

Tolerance of Antarctic cyanobacterial mats to enhanced UV radiation

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Abstract

To assess the biological implications of ozone depletion over the Antarctic Peninsula, the ultraviolet (UV) regime of two Antarctic cyanobacterial communities (composed of *Leptolyngbya* sp. and *Phormidium* sp.) was manipulated using screens that cut out UV radiation and a lamp which enhanced the dose of UV-B radiation (280–315 nm). The biological response of the cyanobacterial mats was monitored by measurement of chlorophyll fluorescence and pigment concentrations. The *Leptolyngbya* mat showed significant photochemical inhibition due to increased UV-B relative to photosynthetically active radiation (400–700 nm). The effect of UV on the *Phormidium* mat was less pronounced and dependent on the method of analysis: significantly lower photochemical yields were observed in UV-enhanced *Phormidium* mats compared to UV-excluded treatment, but if the yield data relative to the time zero control were considered then no effect of UV enhancement was observed. The *Phormidium* mat contained over 25 times the absolute concentration of UV-protecting mycosporine-like amino acid (MAA) and double the concentration of carotenoids compared to the *Leptolyngbya* mat, but the latter contained a higher ratio of carotenoids+MAAs to chlorophyll. There were no significant treatment-related changes in the concentrations of MAA, carotenoids and chlorophyll *a* in the *Phormidium* mat. The *Leptolyngbya* mat showed significantly lower chlorophyll *a* concentrations under UV enhancement, which could account for the lower photochemical yield in this sample. Our results show that different cyanobacterial species have differing photochemical sensitivity to UV-B radiation, which may confer a subtle advantage to the UV-B tolerant species over the less tolerant type during a period of high UV-B irradiance. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Since the ‘ozone hole’ was first reported by Farman et al. in 1985 [1], there has been a sustained seasonal decline in springtime stratospheric ozone over the Antarctic [2] resulting in increased levels of ultraviolet (UV)-B radiation reaching the Earth’s surface. UV-B radiation comprises

wavelengths between 280 and 315 nm, though even under severe ozone depletion wavelengths below 290 nm are rarely detected. UV-B is a harmful component of solar radiation for living organisms exposed to sunlight and can cause damage to DNA, proteins and the photosynthetic apparatus [3]. To avoid or reduce UV-B damage, organisms can employ a variety of protective mechanisms. The synthesis of UV-absorbing compounds (mycosporine-like amino acids (MAA) and scytonemin) provides screening for cellular fractions from UV damage [4,5]. Carotenoids quench the highly reactive oxidants produced by UV radiation [6]. Some organisms avoid UV exposure by retreating to habitats with reduced exposure (for example, deeper into mat communities or lower in the water column [7]), whilst others have efficient repair mechanisms for the reconstruction of damaged molecules such as enzymes and DNA [8].

The biological consequences of increasing UV-B irradiation are the subject of much speculation [9,10]. The del-

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Abbreviations: Chl-*a*, chlorophyll *a*; MAA, mycosporine-like amino acid; PAM, pulse amplitude modulated; PAR, photosynthetically active radiation; UV-B, ultraviolet-B radiation; GPD UV-B, ultraviolet-B radiation weighted for general plant damage

etrious effects of UV-B radiation on cellular processes are consistently demonstrated in laboratory culture studies [11–13], even when realistic ambient spectral conditions are mimicked [14]. However, it is not clear whether these laboratory-based observations can be extrapolated into the natural environment. If so, what implications are there for Antarctic ecosystems? If UV-B effects do exist in natural ecosystems, Antarctic environments, which are naturally exposed to elevated levels of UV-B, represent the ideal location in which to identify them.

Two approaches can be employed in field experiments to determine the effects of UV-B radiation on organisms: (i) screens can be used to exclude certain wavelengths of ambient UV radiation and to determine biological responses to specific wavelength bands [15] and (ii) UV-B lamps can be used to increase the UV:photosynthetically active radiation (PAR) ratio [16]. There are a number of problems with both approaches. The first approach, because it reduces UV radiation incident on target organisms, does not inherently reveal effects of increased UV-B radiation associated with increased ozone depletion. However, UV lamps do not mimic the solar spectrum and are the subject of some speculation as to their effectiveness [17], but are currently the only realistic means of enhancing the UV:PAR ratio in field studies. Both of these approaches were employed in this study.

Cyanobacteria are a major component of terrestrial and freshwater habitats of Antarctica [18]. They form extensive mats which can completely cover benthic substrates [19]. Despite their importance as pioneers in extreme environments and their potential as indicators of global climate change [20], surprisingly little research has focussed on the ecophysiology of cyanobacteria and their response to elevated UV-B radiation.

Given the limited availability of cyanobacterial mats, it was important to select a non-destructive means to monitor the response of mats to experimental conditions. The measurement of chlorophyll fluorescence is a non-invasive, reliable and near-instantaneous method to assess photosynthetic performance [21]. As the photosynthetic system of cyanobacteria is closely connected to their major metabolic pathways, chlorophyll fluorescence signals also provide real-time information about the overall acclimation status of cyanobacteria [22]. This technique has been used in many studies on the ecophysiology of cyanobacteria, from assessing the toxicity of mercury [21] to on-line monitoring of photoinhibition at high oxygen concentration and low temperature [23]. In this study pulse amplitude modulated chlorophyll fluorescence was used to assess the acclimation of Antarctic cyanobacteria to experimental conditions.

The aim of this study was to analyse the effects of different wavelengths of natural and artificially enhanced UV radiation on the photosynthetic efficiency and pigment composition of cyanobacterial mats in Antarctica.

2. Materials and methods

Cyanobacterial mats ($\sim 20 \text{ cm}^2$) were collected from Léonie Island ($67^\circ 36'S$, $68^\circ 20'W$), close to the western Antarctic Peninsula on 10 March 1998. Portions of mat were placed in petri dishes secured to a platform adjacent to the laboratories at nearby Rothera Research Station, Adelaide Island ($67^\circ 34'S$, $68^\circ 08'W$) and left overnight to acclimate before duplicate samples of each mat were placed under treatments simulating differing UV environments: (i) enhanced UV-B radiation and (ii) UV-excluded. Control samples of *Phormidium* mat were also incubated under a screen that was transparent to all wavelengths of solar radiation, but these mats blew away soon after the start of the incubation.

