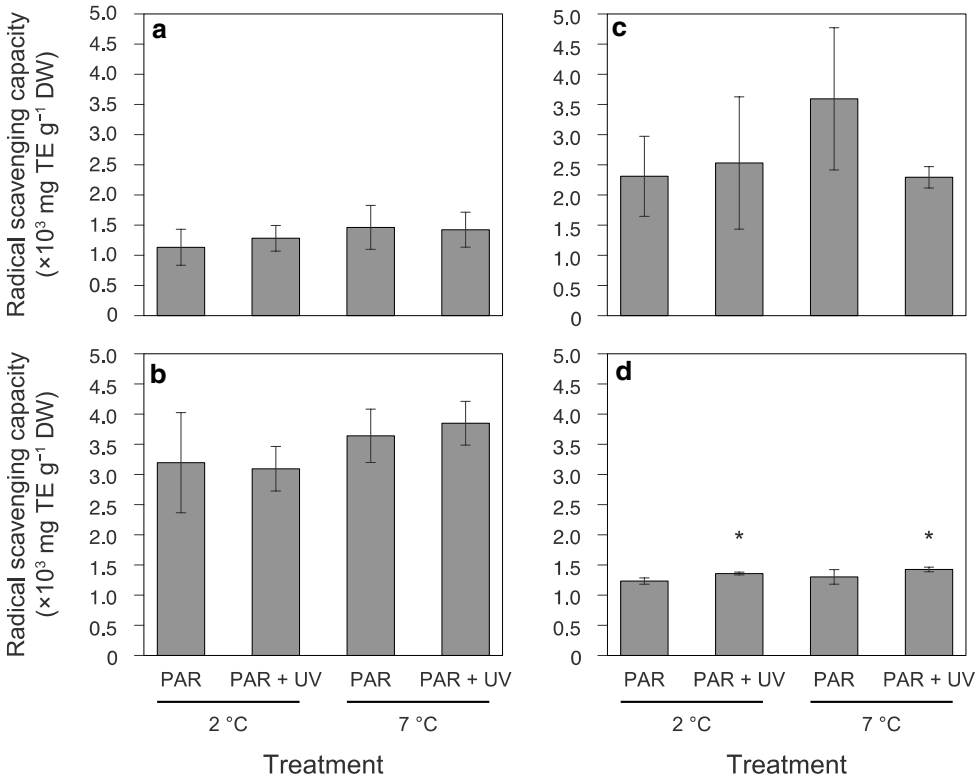

Fig. 4 Radical scavenging capacities of the brown macroalgae, **a** *D. anceps*, **b** *D. menziesii*, **c** *H. grandifolius*, and **d** *A. mirabilis* after 6 h of UV/temperature treatments. Data are means ($n = 4$) and *error bars* represent SDs. *Asterisks* denote statistical differences ($P < 0.05$). TE–Trolox Equivalent, DW–macroalgal dry weight

UV stress was significantly slower in *D. anceps* collected from the lower than in conspecifics from the upper and mid subtidal (Rautenberger et al. 2013). This is supported by previous studies in which UV-B radiation affected the PSII repair cycle in natural Antarctic, temperate, and tropical phytoplankton communities rather than causing

