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Antarctic moss: surviving ultraviolet radiation in a changing climate

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ANTARCTIC MOSS: SURVIVING ULTRAVIOLET RADIATION IN A CHANGING CLIMATE

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I, Johanna Dorothy Turnbull, declare that this thesis which is submitted to fulfil the requirements of the degree of Doctor of Philosophy from the School of Biological Sciences, Faculty of Science, Medicine and Health at the University of Wollongong has not been submitted for qualifications at any other academic institution. This thesis comprises two published papers.

Chapter 3 is published as Turnbull, J. D. and S. A. Robinson (2009). "Accumulation of DNA damage in Antarctic mosses: correlations with ultraviolet-B radiation, temperature and turf water content vary among species." Global Change Biology **15**: 319-329.

Chapter 4 is published as Turnbull J. D., Leslie S. J., and S. A. Robinson (2009). "Desiccation protects two Antarctic mosses from ultraviolet-B induced DNA damage." Functional Plant Biology **36**: 214-221.

The original ideas for both chapters were generated by my supervisor. For the work presented in Chapter 3, I was responsible for the experimental design, sample collection, and all laboratory and statistical analyses. The data in Chapter 4 extends an experiment from Simon Leslie's honours project (2002). I supervised his sample collection and irradiation experiment in Antarctica, while Simon completed all DNA extractions and conducted preliminary analyses and optimisation for the ELISAs used to quantify DNA damage. I subsequently reran ELISAs to generate a new data set using Simon's extracted DNA and performed subsequent statistical analyses.

I wrote first drafts for both manuscripts which were subsequently altered by Professor Sharon Robinson and in response to reviewers, prior to publication. The rest of the thesis is wholly my own work, except where acknowledged or referenced.

Johanna Dorothy Turnbull
August 2015

I dedicate this thesis to mindfulness of small things

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ABSTRACT

The Antarctic ozone hole increases ultraviolet-B radiation (UV-BR) at the Earth's surface, and is linked to increased wind speed, altered precipitation and snow deposition patterns. The influence of UV-BR was investigated in three co-occurring moss species from East Antarctica: two cosmopolitan (*Ceratodon purpureus* (Hedw.) Brid. and *Bryum pseudotriquetrum* (Hedw.) Gaertn., B. Mey. & Scherb) and the endemic (*Schistidium antarctici* (Cardot) L.I. Savicz & Smirnova). A comprehensive field study concurrently measured a range of physiological parameters, indicative of both plant stress and photoprotection, over summer 2002/03. Changes in these parameters were compared to natural fluctuations in environmental variables, including incident UV-BR, water availability and temperature. To complement the field study, and assess interactions between UV-BR and water, UV tolerance of the three species was compared in both dry and hydrated moss in a light-box irradiation study.

UV tolerance was assessed as DNA damage; cyclobutane pyrimidine dimers (CPDs) and (6-4)-photoproducts were determined using enzyme linked immunosorbent assays. Bulk UV absorbing compounds (UVACs), including anthocyanins, were measured spectroscopically, using acidified methanol and alkaline hydrolysis to extract from the cytosol and cell wall respectively. Photosynthetic rates were calculated from chlorophyll fluorescence data, and chloroplastic pigments were quantified using high performance liquid chromatography.

Photosynthesis and pigment accumulation were strongly seasonal. In the cold, low light conditions of early season, mosses shifted from a photoprotected state (low photosynthesis and high zeaxanthin conversion) to higher photosynthetic efficiency and rate as the season warmed. Low temperatures limit photosynthesis in all species, but only the cosmopolitan species maintained photosynthesis at higher temperatures. High light adaptation is suggested in *B. pseudotriquetrum,* with high photosynthetic efficiency, low xanthophyll pigments and a higher chlorophyll a/b than the other two study species.

The field season had unusually low springtime ozone losses and subsequent results likely underestimate the response in a more typical year. Despite low ambient UV-BR all species accumulated DNA damage in field samples, with repair occurring in warmer, wetter conditions in *B. pseudotriquetrum*. The endemic *S. antarctici* is most vulnerable to UV-BR, with sustained DNA damage in the field and the highest accumulation of photoproducts under elevated UV-BR (4 h UV-BR: 8.5 W m^{-2}). In contrast, the cosmopolitan species were protected from DNA damage, particularly when dry, with CPDs reduced by at least 60% relative to hydrated moss. Since mosses are metabolically inactive when dry, this is likely due to passive protection, such as provided by UVACs. The UV vulnerability of *S. antarctici* is likely due to its relatively low concentrations of UVACs compared to the other species. All species accumulate anthocyanins with concentrations peaking in midsummer. However, a correlation between these compounds and ambient UV-BR was only apparent in the highly tolerant *C. purpureus,* suggesting a UV protective role for anthocyanins in this species. Other UVACs did not accumulate in ambient UV-BR, perhaps due to the atypically low UV-BR. However, concentrations of cell wall UVACs reduced with warmth, possibly as new cell walls are produced during growth. Anthocyanins and cell wall UVACs may also play complementary protective roles in actively growing cells. Furthermore, the relatively fast growing *B. pseudotriquetrum* accumulates mostly intracellular UVACs, whereas the slower growing *S. antarctici* and *C. purpureus* have predominantly cell wall UVACs. So, the preferred location for sequestration of UVAC may be considered as a functional leaf trait that relates to the productivity and life history strategy of each species.

Elevated UV-BR should wane as ozone recovers from mid century onwards. However, its influence on regional weather patterns may be supplanted by greenhouse gases, so desiccating high winds may persist. The cosmopolitan species' remarkable ability to endure UV-BR, especially when dry, suggests they will tolerate future UV-BR better than the endemic species. Since relative UV and desiccation tolerance co-occur, community composition could shift towards *C. purpureus* if the

high UV-BR and the drying trend identified in this region persist. Both cosmopolitan species may also benefit from future warming. The endemic *S. antarctici* is most vulnerable to higher UV-BR and temperature, and low water availability, raising biodiversity concerns for this species. The desiccated state provides protection from UV-BR in the cosmopolitan mosses, but photosynthetic activity, enzymatic repair and synthesis of protective compounds all require water and warmth. Thus the timing of high UV-BR across the season will alter its impact, with amelioration in warmer and wetter midsummer conditions. Currently, snow cover protects mosses during springtime ozone depletion. However, if global warming causes earlier snowmelt, higher UV-BR could occur when mosses have rehydrated but cold temperatures limit repair. Under this scenario mosses may be particularly vulnerable to UV-BR.

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I am forever indebted to the other 'moss girl' Jane Wasley for taking me to Antarctica with her and launching me into the captivating world of moss physiology. My three supervisors Jane Wasley, Kris French and Sharon Robinson have all been inspiring and heartening, with crucial contributions at different stages. Jane Wasley was invaluable as Antarctic field support with thoughtful input into the experimental design and feedback on the manuscript. Kris French was my statistical redeemer at vital points. Thank you! And I am indebted to my principal supervisor Sharon for her enduring personal and scientific support. Her energy, enthusiasm, humanity and savant capacity to intuitively understand data are an inspiration.

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Australian Antarctic Division (Commonwealth of Australia). The data are described in the State of the Environment indicators 1 *Monthly mean air temperatures at Australian Antarctic Stations* and 10 *Daily broad-band ultra-violet radiation observations using biologically effective UVR detectors.* Data were accessed in 2006/07 and 2013.

Finally I would like to thank my children for noticing that Mummy likes moss and cheering me with their belief that when I am not home, I am WORKING in the "UNIVERSE"(ity). I thank my parents for their endless encouragement. And for Dave Hendren, I am eternally grateful for your constant material and emotional support and unfaltering good cheer. You make my life better every day.

ABBREVIATIONS

ASPA, Antarctic Specially Protected Area

AT, air temperature

ATMIN, minimum daily air temperature

AT10, ten day mean air temperature

CPD, cyclobutane pyrimidine dimer

DU, Dobson unit

ELISA, enzyme linked immunosorbent assay

GAMs, Generalized additive models

iUVAC, intracellular UV absorbing compounds

MT, moss turf temperature

NPQ, nonphotochemical quenching

ozone, ozone column depth

PAR, photosynthetically active radiation

6-4pps, pyrimidine (6-4) pyrimidone dimers

PSI, photosystem I

PSII, photosystem II

RH, relative humidity

RuBisCo, Ribulose bisphosphate carboxylase- oxygenase

Tchl, Total chlorophyll

TUV, total ultraviolet radiation

UV, ultraviolet

UV-BR, ultraviolet-B radiation

VAZ/ Tchl, violaxanthin+ antheraxanthin + Zeaxanthin/Total chlorophyll ratio

WUVAC, Cell wall UV absorbing compounds

wUVR, weighted UV radiation

WC, turf water content

Z/VAZ, acute plant stress, zeaxanthin/ violaxanthin + antheraxanthin + Zeaxanthin ratio

1 Introduction

1.1 Antarctic growth environment

Antarctica has the most extreme climate on Earth. The severity of the climate is caused by a high degree of thermal isolation due to strong westerly circumpolar winds (Antarctic polar vortex) and ocean currents (Antarctic circumpolar current; Turner et al. 2014b). The unique climatic conditions can be described in superlatives: coldest, driest, windiest, highest and most isolated.

Most of the continent, excluding the Antarctic peninsula, is below the Antarctic Circle (66.5622°S) and so receives near continuous daylight in summer and continuous darkness in winter. The continent is vast, 13 million km^2, but only 0.32% is ice free (Ugolini and Bockheim 2008). Permanent ice sheets surround rare oases where rocky outcrops emerge from snow cover in summer to support a largely cryptogamic terrestrial ecosystem. The vegetation is limited by the short summer growing season, and includes algae, fungi, lichens, bryophytes (non-vascular plants eg. liverworts and mosses) and two vascular plant species which are found only on the Antarctic peninsula. Mean temperatures in summer are below freezing, which is near biological thresholds for survival (Convey and Smith 2006). Strong winds increase evaporative water loss, rapidly reduce surface temperatures, increase the number of freeze/thaw events and also may disturb CO_2 concentrations in the boundary layer above moss turves.

Water is a major factor determining the distribution of Antarctic taxa and limiting the extent of terrestrial plant communities (Adamson and Adamson 1992; Schwarz et al. 1992; Kennedy 1993a; Clarke et al. 2012; Wasley et al. 2012; Bramley-Alves 2015). The availability of water in Antarctica is related to ambient temperatures and, despite its abundance as ice, water is only biologically available during the summer melt. In summer, water availability is variable as high light, low humidity and strong winds can lead to high evaporative rates and temperatures can rapidly plunge below the freezing point of water.

Another limitation to plant growth is increasing ultraviolet radiation (UV) due to anthropogenic ozone depletion. Complex climatic interactions occur between warming from elevated greenhouse gases (GHGs) and ozone depletion that have substantial impacts on terrestrial biota (Robinson and Erickson 2015).

1.2 Climate change

1.2.1 Depletion of the ozone layer and increasing ultraviolet radiation

The stratospheric ozone layer screens the Earth's surface from the damaging components of sunlight. Major springtime ozone depletion above Antarctica became evident in the 1980s (Farman et al. 1985), resulting in a spectral shift toward the biologically damaging shorter wavelength ultraviolet-B radiation (UV-BR; wavelength 280-315 nm) components of solar radiation (Frederick and Snell 1988). Prior to this time, high latitudes received low UV-BR fluxes due to the low solar angle and the thickness of the ozone layer (>300 Dobson Units; DU). The 'ozone hole' is defined as the area with an ozone column depth below 220 DU (Roy et al. 1994). Elevated UV-BR impacts terrestrial and aquatic ecosystems with reduced productivity and changes in species assemblages and trophic interactions (Bornman et al. 2015; Hader et al. 2015).

The ozone hole progressively increased in size from the early 1980s until 1995, then stabilised with considerable interannual variation (Figure 1.1). The emissions of ozone depleting substances (ODS) have reduced, owing to successful international co-operation and regulation through The Montreal Protocol (1987; Bais et al. 2015). Since ODS are also potent greenhouse gasses, the curbing of emissions has also slowed global warming (Estrada et al. 2013). Without the swift and effective regulation provided through The Montreal Protocol, by the year 2100 the Earth's ozone layer may have been completely destroyed, rendering most ecosystems uninhabitable (Newman et al. 2009; Egorova et al. 2013). The long life spans of ODS in the atmosphere delays ozone recovery (McKenzie et al. 2011; Turner et al. 2014b). Currently ozone depletion has increased the mean noon time UV index by 40% above Antarctica since the early 19080s, and this is anticipated to reverse by

2100 as ozone recovery proceeds through the mid 21st century (Andrady et al. 2015).

The ozone hole is fluid and fluctuates with atmospheric currents leading to considerable daily variation in ozone column thickness, especially at the edge of the ozone hole (Rousseaux et al. 2001). Whilst the centre of Antarctica usually experiences pronounced ozone depletion throughout this period, the edge of the continent, where the majority of ice-free habitats are found, is exposed to highly variable UV-BR. The combination of variable ozone depth and other factors such as cloud cover, microsite geometry, albedo and snow cover, leads to high daily variability and unpredictability in the levels of damaging UV radiation reaching Antarctic plants (Robinson et al. 2003).

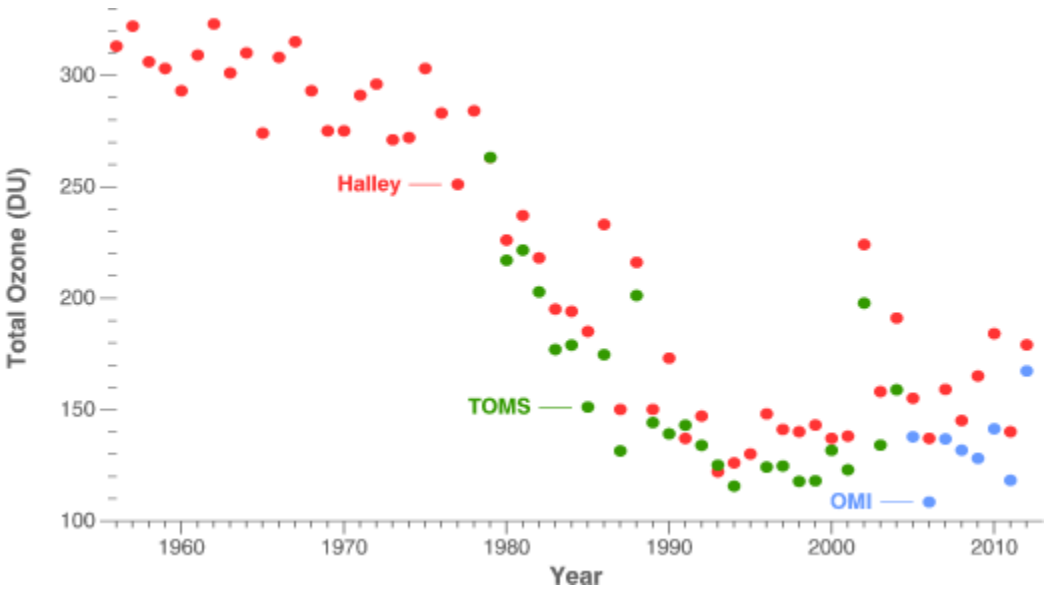

Figure 1.1: Total atmospheric ozone (Dobson units; DU) above Antarctica in October over 60 years. Measurements are from instruments on the ground (at Halley research station, Antarctica) and from satellites: Total Ozone Monitoring Spectrophotometer (TOMS) and Ozone Monitoring Instrument (OMI). Figure from NASA Ozone Watch.

Ozone depletion has caused complex and often subtle changes in terrestrial and aquatic ecosystems. Ultraviolet-B radiation impacts interspecific competition between plants (Barnes et al. 1995) and trophic interactions between plants and herbivores, pathogens and detritivores (Caldwell et al. 2007; Ballaré et al. 2011;

Bornman et al. 2015). Elevated UV-BR reduces growth of Antarctic vascular plants, mosses, fungi, terrestrial algae and soil microalgae (Ballare et al. 2001; Hughes et al. 2003; Robinson et al. 2003; Hughes 2006). The plant rhizosphere and associated soil microbial and microinvertebrate communities are also impacted (Caldwell et al. 2007). The magnitude of ecosystem changes from the last 30 years of ozone depletion is hard to assess without baseline studies. The vulnerability of Antarctic plants to UV-BR is likely to be both species specific, and to depend on exposure to other abiotic stressors. Increases in desiccating winds around the Antarctic continent are associated with ozone depletion, and this provides a further stress to Antarctic vegetation (Perlwitz et al. 2008; Clarke et al. 2012; Robinson and Erickson 2015).

1.2.2 Interactions between ozone depletion and climate change

Complex interactions exist between ozone depletion and global warming (Bais et al. 2015). Additionally, both ozone deletion and global warming strongly affect Antarctic atmospheric circulation (Robinson and Erickson 2015 and references therein). Temperature records are scarce across Antarctica, but as a whole it appears to have warmed at approximately 0.1 °C decade^{-1} for the past 30–50 years (Steig et al. 2009; O'Donnell et al. 2011; Schneider et al. 2011). However, this figure conceals regional and seasonal differences and may underestimate the substantial climatic change in marine and terrestrial ecosystems. Temperature changes, where they occur close to the freezing point of water, are likely to impact most on terrestrial ecosystems (Convey et al. 2014).

In situ temperature observations indicate the Antarctic Peninsula is one of the most rapidly warming places on Earth (Vaughan et al. 2003). In contrast, other Antarctic locations show stable or even decreasing temperatures, such as the cooling reported from Amundsen Scott station at the Pole (Turner et al. 2014b). Assessment of Antarctic climate change is hampered due to sparse, incomplete and mainly coastal long-term records (Turner et al. 2014b). However, in East Antarctica a warming of 0.1–0.2 °C is suggested by both ice cores and temperature

reconstructions interpolating ground based data with satellite measurements over the past 20–50 years (Steig et al. 2009; Muto et al. 2011). In West Antarctica ice cores indicate warming from 1800 with a sharp increase to 0.7 °C decade^{-1} in the past 20 years (Turner et al. 2014b). Such warming trends are also supported by ice loss from the Antarctic ice sheet (Chen et al. 2009).

Both rapid warming on the peninsula and the weak change in East Antarctica are attributed to the influence of ozone depletion on climate, with a minor but increasing contribution from increased greenhouse gasses (Figure 1.2; Robinson and Erickson 2015 and references therein). Ozone depletion strongly affects the climate of the Southern hemisphere by shifting air circulation patterns. The stratosphere cools as ozone depletion reduces the absorption of UV-BR in this region (Thompson et al. 2011). This cooling lowers air pressure and causes the tropopause to lift and strengthens the polar jet stream and shifts it south by 1-2^{o} latitude (Turner et al. 2014b). This effect on climate is described by the Southern Annular Mode (SAM) which is defined as the difference in zonal mean sea level pressure between 40°S and 65°S. This large-scale climatic pattern is the major contributor (35%) to climate variability in the Southern Hemisphere (World Meteorological Organization 2014; Robinson and Erickson 2015). Its current positive phase is at the highest level in the past thousand years (Abram et al. 2014).

The contraction of the polar jetstream south towards Antarctica has increased mean windspeeds by 7% and up to 27% in some regions between latitude 50–65 ^{o}S since the late 1970s (Korhonen et al. 2010; Son et al. 2010). These winds increase the thermal isolation of the Antarctic continent, maintaining a cold mass of air above Antarctica and reducing incursions of tropical warm air. (Perlwitz et al. 2008; Son et al. 2010; Ding et al. 2011). This results in a cooling of the Antarctic continent with a warming of the surrounding region particularly in summer and autumn (Turner and Overland 2009). This has restrained warming rates in coastal East Antarctica resulting in less melt in coastal regions (Turner et al. 2014b and references therein). The increase in westerly winds is likely to abate as ozone

recovers and into the future warming is modelled to increase by a further 0.34 °C decade^{-1} (or about 3 °C in total) by the end of the 21st Century (Bracegirdle et al. 2008; Robinson and Erickson 2015).

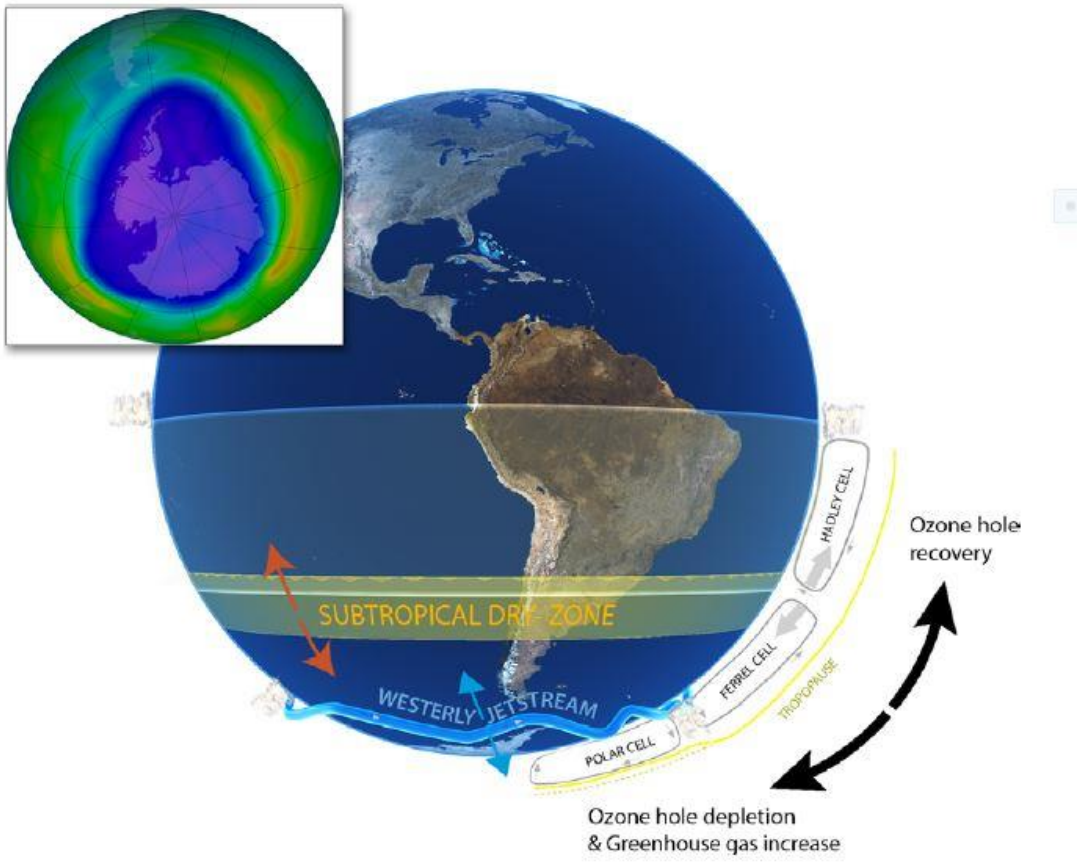

Figure 1.2: Schematic showing the impact of Antarctic ozone depletion (inset) on Southern Hemisphere atmospheric circulation. Stratospheric ozone depletion and associated cooling over Antarctica has lifted the tropopause, facilitating a southwards shift of the Hadley cell, polar jet stream and the tropical dry zone to the south as shown. These changes are associated with a more positive phase of the Southern Annular Mode (SAM) forced by both ozone depletion and increased greenhouse gasses (black arrows). Image reproduced with permission from Robinson and Erickson (2015).

Climate change has caused substantial changes in marine and terrestrial ecosystems. The rapid warming on the Antarctic peninsula is associated with changes in penguin numbers, krill recruitment, phytoplankton and zooplankton communities and expansion of vascular plant communities (Convey and Smith 2006; Ducklow et al. 2007). Drying trends in the McMurdo Dry Valleys (Doran et al. 2002)

and Windmill Islands region of East Antarctica are attributed to changes in the SAM due to ozone depletion. This drying trend impacts both terrestrial vegetation (Clarke et al. 2012; Wasley et al. 2012) and lake communities (Hodgson et al. 2006).

The impact of temperature change is particularly pronounced when it results in a shift from negative to positive temperatures as this controls the availability of water. Whilst temperatures remain below 0˚C increases (or decreases) will likely have minimal effect, but a very small change in temperature that results in melt will have a far greater impact on Antarctic biota. If there is an increase in the frequency of metabolically costly freeze-thaw events this may result in reduced energy budgets and is likely to be deleterious for mosses. Once temperatures are above freezing then increases are likely to be favourable as long as water is still available (Robinson et al. 2003; Convey and Smith 2006).

1.2.3 Changes in water availability

Water is a key factor determining terrestrial productivity (Convey et al. 2014). In Antarctica water is mostly locked away as ice, and biologically unavailable, except during the short summer melt (Convey et al. 2014). It is also the most difficult parameter to measure accurately for a number of reasons. On the continent almost all the precipitation falls as snow, which is harder to measure accurately than rain. It is likely underestimated in current meteorological reporting due to blowing snow and sublimation. In addition, available water for any particular community varies depending both on how much precipitation falls over the community and also inputs from the melt of (permanent) upstream snow banks (Lucieer et al. 2014). Temperatures above zero will produce more melt but water is also lost from moss beds on warm sunny days through evaporation into the dry air.

More accurate methods for estimating the water available at various microsites are urgently required and one method that shows potential in this regard is the use of stable isotopes. The ratio of the two stable isotopes of carbon ($^{12}C/^{13}C$) laid down as plants photosynthesise shows promise as a proxy for water availability to moss beds

(Bramley-Alves 2015). To date this method has been used to show that sites in the Windmill Islands, East Antarctica appear to be drying (Clarke et al. 2012; Bramley-Alves 2015) whilst confirming that Signy Island has become warmer and wetter in recent decades (Royles et al. 2012). The drying trend in the Windmill Islands is associated with altered community structure: from bryophytes towards more desiccation tolerant lichens. Regional drying may also be causing shifts within the bryophyte community towards more desiccation tolerant moss species (Figure 1.3; Wasley et al. 2012).

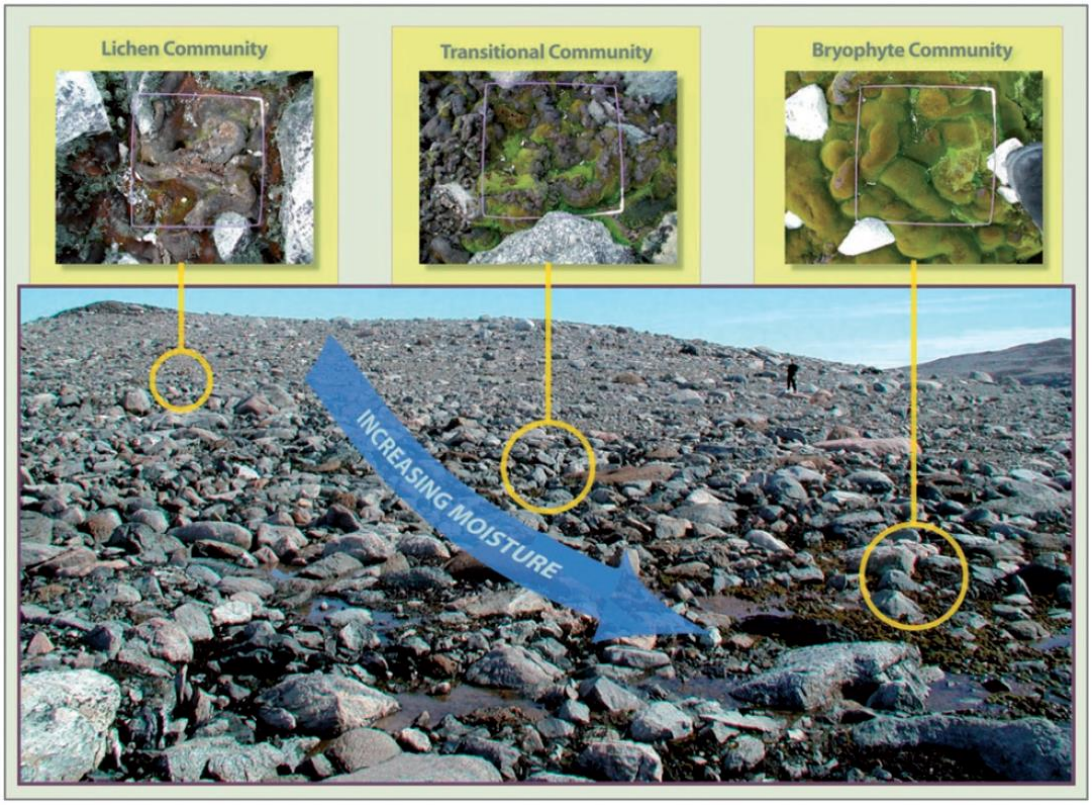

Figure 1.3: Vegetation biomonitoring along a moisture gradient showing representative quadrats from the moist bryophyte community, transitional community and drier lichen community. This site is in the Windmill Islands region of East Antarctica where a prevalence of lichen encrusted moribund moss indicates a shift in broadscale vegetation patterns. Image reproduced with permission from Wasley et al. (2012).

The difficulty measuring water inputs currently adds to the complexity of accurately predicting future water. Climate models predict future increases in precipitation in coastal regions (Turner et al. 2014b) and this has been observed on the plateau

(Morgan et al. 1991; van Ommen and Morgan 2010). But increased precipitation will only lead to increased water availability in terrestrial communities if there is more melt (Convey and Smith 2006). Increased melt can also lead to the exhaustion of previously permanent snow banks and cut off melt streams. More wind may reduce water availability by blowing snow away or increasing evaporative water loss from moss beds (Clarke et al. 2012), lakes and streams (Hodgson et al. 2006). It is currently unclear how these changes in water availability will impact on the stress experienced by plants.

Antarctic bryophytes owe their survival in such inhospitable conditions to their poikilohydric lifestyle. The following section compares poikilohydry with the homoihydric strategy of vascular species.

1.3 Homoiohydry vs poikilohydry

Our understanding of plant responses to light derives mostly from research into vascular plants. This section compares vascular and bryophyte species which share a common evolutionary history but have contrasting strategies for surviving the inconsistent water supply on land (Proctor and Tuba 2002). Vascular plants are homoiohydric and regulate internal water levels with lignified water conducting vessels, impervious surfaces and stomatal closure to reduce water loss. In contrast, the small stature of mosses facilitates a poikilohydric lifestyle, where internal water content equilibrates with that of the surroundings. Many mosses are thus desiccation tolerant and rapidly recover from losing virtually all cellular free water (as reviewed in Proctor et al. 2007). Poikilohydry influences all aspects of bryophyte existence and forms the basis for differences between bryophytes and vascular species in anatomy, photosynthesis and stress tolerance (summarised in Table 1.1).