The samples exposed to enhanced UV levels were placed under a UV-B lamp with peak output at 313 nm (Cole-Palmer Instrument Co., London, UK), with two Sanalux glass panels to absorb wavelengths below 280 nm. A typical solar spectrum from a sunny day at Rothera during the experimental period (11/3/98) together with the enhanced radiation from the lamp is shown in Fig. 1a. The lamp also emitted spikes in the visible spectrum (393 nm,

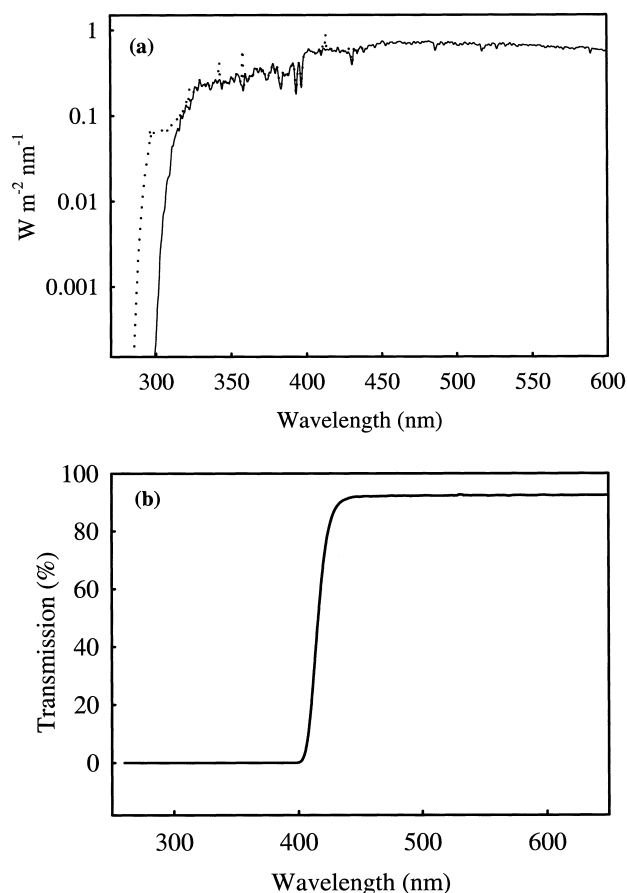


Fig. 1. a: Typical solar spectrum from a sunny day at Rothera during the experimental period (11/3/98) (solid line), and with the enhancement due to lamp added (dotted line). b: Transmission properties of UV-excluding (VE) Perspex.

436 nm, 543 nm and 579 nm). The UV-B lamp and filter were positioned to provide a uniform UV-B dose over the test area, but to minimise shading of the natural radiation, with a distance of 30 cm between lamp and samples. This enhancement was given for up to 5 h day⁻¹ around solar noon, and the UV-B lamp was only operated when natural irradiance exceeded 50 W m⁻². The experiment was continued for 7 days.

Duplicate samples were also incubated under UV-opaque Perspex screens (0.5 m², 8 cm height, Du Pont Polyesters Group, Middlesbrough, UK). Lack of sample material did not allow any further replication of test conditions. The transmission properties of the UV-excluding screen are shown in Fig. 1b. To ensure the mats were water saturated, they were sprayed with water 1 h prior to chlorophyll fluorescence measurement.

2.1. Taxonomic analysis

Immediately after collection, 100 µl of homogenised mat sample was placed on plates of BG-11 cyanobacterial culture medium [24] and incubated at 20°C in an illuminated incubator (Vindon, model 513S, Oldham, UK). Single-species cultures were obtained by successive rounds of sub-culturing. These, and desiccated samples of the original mat were identified by microscopic analysis by Dr. Paul Broady, University of Christchurch, New Zealand.

2.2. Measurement of ambient solar radiation, temperature and ozone depth

A Bentham DM150 scanning spectroradiometer (Bentham Instruments Ltd., Reading, UK) adjacent to the experimental site was used to measure the spectral global irradiance at Rothera Research Station. Spectral scans were taken from 280 to 700 nm at 0.5 nm intervals, with a resolution of 1 nm, at 30 min intervals. The same instrument was used to measure the output of the UV lamp, and to cross-calibrate broad-band UV-A and UV-B sensors (Delta-T Devices, Cambridge, UK) and PAR quantum sensors (Skye Instruments Ltd., Powys, UK) which were used to measure conditions at the experimental site. Irradiance data were transformed to general plant damage (GPD)-effective doses using the action spectra of Caldwell et al. [25]. The air temperature at the experimental site, adjacent to the cyanobacterial mats, was measured using temperature probes (Cambell Scientific Ltd., Loughborough, and a Cambell 21X logger). Ozone column depth measurements were obtained from the NASA Earth Probe Total O3 Monitoring Satellite, which overpassed Rothera Research Station at approx. 13.45 h (local time) each day.

2.3. Determination of photosynthetic efficiency

The effective photosystem II (PS II) quantum yield of the cyanobacterial mats was measured using pulse ampli-

tude modulated (PAM) fluorescence. Measurements were made in the early morning and at intervals until nightfall under natural light conditions. A portable OS100 modulated chlorophyll fluorometer (Opti-Sciences Inc., MA, USA) with a 65° open body cuvette guide was used to obtain steady-state yield values from the surface of the mats. Five or six yield measurements were made for each mat portion (depending on the size of the mat) and the sample dishes were marked to ensure positioning of the fluorometer probe at the same location for each measurement. The relative amplitude of the modulated light was set at 1 with an 0.7 s pulse duration. These conditions were found to give the optimal response. The effective quantum yield of PS II ($\Delta F/F_m'$) was determined from the fluorescence signals using the following equation:

$$\Delta F/F_m' = (F_m' - F)/F_m'$$

where F_m' is the maximal yield in the light-adapted state and F is the fluorescence yield [26].

