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Stress tolerance of Antarctic macroalgae in the early life stages

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Abstract

Background: Early life stages of macroalgae, especially from polar species, can be highly vulnerable to physical stressors, leading to important consequences for the fate of the whole population in scenarios of changing environmental variability. In the present study, tolerance to UV and temperature stress, as measured by rapid adjustment of photochemistry, F_v/F_m, and photosynthetic characteristics based on P-E curves (ETR_{max}, α and E_k), was assessed in the early life stages of six Antarctic macroalgal species from eulittoral (*Pyropia endiviifolia*, *Iridaea cordata*, *Adenocystis utricularis* and *Monostroma hariotii*) and sublittoral (*Ascoseira mirabilis* and *Gigartina skottsbergii*).

Results: Reproductive cells of eulittoral species showed the highest light demands (E_k >45 μmol photon m^{-2} s^{-1}) when compared to those from sublittoral species (E_k < 30 μmol photon m^{-2} s^{-1}). Short-term experiments of 1 h revealed that reproductive cells of *P. endiviifolia*, *A. utricularis* and *M. hariotii* had the highest temperature tolerance with a decrease of F_v/F_m observed only at 30 °C, while carpospores of *G. skottsbergii* exhibited the highest sensitivity to temperature increase with a decrease of F_v/F_m, which could be observed at 5 °C. UV tolerance was observed in reproductive cells of the eulittoral species with < 20 % inhibition in F_v/F_m from UV after four hours of exposure, while sublittoral species were more sensitive with >30 % inhibition in F_v/F_m in the same condition. Enhanced temperature (7 and 12 °C) improved the tolerance of *I. cordata* compared to 2 °C, but exacerbated the detrimental effects of UV on *A. mirabilis*.

Conclusion: Results showed that photosynthetic characteristics varied among reproductive cells of different species, reflecting the vertical zonation of parental thalli. Otherwise, these differences appear to underlie biogeographical and evolutionary components. In addition, UV tolerance was modulated by temperature increase, while temperature increase, in turn, ameliorated the detrimental effects of stress treatments in some eulittoral species (*I. cordata* tetraspores). In sublittoral *A. mirabilis* gametangia, temperature exacerbated the reduction of photosynthetic efficiency.

Keywords: Antarctica, Reproductive cells, Seaweeds, Temperature, UV tolerance

Background

The Antarctic environment is characterized by low temperatures and extremes in seasonal underwater light conditions. Macroalgae thrive by virtue of their highly efficient metabolic adaptations to photosynthesize and grow at low temperature and almost permanent low light conditions. However, during maturation and development of early phases, e.g., spores, gametes and embryonic thalli, Antarctic macroalgae can be vulnerable, especially to episodic enhanced solar radiation during late winter-spring, following break-up of the ice cover (reviewed by [1]). Although studies have reported on the effects of UV radiation, temperature and their interactive effects on Antarctic macroalgae, they have mostly focused on the adult stages (e.g., [2, 3]), while studies on early life stages are scarce. However, during the last decade, photosynthetic characteristics and UV effects on microscopic developmental stages, e.g., spores, gametes, propagules and plantlets, of some selected Antarctic macroalgae have been published [4–6] (see Table 1). These studies report that early life stages are also extremely shade-adapted and susceptible to environmental stresses, such as exposure to UV radiation [4, 7]. Even though the effect of UV on microscopic life history

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Table 1 Photosynthetic characteristics (E_k: [μmol photon $m^{-2}s^{-1}$], ETR_{max}: [μmol e^- m^{-2} s^{-1}]; α_{ETR} [μmol e^- m^{-2} s^{-1}]. [μmol photon m^{-2} $s^{-1}]^{-1}$), inhibition by UV radiation compared to PAR, and subsequent recovery in the early developmental stages of Antarctic macroalgae

