UV-B screening potential is higher in two cosmopolitan moss species than in a co-occurring Antarctic endemic moss – implications of continuing ozone depletion

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UV-B screening potential is higher in two cosmopolitan moss species than in a co-occurring Antarctic endemic moss – implications of continuing ozone depletion.

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Running title. UV SCREENING POTENTIAL OF 3 ANTARCTIC MOSSES

Abbreviations: ASPA, Antarctic Specially Protected Area; DU, Dobson unit; RR, Robinson Ridge; TSR, total solar radiation; TUV, total ultraviolet; UV, ultraviolet; UV-B, ultraviolet-B.

Abstract

Concentrations of UV-B absorbing pigments and anthocyanins were measured in three moss species, over a summer growing season in Antarctica. Pigment concentrations were compared with a range of climatic variables to determine if there was evidence that pigments were induced by UV-B radiation, or other environmental parameters, and secondly if there were differences between species in their pigment responses. Significant seasonal differences in the potential UV-B screening pigments were found, with the two cosmopolitan species *Bryum pseudotriquetrum* and *Ceratodon purpureus* appearing better protected from the potentially damaging effects of ozone depletion than the Antarctic endemic *Schistidium antarctici*. *Bryum pseudotriquetrum* accumulated the highest concentration of UV-B screening pigments and showed positive associations between UV-B radiation and both UV-B absorbing and anthocyanin pigments. The negative associations between water availability measures and UV-B absorbing and anthocyanin pigments also suggest that *B. pseudotriquetrum* is well protected in the desiccated state. This could offer *B. pseudotriquetrum* an advantage over the other species when high UV-B radiation coincides with low temperatures and low water availability, thus limiting physiological activity and consequently, active photoprotective and repair mechanisms. Since these pigments could act as either direct UV-B screens or antioxidants, the results suggest that *B. pseudotriquetrum* is best equipped to deal with the negative effects of increased exposure to UV-B radiation due to ozone depletion. The most exposed species, *C. purpureus*, has intermediate and stable concentrations of UV-B absorbing pigments suggesting it may rely on constitutive UV-B screens. Anthocyanin pigments were more responsive in this species and could offer increased antioxidant protection during periods of high UV-B radiation. *Schistidium antarctici* appears poorly protected and showed no evidence of any UV photoprotective response, providing additional evidence that this endemic is more vulnerable to climate change.

Introduction

Depletion of stratospheric ozone, resulting from anthropogenic, atmospheric pollution has led to increased ultraviolet (UV) radiation at the Earth's surface as well as a spectral shift to the more biologically damaging shorter wavelengths (Frederick & Snell, 1988). The decrease in ozone has been most pronounced and consistent over Antarctica with record levels of austral ozone depletion in the last decade (Bodeker *et al.*, 2001, McKenzie *et al.*, 2003, Robinson *et al.*, 2003). As a consequence Antarctica now experiences unseasonably high UV-B radiation through much of the spring, caused by the combined effects of the 'ozone hole' and the approach of the natural annual radiation peak, the summer solstice (Frederick & Snell, 1988, Roy *et al.*, 1994). Recovery of the Antarctic ozone hole is currently predicted by 2050, but remains a topic of intense research interest (McKenzie *et al.*, 2003).

Currently less than 2% of Antarctica is ice free, severely limiting options for plant colonisation. Furthermore, the harsh climate of continental Antarctica, where even summer temperatures barely reach above zero, restricts terrestrial vegetation to cryptogams. These organisms are desiccation and freezing tolerant, and able to survive frozen beneath snow during the long polar winter. The emergence from snow and the start of the short growing season currently coincides with peak levels of UV-B radiation due to ozone depletion. Since cryptogams are a major component of the vegetation in both polar regions their response to elevated UV-B radiation is of particular interest.

In general, organisms which are native in habitats with naturally high UV-B exposure (e.g. alpine and tropical areas) tend to have better developed mechanisms for UV-B tolerance than natives of lower UV environments (Caldwell *et al.*, 1982, Barnes *et al.*, 1987, Ziska *et al.*, 1992). Historically, Antarctic plants were growing under the lowest UV-B levels on Earth, now, as a result of ozone depletion, they are exposed to some of the highest, with little time for evolutionary adjustment and acclimation (Madronich *et al.*, 1995).

Bryophytes may be particularly susceptible to UV-B damage because of their simple structure, with most lacking differentiation and the protective cuticle or epidermal layer of higher plants. Combined with the physiologically stressful effects of repeated freeze/thaw cycles, an intermittent water supply and limiting nutrients, polar bryophytes are likely to be sensitive to the additional stress imposed by elevated UV-B radiation (Robinson *et al.*, 2003, Wasley *et al.*, 2006a, Wasley *et al.*, 2006b). The survival of Antarctic bryophytes under ozone depletion depends on their ability to acclimate to increasing UV-B radiation by employing photoprotective mechanisms to avoid or repair UV-B damage (Jansen *et al.*, 1998). UV-B absorbing pigments are widespread across the plant kingdom, due to their ability to absorb biologically damaging UV-B radiation while transmitting essential photosynthetically active radiation (Cockell & Knowland, 1999). A meta-analysis of field studies revealed that the most striking and consistent response of plants to increased UV-B radiation was an increase in UV-B absorbing pigments, on average by 10% (Searles *et al.*, 2001). A similar study of Arctic plants also showed increases in UV-B screening or radical scavenging compounds as the major response to increasing UV-B radiation (Dormann & Woodin, 2002). However, high latitude, southern hemisphere vascular plants do not show such consistent accumulation of UV-B absorbing compounds (Day *et al.*, 2001, Giordano *et al.*, 2003). The accumulation of UV-B absorbing pigments could be particularly useful in polar and alpine bryophytes, since when such plants are physiologically inactive during desiccation or freezing, passive screens would provide more effective protection from UV-B damage than repair mechanisms which require an active metabolism (Cockell & Knowland, 1999).

Flavonoids are important UV-B absorbing pigments, which can be induced within hours in response to UV-B radiation, and are ubiquitous in higher plants (Cooper-Driver & Bhattacharya, 1998). They have been extracted from about half of the bryophyte species examined (Markham, 1990). Flavonoids from herbarium specimens of Antarctic *Bryum* *argenteum* were also shown to correlate with historical ozone levels suggesting the possibility that these were actively induced UV-B screens (Markham *et al.*, 1990). Recently, high concentrations of UV-B absorbing pigments have also been reported in two Antarctic mosses, *Sanionia uncinata* and *Andreaea regularis,* and one liverwort, *Cephaloziella varians*, with positive correlations between pigment accumulation and flux of natural solar UV-B radiation (Newsham *et al.*, 2002, Newsham, 2003, Newsham *et al.*, 2005). Conversely, UV-B absorbing pigments decreased or showed no change in response to elevated UV-B levels in seven European, Arctic and South American moss species (Barsig *et al.*, 1998, Gehrke, 1998, Gehrke, 1999, Searles *et al.*, 1999, Niemi *et al.*, 2002a, Niemi *et al.*, 2002b, Martínez-Abaigar *et al.*, 2003). As a result of these studies it has been suggested that mosses are less likely to synthesise UV-B absorbing pigments than other plant groups and are potentially more vulnerable as a functional type (Gwynn-Jones *et al.*, 1999). Although relatively few mosses have been studied, negative effects of UV- radiation on moss growth and morphology have been reported for some high latitude species (Sonesson *et al.*, 1996, Searles *et al.*, 1999, Searles *et al.*, 2002, Robson *et al.*, 2003, Robinson *et al.*, 2005,).

Anthocyanins are a type of flavonoid, which absorb strongly in the visible region of the spectrum with a tail in the UV. There is debate about their effectiveness as UV-B screens (Cockell & Knowland, 1999, Gould, 2004), although recent studies have shown that polyacylated anthocyanins can offer protection from UV-B radiation (Mori *et al.*, 2005). It has also been suggested that anthocyanins may reduce the rate of DNA photoproduct repair by filtering out the blue light needed to activate the photolyases that catalyse such repair (Hada *et al.*, 2003). Anthocyanins, like other flavonoids, have antioxidant activity and thus may indirectly increase tolerance to UV-B radiation by neutralising free radicals (Husain *et al.*, 1987). High levels of anthocyanin-like pigments have previously been measured in one of the Antarctic mosses examined in this study, *Ceratodon purpureus,* and in the liverwort *Cephaloziella varians* (Post, 1990, Newsham *et al.*, 2005).