Table 1.1: Summary of differences between typical vascular species and typical bryophytes. These successful adaptations to water limitation in terrestrial ecosystems are inextricably linked to the scale of the organisms

Vascular plants	**Bryophytes**	**References**
Homoiohydric and can grow large	Poikilohydric and always small	(Proctor 2000; Proctor and Tuba 2002)
Complex anatomy, with roots, stem and proper leaves	No root system for energy storage or absorption of water and nutrients	
Lignified conducting vessels transport water	Limited conducting vessels	(Ochyra et al. 2008)
Thick leaves with waterproof surfaces	Simple leaflets often one cell thick and lacking complex surface layers	(Ochyra et al. 2008)
Lower ratio of cell wall: cytoplasmic cell contents	Higher ratio of cell wall: cytoplasmic cell contents	(Marschall and Proctor 2004)
Photosynthesis		
Stomata facilitate CO_2 diffusion into leaves containing air spaces	Water inundation limits CO_2 diffusion into single celled leaflets	(Rice and Cornelissen 2014)
Non-photochemical quenching requires PsbS protein	Non-photochemical quenching utilises either PsbS protein or LHCSR	(Niyogi et al. 2005; Gerotto et al. 2012)
Usually light dependent zeaxanthin accumulation	Light and/or desiccation induces zeaxanthin accumulation	(Garcia-Plazaola et al. 2012)
Chla/b ratio is high in sun (3.4) and low in shade (2.4)	Overlaps with vascular species but usually <2.3	(Demmig-Adams and Adams 1992a; Marschall and Proctor 2004)
UV absorbing compounds		
Soluble UVACs typically accumulate under UV-BR	Particular species have UV-BR responsive intracellular UVAC, other species may rely more on cell wall UVAC	(Day et al. 1993; Vogelmann 1993; Clarke and Robinson 2008)
Anthocyanins accumulate in vacuoles or free within cytoplasm	Anthocyanins or anthocyanin-like compounds in cell wall of some bryophytes	(Gould and Lister 2006)

Evolution has of course provided a spectrum between poikilohydry and homoiohydry (Proctor and Tuba 2002). Desiccation tolerance occurs in some vascular species and certain mosses have rudimentary water conducting structures which range in efficiency (Proctor and Tuba 2002). However, whilst homoiohydric vascular species have desiccation tolerant seeds, less than 0.15% of species are desiccation tolerant in the adult vegetative phase (Proctor et al. 2007). In contrast, poikilohydric mosses have more than 15,000 desiccation tolerant species (Yamakawa et al. 2012 and references therein). Vascular plants tolerate drought stress and continue metabolic activity at reduced turgor, however mosses avoid drought by entering a desiccated and metabolically inactive state (Proctor and Tuba 2002). Moss and vascular species share common biochemical pathways for photosynthesis and photoprotection. However, the small scale and simple anatomy of mosses imposes variations on the survival strategies employed by vascular plant species.

Bryophytes have simple leaflets, no root system to store reserves, and limited water conducting structures (Table 1.1). This simplicity was originally thought to increase their UV-BR vulnerability, but many mosses are highly tolerant (Boelen et al. 2006; Wolf et al. 2010). The thick leaves of vascular species are compared with the thin leaflets of moss in Figure 1.4. In bryophytes, uptake of water and nutrients primarily occurs across the surface of these thin leaflets (Waite and Sack 2011) which are typically one cell thick. Bryophytes also have higher cell wall to cell content ratios than vascular species (Marschall and Proctor 2004 and references therein; Table1.1) and allocate more resources to metabolic processes than to non photosynthetic tissues (Wang et al. 2014).

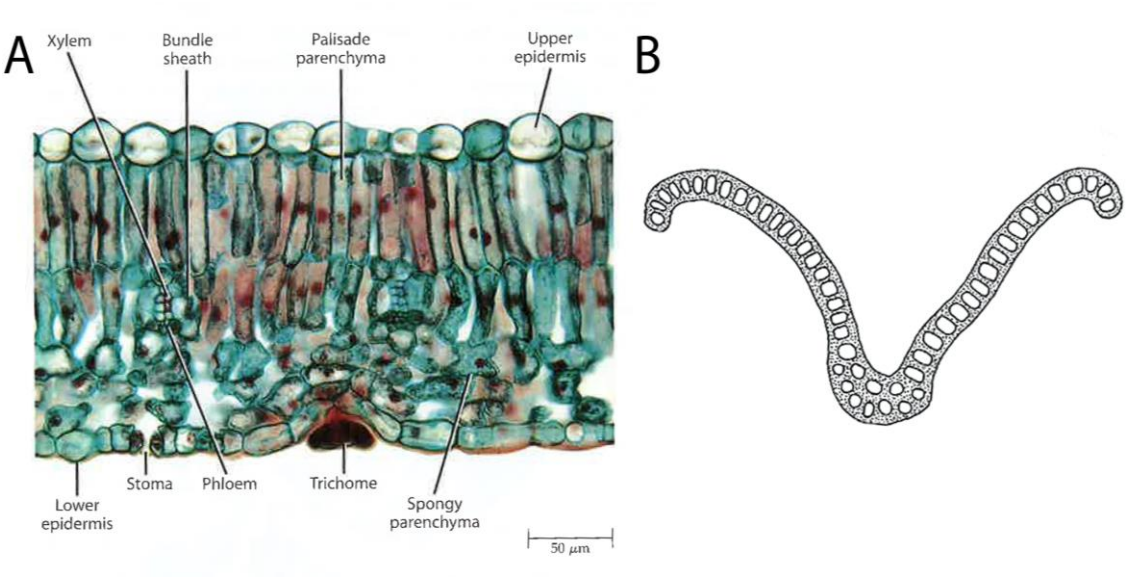

Figure 1.4: A comparison of cross sections of a portion of a typical vascular plant leaf and a complete moss leaflet. The transverse section of the lilac leaf (A; *Syringa vulgaris*), shows the differentiated tissue layers, including palisade and spongy parenchyma, vascular bundles (xylem, phloem and associated bundle sheath cells) and upper and lower epidermis. The latter containing stomata (Image reproduced from Raven et al. 2005). By contrast, the cross section of a leaf blade of *Schistidium antarctici* demonstrates the simplicity and relative size of the thin leaflets of mosses, which are often only one cell thick (Image reproduced from Ochyra et al. 2008).

Plants can adjust to excess and damaging light by minimising light absorption into the leaf. Higher plants have more capacity to attenuate light through either leaf thickening or by changes in leaf surface structures usually absent in mosses such as the epidermis, cuticle, surface waxes and leaf hairs (Table1.1; Figure1.4; Day et al. 1993; Vogelmann 1993). In gymnosperms and angiosperms UV absorbing compounds (UVACs) often accumulate at the leaf surface in trichomes, or in cuticular or epidermal cells and these can reduce UV transmission by up to 99% (Robberecht and Caldwell 1978). Mosses employ a sub-set of the strategies available to vascular plants including cell wall thickening, chloroplast movement, changes in leaf orientation and shape, and self shading within the canopy (Robinson and Waterman 2014). Likewise, the response of photosynthesis to environmental conditions is largely similar in bryophytes and vascular species.

Bryophytes, vascular plants and Charophyte green algae share the same photosynthetic apparatus and have remarkably similar chloroplastic pigments, utilising chlorophylls a and b, carotenoids and xanthophylls (Esteban et al. 2015). Photosynthetic rates standardised on a chlorophyll basis are similar in bryophytes and vascular species but are two to tenfold higher in vascular species when expressed on a mass basis (Rice and Cornelissen 2014). This is because bryophyte photosynthetic measurements usually use whole moss canopies, rather than individual leaves. Canopy measurements include more structural and storage components and have a vertical decreasing gradient in chlorophyll concentrations (Rice and Cornelissen 2014).

Photosynthesis in bryophyte and vascular species share similar responses to light and temperature but thin bryophyte leaves impose additional limitations on photosynthesis. Photosynthesis drops sharply to zero as mosses desiccate, but is also limited when water inundation slows CO_2 diffusion into the leaflet (Table 1.1; Wasley et al. 2006b; Rice and Cornelissen 2014). At full water content photosynthetic rates can be half to a third of the maximal rates (Rice and Cornelissen 2014). The capacity of moss to cease metabolism when dry, and enter a dormant state enables them to survive prolonged periods of inhospitable climatic conditions.

Mosses are an ancient life form and flourish in some of the most exposed and marginal ecosystems on Earth. In the desiccated state mosses are highly resistant to extreme environmental stress and for example can survive temperatures from -196 to +100 ˚C (Alpert 2000). This desiccated state is crucial to the survival of Antarctic mosses throughout the extreme cold of winter. As temperatures drop, water diffuses out of moss leaflets in the presence of external ice (Lenne et al. 2010). This is because the water potential of ice is lower than that of water (Lenne et al. 2010). Actual freezing (ice formation) is avoided unless the temperature drops too rapidly for frost formation to occur, which is unlikely in the natural setting (Lenne et al. 2010). Freeze-thaw and desiccation events are therefore physiologically

synonymous in Antarctic moss. However the impact of the UV-BR component of solar radiation in mosses on the desiccated state has not yet been investigated to my knowledge.

1.4 Plant responses to UV-BR and visible light

This section reviews progress and different experimental methodologies used to investigate plant response to damaging light levels which are similar in bryophyte and vascular species (Rozema et al. 2005). The known differences between these groups are highlighted and bryophyte examples are used where available. Damage and protection from UV-BR and excess light are reviewed. Ultraviolet absorbing compounds and the role of protective pigments within the chloroplast are discussed in detail.

1.4.1 Probing plant response to UV-BR; shifting paradigms

Since ozone depletion sparked a wave of UV-BR research in the 1980s, ideas have progressed from UV-BR as extremely damaging for all plants to the view that UV-BR damage is rare, particularly for plants in tropical and temperate mesic outdoor settings (Searles et al. 2001; Dormann and Woodin 2002; Ballaré et al. 2011; Bornman et al. 2015). Damaging light levels can harm plants by targeting sensitive molecules directly or indirectly through the production of reactive oxygen species (ROS; Hideg et al. 2013). Laboratory studies have advanced our understanding of the numerous factors influencing the plant UV-BR response, but can exaggerate damage (Searles et al. 2001; Dormann and Woodin 2002; Ballaré et al. 2011). Field studies generally report more subtle impacts of UV-BR. This is because the recently discovered UV-BR receptor (UVR8) induces efficient protective responses (Caldwell et al. 2007; Newsham and Robinson 2009; Rizzini et al. 2011). As the UV-BR dose increases, the response changes from a eustress (positive) to a distress (negative) (Kranner et al. 2010) and the transition point between these two states is dependent on numerous other factors which are discussed below.

All plants must balance the need to harvest sufficient sunlight for growth and reproduction with the protection of their tissues from the damaging components of solar radiation. Ultraviolet-B radiation and excess photosynthetically active radiation (PAR) damage key components of cells, including DNA, pigments, proteins and lipids, either through direct absorption, or indirectly through the production of ROS (Jansen et al. 1998; Ulm and Nagy 2005; Jenkins 2009). Excess radiation generates highly destructive ROS within the chloroplast, including, for example, superoxide, hydrogen peroxide and singlet oxygen.

Some forms of damage are specific to UV-BR and created by direct absorption. Direct UV damage to DNA and proteins occurs because of their absorbance in the UV spectrum (Davies 1995). This includes UV-BR induced DNA damage, and probably damage to the protein subunits of PSI and PSII (photoinhibition). Takahashi and co-workers (2010) suggest a two step model for photoinhibition of PSII; firstly the manganese cluster in PSII is damaged by direct absorption of UV-BR, then ROS created from excess PAR and UV-BR inhibit repair of PSII by blocking the protein translation required for repair. Moreover, direct absorption of UV-BR by DNA creates cyclobutane pyrimidine dimers (CPDs), which can also block transcription. Recently Vass et al. (2013) used cyanobacterial mutants deficient in CPD repair to demonstrate higher concentrations of CPDs reduced the transcription of the gene *PsbA3,* which encodes the D1 protein from PSII. Rapid turnover of the D1 protein is crucial to the repair of damage to PSII from natural sunlight. Slowing this important repair cycle may inhibit photosynthesis and cause extended photoinhibition (Matsubara et al. 2012). Strategies that reduce UV light absorption, such as UVACs are therefore particularly important in preventing direct damage from UV-BR (Robinson and Waterman 2014).

High doses of PAR are damaging to plants when they exceed the level that can be utilised (or quenched) by photosynthesis. Other stressors, such as cold and drought, exacerbate damage by slowing the enzymatic reactions of carbon fixation and protective photochemical processes such as photorespiration, and thus reduce

thylakoid electron transport (Takahashi and Murata 2008; Garcia-Plazaola et al. 2012). Therefore PAR can be harmful, even at low light levels when photosynthesis is impaired.

Likewise plant response to UV-BR is complex and as with PAR, varies with a number of factors. This initially led to some conflicting UV-BR effects reported in the literature. Deleterious impacts of UV radiation increase with quantity (fluence) and with shorter UV wavelengths, and are also influenced by the nature of the background radiation (Brosche and Strid 2003; Newsham and Robinson 2009). Plant response is also dependent on prior exposure (Frohnmeyer and Staiger 2003), the developmental stage of the plant (Jordon et al. 1994) and other abiotic factors (Ballaré et al. 2011; Bornman et al. 2015). Herbaceous plants are more UV-BR sensitive than woody plants (Li et al. 2010), but generalisations between broad functional groups have not been useful (Dormann and Woodin 2002; Newsham and Robinson 2009). UV-BR sensitivity varies both between species (Lovelock and Robinson 2002) and within species (ecotypes; Hofmann et al. 2001; Kalbina and Strid 2006; Jansen et al. 2010) and this impacts on plant-plant competition (Newsham and Robinson 2009; Snell et al. 2009). Rapid environmental changes and/or simultaneous stressors are most damaging (Robinson and Waterman 2014). Whilst high radiation levels are potent stressors for plants, low doses of both UV-BR and PAR are essential regulators of plant architecture and acclimative protective mechanisms (Jenkins 2009).

A UV-BR receptor has recently been discovered and the constructive role of low doses of UV-BR as a signalling agent is being investigated (Rizzini et al. 2011; Hideg et al. 2013). Sunlight perception and signalling pathways are vital for plant survival. Photoreceptors sense specific wavelengths of light and control the growth, development and biochemistry of plants to optimise survival in the prevailing conditions. These include phytochrome that absorbs in the red and far-red (600-800 nm), phototropin and cryptochrome that both absorb UVA/blue light (320-500 nm) and the UV-BR receptor (UVR8; Rizzini et al. 2011). The latter responds to low UV-

BR doses causing alterations to the expression of hundreds of genes (Ulm and Nagy 2005). In addition to plant architecture, UVR8 regulates genes involved in acclimative protective mechanisms such as UVAC synthesis, DNA damage repair, enzyme production, and antioxidant activity (Izaguirre et al. 2003; Brown et al. 2005).

Laboratory studies have been crucial to understanding the molecular mechanisms underlying the UV-BR response of plants. Yet such studies do not predict consequences for plants in natural settings. Sunlight is difficult to emulate in the laboratory and irradiation treatments often contain unrealistically high UV-BR or high ratios of UV-BR to PAR. Low PAR inhibits specific repair processes (eg. DNA damage photoreactivation) and thus inflates damage (Rozema et al. 1997). Prior growth of experimental plants in unnatural radiation also amplifies damage by impairing acclimation responses (eg. UVAC accumulation; Searles et al. 2001; Dormann and Woodin 2002; Ballaré et al. 2011). In field studies background solar radiation and climatic conditions induce efficient protective mechanisms. Thus, less exaggerated responses are generally found than in controlled environment experiments (Searles et al. 2001).

Outdoor studies report subtle impacts of UV-BR on plant growth and biochemistry, with important ecosystem ramifications (Ballaré et al. 2011). Field studies report modest growth reductions (<20%), increased UVAC, and low levels of DNA damage (Searles et al. 2001; Newsham and Robinson 2009). Subtle changes delaying development and altering reproduction and phenology also occur (Day et al. 2001; Feng et al. 2007; Comont et al. 2012). These changes impact plant-plant, plant-herbivore, plant-pathogen and plant-detritivore interactions, and lead to complex ecosystem effects (Caldwell et al. 2007; Ballaré et al. 2011; Bornman et al. 2015).

In field studies, solar UV-BR is typically either decreased using filters or supplemented with light banks (Searles et al. 2001; Boelen et al. 2006; Newsham and Robinson 2009). Each approach has inherent problems and the methodology

appears to influence the conclusions about the UV-BR tolerance of plants (Newsham and Robinson 2009). Screens alter other abiotic variables like temperature, water and humidity and can produce unnaturally large differences between the control and treatment (Newsham and Robinson 2009). But UV-BR supplementation may have unstable outputs in cold environments (Newsham and Robinson 2009). Other studies have compared plant response to natural variation in incident UV-BR caused by altitude (Hespanhol et al. 2014) or ozone depletion (Newsham et al. 2002; Newsham 2003).

1.4.2 Plant protection from damaging light

Sophisticated protective strategies from damaging light are essential to plant survival (Figure 1.5). Such strategies limit impacts by reducing light absorption, mitigating its impact or by repairing damage (Robinson et al. 2003). Plant pigments play vital roles as screens, antioxidants and in dissipating excess absorbed light energy safely as heat within the chloroplast (Gould and Lister 2006; Nichol et al. 2012). Where protection is insufficient then the ensuing damage, for example, to the photosynthetic apparatus and DNA can also be repaired. Pathways such as PSII repair and DNA damage repair can be energetically costly and error-prone in the latter case, and so protective strategies are preferable (Bray and West 2005). As photosynthesis is vital in plants, several pathways exist within the chloroplast to avoid damage from solar radiation and maintain photosynthesis.

1.4.2.1 Photoprotection within the chloroplast

Both visible and UV-BR wavelengths can damage the photosynthetic apparatus (Takahashi and Badger 2011). Carotenoids, including β–carotene and xanthophyll pigments can mitigate such damage through ROS scavenging (Havaux et al. 2007). The xanthophylls have also received much attention for their role in dissipating excess light energy safely as heat in a process called non-photochemical quenching (NPQ) or reversible photoinhibition (Demmig-Adams and Adams 1992a).

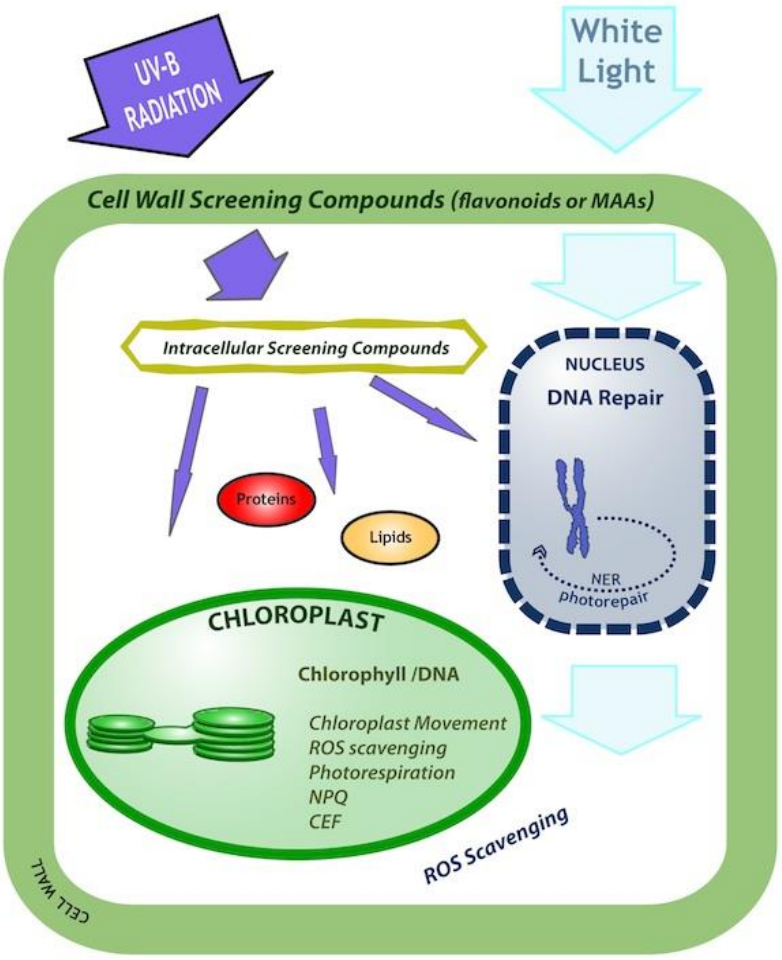

Figure 1.5: Possible consequences of UV-BR and high light on plant cells are shown. Potential sites of damage include molecules such as proteins, lipids and DNA. Incident UV-BR can be reduced by cell wall and intracellular UV screening compounds. The nucleus and chloroplast are particularly vulnerable. Damage to DNA can be repaired via photorepair or nucleotide excision repair. Within the chloroplast, damage can be avoided through chloroplast movement, reactive oxygen species (ROS) scavenging, photorespiration, non photochemical quenching (NPQ) and cyclic electron flow (CEF). Reproduced with permission from Robinson and Waterman (2014).

Zeaxanthin (Z) dependent NPQ occurs in both bryophytes and vascular plants (Demmig-Adams and Adams 1996; Garcia-Plazaola et al. 2012). In vascular plants this is generally thought to be regulated by light but in bryophytes also appears to be triggered by the desiccation process and uses additional proteins (Table 1.1; Garcia-Plazaola et al. 2012; Gerotto et al. 2012).

In vascular species two xanthophyll cycles mediate the conversion of excess excitation energy to heat (Figure 1.6). The first cycle interconverts violaxanthin (V) to Z via antheraxanthin (A) (Demmig-Adams and Adams 1996) and the second cycle converts lutein epoxidase (Lx) to lutein (L) (Garcia-Plazaola et al. 2007). In the presence of V or Lx light energy passes to photochemical processes, whilst Z and/ or L facilitate thermal deactivation of light energy absorbed by chlorophylls. The enzymatic production of Z by violaxanthin de-epoxidase occurs when high light levels generate a pH gradient across the thylakoid membrane (Nichol et al. 2012). Non-photochemical quenching is rapidly responsive to fluctuating light levels; as light levels ease, ΔpH relaxes and photosynthetic efficiency is rapidly restored, even while Z is still present (Niyogi et al. 2005; Demmig-Adams et al. 2012; Gerotto et al. 2012; Nichol et al. 2012).

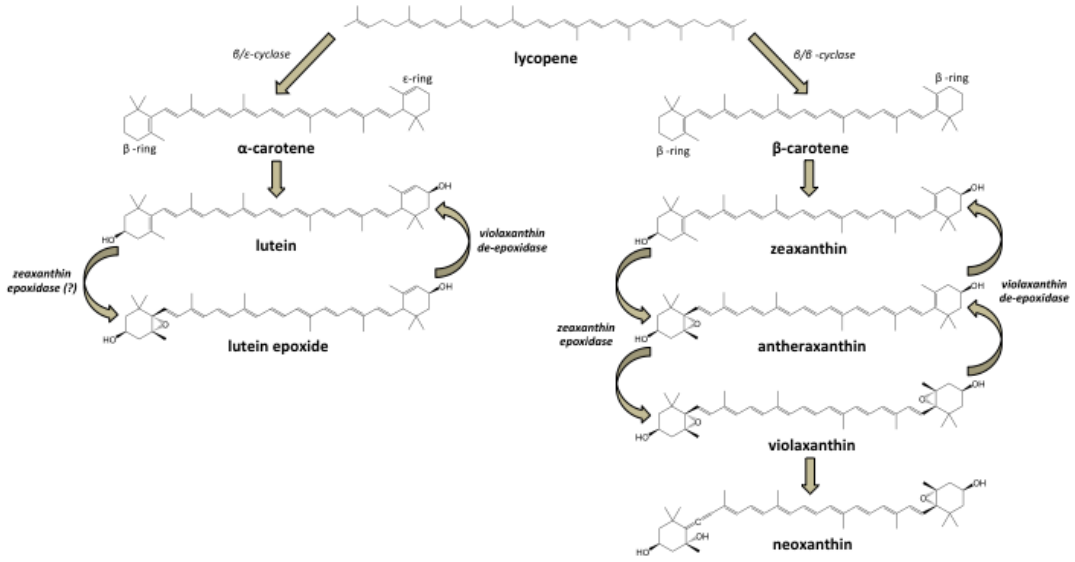

Figure 1.6: The xanthophyll pathway. These carotenoids are derived from lycopene. Violaxanthin de-epoxidase converts violaxanthin or lutein epoxidase to zeaxanthin or lutein, which facilitate thermal deactivation. Reproduced with permission from Robinson and Waterman (2014).

In vascular species Z dependent NPQ is regulated by three components; strong light which forms a proton gradient across the thylakoid membrane, interconversion of xanthophyll pigments (to Z) and the protonated PsbS protein (Demmig-Adams and Adams 1992b; Niyogi et al. 2005; Garcia-Plazaola et al. 2007).

However, cryptogams such as bryophytes and lichens, are substantially metabolically inactive when dry (Fernandez-Marin et al. 2013). In this state they cannot generate the pH gradient required to convert V to Z, but are still vulnerable to photo-oxidative damage from excess light (Heber et al. 2006a). To overcome this, desiccation tolerant cryptogams appear to produce Z as a result of the desiccation process (Table 1.1). Other types of quenching may be involved, and controversy exists over the role of Z in this process (Heber et al. 2006a; Fernandez-Marin et al. 2009; Fernandez-Marin et al. 2011). Nevertheless, the capacity to engage NPQ when dry is critical to the survival of desiccation events.

In some vascular species, such as overwintering conifers, cold temperatures can similarly result in sustained Z accumulation, which is not reversed in overnight darkness but requires warming. Such light independent Z accumulation in response to abiotic stress may be more common than previously thought (Fernandez-Marin et al. 2011). Verhoeven (2014) highlights similarities in the fluorescence kinetics of desiccation induced NPQ in mosses and the sustained non-photochemical quenching in overwintering conifers. Since mosses lose cellular water as they freeze, similar processes likely operate (Lenne et al. 2010; Verhoeven 2014).

Vascular plants, mosses and chlorophyte algae have similar capacities for NPQ, but rely on different light harvesting centre proteins (Table 1.1; Gerotto et al. 2011). Vascular plants exclusively use the PsbS protein (Li et al. 2004; Niyogi et al. 2005) and chlorophyte algae exclusively use the LHCSR protein (Peers et al. 2009). Yet both proteins occur in the moss *Physcomitrella patens* and these appear to function independently (Gerotto et al. 2012). The physiological relevance of these

differences are currently unclear but may provide mosses with more quenching capacity (Gerotto et al. 2012).

1.4.2.2 UV protection in plants

The accumulation of UV sunscreens is a widespread response of plants to UV-BR (Searles et al. 2001; Newsham and Robinson 2009). These secondary metabolites are a diverse group of compounds which screen damaging UV-BR wavelengths but transmit PAR. This filters UV-BR before it reaches vulnerable molecules, but does not attenuate PAR so allows photosynthesis to proceed (Jansen et al. 1998; Cockell and Knowland 1999). Such compounds in herbarium samples, spores, pollen, fossils and in long shoots of extant moss hold promise as proxies for extending the brief record of ozone column depth and UV-BR levels (Bjorn and McKenzie 2007; Bornman et al. 2015). Methanol soluble UVAC are easily extracted from the cytoplasm but UVAC from the cell wall are less commonly investigated. Interspecific differences in the location of UVAC within the leaf (cell wall or intracellular) may alter the effectiveness and metabolic cost of UV-BR protection (Semerdjieva et al. 2003).

The chalcone synthase pathway that gives rise to UVAC, including anthocyanins, is extant in mosses, green algae and vascular plants, thus has approximately 470 million years of evolutionary history (Gould and Lister 2006; Jiang et al. 2006). UV absorbing compounds contain aromatic ring(s) and hydroxyl group(s). In plants, they include flavonoids (including anthocyanins), hydroxycinnamic esters and phenolic acids. Flavonoids have been identified from the cytoplasm of mosses and biflavones and hydroxycinnamic acids from the cell walls of mosses (Fabon et al. 2012a; Waterman 2015).

Rapid accumulation of methanol soluble UVAC occurs in elevated UV-BR, diurnally or over longer periods (Gwynne-Jones and Johanson 1996; Veit et al. 1996). In addition to screening, particular UVAC (eg. flavonoids) also reduce indirect UV-BR damage by scavenging reactive oxygen species (Gould 2004; Gould and Lister 2006;

Cash et al. 2007; Agati et al. 2012). Whilst the UV-BR protective role is often emphasised, certain flavonoids also have roles in plant signalling and in protection from other biotic and abiotic stressors (Manetas 2006; Jansen et al. 2008; Agati and Tattini 2010; Jaakala and Hohtola 2010). Hypersensitivity to UV-BR occurs in *Arabidopsis* mutants deficient in phenolic compounds confirming their role in UV-BR protection (Li et al. 1993; Lois and Buchanan 1994).

Most field studies employ methanol extractions, a cheap and effective tool for assessing changes in the bulk intracellular pool of these compounds. However, this technique does not quantify interconversion between different compounds (Jaakala and Hohtola 2010 and references therein) nor cell wall bound insoluble pigments (Bornman and Teramura 1993; Ruhland and Day 2000; Semerdjieva et al. 2003; Clarke and Robinson 2008; Monforte et al. 2015).

Anthocyanins are a fascinating class of methanol soluble flavonoids which are widely distributed across taxa and responsible for red or purple pigmentation in stems and leaves. They often occur in leaves in cold climates, temperate seedlings, juvenile tropical red flushes and in senescent leaves (Robinson and Russell 1998; Gould and Lister 2006; Hatier and Gould 2009). They often transiently accumulate at different ontological stages, or in response to a variety of stressors, both biotic: herbivory and pathogen infection, and abiotic stress: high light, temperature extremes, drought, heavy metals and nutrient deficiency (Steyn et al. 2002; Close and Beadle 2003; Gould 2004; Gould and Lister 2006; Hughes et al. 2012; Landi et al. 2015). Their functional role has been debated since 1909 (cited in Lee et al. 1987), and whilst convincing arguments exist for a primary role in antiherbivory and insect deterrence (Manetas 2006), this is unlikely in mosses, which are rarely eaten (Haines and Renwick 2009). A body of experimental evidence favours a photoprotective role (reviewed in Steyn et al. 2002; Close and Beadle 2003; Gould 2004; Gould and Lister 2006; Landi et al. 2015).

Anthocyanins possess antioxidant activity and also absorb both visible and UV wavelengths of light (Gould et al. 2002; Neill and Gould 2003; Mori et al. 2005). In vascular plants, anthocyanins are considered more important for protection from excess visible light than from UV-BR. They can reduce photoinhibition and assist recovery from excess light (Hughes et al. 2012 and references therein). This conclusion is partly due to their location; in vacuoles or the cytoplasm of mesophyll cells, and not at the leaf surface, which is the optimal location for UV screening (Gould and Lister 2006).

However, anthocyanins accumulate in response to UV-BR in both vascular species (Bandurska 2012) and bryophytes (Snell et al. 2009). They also reduce UV-BR damage to photosynthesis (Burger and Edwards 1996) and DNA (Takahashi et al. 1991; Hada et al. 2003). Conversely, studies in rice and petunia mutants have actually shown a detrimental impact of purple anthocyanins to UV tolerance, with reduced growth and yield in sustained supplemented UV-BR (1 W m^{-2}; Ryan et al. 2002; Hada et al. 2003). This is because anthocyanins screen UVA/blue light which is the energy source for photorepair of DNA damage caused by UV-BR (Hada et al. 2003). Recently the importance of UV-BR wavelengths to photoinhibition has been promoted (reviewed in Vass 2012), restoring a possible role for anthocyanic UV-BR protection of chloroplasts, either as screens or antioxidants. Furthermore, bryophytes appear to accumulate anthocyanins in their cell walls as compared to the cytosolic location in vascular plants (Table 1.1). This suggests they may have distinct roles in different plant groups.

Anthocyanins and anthocyanin-like compounds are histologically associated with the cell walls of certain bryophytes (Post and Vesk 1992; Newsham et al. 2005; Gould and Lister 2006; Hooijmaijers and Gould 2007; Hatier and Gould 2009; Snell et al. 2009). In the cell wall, anthocyanins provide an effective screen from both UV-BR and visible light and perhaps screening is the foremost role of these compounds in bryophytes. Although an antioxidant role in the complex matrix of the cell wall is not discounted. Gould (2004) describes anthocyanins as ‘Nature’s Swiss army knife’

and suggests they may have diverse roles in different taxa or multiple functions within the same leaf.

Increases in bulk methanol soluble UVACs is a common response to UV-BR in vascular species but may be less common in bryophytes (Table 1.1; Newsham et al. 2002; Newsham 2003; Dunn and Robinson 2006; Clarke and Robinson 2008; Lappalainen et al. 2008). Bryophytes lack the complex leaf surface structures (eg. epidermis) where methanol soluble UVAC typically accumulate in vascular species (Day et al. 1993; Vogelmann 1993). Bryophytes may rely more on cell wall UVAC (Table 1.1). The latter are rarely quantified so UV-BR screening capacity in bryophytes may be profoundly underestimated (Clarke and Robinson 2008).