Species/Stage	Depth	Ek	ETR_{max}	α_{ETR}	F_v/F_m	UV inhibition	Recovery	Reference
Ascoseira mirabilis								
Gametangia	**Deep sublittoral (17–30 m)**	**17 ± 5c**	**3.6 ± 0.4**	**0.21 ± 0.03**	**0.48 ± 0.02**	**30–60 % (4 h)[a]**	**73–83 % (4 h)**	**Present study**
Gametangia	Upper sublittoral	52	5	0.09	0.40 ± 0.06	20–25 % (1 h)[b]	100 % (2 d)	[5]
Conceptacles	Shallow sublittoral (1 m)	52 ± 14	10.6 ± 4.2	0.20 ± 0.04	~0.6	0 % (6 h)[d]	100 % (14 h)	[12]
Adenocystis utricularis								
Zoospores	**Eulittoral**	**59 ± 6c**	**27 ± 3**	**0.45 ± 0.01**	**0.64 ± 0.02**	**5 % (4 h)[a]**	**100 % (4 h)**	**Present study**
Zoospores	Eulittoral	64	9	0.14	0.46 ± 0.11	37 % (8 h)[c]	100 % (48 h)	[4]
Cystosphaera jacquinotii								
Receptacles	Deep sublittoral	13 ± 6	3.8 ± 1.7	0.28 ± 0.01	~7.2	15–21 % (2 h)[e]	~90 % (4 h)	[12]
Gigartina skottsbergii								
Carpospores	**Sublittoral (5–8 m)**	**27 ± 9c**	**7 ± 2**	**0.27 ± 0.05**	**0.42 ± 0.32**	**30–60 % (4 h)[a]**	**50–60 % (4 h)**	**Present study**
Carpospores	Sublittoral	54 ± 2	6.87 ± 0.18	0.14	0.40 ± 0.03	1–3 % (UV-B; 8 h)[b] 11–18 % (UV-A; 8 h)[b]	100 % (2 d)	[6]
Tetraspores	Sublittoral	44 ± 21	5.60 ± 0.08	0.14	0.31 ± 0.07	4–7 % (UV-B; 8 h)[b] 11–21 % (UV-A; 8 h)[b]	100 % (2 d)	[6]
Iridaea cordata								
Tetraspores	**Eulittoral**	**46 ± 6bc**	**7.4 ± 0.3**	**0.17 ± 0.03**	**0.38 ± 0.03**	**20 % (4 h)[a]**	**100 % (4 h)**	**Present study**
Tetraspores	Upper sublittoral	57	6.9	0.12	0.47 ± 0.04	~25 % (UV-B; 8 h)[b] ~30 % (UV-A; 8 h)[b]	100 % (2 d)	[35]
Porphyra endiviifolia								
Carpospores	**Eulittoral**	**101 ± 19b**	**14 ± 2**	**0.14 ± 0.01**	**0.35 ± 0.01**	**20 % (4 h)[a]**	**100 % (4 h)**	**Present study**
Monospores	Upper eulittoral	33	4	0.12	0.49 ± 0.04	0 % (8 h)[c]	100 % (48 h)	[4]
Monostroma hariotii								
Gametes	**Eulittoral**	**295 ± 84a**	**25 ± 5**	**0.09 ± 0.01**	**0.26 ± 0.01**	**20 % (4 h)[a]**	**100 % (4 h)**	**Present study**
Gametes	Eulittoral	83	5	0.065	0.29 ± 0.04	0 % (8 h)[c]	100 % (48 h)	[4]
Urospora penicilliformis								
Zoospores, gametes	Upper eulittoral	87	14	0.16	~0.50	~37 % (UV-B; 6 h)[b] ~33 % (UV-A; 6 h)[b]	100 % (24 h)	[37]
Gametophytes	Upper eulittoral	252	44	0.18	~0.51	~26 % (UV-B; 6 h)[b] ~25 % (UV-A; 6 h)[b]	94 % (1 h)	[37]

Results of the present study are marked in bold, and the differences among photosynthetic parameters of the studied species are denoted by different letters (Tukey test; $P < 0.05$). In all studies, photosynthetic activity was determined by measuring the variable chlorophyll fluorescence of PSII. Radiation levels used [a]: UV-B = 0.26, UV-A = 0.15, PAR = 2.79; [b] UV-B = 0.40, UV-A = 4.34, PAR = 4.73; [c] UV-B = 0.35, UV-A = 4.05, PAR = 4.73; [d] UV-B = 0.40, UV-A = 4.34, PAR = 2.79; [e] UV-B = 0.26, UV-A = 1.5, PAR = 2.79

stages has attracted more research attention, assessments of the combined impact of UV radiation and temperature are lacking. The importance of evaluating the impact of these factors on early stages lies in the fact that the life cycle and, in general, the whole fate of the macroalgal population depend on both reproductive output of parental thalli and the environmental tolerance of propagules [5, 7–11]. A recent study demonstrated that reproductive parental sporophytes show different UV sensitivity compared to vegetative individuals and, moreover, that algae invest considerable energy in synthesis and allocation of photoprotective substances [12]. In general, these are adaptive responses that can have important consequences for survival and establishment processes in scenarios of enhanced environmental variability driven by global climate change.

Temperature has become an environmental factor that could modify physiological responses of algae. In fact, temperature, both directly and indirectly, impacts seaweed biology from subcellular to community-level processes. In general, few studies have focused on thermal tolerance of photosynthesis in early phases of Antarctic macroalgae. However, some comparative studies at 0 °C indicate that microscopic gametophytes and embryonic sporophytes of the endemic brown alga *Desmarestia menziesii* show higher photosynthesis than adult sporophytes [13]. Overall, upper temperatures for development of early phases appear to be lower than those for macro thalli, showing a remarkable capacity to tolerate temperatures well above those measured *in situ* [1]. For example, some spores and gametophytes of Antarctic Desmarestiales grow in conditions reaching 15 °C with upper survival temperatures as high as 18 °C [14]. In adult thalli, enhanced temperature close to their thermal limit can exacerbate the detrimental UV effects of different physiological parameters [15–19]. However, it was demonstrated recently that increased temperature could also mitigate the damaging effects of UV radiation to photosystem II (PSII) in four Antarctic macroalgal species, thus improving their tolerance to UV [3]. Also, in the Artic kelp *Saccharina latissima*, which shows a physiological optimum at around 7 °C, UV damage of the photosynthetic D1 protein was less severe at 12 °C than at 2 °C, emphasizing the importance of temperature shifts on the photobiological responses of algae [20]. The question then arises whether enhanced temperature influences the photosynthetic responses of early phases, including spores and gametes, of Antarctic seaweeds and, if so, to what extent these developmental phases exhibit physiological mechanisms able to cope with both acute and chronic thermal stress.