In this study we measured concentrations of UV-B absorbing pigments and anthocyanins in three Antarctic moss species over a summer growing season. Pigment concentrations were compared with a range of climatic variables to determine if there was evidence that pigments were induced by UV-B radiation, or other environmental parameters, and secondly if there were differences between species in their pigment responses. The Antarctic endemic moss *Schistidium antarctici,* was compared with the other two native mosses of the Windmill Island region of East Antarctic, *Bryum pseudotriquetrum* and *C. purpureus.* Our hypothesis was that the endemic species *S. antarctici* would have less protection from UV-B radiation than co-occurring cosmopolitan species since this moss has been shown to be vulnerable to current levels of UV-B radiation (Robinson *et al.*, 2005).

Materials and Methods

Study sites and sampling

The Windmill Islands region (centred at 66˚22' S, 110˚30' E) consists of a series of ice-free islands and peninsulas along the eastern coastline of Antarctica. The landscape consists of low rounded hills and rocky outcrops, with a maximum altitude of 109 m a.s.l., separated by intervening valleys filled with snow or glacial moraine. The climate is classified as frigid Antarctic *(sensu* Longton, 1988*)*. Meteorological data from Casey Station, the Australian base located within this region, reports the mean temperature for the warmest and coldest months as 0.3 ˚C and – 14.9 ˚C respectively, with extremes of 9.2 ˚C to – 41 ˚C. The dry mean annual snowfall is 224.6 mm (rainfall equivalent) and there are frequent gale force winds (mean 96 days pa) predominantly blowing in an easterly direction off the polar ice cap (Melick & Seppelt, 1997). Despite this harsh climate, the Windmill Islands region supports some of the most extensive and complex bryophyte communities on the Antarctic continent.

Three moss species are found in the region, *Schistidium antarctici* Card. (previously known as *Grimmia antarctici*) is endemic to the Antarctic continent, while both *Bryum pseudotriquetrum* (Hedw.) Gaertn. and *Ceratodon purpureus* (Hedw.) Brid. have widespread cosmopolitan distributions. Within the bryophyte community the species distribution generally follows the moisture gradient, with *S. antarctici* forming extensive turves in the low lying wetter areas, *C. purpureus* more common in the higher, drier areas and *B. pseudotriquetrum* co-occurring with both. This distribution relates to the desiccation tolerance of each of the three species (Robinson *et al.*, 2000, Wasley *et al.*, 2006a, Wasley *et al.*, 2006b).

Samples were collected from three sites in the Windmill Islands region, where well-developed moss turves containing all three species could be identified. Two sites were within the

Antarctic Specially Protected Area (ASPA) 135 on Bailey Peninsula about 1 km from Casey Station. The most easterly site, designated ASPA 1, was located along the western edge of a large melt lake (66˚16.03' S, 110˚32.53' E). One hundred metres west over a rocky ridge the second site, designated ASPA 2, lay in a slight depression with a small melt lake forming in the centre (66˚16.92'S, 110˚32.36'E). The third site was located 10 km south-southwest at Robinson Ridge (RR) on a gentle slope with a north-easterly aspect (66˚22.1'S, 110˚35.2'E).

Each species was sampled six times from each site over the 1999/2000 summer season (total 14 sampling dates). Samples were collected as close to solar noon as possible. The first samples were taken in late November, as soon as substantial turf was exposed from under winter snow cover, and then every two to four weeks until the end of the season (28th, 30th November, 2nd, 6th, 17th, 21st December, 4th, 6th, 20th, 24th, 31st January, 5th February, 10th, 13th March). A total of 143 samples, (n=49 for *S. antarctici;* n=47 for *B. pseudotriquetrum* and n=47 for *C. purpureus*) were taken. Each sample was obtained by removing a 1 cm^2 plug of moss from the turf and cutting off the top 2-6 mm (the photosynthetically active apices). The base was returned to the moss turf to minimise destructive impact.

Plant analysis

All samples were weighed and dried to constant weight over desiccant. UV-B absorbing and anthocyanin pigments were then extracted from these dry samples. UV-B absorbing pigments were extracted in acidified methanol (methanol:H_2O:HCl; 79:20:1) and analysed using the method described in Lovelock & Robinson (2002). Anthocyanin concentrations were determined from a subset of samples (n=40 for *S. antarctici*; n=38 for *B. pseudotriquetrum* and n=42 for *C. purpureus* total=120). Pigments were extracted in 1% HCl in methanol (Lovelock & Robinson, 2002) and quantified using the differential pH method of Francis (1982) as modified by Lovelock & Robinson (2002). Turf water content of each sample was determined as described in Robinson *et al.* (2000).

Climate data

Climatic data covering October 1999 through to March 2000 was obtained from the Australian Bureau of Meteorology (Casey Station). This data included three hourly observations of air temperature (˚C), cloud cover (eighths of the sky), relative humidity (%) and wind speed (km hr^{-1}). Additional daily data included total sunshine (hrs), minimum and maximum air temperature (˚C), maximum wind speed (km hr^{-1}) and snowfall amount (mm - rainfall equivalent). Day length, measured as the time between sunrise and sunset, was obtained from the Auroral Space Physics (ASP) department at Casey Station.

Radiation measurements were obtained from Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) sensors situated on the roof of the station's accommodation building. The sensors used provided UV-B (International Light UVB radiometer, wavelength range 280 - 315 nm), total ultraviolet (TUV; Eppley total UVR radiometer; 290 - 400 nm) and total solar (TSR; Eppley precision spectral pyranometer; 285 - 2800 nm) radiation measures. Data from each sensor were downloaded as counts 100 times a minute, averaged every 10 minutes and then corrected for drift and error and converted to daily means (W m^{2}) by John Javornisky of ARPANSA (for details see Roy *et al.,* 1998). Data on the thickness of the ozone layer above the Windmill Islands region were obtained from the National Aeronautical and Space Administration web site (NASA, 2006).

Data analysis

Regression analyses were performed to determine which individual and combined parameters best predicted the concentrations of UV-B screening or anthocyanin pigments. Pigment data was analysed for each species separately. Linear regression was used to build a model for pigment changes over the season, to determine differences in the direction and magnitude of the response of the three moss species to various climatic parameters. Environmental factors were considered individually and as part of multiple regression models. Site was a common factor in all multiple regression models and was fitted first. For each environmental factor, the means and maxima for the 24 h, 5 d and 10 d preceding moss sampling were calculated and fitted into models. The best fit for each individual environmental parameter (1, 5 or 10 d) was then included in the respective models. The environmental predictor factors that were common to models could be separated into two groups. The first group contained factors related to radiation flux and included 10 d means and maxima of radiation parameters, ratios of radiation parameters (such as UV-B/TUV radiation, UV-B radiation/TSR) and factors which could influence radiation such as cloud cover and ozone layer thickness. The second group were parameters which either measured or were likely to affect water availability, including turf water content, 10 d means of air temperature, snowfall, relative humidity and wind speed. Due to the high degree of correlation between radiation parameters, all could not be included in the model, thus a substitution process was utilised. Similarly only one measure (1, 5 or 10 d mean) per environmental factor was included in any particular model. Due to the high number of environmental variables, stepwise regression was employed initially to determine which parameters were most significant. A process of substitution then compared all possible combinations of multiple regression models and the best selected by optimising r^2 *adj*.

Anthocyanin and UV-B absorbing pigment data were transformed (log or square root respectively) to achieve homoscadascity and normality. Statistical analyses were conducted using JMP 5.1 and SAS 10 (SAS Institute, Cary, NC, USA) computer packages.

Results

Variation in radiation, and related parameters, across the season

Ozone layer thickness above the Windmill Islands oscillated about a mean of 313 Dobson Units (DU) over the season (Figure 1A). These oscillations were greatest in October, with ozone layer thickness varying from the season minimum of 187 DU to the maximum of 427 DU over a couple of weeks. These oscillations decreased in amplitude through November and December with much more stable values, ranging between 270 and 340 DU, throughout January to March.

Cloud cover was quite high, averaging 75% for the season, but varying widely between days from clear sky (0) to overcast (8) and displaying no seasonal trend (Figure 1B). There were however periods from a few days to a couple of weeks where conditions were consistently cloudy such as early October, late December to early January and late January, or consistently clear as in early November, early December and late January. Day length increased from 13 h in mid October to a maximum of almost 23 h around the summer solstice, December 21-23, before decreasing to 11 h in late March.