Accumulation of cell wall UVAC in response to UV-BR occurred in the liverwort *Jungermannia exsertifolia* subsp. *cordifolia* (Fabon et al. 2010) but not in the mosses *Bryum pseudotriquetrum* and *Fontinalis antipyretica*. (Fabon et al. 2012a). Possibly protection provided by cell wall UVAC is constitutive in mosses, or like soluble UVAC, only particular species increase these compounds in UV-BR. This is important since cell wall UVAC are more stable than methanol soluble UVAC and thus potentially more useful as proxies for past UV-BR levels (Monforte et al. 2015).

Differences between taxa in location (cell wall or intracellular) and quantity of UVAC and anthocyanin concentrations may reflect different UV-BR protective and life history strategies (see Section 5.1 for details on anthocyanins). The location, concentration and UV-BR responsiveness of UVAC was shown to be markedly different in three sub-Arctic *Vaccinium* species – surprising in such closely related plants (Semerdjieva et al. 2003). These authors hypothesise that different strategies may relate to leaf longevity. Deciduous leaves may rely on intracellular UVAC, which can be remobilised before leaf drop, thus saving metabolic costs. In contrast, long-lived evergreen leaves have higher levels of UVAC in cell walls of epidermal cells. The latter provides optimal UV screening but at a higher metabolic investment since these compounds are unlikely to be retrieved from the cell wall. The location of

UVAC may be a bryophyte leaf trait that reflects a higher (cell wall) or lower (intracellular) investment and may relate to the life history strategy of the species.

In vascular species, the Worldwide Leaf Economics Spectrum (WLES) describes a markedly consistent set of correlations between leaf functional traits, indicating strong constraints on metabolic costs and returns over the lifetime of the leaf (Wright et al. 2004). At one end of the continuum, slow return species have long-lived expensive leaves, with high leaf mass per area and respiratory rates, low nitrogen and phosphorus content, and low photosynthetic rates. At the other end, quick return species have short-lived, structurally inexpensive leaves, with low leaf mass per area, but a rapid return on investment, with high Nitrogen and Phosphorus content to facilitate high photosynthetic rates and rapid growth.

In bryophytes leaf traits can be substituted for more easily measured traits at the canopy or shoot scale (Rice et al. 2008; Waite and Sack 2010; Waite and Sack 2011; Rice and Cornelissen 2014; Wang et al. 2014). Photosynthesis is usually measured at the canopy scale in bryophytes, with the leaf layers considered analogous to the layers in the mesophyll (Waite and Sack 2010 and references therein).

This approach has been tested in bryophytes in Hawaii (Waite and Sack 2010; Waite and Sack 2011) and within boreal *Sphagnum* species (Rice et al. 2008; Laing et al. 2014). Like vascular species, maximal photosynthesis increased with colony mass per area. However, bryophyte canopies are not leaves and not all trait relationships found in vascular species were observed (Rice and Cornelissen 2014). The strong relationship in angiosperms between increasing leaf Nitrogen and maximum photosynthetic rates does not occur in bryophytes. This is because allocation to structural components, such as costa and hyaline cells which are important for water balance, will reduce photosynthetic efficiency on a mass basis (Rice and Cornelissen 2014).

Accumulation of phenolics have been demonstrated to reduce fitness when UV-BR is low, implying a metabolic cost in their production (Weinig et al. 2004). The

relative size of these costs may increase in low quality environments where energy budgets are reduced (Cipollini et al. 2003; Snell et al. 2009). However, photosynthetic and genetic integrity are paramount to plant survival. If the UV-BR dose exhausts the plant's protective mechanisms then damage to photosynthesis and DNA can occur.

1.4.3 Damage to the photosynthetic pathway

Ultraviolet-B radiation can reduce photosynthesis through the inactivation of photosystem I (PSI) and photosystem II (PSII), chlorophyll bleaching (photo-oxidation) and decreased Rubisco activity (Ballaré et al. 2011). Photosynthesis is largely protected from UV-BR in natural conditions (Searles et al. 2001; Newsham and Robinson 2009). However, in stressful polar and desert environments, particular species appear vulnerable showing decreased photosynthesis or reduced photosynthetic pigments in response to UV-BR. These include studies using either UV-BR enhancement (Gehrke 1998; Gehrke 1999; Belnap et al. 2008; Arroniz-Crespo et al. 2011) or UV-BR filtration (Montiel et al. 1999; Xiong and Day 2001; Newsham et al. 2005; Robinson et al. 2005; Albert et al. 2008; Albert et al. 2011).

Enhanced UV-BR decreased chlorophyll pigments in the sub-Arctic mosses *Polytrichum commune* and *Sphagnum fuscum*, with a non-significant trend to decreasing chlorophyll in a third species *Hylocomium splendens* (Gehrke 1998). Despite this, *P. commune* and *S. fuscum* were more UV-BR tolerant than *H. splendens* which had reduced photosynthetic efficiency, reproductive output and growth after long term enhanced UV-$BR_{BE\ Caldwell}$ (1.2 kJ m^{-2} day^{-1})(Gehrke 1998; Gehrke 1999; Arroniz-Crespo et al. 2011). This suggests this species is less able to adapt to UV-BR. Reduced photochemical yield occurred in UV-BR_{BE} (enhancement; 0.74 W m^{-2}) in the Antarctic moss *Sanionia uncinata* but not in the two co-occurring vascular species (Montiel et al. 1999). Additionally, we found UV-BR affected the Antarctic moss *Schistidium antarctici* with morphological changes and reduced chlorophyll concentrations concomitant with increased protective carotenoids including zeaxanthin and β-carotene (Figure 1.7; Robinson et al. 2005).

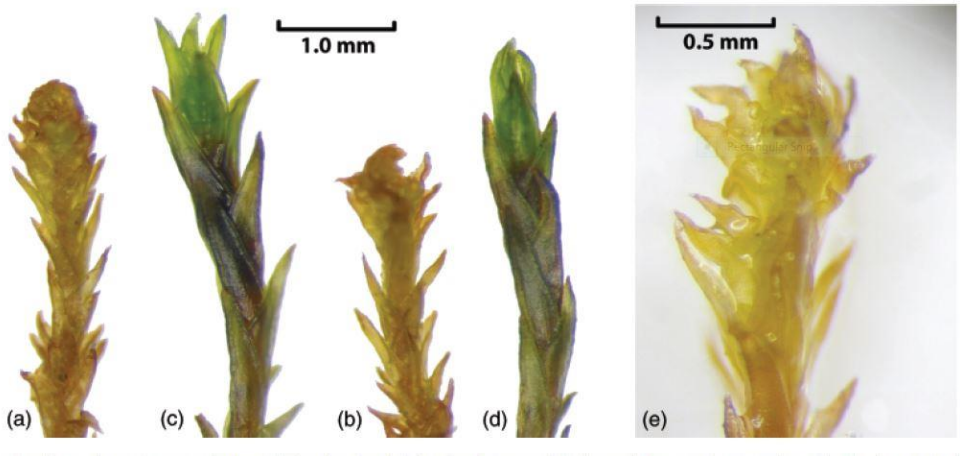

Figure 1.7: Comparison of gametophytes of *Schistidium antarctici* contrasting the normal green leaves found when ultraviolet-B radiation was reduced by screening (c,d) with the atypical morphology with shorter blunted leaf tips and yellow colouration which was more commonly found in natural UV-BR (a,b) and enlarged (e). Image reproduced with permission from Robinson et al. (2005).

Enhanced UV-BR (simulating 30% ozone depletion) reduced photosynthetic efficiency in biological soil crusts containing cyanobacteria, lichens and mosses in the Mojave Desert (Belnap et al. 2008). This effect was exacerbated in hot conditions (Belnap et al. 2008). Interestingly, UVAC failed to accumulate in this study and actually declined over the hot field season. The authors suggest that the extreme heat caused a carbon deficit, reducing the resources available for the synthesis of UVAC. The Antarctic liverwort *Cephaloziella varians* synthesised the protective anthocyanin riccionidin A within 48 hours of an abrupt increase in UV-BR. The minimum metabolic cost was 1.85% of carbon fixation in the same 48h period (Snell et al. 2009). Whilst this figure is low, for plants in low energy ecosystems with intermittent photosynthesis, the cost of UV-BR protection may be a significant burden on overall energy budgets. Differences in the effectiveness of UV-BR protection between species will be reflected in the DNA damage load.

1.4.4 DNA damage and repair

DNA damage is a useful parameter for comparing UV-BR tolerance between species. DNA absorbs strongly in the UV-BR spectrum (with absorbance peaking at 260 nm)

and so is a primary target of damage (Davies 1995). Pyrimidine dimers are shown in Figure 1.8. They comprise 98% of UV-BR induced DNA damage, mainly as cyclobutane pyrimidine dimers and pyrimidine (6,4)-pyrimidone dimers (6-4pps; Mitchell and Karentz 1993). Since accumulation of DNA damage balances the ability to specifically screen UV-BR with the potential for enzymatic DNA repair it can provide a short-term indication of a species' relative genetic UV-BR sensitivity (Hidema *et al.* 2007). High numbers of photoproducts affect DNA function and are associated with reduced growth (Rousseaux et al. 1999b; Giordano et al. 2003).

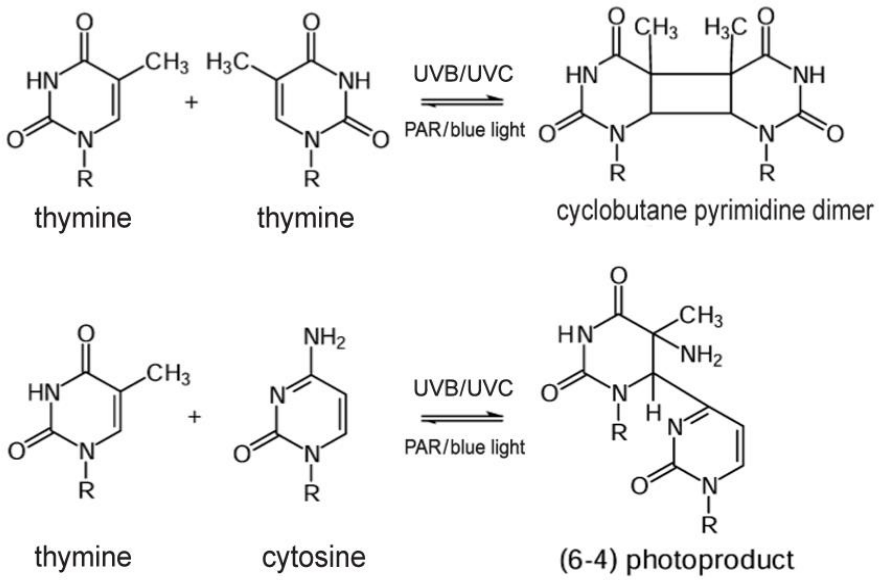

Figure 1.8: Cyclobutane pyrimidine dimers and 6-4-photoproducts form when UV-BR induces a covalent bond between adjacent pyrimidines on the DNA strand. Photorepair is rapid and accurate repair, using photolyases. These enzymes are activated by blue light or UV-A. Adapted from Beukers (2008).

DNA photoproduct accumulation is the balance between rates of induction and enzymatic repair. Thus higher DNA damage can occur under low UV-BR where enzymatic repair is reduced (e.g. from cold or drought; Li et al. 2002; Buffoni Hall et al. 2003). Both enzymatic repair activity, and UVACs, which limit UV-BR penetration into the leaf are likely to be important determinants of UV-BR sensitivity.

In high numbers, CPDs and 6-4pps can cause cell death and DNA mutation and are implicated in cell cycle checkpoints (Callegari and Kelly 2007; Tuteja et al. 2009). They also distort the structure of DNA, blocking DNA transcription and replication. (Figure 1.8; Ulm and Nagy 2005; Callegari and Kelly 2007). Such reductions in transcription of genes important to the PSII repair cycle may impair photosynthesis (see Section 1.4.1 above; Vass et al. 2013).

Slight reductions in growth are a consistent response to elevated UV-BR in field studies (Newsham and Robinson 2009). Growth reductions in the Southern Patagonian herb *Gunnera magellanica*, during ozone depletion events are suggested to be due to increased CPD accumulation (see Section 3.4; Rousseaux et al. 1999b; Giordano et al. 2003). So whilst plants can tolerate a low level of CPDs in their genome these may still impact on the fitness of the whole organism.

1.4.5 Plant response to combined abiotic stress

Plants rarely experience environmental stressors in isolation. Climate change is likely to affect several parameters at once. For example, in East Antarctica ozone depletion is linked to increased wind speeds as well as increased UV-BR (Ballaré et al. 2011; Clarke et al. 2012; Robinson and Erickson 2015). This section explores how the interactions between UV-BR and other abiotic variables can affect plants, particularly drought and cold temperatures.

Concurrent multiple stressors generally aggravate plant damage. For example, water availability and temperature affect all aspects of cell function. Reductions in photosynthesis will limit the metabolic resources available for protection and repair. However, cross-tolerance also occurs between environmental variables such as UV-BR, temperature and drought. Acclimation to one stress can reduce the impact of another simultaneous or subsequent stress. UV-BR in this case, can be considered a eustress, invoking an acclimative response that is protective against several stressors (Izaguirre et al. 2003; Bornman et al. 2015).

Ultraviolet-B radiation may be a signal that invokes a multipurpose stress response (Izaguirre et al. 2003). This involves common genes and complex signalling pathways. Cross-talk between plant hormone signalling pathways; jasmonic acid, salicylic acid and particularly abscisic acid, orchestrates the stress response. Heat shock proteins, small RNAs, transcription factors, kinase cascades and ROS also contribute to this regulatory crosstalk (reviewed in Atkinson and Urwin 2012).

Transcriptome profiling has demonstrated an overlap in the expression of genes differentially regulated when various stress factors such as cold, drought and UV-BR, are applied in isolation (Izaguirre et al. 2003; Kilian et al. 2007). In addition to these common genes, simultaneous stressors, such as heat or cold, and drought, activate a unique set of genes, distinct from those responding to the individual stress (Rizhsky et al. 2004; Mittal et al. 2012). Rasmussen and co-workers (2013) found that up to 60% of transcriptomes responding to combinations of stress cannot be predicted from single stress studies. This has profound implications for the discipline of plant stress biology and highlights the importance of field studies where simultaneous stressors are frequent.

Most studies investigating cross-tolerance are laboratory based. Growth chamber studies typically have no UV-BR in the control so actually investigate UV-BR as a eustress (eg. Comont et al. 2012). Some UV-BR is required for healthy growth and development and it promotes efficient protective responses via the UV-BR receptor UVR8 (Rizzini et al. 2011; Jenkins 2014). This is highly relevant to agriculture, for example, in hardening of seedlings, but these findings may not be relevant to a wild plant's response to increased UV-BR under ozone depletion. Such studies are therefore not considered further here.

Plants in low productivity environments such as deserts, alpine and polar regions experience multiple abiotic distress. Often desiccation tolerant, these plants have discontinuous photosynthesis, ceasing metabolism in unfavourable conditions. The metabolic costs of UV-BR protection are likely to comprise a relatively larger portion

of their energy budget. In these locations, simultaneous stressors are more likely to have additive and deleterious impacts on plants (Gehrke 1999; Belnap et al. 2008).

1.4.5.1 UV-BR and drought

Water availability is a major limiting factor for Antarctic mosses (Clarke et al. 2012; Wasley et al. 2012). Field studies of agricultural plants show UV-BR exposure may improve drought tolerance, but additive impacts can also be negative. The response is dependent on the taxa involved, and also on other environmental stressors which impact on the energy budget of the plant. A major difference between moss and most vascular plants is the ability to recover from desiccation (Proctor and Tuba 2002).

In vascular species, cross-tolerance has been observed between UV-BR and drought which often occur simultaneously (Ballaré et al. 2011). Growth in elevated UV-BR outdoors, appears to confer protection from drought and vice versa (Petropoulou et al. 1995; Manetas et al. 1997; Nogues et al. 1998). Features of UV-BR acclimation that may assist in improving drought tolerance include thicker leaves and cuticles, shorter plant height, reduced leaf area and stomatal conductance and increased branching, cuticular wax, antioxidants and capacity for nonphotochemical quenching (Manetas et al. 1997; Gitz and Liu-Gitz 2003; Poulson et al. 2006). However, whilst UV-BR can improve growth during a subsequent drought challenge, drought may not increase resistance to UV-BR. Apparent tolerance to UV-BR can occur if drought induces large reductions in growth which then mask the smaller UV-BR effects (eg. Duan et al. 2008; Arroniz-Crespo et al. 2011).

Bryophytes are desiccation tolerant so have a fundamentally different strategy for drought survival than vascular plants (Proctor and Tuba 2002). Despite this, a similar cross tolerance between water and UV-BR may occur. Moss species with higher relative desiccation tolerance have improved tolerance and recovery from both excess PAR and UV-BR (Seel et al. 1992a; Tákacs et al. 1999; Csintalan et al. 2001). Enhanced UV-BR had species specific impacts on Arctic bryophytes with stronger

UV-BR effects in mesic than xeric sites in a long-term outdoor study (Arroniz-Crespo et al. 2011). This could be due to masking of small UV-BR growth effects by large reductions in growth from the lack of water in the xeric site (Arroniz-Crespo et al. 2011). Desiccation tolerant mosses are protected from other forms of extreme stress in the desiccated state so this may also confer protection from UV-BR. This has not been investigated in bryophytes. However, UV-BR induced DNA damage increased in desiccated samples of the lichen *Cladonia arbusculata* and the authors ascribed this to impairment of enzymatic repair in the dry state (Buffoni-Hall et al. 2003). Desiccation events are metabolically costly (Proctor et al. 2007), and frequent dry episodes may also reduce the resources available for UV-BR protection.

Much of this work has been done using plants in relatively amenable environments prior to a drought challenge. These plants are well equipped to reallocate resources to protection. In plants from extreme environments, drought may also increase UV-BR sensitivity. As described above, cryptogamic mats in the desert have increased sensitivity to UV-BR during drought treatments, particularly in hotter years (Section 1.4.3; Belnap et al. 2008). Two species of Arctic moss, actually reduced UVAC in supplemented UV-BR, possibly because this species lacked the metabolic capacity to upregulate protection (Gehrke 1999). These examples illustrate that reduced energy budgets in extreme environments, such as deserts, alpine and polar regions, may lower the capacity to upregulate UV-BR protection such as UVAC (Drilias et al. 1997; Belnap et al. 2008).

1.4.5.2 UV-BR and temperature

Temperature is a major limiting factor for Antarctic mosses. Cold reduces all cellular processes including protection and repair from UV-BR, for example repair of PSII and CPDs (Buffoni Hall et al. 2003; Takahashi and Murata 2008). Moss can survive freezing, but frequent freeze-thaw events are metabolically costly and can damage membranes leading to solute leakage (Melick and Seppelt 1992). Vascular plants appear best able to cope with UV-BR when functioning near to their temperature

optima (Giordano et al. 2003), but few studies investigate the combined effects of cold and UV-BR. Cold temperatures can increase the impact of UV-BR (Yang et al. 2007b), conversely other studies show a eustress response with UV-BR increasing frost tolerance (Chalker-Scott and Scott 2004; Yang et al. 2007a).

Enhanced UV-BR increased frost tolerance in *Rhododendron sp.* (Chalker-Scott and Scott 2004) but decreased frost tolerance in one of three species of Arctic dwarf shrubs tested (Taulavuori et al. 2011). Taulavuori (2005) suggests the Arctic species may be resource limited and postulates a trade-off between cryoprotectants required for frost hardiness and UVAC which afford protection from UV-BR.

Prior growth in UV-BR improved frost tolerance in wheat seedlings compared to control plants (0 kJ m^{-2} d^{-1} $UV_{BE\ Caldwell}$), but a low UV-BR dose (4.2 kJ m^{-2} d^{-1} $UV_{BE\ Caldwell}$) was more effective at improving seedling survival than a higher UV-BR dose (7.0 kJ m^{-2} d^{-1} $UV_{BE\ Caldwell}$). This indicates the higher UV-BR dose was moving towards a distress (Yang et al. 2007a). In a similar study, reduced photosynthesis and yield occurred in wheat seedlings under a high UV-BR dose (7.0 kJ.m^{-2}.d^{-1} $UV_{BE\ Caldwell}$). This was exacerbated in cooler temperatures. Additionally, in the lower UV-BR dose (4.2 kJ.m^{-2}.d^{-1} $UV_{BE\ Caldwell}$) these UV-BR impacts only occurred when temperatures were reduced (from 25/20 ^{o}C to 10/5 ^{o}C; Yang et al. 2007b). The inclusion of more than one UV-BR dose is instructive and demonstrates that the lower UV-BR dose changes from a eustress (inducing acclimation) to distress (damaging) as temperature decreased from optimal. The line at which each abiotic variable changes from eustress to distress impacts on the plant response to other variables. This line is also likely to vary between taxa and with the acclimation state.

1.5 Thesis Outline

This thesis explores the response of Antarctic moss to UV-BR using a combination of field and laboratory studies. This comprehensive study simultaneously assesses the inherent seasonal variability in multiple plant physiological parameters. These reflect damage and protection in ambient sunlight. This gives insight to the varied

protective strategies provided by plant pigments and facilitates a comprehensive assessment of the UV vulnerability in three co-occurring species.

In this thesis I sought to address the following questions using three species of co-occurring Antarctic moss from the Windmill Islands, East Antarctica.

1. Are these Antarctic mosses damaged by increased UV-BR from ozone depletion?
2. Are there differences in the effectiveness of protective strategies between co-occurring species?
3. What is the inherent variability in photosynthesis and protective pigments within one summer growing season?

Chapter 2 of this thesis describes the general characteristics of the region and more specifically the conditions of the 2002/03 summer season. The ecology, anatomy and physiology of the three study species is also reviewed to facilitate comparisons of the survival strategies used by each species.

The four data chapters (3–6) are formatted as manuscripts with chapter 3 and 4 already published.

Chapter 3 considers if these mosses accumulate DNA damage in the field, across the 2002/04 growing season. It also assesses relationships between DNA damage and environmental factors which may induce DNA damage or affect its repair.

Chapter 4 investigates the extent to which DNA damage can be induced in a light box experiment designed to test the UV resilience of the three species in desiccated and hydrated conditions. This was an attempt to separate some of the many abiotic factors present in the field by way of a controlled experiment.

Chapter 5 investigates the variability in UVACs, including anthocyanins, in the three species across the 2002/03 growing season. The environmental factors driving accumulation are explored. The role of UV-BR in UVAC induction is of particular interest to the use of these compounds as proxies for past UV-BR levels.

In Chapter 6 I assess the inherent variation in photosynthesis and its associated pigments within the 2002/03 growing season. I use a novel approach to generate photosynthetic temperature and water optima across the season for the three species from field chlorophyll fluorescence data. Photoprotective strategies in the three species are compared combining UVACs and photosynthetic and photoprotective pigments within the chloroplast, with interrelationships between pigments also considered.

Chapter 7 provides a synthesis of the preceding chapters.

2 Study Site and species

2.1 Antarctic biogeographic zones and climate

Antarctica has traditionally been broadly divided into three biogeographic zones: subantarctic, maritime and continental (Convey 2011). This practical classification system separates the different climates and ecosystems of the subantarctic, the Antarctic peninsula, and the main body of the Antarctic continent (Figure 2.1; Convey 2011). However, it may oversimplify the genetic history of this region. Previously, Antarctic terrestrial ecosystems were thought to have colonised since the last glacial maxima, but considerable endemism and advances in describing bioregional phylogenies support the existence of Antarctic refugia, extending to the Pliocene and Miocene and possibly further (Terauds et al. 2012 and references within; Convey et al. 2014; Chown et al. 2015). The Transantarctic Mountains are a strong geographic boundary to colonisation into continental Antarctica. Reflecting this, the Gressitt line has been proposed, and lies close to the boundary between the maritime and continental Antarctica (Chown and Convey 2007). Furthermore, fifteen distinct biogeographic regions recognising distinct climate and taxa, are also proposed for the Antarctic Continent (Figure 2.1; Terauds et al. 2012). Following Terauds and co-workers (2012), my study was conducted in Antarctic Conservation Biogeographic Region 7, East Antarctica.

Across the Antarctic continent, rare ice-free oases support fellfield ecosystems, where the extreme climate limits vegetation to entirely cryptogamic populations (lichens, moss, algae and cyanobacteria). These ecosystems have low biodiversity, productivity and in mosses sexual reproduction is rare. The main constraints are the cold temperatures and associated water deficit which are discussed below (Kennedy 1993a; Wasley et al. 2006a).

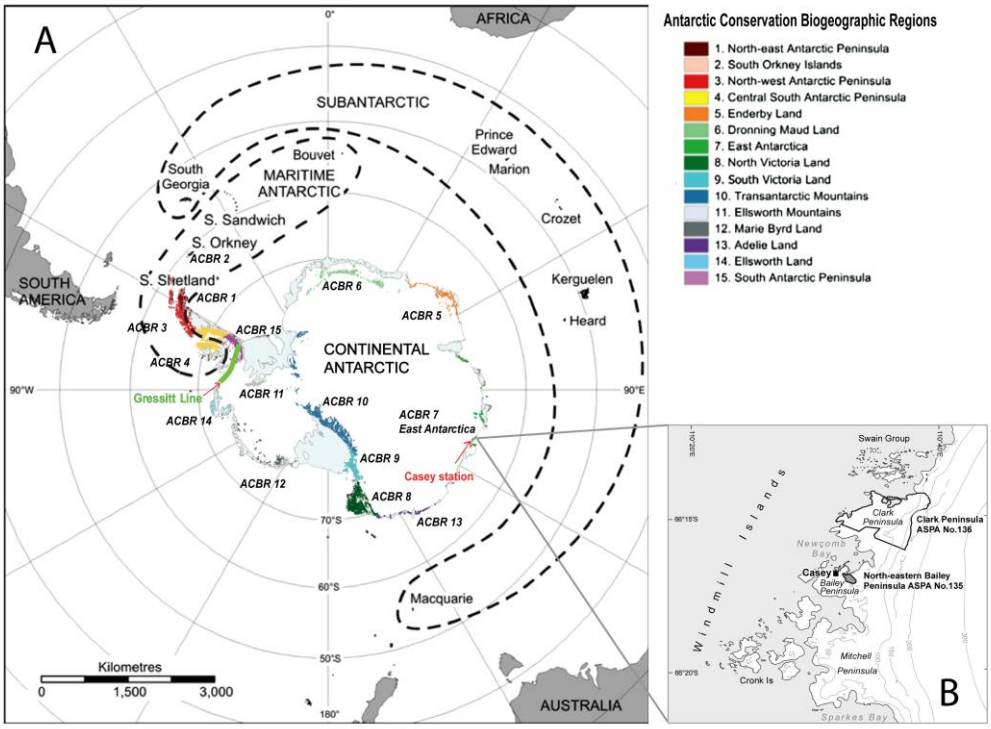

Figure 2.1: Map of the Antarctic (A) showing boundaries of the broad phytogeographic zones: the continental Antarctic, subantarctic and maritime Antarctic (bounded by dashed line; figure adapted from Convey 2011). The Gressitt line is shown (solid green line; redrawn from Chown and Convey 2007). Fifteen Antarctic Conservation Biogeographic Regions (ACBRs) are shown in colour according to the legend adapted from Terauds et al. (2012). Inset (B) shows the Windmill Islands region which is located in ACBR 7 (East Antarctica) following Terauds et al. (2012). The study site was located within Antarctic Specially Protected Area 135.

Antarctic mosses primarily reproduce vegetatively, although sporophytes have been reported as far south as 77° 55' S (Seppelt et al. 1992). The extreme climate limits sexual reproduction and *Ceratodon purpureus* populations show reduced genetic variation relative to temperate populations of the same species (Clarke et al. 2008). These microsatellite studies contradict previous reports of high levels of somatic variation in these mosses attributed to UV-BR induced DNA damage (Skotnicki et al. 2000). This low genetic diversity, combined with low gene flow due to geographical isolation may make these bryophytes especially susceptible to climate change (Clarke et al. 2009).

The simple structure of the terrestrial ecosystem, limited trophic interactions and low human impacts provide an excellent system to study the impact of environmental variables on plants and to assess impacts of global climate change.

2.2 Study site

2.2.1 Windmill Islands region

The Windmill Islands, on the Budd Coast, Wilkes Land, East Antarctica (66° 22', 110° 30') consist of a chain of low lying rocky peninsulas and islands (Figure 2.1 B). The northern end of this region deglaciated 8200 years BP (Gasparon et al. 2007 and references therein). The metamorphic geology of the Bailey and Clarke peninsulas are a stable substrate for vegetation (Blight and Oliver 1977; Melick et al. 1994). This region is frequently sheltered from katabatic winds by Law dome, a large ice dome to the west of the region (Melick and Seppelt 1997). The extensive cryptogamic communities are amongst the best developed in continental Antarctica and contain at least 27 macrolichens, 3 mosses and one liverwort (Lewis Smith 1986; Lewis Smith 1988b; Wasley et al. 2012).

This study was conducted on Bailey Peninsula in Antarctic Specially Protected Area 135; an area of approximately 0.5 km^2 set aside to protect the vegetation (Figure 2.1 B). Bryophytes grow in seepage areas and on the periphery of melt lakes and can form carpets as large as 50 m^2 (Lewis Smith 1986). Ancient penguin colonies provide a nitrogen source (Emslie and Woehler 2005; Wasley et al. 2012). Detailed descriptions of this site are published (Lewis Smith 1988b; Wasley et al. 2012).

2.2.1.1 Environmental conditions in 2002/03 compared to average

In the 2002/03 season the Antarctic ozone hole dissipated early in the season leading to an unusually high ozone column depth and lower UV-BR over Spring and Summer (Figure 3.1 and 3.2). This season also had low wind speeds (Section 3.3.5), warmer air temperatures in November (Figure 3.2) and an early start to a protracted and extensive summer melt.

Moss microsite data collected with samples in the 2002/03 season are presented here in the context of long term climate averages recorded at Casey station, on the Bailey peninsula (data collection described in Section 3.2.1, 6.2.1). Instantaneous measurements of photosynthetically active radiation (PAR), moss turf temperature (MT) and turf water content (WC) were collected with each sample.

The strong seasonality in Polar environments is driven by solar radiation. The Instantaneous PAR was highly variable between days but peaks around the summer solstice, consistent with high solar angles and longer days (solstice at day 43; Figure 2.2, A,B,C). There were few differences between species in the incident light levels measured at the moss turf. Days with clear skies were preferred for measurements but some days had thin high clouds or broken cloud cover. Cloud cover is more influential to five day weighted UV radiation doses than daylength and solar angle, causing a lack of correspondence between instantaneous measurements of noon time PAR and one and five day doses of UV radiation (Figure 2.2).

In ice free areas microhabitat conditions are often less extreme than ambient conditions and crucial in facilitating growth (Cannone et al. 2013). High absorption of solar radiation in summer regularly increases moss turf temperatures to 15 – 20˚C above ambient air temperatures (Lewis Smith 1988c; Green et al. 1999; Bramley-Alves et al. 2014). Extreme high temperatures can also occur with continental *Schistidium antarctici* recording temperatures as high as 43˚C (Lewis Smith 1988c). High, as well as low temperatures are likely to cause stress, particularly as high temperature occurs during periods of high insolation.