Relative electron transport rates in the cyanobacterial mats were calculated by the multiplication of $\Delta F/F_m'$ by the photosynthetically active light intensity (300–700 nm) [26].

2.4. Pigment and UV-absorbing compound extraction

To measure pigment concentrations, three subsamples of each mat (~1 cm²) were taken at the start, and after 41.5, 108 and 180 h of treatment, lyophilised and stored at -80°C prior to spectrophotometric analysis. To measure concentrations of hydrophilic UV-absorbing compounds, mat samples were extracted with 30% (v/v) methanol at 40°C for 1 h without perturbation, based on the protocol of Garcia-Pichel and Castenholz [4], who demonstrated that this extraction procedure was able to extract 98% of UV-absorbing compounds. To extract carotenoids, chlorophyll and scytonemin for spectrophotometric analysis, samples were ground to a powder in liquid nitrogen, then homogenised with 95% (v/v) acetone, and left at 4°C in the dark overnight, with occasional agitation. The extracts were clarified by centrifugation and filtration (0.45 µm). To extract hydrophobic pigments for HPLC analysis, lyophilised mat portions were weighed, then homogenised in 90% (v/v) acetone, and then agitated at 4°C in the dark for 1 h. The cells were pelleted by centrifugation, then re-extracted with 100% acetone. The extracts were pooled, filtered (0.45 µm), and stored at -80°C until analysis.

2.5. Determination of pigment and UV-absorbing compound content

UV-visible spectra were obtained using a Unicam spectrophotometer (Unicam, Cambridge, UK) immediately after pigment or UV-absorbing compound extraction. Spectra from the acetone extract were transformed using trichromatic equations of Garcia-Pichel and Castenholz

[27] to give the concentrations of scytonemin, total carotenoids and chlorophyll *a* (chl-*a*). The UV-absorbing compound content was determined from the methanol extract scan using the transformation of Garcia-Pichel and Castenholz [4], with a generic extinction coefficient of $120 \text{ l g}^{-1} \text{ cm}^{-1}$.

Chromatography was performed with an automated gradient HPLC system (Kontron Instruments Ltd., Watford, UK), comprising a ternary pump system, autosampler and diode array detector. Pigments in the acetone extract were separated using a Waters Spherisorb, ODS-2 column with a particle size of $5 \mu\text{m}$ (Alltech Associates, Carnforth, UK). The chromatography protocol of Wright et al. [28] was used. UV-absorbing compounds in the methanol extract were separated with a Brownlee RP8 (Spheri-5 $5 \mu\text{m}$) column (Alltech Associates, Carnforth, UK), based on the isocratic HPLC protocol of Garcia-Pichel and Castenholz [4], with 4% (v/v) methanol, 0.2% (v/v) acetic acid as the mobile phase.

A 1 ml min^{-1} flow rate was used with the column heated to 30°C (acetone extracts) or unheated (methanol extracts). Pigments in the eluate were measured by diode array detector (Kontron Instruments Ltd.). Spectral scans were obtained for all compounds from 300 to 700 nm (acetone-extracted pigments) and from 240 to 600 nm (methanol-extracted UV-absorbing compounds). The system was calibrated to reference standards following SCOR protocols [29] and US Environmental Protection Agency Standards. Pure standards were not available to identify the methanol-extracted UV-absorbing compounds.

2.6. Statistics and mathematical modelling

For each time point, means and standard error were calculated from a minimum of nine PAM fluorescence measurements on the two mat pieces under a particular treatment. Mean pigment concentrations were calculated from extracts of three mat samples.

To test the significance of environmental conditions and treatment on $\Delta F/F_m'$ a mathematical model was fitted to the light response curves shown in Fig. 2, using the Genstat statistical package (version 5.4.1). This model allowed a linear effect of temperature on yield and a spline curve with 5 degrees of freedom to model the effect of PAR on the yield values. Treatment, time, cumulative hours and replicates within time were all included as intercept terms in the model. All terms in the final model were statistically significant (all at least at $P < 0.05$, many at $P < 0.01$) as judged by an analysis of variance. This modelling procedure was also applied to relative $\Delta F/F_m'$ data ($[\Delta F/F_m']_t - [\Delta F/F_m']_0$) generated by subtracting the $\Delta F/F_m'$ of mat samples prior to treatment ($[\Delta F/F_m']_0$) from $\Delta F/F_m'$ measurements from the various time points under the treatments ($[\Delta F/F_m']_t$).

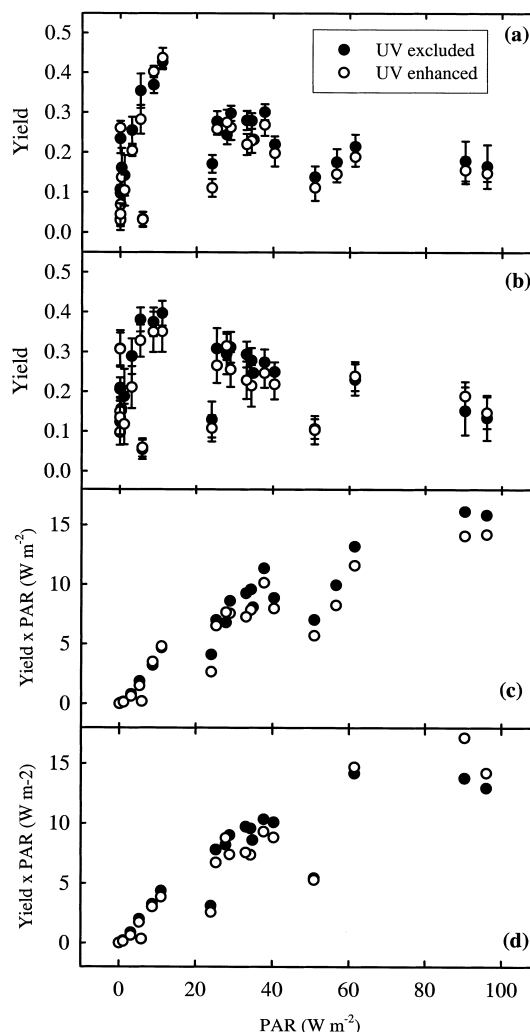


Fig. 2. Light response curves. Variation of effective PS II quantum yield with PAR in (a) *Phormidium* mat, (b) *Leptolyngbya* mat under different UV treatments. The points are the average of a minimum of nine independent measurements and the error bars represent the standard deviation of the $\Delta F/F_m'$ readings for mat samples under a given treatment at a given time. Relative electron transport rates versus PAR in (c) *Phormidium* mat, (d) *Leptolyngbya* mat.