To address these questions, the present study examines P-E-based photosynthetic characteristics and short-term responses to different treatments of UV radiation and elevated temperature in reproductive cells, i.e., spores and gametes, of six species of macroalgae from the eulittoral and sublittoral of Fildes Peninsula (King George Island, Antarctica). We hypothesize that increased temperature will mitigate the detrimental effects of UV on photosynthesis in a manner similar to that reported in adult thalli. Since most Antarctic seaweeds show broad vertical distribution in a range between 5 and 30 m, it could be expected that a) spores from populations of a species living at the upper limit of distribution will be more tolerant to temperature increase and UV radiation than their deeper counterparts, which should also show more shade-adapted characteristics, and b) enhanced temperature will improve the UV tolerance of early stages, especially in those algae with upper distribution.

Methods

Algal collection and processing

The brown algae *Ascoseira mirabilis* Skottsberg and *Adenocystis utricularis* (Bory) Skottsberg; the red algae *Iridaea cordata* (Turner) Bory, *Gigartina skottsbergii* Setchell & Gardner and *Pyropia endiviifolia* (A & E. Gepp H.G. Choi & M.S. Hwang) (formerly *Porphyra endiviifolium*) (Rhodophyta); and the green alga *Monostroma hariotii* Gain (Chlorophyta) were collected from Fildes Bay (King George Island, Antarctic) during January and February 2015. During this period, records from Onset HOBO Datalogger (Onset Computer Corporation, Bourne, MA) indicated that mean seawater temperature in Fildes Bay was 1.0 ± 0.1 ° C in the subtidal zone (15 m), while surface water temperature averaged 2 °C. During low tide, intertidal species were exposed to temperatures close to 10 °C, especially during calm and sunny conditions. The zonation of Antarctic macroalgae and the most relevant photobiological scenarios at this locality have been previously described by [2]. After sampling, algae were transferred to the laboratory at the Chilean Antarctic Base Station Profesor Julio Escudero where they were cleaned of epiphytes and induced to release their spores and/or gametes.

Release of spores and gametes

Propagule release was induced by dehydration of reproductive fronds under a temperature of 2 ± 0.5 ° C. Spores and/or gametes were counted using a Neubauer counting chamber under a stereomicroscope (Motic Inc., Ltd.) before transfer to cell culture plate incubators (TrueLine, USA) with filtered (0.2 μm) seawater. The spores were photographed under a light microscope (Motic BA310; Motic Inc., Ltd) in order to obtain cell diameters (Fig. 1). The density of cells in the suspension of each species was adjusted with filtered seawater to obtain 130 mL of stock suspension to obtain the desired background fluorescence for photosynthetic measurements. Thus, the reproductive cell density used in the experiments were as follows: 4.7 x

10^5 cells mL^{-1} of *Adenocystis utricularis* zoospores (mean diameter 6 ± 1 μm), 6 x 10^4 cells mL^{-1} of *Iridaea cordata* tetraspores (mean diameter 18 ± 2 μm), 2.25 x 10^4 cells mL^{-1} of *Gigartina skottsbergii* carpospores (mean diameter 25 ± 2 μm), 1.09 x 10^5 cells mL^{-1} of *Pyropia endiviifolia* carpospores (mean diameter 12 ± 1 μm), 1.36 x 10^6 cells mL^{-1} of *Monostroma hariotii* gametes (length around 5 ± 0.5 μm), and 1.25 x 10^5 cells mL^{-1} of *Ascoseira mirabilis* gametangia (mean diameter 22 ± 4 μm).

Photosynthetic characteristics

Immediately after spore release, photosynthesis was measured through relative electron transport rate (rETR)-based P-E curves by exposing the cells to increasing (every 20 s) actinic light intensities (8 points between 0–446 μmol photon m^{-2} s^{-1}) with a Water-PAM fluorometer (Walz, Effeltrich, Germany). rETR values were estimated by multiplying the effective quantum yield of photosystem II (Φ_{PSII}) with the corresponding intensity of the actinic irradiance [21], as

$$\mathbf{rETR} = \Phi_{\mathbf{PSII}} * \mathbf{E} * \mathbf{0.5},$$

where E is the incident irradiance of actinic light. The factor 0.5 was derived assuming that 4 of the 8 electrons required to assimilate one CO_2 molecule are supplied by PSII.