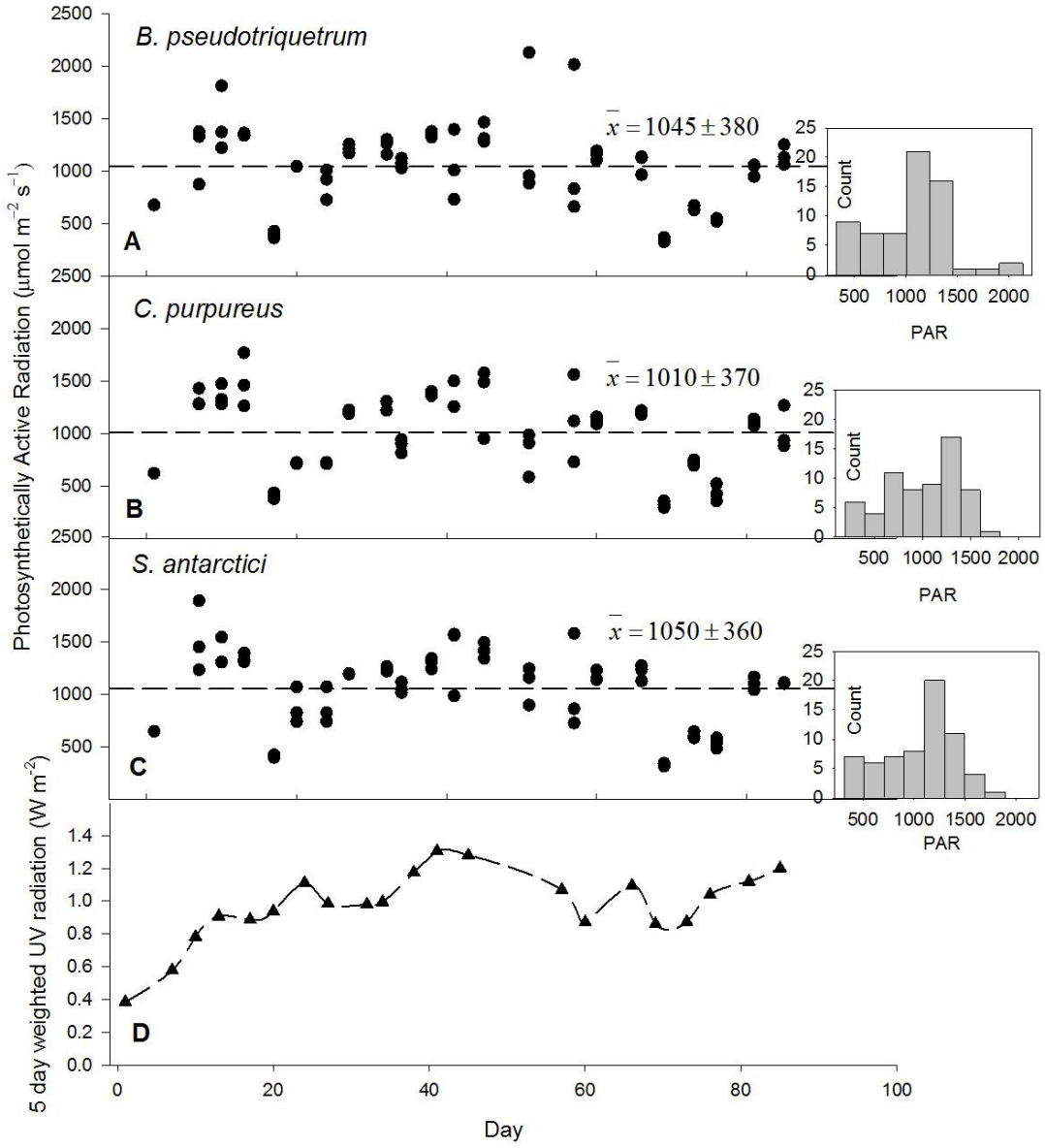

Figure 2.2: Photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}) incident on *Bryum pseudotriquetrum* (A), *Ceratodon purpureus* (B) and *Schistidium antarctic*i (C) measured at the time of sampling using the mini-PAM leaf clip attachment between 9 November 2002 and 1 February 2003. Dashed lines show season means for collected data. Frequency histograms for PAR are inset for each species as counts (n=64). Five day mean weighted UV radiation (D) is shown for the sampling days. This is weighted to human exposure and was converted from standard erythemal doses (Solar Light UV Biometer, SL501). Day 0 = 8 November.

Moss turf temperatures in 2002/03 were considerably above the maximum air temperatures, as is typical for continental Antarctic mosses (Figure 2.3). This is due to the strong absorption of solar radiation and thick boundary layers above the moss turf (Bramley-Alves et al. 2014). The lack of correspondence between minimum and maximum air temperatures is likely due to cloud; on clear sky days mosses absorb more solar radiation, but clear sky nights have increased radiative heat loss. The moss turf of *S. antarctici* appears to have fewer low temperatures than the other two species (Figure 2.3, inset in C).

Cold temperatures limit metabolic activity and increase the risk of freezing during the growing season (Bramley-Alves et al. 2014). Whilst moss can survive recurring freeze thaw events, they are metabolically expensive and most deleterious when there is no opportunity for positive carbon gain in the intervening period (Lovelock et al. 1995a; Lovelock et al. 1995b; Bramley-Alves et al. 2014).

Average monthly temperatures in the Windmill Islands range from 0.3 to -14.9 ^{o}C in the warmest and coldest months with recorded temperature extremes of -41 and 9.2 ^{o}C (Melick and Seppelt 1997). In the summer months (November to February) the average monthly maximum temperature ranges from -0.1 to 2.3 ^{o}C while the minimum ranges from -8.8 to -2.5 ^{o}C (Bureau of Meteorology 2015).

Both high and low temperatures reduce water availability to Antarctic mosses through increased desiccation or freezing respectively. High wind can also be both cooling and desiccating.

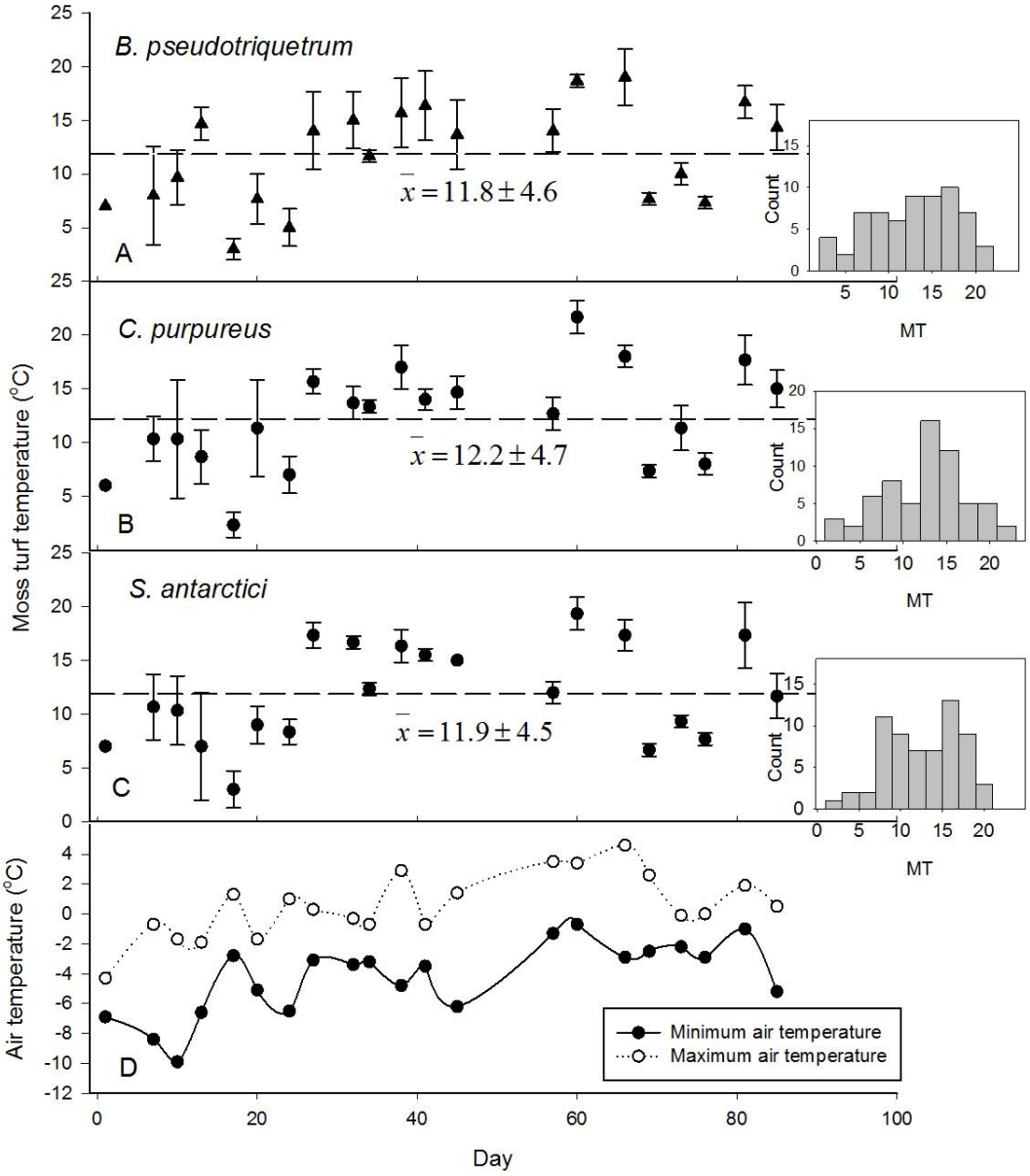

Figure 2.3: Daily mean moss turf temperature (°C) in *Bryum pseudotriquetrum* (A), *Ceratodon purpureus* (B) and *Schistidium antarctici* (C) measured at the time of sampling (mean ± sd, n=3 except day 1 where n=1). Dashed lines show season means and standard deviation. Moss turf temperature frequency histograms are inset for each species as counts (n=64). Daily minimum and maximum air temperature (D) provided by the Bureau of Meteorology, Casey station. Note the different scale of the axes between air temperature (D) and moss turf temperature (A,B,C). See Figure 2.2 for dates. Day 0 = 8 November.

This region is relatively sheltered from the regular katabatic winds which are typical in contintental Antarctica. However it still has an average of 96 days of gale force winds per year, usually from the East (Melick and Seppelt 1997). The annual mean daily wind run is 606 km and the maximum recorded wind gust is 241 km/hr (Bureau of Meteorology 2015). This season had lower than average wind speeds (Section 3.3.5).

In Antarctic mosses, habitat hydrology determines growth and productivity, and so defines plant community extent and structure (Clarke et al. 2012; Wasley et al. 2012). This area has low precipitation, usually falling as snow, with a mean annual water equivalent of 221 mm yr^{-1} (Bureau of Meteorology 2015). During the summer growing season (November to February) the mean number of days with a water equivalent ≥ 1mm ranges from 2.7 to 3.7 (Bureau of Meteorology 2015).

In the 2002/03 season water was only limiting in early season, before the widespread melt began (Figure 2.4). Daily mean turf water contents were lowest at the beginning of the season and generally increased until the summer solstice (day 43) and remained high thereafter. Water contents were lower in *C. purpureus* than in the other two species and *B. pseudotriquetrum* had fewer values below 2 gH_20 gdw^{-1} (Figure 2.4).

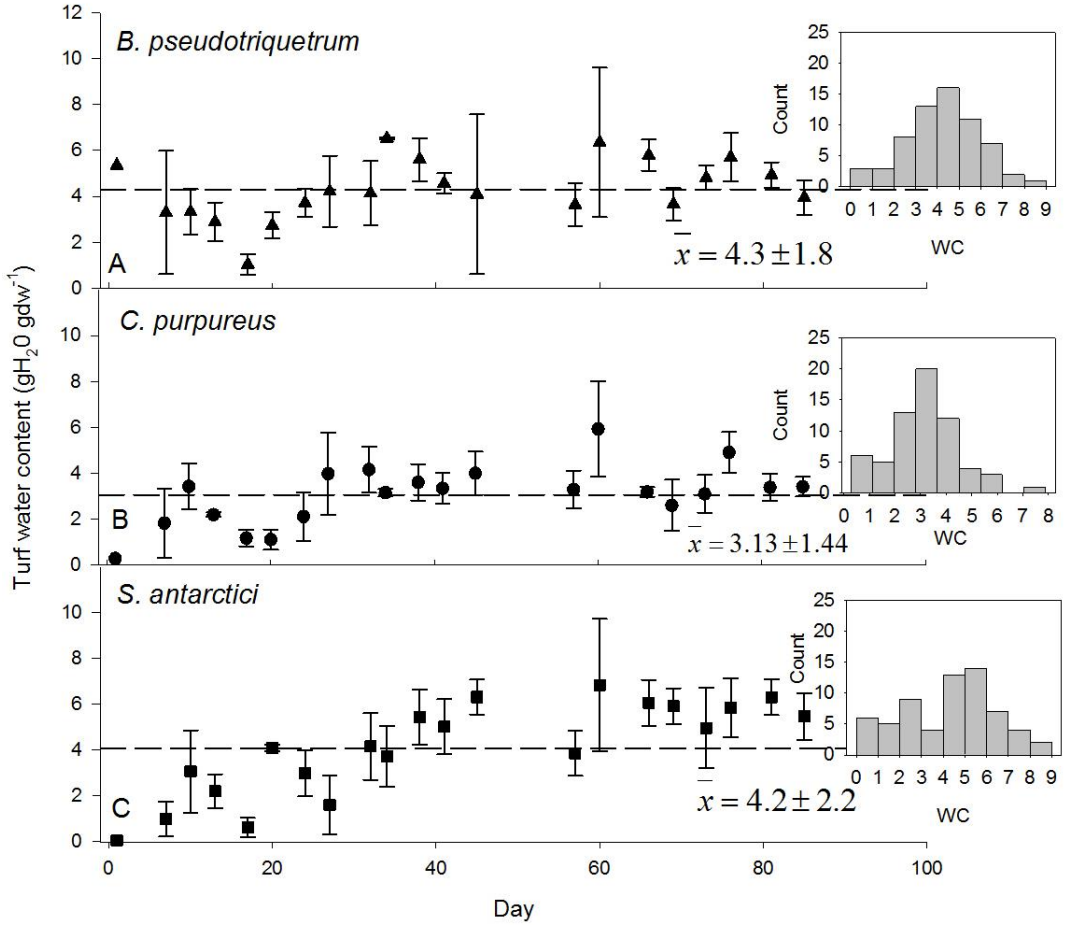

Figure 2.4: Daily mean turf water content ($gH_20\ gdw^{-1}$) for (A) *Bryum pseudotriquetrum*, (B) *Ceratodon purpureus* and (C) *Schistidium antarctici* (mean ± sd, n=3 except for day 1 where n=1). Dashed lines are season means and standard deviation. Moss turf water content frequency histograms are inset for each species as counts (n=64). See Figure 2.2 for dates. Day 0 = 8 November.

2.3 Study Species

The study species are *Schistidium antarctici* (Cardot) L.I. Savicz & Smirnova (syn. *Grimmia antarctici* Card.), *Bryum pseudotriquetrum* (Hedw.) Gaertn., B. Mey. & Scherb. and *Ceratodon purpureus* (Hedw.) Brid (see Figure 2.5). The latter two species have cosmopolitan distributions but *S. antarctici* is endemic to Antarctica and locally most abundant (Wasley et al. 2012).

Numerous studies have compared ecological, morphological and physiological traits in the study species in the Windmill Islands. The summary in Table 2.1 facilitates a comparison of the survival strategies employed by each species. Each species has a suite of adaptations, particular to its ecological niche and these appear largely driven by water relations. This summary is an important context for subsequent chapters, where the different strategies utilised by each species to protect from UV-BR are compared.

Hydrology is the prime influence on distribution of these three species and their relative tolerance of desiccation, as measured by chlorophyll fluorescence, reflects their ecological niche (Table 2.1; Selkirk and Seppelt 1987; Lewis Smith 1999; Wasley et al. 2012). *Schistidium antarctici* occurs predominantly in low-lying wetter sites and has a high tolerance of submergence but limited photosynthesis at low water contents (Robinson et al. 2000; Wasley et al. 2006b). *Ceratodon purpureus* is more common in higher, drier sites and is intolerant of submergence (Robinson et al. 2000; Wasley et al. 2006b). *Bryum pseudotriquetrum* is distributed throughout wet and dry sites. This species can tolerate submergence and has a highly plastic response to water (Robinson et al. 2000; Wasley et al. 2006b).

Table 2.1: Comparison of ecological, physiological and morphological traits in the three moss species found at the Windmill Islands.

	Trait	*B. pseudotriquetrum*	*C. purpureus*	*S. antarctici*	Citation
ecology	Habitat	Throughout wet and dry sites	Drier exposed sites	Wetter soaks and hollows	(Melick and Seppelt 1997; Wasley et al. 2012)
water	Desiccation tolerance; critical water contents below which photosynthesis drops sharply ($gH_20.gdw^{-1}$)	0.6-2.0 Decreased over the season	0.25-1.25	1.2-2.0	(Wasley et al. 2006b)
	Tolerance of submergence: effective yield (ΦPSII) after 6 weeks submergence	50% decrease	85 % decrease after two weeks	40% decrease	(Wasley et al. 2006b)
	Bulk $\delta^{13}C$ ratios	-26.0 ± 1.2 ‰	-25.2 ± 1.6 ‰	-24.2 ± 1.5‰	(Bramley-Alves et al. 2015)
morphology	Shoot density (cm^{-2})	550±70	888±47.5	550±52.5	(Wasley et al. 2006b)
	Rhizoids (Windmill Islands study)	dense to sparse	not reported	Non-rhizoidal	(Lewis Smith 1988a)
	Rhizoids across Antarctic	dense to sparse	various	Sparse to absent	(Ochyra, 2008)
	Hydroids in stem (central strand)	large	present	Indistinct to absent	(Ochyra, 2008; Lenne et al., 2010)
	Cell wall width	Thin <5 μm	~10 μm	>20 μm, higher in dry than wet sites	(Bramley-Alves 2015)
Freezing tolerance	Tolerance of 16 freeze thaw cycles whilst hydrated (% loss of total sugar pool)	66%	<29%	<31%	(Melick and Seppelt 1992)

	Trait	*B. pseudotriquetrum*	*C. purpureus*	*S. antarctici*	Citation
Photosynthetic capacity	O_2 evolution at 20 ºC ($\mu mol\ O_2\ mgchl^{-1}\ min^{-1}$)	4.2	2.8	NA	(Lewis Smith 1999)
	ETR max ($\mu mol\ e\ m^{-2}\ s^{-1}$)	190	180	85	(McKinley 2012)
Relative growth rates	Growth rates	high	medium	low	(Selkirk and Skotnicki 2007; Clarke et al. 2012)
Photosynthetic pigments Mean ± se	Total chlorophyll ($nmol.gfw^{-1}$)	475±60	241±40	453±44	(Lovelock and Robinson 2002)
	Chla/b	3.2±0.2	3.6±0.3	3.2±0.1	(Lovelock and Robinson 2002)
	VAZ/total chlorophyll ($mmol\ mol^{-1}$)	64±5	132±36	106±6	(Lovelock and Robinson 2002)
	%AZ/VAZ	18±3.9	39.4±4.9	37.4±2.5	(Lovelock and Robinson 2002)
	Total carotenoids / Total chlorophyll ($mmol\ mol^{-1}$)	443±15	643±96	631±31	(Lovelock and Robinson 2002)
metabolites	Fatty acids	medium	high	low	(Wasley et al. 2006b)
	Total soluble carbohydrates ($mg\ gdw^{-1}$)	Highest at season's start (≥270) but largest decrease over the season. seasonal mean = 90	Low throughout season (<70)	Medium, decreased over the season: seasonal mean =120	(Wasley et al. 2006b)
	Ratio of fatty acids to identified soluble carbohydrates	0.158	0.756	0.145	(Wasley et al. 2006b)

	Trait	*B. pseudotriquetrum*	*C. purpureus*	*S. antarctici*	Citation
Intracellular UV absorbing compounds	Mean ± se ($A_{280-315}$ gdw^{-1})	13,300±800	3,800±200	1,500±100	(Clarke and Robinson 2008)
Cell wall UVAC	Mean ± se ($A_{280-315}$ gdw^{-1})	13,800±4000	24,000±2000	14,000±1000	(Clarke and Robinson 2008)
Anthocyanins Mean ± se	Mean ± se ($A_{\Delta526}$ gfw^{-1})	1.2±0.2	1.3±0.2	1.2±0.2	(Lovelock and Robinson 2002)
	Anthocyanins /Tchl ($A_{\Delta526}$ mol^{-1})	2.9±0.3	5.7±1.2	3.0±0.2	(Lovelock and Robinson 2002)

This hydrological species distribution is partially reflected in field carbon isotope ratios which indicate water availability during growth. Water inundation during growth limits CO_2 diffusion to the carbon fixing enzyme RuBisCO. In conditions of saturating CO_2, this enzyme preferentially utilises the lighter isotope ^{12}C, but ^{13}C is increasingly sequestered as CO_2 becomes limiting (Royles et al. 2014; Bramley-Alves 2015). Field samples of the study species show *S. antarctici* has least negative $\delta^{13}C$ ratios indicating frequent submergence and limiting CO_2, while *B. pseudotriquetrum* has the most negative ratios, thus is least affected by CO_2 limitations (Table 2.1; Bramley-Alves 2015). *Ceratodon purpureus* has intermediate values, which was not predicted from its preference for dry sites (Wasley et al. 2012; Bramley-Alves 2015). In the natural setting, *B. pseudotriquetrum* may avoid water inundation and related CO_2 limitation through its taller, more emergent growth form, allowing growth above the water level (Table 2.1; Bramley-Alves 2015).

Such differences in morphology and growth form of moss turf can influence the rate of water uptake and loss, and also the transmission of light into leaves (Lewis Smith 1988a; Wasley et al. 2006b; Malenovsky et al. 2015). Morphological differences between species span a range of scales; from whole turf structure, through gametophytes and leaves, to cellular and subcellular morphology. Morphological features of many moss species can also vary with environmental conditions, such as water availability at the sampling site (Ochyra et al. 2008).

The dense turf structure found in Antarctic mosses probably minimises turbulence of the boundary layer, lowers water loss and increases self-shading. Increased shoot density reduces evaporative water loss and is highest in *C. purpureus*, and similar in the other two species. However, the large gametophytes of *B. pseudotriquetrum* are more tightly packed than in the looser turf of *S. antarctici* (Table 2.1; Figure 2.5; Wasley et al. 2006b). *Bryum pseudotriquetrum* and *S. antarctici* have higher water holding capacities than *C. purpureus* (Wasley et al. 2006b), but in *B. pseudotriquetrum* larger leaves increase internal water holding capacity while

S. antarctici holds more external water in its loosely packed shoots (Table 2.1; Figure 2.5; Wasley et al. 2006b).

Mosses have a limited capacity for water conduction using rudimentary water conducting cells called hydroids. These structures are analogous to the xylem found in vascular plants and their prominence differs between species and with growth conditions (Ochyra et al. 2008). In the stem, hydroids form the central strand which is reported in the three study species (Table 2.1). Ochyra (2008) describes hydroids as large in Antarctic *B. pseudotriquetrum,* but indistinct to absent in *S. antarctici.* Lenne and co-workers (2010) report their presence in *C. purpureus.* Hydroids redistribute water throughout the plant and may facilitate greater control of water gain and loss (Lenne et al. 2010; Stanton et al. 2014).

As mosses dry (or freeze), the central strand in the stem may cavitate and block water loss through this pathway (Stanton et al. 2014). This is thought to explain slower drying rates in individual shoots of *C. purpureus* compared to rapid drying of whole clumps of moss turf (Stanton et al. 2014). Slower drying prolongs the period available for photosynthetic activity, but may also influence the capacity for photoprotection in the desiccated state (Wasley et al. 2006b; Fernandez-Marin et al. 2013). Slower drying resulted in higher final water contents in the dry state, and this was suggested to facilitate some enzymatic activity, such as conversion of V to A and Z thus promoting protection of the photosynthetic apparatus from excess light (Fernandez-Marin et al. 2013).

Morphological features of the shoot, leaflets and cells are also important to water balance. Water holding capacity improves with increased rhizoid density in *B. pseudotriquetrum* (Lewis Smith 1988a). *Schistidium antarctici* from the Windmill Islands has been described as non-rhyzoidal (Lewis Smith 1988a). Across Antarctica, rhizoids are described in *S. antarctici* as “scattered to nearly absent”, whilst rhizoids in *C. purpureus* and *B. pseudotriquetrum* are reported to be “various”, and “sparse to dense”, respectively (Table 2.1; Figure 2.5; Ochyra et al. 2008). In *S. antarctici* hyaline hair points, a feature important to water balance, may be “nearly absent or

0.5 mm" long (Ochyra et al. 2008), but are not reported from the other two species. Cell wall thickness may influence rates of water uptake and loss and also diffusion of CO_2 into the cell (Bramley-Alves 2015). Cell walls are thickest in *S. antarctici* and thinnest in *B. pseudotriquetrum* with *C. purpureus* intermediate (Table 2.1; Bramley-Alves 2015). The capacity of each species to maintain an optimal water balance for photosynthesis determines the ecological niche.

Figure 2.5: Scale drawing of the three study species *Bryum pseudotriquetrum* (wet habit)*, Schistidium antarctici* and *Ceratodon purpureus.* When hydrated the leaflets of *S. antarctici* extend horizontally so the adaxial surfaces are exposed to sunlight. This is less pronounced in the other two species, where abaxial surfaces may receive more light. Images adapted from Ochyra et al. (2008).

In continental Antarctica *S. antarctici* is found in moist habitats, but in the maritime Antarctic it occupies xeric habitats, such as rock surfaces (Ochyra et al. 2008). Stanton and co-workers (2014) suggest this bimodal distribution may be due its capacity to tolerate submergence combined with its preference for rapid drying. Mosses are more susceptible to freezing damage when hydrated (Kennedy 1993b;

Lovelock et al. 1995b) and avoid potentially damaging internal ice formation by controlled water loss in the presence of external ice (Lenne et al. 2010).

In continental Antarctica the wetter habitat of *S. antarctici* may also afford some protection from freezing events, since the latent heat of freezing disengages the water temperature from air temperature. Additionally, melt streams reduce water flow when temperatures drop and upstream water freezes. *Bryum pseudotriquetrum* appears less tolerant of frequent freeze thaw events with nearly double the solute leakage of the other two species (Table 2.1; Melick and Seppelt 1992).

In optimal conditions, higher photosynthetic rates occur in *B. pseudotriquetrum* than in the other two species (Table 2.1; Lewis Smith 1999; McKinley 2012). This species also appears to have a higher field growth rate (Clarke et al. 2012) and is particularly able to take advantage of good growing conditions, with rapid comparative growth in chamber experiments (Bramley-Alves 2015). *Bryum pseudotriquetrum* has lower concentrations of total carotenoids and xanthophyll pigments and a lower conversion of violaxanthin to zeaxanthin (acute light stress response; Table 2.1; Lovelock and Robinson 2002). These comparatively low levels of protective pigments within the chloroplast suggest a lower requirement for quenching of excess light. *Ceratodon purpureus* has half the amount of chlorophyll of the other two species (Lovelock and Robinson 2002) and a heavy investment in photoprotection (Table 2.1).

Soluble carbohydrate and lipid composition varies in the three moss species (Table 2.1). Particular compounds are implicated in both desiccation tolerance and/or cryoprotection, such as stachyose, trehalose and the sugar alcohols (Buitink and Leprince 2004; Wasley et al. 2006b; Yobi et al. 2013). *Bryum pseudotriquetrum* has the highest levels of stachyose and trehalose and total soluble carbohydrates, with the latter halving from the beginning of December to late February (Wasley et al. 2006b). *Ceratodon purpureus* has the lowest total soluble carbohydrates but highest lipid concentrations and high ratios of unsaturated to saturated fats (Wasley et al.

2006b). These unsaturated fats may maintain membrane fluidity and facilitate the high desiccation tolerance in this species (Wasley et al. 2006b).

Tolerance of UV-BR may be determined by the effectiveness of the UV screening strategy. Interspecific differences in the abundance and location (cell wall or intracellular) of UV absorbing compounds (UVACs) may contribute to UV-BR tolerance (Clarke et al. 2008). This is discussed in detail in Chapter 6. Of the study species, only *B. pseudotriquetrum* has demonstrated the capacity to accumulate intracellular methanol extractable UVACs in response to UV-BR (Dunn and Robinson 2006; Nunez-Olivera et al. 2010; Fabon et al. 2012a). In the Windmill Islands, intracellular UVACs are typically between 2 and 10 times higher in field samples of *B. pseudotriquetrum* than in *S. antarctici* and 2-3 times higher than that of *C. purpureus* (Table 2.1). Variation in UVACs, including anthocyanins occurs between sites, but the environmental drivers of these changes are poorly understood (Lovelock and Robinson 2002; Robinson et al. 2005; Dunn and Robinson 2006).

Anthocyanin concentrations in the three moss species from the Windmill Islands did not vary seasonally but large differences occurred between sites (Dunn and Robinson 2006). All three species had similar anthocyanin concentrations on a per gram basis (Table 2.1) but lower chlorophyll levels in *C. purpureus* mean that anthocyanins are higher per chlorophyll in this species (Table 2.1; Lovelock and Robinson 2002).

In summary, *B. pseudotriquetrum* has a higher photosynthetic capacity (McKinley 2012) which facilitates a faster growth rate, particularly in good growing conditions (Clarke et al. 2012; Bramley-Alves 2015). This species appears to have the most capacity to adjust UVAC and photosynthesis to its growing conditions (Dunn and Robinson 2006; Wasley et al. 2006b) and experiences less CO_2 limitation in the field (Bramley-Alves et al. 2015). It may experience less light stress than the other species, suggested by the lower levels of protective carotenoids (Lovelock and Robinson 2002). However, whilst *B. pseudotriquetrum* appears able to rapidly take

advantage of good growing conditions it may be more vulnerable in cold temperatures as it loses more soluble carbohydrates during freezing events (Melick and Seppelt 1992). *Ceratodon purpureus* has high concentrations of lipids and low chlorophyll concentrations (Lovelock and Robinson 2002; Wasley et al. 2006b). It is highly desiccation tolerant and appears to invest heavily in protection, rather than growth (Wasley et al. 2006b; Clarke et al. 2012). *Schistidium antarctici* is the only endemic and locally the most abundant species (Wasley et al. 2012). It has similar high chlorophyll concentrations as *B. pseudotriquetrum* (Lovelock and Robinson 2002), yet the lowest photosynthetic rates in optimal conditions (McKinley 2012). Its high tolerance of submergence and freeze thaw cycles likely means it can survive at sites in the many melt lakes and streams, which are periodically submerged.

The following chapter investigates the tolerance of the three study species to ambient UV-BR during one summer growing season. Tolerance is assessed as DNA damage that is rapidly responsive and specific to UV-BR.

3 Accumulation of DNA damage in Antarctic mosses: correlations with ultraviolet-B radiation, temperature and turf water content vary amongst species.

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3.1 Introduction

Ozone depletion above the Antarctic has resulted in large increases in springtime ultraviolet-B radiation (UV-BR) over the last four decades and full recovery of the austral ozone layer is not expected until after 2060 (McKenzie et al. 2007). Ultraviolet-B radiation is damaging to biological molecules including DNA, proteins, lipids and photosynthetic pigments. Plants can protect themselves from UV-BR induced damage by screening UV-BR before it reaches these molecules (Cockell and Knowland 1999) or by repairing damage once it has occurred (Robinson et al. 2003; Britt 2004). Whilst UV-BR screening compounds and structures are a feature of the epidermal layers of many higher plants, the simple structure of bryophytes, particularly the lack of epidermal layers, was assumed to render them particularly vulnerable to UV-BR damage (Gwynn-Jones et al. 1999). However, recent work suggests that some bryophyte species have high resilience to UV-BR (Lud et al. 2002; Newsham et al. 2002; Newsham 2003; Boelen et al. 2006; Dunn and Robinson 2006; Clarke and Robinson 2008).