UV-B radiation varied widely between days, however there was an obvious seasonal trend of rapid increase in October (0.03 Wm^{-2} to 0.24 Wm^{-2}), sustained high levels through November and December and then a decrease to low levels in February (Fig 1C). The maximum UV-B (0.32 Wm^{-2}) was measured on December 14. The ratio of UV-B to total UV radiation (UV-B/TUV) increased rapidly in October, then remained relatively constant before declining more slowly during February (Fig 1D).

Seventy seven percent of the variability in UV-B radiation over the season was explained by a multiple regression model with three terms; day length, cloud cover and ozone layer thickness (Table 1). UV-B radiation showed a significant positive association with day length and significant negative associations with cloud cover and ozone layer thickness. A similar three term model explained 80% of the variability in UV-B/TUV, however in this case there were significant positive associations with day length and cloud cover and a significant negative association with ozone layer thickness. For TUV radiation, cloud cover (negative association) and daylength (positive association) explained 78% of the variability and the addition of ozone depth did not improve the model.

Variation in other environmental factors across the season

Air temperature was variable within and between days, however a seasonal trend was evident with air temperatures lowest in October, increasing to a peak in January and then declining during March (Fig 2A). In early October daily mean air temperatures were less than –10 ˚C, with a minimum of –22.8 ˚C. Variability of air temperature was particularly high during mid-late October, with the daily mean ranging between –4 ˚C and –17 ˚C. Throughout November and December this variability decreased and air temperature steadily increased. The maximum temperature over the season occurred on January 4 (4.8 ˚C). The threshold for snowmelt, air temperatures above 0 ˚C, was reached on almost half the days between December and February. By March, variability had increased and daily mean air temperatures fluctuated between –2 ˚C and –13 ˚C with a minimum of –17 ˚C on March 8. In Antarctica the temperature of moss beds is often 10 - 20 ˚C higher than the air temperature, especially on clear days with high radiation load, see Beyer and Bölter (1998) for measurements at Casey, and Newsham *et al.* (2002) for maritime Antarctica. Relative humidity varied over the summer ranging from 40-100% but there was little seasonal pattern apart from a period of low relative humidity in early December (Figure 2B).

Wind speed was high and variable over the season and showed no seasonal trend (Figure 2C). Mean daily wind speed was 24 km hr^{-1} for the season and ranged from 6 to 98 km hr^{-1}. On 15 days throughout the season daily mean wind speeds were greater than 50 km hr^{-1}. Gale force winds (>63 km hr^{-1}) occurred on 62 days with gusts greater than 100 km hr^{-1} on 30 days. A maximum gust speed of 172 km hr^{-1} was measured on the 13th October. Wind direction was predominantly easterly.

Snowfall occurred on 56 days throughout the season with 19 days of blowing snow, and blizzard conditions on 11 days (data not shown). Monthly total snowfall displayed a seasonal trend with most snowfall early and late in the season (Figure 3A).

Turf water content, which gives a spot measure of water availability to individual moss samples, ranged between 0.13 gH_20 g^{-1}dw and 4.21 gH_20 g^{-1}dw but was not significantly different between species (mean values 1.89 ± 0.14, 1.78 ± 0.13, 1.91 ± 0.17 for *B. pseudotriquetrum*, *C. purpureus* and *S. antarctici,* respectively). Turf water content was similar across sites early in the season but the ASPA1 site maintained a high water content until snow covered the site in early March, whilst the other two sites remained exposed and thus dried out during February (Fig 3B, site by sampling date interaction, $F_{2,51} = 6.32$, $P = 0.004$).

Variation in plant pigment concentration across the season.

Concentrations of UV-B absorbing pigments were significantly different between all three species, with *B. pseudotriquetrum* having four- and two- fold higher concentrations than *S. antarctici* and *C. purpureus* respectively (Fig 4, $F_{2,51} = 574$, $P< 0.0001$). Highest concentrations of UV-B absorbing pigments were measured in *B. pseudotriquetrum* in early December (693 $A_{280\text{-}320}$ g^{-1} dw) and concentrations declined by more than half through the remainder of the season (March minimum 305 $A_{280\text{-}320}$ g^{-1} dw ^{1}). Concentrations of UV-B absorbing pigments ranged between 54.9 and 179 $A_{280\text{-}320}$ g^{-1} dw in *S. antarctici* and between 150 and 347 $A_{280\text{-}320}$ g^{-1} dw ^{1} in *C. purpureus* but there was no evidence of a seasonal trend in either of these species.

UV-B absorbing pigment concentration also varied across the three sites (site x species interaction $F_{4,49} = 7.68$, $P < 0.0001$). In *B. pseudotriquetrum* UV-B absorbing pigments were higher at ASPA1 than at RR but the decline in pigment concentration over the season was apparent at all locations (Fig 4). In *S. antarctici* pigment concentration was higher at ASPA2 than at ASPA1, whilst concentration at the RR site was intermediate. *Ceratodon purpureus* had similar concentrations of UV-B absorbing pigments across all sites.

Anthocyanin concentration in all three species was highly variable but there was no evidence of seasonal trends. Anthocyanin concentrations in *S. antarctici* were significantly higher at ASPA2 than at the RR or ASPA1 sites (Table 2, site by species interaction $F_{4,48} = 5.55$, $P = 0.001$) and a similar, but not significant pattern was observed for *C. purpureus*. *Bryum pseudotriquetrum* had similar concentrations of anthocyanin across all sites.

Interactions between environmental factors and potential UV-B screening pigments

Bryum pseudotriquetrum

The best single predictors for UV-B absorbing pigments in *B. pseudotriquetrum* were a negative association with 10 d mean humidity, which explained 47% of the variability (Figure 5A, Table 3), and a positive association with 10 d mean TUV radiation, which explained 39% of the variability (Figure 5B, Table 3). Amongst the radiation parameters the 10 d means of UV-B radiation and TSR and the 10 d maximum UVB/TUV showed very similar regression relationships ($r^2 > 36\%$) whilst the 10 d maximum UVB/TSR and 5 d mean day length also gave strong positive correlations ($r^2 > 32\%$, Table 3). Other radiation parameters were tested, including 1 and 5 d mean and maximum values and their respective ratios, but although significant, these were all worse predictors ($r^2 < 27\%$). The 10 d mean snowfall was the only other non-radiation parameter that gave a significant relationship ($r^2=27\%$, Table 3). Radiation parameters were positively correlated with UV-B pigment concentration whilst water related environmental parameters, including snowfall, gave negative correlations (Table 3).

The multiple regression model for UV-B absorbing pigments in *B. pseudotriquetrum* included six terms (site, turf water content and 10 d means of UV-B (or TUV) radiation, relative humidity and cloud cover, Table 4) and explained 96% of the variability. The model described positive relationships with UV radiation and cloud cover and negative associations with the water availability parameters (relative humidity and turf water content). Site was significant, as described above, with pigment concentration highest at the ASPA1 and lowest at the RR site. Substituting the other radiation parameters shown in Table 3 into the model gave lower r^2 values.

None of the environmental variables tested were significant single predictors for anthocyanin concentration in *B. pseudotriquetrum*. The multiple regression model for anthocyanin concentration included four terms (site, turf water content and 10 d mean of air temperature and 10 d maximum UV-B/TSR, Table 5) and explained 63% of the variability. Significant factors in this model were air temperature (negative relationship) and maximum UV-B/TSR (positive relationship).

Ceratodon purpureus

There were no significant single predictors for UV-B absorbing or anthocyanin pigments in *C. purpureus.* The multiple regression model for UV-B absorbing pigments included five terms (site, turf water content and 10d means of relative humidity and wind speed, Table 4) and explained 72% of the variability. Site differences were the only significant factor in this model with UV-B pigment concentration lower at RR than the ASPA sites. The environmental predictors all showed slight (but not significant) positive relationships to pigment concentration.

For anthocyanins the multiple regression model included five terms (site, 10 d mean ozone depth, cloud cover and wind speed, Table 5) and explained 85% of the variability. Significant factors included a positive association with wind speed and negative associations with cloud cover and ozone depth. Site was a significant predictor with higher anthocyanin concentration in *C. purpureus* from the ASPA2 site (Table 2).