ETR parameters were obtained by fitting the ETR-irradiance curve according to the modified nonlinear function of [22], as

$$\mathbf{ETR} = \mathbf{rETR_{max}} * \mathbf{tanh}\left(\boldsymbol{\alpha} * \mathbf{E}/\mathbf{rETR_{max}}\right),$$

where $rETR_{max}$ is the maximal rETR, tanh is the hyperbolic tangent function, α is the efficiency of electron transport, i.e., initial slope of rETR versus irradiance curve, and E is the incident actinic irradiance. Finally, the saturating irradiance (E_k) was calculated as the intersection between α and $rETR_{max}$.

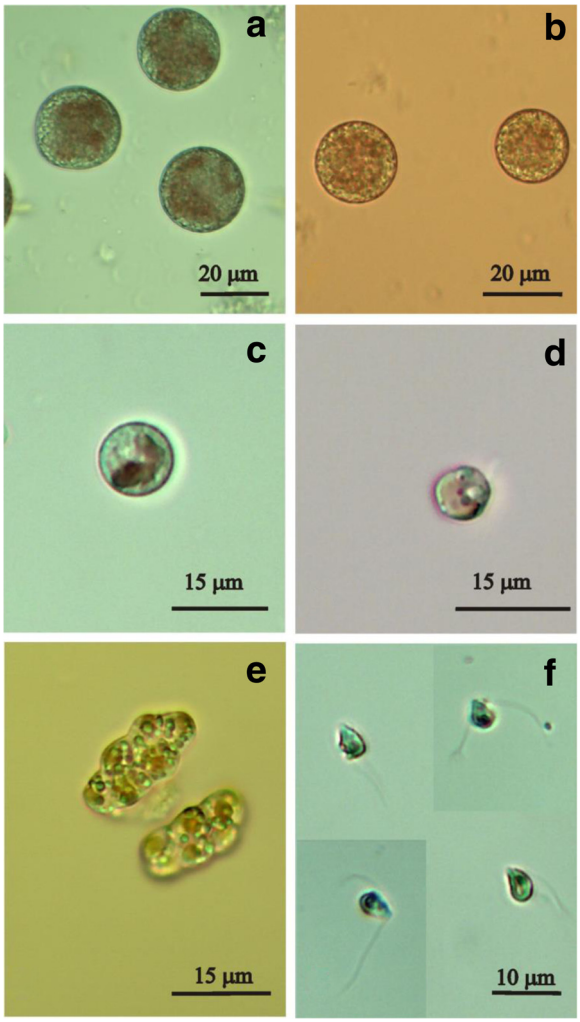

Fig 1 Reproductive cells of the six studied macroalgal species from Fildes Peninsula (King George Island, Antarctica). **a**: *Gigartina skottsbergii* carpospores; **b**: *Iridaea cordata* tetraspores; **c**: *Pyropia endiviifolia* carpospores; **d**: *Adenocystis utricularis* zoospores; **e**: *Ascoseira mirabilis* gametangia; **f**: *Monostroma hariotii* gametes

Exposure to increasing temperatures

Aliquots (4 mL) from the suspension of reproductive cells were put inside cell culture plates ($n = 5$) and immediately submitted to a temperature gradient (0, 5, 10, 15, 20, 25 and 30 °C) using a heating unit (Digit-Cool, J.P. Selecta, Spain). After 1 h at 0 °C, the effect of temperature on photosynthesis was assessed as rapid adjustment of photochemistry through changes in maximal quantum yield (F_v/F_m) in algal samples previously kept in the darkness for 5 min at 0 °C. F_v/F_m is regarded as a reliable estimator of photosynthetic response to stress. After measuring, the samples were placed back into the heating system, and the temperature was increased at intervals of 5 °C. Once the temperature rose to 5 °C, the samples remained at that temperature for one hour, and F_v/F_m was measured again. This procedure was carried out until reaching 30 °C. During the experiments, samples were illuminated with 13 μmol m^{-2} s^{-1}. Control measurements of F_v/F_m were obtained at time zero.

Exposure to UV radiation under different temperatures

Cell culture plates containing spores or gametes were exposed to PAR + UV and PAR treatments for 4 h at temperatures of 2, 7 and 12 °C, followed by a 4-h recovery period under dim light (<5 μmol photon m^{-2} s^{-1}). The three temperature treatments were obtained using heating units. For illumination, a combination of UV (Q-Panel-313 and 340 nm fluorescent tubes; Q-Panel Co., Cleveland, OH) and PAR lamps (Daylight, Philips; Amsterdam, the Netherlands) was used. The cell culture plates were

covered with different cut-off filters in order to establish the two irradiation treatments: PAR only achieved by Ultraphan 395 nm (Digefra; Munich, Germany) and PAR + UV using Ultraphan 295 nm. The levels of irradiation were measured with a RAMSES-ACC2-UV–VIS hyperspectral radiometer (Trios Optical Sensors; Oldenburg, Germany), and the spectra were weighted using the action spectrum for DNA damage [23] and photoinhibition of photosynthesis [24]. The experimental levels of UV-B radiation (0.26 W m^{-2}) matched values measured *in situ* at Fildes Bay during the summer season [2]. However, UV-A levels (1.5 W m^{-2}) and UV-A:UV-B ratios were lower than those occurring under natural conditions. The weighted values were 0.08 (DNA damage) and 0.19 Wm^{-2} (photoinhibition of photosynthesis). The irradiance of PAR (13 μmol m^{-2} s^{-1}) remained low to avoid masking UV effects [2].