Since bryophytes represent a major component of vegetation in polar ecosystems where ozone depletion is most severe, the increase in UV-BR exposure might be expected to have a negative impact on the Antarctic flora. Previous studies have shown that several Antarctic moss species accumulate UV-BR screening compounds (Lovelock and Robinson 2002; Newsham et al. 2002; Newsham 2003; Dunn and Robinson 2006) and in a few polar and alpine bryophytes concentrations of these compounds correlate positively with exposure to UV-BR (Newsham 2003; Arróniz-Crespo et al. 2006; Dunn and Robinson 2006; Lappalainen et al. 2008). However,

other species show a limited capacity to acclimate to UV-BR. Of the three moss species dominant in the Windmill Islands of East Antarctica, the two cosmopolitan species, *Bryum pseudotriquetrum* and *Ceratodon purpureus,* were found to accumulate two-fold higher concentrations of total (methanol soluble and cell wall) UV-B absorbing compounds than the endemic *Schistidium antarctici* (Lovelock and Robinson 2002; Dunn and Robinson 2006; Clarke and Robinson 2008). Damage, in the form of abnormal morphology and loss of photosynthetic pigments, under ambient UV-BR has also been reported in this endemic species (Robinson et al. 2005). If UV-BR screening capacity in *S. antarctici* is insufficient to protect chlorophyll we postulated that DNA damage might also accumulate as a result of UV-BR exposure.

The most common type of UV-BR induced DNA damage is pyrimidine dimers (Britt 2004). These photoproducts distort the structure of DNA, blocking DNA transcription and replication and causing reductions in plant growth rate (Jiang et al. 1997; Britt 2004). In high numbers they are cytotoxic and mutagenic (Taylor et al. 1997; Britt 2004). DNA photoproducts that form in mature plant cells are repaired by photoreactivation, a light-dependent process that requires UV-inducible enzymes. Nucleotide excision repair is a light-independent process that is prevalent in proliferating cells (Kimura et al. 2004). Since both of these repair methods are enzymatic processes, their effectiveness could be limited when plants are exposed to low temperatures (Pakker et al. 1999; MacFadyen et al. 2004) or desiccation (Buffoni Hall et al. 2003) as is frequently the case for polar mosses.

UV-induced DNA damage has been measured in a few terrestrial polar organisms including the mosses, *Sanionia uncinata* (Lud et al. 2002; Boelen et al. 2006), *Chorisodontium aciphyllum, Warnstorfia sarmentosa* and *Polytrichum strictum* (Boelen et al. 2006), the alga *Prasiola crispa* (Lud et al. 2001) and the Patagonian herb, *Gunnera magellanica* (Rousseaux et al. 1999a; Giordano et al. 2003). Whilst ambient UV-BR failed to produce significant levels of DNA damage in the Antarctic mosses (Lud et al. 2002; Boelen et al. 2006), damage was detected in both the alga

and the herb (Rousseaux et al. 1999a; Lud et al. 2001; Giordano et al. 2003). Supplementing the UV-BR dose, approximately 10-fold, resulted in measurable DNA damage in *Sanionia uncinata* but not in the other mosses, and all such damage to mosses was repaired overnight (Lud et al. 2002; Boelen et al. 2006).

Accumulation of DNA damage is a useful parameter to compare UV tolerance between Antarctic moss species. Long term field experiments are logistically challenging in continental Antarctica and measurements of growth and reproduction difficult in these tiny, slow growing and clonally reproducing cryptogams (Selkirk and Skotnicki 2007; Clarke et al. 2009; Clarke et al. 2012) Commonly measured parameters in UV-BR studies, such as growth, morphology or production of protective pigments exhibit great interspecies variation and may be difficult to attribute specifically to UV-BR (Boelen et al. 2006). Lack of information on the functional significance of some of these parameters can likewise hamper conclusions about the UV tolerance bestowed. Since accumulation of DNA damage balances the ability to specifically screen UV-BR with potential for DNA repair it provides a short-term indication of a species' relative genetic UV-BR sensitivity (Hidema et al. 2007).

This study measured UV-BR induced DNA damage as accumulation of cyclobutane pyrimidine dimers (CPDs), the most common type of UV-induced DNA damage (Britt 2004), using an enzyme-linked immunosorbent assay (ELISA). We determined CPD accumulation in three Antarctic moss species under natural UV-BR irradiation across a summer season and examined the relationship between DNA damage and environmental and turf parameters. Our hypotheses were that 1) *S. antarctici* would accumulate higher concentrations of photoproducts due to its lower capacity to screen UV-BR at the cellular level, and 2) that both UV-BR dose, and factors which slow enzymatic repair, such as reduced temperature or desiccation, would increase the accumulation of photoproducts in these mosses.

3.2 Materials and Methods

3.2.1 Study sites and sampling

The Windmill Islands (centred at 66˚22’ S, 110˚30’ E) is a region of ice-free islands and peninsulas on the eastern coastline of Antarctica. The climate is classified as frigid Antarctic (sensu Longton 1988) and further described in Melick & Seppelt (1997) and Dunn & Robinson (2006). Despite this harsh climate, the Windmill Islands region supports some of the most extensive and complex bryophyte communities on continental Antarctica.

Three moss species are found in the region; *Schistidium antarctici* (Cardot) L.I. Savicz & Smirnova (formerly known as *Grimmia antarctici*) is endemic to the Antarctic continent, while both *Bryum pseudotriquetrum* (Hedw.) Gaertn., B. Mey. & Scherb and *Ceratodon purpureus* (Hedw.) Brid. have cosmopolitan distributions. Species distribution within the bryophyte community generally follows the moisture gradient, with *S. antarctici* associated with 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. 2006b). Samples were collected from within Antarctic Specially Protected Area (ASPA) 135 on Bailey Peninsula from the site designated ASPA 1 in Dunn & Robinson (2006).

Each moss species was sampled on 20 separate dates during the summer season of 2002/03 (180 samples in total, n=60 per species) from randomly selected sampling sites. The first samples were taken as soon as substantial turf was exposed from under winter snow cover (9 November 2002), and then every three to six days until 1 February 2003. Prior to sampling, turf surface temperature was measured using an Infrared thermometer (Scotchtrack T Heat tracer IR1600L; 3M, Austin TX, USA). Moss samples (2 cm^2) were removed from the turf at midday. Care was taken not to shade moss during sampling and samples were immediately put in the dark on ice and transported to the Station Science Building. Within 3 h of sampling the photosynthetically active shoot tips (3-5 mm) were removed from each sample in a

–20 °C freezer room. Samples were frozen in liquid N_2 and transported to Australia for DNA extraction and analysis of DNA photoproducts. A subset of each sample was dried to constant weight, extracted in acidified methanol (methanol:H_2O:HCl; 79:20:1) and analysed for UV-B absorbing pigment concentration using the method described in Lovelock & Robinson (2002). Remaining gametophyte material was weighed and oven-dried for turf water content (WC) determination as described in Robinson *et al.* (2000).

3.2.2 DNA Extraction

DNA was extracted from all samples using a modification of the method of Mason & Schmidt (2002). Each sample (50-100 mg fresh weight) was frozen in liquid nitrogen and ground to a fine powder. The homogenate was resuspended in 1.5 mL DNA extraction buffer and DNA subsequently purified as described in Mason & Schmidt (2002). DNA concentration and purity for each sample were determined spectrophotometrically (UV-1601 UV visible spectrophotometer, Shimadzu, Melbourne, Australia). Samples were then diluted in TE [10 mM Tris (pH 8), 1mM EDTA] buffer to achieve a concentration of 4 ng/µl.

3.2.3 Quantification of DNA Photoproducts by ELISA

The concentration of CPDs was quantified by ELISA in a method modified from Taylor *et al*. (1996) using TDM-2 monoclonal antibodies specific for CPDs (O. Nikaido, Kanazawa University, Japan; Mori et al. 1991). The detection system used in the ELISA was modified from the 1,2-orthophenylenediamine method used by Taylor *et al*. (1996) with an Amplex red system substituted in order to optimise sensitivity (Leslie 2003).

Fluorotrac™ microtitre plates (Greiner Bio-one, Austria) were coated with protamine sulphate (Sigma, Australia) to reduce non-specific binding of the primary antibody to the plate. 200 µL of a 1% (w/v) solution of protamine sulphate was added to each well. Plates were incubated for 2 h at 37 °C then washed twice with sterile water and left to dry overnight at 30 °C. 200 ng of moss DNA was placed in

each well (50 µL of 4 ng/µL moss DNA solution) and left to dry for 48 h at 37 °C. Plates were then washed four times in phosphate buffered saline (0.8 M disodium hydrogen orthophosphate, 0.2 M sodium dihydrogen orthophosphate, 1 M sodium chloride pH 7.5) with 0.02% Tween-20 (PBS-T). 200 µL of blocking solution (1% heat denatured casein and 0.01 % thimerosal in PBS-T, pH 7.4) was added to each well and plates incubated for 2 h in the dark at room temperature. Plates were washed five times in PBS-T. Primary antibody against CPDs (TDM-2) was diluted 1:1000 in PBS and 50 µL added to each well. Following incubation at room temperature in the dark for 90 min, plates were washed five times with PBS-T. Secondary antibody (biotin goat anti-mouse IgG, Sigma- Aldrich, Sydney, Australia) was diluted 1:1000 (v/v) in blocking solution and 50 µL added to each well. Plates were incubated for 90 min in the dark at room temperature and then washed five times in PBS-T. HRP conjugated Streptavidin enzyme amplification reagent was prepared by diluting 1 mg/mL streptavidin and 1 mg/mL HRP-biotin conjugate 1:400 (v/v) in blocking solution. 50 µL of this solution was added to each well and plates were again left in the dark at room temperature for 90 min. 100 µl of developer solution (50 µM Amplex® Red reagent [10-acetyl-3,7-dihydroxyphenoxazine; Molecular Probes, USA]), 200 µM hydrogen peroxide, 0.05 M sodium phosphate buffer, pH 7.4) was then added to each well. After incubation for 30 min in the dark, fluorescence was measured using a fluorescence plate reader (Fluostar Optima, BMG Labtech Pty Ltd., Mornington, VIC, Australia) equipped with a filter set for excitation and emission at 540 and 590 nm respectively, to give a quantitative assay of CPD concentration.

DNA from each individual moss sample was plated into four wells of a 96 well plate. Standards containing pre-irradiated and un-irradiated calf thymus DNA (0.6 ng DNA) were also plated onto each ELISA plate and used as positive and zero internal controls, respectively, to set the gain on the plate reader and allow comparisons between plates. The relative fluorescence value for each moss sample was calculated by normalising to the pre-irradiated and unirradiated calf thymus controls on the respective plates. The relative standard deviation of the mean for

calf thymus values across all experiments was 0.070 and 0.035 for pre-irradiated and un-irradiated DNA respectively (n=180).

3.2.4 Climate data

Climatic data (including temperature, wind speed and precipitation) covering October 2002 to February 2003 was obtained from the Australian Bureau of Meteorology (Casey Station) as detailed in Dunn & Robinson (2006). The metadata record for station air temperature (Barnes-Keogahn 2007) was used to calculate the 38 year mean for each of the summer months from 1969-2007. The position of instruments collecting meteorological data changed in 1989 but there is no obvious effect of this move on the air temperature data.

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 provided UV-BR (International Light UVB radiometer, wavelength range 280 - 315 nm), total ultraviolet (TUV; Eppley total UVR radiometer; 290 - 400 nm) and biologically effective UV (Solar Light UV Biometer, SL501) radiation data (for details see Roy et al. 1998). Data were downloaded and converted as described in Dunn & Robinson (2006). The biometer sensor data was converted to standard erythemal dose and this metadata (Gies and Roy 2001) was used to construct the 10 year means for the summer months from 1996-2006. Total UV radiation was used as a proxy for Total Solar Radiation (TSR) as the station TSR sensor was not calibrated in 2002/03. Dunn & Robinson (2006) found this parameter responded similarly to changes in cloud and daylength and was a suitable substitute for TSR. No radiation data were collected between 25th December 2002 and 2nd January 2003. 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).

3.2.5 Statistical analysis

Means for the various climate, radiation and moss physiology parameters were calculated for the pre-solstice (9 November - 23rd December) and post-solstice (24th December - 1 February) periods of the sampling season and were compared using ANOVA. The accumulation of CPDs for each independent moss sample was calculated as the mean relative fluorescence of its four replicate DNA samples. Means for each sampling date where then calculated from these individual sample means. The effect of species and time (pre- or post-solstice) on CPD accumulation was analysed using two-way ANOVA. Where significant differences were observed *post hoc* Tukey-HSD tests were performed.

Regression analyses were performed to determine which individual and combined parameters best predicted the accumulation of CPDs for each species. Environmental factors were considered individually and as part of multiple regression models (see Dunn & Robinson 2006). 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. 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 adjusted r^2. Statistical analyses were conducted using JMP 5.1 and SAS 10 (SAS Institute, Cary, NC, USA) computer packages.

3.3 Results

3.3.1 The 2002 'ozone hole' and incident UV-B radiation

In September 2002 atypical atmospheric conditions caused the ozone hole to split into two, one part of which rapidly dissipated while the second returned to the pole as a much smaller vortex (Allen et al. 2003). This led to an unusually small 'ozone hole' both in depth and area with a seasonal (November 2002 to February 2003) mean ozone column depth above Casey of 339 Dobson units (DU). Variation in ozone depth above Casey was still evident with periods of low ozone (<300 DU) in late November and January (Figure 3.1 A). The minimum and maximum ozone depth for the season were 260 DU and 440 DU on 30th January and 20th February respectively. Unusually for recent years, mean ozone depth was significantly higher in the pre-solstice period than post-solstice (Table 3.1).

Table 3.1: Difference in mean environmental parameters measured pre-solstice (9 November–- 23 December) and post- solstice (24 December – 1 February) over the 2002/03 summer season in the Windmill Islands region, East Antarctica. Data are means ± SEM and ANOVA summary results are shown.

Parameter	Pre-solstice (Mean ± SEM)	Post-solstice (Mean ± SEM)	F	P
Daily UV-BR dose (KJ m^{-2})	14.7±1.47	8.6±1.11	$F_{1,76}$=10.6	**0.002***
Daily TUV dose (KJ m^{-2})	1926±88.9	1754±88.1	$F_{1,73}$=1.8	0.19
UV-BR/TUVR daily dose	0.007±0.0006	0.004±0.0005	$F_{1,74}$=11.1	**0.001***
Ozone daily mean (DU)	342±4.4	309±2.8	$F_{1,74}$=39.1	**<0.0001***
Air temperature daily mean (˚C)	-3.12±0.22	-0.36±0.22	$F_{1,85}$=79.3	**<0.0001***
Wind speed daily mean (km h^{-1})	21.3±2.5	14.8±1.1	$F_{1,83}$=5.22	**0.025***

***p<0.05**

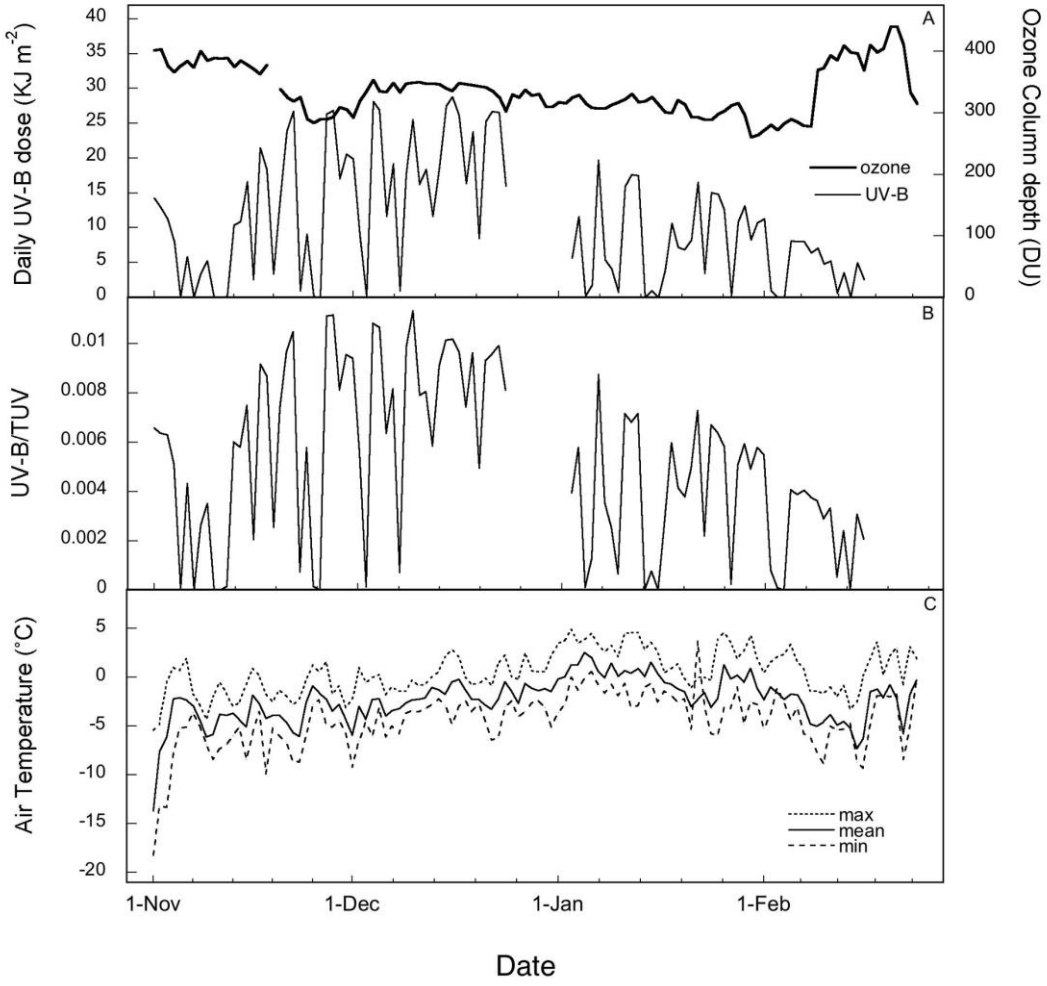

Figure 3.1: Daily variation in (A) UV-B radiation (280-315 nm) and ozone layer thickness, (B) the proportion of UV-BR to total UV radiation (UV-B/TUV) and (C) air temperature from November 2002 to February 2003 at Casey Station in the Windmill Islands region, East Antarctica. No radiation data were collected from 25 December 2002 to 2 January 2003.

Daily UV-BR dose is highly affected by cloud cover, solar declination and daylength which can mask the effect of ozone levels on incident UV-BR. Daily UV-BR dose increased from mid November with highest values recorded in the broad peak before the solstice. The highest UV-BR of the season (10 min. dose) was recorded on 28th November during a period of ozone depletion, whilst the highest daily UV-BR dose occurred on 16th December (Figure 3.1 A). The ratio of UV-BR to TUV radiation (Figure 3.1 B) confirms that UV-BR was high relative to other wavelengths pre-solstice. Since daily UV-BR dose was highest before the solstice (*P*=0.002; Table

3.1) but daily TUV dose did not change significantly, the ratio of UV-BR/TUV radiation was 75% higher in the pre-solstice than post solstice period (*P*=0.001; Table 3.1).

3.3.2 Interannual comparison of monthly effective UV-B radiation

Although standard erythemal dose is weighted to the more damaging parts of the UV-BR spectrum for human exposure rather than plant damage, the accuracy and long term nature of this data set enables a comparison between seasons, as a reliable record for Casey is available since 1996. Figure 3.2A compares the monthly mean SED for the 2002/03 season with the 10 year mean from 1997 to 2007. SED in 2002/03 was lower than the long term mean in all months except January. Between September and November the 2002 SED was 75% or less of the 10 year mean value for these months.

3.3.3 Air temperature

Air temperature (AT) increased from November to January then decreased through February and March (Figure 3.1 C; Figure 3.2 B). The AT was significantly cooler, by almost 3 ˚C, in the pre-solstice period than after the solstice (P<0.0001, Table 3.1). The season daily minimum AT of –18.2 ˚C occurred on 1 November, after which, AT remained above -10 ˚C throughout the summer. The daily maximum AT exceeded 0 ˚C on 33% of the pre-solstice days compared with 90% of post-solstice days, with the season maximum of 4.9 ˚C recorded on 3rd January (Figure 3.1 C). Minimum AT dropped below zero overnight on all but two days in January.

3.3.4 Interannual comparison of air temperature

Figure 3.2B compares the monthly mean AT in 2002/03 with the 38 year mean (1969-2006). The 2002/03 season was cooler by 0.9 and 1.3 ˚C in October and December respectively but 1.5 ˚C warmer than the long term mean in November. The temperatures for September and January to March were typical for the region.

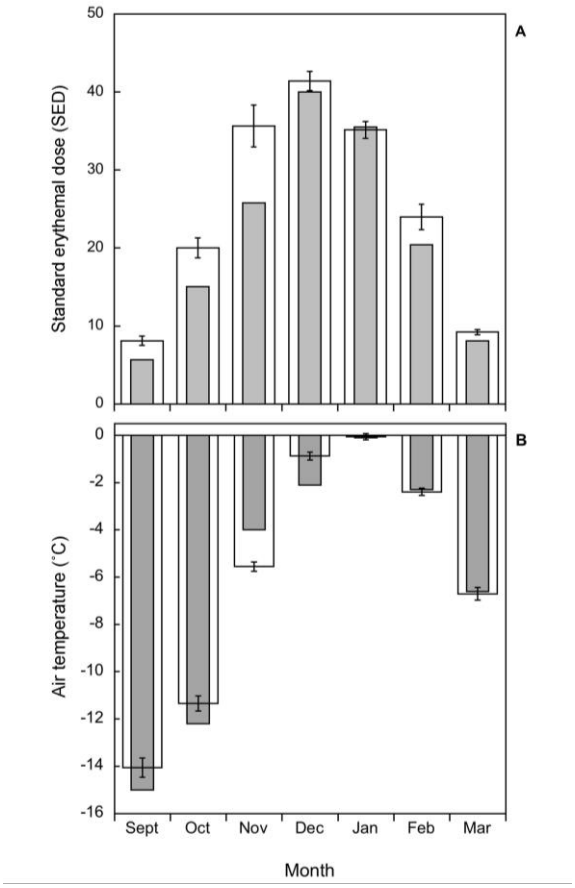

Figure 3.2: Monthly variation in UV-B radiation (A; standard erythemal dose) and mean air temperature (B), in the 2002/03 summer season (grey bars) relative to the long term means (10 years for SED data, 38 years for air temperature; white bars) at Casey Station in the Windmill Islands region, East Antarctica. For long term means error bars represent ±SEM.

3.3.5 Other climate factors

The 2002/3 season was also characterised by low wind speeds. Just one in ten days experienced gales (>60 km.hr^{-1}) compared to a long term annual mean of one in four days (Melick and Seppelt 1997). It was significantly windier in the pre-solstice period than after the solstice (P=0.025, Table 3.1). Mean precipitation from November to March was low compared to the long term, averaging 0.6 mm d^{-1}.

3.3.6 Accumulation of DNA damage over the 2002/3 season

The seasonal accumulation of CPDs in the three species of moss is shown in Figure 3.3. Seasonal mean CPDs (relative fluorescence units per 200 ng DNA) for the three moss species were similar, *S. antarctici* (0.155±0.013), *B. pseudotriquetrum* (0.151±0.012), and *C. purpureus* (0.146±0.012). Both *C. purpureus* and *B. pseudotriquetrum* showed significantly lower accumulation of CPDs in samples collected after the solstice compared with pre-solstice samples (Figure 3.4 A) whereas CPDs in *S. antarctici* showed no seasonal trend (species by time interaction $F_{5,174}$ = 3.5, p=0.033). Post-solstice CPD accumulation was 53% and 73% of pre-solstice accumulation in *B. pseudotriquetrum* and *C. purpureus* respectively.

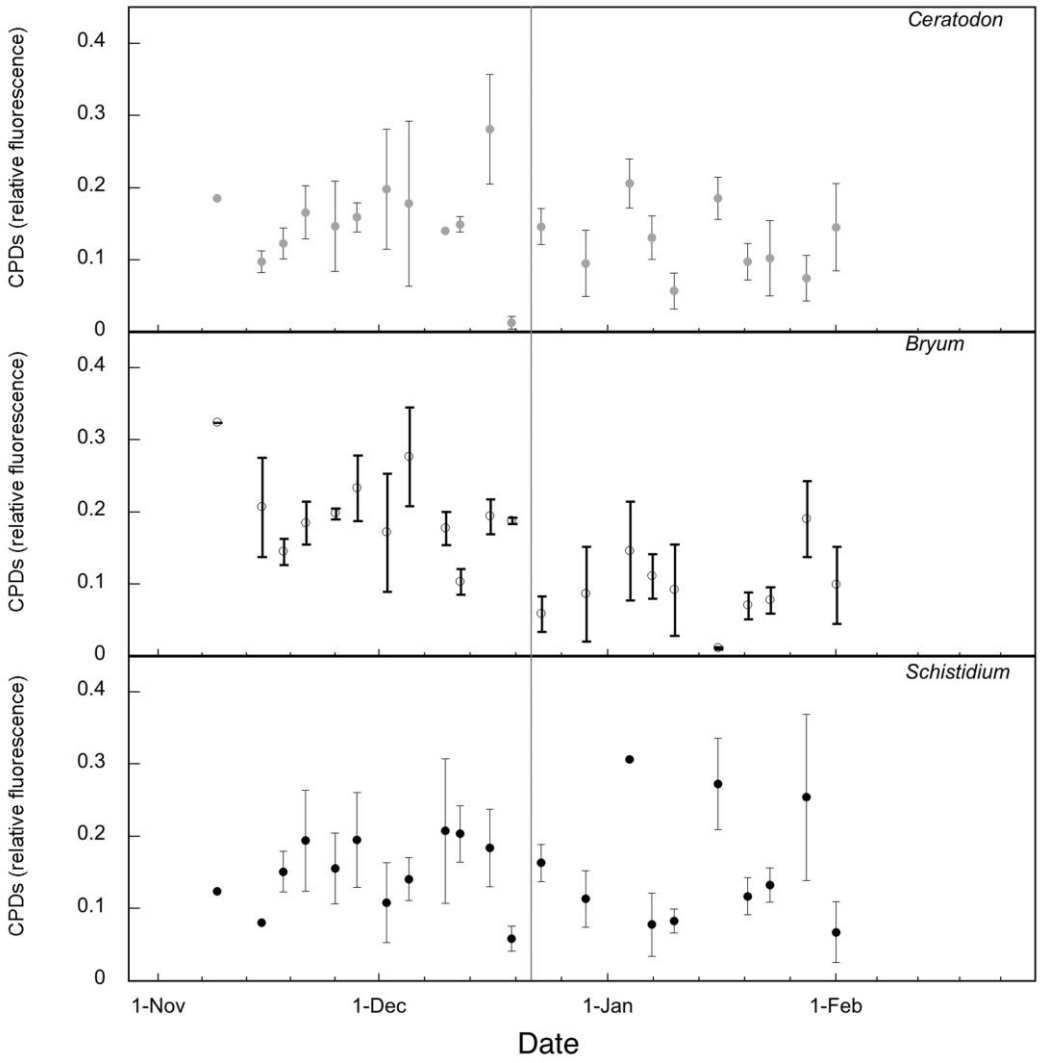

Figure 3.3: Accumulation of cyclobutane pyrimidine dimers (CPDs) in field samples of *Ceratodon purpureus*, *Bryum pseudotriquetrum* and *Schistidium antarctici* over the 2002/03 summer season. The line indicates the summer solstice (23rd December). Data are means ± SEM of three separate moss samples collected on each sampling date, except for the first sampling date where n=1.

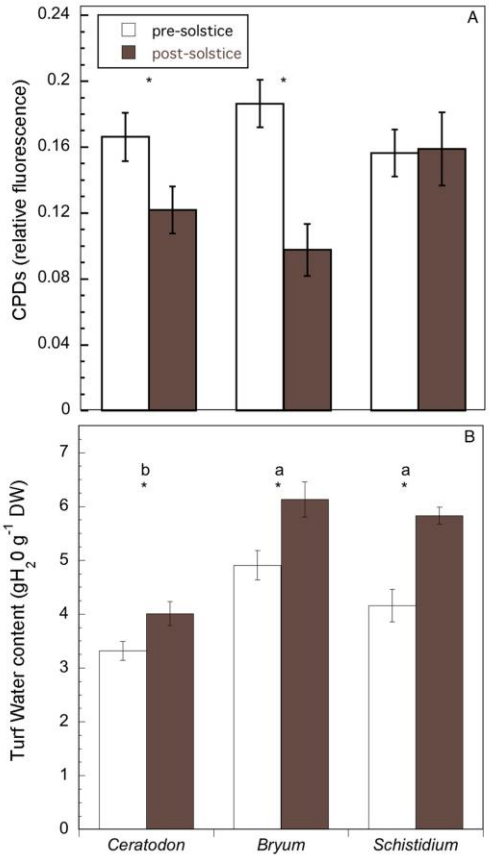

Figure 3.4: Pre- and post-solstice mean accumulation of cyclobutane pyrimidine dimers (A; CPDs) and turf water content (B) in field samples of *Ceratodon purpureus, Bryum pseudotriquetrum* and *Schistidium antarctici* for the 2002/03 summer season. Significant differences ($P<0.05$) between early and late season means within a species are indicated by asterisks, whilst different letters denote species that show different turf water means throughout the season. For all species data are means ± SEM, n=33-37 pre-solstice and n=26-28 post-solstice.

3.3.7 Turf water content, turf temperature and UV absorbing compounds

Turf water content (WC) was significantly higher in *B. pseudotriquetrum* and *S. antarctici* than in *C. purpureus* (Figure 3.4 B). All species had a significantly lower WC early in the season (Figure 2.4). No significant difference in turf temperature was observed either between species or across the season (Figure 2.3). Throughout the season turf temperatures for all species were consistently more than 11 ˚C above the corresponding air temperatures on sampling days. Methanol soluble UV-B absorbing compounds were significantly higher in *B. pseudotriquetrum* (mean±SEM, 500 ± 30 $A_{280\text{-}320}\,g^{-1}$ DW), intermediate in *C. purpureus* (269 ± 16 $A_{280\text{-}320}\,g^{-1}$ DW) and lowest in *S. antarctici* (143 ± 7 $A_{280\text{-}320}\,g^{-1}$ DW; see Chapter 5). There was no seasonal change in these compounds.

3.3.8 Relationship between accumulation of DNA damage and UV-BR exposure

Both *B. pseudotriquetrum* and *C. purpureus* showed significant positive associations between the accumulation of CPDs and ambient UV-BR (Table 3.2). For

B. pseudotriquetrum the strongest associations were observed between DNA damage and the daily radiation parameters (Daily UV-BR and TUV doses) with the best fit achieved with UV-B/TUV radiation (r^2= 53%, Figure 3.5 A). For *C. purpureus* radiation parameters over the preceding 5 days gave better fits than those for daily dose (Table 3.2). Five day mean dose of both UV-BR and TUV showed strong positive associations, with the ratio of these two parameters again providing the strongest fit (r^2= 60%, Figure 3.6). Turf water content and the 10 d mean of air temperature were both negatively associated with the accumulation of CPDs in *B. pseudotriquetrum* (r^2= 47 and 51%, respectively; Table 3.2, Figs. 3.5 B,C). CPD accumulation in *S. antarctici* was not significantly associated with any of the environmental parameters tested, either as single or multiple factors in models.