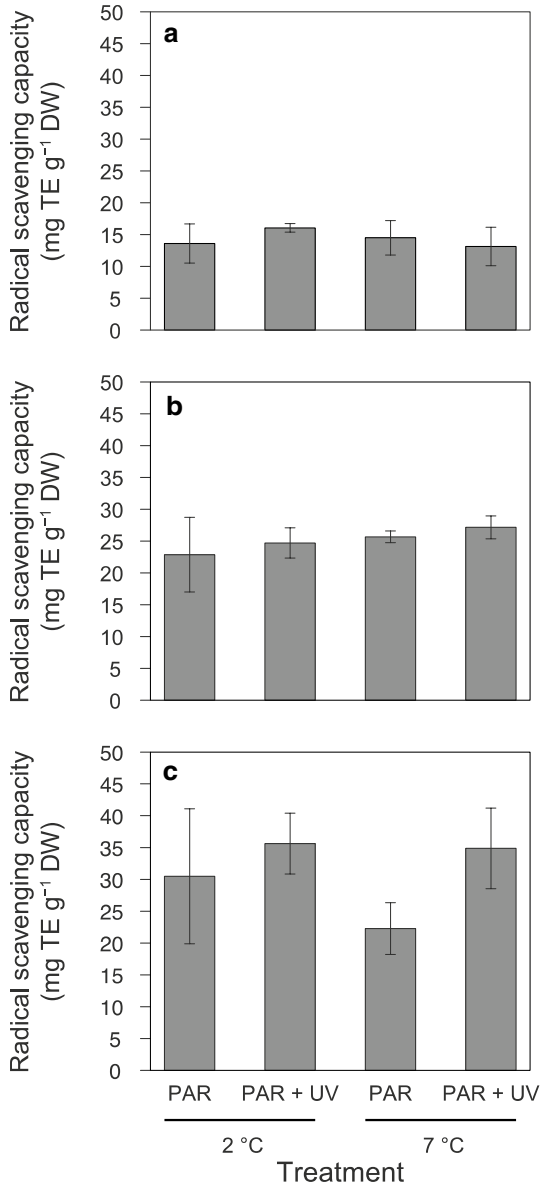

Fig. 5 Radical scavenging capacities of the red macroalgae, **a** *I. cordata*, **b** *T. antarcticus*, and **c** *P. decipiens* after 6 h of UV/temperature treatments. Data are means (n = 3–4) and *error bars* represent SDs. *Asterisks* represent statistically significant differences of means between treatments ($P < 0.05$). TE–Trolox Equivalent, DW–macroalgal dry weight

damage to PSII by D1 degradation (Bouchard et al. 2005a, b). The inhibition of the PSII repair cycle exacerbates the loss in PSII activity since the D1 protein biosynthesis cannot compensate for the inactivation of PSII. Therefore, an active, ongoing *de novo* synthesis of the D1 proteins is crucial to maintain the activity of PSII (Bouchard et al. 2005a, b). Further studies need to be conducted to investigate this specific effect on Antarctic macroalgae such as *D. anceps*.

The role of UV-absorbing compounds

Another important factor that can contribute to macroalgal UV tolerance is the protection of biological structures such as the photosynthetic apparatus by UV-absorbing compounds, which can be specifically induced by UV radiation (Pavia et al. 1997; Swanson and Druehl 2002; Gómez and Huovinen 2010). The present study suggests that the induction of soluble phlorotannins by UV radiation could be related to their basic levels: UV induction was predominantly detected in macroalgae with low rather than high contents as previously assumed by Huovinen and Gómez (2013). In *A. mirabilis* from Antarctica, which shows relative low contents (<10 mg g^{-1} DW), UV radiation induced the biosynthesis of soluble phlorotannins at both temperatures. The effects of these slight changes to the high UV tolerance of *A. mirabilis*, which can be largely influenced by its optically dark-pigmented leathery fronds (Rautenberger and Bischof 2008), still need to be determined by future studies. In the temperate kelp *Lessonia nigrescens*, which has both a similar morphology and comparable contents of soluble phlorotannins (5–10 mg g^{-1} DW) to *A. mirabilis*, rapidly increased contents of phlorotannins triggered by UV radiation sustained the structural and functional integrity of PSII and DNA (Gómez and Huovinen 2010). In contrast, the constitutively high contents of phlorotannins in Antarctic Desmarestiales (40–100 mg g^{-1} DW), which correspond well to those of other studies (Fairhead et al. 2005, 2006; Iken et al. 2007; Huovinen and Gómez 2013; Schoenrock et al. 2015), did not change due to UV exposure. However, UV tolerance did not seem to benefit from such high contents. For example, the 42 % decrease in F_v/F_m in *H. grandifolius* at 2 °C indicates that phlorotannins are not particularly relevant in protection of photosynthesis against UV radiation. This appears to confirm that the high contents of phlorotannins found in *D. anceps*, *D. menziesii*, and *H. grandifolius* have biological functions other than direct UV shielding, e.g. herbivore deterrence or supporting cell growth (Fairhead et al. 2006; Iken et al. 2007; Huovinen and Gómez 2013). In fact, insoluble phlorotannins, which are formed from soluble phlorotannins, primarily have important structural functions in strengthening cell walls and are related to macroalgal growth (Schoenwaelder 2002; Arnold and Targett 2003). Nevertheless, due to their UV-absorbing properties and accumulation in outer cell layers such as the cortex and meristoderm, they can act as a 'general UV shield' as well (Gómez and Huovinen 2010; Cruces et al. 2013). Moreover, it is striking that the content of insoluble phlorotannins in *A. mirabilis* is twice as high as the content of the soluble phlorotannins. This can be explained by the fact that *A. mirabilis* exudes high amounts of phlorotannins into the surrounding seawater, which

can—in addition to other functions—provide a certain level of extracellular UV protection (Bischof et al. 2006).