Schistidium antarctici

There were no significant single predictors for either UV-B absorbing or anthocyanin pigments in *S. antarctici.* The multiple regression model for UV-B absorbing pigments included three terms (site and 10 d mean snowfall, Table 4) and explained 71% of the variability. The relationship between pigment concentration and snowfall was positive.

For anthocyanins, the multiple regression model included three terms (site and 10 d mean air temperature, Table 5) and explained 71% of the variability. Site differences were the only significant factor in this model with anthocyanin concentration highest at the ASPA2 site (Table 2). There was a slight ($P<0.1$) negative association with air temperature.

Discussion

The ozone layer thickness was highly variable during October, November and to a lesser extent, December. The circulation of the polar vortex combined with the coastal location, resulted in the edge of the often elliptical shaped ‘ozone hole’ passing over the Windmill Islands region three times during October and November. These low ozone levels were reflected in elevated UV-B receipt at Casey. During October, day length was relatively short and cloud cover high, thus UV-B flux was variable but relatively low. However, ozone depletion and clear sky conditions early in November resulted in high UV-B levels which were maintained throughout the month and into December as the solar angle increased with the approach of the summer solstice. These results emphasise that ozone depletion leads to an extended season of elevated UV-B flux superimposed over a highly variable light environment. As expected, the flux of UV-B radiation was strongly associated with the depth of the ozone column, cloud cover and day length (which is a proxy for solar zenith angle; McKenzie *et al.*, 2003).

These three moss species are at the physiological and geographical limits of their distribution and might be expected to be extremely sensitive to additional climatic variation. The climate parameters that were included in the models could be separated into five groups; radiation, water availability, air temperature, wind speed and snowfall. The first three of these are likely to be the most important factors determining growth, productivity and ultimately survival of these plants. Wind speed and snowfall are also likely to influence water availability but, in addition, physically affect the exposure of the plants. In the extreme Antarctic environment these climatic factors can also be a source of plant stress, alone or in combination. The parameters influencing radiation (including cloud cover) and water availability (humidity/TWC) were each common to three of the four models involving the cosmopolitan species (*B. pseudotriquetrum* and *C. purpureus*) but were not included in either model for *S.*

antarctici. Air temperature was included in two models (*B. pseudotriquetrum* and *S. antarctici*), whilst wind speed was common to both *C. purpureus* models and snowfall to the UV-B pigment model for *S. antarctici*. Pigments also varied between the three sites in both *B. pseudotriquetrum* and *S. antarctici* as described previously for the same or closely located sites (Lovelock & Robinson, 2002, Robinson *et al.*, 2005). Given that the RR site was further away from the station, and thus from the site of meteorological and radiation measurements, it is likely that these parameters were less accurate for this site. This may have introduced greater variation and could confound the site differences, since weather patterns tend to move along the coast and there would be slight temporal differences in daily weather between the RR and ASPA sites. Despite this, excluding the RR data gave similar results for other environmental parameters.

The importance of free water to poikilohydric Antarctic bryophytes is highlighted by water availability parameters being common to three pigment models. Water availability is a major factor determining moss distribution in the Antarctic, both on a broad scale relative to lichens, and at a micro scale determining species distribution within moss beds (Lewis Smith, 1999). Melt water is the only water available to these plants and consequently moss water content varies as a result of climatic factors and topographical site effects. Snow cover over the moss beds started melting from all three sites during late November (Dunn *pers. obs.*) resulting in transiently high water contents. Major melt of the remaining snow occurred in January once temperatures rose sufficiently providing a more reliable water source. The timing and extent of snowmelt is known to have major impacts on polar and alpine plants (Callaghan *et al.*, 2004, Wahren *et al.*, 2005). In addition to being the only source of water, snow banks may protect moss beds from UV-B radiation during springtime ozone depletion.

The two cosmopolitan species had higher concentrations of UV-B absorbing compounds with *B. pseudotriquetrum* having the highest overall, *C. purpureus* intermediate levels and the endemic *S. antarctici* showing the lowest concentration. Associations between pigment concentrations and environmental parameters were strongest in *B. pseudotriquetrum* and weakest in *S. antarctici*.

Bryum pseudotriquetrum was the only species that showed an unequivocal change in potential screening pigments over the season with UV-B absorbing pigment concentration positively associated with UV radiation. The UV radiation parameters and site variability produced the best 3-term model, explaining 84% of the variability and supporting radiation as a strong environmental predictor. Although all the radiation parameters tested were highly co-correlated, UV-B and TUV radiation consistently gave better results than TSR supporting our hypothesis that the UV component of solar radiation is responsible for this effect rather than radiation *per se*. This provides strong evidence that these pigments play a specific role in UV-B protection in *B. pseudotriquetrum*. These pigments are most likely flavonoids, since these have been measured previously in *Bryum* species (Markham & Given, 1988).

The concentration of UV-B absorbing compounds in *B. pseudotriquetrum* displayed a seasonal decline. Particularly high concentrations were measured early in the season, when ozone depletion and the high solar angle result in high UV-B radiation levels. High UV-B radiation induces synthesis of flavonoid compounds in higher plants (Lois, 1994, Cuadra & Harborne, 1996). The high UV-B absorbing pigment concentration in *B. pseudotriquetrum* at the start of measurements suggest that accumulation of screening pigments occurs rapidly at the start of the season, possibly even prior to total snow melt. For this study the earliest samples were taken once snow had receded from the entire moss bed and they would have experienced several days exposure to radiation prior to the initial sampling. There is some evidence of lower concentrations of UV-B absorbing pigments at the very first sampling time (Nov 28) but earlier sampling, possibly from under snow, would be required to confirm this trend. Plants can synthesise flavonoids in less than a day (Markham *et al.*, 1990) and similarly rapid increases in UV-B absorbing pigments have been shown in other Antarctic bryophytes (Newsham *et al.*, 2002). The half-life for destruction of flavonoids is much slower (3-15 days, Markham *et al.*, 1990), which is consistent with our finding that the 10 d means for radiation and other environmental parameters gave the best relationship in models fitted whilst pigment concentration was declining. In a similar study of UV-B absorbing pigments in Antarctic bryophytes, Newsham (2002) found that daily radiation parameters gave the best fit, but these changes were measured for a month at the start of the season and likely determined synthesis rates for UV-B pigments. Anthocyanin pigments also showed a positive association with the radiation parameter (UV-B/TSR) in *B. pseudotriquetrum*, suggesting that these pigments might offer additional screening or antioxidant protection during high UV-B exposure. Markham *et al.* (1990) reported that flavonoid concentration was higher in herbaria samples of *Bryum argenteum* collected during seasons with greatest ozone depletion. This study confirms these results for live specimens of *B. pseudotriquetrum*, and shows that there is also a seasonal response to UV-B radiation.

UV-B absorbing pigments and anthocyanins also responded to water availability and air temperature parameters in *B. pseudotriquetrum* with highest concentrations of pigments under low humidity and TWC, or low temperature respectively. Accumulation of anthocyanins at low temperature is common in many plant species (Chalker-Scott, 1999). However, in Antarctica the role of temperature in determining water availability is an important factor and we cannot rule out the possibility that this response is primarily related to water availability. It is likely that both of these pigment groups play an antioxidant role under desiccated or frozen conditions, a role that would enhance protection from UV-B radiation. Flavonoids are effective at neutralising free radicals produced by both excess photosynthetically active- and UV-B - radiation (Husain *et al.*, 1987). At low water content, moss photosynthetic capacity will be reduced and excess light absorption becomes more problematic. The response of these pigments to water availability in *B. pseudotriquetrum* suggests that they may play an important antioxidant role in this species. The positive association between cloud cover and UV-B absorbing pigments is puzzling, it maybe related to increased scattering of UV-B radiation between clouds and snow cover, which is known to affect the incident flux of UV-B radiation in sites with strong surface albedo (Nichol *et al.*, 2003), but this was not an expected response. Given that radiation and humidity are strong single predictors for UV-B absorbing pigments it is not surprising that these contribute most to the model, with cloud cover and TWC only increasing r^{-2} from 87 to 96%.