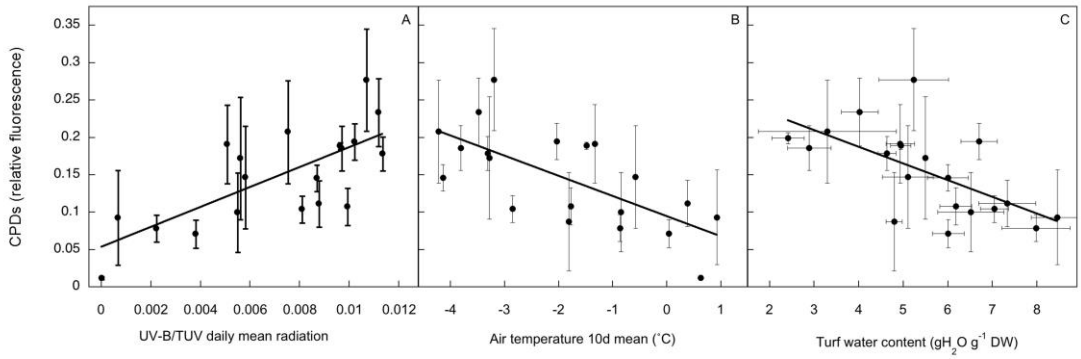

Figure 3.5: Mean concentration of cyclobutane pyrimidine dimers (CPDs) in *Bryum pseudotriquetrum* as a function of the daily ratio of UV-B to total UV radiation (A), the 10 d mean of air temperature (B) and the turf water content (C). Data are mean ± SEM, n=3 except for symbols with no error bars where n=1. Regression details; A) r^2 = 53%, CPD = 0.057 + 12.2 UVB/TUV daily mean radiation; B) r^2 = 51%, 0.094 - 0.026 AT10; C) r^2 = 47%, CPDs = 0.27 - 0.022WC**.**

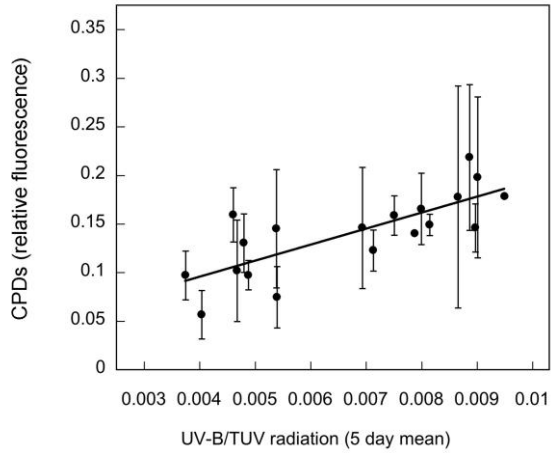

Figure 3.6: Mean concentration of cyclobutane pyrimidine dimers (CPDs) in *Ceratodon purpureus* as a function of the ratio of UV-B to total UV radiation (5 d mean). Data are mean ± SEM, n=3 except for symbols with no error bars where n=1. Regression details r^2 = 60%, CPDs = 0.03 + 16.5UV-B/TUV 5 d mean radiation.

Table 3.2: Data from linear regression analyses showing associations between environmental parameters and accumulation of cyclobutane pyrimidine dimers in two Antarctic mosses over the 2002/03 summer season in the Windmill Islands region, East Antarctica. For *Bryum pseudotriquetrum* only those parameters that gave r^2 values > 20% are shown. All parameters that gave significant regressions (P > 0.1) are shown for *Ceratodon purpureus*. No significant regressions were obtained for *Schistidium antarctici*.

Environmental variable	r^2 (%)	Slope	F	P
Bryum pseudotriquetrum				
Air temperature (10 day mean)	51	-0.026	$F_{1,19}$=20.1	**0.0003***
Turf Water content†	47	-0.022	$F_{1,18}$=16.3	**0.0008***
Daily UV-B dose†	49	4.3×10^{-3}	$F_{1,17}$ = 15.6	**0.001***
Daily TUV dose†	47	8.1×10^{-5}	$F_{1,17}$ = 14.9	**0.001***
UV-B/TUV daily dose†	53	12.2	$F_{1,17}$ = 19.2	**0.0004***
5 day UV-B dose	22	4.9×10^{-3}	$F_{1,17}$ = 4.53	**0.049***
5 day UV-B/TUV	31	17.2	$F_{1,17}$ = 7.28	**0.016***
Ceratodon purpureus				
Daily UV-B dose	19	1.9×10^{-3}	$F_{1,18}$=4.2	0.055
Daily UV-B/TUV	17	4.62	$F_{1,18}$=3.6	0.075
5 day UV-B dose	53	5.4×10^{-3}	$F_{1,18}$=18.8	**0.0004***
5 day TUV dose	33	8.1×10^{-5}	$F_{1,18}$ = 8.4	**0.01***
5 day UVB/TUV	60	16.5	$F_{1,18}$=25.3	**0.0001***

*p<0.05, †1 outlier was excluded from these analyses to achieve homogeneity of variance.

The best two factor multiple regression model for CPDs in *B. pseudotriquetrum* included WC and daily UV-B dose (Table 3.3) and explained 72% of the variability ($F_{2,15}$ = 19.3; P<0.0001). The model described a positive association with UV-B and a negative association with WC. The best three factor multiple regression model for CPDs in this species included WC and daily UV-B dose and wind speed (Table 3.3) and explained 77% of the variability ($F_{3,14}$ = 15.4; P<0.0001). The model described a positive association with UV-BR and a negative association with WC. Substituting

the radiation parameters UV-B/TUV, UV-B maximum or TUV radiation, into these models produced similar but marginally lower r^2 values. Additional environmental factors provided no significant improvement on the single regressions for 5 d radiation means for *C. purpureus* described above.

Table 3.3: Summary data for 2 and 3-factor multiple regression models showing associations between climate factors and cyclobutane pyrimidine dimers in *Bryum pseudotriquetrum* over the 2002/03 summer season in the Windmill Islands region, East Antarctica. Environmental variables, direction of the association and significance of each effect in the model are shown.

Model terms	r^2	Predictor variable (slope)	F	P
2-factor	72%	Turf Water content (-)	$F_{1,15} = 22.8$	**<0.001***
($F_{2,15} = 19.3$; p<0.0001)		Daily UV-B dose† (+)	$F_{1,15} = 8.0$	**0.013***
3-factor	77%	Turf Water content (-)	$F_{1,14} = 11.2$	**0.005***
($F_{3,14} = 15.4$; p<0.0001)		Daily UV-B dose† (+)	$F_{1,14} = 11.9$	**0.004***
		Wind speed (daily mean) (+)	$F_{1,14} = 2.9$	0.11

* p<0.05, †Substituting daily UV-B dose with daily TUV dose or UV-B/TUV produced similar but marginally worse models.

3.4 Discussion

This field study coincided with anomalous stratospheric dynamics in 2002, which caused warming of the polar vortex and an early dissipation of the Antarctic 'ozone hole' (Allen et al. 2003). The resultant springtime UV-BR (SED) flux was 25% lower than the 10 year mean (Figure 3.2 A). Despite this DNA photoproducts accumulated in all three moss species examined and, given the unusually deep ozone layer in 2002, this may well underestimate the damage possible in seasons with a more typical 'ozone hole'. In addition there was less DNA damage in the two cosmopolitan species, *B. pseudotriquetrum* and *C. purpureus,* sampled later in the season compared to pre-solstice (Figure 3.4 A) and a positive association between photoproduct accumulation and incident UV-BR in both these species (Figure 3.5; Figure 3.6). For *B. pseudotriquetrum* negative associations were also observed between photoproduct accumulation and both turf water content (WC) and the 10 d mean air temperature (Figure 3.5 B, C). Photoproduct accumulation in the

endemic species *S. antarctici* was similarly high across the season and no significant association with environmental variables was found. These results contrast with those involving four other Antarctic mosses where ambient UV-BR resulted in no significant DNA damage (Lud et al. 2002; Boelen et al. 2006). The only other report of DNA damage in an Antarctic moss (*S. uncinata)* required supplementation to 2.5 W m^{-2} UV-BR and CPDs were transient and were repaired overnight (Lud et al. 2002).

The observation that both *B. pseudotriquetrum* and *C. purpureus* accumulate fewer DNA photoproducts in the later part of the season could be due to either reduced initial damage or increased repair of damage. The positive association with UV-BR suggests that the lower DNA damage in these species post-solstice is due to the 70% reduction in daily UV-BR (Table 3.1). However, several environmental factors that could influence the rate of DNA repair were also more favourable later in the season. Post-solstice, air temperatures were almost 3 ˚C warmer, wind speeds were reduced by 30% and turf water content was 21-38% higher (Table 3.1; Figure 3.4). Given that no other environmental parameters showed significant associations with photoproduct accumulation in *C. purpureus*, reduced UV-BR post-solstice remains the strongest argument for the 27% decrease in DNA damage. In *B. pseudotriquetrum* the decrease in DNA damage was larger (47%) and in this species strong negative associations were observed between photoproduct accumulation and both WC and the preceding 10 d mean air temperature. The association of lower CPD content with high WC and higher air temperatures suggests that repair of DNA damage is also enhanced later in the season.

In studies of algae, lichens and *Daphnia*, UV-BR exposure at low temperatures (0–5 ˚C) led to higher net accumulation of DNA damage compared with that at warmer temperatures (12-25 ˚C), due to reduced rates of repair at the lower temperatures (Pakker et al. 1999; Buffoni Hall et al. 2003; MacFadyen et al. 2004). Similarly in this study *B. pseudotriquetrum* may be able to sustain higher rates of photoproduct repair later in the season and this would contribute to the reduced accumulation of

CPDs. Thus, the combination of reduced damage and enhanced repair might explain why this species had the lowest level of CPDs in the post-solstice period.

In general effects of ambient levels of UV-BR on plants have been relatively minor but a few high latitude studies have found larger growth reductions (Searles et al. 2001; Rozema et al. 2006; Caldwell et al. 2007). Caldwell and co-workers (2007) suggest that low capacity for DNA repair may be a feature of high latitude regions where species have evolved under low levels of ambient UV-BR. Some studies have demonstrated CPD accumulation under ambient UV-BR, for example in the South American herb, *Gunnera magellanica* (Rousseaux et al. 1999a) and in an Antarctic terrestrial alga *Prasiola crispa* (Lud et al. 2001). In *G. magellanica* a positive dose response between UV-BR and CPD accumulation was observed (Rousseaux et al. 1999a) and growth reductions in this species most likely result directly from DNA damage, rather than via indirect effects of UV-BR such as reactive oxygen species production and associated lipid peroxidation (Giordano et al. 2004). The South American study included ozone depletion events much greater than observed in our study, with a minimum ozone depth of 170 DU. This resulted in a 65% increase in CPD levels compared to samples taken when the overhead column ozone was 300 DU. Such large fluctuations in stratospheric ozone were not observed at Casey during 2002/03, but if the association between CPD accumulation and UV-B/TUV radiation is extrapolated to the radiation experienced at Casey during the 1999/2000 season, CPD accumulation would have more than doubled.

Although our results do not definitively show that any of the species is more sensitive to UV induced DNA damage, the lower CPD accumulation post-solstice is an indication of enhanced resistance to UV-BR in the two cosmopolitan mosses. This is consistent with an accompanying laboratory study using enhanced UV-BR (8.5 W m^{-2}) where *S. antarctici* accumulated twice the CPDs of *B. pseudotriquetrum* and 4 times those of *C. purpureus* (see Chapter 4; Figure 4.2). In addition a long term screening experiment has also shown *S. antarctici* to be negatively affected by ambient UV-BR (see Chapter 1; Figure 1.7; Robinson et al. 2005).

Both of the cosmopolitan species in this study show equivalently high overall concentrations of UV-B screening compounds, more than 2 fold higher than *S. antarctici*. *Bryum pseudotriquetrum* accumulates more methanol soluble compounds (Lovelock and Robinson 2002; Dunn and Robinson 2006) whilst *C. purpureus* has a higher concentration of wall-bound UV absorbing compounds (See Chapter 5; Clarke and Robinson 2008). Higher concentrations of UV absorbing compounds should provide better screening and thus reduce DNA damage (Schmitz-Hoerner and Weissenbock 2003). Soluble UV-B absorbing compounds in *B. pseudotriquetrum* and anthocyanins in *C. purpureus* can also respond to changing UV-BR (Post 1990; Lewis Smith 1999; Dunn and Robinson 2006) although this was not apparent in the present study either because the season was too short or ozone depletion was insufficient to induce acclimation.

3.5 Conclusions

Given that moss growth is strongly dependent on water, the availability of which is related to air temperature, 2002/03 was a benign season with the lowest incident UV-BR and most abundant melt of the last decade. Despite these favourable conditions DNA photoproduct accumulation was apparent in the three moss species. Accumulation of DNA damage in both cosmopolitan moss species was positively associated with UV-BR dose. In one species *B. pseudotriquetrum* we also found negative associations between CPDs and both WC and air temperature perhaps indicating a greater role for enzymatic repair when conditions were warmer and wetter. This study provides further evidence that the endemic moss *S. antarctici* is more susceptible to UV-BR induced DNA damage.

This chapter identified several abiotic variables which may be affecting DNA damage accumulation in the field. The next chapter explores the influence of the hydration state of samples on damage accumulation and compares the relative UV tolerance of the three species in controlled conditions.

4 Desiccation protects two Antarctic mosses from ultraviolet-B induced DNA damage.

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4.1 Introduction

Antarctic plants, of which mosses are a dominant component, have been exposed to large increases in springtime ultraviolet-B radiation (UV-BR) over the last three decades as a result of austral ozone depletion (McKenzie et al. 2007). Living in a frozen desert, Antarctic mosses are characterised by the ability to survive both desiccation and freezing and can tolerate multiple freeze-thaw and desiccation-rehydration events over the summer growing season (Lovelock et al. 1995b). As a result of recent ozone depletion, these mosses are currently exposed to multiple stressors, in particular the combination of UV-BR and water stress. Full recovery of the ozone layer is not expected until after 2060, and the largest ozone holes have occurred in the last decade (McKenzie et al. 2007). Therefore, it is important to understand how these combined stressors impact on Antarctic mosses.

Ultraviolet-B radiation is damaging to biological molecules including DNA, proteins, lipids and photosynthetic pigments. Plants can protect themselves from UV-BR induced damage by screening UV-BR before it reaches these molecules (Cockell and Knowland 1999) or by repairing damage once it has occurred (Britt 2004). Pyrimidine dimers are the most common type of UV-BR induced DNA damage, with cyclobutane pyrimidine dimers (CPDs) forming the bulk of these photoproducts (approximately 75%) whilst pyrimidine (6-4) pyrimidone dimers (6-4pps) account for the remainder (Britt 2004). These photoproducts distort the structure of DNA, blocking transcription and replication and are potentially cytotoxic and mutagenic (Jiang et al. 1997; Taylor et al. 1997; Britt 2004). DNA photoproducts that form in mature plant cells are repaired by photoreactivation, a light-dependent process that requires UV-inducible enzymes (Britt 2004). Nucleotide excision repair is a light-independent process that is prevalent in proliferating cells (Kimura et al. 2004).

Since both of these repair methods are enzymatic processes, their effectiveness could be limited when plants are exposed to low temperatures (MacFadyen et al. 2004) or desiccation (Buffoni Hall et al. 2003).

Ultraviolet-induced CPD accumulation has been measured in a few terrestrial polar organisms including seven mosses (Chapter3; Lud et al. 2002; Boelen et al. 2006), the alga *Prasiola crispa* ssp. *antarctica* (Kützing Knebel (Lud et al. 2001) and the Patagonian herb, *Gunnera magellanica* Lam., (Rousseaux et al. 1999a). Ambient UV-BR failed to produce significant levels of DNA damage in four of the Antarctic mosses (Lud et al. 2002; Boelen et al. 2006) but damage was detected in *Ceratodon purpureus, Bryum pseudotriquetrum, Schistidium antarctici*, and in both the alga and the herb (Chapter 3; Rousseaux et al. 1999a; Lud et al. 2001; Giordano et al. 2003). Supplementing the UV-BR dose 10-fold resulted in measurable DNA damage in *Sanionia uncinata* (Hedw.) Loeske (Lud et al. 2002) but not in *Chorisodontium aciphyllum* (Hook. f. & Wilson) Broth, *Warnstorfia sarmentosa* (Wahlenb.) Hedenas or *Polytrichum strictum* Menzies ex Brid*.* and all such damage to mosses was repaired overnight (Boelen et al. 2006).

Studies of several plant species have demonstrated links between drought and UV-B tolerance, with UV-BR exposure alleviating drought symptoms in several higher plants (Petropoulou et al. 1995). Bryophytes however, have a fundamentally different strategy for drought tolerance and many are desiccation tolerant. To our knowledge the relationship between CPD accumulation and desiccation has only been investigated in the lichen *Cladonia arbuscula* (Wallr.) Flot ssp. *mitis* (Sandst.) Ruoss*,* with more DNA damage accumulating in desiccated samples, presumably due to decreased photoreactivation (Buffoni Hall et al. 2003). Whilst desiccation tolerant bryophytes often show a higher tolerance of both photosynthetically active radiation (PAR) and UV-BR, than desiccation sensitive species (Seel et al. 1992b; Tákacs et al. 1999; Csintalan et al. 2001) we do not know if the desiccated state confers protection from UV-BR induced DNA damage in these plants. Since the accumulation of DNA photoproducts represents the balance of damage and repair,

and desiccation is likely to reduce the capacity for enzymatic repair, mosses could be particularly vulnerable to UV-BR in the desiccated state. However, if the process of desiccation confers greater stability on DNA molecules or the dehydration process results in an increased concentration of UV-B screening compounds, this could confer greater resilience to UV-BR in the desiccated state. If desiccation tolerance predicts UV-BR tolerance we would expect that *C. purpureus* and *B. pseudotriquetrum,* two cosmopolitan species found in East Antarctica, would be more tolerant of UV-BR than the co-occurring endemic, *S. antarctici,* due to their demonstrated higher tolerance of desiccation (Robinson et al. 2000; Wasley et al. 2006b).

Several Antarctic moss species have been shown to accumulate UV-B absorbing compounds (Lovelock and Robinson 2002; Newsham et al. 2002; Newsham 2003; Dunn and Robinson 2006) and concentrations of these compounds correlate positively with exposure to UV-BR in some species (Newsham 2003; Newsham et al. 2005; Arróniz-Crespo et al. 2006; Dunn and Robinson 2006; Lappalainen et al. 2008). For the three co-occurring Antarctic mosses mentioned above, concentrations of UV absorbing compounds also appear to be positively associated with desiccation tolerance as *B. pseudotriquetrum* and *C. purpureus* accumulate two-fold higher concentrations of total UV-B absorbing compounds than *S. antarctici* (Lovelock and Robinson 2002; Dunn and Robinson 2006; Clarke and Robinson 2008). In addition, there was a negative association between UV-B absorbing compounds and turf water content in *B. pseudotriquetrum,* and a positive association between anthocyanins and wind speed in *C. purpureus,* suggesting higher concentrations of potentially protective compounds in desiccated mosses (Dunn and Robinson 2006). In contrast, there was no evidence of changes in concentrations of UV-B absorbing compounds in *S. antarctici*, and damage under ambient UV-BR, in the form of abnormal morphology and loss of photosynthetic pigments, has also been reported for this species (Robinson et al. 2005).

We measured UV-BR induced DNA damage, as accumulation of DNA photoproducts, in three moss species from the Windmill Islands region, East Antarctica. Samples of field-collected moss were subjected to elevated UV-BR in a laboratory experiment designed to test the relative resilience of the three species to the effects of UV-B irradiation, as well as the UV-BR resilience of hydrated versus desiccated moss. Our hypotheses were that 1) *S. antarctici* would accumulate higher concentrations of photoproducts due to its lower capacity to screen UV-BR at the cellular level and 2) that desiccated mosses would accumulate more photoproducts than hydrated mosses, particularly if repair processes were more important than screening ability in these species.

4.2 Materials and Methods

We measured UV-BR induced DNA damage as accumulation of two types of photoproduct; CPDs and 6-4pps using an enzyme linked immunosorbent assay (ELISA). Preliminary experiments were conducted with *Schistidium antarctici* (Cardot) L.I. Savicz & Smirnova (formerly *Grimmia antarctici*) to establish an appropriate radiation dose. An experiment to test whether desiccated and hydrated mosses responded differently in their accumulation of UV-BR induced DNA damage, as well as to test the relative resilience of the three species, was then conducted in Antarctica. Samples of field-collected moss were first subjected to either a desiccation or hydration treatment and then to artificial UV-BR in a light box.

4.2.1 Preliminary experiments

Dose responses of CPD accumulation to artificial UV-BR were examined in *S. antarctici.* Samples were collected in Antarctica and kept frozen at -20˚C for several months before measurement. Prior to the experiment moss samples were thawed in a fridge, rehydrated and maintained moist overnight to restore physiological activity (determined as constant, high maximum quantum yield, F_v/F_m). Two light boxes were used, to allow simultaneous irradiation at different doses, with moss samples placed at different heights to alter the UV-BR dose. Moss samples (50 mg green gametophyte tips, n=4) were floated on a thin layer of

distilled water in metal bottle caps and irradiated in the light boxes for 4 h at one of five light treatments (0, 2, 4, 6, 8 W m^{-2} UV-BR supplied by 4 x 2ft conditioned UV-B lights; Phillips FL20SE; Davis Ultra Violet, Melbourne, Australia). Samples were placed under UV-BR permeable screens (cellulose acetate and plexiglass GS2458; Plastral Pty Ltd, Australia) to remove UV-C radiation. Radiation was measured using a compact radiometer (RM21, Dr Gröbel; UV Electronik GmbH, Ettlingen, Germany) with IP65 UV-B (280-315 nm) and UV-A (315-400 nm) sensors. To maintain normal physiological temperatures (between 0 and 5˚C) the metal bottle caps containing the moss were placed in containers of packed ice and the light box was placed in a 4˚C cold room. Following irradiation samples were frozen in liquid N_2 and stored at -80˚C for subsequent DNA extraction and analysis of DNA photoproducts.

4.2.2 Study sites and sampling

The Windmill Islands (centred at 66˚22’ S, 110˚30’ E) is a region of ice-free islands and peninsulas on the eastern coastline of Antarctica. Three moss species are found in the region; *S. antarctici* is endemic to the Antarctic continent, whilst both *Bryum pseudotriquetrum* (Hedw.) Gaertn., B. Mey. & Scherb and *Ceratodon purpureus* (Hedw.) Brid. have cosmopolitan distributions. Species distribution within the bryophyte community generally follows the moisture gradient, with *S. antarctici* associated with wetter areas, *C. purpureus* more common in 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. 2006b). Samples were collected from within Antarctic Specially Protected Area (ASPA) 135 on Bailey Peninsula from the site designated ASPA 1 in Dunn & Robinson (2006). A total of 72 individual 1 cm^2 samples (24 per species) were collected at midday on 27th December 2002 (a sunny day with a maximum air temperature of 2.5˚C and overhead ozone 337 Dobson Units). Care was taken not to shade moss during sampling and samples were immediately transported on ice to the Station Science Building.

4.2.3 Desiccation and hydration pre-treatments

Immediately on return to the laboratory, gametophyte tips were cut from each moss sample, placed on pre-weighed filter paper (2.5 cm diameter, Whatman, Grade 1, http://www.whatman.com) and weighed to determine fresh weight. Half of the samples (n=12 for each species) were then allowed to desiccate whilst the remaining samples were maintained in a fully hydrated state. Desiccated samples (D) were allowed to dry in a relative humidity of 22% for 6 h and then maintained in the presence of silica gel until constant weight was achieved (6 h). Hydrated samples (H) were maintained during this period by adding filtered water from melted snow to each sample until moss and filter paper were saturated. Hydrated samples were kept in a sealed, clear plastic container to maintain high humidity. All samples were maintained under low light (~10 µmol m^{-2} s^{-1}) at 18˚C.

4.2.4 UV-irradiation treatment

Four samples of each species, from each water pre-treatment (desiccated or hydrated), were irradiated with 8.5 W m^{-2} UV-B and 2.8 W m^{-2} UV-A for 4 h in the light box. Based on the distance between the lamps and the plant material and the radiation spectrum of the lamps measured under similar conditions, we estimate that 8.5 W m^{-2} treatment is equivalent to a UV-B_{BE} dose of 12 kJ m^{-2} (Caldwell 1971) over the 4h. This approximates a two-fold increase in the maximum daily UV-BR measured at various Antarctic stations (Seckmeyer et al. 1995). Throughout the light treatment desiccated moss samples were maintained in metal bottle caps on dry filter paper and were separated from hydrated samples, and surrounded by silica gel to prevent rehydration. Hydrated moss samples were maintained in metal caps, floating on a thin layer of melted snow. To maintain physiological temperatures (between 0 and 5˚C) during the radiation treatment the light box was placed in the cold porch of the Science Building. Following irradiation, samples were frozen in liquid N_2 and transported to Australia for DNA extraction and analysis of DNA photoproducts.

To ensure that all photoproduct accumulation measured resulted from UV-BR a series of controls were also included. Pre-irradiation controls were sampled direct from the field, and samples were also taken after the 12 h hydration and desiccation treatments. Additional samples were included in the light box, with UV-BR reduced by 98% (UV-A controls) with UV-B-blocking plexiglass screens (GS233 Plastral Pty Ltd, Australia) or light eliminated totally by foil covers (dark controls), to test whether DNA damage occurred specifically as a result of UV-BR. Samples are designated by water treatment, either hydrated or desiccated (H and D respectively), followed by light treatment, either with (+) or without (-) UV-B radiation.

4.2.5 DNA extraction

DNA was extracted from all samples using a modification of the method of Mason & Schmidt (2002) as described in Chapter 3. DNA concentration and purity for each sample were determined spectrophotometrically (UV-1601 UV visible spectrophotometer, Shimadzu, Melbourne, Australia).

4.2.6 Quantification of DNA photoproducts by ELISA

The concentration of CPDs and 6-4pps was quantified by ELISA in a method modified from Taylor *et al*. (1996) by substituting an Amplex red detection system described in Section 3.2.2. The ELISA method differed only in that two monoclonal, primary antibodies, specific for CPDs (TDM-2) and 6-4pps (64M-2; O. Nikaido, Kanazawa University, Japan; Mori et al. 1991) were used in separate ELISAs. DNA (4 ng μl^{-1}) from each individual moss sample was plated into four wells each of a 96 well plate for CPD or 6-4pp determinations. Standards containing pre-irradiated and un-irradiated calf thymus DNA (0.6 ng DNA) were plated onto each ELISA plate and used as positive and zero internal controls, respectively. The relative fluorescence value for each moss sample was calculated by normalising to the pre-irradiated and unirradiated calf thymus controls on the respective plates. The relative standard

deviation was 7% of the mean for irradiated calf thymus values for both photoproducts.

4.2.7 Statistical analysis

The accumulation of CPDs and 6-4pps for each independent moss sample was calculated as the mean relative fluorescence of its replicate, plated DNA samples. The mean for each treatment was then calculated from these individual sample means. Differences between means were examined using ANOVA. Initially a two-way ANOVA was conducted to determine if either water pre-treatments (hydration and desiccation) or control irradiation treatments (dark or UV-A only) caused increased photoproduct accumulation relative to field samples. For each species a two-way ANOVA then tested if irradiation (UV-B + UV-A versus UV-A only) or water status (hydrated versus desiccated moss samples) or the interaction term affected accumulation of each photoproduct. Differences between species were also explored in a two-way ANOVA of photoproduct accumulation using only UV-BR irradiated samples (hydrated versus desiccated moss samples). Photoproduct data were fourth root transformed to satisfy the assumptions of the ANOVA. Where significant effects ($P<0.05$) were observed *post hoc* Tukey-HSD tests were performed. Statistical analyses were conducted using JMP 7 (SAS Institute, Cary, NC, USA) computer package.

4.3 Results

Preliminary experiments were conducted to determine the UV-BR dose response of CPD accumulation in Antarctic collected *S. antarctici*. There was no increase in CPD accumulation damage in *S. antarctici* exposed to 2 W m^{-2} UV-BR (Figure 4.1). Above 2 W m^{-2} there was a linear relationship between CPD accumulation and UV-BR dose in this species. In a similar experiment, using cultured, Antarctic *Ceratodon purpureus*, 4 h irradiation with 8 W m^{-2} UV-BR was required to achieve significant accumulation of CPDs and 6-4pps (Venturini 2003).

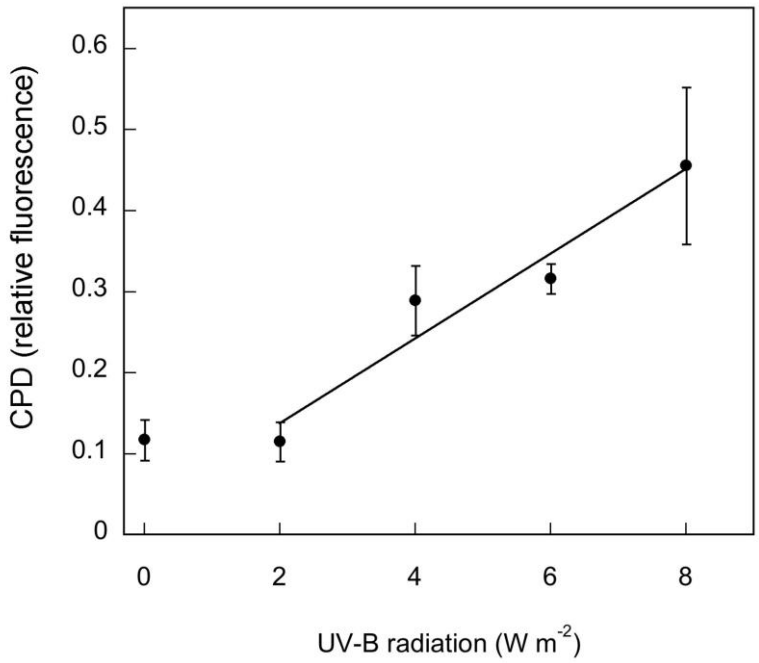


Figure 4.1: The effect of irradiation with five different doses of UV-B radiation on cyclobutane pyrimidine dimer accumulation (CPDs; relative fluorescence) in the moss *Schistidium antarctici*. Data are means ± SEM (n = 4-8) Equation of fitted line: CPD = 0.032 + 0.052 UV-BR dose, R^2adj= 0.94 P<0.03. Zero values were excluded from analysis, as there was no significant change in CPDs below UV-BR fluxes of 2 W m^{-2}.

Samples of the three moss species collected from the field showed similar levels of CPDs and 6-4pps regardless of species (Table 4.1).There was no significant change in concentration of either photoproduct as a result of the initial desiccation/hydration treatments. Although CPDs increased (72%) during the pre-treatments in *S. antarctici*, this increase was not significant and applied equally to hydrated and desiccated treated samples (Table 4.1). The dark and UV-A radiation treatments did not cause significant accumulation above pre-treatment levels in any species (c.f. Table 4.1 with Figures 4.2 & 4.3). Significant photoproduct accumulation was thus restricted to samples that received UV-BR irradiation (Table 4.2). When CPD accumulation in UV-BR irradiated, hydrated samples (H+ treatment) was compared with that in the corresponding UV-AR controls (H- treatment) there was a 3-fold increase in *C. purpureus* and more than a 6-fold increase in both *B. pseudotriquetrum* and *S. antarctici* (Figure 4.2). Similarly, 6-4pp accumulation in

H+ samples was approximately 2-fold higher in *C. purpureus* and *B. pseudotriquetrum* and 4-fold higher for *S. antarctici* than the corresponding H- control samples (Figure 4.3).

Table 4.1: CPD and 6-4pp concentration in three species of Antarctic moss, *Ceratodon purpureus, Bryum pseudotriquetrum* and *Schistidium antarctici;* on collection in the field, after water (desiccation/hydration) pre-treatment and following the dark treatment in the light box. CPDs and 6-4pps are reported in units relative to 4 h irradiated calf thymus DNA and values for different photoproducts are not directly comparable. No significant differences in photoproducts were observed either between species in the field, or within species as a result of desiccation/hydration pre-treatment or radiation controls.