Four mL of each cell suspension were used per replicate ($n = 4$) for each temperature and light treatment. Initial measurements of F_v/F_m were obtained at time zero. The effect of UV radiation under different temperatures was assessed as inhibition of F_v/F_m. Values were determined as a percentage of decrease between samples treated with PAR + UV and samples exposed to PAR. Similarly, the recovery was estimated by comparing the F_v/F_m values of samples exposed to UV treatment with those from PAR treatment.

Statistical analysis

Photosynthetic light requirement (E_k) values of each species were compared using one-way ANOVA. The effect of temperature on F_v/F_m for each species was compared using repeated measures ANOVA (RMANOVA). In the case of the effect of UV on F_v/F_m inhibition under different temperatures, two-way ANOVA was performed. Post-hoc comparisons of means were assessed with Tukey HSD. All analyses were done using the Statistica 7 software (StatSoft, Inc., USA).

Results

Photosynthetic characteristics

Photosynthetic performance of reproductive cells is shown in Fig. 2, while photosynthetic characteristics (ETR_{max}, α and E_k) are shown in Table 1. The reproductive cells of the different species showed marked differences in saturating irradiance of photosynthesis (ANOVA: F = 26.75; $p < 0.00001$) (Table 1, Fig. 2). Reproductive cells of the eulittoral species *A. utricularis*, *M. hariotii* and *P. endiviifoli*a showed the highest light demands ($E_k > 60$ μmol photon m^{-2} s^{-1}) and $rETR_{max}$ (>14 μmol e^- m^{-2} s^{-1}), while the sublittoral species *G. skottsbergii* and *A. mirabilis* showed the lowest light demands ($E_k < 27$ μmol photon m^{-2} s^{-1}) and ETR_{max} (<7 μmol e^- m^{-2} s^{-1}). Tetraspores of the eulittoral red alga *I. cordata* exhibited E_k values significantly different when compared to the other eulittoral species and when compared to sublittoral species (Table 1, Fig. 2).

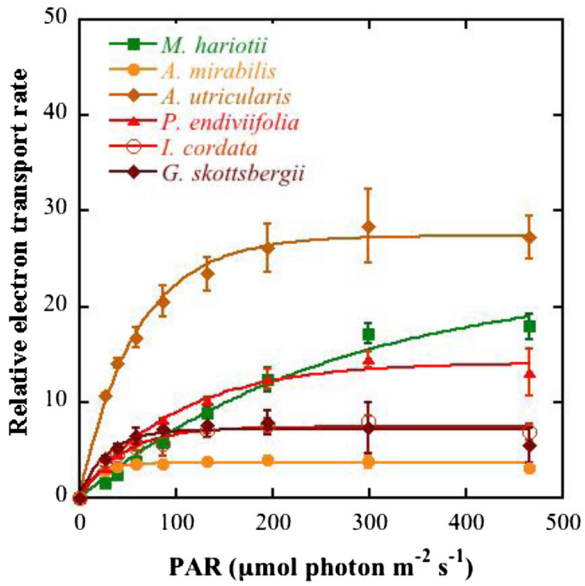

Fig 2 Electron transport rate (rETR) based on P-E curves of reproductive cells of *Adenocystis utricularis*, *Monostroma hariotii*, *Pyropia endiviifolia*, *Iridaea cordata*, *Gigartina skottsbergii*, and *Ascoseira mirabilis* collected in Fildes Peninsula (King George Island, Antarctica). Values are means ± S.D. of 4 measurements

Temperature tolerance

In the six studied species, increases in temperature caused a substantial decrease in F_v/F_m (Fig. 3). In *P. endiviifolia*, *A. utricularis* and *M. hariotii*, the decrease in F_v/F_m was observed only at 30 °C, while in *A. mirabilis*, *I. cordata* and *G. skottsbergii*, it was observed at 25, 20 and 5 °C, respectively. On the other hand, only gametes of *M. hariotii* showed a significant increasing trend in their F_v/F_m values between 0 to 25 °C (Fig. 3).

Tolerance to UV radiation and temperature

UV exposure caused decreases in F_v/F_m of reproductive cells in all studied species (Fig. 4). UV tolerance varied among species. Zoospores of *A. utricularis* were the most tolerant, while *G. skottsbergii* carpospores were the most sensitive. Species from eulittoral showed decreases less than, or equal to, 20 %, while in the sublittoral red alga *G. skottsbergii*, decreases under UV treatment ranged between 30 and 65 %.