Ceratodon purpureus had intermediate levels of UV-B absorbing pigments, approximately half that of *B. pseudotriquetrum* and twice that of *S. antarctici*. Although, this level of UV-B absorbing pigments is likely to offer some constitutive screening, there was no clear evidence of a seasonal trend, suggesting that this species does not regulate UV-B absorbing compounds specifically in response to elevated UV-B radiation. This is perhaps surprising given that *C. purpureus* is the first species to be exposed during snow melt, due to its location in the higher, drier areas (Wasley *et al.*, 2006b), and might be expected to be particularly susceptible to UV-B damage early in the season. Another possibility is that relative concentrations of individual flavonoid species are changing but not the overall concentration of UV-B absorbing pigments, as has been reported for the liverwort *Marchantia polymorpha* and suggested to possibly enhance antioxidant activity (Markham *et al.*, 1998).

Anthocyanin concentration in *C. purpureus* was higher when both cloud cover and ozone depth were low, suggesting that these pigments respond to higher radiation exposure and possibly even increasing UV-B radiation. Accumulation of anthocyanin-like pigments has previously been reported for this species in response to high radiation (Post, 1990) and a role in UV-B protection was postulated. The location of this species leads to greater exposure and it exhibits the highest tolerance of desiccation of the three species (Robinson *et al.*, 2000, Wasley *et al.*, 2006b). Anthocyanin concentration in *C. purpureus* also showed a positive association to wind speed, which is consistent with this species' increased exposure to drying winds and thus greater likelihood of experiencing desiccation stress.

The endemic species, *S. antarctici,* is particularly lacking in UV-B absorbing pigments. This is consistent with a previous two-season study where UV radiation was screened from *S. antarctici* moss beds and UV radiation was found to cause photo-oxidation of chlorophyll and morphological damage but no evidence of accumulation of UV-B screening pigments was observed (Robinson *et al.*, 2005). In general, *S. antarctici* is the last species to be exposed to full sunlight due to the persistence of snow cover in the low-lying areas where it grows (Wasley *et al.*, 2006b). Early in the season it may gain protection from this snow layer but since snow lying over moss beds had melted out by the end of November all mosses would be exposed to significant levels of radiation mid season. The fact that snowfall was the only significant environmental factor in the UV-B absorbing pigment model for *S. antarctici*, lends support to an important protective role of snow for this species. Loss of pigments by photo-oxidation is likely to occur once the snow melts and plants are exposed, thus explaining the positive association between snowfall and UV-B absorbing pigments.

Schistidium antarctici does possess relatively high levels of anthocyanins but the model suggests that these provide more protection from cold than from radiation. This endemic species is obviously well adapted to the cold conditions in continental Antarctica and currently is the dominant species in the Windmill Islands region. However, it appears to be the least well protected under increasing UV-B radiation or a drying climate (Robinson *et al.*, 2000, Robinson *et al.*, 2005, Wasley *et al.*, 2006b).

Recently it was concluded that Arctic and Antarctic bryophytes were no more sensitive to UV-B than vascular plants and are unlikely to be significantly impacted by 15-30% ozone depletion (Rozema *et al.*, 2005). The number of Antarctic bryophytes that have been studied to date is small, but Newsham and coworkers have shown that Antarctic *S. uncinata, A. regularis,* and *C. varians* showed rapid increases in UV-B absorbing pigments in response to natural increases in UV-B radiation and there was no evidence of adverse physiological affects on these species (Newsham, 2003, Newsham *et al.*, 2002, Newsham *et al.*, 2005). The response of *S. uncinata* to ozone depletion has been studied across both polar regions and this species appears to show little evidence of negative effects (Lud *et al.*, 2002). High potential for screening UV radiation, as measured by a UV-A chlorophyll fluorometer, has been demonstrated for Antarctic *Bryum subrotundifolium* and *C. purpureus.* Sun exposed plants of both species showed about 80% UV-A protection as against 48% and 60% for shade forms of *B. subrotundifolium* and *C. purpureus* respectively. Cellular screening of UV-A radiation also increased from 55% to 76% in shaded *B. subrotundifolium* exposed to full sun over 8 days (Green *et al.*, 2005). Lewis Smith (1999) reported that whilst *B. pseudotriquetrum* and *C. purpureus* showed no growth variation, a co-occurring species *Bryum argenteum* showed significantly more growth under reduced rather than ambient UV-B radiation, The latter species, like *S. antarctici*, was commonly found in wetter areas.

Our results for the cosmopolitan species are in agreement with this general finding of tolerance to current UV-B radiation. In contrast, our findings for *S. antarctici*, both here and previously (Robinson *et al.*, 2005), suggest poor tolerance of UV radiation. Although *S. antarctici* is the only endemic moss studied to date, we know too little regarding the colonisation history of these species to conclude much from this observation. Given the species-specific nature of the UV-B response in terrestrial plants (Zaller *et al.*, 2004) there is little reason to suppose that mosses behave as a consistent functional group with respect to UV-B radiation. The difference between the species may be related to their relative desiccation tolerance. Our results support the finding that high UV-B -tolerance is coincident with strong desiccation tolerance in bryophytes (Csintalan *et al.*, 2001, Lud et al., 2002) since

C. purpureus and *B. pseudotriquetrum* also show higher tolerance of desiccation than *S. antarctici.* In addition, *B. pseudotriquetrum* shows plasticity in its desiccation tolerance (Robinson *et al.*, 2000, Wasley *et al.*, 2006b), and in its production of UV screening pigments.

Conclusions

This study demonstrates significant seasonal differences in the potential UV-B screening pigments of three Antarctic moss species. The two cosmopolitan species appear better protected from the potentially damaging effects of ozone depletion than the endemic.

Bryum pseudotriquetrum was the only species that showed positive associations between UV radiation parameters and both UV-B absorbing and anthocyanin pigments. These pigments could act as direct UV-B screens or as antioxidants. The negative correlations between water availability measures and UV-B absorbing and anthocyanin pigments also suggest that *B. pseudotriquetrum* is well protected when it is in the desiccated state, and potentially most vulnerable. Our results suggest that only *B. pseudotriquetrum* responds to elevated UV radiation early in the season by accumulating UV-B screening pigments. This could offer *B. pseudotriquetrum* an advantage over the other species at a time when high UV-B radiation coincides with frequent low temperatures and low water availability, thus limiting physiological activity and consequently, active photoprotective and repair mechanisms. This species appears to be best equipped to deal with the negative effects of increased exposure to UV-B radiation due to ozone depletion.

Ceratodon purpureus had intermediate levels of UV-B absorbing pigments which were constant throughout the season. Its location in the most exposed conditions may thus lead to reliance on constitutive UV-B screens. Anthocyanin pigments are more responsive in this species and could offer increased antioxidant protection during periods of high UV-B radiation.

Schistidium antarctici appears poorly protected and shows no evidence of any UV photoprotective response. Our findings here support our UV-B exclusion and desiccation tolerance studies showing that this endemic species appears most vulnerable to climate change (Robinson *et al.*, 2005, Robinson *et al.*, 2000, Wasley *et al.*, 2006b).

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Table 1. Summary data from multiple regression analyses showing associations between mean daily ozone column thickness, cloud cover, daylength and radiation parameters (mean daily UV-B radiation (three terms), TUV radiation (two terms) and UV-B/TUV radiation (three terms)) from mid November 1999 through to mid March 2000, at Casey Station in the Windmill Islands region, Antarctica. Displayed are the significant parameters and significance of each effect in the model.

Radiation parameter	R^2	**Predictor variable**	$F_{1,94}$	**P-value**
UV-B/TUV	80%	Day length	258.4	<0.001
		Cloud cover	23.9	<0.001
		Ozone layer thickness	89.47	<0.001
UV-B	77%	Day length	222.8	<0.001
		Cloud cover	76.6	<0.001
		Ozone layer thickness	10.98	0.0013
TUV	78%	Day length	192.0	<0.001
		Cloud cover	116.5	<0.001

Table 2 Anthocyanin concentration in *Schistidium antarctici*, *Bryum pseudotriquetrum* and *Ceratodon purpureus* across three sites in the Windmill Islands region of East Antarctica (Date are means ± SEM, n=5,6). Values not connected by the same letter are significantly different, P = 0.05). Sites: ASPA = Antarctic Specially Protected Area, RR = Robinson Ridge.