Photoproduct and species	Field samples (Mean fluorescence ± SEM, n=4)	Water pre-treatment (Mean fluorescence ± SEM, n=8)	Dark treatment (Mean fluorescence ± SEM, n=8)
CPDs			
B. pseudotriquetrum	0.034±0.006	0.034±0.014	0.039±0.005
C. purpureus	0.041±0.005	0.030±0.006	0.040±0.003
S. antarctici	0.036±0.005	0.062±0.009	0.116±0.011
6-4pps			
B. pseudotriquetrum	0.700±0.030	0.689±0.029	0.553±0.031
C. purpureus	0.556±0.170	0.752±0.087	0.553±0.049
S. antarctici	0.630±0.073	0.737±0.043	0.663±0.087

CPDs, cyclobutane pyrimidine dimers; 6-4pps, pyrimidine (6-4) pyrimidone dimers.

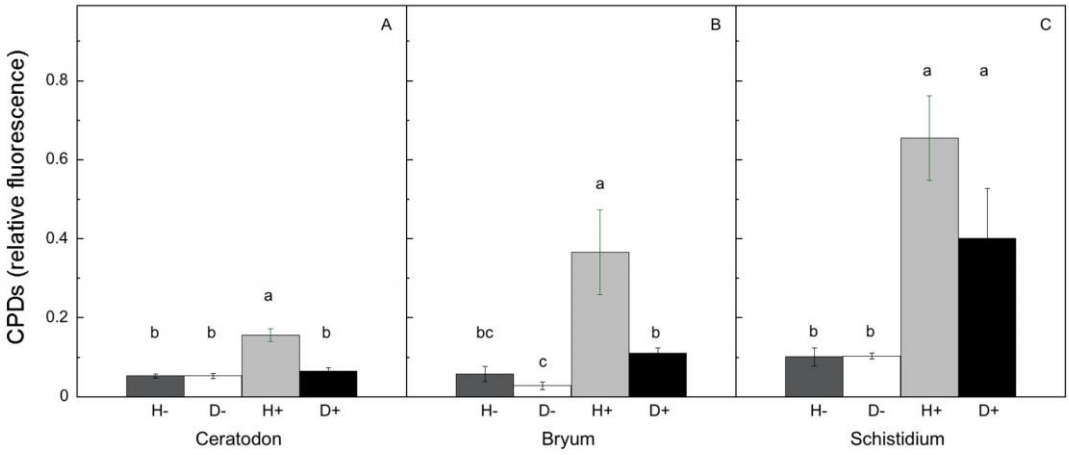


Figure 4.2: Accumulation of CPDs in three species of Antarctic moss, *Ceratodon purpureus* (A)*, Bryum pseudotriquetrum* (B) and *Schistidium antarctici* (C) after 4 h exposure to radiation with (+) or without (-) UV-B wavelengths. Moss samples were irradiated in either a hydrated (H) or desiccated (D) state. Data are means for individual moss samples ± SEM (n = 4). CPDs are reported in units relative to 4 h irradiated calf thymus DNA. Different letters indicate mean photoproducts are significantly different within species at $P<0.05$.

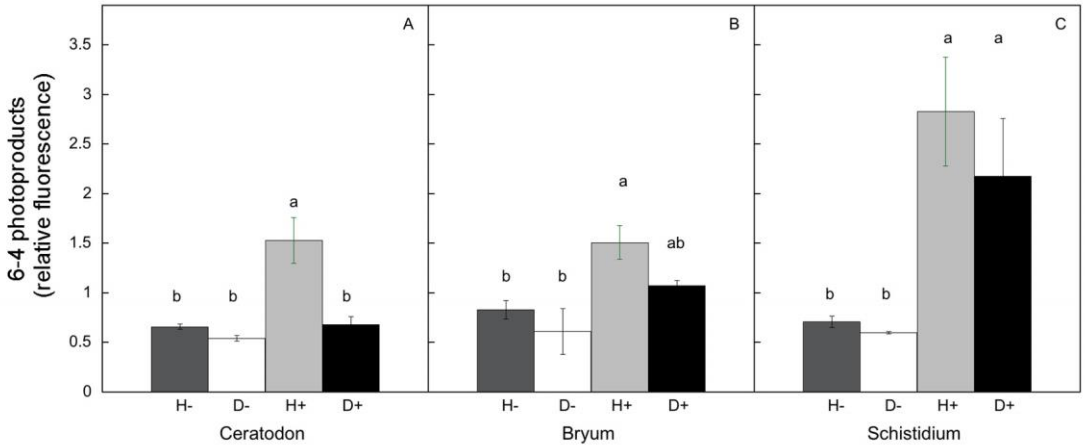


Figure 4.3: Accumulation of 6-4pps in three species of Antarctic moss, *Ceratodon purpureus* (A)*, Bryum pseudotriquetrum* (B) and *Schistidium antarctici* (C) after 4 h exposure to radiation with (+) or without (-) UV-B wavelengths. Moss samples were irradiated in either a hydrated (H) or desiccated (D) state. Data are means for individual moss samples ± SEM (n = 4) except for *S. antarctici* H+ where n=3. Photoproducts are reported in units relative to 4 h irradiated calf thymus DNA. Different letters indicate mean photoproducts are significantly different within species at $P<0.05$.

Table 4.2: Results of two-way ANOVA to test the effects of irradiation (UV-B + UVA or UVA alone) and water status (hydrated or desiccated) treatments on CPD and 6-4pp accumulation in Antarctic mosses. ANOVAs were conducted separately on three species of Antarctic moss, *Ceratodon purpureus, Bryum pseudotriquetrum* and *Schistidium antarctici.* Mean values are shown in Figure 4.2 (CPDs) and Figure 4.3 (6-4pps).

Species and factors	CPDs		6-4pps	
	F stat	*P*-value	*F* stat	*P*-value
Ceratodon purpureus				
Light	33.0	**<0.0001**	22.9	**0.0004**
Water	18.0	**0.001**	21.6	**0.0006**
Light * water	17.4	**0.001**	8.97	**0.011**
Bryum pseudotriquetrum				
Light	31.1	**0.0001**	15.1	**0.002**
Water	10.3	**0.007**	4.98	**0.044**
Light * water	1.34	0.269	0.65	0.433
Schistidium antarctici				
Light	50.5	**<0.0001**	45.4	**<0.0001**
Water	1.87	0.196	1.58	0.234
Light * water	2.69	0.127	0.36	0.561

CPDs, cyclobutane pyrimidine dimers; 6-4pps, pyrimidine (6-4) pyrimidone dimers.

When desiccated and hydrated samples of the three species were irradiated with enhanced UV-BR, desiccation conferred significant protection from UV-B induced DNA damage in *C. purpureus* and *B. pseudotriquetrum*, but not *S. antarctici* (Figure 4.2; Figure 4.3).

For *C. purpureus,* only hydrated samples exposed to UV-B radiation (H+ treatment, Figure 4.2 A; Figure 4.3 A) accumulated significant photoproducts. For both CPDs and 6-4pps there were significant light * water treatment interaction (Table 4.2). When desiccated *C. purpureus* was treated with UV-B radiation (D+ treatment) there was no increase in either photoproduct above the level in the UV-A irradiated control samples (H- and D- treatments; Figure 4.2A; Figure 4.3A).

For *B. pseudotriquetrum,* UV-B irradiation caused significant accumulation of CPDs and 6-4pps (light treatment, Table 4.2, Figures 4.2 B; Figure 4.3 B). There was also a significant effect of water treatment (Table 4.2) with hydrated samples showing

higher levels of both photoproducts than desiccated samples. Desiccation protected *B. pseudotriquetrum* from DNA damage, with desiccated samples (D+) accumulating only 26% of the CPDs in hydrated samples (H+; Figure 4.2 B). Protection from UV-BR by desiccation was less pronounced for 6-4pps, however, with desiccated samples accumulating almost 70% of the photoproducts measured in the hydrated samples (c.f. D+ with H+), a non-significant decline (Figure 4.3 B).

Whilst UV-B irradiation caused significant accumulation of CPDs and 6-4pps in *S. antarctici* (light treatment, Table 4.2, Figure 4.2 C; Figure 4.3 C), desiccation did not provide significant protection against DNA damage in this species. Desiccated samples accumulated 54% of the CPDs and 74% of the 6-4ppsfound in the respective H+ treatments, but variance was high and the decline was not significant (Table 4.2; Figure 4.2 C; Figure 4.3 C).

Photoproduct accumulation in response to enhanced UV-BR differed between the three species. *Schistidium antarctici* accumulated significantly more CPDs and 6-4ppsthan the other two species (CPDs; $F_{2,21}$=28.0, P<0.0001; (6-4) photoproducts; $F_{2,20}$=28.0, P=0.0004). *Ceratodon purpureus* also accumulated significantly fewer CPDs than *B. pseudotriquetrum* (Figure 4.2) but (6-4) photoproduct accumulation was similar in the two cosmopolitan species (Figure 4.3).

4.4 Discussion

The main findings of this study are that 1) desiccation confers protection from UV-BR induced DNA damage in two cosmopolitan species of Antarctic moss and 2) whilst all the species in this study have relatively high tolerance to enhanced UV-BR, the endemic *S. antarctici* is the least tolerant species.

4.4.1 Desiccation confers protection from UV-B induced DNA damage in two moss species

Although tolerance of solar radiation and either drought stress or desiccation are associated in many plants, this study is the first to demonstrate that bryophytes are protected from UV-BR induced DNA damage in the desiccated state and this finding

was unexpected, since a previous study had shown that lichen thalli accumulate more damage when desiccated (Buffoni Hall et al. 2003). Desiccation tolerant mosses and lichens can often tolerate exposure to both high PAR and UV-BR (Seel et al. 1992b; Seel et al. 1992c; Tákacs et al. 1999; Heber et al. 2000) and this tolerance can manifest differentially in the hydrated and desiccated state. For example, the photosynthetic apparatus of the desiccation tolerant moss species, *Tortula ruralis*, is more tolerant of photoinhibition when the moss is desiccated than when it is hydrated (Seel et al. 1992b; Seel et al. 1992c), but even when hydrated this species was able to tolerate elevated UV-BR for 8 days with no significant decline in F_v/F_m (Tákacs et al. 1999). In the Antarctic mosses studied here, both the tolerance to UV-B induced DNA damage, and the extent to which desiccation is protective, fits with the degree of desiccation tolerance and hence the hydrological habitat of each species (Robinson et al. 2000; Wasley et al. 2006b), as well as with its relative accumulation of UV-B absorbing compounds (Lovelock and Robinson 2002; Dunn and Robinson 2006; Clarke and Robinson).

The fact that these mosses are so well protected when dry is suggestive of passive protection, as enzymatic repair processes are unlikely to be active in desiccated organisms (Buffoni Hall et al. 2003). Passive protection mechanisms would also be effective when these mosses are frozen and could thus be particularly beneficial to polar and alpine plants. Protection from UV-BR when desiccated could be due to morphological changes upon drying, which reduce light levels in the cell. Desiccation tolerant plants typically reduce exposed leaf area when dry by folding or curling of leaves (Davey and Ellis-Evans 1996; Proctor and Tuba 2002). This reduces transmission of PAR into the cell by between 40-60% in a range of moss species including *B. pseudotriquetrum* and *T. ruralis* (Seel et al. 1992b). UV-BR is likely to be similarly reduced which would contribute considerable protection at the molecular level. Based on relative turf densities, desiccation of these three mosses results in reductions in size ranging from 25% in *S. antarctici* to 40 to 50% in *B. pseudotriquetrum* and *C. purpureus* respectively (Wasley et al. 2006b). When

moss cells shrink upon desiccation, cytoplasm volume is reduced, concentrating cellular contents including UV screening compounds and possibly increasing the attenuation of UV-BR.

In most ecosystems, periods of high insolation (and associated UV-BR stress) cause desiccation in bryophytes as they equilibrate leaf turgor with that of their surroundings (Gehrke 1999). The reverse is true in the Antarctic environment however, where the major water source is snow-melt, which is maximal during periods of high insolation, and can coincide with elevated UV-BR as a result of ozone depletion. Thus if desiccation is a major strategy for protection from UV-BR these plants may still be at risk from high UV-BR during ozone depletion, especially when this coincides with spring melt.

4.4.2 UV-B irradiation induces more DNA damage in an Antarctic endemic than two cosmopolitan moss species

This research confirms an earlier field study that showed *S. antarctici* was sensitive to ambient UV-BR (Robinson et al. 2005). The difference in tolerance between the three species could be the result of a number of factors since these species vary in their desiccation tolerance, morphology and concentrations of UV-B absorbing compounds and may differ in their ability to repair DNA damage. Whilst the morphology of the three species is different; the leaves of *C. purpureus* and *B. pseudotriquetrum* adhere to the 'stem' whereas the leaves of *S. antarctici* are flat and more exposed (Robinson et al. 2000), UV-BR reflectance is uniformly low in all three species and so reflectance is less likely to be an important factor in protection (Lovelock and Robinson 2002).

Higher concentrations of UV absorbing compounds could explain the decreased DNA damage in the two cosmopolitan species relative to *S. antarctici* since the latter species contains the lowest concentration of these screens. The location of UV absorbing compounds also differs, with *B. pseudotriquetrum* accumulating more of these compounds inside the cell compared to *C. purpureus* and *S. antarctici*, in

which the bulk of these compounds are bound to the cell walls (see chapter 5; Lovelock and Robinson 2002; Dunn and Robinson 2006; Clarke and Robinson 2008). If cell wall-bound UV absorbing compounds make more effective screens this could also explain the particularly high resilience of *C. purpureus* to UV-BR induced damage.

In the related field study in Chapter 3, DNA damage from ambient UV-BR was relatively low for all three species but there were indications that repair of DNA damage might be enhanced by warmer, wetter conditions. If the three species differ in their ability to repair DNA damage this might also explain the difference in UV sensitivity of the hydrated samples.

4.4.3 Limitations of the study

The UV-BR dose used in this experiment was artificially high, almost double that currently experienced by plants anywhere at the Earth's surface (Kinzie et al. 1998). This dose was chosen because; preliminary experiments showed that below this dose no significant DNA damage was observed in cultured, Antarctic *C. purpureus* (Venturini 2003) and, due to restrictions on the number of samples that can be collected in the Antarctic, multiple UV-BR doses could not be justified. Our preliminary study using *S. antarctici* showed that DNA photoproducts did not accumulate at or below 2 W m^{-2} (UV_{BE} dose approximately 3 kJ m^{-2}), suggesting a threshold for damage accumulation, but a linear relationship with UV-BR from 4 to 8 W m^{-2} was observed. This is similar to experiments using Antarctic *Sanionia uncinata,* where ambient UV-BR of 2.1 W m^{-2} failed to produce measurable CPD accumulation, and a 10-fold increase in UV_{BE} dose was required to induce significant photoproduct accumulation.

Repair of DNA photoproducts occurs predominantly via photolyases in plants. These enzymes are induced by visible light and require blue or UV-A light for photoreactivation to occur (Kimura et al. 2004). The induction of UV absorbing compounds can also require prior exposure to solar radiation. Since these plants

were collected from the field during mid summer, they are likely to have induced photolyases and protective compounds. However, their capacity for repair of DNA damage during the experiment could have been limited by the relatively low levels of photoreactivating light, leading to a possible overestimate of photoproduct accumulation in hydrated samples.

4.5 Conclusions

Although the high UV-BR dose limits extrapolation of these results to the field situation, our study has highlighted the remarkable tolerance of the three species whilst in the desiccated state. The high resilience of desiccated mosses to DNA damage suggests that passive screening maybe more important than repair in these species. Differences in UV-BR tolerance between the three species match their desiccation tolerance, with *C. purpureus* most tolerant of both stressors, *B. pseudotriquetrum* intermediate and *S. antarctici* the least. The finding that the two cosmopolitan species are likely to be more resilient in the face of continued ozone depletion raises biodiversity concerns for the endemic species *S. antarctici.*

The following chapter investigates interspecific differences in field concentrations of UV absorbing compounds including anthocyanins. These passive screens reduce UV-BR light penetration and may explain the differences in UV tolerance found between species.

5 Environmental drivers of UV absorbing compounds in Antarctic moss.

5.1 Introduction

Ozone depletion became evident in the 1980s (Farman et al. 1985) and has caused a doubling of incident UV-BR in late Spring in Antarctica (McKenzie et al. 2011). The long term natural variability in UV-BR is an important context for understanding the impact of recent anthropogenic changes, but the reliable record of ozone column depth is brief. Records of ozone column depth began in 1926, with very limited geographic coverage (Bjorn and McKenzie 2007 and references therein; Bornman et al. 2015). This record can be reliably extended to 1900 through estimates based on ozone spectral traces retrieved from photographs of stars (Bjorn and McKenzie 2007 and references therein). However, incident UV-BR is more difficult to determine. It is affected not only by ozone depth, but by multiple other factors, including solar elevation, cloud cover, albedo, altitude and aerosols (Bais et al. 2015). Plant UV absorbing compounds (UVACs) respond to incident UV-BR and may be useful proxies for past UV-BR (for reviews see Bjorn and McKenzie 2007; Bornman et al. 2015)

The most common response of plants to elevated UV-BR in field studies is the accumulation of intracellular (methanol soluble) UVACs (iUVACs; Searles et al. 2001; Newsham and Robinson 2009). The use of such compounds as proxies in herbarium samples, spores and pollen has met with varying success (Markham et al. 1990; Huttunen et al. 2005a; Huttunen et al. 2005b; Lomax et al. 2008; Otero et al. 2009; Rozema et al. 2009; Ryan et al. 2009; Willis et al. 2011; Lomax et al. 2012). In the relatively unpolluted and undisturbed Antarctic environment, mosses remain *in situ,* facilitating the analysis of environmental factors which influence UV-BR at the scale of the plant such as shade, slope and aspect. Long shoots of moss can now be dated using radiocarbon from atomic bomb testing in the 1950s and 1960s which allows such compounds to be traced over decades to centuries (Clarke et al. 2012; Bramley-Alves et al. 2015). Alkali extractable cell wall UVACs (WUVACs) are more stable than intracellular compounds (Monforte et al. 2015) and are better suited as

a UV-BR proxy. But it is unclear if they are induced by UV-BR in Antarctic mosses. Unlike the better characterised iUVAC, there is currently little understanding of the factors driving the accumulation of WUVACs.

In mosses UVAC form via the phenylpropanoid pathway and typically include hydroxycinnamic acids and flavonoids, including anthocyanins (Fabon et al. 2012a; Waterman 2015). These compounds may have multiple functions within the leaf, eg. as screens, antioxidants, regulating signal transduction or mediating plant immunity (as reviewed in Gould and Lister 2006; Jansen et al. 2008; Agati et al. 2012). Studies in mutants lacking phenolic compounds demonstrate that UVAC can protect against both UV-BR (Landry et al. 1995) and visible light (Havaux and Kloppstech 2001). Mosses may rely more on WUVACs as they lack the complex leaf surface structures of vascular plants, where iUVAC can absorb up to 99% of incident UV-BR (eg. epidermis, trichomes, cuticle; Robberecht and Caldwell 1978; Markham 1990; Day et al. 1993; Vogelmann 1993; Gould and Lister 2006).

Accumulation of iUVAC in UV-BR is less common in bryophytes than vascular species (Boelen et al. 2006; Newsham and Robinson 2009). Yet does occur in certain moss taxa, for example, in the genera *Sanionia, Plerozium, Bryum* and *Andreaea* and in the liverwort *Jungermannia* (Newsham et al. 2002; Newsham 2003; Dunn and Robinson 2006; Otero et al. 2009; Fabon et al. 2012a; Schroeter et al. 2012; Singh et al. 2012). However WUVAC, located in the cell wall, are rarely studied and UV protection in bryophytes may be seriously underestimated (Clarke and Robinson 2008; Hespanhol et al. 2014).

Interspecific differences in the location of UVAC represent different photoprotective strategies, which may affect the effectiveness and metabolic costs of UV-BR protection (Semerdjieva et al., 2003). Intracellular UVAC can be remobilised when not required, which is particularly advantageous in carbon limited plants. However, WUVACs are incorporated outside the living cell, so are unlikely to be remobilised. This leads to a higher resource investment per leaf.

Differences in the ratio of WUVAC:iUVAC were found between co-occurring moss genera in a survey of 23 species from alpine Portugal and in my three Antarctic study species (Clarke and Robinson 2008; Hespanhol et al. 2014). Possibly this relates to leaf resource investment, but further research is required to understand these differences in UV protective strategies between taxa and how this relates to life history attributes.

Different UV protective strategies are also reported in the three study species *Bryum pseudotriquetrum, Ceratodon purpureus* and *Schistidium antarctici* (Clarke and Robinson 2008). The total concentrations of UVAC and the ratio of WUVAC:iUVAC vary between these species. In our irradiation study, UV-BR tolerance was highest in *C. purpureus* and intermediate in *B. pseudotriquetrum* (Figure 4.2; Figure 4.3). *Schistidium antarctici* was least tolerant of UV-BR (Figure 4.2; Figure 4.3) and may be particularly susceptible. Our previous work also reports morphological changes and chlorophyll bleaching in *S. antarctici* in ambient UV-BR (Figure 1.7; Robinson et al. 2005). DNA damage was also reduced in desiccated metabolically inactive samples of all three species suggesting passive protection, such as screening through UVACs.

Intracellular UVACs can change rapidly and correlate with daily UV-BR in certain Antarctic bryophytes (Newsham et al. 2002; Newsham 2003). A diurnal UV-BR response was also reported in a study of a tropical tree and a high altitude fern; UV-BR induced intracellular flavonoids increased at noon and decreased to basal levels overnight, but were maintained at low concentrations under UV-BR filters (Veit et al. 1996). Precise induction and relaxation times are unclear and may differ for the individual compounds, between taxa and with other environmental variables.

The question of whether the WUVACs are inducible or constitutive in Antarctic moss is complex, as these stable pigments are tightly bound to the cell wall and may remain in place for the life of the leaf. As for iUVACs, the UV history of the samples is important, as samples collected with already high UVACs may not demonstrate additional increases on UV treatment (Martinez-Abaigar et al. 2009). In Norway

Spruce, iUVACs declined with disease while WUVACs remained constant, suggesting that iUVACs in epidermal tissue are variable and actively mediated whereas WUVACs are a constitutive passive screen (Hoque and Remus 1999). These authors also report that WUVACs increased with needle age, indicating the importance of factors other than UV-BR radiation exposure to UVAC accumulation.

Cell wall UVACs do accumulate in response to UV-BR in some plant taxa. Semerdjieva and co-workers (2003) investigated three closely related Arctic dwarf shrubs and found differences in the WUVAC:iUVAC ratio. Only the evergreen species increased WUVACs in response to field UV-BR treatment (1.2 kJ m^{-2} d^{-1} $UV_{BE\ Caldwell}$) with unchanged or decreased concentrations in the deciduous species. These differences were attributed to contrasting leaf longevity. In bryophytes, a leafy liverwort increased WUVACs in a laboratory UV-BR study (1.21 W m^{-2} for 31 days), whilst under the same conditions two moss species, *B. pseudotriquetrum* and *Fontinalis antipyretica* had fluctuating concentrations of WUVACs which were unrelated to the UV-BR treatment (Fabon et al. 2010; Fabon et al. 2012a). Cell wall UVAC protection may be constitutive in mosses, or possibly like soluble UVACs, only particular species have the capacity to increase concentrations in response to UV-BR.

Fabon and co-workers (2012b) investigated the liverwort *Jungermannia exsertifolia* and found the concentrations of bulk WUVACs, and the hydoxycinnamic acids *p*-coumaric acid and ferulic acid isolated from the cell wall increased within hours of a noon UV-BR challenge. Slight reductions occurred overnight, but all changes were less pronounced on the second day of measurement.

Anthocyanins are a class of flavonoid widely distributed across taxa and ecosystems, but the role of these compounds in vegetative tissue is poorly understood (for reviews see Steyn et al. 2002; Gould 2004; Gould and Lister 2006; Manetas 2006; Landi et al. 2015). Anthocyanins can protect against photoinhibition (Nielsen and Simonsen 2011; Ranjan et al. 2014) either as anti-oxidants or by filtering UV and visible wavelengths and may can perform these functions simultaneously (Gould et

al. 2002; Neill and Gould 2003; Mori et al. 2005). They often accumulate transiently at different developmental stages, in dormant mature leaves or in response to stress, particularly when light levels exceed photosynthetic capacity (Steyn et al. 2002; Gould 2004; Gould and Lister 2006). Transience is advantageous, since such permanent light filters would otherwise reduce photosynthesis under low or fluctuating light conditions (Steyn et. al. 2002).

In vascular species, anthocyanins accumulate transiently in juvenile expanding leaves (Lee et al. 1987; Tuohy and Choinski 1990; Oberbauer and Starr 2002; Hughes et al. 2007). Their presence precedes the synthesis of chlorophyll and photoprotective xanthophyll (VAZ pool) pigments, and so anthocyanins may protect against excess light as the photosynthetic apparatus develops (Robinson and Russell 1998; Gamon and Surfus 1999; Hughes et al. 2007; Liu et al. 2009). Since WUVACs are rarely measured concomitantly with anthocyanins, little is known about their relative roles.

Anthocyanins require light for accumulation and increase under a range of other abiotic and biotic stressors, for example: low temperature, nitrogen starvation, or wounding and pathogen attack (Steyn et al. 2002; Gould 2004; Gould and Lister 2006). In some Antarctic bryophytes anthocyanin concentrations respond to solar radiation, particularly UV-BR wavelengths, but other environmental factors may also be influential. Anthocyanins accumulated in samples exposed to high solar radiation (Post and Vesk 1992), decreased when UV-BR was screened (Snell et al. 2009) and accumulated faster in unfiltered than in UV-BR screened solar radiation in *Cephaloziella varians* (Newsham et al. 2005). Anthocyanin concentrations also showed relationships with ambient UV-BR and ozone column depth in multiple regression models for *B. pseudotriquetrum* and *C. purpureus* respectively (Dunn and Robinson 2006), however no relationship was apparent for *S. antarctici*. The relationship with UV-BR could be masked by the influence of other climatic factors.

In our Antarctic screening study using *S. antarctici*, anthocyanins and iUVAC were higher in shaded treatments and in valleys within the undulating moss

microtopography (Figure 1.7; Figure 5.1), but were unresponsive to UV-BR treatment (Robinson et al. 2005). This is contrary to what might be expected if these compounds responded only to light. It remains unclear which climatic factors are most important. Furthermore, Anthocyanic red colouration can also be easily assessed photographically for biomonitoring of Antarctic moss (King 2015 pers.comm). However, the interpretation of these transient changes is confounded by a lack of understanding of the environmental drivers of anthocyanin accumulation.

Figure 5.1: Antarctic moss turves within the study site ASPA 135, showing distinctive microtopography thought to be caused by cryoturbation. The ridges are more exposed to both light and wind and have lower water availability whereas the valleys provide wetter, shadier conditions. Photo: J. Turnbull.

Climatic factors which reduce photosynthesis may lower the metabolic resources available for the synthesis of UVAC, particularly in low energy environments (Belnap et al. 2008). Bandurska and co-workers (2012) found low water availability during a UV-BR challenge reduced UVACs and anthocyanin production in droughted barley. This did not occur in well watered seedlings. In the Antarctic liverwort *Cephaloziella varians* the anthocyanin Riccionidin A almost doubled in 48 hours, using an estimated 1.85% of the carbon gained from photosynthesis over this time (Snell et al. 2009). Whilst this figure may seem low, decreased photosynthesis may lower the resources available for pigment synthesis. Yet this is when such pigments are most needed. Low photosynthesis, for example, due to low temperature, leads to a higher need for general photoprotection from both UV and visible light (see Section 1.4.1).

The 2002/03 summer season at Casey station had atypically low ozone depletion, and an early and protracted summer melt (Section 3.3.1 to 3.3.5). The ozone hole broke up early in late September 2002, and while there was still variation in incident UV-BR (Figure 3.1), the Springtime UV-BR was 25% lower than the longterm average (Figure 3.2). This season provides a useful baseline as it allows characterisation of moss pigments in the early part of the season, without additional UV-BR from ozone depletion, and may represent pre-ozone depletion seasonal changes.

This study aims to characterise WUVAC, iUVAC and anthocyanin concentrations to compare photoprotective strategies in the three Antarctic moss species to determine which climatic factors are most important to their accumulation over a summer season. The response of UVAC to UV-BR is of particular interest to the development of proxies for past UV-BR. The climatic factors driving anthocyanin accumulation are relevant to the use of this pigment in biomonitoring. The aims are to determine; 1) If UVACs including anthocyanins, are induced by UV-BR and 2) if accumulation is influenced by cold temperatures and factors reducing water availability to the mosses. In addition, species differences in the abundance and location of UVACs, including anthocyanins, were investigated.

5.2 Methods

5.2.1 Data collection

Sample collection is described in Section 3.2.1. However, an expanded data set is used in Chapter 5 and 6. This comprises 192 samples in total, n=64 per species, including 3 samples per species on 22 sampling days, (except day 1 where n=1). Daily means were calculated for pigments and physiological data, collected with each sample, and used in subsequent analysis. To achieve factorial balance within early, mid and late season, samples with missing light data (n=1 per species) were excluded from analysis. Nine missing data for MT and ETRs were filled with season means. Missing light data (wUVR5) was modelled using data from each day of the season (see Appendix A). Care was taken to sample from ridges within the microtopography with full solar exposure (see Figure 5.1).

5.2.2 UVAC analysis

5.2.2.1 Intracellular UVAC Extraction

Freeze dried moss apices (10–30 mg) were homogenised in a microfuge tube for 2 minutes, with a 3mm Tungsten- Carbide bead at 30 Hz (tissue lyser; Qiagen, Retsch www.retsch.com). The resultant powder was extracted in 1.5 mL of 1% acidified methanol for three hours on ice in the dark. The supernatant was stored overnight at 4 °C, prior to spectrophotometric analysis.

5.2.2.2 Cell Wall UVAC extraction

Cell wall bound phenolics were extracted by alkaline hydrolysis technique as per Clarke and Robinson (2008), with modifications (Melinda Waterman pers. comm. 2013). The moss residue from the intracellular extraction was washed twice in 100% methanol, and incubated for 1 hour at room temperature. The pellet was washed twice in 1 mL 1 M NaCl for 20 minutes, once in 1 mL 100% methanol, then twice in 1 mL of 1:1 methanol: chloroform mixture leaving the suspension for 1 hour at room temperature the first time and ten minutes the second time. The pellet was washed again in 1 mL 100% methanol and air dried at room temperature. Then alkali

extraction in 1 mL 1M NaOH continued for 16 hours in the dark at room temperature. After centrifuging, 0.8 mL of supernatant was added to 0.5 mL of 1.5 M formic acid, allowing two hours for full neutralisation, with centrifuging prior to spectrophotometry.

5.2.2.3 Anthocyanin extraction

The intracellular UVAC supernatant was used to quantify anthocyanin concentrations ($A_{\Delta 526}$ gdw^{-1}), using a differential pH method modified from Lovelock and Robinson (2002). Preliminary scans (400–800 nm) revealed three peaks at 526 nm, 567 nm, and 600 nm in all three moss species (Figure 5.2). Absorbance was measured at these wavelengths in acidified methanol (pH 1), then after adding 100 μL of 2.0 M sodium acetate buffer to 500 μL of supernatant, at pH 4.5. Absorbance was zeroed to 700 nm. Comparison of the absorbance at pH 4.5 and pH 1 suggests peaks at 526 nm and 600 nm are pH colour stable, and may be compounds based on 3-desoxyanthocyanidins, reported from bryophyte cell walls, whereas the peak at 567 nm is pH labile and more likely to be an anthocyanidin (Andersen and Jordheim 2006). Since all three peaks were correlated (ρ= 0.86–0.98 p<0.0001 n=192), the peak at 526 nm was analysed as it has precedence in the literature (Lovelock and Robinson 2002; Dunn and Robinson 2006).