UV tolerance was affected by temperature in *A. utricularis* (F = 4.9; $p < 0.04$), *I. cordata* (F = 11.3; $p < 0.005$) and *A. mirabilis* (F = 19.1; $p < 0.005$). In *I. cordata*, tolerance increased with temperature, while in *A. mirabilis*, gametes were more sensitive to UV radiation under 12 °C. In *A. utricularis*, exposure to 7 °C increased F_v/F_m.

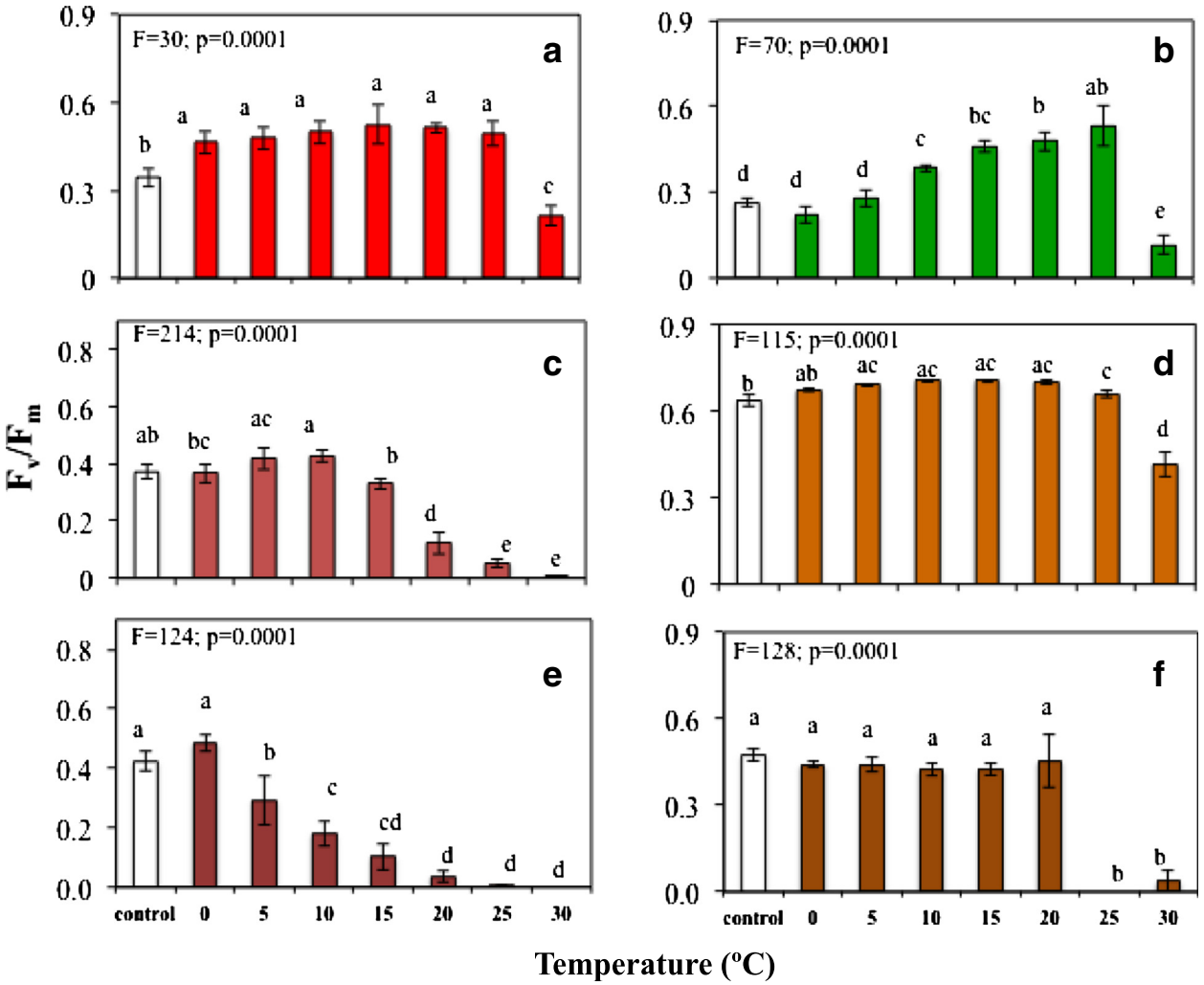

Fig 3 Short-term (1-h) responses of maximum quantum yield of fluorescence (F_v/F_m) to temperature in reproductive cells of *Pyropia endiviifolia* carpospores (**a**), *Monostroma hariotii* zoospores (**b**), *Iridaea cordata* tetraspores (**c**), *Adenocystis utricularis* zoospores (**d**), *Gigartina skottrbergii* carpospores (**e**), and *Ascoseira mirabilis* gametangia (**f**) collected in Fildes Peninsula (King George Island, Antarctica). Values are means ± S.D. (4–5 measurements). Different letters denote significant differences between mean values according to Tukey test ($P < 0.05$)

After 4 h under dim light, the eulittoral species recovered almost completely (95–100 %) in all temperature treatments. In sublittoral species, the recovery reached values between 50 and 80 %, with the exception of *G. skottsbergii* whose carpospores recovered completely at 12 °C.