	Anthocyanin concentration (A_{526}diff g^{-1}dw)		
Species	**ASPA1**	**ASPA2**	**RR**
S. antarctici	3.92 ± 0.59^{B}	9.24± 0.69^{A}	5.67 ± 0.92^{B}
B. pseudotriquetrum	6.24 ± 0.42^{AB}	5.49 ± 0.83^{B}	6.17± 0.71^{B}
C. purpureus	3.47 ± 0.50^{B}	6.32 ± 0.66^{AB}	3.78 ± 0.48^{B}

Table 3 Data from linear regression analysis showing associations between environmental parameters and concentrations of UV-B absorbing pigments in *Bryum pseudotriquetrum.* Only those radiation parameters that gave r^2 values > 27% are shown.

Predictor variable	**r^{-2} (%)**	**Slope**	**$F_{1,16}$**	**P-value**
Relative Humidity (10 day mean)	47.4	-19.2	14.4	0.002
Total UV radiation (10 day mean)	39.1	19.1	10.26	0.006
UV-B/total UV radiation (10 day max.)	38.1	575	9.85	0.006
UV-B radiation (10 day mean)	37.5	1010	9.60	0.007
TSR radiation (10 day mean)	36.9	1.46	9.34	0.008
UV-B/TSR(10 day max.)	32.1	335	7.55	0.014
Day length (5d mean)	33.2	474	7.99	0.012
Snowfall (10 day mean)	26.6	-236	5.81	0.028

Table 4 Summary data from multiple regression models showing associations between climate factors and UV-B absorbing pigment concentration in *Bryum pseudotriquetrum* (six terms), *Ceratodon purpureus* (four terms), and *Schistidium antarctici* (three terms), over the 1999/2000 summer season in the Windmill Islands Region, Antarctica. Displayed are the significant parameters and significance of each effect in the model. Sites: ASPA = Antarctic Specially Protected Area, RR = Robinson Ridge.

Species	R^2	**Predictor variable**	$F_{6,11}$	**P-value**
B. pseudotriquetrum	96%	Site (RR & ASPA2 v ASPA1)	77.8	<0.001
		Site (RR v ASPA2)	49.6	<0.001
		Relative humidity (10 d mean)	23.2	<0.001
		UV-B or (TUV) radiation (10 d mean)*	26.9	<0.001
		TWC	19.4	0.001
		Cloud cover (10 d mean)	15.9	0.002
C. purpureus	72%	Site (RR v ASPA1 & ASPA2)	13.3	0.003
		Site (ASPA1 v ASPA2)	3.66	0.08
		Relative humidity (10 d mean)	3.29	0.095
		Wind speed (10 d mean)	2.84	0.118
		TWC	2.69	0.127
S. antarctici	70%	Site (ASPA1 v RR & ASPA2)	14.0	0.002
		Site (RR v ASPA2)	8.90	0.010
		Snowfall (10 d mean)	7.31	0.017

*Substituting UV-B radiation (10 d mean) with TUV (10 d mean) produced the same result.

Table 5 Summary data from multiple regression models showing associations between climate factors and anthocyanin pigment concentration in *Bryum pseudotriquetrum* (four terms), *Ceratodon purpureus* (five terms), and *Schistidium antarctici* (three terms), over the 1999/2000 summer season in the Windmill Islands Region, Antarctica. Displayed are the significant parameters and significance of each effect in the model. Sites: ASPA = Antarctic Specially Protected Area, RR = Robinson Ridge.

Species	R^2	**Predictor variable**	$F_{6,11}$	**P-value**
B. pseudotriquetrum	63%	Site (RR & ASPA2 v ASPA1)	1.29	0.279
		UV-B/TSR (10 d max)	16.1	0.002
		Air temperature (10 d mean)	8.39	0.013
		TWC	1.14	0.306
C. purpureus	85%	Site (ASPA1 & RR v ASPA2)	39.3	<0.001
		Site (ASPA1 v RR)	2.61	0.132
		Wind speed (10 d mean)	18.7	0.001
		Cloud cover (10 d mean)	15.5	0.002
		Ozone layer thickness (10 d mean)	5.48	0.037
S. antarctici	71%	Site (ASPA1 & RR v ASPA2)	24.10	<0.001
		Site (ASPA1 v RR)	3.27	0.092
		Air temperature (10 d mean)	3.47	0.084

Figure Legends

Figure 1. Daily variation in ozone layer thickness (A), mean cloud cover (B) UV-B radiation (280-315 nm; C) and the proportion of UV-B to total UV radiation (UV-B/TUV; D) from October 1999 through to March 2000 at Casey Station in the Windmill Islands region, East Antarctica.

Figure 2. Daily variation in minimum, maximum and mean air temperature (A), relative humidity (B) maximum and mean wind speed (C) from October 1999 through to March 2000 at Casey Station in the Windmill Islands region, East Antarctica.

Figure 3. Cumulative monthly snowfall at Casey Station (A) and mean turf water content of moss collected from three sites (B); ASPA1 (circles), ASPA2 (squares) and Robinson Ridge (triangles) within the Windmill Islands region, East Antarctica, between November 1999 and March 2000. Data for all species at each site were pooled and represent mean ±SEM, n = 8-9.

Figure 4. Mean concentrations of UV-B absorbing pigments in *Schistidium antarctici* (open symbols), *Bryum pseudotriquetrum* (filled black symbols), and *Ceratodon purpureus* (filled grey symbols) collected from three sites within the Windmill Islands region, ASPA1 (circles), ASPA2 (squares) and Robinson Ridge (triangles) between November 1999 and March 2000. Data are mean ±SEM, n = 3 except for symbols with no error bars where n=1.

Figure 5. Mean concentration of UV-B absorbing pigments in *Bryum pseudotriquetrum* as a function of 10 d means of (A) relative humidity and (B) total UV radiation (295-385nm). Data are mean ±SEM, n = 3 except for symbols with no error bars where n=1. Regression details A) $r^{-2} = 47\%$, UVP = 1927 - 19.2RH; B) $r^{-2} = 39\%$, UVP = 258 + 19.1TUV.