3. Results

3.1. Cyanobacterial mats

Two types of cyanobacterial mat were found on Léonie Island. (i) A mat with orange surface colour and green underside was found in small pools of meltwater between rocks and extending out into runnels emerging from the pools. This mat was dominated by thin filamentous cyanobacteria, of the genus *Leptolyngbya* Anagnostidis and Komarek, and also contained diatoms and green and red algae. (ii) The other mat was black, and of more homogeneous composition than (i). It was found in meltwater runnels over bare rock, forming a smooth film of up to

10 mm thickness and was composed of *Phormidium* sp., probably *Phormidium autumnale*. Casual observations over the course of 3 years showed that the mats formed when meltwater became available on Léonie Island, probably under the existing snow cover, in January–February. When the water source was exhausted, the mats rapidly desiccated and were blown away. In a year of particularly heavy snow cover, they were not observed at all. The appearance of the mats did not alter noticeably during the course of the experiment.

3.2. Light and temperature conditions

The mats were incubated under ambient temperature conditions, which ranged from -3.2°C to $+4.9^{\circ}\text{C}$. The weather was generally cloudy during the experimental period. UV enhancement data for the period when the UV lamp was switched on are given in Table 1, calculated from the spectral data generated by the scanning spectrophotometer integrated over the time period that the lamp was switched on. For comparison, data are also given for a cloudless day under severe ozone depletion (ozone depth = 133.5 DU) later in the same year (20/10/98). The average ozone depth during this study was 285.3 DU. The lamp enhanced the dose of UV-B to 1.5–2.8 times that of the ambient UV-B radiation. The UV-B lamp increased the dose of biologically damaging (GPD) UV-B between 3.1 and 5.8 times, compared to ambient levels. The total levels of GPD-weighted UV-B h^{-1} (ambient+lamp) were approximately half those measured under ozone hole conditions (20/10/98). The UV-B/PAR ratios during enhancement with the UV lamp ranged from 0.007 to 0.013, generally higher than that observed during ozone hole conditions on 20/10/98 (0.0077). The GPD-weighted UV-B/PAR ratios during the experimental period ranged between 0.0013 and 0.0027, whereas that measured on 20/10/98, under ozone hole conditions, was 0.0018. The PAR values measured during the experimental period ranged between 189 and $447 \text{ kW m}^{-2} \text{ h}^{-1}$, whereas the average

PAR h^{-1} at peak PAR hours on 20/10/98 was $592 \text{ kW m}^{-2} \text{ h}^{-1}$.

3.3. Photochemical yield

The effective PS II quantum yields of the cyanobacterial mats at ambient PAR levels are shown in Fig. 2a,b. Up to a PAR value of $\sim 16 \text{ W m}^{-2}$, an increase in PAR resulted in an increase in $\Delta F/F_m'$ in both mat types. Above this threshold, photoinhibition was observed, and increased light intensity did not result in an increased $\Delta F/F_m'$. The exclusion or enhancement of UV did not affect the threshold of this photoinhibition effect. Both mats responded in similar ways to the prevailing environmental conditions, and showed similar yields at each sampling time. Relative electron transport rates for each mat under each treatment were plotted against PAR (Fig. 2c,d). The light dependences of the relative electron transport rates saturated at $\sim 62 \text{ W m}^{-2}$.

To investigate the effect of experimental conditions and UV regime on the photochemical yield of each mat, a non-parametric mathematical model (see Section 2) was fitted to the $\Delta F/F_m'$ data for each mat, and the relative $\Delta F/F_m'$ data (Fig. 3). The model generated data for each time point with the systematic variation (due to time, temperature, PAR) removed. The model gave a close fit to the observed data (Fig. 3a,b). Analysis of variance of the $\Delta F/F_m'$ data generated by the model (with the systematic variation removed) showed that in both mats, UV enhancement caused a significant ($P < 0.05$) inhibition of effective PS II yield. The model also showed that temperature, PAR value, treatment, time of day and the cumulative hours that the experiment had been running all had significant effects on $\Delta F/F_m'$ ($P < 0.05$).

The mathematical modelling revealed that $\Delta F/F_m'$ was strongly influenced by the ambient PAR and temperature, but that this influence decreased above the photoinhibition threshold of 16 W m^{-2} (Table 2). Below this threshold, PAR and temperature exerted strong influences over the

Table 1
UV-B enhancement data for the time period when the UV lamp was switched on

Date	Duration of UV-B enhancement (h)	UV-B enhancement (UV-B _{LAMP} /UV-B _{AMB})	GPD UV-B enhancement (GPD UV-B _{LAMP} /GPD UV-B _{AMB})	UV-B h^{-1} during enhancement	GPD UV-B h^{-1} during enhancement	UV-B _{AMB} +LAMP/ PAR for enhancement period	GPD UV-B _{AMB} +LAMP/ PAR for enhancement period
10/3/98	5	2.1	4.4	2648	525	0.0097	0.0019
11/3/98	2.5	1.5	3.1	2978	567	0.0066	0.0013
12/3/98	2	nd	nd	nd	nd	nd	nd
13/3/98	2.5	2.8	5.8	2443	502	0.0099	0.002
14/3/98	2.5	2.5	5.3	2516	508	0.0133	0.0027
15/3/98	1.5	2.1	4.6	2646	520	0.0076	0.0015
16/3/98	4	nd	nd	nd	nd	nd	nd
20/10/98	–	–	–	(4555)	(1090)	(0.0077)	(0.0018)

The data in parentheses are included for comparative purposes, and were obtained over a 3 h period from 12.00 to 15.00 h on a cloudless day under severe ozone depletion on 20/10/98, with no lamp enhancement of UV-B. UV-B_{AMB}, dose of ambient UV-B radiation; UV-B_{LAMP}, lamp-enhanced dose of UV-B radiation; nd, no data available.