Discussion

Photosynthetic characteristics

Differences in photosynthetic performance among reproductive cells, such as spores and/or gametes, of the studied species were observed. These differences could be related to the type of reproductive cell, which can have different features, such as size, motility or pigment composition. Notwithstanding such species-specific differences, photosynthetic characteristics of reproductive cells allowed us to distinguish between eulittoral and sublittoral algae, the former having higher irradiance saturation. These results are in agreement with the actual position of these species on the shore, and they confirm previous reports of [4].

Among eulittoral species, reproductive cells of *I. cordata* and *A. utricularis* seem to be strongly adapted to low light, while *M. hariotii* and *P. endiviifolia* are less shade-adapted, even though *M. hariotii* is distributed in the lower eulittoral [2]. In contrast, [4] reported that spores of *P. endiviifolia* are strongly shade-adapted and that propagules of *A. utricularis* and *M. hariotii* seem to be less shade-adapted (for comparison, see Table 1). Differences between these studies could result from the number of spores used in the measurements, time of release of spores or reproductive season. In this context,

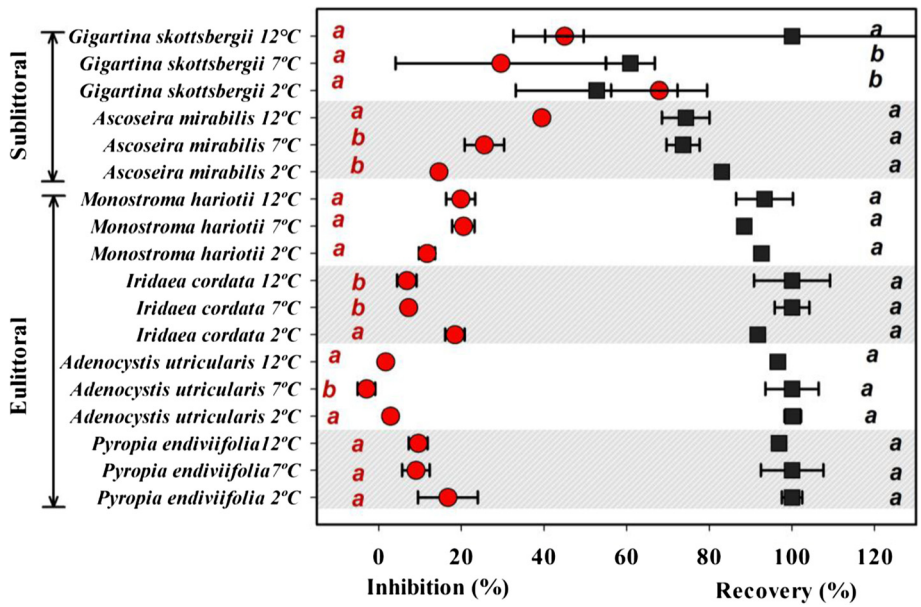

Fig 4 Inhibition of maximum quantum yield of fluorescence (F_v/F_m) after a 4-h exposure to UV radiation and subsequent 4-h recovery in reproductive cells of *Adenocystis utricularis*, *Monostroma hariotii*, *Pyropia endiviifolia*, *Iridaea cordata*, *Gigartina skottsbergii*, and *Ascoseira mirabilis* collected in Fildes Peninsula (King George Island, Antarctica). Values are mean ± S.D. (4–5 measurements) and represent the percentage reduction in F_v/F_m of UV treatment relative to PAR exposure. Different letters denote significant differences between mean values within temperature treatments according to Tukey test ($P < 0.05$)

the photosynthetic capacity of sporelings in suspension could partially reflect the depth distribution of the parental thalli because macroalgal zonation is ultimately defined by the ability of algae to tolerate environmental stress through adaptations of more advanced developmental processes, such as germination, settlement and growth [9–10].

Temperature tolerance

In our study, adaptation of the studied species to cold was confirmed by the high photosynthetic efficiency under 0 °C. However, a remarkable photosynthetic capacity was also observed at high temperatures, mainly in the eulittoral species *A. utricularis*, *M. hariotii* and *P. endiviifolia*, a phenomenon previously been observed in propagules, gametophytes and embryonic sporophytes of several Antarctic and Arctic species of macroalgae (reviewed in [1]. These results suggest that reproductive cells of these three species are thermally well adapted, at least for short periods of time, allowing them to develop in a highly variable environment. This ability apparently forms part of a suite of adaptive mechanisms displayed by these species, enabling their spread and colonization of different biogeographic regions. In fact, *A. utricularis* and *M. hariotii* are widely distributed in subantarctic and temperate coasts of South America [25]. In contrast, carpospores of *G. skottsbergii* exhibited a high sensitivity to enhanced temperature, which agrees with the ranges for growth reported for this species (0 and 5 °C; [26, 27]. Interestingly, gametes of *A. mirabilis*, an endemic Antarctic species, showed high temperature tolerance. This can be partially explained by the upper vertical distribution of the parental sporophytes. It should be noted that *A. mirabilis* can colonize the infralittoral fringe during spring-summer. Alternatively, tolerance to high temperatures could be a conserved trait related to the fact that the species is probably a relict of Mesozoic (Gondwana) marine flora, which was highly diverse when the average water temperatures were close to 12 °C [28].