Figure 1

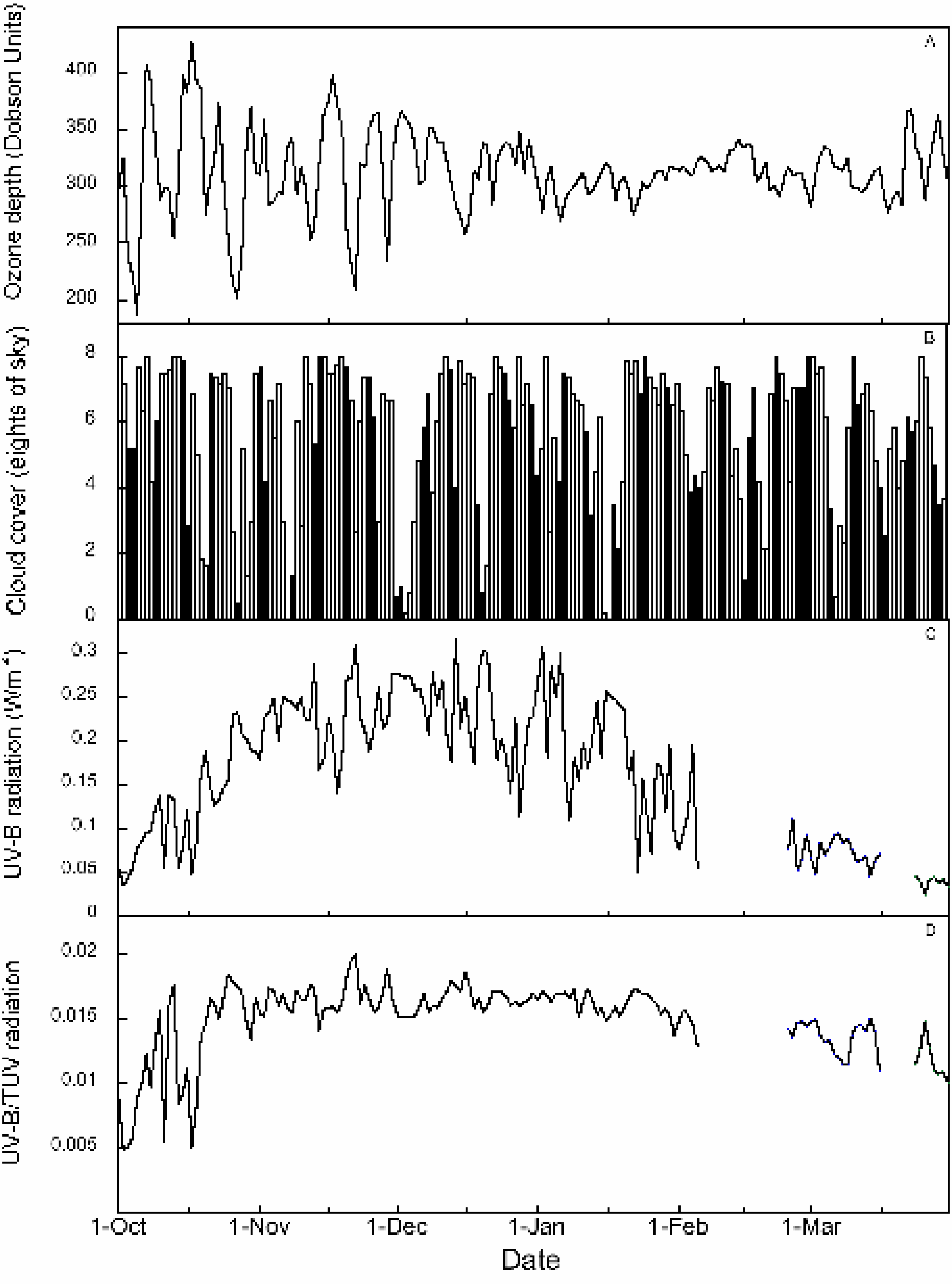

A
B
C
D
Ozone depth (Dobson Units)
200
250
300
350
400
Cloud cover (eights of sky)
0
2
4
6
8
UV-B radiation (Wm^{-2})
0
0.05
0.1
0.15
0.2
0.25
0.3
UV-B/TUV radiation
0.005
0.01
0.015
0.02
1-Oct
1-Nov
1-Dec
1-Jan
1-Feb
1-Mar
Date

Figure 2

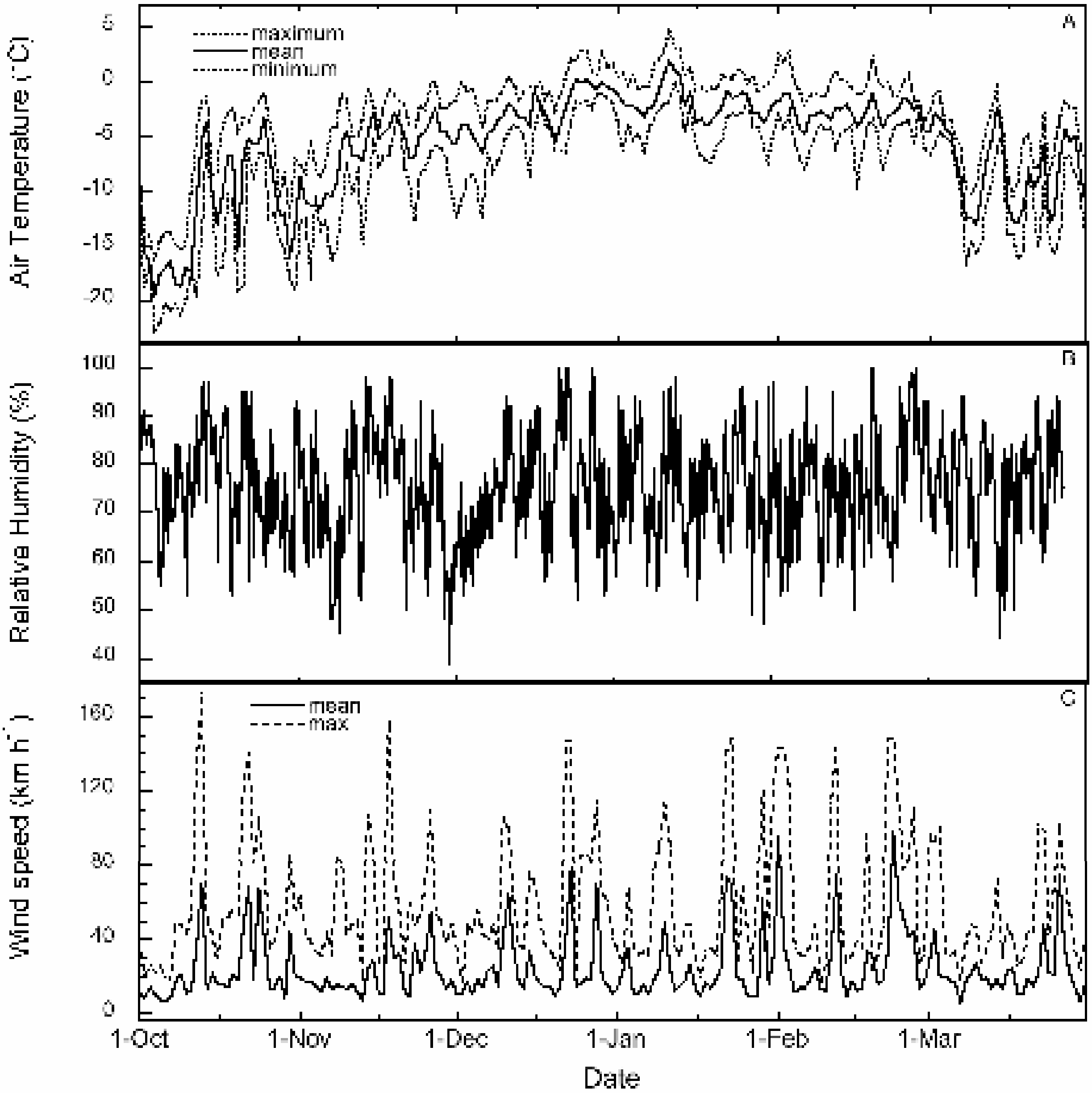

Air Temperature (°C)
5
0
-5
-10
-15
-20
maximum
mean
minimum
A
Relative Humidity (%)
100
90
80
70
60
50
40
B
Wind speed (km h⁻¹)
160
120
80
40
0
mean
max
C
1-Oct
1-Nov
1-Dec
1-Jan
1-Feb
1-Mar
Date