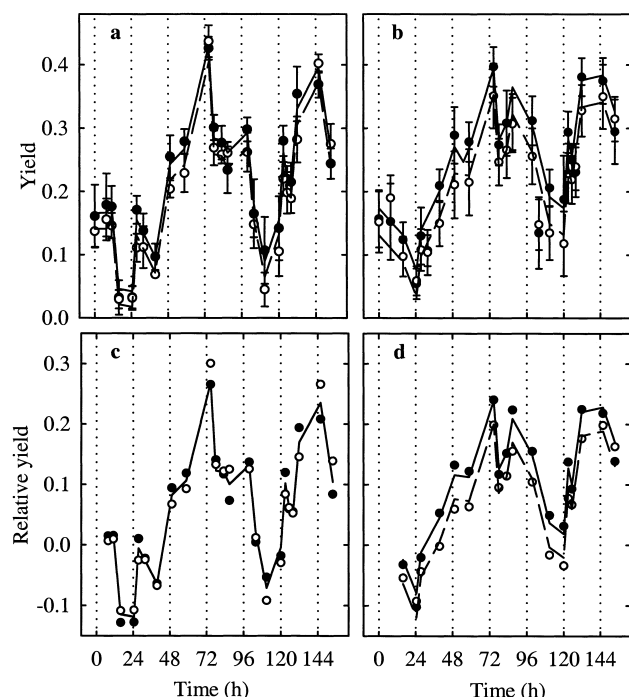


Fig. 3. Modelling of $\Delta F/F_m'$. \circ , UV-enhanced; \bullet , UV-excluded. The lines join data points generated by the mathematical model fitted to the UV-excluded mat (—) or UV-enhanced mat (---); see text for details. Observed $\Delta F/F_m'$ data: (a) *Phormidium* mat, (b) *Leptolyngbya* mat. Relative $\Delta F/F_m'$ data: (c) *Phormidium* mat, (d) *Leptolyngbya* mat. The error bars represent the standard deviation. The grid lines show the 24 h periods starting from the first measurement at 07.10 h. NB, the lines joining the modelled data in (c) superimpose almost exactly.

variation in $\Delta F/F_m'$ with time, accounting for 83.1% and 69.1% of the variation in the *Phormidium* and *Leptolyngbya* mats, respectively. Temperature was the stronger influence on $\Delta F/F_m'$ in both mats, accounting for over half of the variation in $\Delta F/F_m'$ with time when PAR < 16 W m⁻². This influence reduced to ~25% at light intensities above this threshold value. PAR had a greater influence over the variation in $\Delta F/F_m'$ in the *Phormidium* mat than in the *Leptolyngbya* mat, accounting for 29.1 and 10.6% of the variation in yield respectively when light intensity was below 16 W m⁻². Above this threshold, PAR accounted for approx. 10% of the variation in $\Delta F/F_m'$ in both mats.

The mathematical model was also used to generate data with systematic error removed for the relative $\Delta F/F_m'$ values. This accounts for any differences in the mat samples before treatment commences. The relative $\Delta F/F_m'$ values,

together with those generated by the mathematical model, are shown in Fig. 3c,d. A different outcome was observed compared to that from the absolute $\Delta F/F_m'$ data (Fig. 3a,b). For the *Phormidium* mat, the modelling procedure using the relative $\Delta F/F_m'$ data showed no significant difference between UV-enhanced and UV-excluded mat (Fig. 3c). The model predicted very similar values for the UV-enhanced and UV-excluded *Phormidium* mats, hence only one line can be seen in Fig. 3c. However, the relative $\Delta F/F_m'$ in the UV-enhanced *Leptolyngbya* mat was significantly lower ($P < 0.05$) than that of the UV-excluded *Leptolyngbya* mat (Fig. 3d).

3.4. Pigment and UV-absorbing compound concentrations

The two mat types differed greatly in their pigment and UV-absorbing compound concentrations. The *Phormidium* mat contained over 6 times as much chlorophyll *a* as the *Leptolyngbya* mat (6.1 $\mu\text{g mg}^{-1}$ compared to 0.93 $\mu\text{g mg}^{-1}$, respectively), twice the concentration of carotenoids (2.5 $\mu\text{g mg}^{-1}$ and 0.99 $\mu\text{g mg}^{-1}$, respectively), and 25 times the concentration of UV-absorbing compounds (7.1 $\mu\text{g mg}^{-1}$ versus 0.28 $\mu\text{g mg}^{-1}$) (Fig. 4). The ratio of total carotenoids to chlorophyll *a* in the *Leptolyngbya* mat was ~2.5 times higher than that for the *Phormidium* mat (Fig. 5), but the ratio of UV-absorbing compound to chlorophyll *a* was 3.8 times higher in the *Phormidium* mat compared to the *Leptolyngbya* mat (Fig. 5). However, in both mat types the ratio of photoprotective pigments (UV-absorbing compound plus carotenoids) to chlorophyll *a* was similar (1.56 and 1.36 for the *Phormidium* and *Leptolyngbya* mats, respectively).

3.5. Pigment and UV-absorbing compound types

HPLC analysis of acetone extracts of the two mats (Table 3) showed that they both contained myxoxanthophyll, zeaxanthin, echinone and β -carotene. The *Phormidium* mat contained an extra unidentified carotenoid while the *Leptolyngbya* mat also contained fucoxanthin and diadinoxanthin. Neither mat contained the sheath pigment scytonein.