Temperature and UV tolerance

Our results indicated an interactive effect of UV and temperature on F_v/F_m of reproductive cells of some Antarctic algae. For example, a decline in photochemical yield by UV radiation was found in *G. skottsbergii* carpospores, and such decrease was exacerbated under exposure to 2 °C. In contrast, inhibition of photosynthesis decreased at temperatures of 12 °C. A similar tendency, although less marked, was observed in *I. cordata* and *P. endiviifolia*. It is known that various processes related to photoprotection, e.g., D1 protein turnover, enzyme repair mechanisms, and dissipative quenching, operate more efficiently at higher temperatures [29, 30]. Thus, the lower inhibition of photosynthesis observed at 12 °C compared to 2 and 7 °C could be regarded as an efficient acclimation of photosynthesis in these macroalgae. For

example, the UV-mediated inhibition of photosynthesis at 10 °C of the Antarctic *Ulva bulbosa* was close to 10 %, comparable to its subantarctic counterpart *Ulva clathrata*. However, at 0 °C, decreases in photosynthesis reach a peak at 37 and 50 % relative to control, respectively [31]. Compared to enzymatic processes, photochemical reactions, such as CO_2 fixation or ATPase reactions [32], are relatively unaffected by temperature, while, on the other hand, chlorophyll turnover and PSII-related reactions are highly sensitive to low temperature, thus exacerbating inhibition and photochemical damage [33]. This apparently explains why photodamage of PSII under high solar radiation is enhanced at low temperatures [34].

Decreases in F_v/F_m, as measured in *A. mirabilis* gametangia at 2 °C, were comparable to those observed in *P. endiviifolia* and *I. cordata*. It must be emphasized that differences in UV tolerance between propagules of different species are a reflection of both the capacity for stress tolerance, as well as other morphofunctional aspects related to cell size, pigmentation, previous UV exposure history within gametangia/sporangia [12], and even the levels and time of UV exposure used in experiments (see Table 1). In the case of size, UV susceptibility has been shown to decrease with increasing cell size in terms of F_v/F_m [6] and DNA damage [4, 6]. In larger propagules observed in our study, such as carpospores of *Iridaea* (18 ± 2 μm) and *Pyropia* (12 ± 1 μm) and gamentangia of *Ascoseira* (22 ± 4 μm), direct UV effect was lower, most likely resulting from the longer pathway for UV penetration [8]. Furthermore, the highest UV tolerance of some reproductive cells has been associated with the presence of UV-absorbing compounds [6, 35, 36]. On the other hand, cell walls of gametangia and auxiliary structures in conceptacles of *A. mirabilis* could offer UV protection to the gametes inside [5, 12].

In the context of global climate change, the ability of early developmental stages to withstand environmental variability is essential for recruitment, especially for those species subject to episodic perturbations from, for example, ice scouring, as their recovery over time depends almost entirely on reproductive output and capacity for rapid settlement.

To the best of our knowledge, only a few studies have addressed the response of spores of Antarctic macroalgae to UV radiation, while no data have thus far been published on the response to different temperatures or the interaction between UV exposure and temperature. Thus, our study is among the first to provide evidence that reproductive cells of Antarctic macroalgae are relatively tolerant to high temperature and that temperature increase can modulate UV tolerance, at least under laboratory conditions.

Conclusions

- Photosynthetic characteristics among reproductive cells of the six studied species varied according to the bathymetric distribution of parental adult stages. As such, eulittoral species exhibited higher light requirements for photosynthesis than sublittoral algae. However, other factors related to biogeographic distribution or evolutionary divergences could affect the patterns determined in this study.
- Reproductive cells of eulittoral species exhibited higher temperature tolerance than sublittoral algae, at least in the short term.
- UV tolerance was ameliorated by enhanced temperature in three of the six studied species. Moreover, reproductive cells of the six studied species could recover within 4 h after UV exposure in a manner unrelated to temperature.
- Although these results indicating acclimation to short-term exposure point to an ecological advantage of eulittoral over sublittoral species, further research based on long-term incubation will provide new insights into the potential mechanisms that permit these algae to cope with large-scale environmental variability.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
NPN: Laboratory or fieldwork, Data analysis and interpretation, Manuscript preparation. PH: Framing hypotheses/experi-mental design, Data analysis and interpretation, Manuscript preparation. IG: Framing hypotheses/experi-mental design, Data analysis and interpretation, Manuscript preparation. All authors read and approved the final manuscript.

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