Figure 3

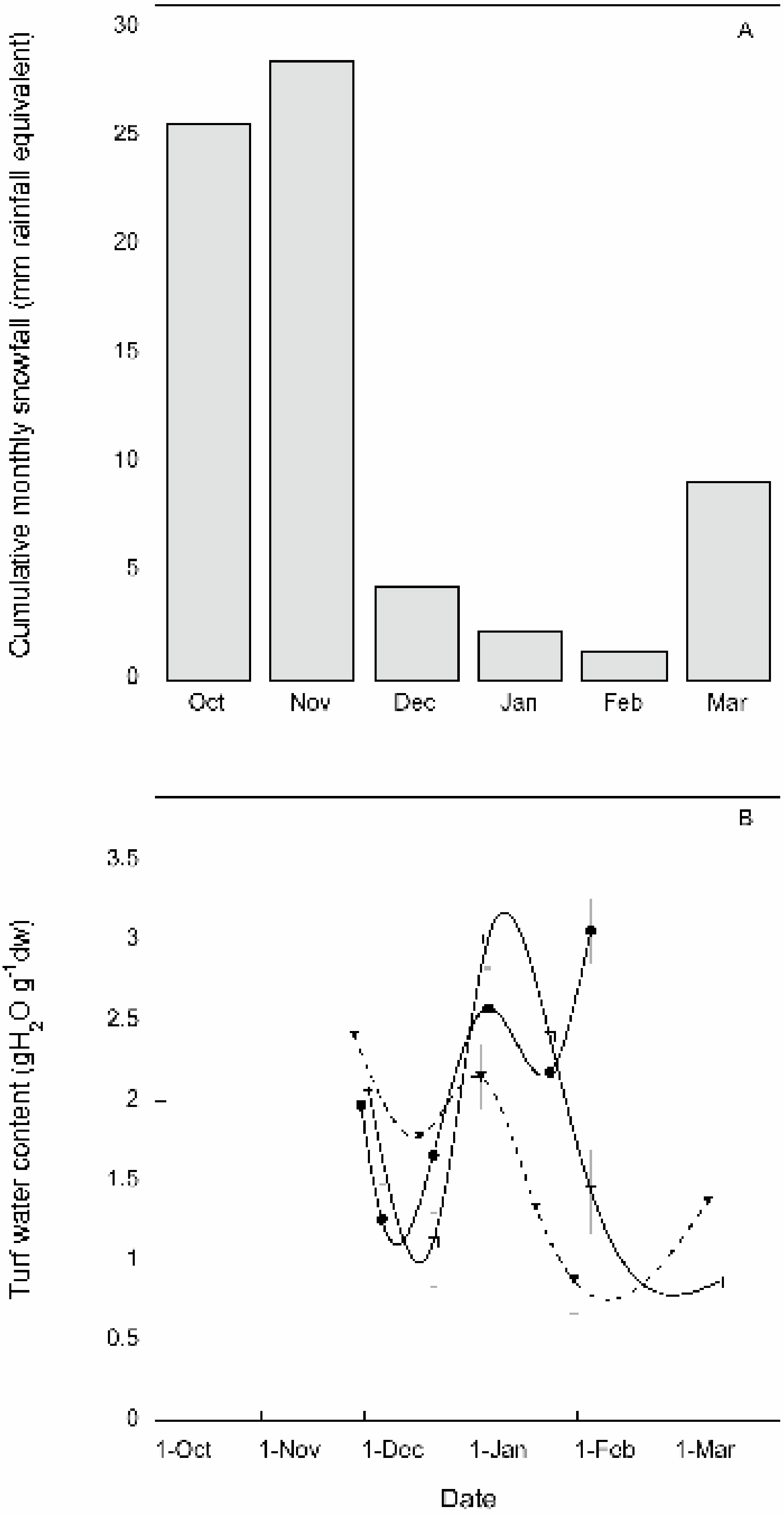

A
Cumulative monthly snowfall (mm rainfall equivalent)
0
5
10
15
20
25
30
Oct
Nov
Dec
Jan
Feb
Mar
B
Turf water content (gH$_2$O g^{-1}dw)
0
0.5
1
1.5
2
2.5
3
3.5
1-Oct
1-Nov
1-Dec
1-Jan
1-Feb
1-Mar
Date

Figure 4

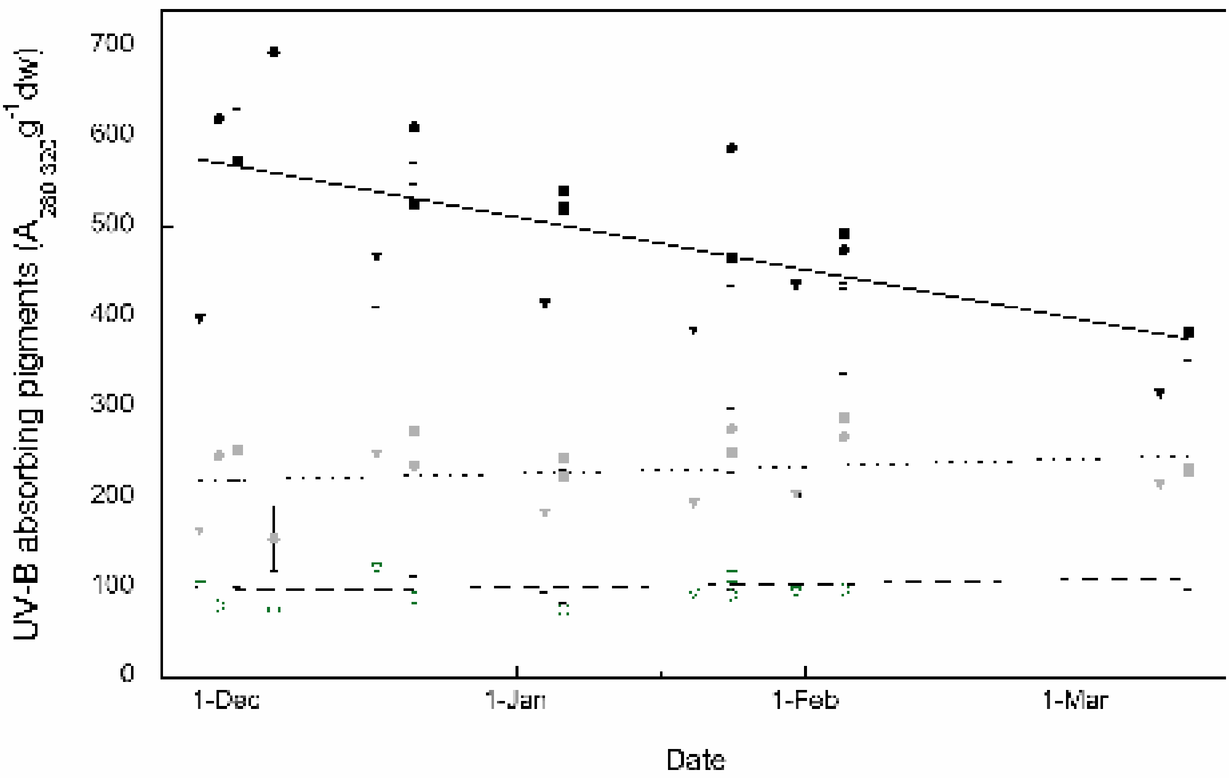
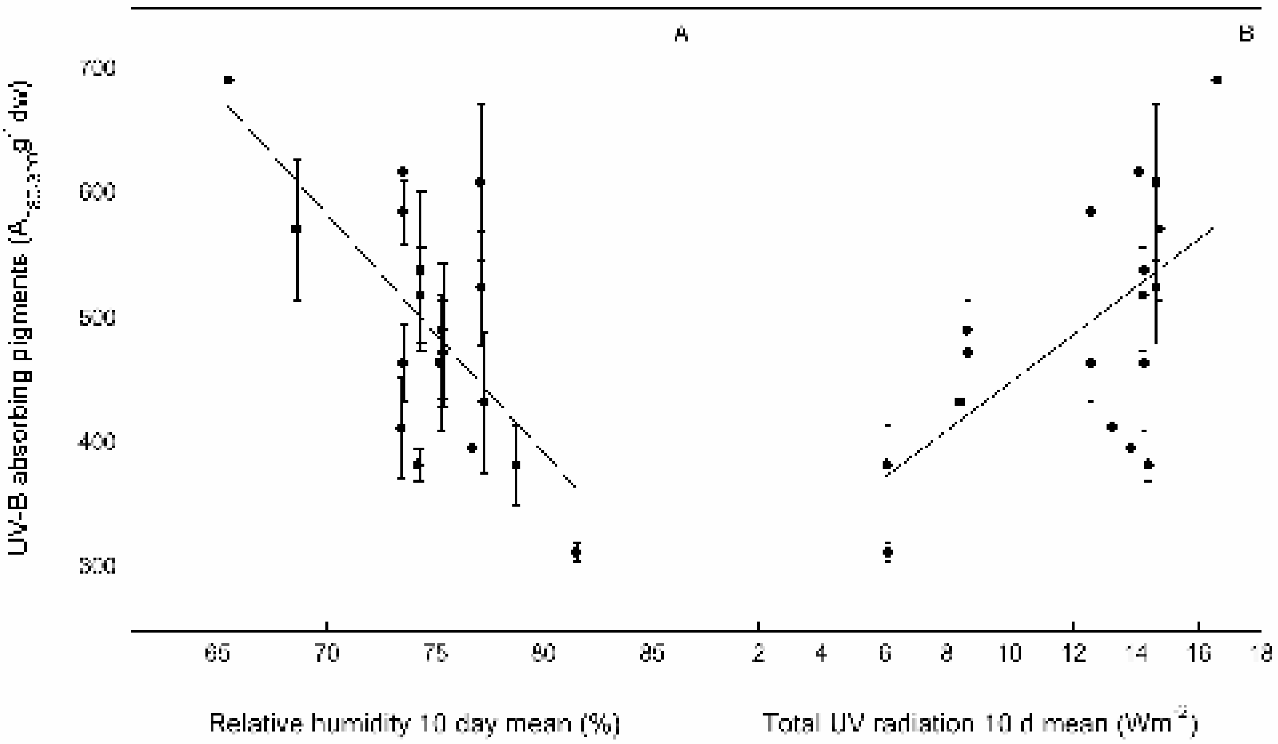

UV-B absorbing pigments ($A_{280\ 322}$ g^{-1} dw)
0
100
200
300
400
500
600
700
1-Dec
1-Jan
1-Feb
1-Mar
Date

Figure 5


A
B
UV-B absorbing pigments (A g-1 dw)
700
600
500
400
300
65
70
75
80
85
2
4
6
8
10
12
14
16
18
Relative humidity 10 day mean (%)
Total UV radiation 10 d mean (Wm-2)