Spectra obtained from both HPLC and spectrophotometer analysis showed very similar absorbance profiles to those published for MAAs [4]. Spectrophotometric analysis of the 30% methanol extracts of the *Phormidium* mat

Table 2

The effect of PAR and temperature on the effective PS II quantum yield of Antarctic *Phormidium* and *Leptolyngbya* cyanobacterial mats, as determined by fitting the mathematical model described in Section 2 to the light response curves shown in Fig. 2

	<i>Phormidium</i> mat	<i>Phormidium</i> mat	<i>Leptolyngbya</i> mat	<i>Leptolyngbya</i> mat
% variation accounted for by a model including:	PAR < 16 W m ⁻²	PAR > 16 W m ⁻²	PAR < 16 W m ⁻²	PAR > 16 W m ⁻²
PAR and temperature	83.1	35.2	69.1	30.9
Temperature	54	24.9	58.5	22.5
PAR	29.1	10.3	10.6	8.4

All terms were significant at $P < 0.05$ or lower.

showed a maximal absorbance at 335 nm. HPLC analysis of a 30% methanol extract of the same mat showed that it contained only one MAA-like substance with a λ_{max} at 332 nm. Spectrophotometric analysis of the 30% methanol extracts of the *Leptolyngbya* mat gave a wavelength of maximal absorbance in the range of 327–331 nm, while HPLC analysis of a 30% methanol extract of the same mat again showed that it contained only one MAA-like compound with a λ_{max} at 332 nm.

3.6. Changes in pigment and UV-absorbing compound concentrations during the experiment

Concentrations and ratios of chlorophyll *a*, total carotenoids and UV-absorbing MAA-like compounds in both of the mats during the course of the experiment are shown in Figs. 4 and 5, respectively. The significance of any changes in pigment concentrations with time and treatment was assessed using a two-way ANOVA test. There was no significant effect of the different treatments on the concentrations of chlorophyll *a*, carotenoids and MAA-like compounds in the *Phormidium* mat. A significant ($P < 0.05$) decrease of all three pigment types, independent of treatment, was observed during the course of the experiment, compared to the time zero control (Fig. 4a–c). However, in the UV-excluded samples of the *Phormidium* mat, there was a significant increase in both carotenoids and chlorophyll *a* between the 110 h and 180 h time points. Carotenoid concentrations in the *Leptolyngbya* mat were significantly lower ($P < 0.05$) in the UV-enhanced samples compared to the UV-excluded samples after 41 h of treatment, but after 108 h no difference was observed (Fig. 4d). There was no significant effect of treatment on the concentration of MAA-like compounds (Fig. 4e). There was a significant effect ($P < 0.05$) of UV treatment on the chlorophyll *a* concentrations of the *Leptolyngbya* mat, with lower concentrations of chlorophyll *a* in the samples irradiated with enhanced UV-B after 41.5 and 108 h of incubation (Fig. 4f).

The ratio of total carotenoids to chlorophyll *a* in the *Phormidium* mat remained constant irrespective of time

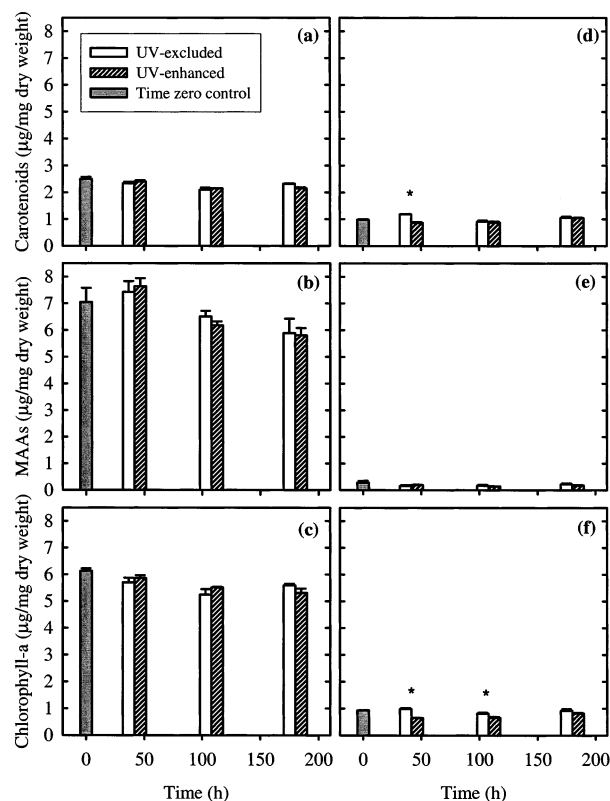


Fig. 4. Concentrations of carotenoids (total), MAA-like compounds and chlorophyll *a* in the *Phormidium* mat (a–c) and the *Leptolyngbya* mat (d–f). The error bars represent the standard deviation. Samples where the UV-enhanced samples are significantly different ($P < 0.05$) from the UV-excluded samples at the same time point are marked with an asterisk.

and treatment (Fig. 5b), whereas the ratio of MAA-like compounds to chlorophyll *a* showed a decrease over time (Fig. 5a), but with no overall effect of treatment. The ratio of MAA-like compounds to chlorophyll *a* in the UV-B-enhanced *Leptolyngbya* mat was higher than the UV-excluded mat after 41 h of treatment, but this ratio had equalised after 108 h of the experiment (Fig. 5c). The ratio of total carotenoids to chlorophyll *a* in the *Leptolyngbya*

Table 3
HPLC analysis of acetone extracts of *Phormidium* and *Leptolyngbya* cyanobacterial mats

Pigment (in elution order)	Retention time (min)	Relative abundance	
		<i>Phormidium</i> mat (%)	<i>Leptolyngbya</i> mat (%)
1. Chlorophyll <i>c</i>	7.7	–	0.8
2. Fucoxanthin	10.3	–	6.6
3. Unidentified carotenoid (peaks at 470, 497, 530 nm)	13.4	1.7	–
4. Diadinoxanthin	14.6	–	3.3
5. Myxoxanthophyll	15.9	11.2	6.1
6. Zeaxanthin	18.3	13.3	12.4
7. Chlorophyll <i>b</i>	20.1	1.6	–
8. Chlorophyll <i>a</i>	21.6	39.5	36.5
9. Echinone	22.5	14.5	14.1
10. β -Carotene	26	16.7	18.9

The relative abundance (in terms of peak area) is given for each mat.