Phlorotannins can additionally act as ROS scavengers to protect cell structures from oxidative stress. The simultaneous UV-induced increase in the content of soluble phlorotannins and radical scavenging capacity in *A. mirabilis* indicates the antioxidative role of phlorotannins under UV stress and raises the question for their specific function in UV protection. Such simultaneous stimulation between both parameters led to the conclusion that soluble phlorotannins in brown macroalgae (mainly Fucales and Laminariales) with low basic levels act as 'primary metabolic antistress agents' to detoxify ROS produced due to UV stress (Cruces et al. 2012, 2013). In Antarctic Desmarestiales, the exposure to the different combinations of temperature and UV radiation did not result in a significant change in ROS scavenging capacities in the present study. Their constitutively high contents of soluble phlorotannins shown here and elsewhere along with high, unchanged radical scavenging capacities (this study) support the assumption that ROS scavenging by soluble phlorotannins is a general rather than a specific response in these algae (Huovinen and Gómez 2013). In the case of the insoluble, cell wall-bound phlorotannins, their role as active ROS scavenging agents remains unclear by the present study as they show negligible changes in response to the different experimental treatments. Although an antioxidant role of cell wall phenolics has been described in higher plants (Agati et al. 2012), a comparable role in macroalgae is not conclusive as the transition of soluble to insoluble phlorotannins in the cell wall interphase is still unclear. Hitherto, it has been suggested that structural changes of soluble phlorotannins (e.g. by phenolsulphatase) can allow them to react with ROS in the cytoplasm after being released from the physodes. High phenolsulphatase activities in light-exposed thallus parts of *Cystoseira tamariscifolia* (Fucales) were related to an increased ROS scavenging activity of soluble phlorotannins (Abdala-Díaz et al. 2014).

Although MAA concentrations were not determined in this study, they are inducible by UV radiation in the red macroalgae studied (Hoyer et al. 2002; Zacher et al. 2009b). This UV-specific adaptation protects the photosynthetic apparatus and DNA from UV stress at low temperatures, which results in a high UV tolerance (Karentz et al. 1991). Although it has been demonstrated that field-grown red macroalgae can have high MAA concentrations in summer (Hoyer et al. 2001), currently it is unknown whether or not temperature can influence the synthesis of MAAs to improve their UV protection. Based on our results of F_v/F_m, it could be concluded that MAAs did not shield PSII completely from UV radiation at 2 °C. It is possible that UV tolerance of rhodophytes ameliorated at 7 °C, and the PSII repair cycle can be related to a putative role of MAAs in these species. However, further studies are required to gain insights into the responses of MAAs to multiple environmental factors.

Ecological remarks

The high UV tolerance of the Antarctic macroalgae shown in this study can be attributed to their high acclimation potential to the predominant UV radiation regimes in their habitats (Bischof et al. 1998; Rautenberger et al. 2013). The highly UV-tolerant brown macroalgae *D. menziesii* and *A. mirabilis*, which mainly occur in the upper and mid subtidal, are frequently exposed to significant irradiances of UV radiation, e.g. under clear skies in spring and summer (Quartino et al. 2001; Richter et al. 2008; Huovinen and Gómez 2013). For example, in the mouth of Potter Cove (King George Island), 10 % of surface UV-A and 1 % of surface UV-B radiation were measured at 8 and 12 m water depth, respectively, in summer (R. Rautenberger unpublished data). In the lower subtidal (>12 m depth), however, (almost) no UV-B radiation reaches the fronds of the less UV-tolerant *H. grandifolius*. In contrast, the wide distribution of *D. anceps* and the red macroalgae studied across the subtidal can be attributed to their high phenotypic plasticity, which allows them to adjust their metabolism to different UV conditions (Rautenberger et al. 2013).

The ability of *H. grandifolius*, *I. cordata*, *T. antarcticus,* and *P. decipiens* to ameliorate the UV tolerance by a short-term increase in seawater temperature raises the question whether or not these macroalgae can tolerate UV stress under ocean warming. Taking into account that high light and UV radiation, among other factors, control the upper distribution of macroalgae in coastal ecosystems (Hanelt et al. 1997; Bischof et al. 1998), an enhanced UV tolerance at higher seawater temperatures can potentially become a relevant physiological trait that influences the distribution of macroalgae along the shores of Antarctica. For example, a more efficient UV stress management (e.g. by the PSII repair cycle) could allow *H. grandifolius* to leave the lower subtidal to grow closer to the water surface in coastal areas where factors such as sea ice scouring do not interfere.

In summary, the present study suggests that strongly cold-adapted Antarctic macroalgae are generally tolerant to short-term UV stress at both current polar and increased seawater temperatures. This UV tolerance can be ascribed to the activity of the PSII repair cycle to restore the UV-damaged photosynthetic D1 protein. Increased UV shielding and ROS detoxification by soluble phlorotannins is an important mechanism to enhance UV tolerance in brown macroalgae with low contents of phlorotannins. Since an increased seawater temperature was not stressful to Antarctic macroalgae, it was able to modulate their metabolic activity such that the PSII repair cycle was more effective which resulted in a higher UV tolerance.

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