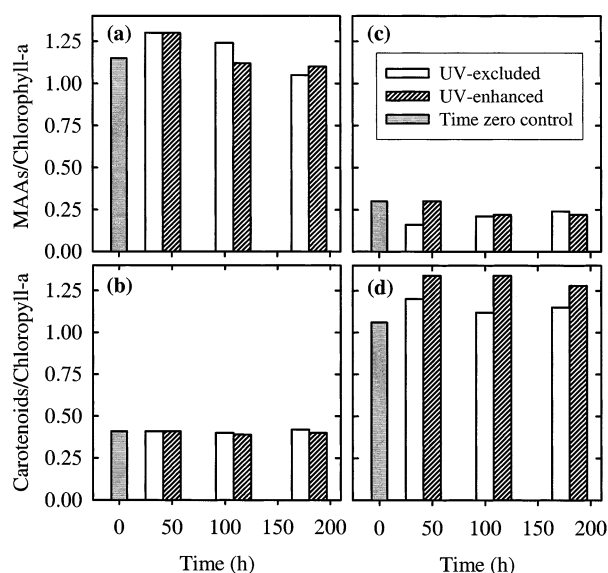


Fig. 5. Ratio of MAA-like compounds and carotenoids (total) to chlorophyll *a* in the *Phormidium* mat (a,b) and the *Leptolyngbya* mat (c,d) under differing UV regimes.

mat was always lower in the UV-excluded samples compared to those that were UV-enhanced (Fig. 5d).

4. Discussion

This study demonstrates that an elevation of UV-B radiation to levels observed during Antarctic ozone depletion caused a significant inhibition of effective PS II quantum yield in Antarctic *Leptolyngbya* mat. The effect of enhanced UV-B on $\Delta F/F_m'$ in Antarctic *Phormidium* mat was less pronounced, and the significance of effect was dependent on the method by which the data were analysed. If the absolute data were used, a significant UV-B-induced photoinhibition was observed in UV-enhanced samples compared to UV-excluded samples. However, if the *Phormidium* mat yield data relative to the time zero control were considered then there was no effect of UV enhancement compared to UV exclusion. This highlights that differences in photochemical response from adjacent portions of the same mat communities could lead to false conclusions about UV sensitivity if not properly accounted for.

Few studies have been conducted on the possible effects of UV radiation on Antarctic terrestrial cyanobacteria. An experiment to measure the effects of different UV regimes on Antarctic cyanobacterial mats from the McMurdo Sound region showed that photosynthetic rates (as measured by ^{14}C uptake) were, on average, 25% lower in mats exposed to the whole UV spectrum compared to those exposed to PAR alone, but these differences were not statistically significant [15]. No significant differences in concentrations of carotenoids or chlorophyll were observed over an 11 day study period, the carotenoids/chl-*a* ratio

remained constant under the different treatments and there was a loss of MAA with time.

In this study, UV enhancement resulted in no significant changes in pigment concentrations in the *Phormidium* mat compared to the UV-excluded mats, though a general loss of all pigment types was observed with time under all treatments (by comparison to the time zero control). This indicates that under the experimental conditions, the *Phormidium* mat was not able to sustain pigment concentrations to the same levels as observed when it was growing naturally. However, the fact that both the chlorophyll *a* and carotenoid concentrations had significantly increased after 180 h compared to the 110 h in the UV-excluded mats suggests that, without UV stress, the mats were able to become acclimatised to the new conditions. Our analysis indicated some effect of UV enhancement on the pigment concentrations of the *Leptolyngbya* mat, most noticeably a significant loss of chlorophyll *a* at the 41.5 and 108 h time points. It is possible that this loss of chlorophyll may be the cause of the reduced photochemical yield under UV treatment, as the cellular concentration of chlorophyll is known to affect the magnitude of the PAM fluorescence signal [30].

Contrasting results were observed in a 60 day study of the effect of UV on bryophyte communities growing at Rothera Research Station, from mid-November to mid-December 1998 (K. Newsham, unpublished data) in which an elevated ratio of UV-B/total solar irradiance due to ozone depletion was correlated with an increase in concentrations of UV-B-absorbing compounds and carotenoids, but no effect on photosynthetic yield was observed. It was concluded that the rapid synthesis of photoprotective pigments protected the photosynthetic apparatus from damage by UV-B radiation.

However, field experiments on the effects of altered UV-B radiation on an Antarctic grass (*Deschampsia antarctica*) and lichen (*Turgidosculum complicatulum*) at Léonie Island (~5 km from Rothera Research Station), including UV-B enhancement using a UV lamp system, showed no evidence that altered UV-B levels had an effect on PS II yield in either species [31]. UV-B reduction did not influence the concentrations of chlorophyll, carotenoids or methanol-soluble UV-absorbing compounds in *D. antarctica*. A separate field experiment on Léonie Island demonstrated that UV-B enhancement had no effect on the photochemical yield of *Colobanthus quitensis*, but produced a significant negative effect on the photochemical yield of the moss *Sanionia uncinata* [16]. A long term experiment (two summer seasons) using screens to manipulate the UV environment of the higher plants *C. quitensis* and *D. antarctica* at Palmer Station, Antarctic Peninsula, showed that reducing incident UV-B radiation had no consistent effect on the concentrations or ratios of pigments, but a strong influence on growth and development was observed (photosynthesis was not measured) [32,33].

Thus it appears that UV-B radiation can have no mea-

surable effect on some Antarctic biota, but induces changes in pigmentation, photosynthesis or growth in others. This could be due to the inherent resistance of an organism to UV-B radiation, or reasons of experimental design. For example, organisms may initially respond to an additional dose of UV-B by increased photoprotective pigment production or an inhibition of photochemical yield, which would be measurable over a short time period, but over a whole season recovery would occur, and no net effects of UV-B radiation be observed. In this study we have demonstrated that two different types of cyanobacterial mat growing adjacently have very different photoprotective pigments and show differing sensitivity to enhanced UV radiation. However, we have also shown that the choice of experimental control can lead to different interpretations of UV-related biological effects.

Although UV-B radiation had a negative effect on the photochemistry of the *Leptolyngbya* mat, by far the greatest influences on chlorophyll fluorescence in this study were the ambient light and temperature conditions. The relationship is complex, with a light saturation effect at relatively low PAR intensities, and a linear effect of temperature. The effective PS II quantum yield values presented here for Antarctic terrestrial cyanobacterial mats (0 to ~ 0.45) are similar to those measured under natural field conditions in Venezuela by Lüttge et al. [34] despite the temperatures in the Venezuelan study being up to 30°C higher. The decrease in $\Delta F/F_m'$ above a certain light intensity is commonly observed in cyanobacteria [35]. The photosynthesis–irradiance curves shown in Fig. 2c,d show that saturation irradiance for electron transport rates ($\sim 62 \text{ W m}^{-2}$) is over twice the irradiance measured for the saturation of oxygen exchange for sun-adapted microbial mats from the McMurdo region of Antarctica [36]. It is to be expected that the cyanobacterial mats from Léonie Island are acclimatised to high light intensities, as they were obtained from non-shaded environments (though for much of the year in the Antarctic, low light intensity prevails). More surprising are the similarities of the light responses of the cyanobacterial mats used in this study, despite being of different species composition, containing different carotenoids, and different concentrations of chlorophyll *a*.

Although the spectral output of UV-B lamps is not identical to that of solar radiation [17], an analysis of total UV-B radiation dose (ambient plus UV lamp) received by the cyanobacterial mats showed that the enhancement regime gave the mats approximately half the dose of biologically damaging radiation (as calculated using the GPD action spectrum) as they would receive on a cloudless day later in the same year, under severe ozone depletion. However, due to the relatively low PAR levels during the experimental period, the UV-B:PAR ratios were comparable or higher than those observed under severe ozone depletion.

A study of the pigments in a range of cyanobacterial

mats from the McMurdo Sound region of Antarctica found ratios of carotenoids/chl-*a* ranging from 0.4 to 2.72, comparable with the range measured here (~ 0.4 for *Phormidium*, ~ 1.2 for *Leptolyngbya*) [19]. The concentrations of chl-*a* in the *Phormidium* mat were similar to those measured in cyanobacterial culture studies (~ 7 – $10 \mu\text{g mg}^{-1}$ dry weight chl-*a* was found in *Nostoc* cultures [37], and in cultures of *Phormidium murrayi* isolated from Antarctica, chl-*a* concentrations ranged from 3 to $10 \mu\text{g mg}^{-1}$ dry weight). The *Leptolyngbya* mat contained lower concentrations of chl-*a* ($\sim 1 \mu\text{g mg}^{-1}$ dry weight) than this, but the ratio of total photoprotective pigments (MAAs and carotenoids) to chl-*a* in both the mats was similar (~ 1.4 – 1.5).

MAA concentrations of 0.9 – $8.4 \mu\text{g mg}^{-1}$ have been measured in cyanobacterial isolates [4], and ratios of MAA to chl-*a* in the range of 0.04 – 0.19 were reported in cyanobacterial mats from the benthic environment of lakes, ponds and streams of the Canadian high Arctic [38]. The *Phormidium* mat contained comparable concentrations to the cyanobacterial cultures, with a far higher ratio to chl-*a* (~ 1.3) than the Arctic mats. The ratio of MAA-like compounds to chl-*a* in the *Leptolyngbya* mat was comparable with the highest observed ratio in the Arctic cyanobacterial mats. However, this high ratio is a function of the low chl-*a* concentrations in the *Leptolyngbya* mat, rather than a high concentration of MAA-like compounds. The relatively low concentration of MAA-like compounds (which absorb radiation in the UV-B region despite showing maximal absorbance at 330 nm in the UV-A region) may be responsible for the sensitivity to UV-B of the *Leptolyngbya* mat compared to the *Phormidium* mat.

The carotenoids myxoxanthophyll, zeaxanthin, echinone and β -carotene are commonly found in cyanobacteria [39], and were present in both the *Phormidium* and *Leptolyngbya* mats (Table 3). The presence of chlorophyll *b* in the *Phormidium* mat indicates a small algal component. The presence of significant amounts of the diatom pigments fucoxanthin and diadinoxanthin (6.6% and 3.3% of the total acetone-extracted pigment content, respectively) reflect the high number of diatoms found in the *Leptolyngbya* mat, and chlorophyll *c* again indicates that some algae were present [29]. The *Phormidium* mat did not contain significant numbers of diatoms even though it was collected from runnels emerging from the pool in which the *Leptolyngbya* mat was found.

In a study of the MAA content of 20 strains of cyanobacteria isolated from habitats exposed to strong insolation, belonging to 13 genera, most contained at least two different types of MAA, with some up to four [4], so it is notable that each mat from Léonie Island used in this experiment contained only one type of MAA-like compound.

This study presents evidence that the *Phormidium*-dominated cyanobacterial mat from Léonie Island shows

greater tolerance to elevated UV-B:PAR ratios compared to a *Leptolyngbya* mat growing adjacently (Fig. 3a,b). The *Leptolyngbya*-dominated mat contained much lower levels of photoprotective pigments, but a relatively high ratio of protective pigments to the concentration of chlorophyll *a*. As these two mats often grow in adjacent sites, during a period of elevated UV-B the enhanced UV tolerance of the *Phormidium* mat might give it some competitive/ecological advantage over the neighbouring community of *Leptolyngbya*. Therefore, the different UV sensitivities demonstrated in this study provide support for the proposal of Vincent and Quesada [6] that subtle shifts in composition and interaction of cyanobacterial communities may occur as a result of change in UV stress.

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