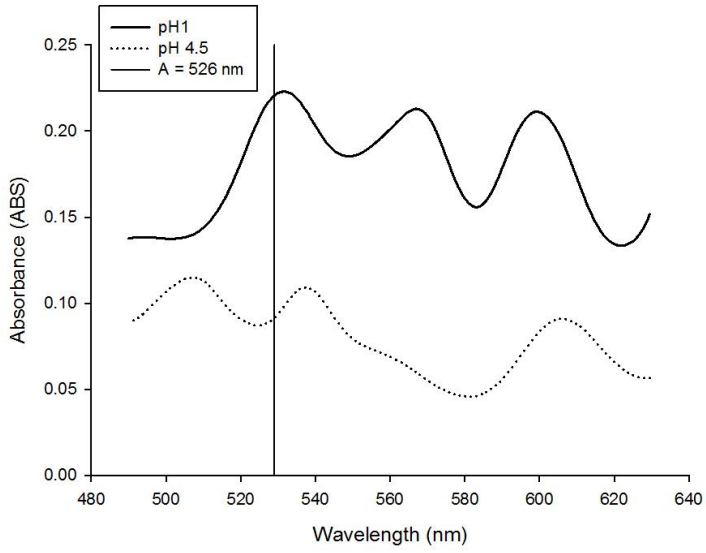


Figure 5.2: Representative absorption spectra *of Schistidium antarctici* extracted in acidified methanol (pH 1) and measured in 1:5 sodium acetate buffer: methanol (pH 4.5) showing three peaks at 526 nm, 567 nm, and 600 nm.

5.2.2.4 Spectrophotometric Analysis

Absorbance was measured between 280–400 nm (UV-visible spectrophotometer; Model 1601, Shimadzu, Australia) and the area under the curve integrated (Shimadzu UVPC1 software; v3.91). Cell wall extracts were blanked with distilled water, methanol extracts with methanol and anthocyanins at pH 4.5 were blanked with 1: 5 sodium acetate buffer: methanol. UVACs are reported in UV-B wavelengths ($A_{280\text{-}315}$ gdw^{-1}).

5.2.3 Climate data

The environmental data included daily, three, five and ten day means calculated from three hourly observations of meteorological data (Australian Bureau of Meteorology, Casey station), overhead ozone column depth (DU; Earth Probe Total Ozone Mapping Spectrophotometer, NASA, 2002) and daylength (hours; Auroral Space physics department, Casey station) and daily doses of solar radiation measurements from sensors at Casey station (Australian Radiation Protection and Nuclear Safety Agency). Radiation measurements are described in Section 3.2.4, except new data for weighted total UV radiation (wUVR: Solar Light UV biometer: SL501) were downloaded as the standard erythemal dose (C.I.E. 1987) on 4 June 2013. This data forms Australia's State of the Environment Indicator 10 (https://www1.data.antarctica.gov.au/aadc/soe/display_indicator.cfm?soe_id=10). The SEDS were then converted to Watts m^{-2} with the assistance of Kerryn King from the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA). This was preferred to other measures of light (Section 3.2.4) as there is a long term data set facilitating comparisons with future work.

The instantaneous environmental variables turf water content (WC; gH_2O gdw^{-1}), turf moss temperatures (MT; ^{o}C), and photosynthetically active radiation (used in Chapter 6; PAR; µmol m^{-2} s^{-1}) were collected at the same time as the samples. These are presented in Section 2.2.1.1. For methods see Section 3.2.1 (WC, MT) and Section 6.2.1 (PAR).

5.2.3.1 Selection of environmental variables

Draftsman's plots were inspected to exclude collinearity (ρ>0.7). Subsequently, ten environmental variables were chosen *a priori* (see Table 5.1). These ten variables were used in univariate and multivariate analysis of pigment accumulation in this, and subsequent chapters.

Daily mean values were preferred over longer time scales (up to 10 days) which tended to become more intercorrelated, and more strongly associated with daylength. Generally, daily values correlated with, and thus were representative of, three day values, which is an appropriate time for pigment induction. Minimum and maximum air temperature did not correlate strongly (ρ<0.7) and were both included.

Light data were highly intercorrelated and five day weighted total UV radiation (weighted to human exposure; wUVR5) was chosen as it has strongest associations with pigments. This also correlated with unweighted 5 day total UV-radiation (TUVR5; ρ=0.83), which was used as a proxy for visible radiation (Dunn 2000). Where significant associations with wUVR5 were identified, these were also tested against TUVR5, in order to check the importance of the UV-B wavelengths to the relationship.

Table 5.1: Environmental variables selected *a priori* for analyses of pigment accumulation

Variable	Biological Relevance
Moss turf water content (WC; $gH_20\ gdw^{-1}$)	Water is especially crucial in poikilohydric mosses which are metabolically inactive when dry. A major limiting factor to growth in terrestrial Antarctic ecosystems.
Ozone column depth (DU)	Low ozone levels are associated with increased damaging UV-BR wavelengths
Five day wind run (WR5; km h^{-1})	High winds disturb moss boundary layers, increasing evaporative water loss, increasing cooling and affecting CO_2 gradients derived from microbial respiration.
Three measures of temperature (°C)	Important limiting factor in Antarctic habitats, affecting metabolic rates and frequency of freeze thaw events. Daily air temperature maxima (ATMAX) and minima (ATMIN) are included in addition to moss turf temperature at time of sampling (MT).
Daylength (h)	Important seasonal factor at high latitudes
Relative humidity (RH; %)	Affects rates of water uptake and loss in moss turves
5 day mean weighted UV radiation (wUVR5; W m^{-2})	Weighted to UV-BR wavelengths, this is an important factor driving the summer melt and photosynthesis and potentially limiting at low and high levels. Had strongest associations with pigment data.
5 day mean total UV radiation (TUVR5; W m^{-2})	This was used as a proxy for visible wavelengths (daily PAR dose; see Dunn, 2000) and compared to wUVR5 to test the importance of the UV-BR wavelengths

5.2.4 Statistical analysis

Physiological variables in this and the subsequent chapter were analysed for variation between species, environmental drivers, and inter-relationships between pigments as described below.

5.2.4.1 Differences between species

Physiological variables were compared between species using two-way ANOVAs and *post-hoc* Tukey's HSD tests (p=0.05). The main effects were species (*B. pseudotriquetrum, C. purpureus,* and *S. antarctici*) and collection dates (fixed

factor; early (9 November to 2 December 2002), mid (5 December 2002 to 4 January 2003) and late season (7 January to 1 February 2003)) and interaction terms. Transformations were performed where necessary to improve normality of residuals and homogeneity of variance, detected through Shapiro-Wilk and Cochran's C tests respectively.

5.2.4.2 Environmental drivers of pigments and relationships between physiological variables

Environmental drivers of pigment accumulation were investigated within species using two way ANCOVAs, with the factors, collection date (as above) and the environmental variable of interest (see Table 5.1), including interaction terms. Linear regressions were conducted over the whole season when the environmental variable was significant (n=21) and within each date category when the interaction was significant (n=7). If light was significant, weighted and unweighted UV radiation was substituted to test for a UV-BR effect.

Inter-relationships between physiological variables (ETR, Z/VAZ, Tchl, anthocyanin and UVAC concentrations) were investigated using three way ANCOVAs. The relationships between anthocyanins and ETR and Z/VAZ are presented in chapter 7. The main effects were collection date, species and the physiological variable of interest (ETR, Z/VAZ, Tchl, anthocyanin, UVAC) with all possible interactions included. If the physiological variable was significant, linear regressions were performed using data from all species and collection date categories (n=63), if the interaction between the physiological variable and collection date was significant linear regression was performed within each collection date category combining data from all three species (n=21). If the interaction between the physiological variable and species was significant linear regression was performed within each species using data from all collection date categories (n=21).

Assumptions were checked as per ANOVAs (see above). Additionally regressions were checked with Cook's D and Durbin-Watson tests. On occasion, Cook's D was

high for samples at the extremes of the range of the environmental variable where data was scarce, but these samples were retained. ANOVAs and the ANCOVAs were conducted in JMP (v. 9 SAS inc. Cary, NC, USA).

5.3 Results

5.3.1 Seasonal and interspecific variation in UV absorbing compounds and anthocyanins

UVAC concentrations (total, cell wall and intracellular), varied between species whilst anthocyanin concentrations did not (Table 5.2; Figure 5.3–5.7). Mean WUVACs were 30-40% higher in both *S. antarctici* (mean±sd = 11000±2500 $A_{280\text{-}315}$ gdw^{-1}) and *C. purpureus* (mean±sd = 12700±3400 $A_{280\text{-}315}$ gdw^{-1}) than in *B. pseudotriquetrum* (mean±sd = 8300±3300 $A_{280\text{-}315}$ gdw^{-1}; Tukey's HSD $p<0.05$; Figure 5.4).

Intracellular UVAC concentrations were four times higher in *B. pseudotriquetrum* (mean±sd = 16700±3600 $A_{280\text{-}315}$ gdw^{-1}) than in *S. antarctici* (mean±sd = 3500±1200 $A_{280\text{-}315}$ gdw^{-1}) and double that of *C. purpureus* (mean±sd = 7800±2250 $A_{280\text{-}315}$ gdw^{-1}; Tukey's HSD $p<0.05$; Table 5.2; Figure 5.5).

The total UV-B screening potential (cell wall + intracellular UVAC) in *B. pseudotriquetrum* (mean±sd = 25000±5000 $A_{280\text{-}315}$ gdw^{-1}), was 20% higher than *C. purpureus* (mean±sd = 20500±4200 $A_{280\text{-}315}$ gdw^{-1}) and 50% higher than *S. antarctici* (mean±sd = 13800±3100 $A_{280\text{-}315}$ gdw^{-1}; Tukey's HSD $p<0.05$; Table 5.2; Figure 5.6).

Table 5.2: Results of two-way ANOVAs for daily mean anthocyanin concentrations, cell wall, intracellular and total UV absorbing pigments for species (*Bryum pseudotriquetrum, Ceratodon purpureus and Schistidium antarctici*) and collection date (early; 9 November to 2 December 2002; mid; 5 December 2002 to 4 January 2003; late; 7 January to 1 February 2003). n=63

Pigments	Factors	DF	F Value	P value	Tukey's HSD p<0.05*-0.10**
Anthocyanins ($A_{\Delta 526}$ gdw^{-1})	Species	2	2.334	0.1066	
	Collection date (E,M, L)	2	7.896	**0.0010***	E<M *
	Collection date*species	4	1.330	0.2708	
	Error	54			
Cell Wall UV absorbing compounds ($A_{280-315}$ gdw^{-1})	Species	2	12.000	**<0.0001***	C,S>B*
	Collection date (E,M,L)	2	3.256	**0.0462***	E>ML **
	Collection date*species	4	1.253	0.2999	
	Error	54			
Intra UV absorbing compounds ($A_{280-315}$ gdw^{-1})	Species	2	141.446	**<0.0001***	B>C>S*
	Collection date (E,M,L)	2	0.0158	0.9843	
	Collection date*species	4	0.632	0.6414	
	Error	54			
Total UV absorbing compounds ($A_{280-315}$ gdw^{-1})	Species	2	41.288	**<.0001***	B>C>S*
	Collection date (E,M,L)	2	1.564	0.2186	
	Collection date*species	4	1.767	0.1490	
	Error	54			

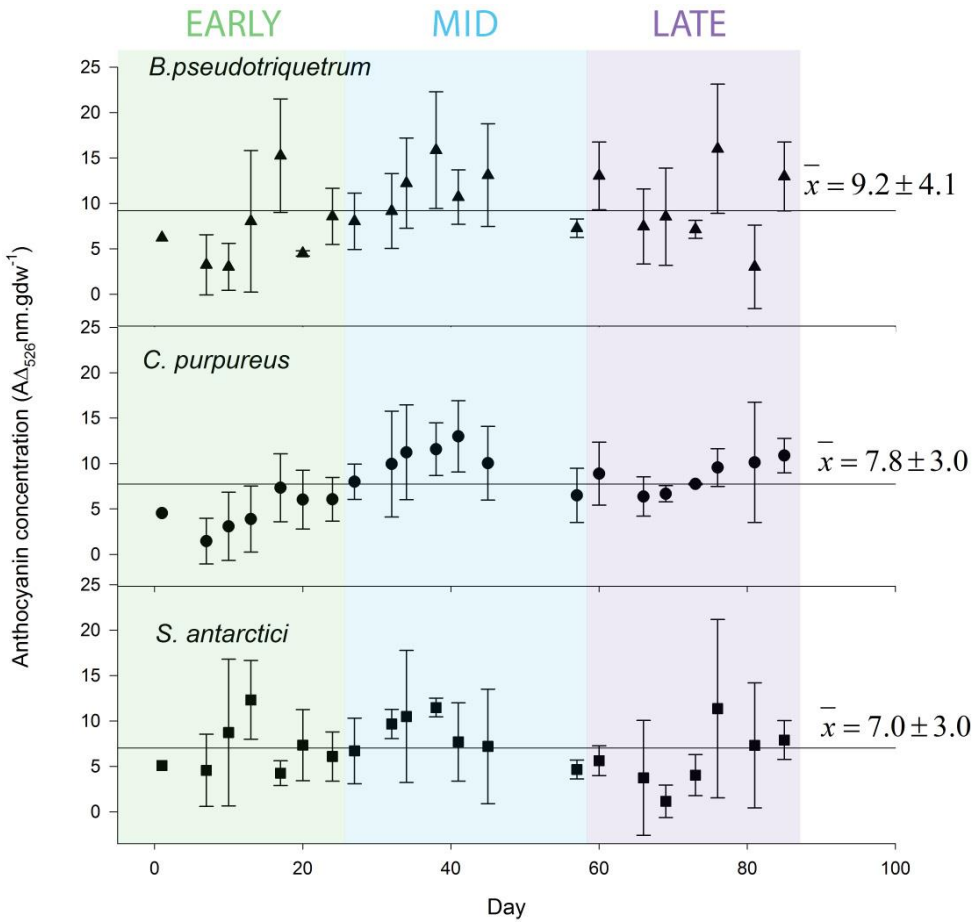


Figure 5.3: Variation in mean intracellular anthocyanin content in the three moss species *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* between 9 November 2002 and 1 February 2003. Data are means ± se, n=3 except for day 1 where n=1. The reference lines show the season mean ± sd. Early (grey), mid (aqua) and late season (violet) dates as per Table 5.2. Day 0 =8 November.

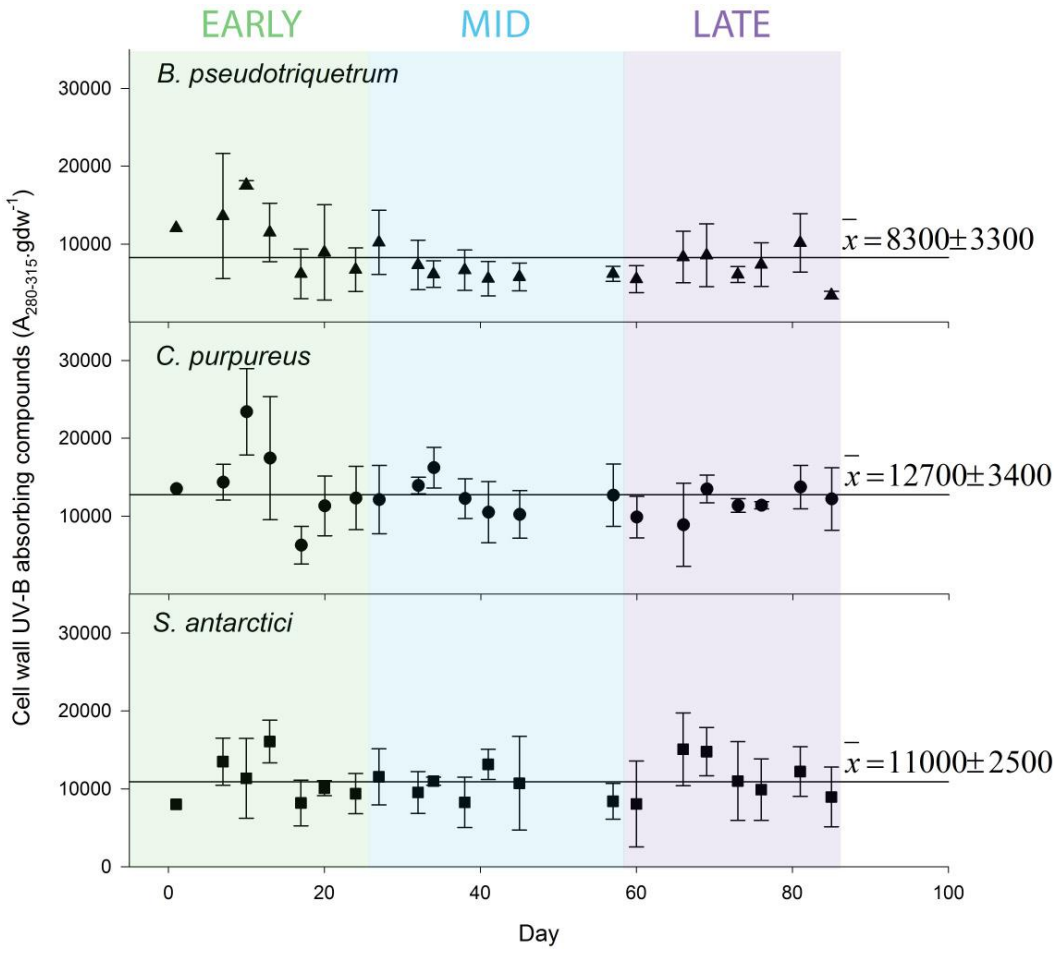


Figure 5.4: Variation in mean cell wall UV absorbing compounds in the three moss species *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* between 9 November 2002 and 1 February 2003. Data are means ± se, n=3 except for day 1 where n=1. The reference lines show the season mean ± sd. Early (grey), mid (aqua) and late season (violet) dates as per Table 5.2. Day 0 =8 November.

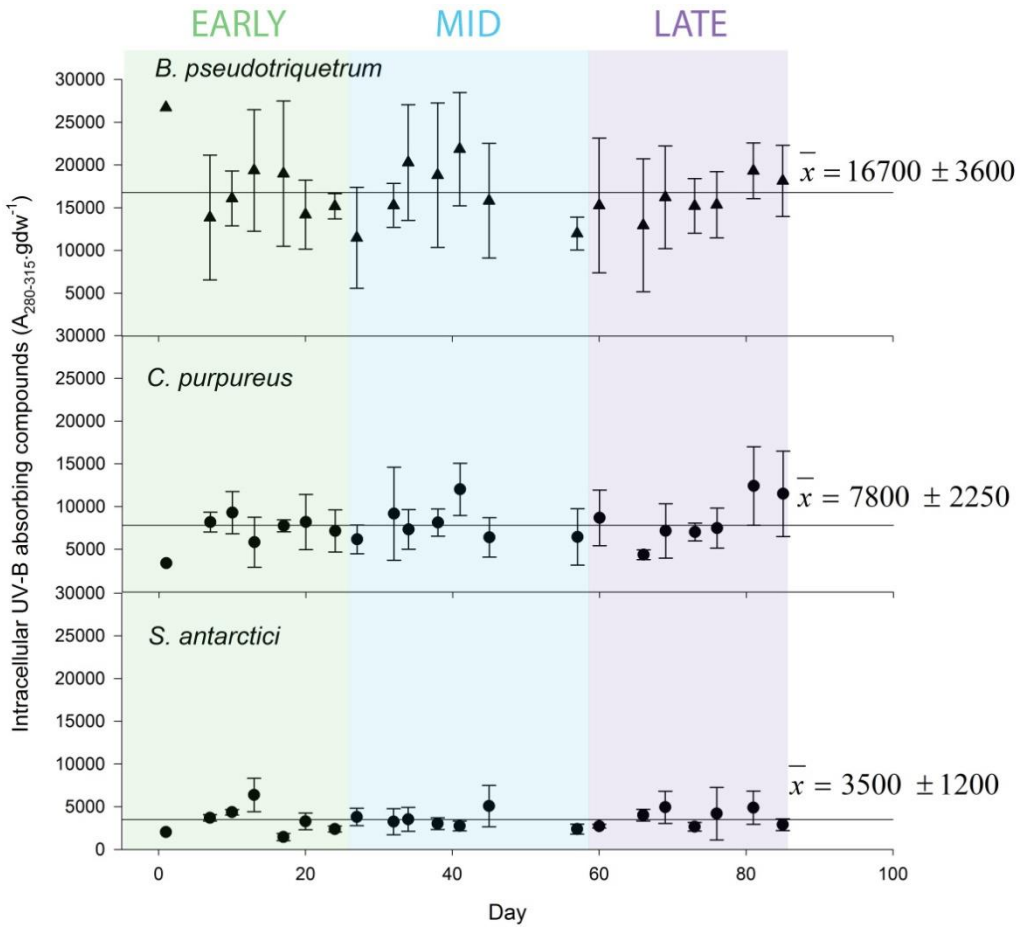


Figure 5.5: Variation in daily mean intracellular UV absorbing compounds in the three moss species *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* between 9 November 2002 and 1 February 2003. Data are means ± se, n=3 except for day 1 where n=1. The reference lines show the season mean ± sd. Early (grey), mid (aqua) and late season (violet) dates as per Table 5.2. Day 0 = 8 November.

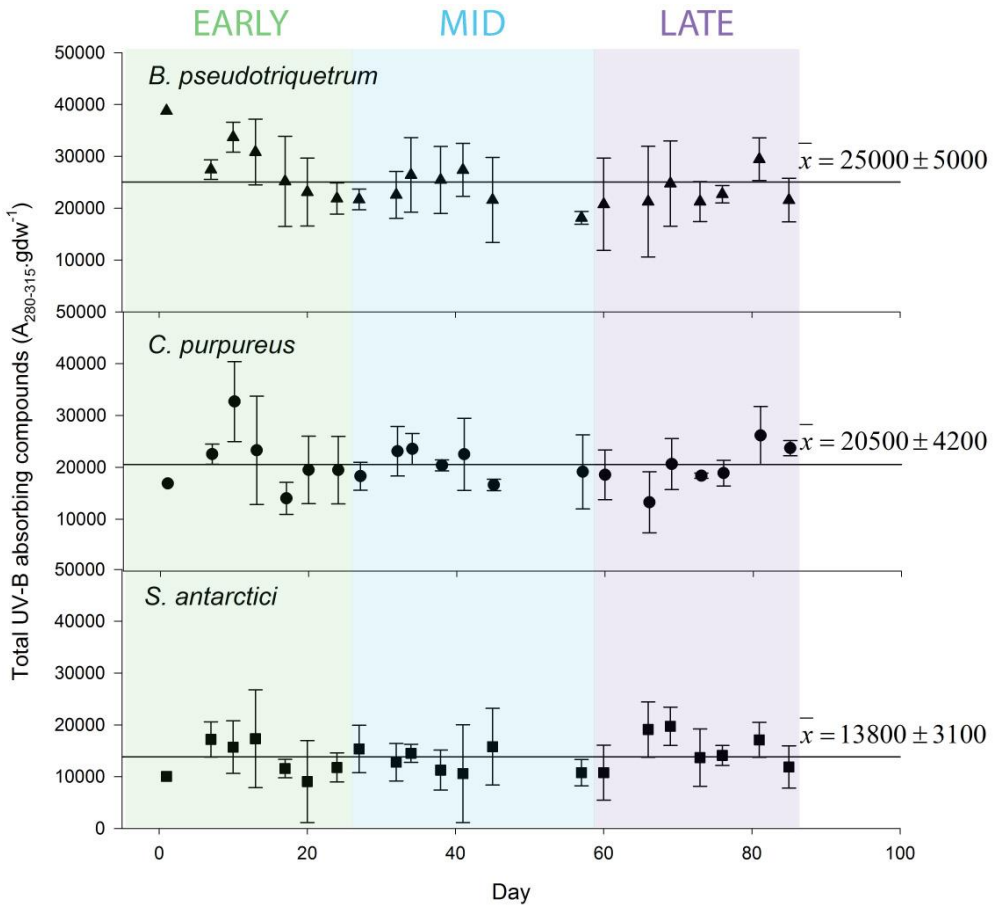


Figure 5.6: Variation in daily mean total UV absorbing compounds (intracellular +cell wall) in the three moss species *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* between 9 November 2002 and 1 February 2003. Data are means ± se, n=3 except for day 1 where n=1. The reference lines show the season mean and standard deviation. Early (grey), mid (aqua) and late season (violet) dates as per Table 5.2. Day 0 = 8 November.

Seasonal changes occurred in anthocyanin concentrations (Table 5.2; Figure 5.3; Figure 5.7 A). For all species anthocyanin concentrations were 40% higher in mid season than at the season's start, with intermediate concentrations in late season (Tukey's HSD $p<0.05$; Table 5.2; Figure 5.7 A). In all species WUVAC concentrations were 20% higher at the beginning of the season than at other times (Tukey's HSD $p<0.10$, Table 5.2; Figure 5.4; Figure 5.7 B). Seasonal variation did not occur in total UVAC nor iUVAC, but the latter was highly variable in *B. pseudotriquetrum* (Table 5.2; Figure 5.5; Figure 5.6).

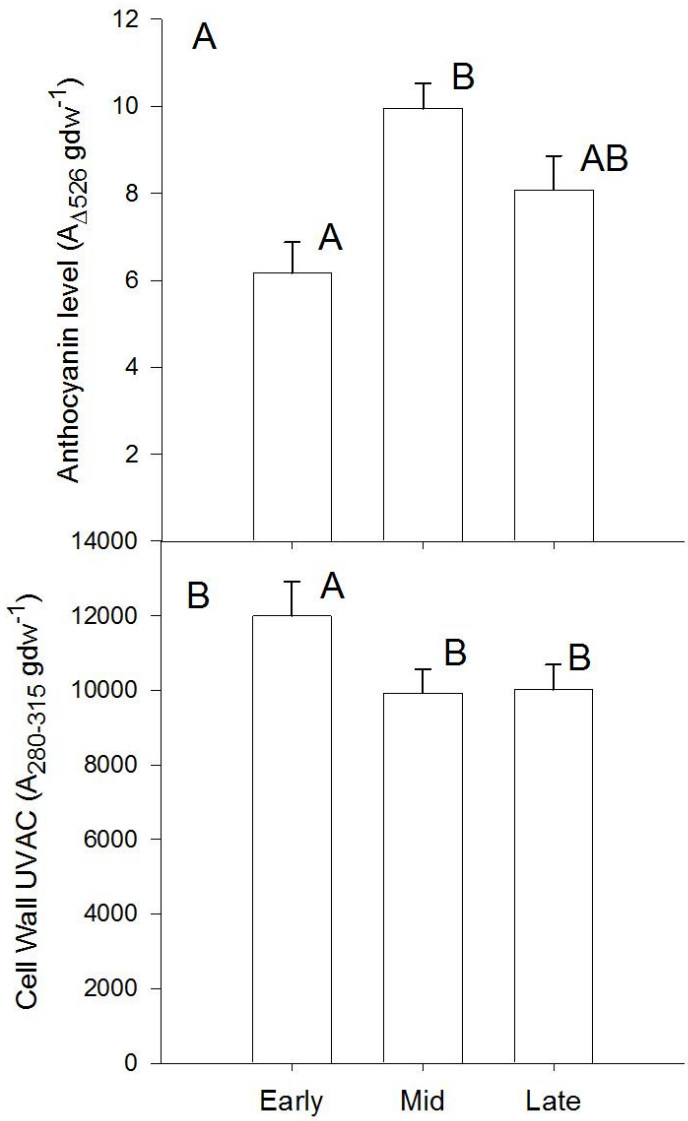


Figure 5.7: Seasonal variation in concentration of (A) anthocyanins and (B) cell wall UV absorbing compounds in early, mid and late season with data pooled from *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* (dates as per Table 5.2; mean ± se, n=21). Bars with different letters indicate significant differences when adjusted for multiple comparisons (Tukey's HSD: $p<0.05$, in (A) in (B) $p<0.10$).

5.3.2 Environmental drivers of UV absorbing compounds

Light and air temperature had a strong influence on UVACs, including anthocyanins. Minimum air temperatures (ATMIN) had stronger associations than moss turf temperatures, daily mean and maximum temperatures, which had looser yet significant associations (data not shown). There were seasonal changes in the relationship between pigments and ATMIN with shifts occurring at ~ -4 °C.

Throughout the low temperatures at the start of the season, anthocyanin accumulation increased with temperature up to about ATmin ~-4 °C, in both *B. pseudotriquetrum* and *C. purpureus* but this relationship dissipated when temperatures remained above ATmin ~-4 °C (Table 5.3; Figure 5.8 A,B). Above this threshold temperature anthocyanin accumulation generally showed no significant response except in the case of *B. pseudotriquetrum* where there was a decrease in anthocyanins with temperature during mid-season (Figure 5.8 A).

In *C. purpureus,* anthocyanin concentration was positively correlated with weighted UV radiation and negatively with ozone column depth (Table 5.3; Figure 5.8 E,F). Early and late in the season high anthocyanin concentrations were associated with low ozone column depth (high UV-BR), but there was no relationship with small changes at thicker ozone column depth in mid season (Figure 5.8 E,F). In *C. purpureus* anthocyanins accumulated with increasing five day mean UV radiation weighted to the UV-BR wavelengths, but had no relationship with unweighted five day mean total UV Radiation (TUVR5; Table 5.3; Figure 5.8 F).

Cell wall UVAC decreased with ATMIN in both *B. pseudotriquetrum* and *C. purpureus,* but only in early season (Table 5.3; Figure 5.8 C,D). This decrease is in the opposite direction to the increases in anthocyanin concentrations (c.f Figure 5.8 A,B, and C,D). In mid and late season, ATMIN is usually above ~-4 °C and WUVAC remained low (Figure 5.8 C,D). In *S. antarctici,* UVAC, including anthocyanins had no associations with the tested environmental variables. These variables also did not explain the accumulation of iUVAC in any species.

Table 5.3: Significant ANCOVA results for daily mean anthocyanin and cell wall UV absorbing compounds in *B. pseudotriquetrum* (B) and *C .purpureus* (C) in response to environmental variables (daily minimum air temperature; ATMIN, ozone column depth; ozone, weighted five day mean UV radiation; wUVR5, five day mean total UV radiation; TUVR5) and collection date; early, mid, and late (E, M, L; dates as per Table 5.2). n=21

Pigments	Climatic variable	Sp	R^2	Effects DF $_{(2,1,2)}$	F value	P value
Anthocyanins ($A_{\Delta 526}.gdw^{-1}$)	ATMIN (°C)	B	0.53	Collection date (E,M,L)	0.1497	0.8623
				ATMIN	0.6742	0.4244
				Collection date*ATMIN	5.7732	**0.0138***
		C	0.75	Collection date (E,M,L)	5.5463	**0.0157***
				ATMIN	0.0843	0.7755
				Collection date*ATMIN	3.9729	**0.0412***
	Ozone (DU)	C	0.79	Collection date (E,M,L)	4.4684	**0.0300***
				Ozone	0.0891	0.7694
				Collection date*Ozone	3.2043	**0.0694****
	wUVR5 ($W\ m^{-2}$)	C	0.72	Collection date (E,M,L)	4.4980	**0.0295***
				wUVR5	5.5016	**0.0332***
				Collection date*wUVR5	0.2086	0.8141
	TUVR5 ($W\ m^{-2}$)	C	0.66	Collection date (E,M,L)	7.1995	**0.0064***
				TUVR5	2.0258	0.1751
				Collection date*wUVR5	0.3762	0.6928
Cell wall UV absorbing compounds ($A_{280-315}\ gdw^{-1}$)	ATMIN (°C)	B	0.74	Collection date (E,M,L)	0.4917	0.6211
				ATMIN	0.4561	0.5097
				Collection date*ATMIN	8.4435	**0.0035***
		C	0.69	Collection date (E,M,L)	3.4453	**0.0587****
				ATMIN	2.9539	0.1062
				Collection date*ATMIN	9.4753	**0.0022***

*p<0.05, **p<0.10.

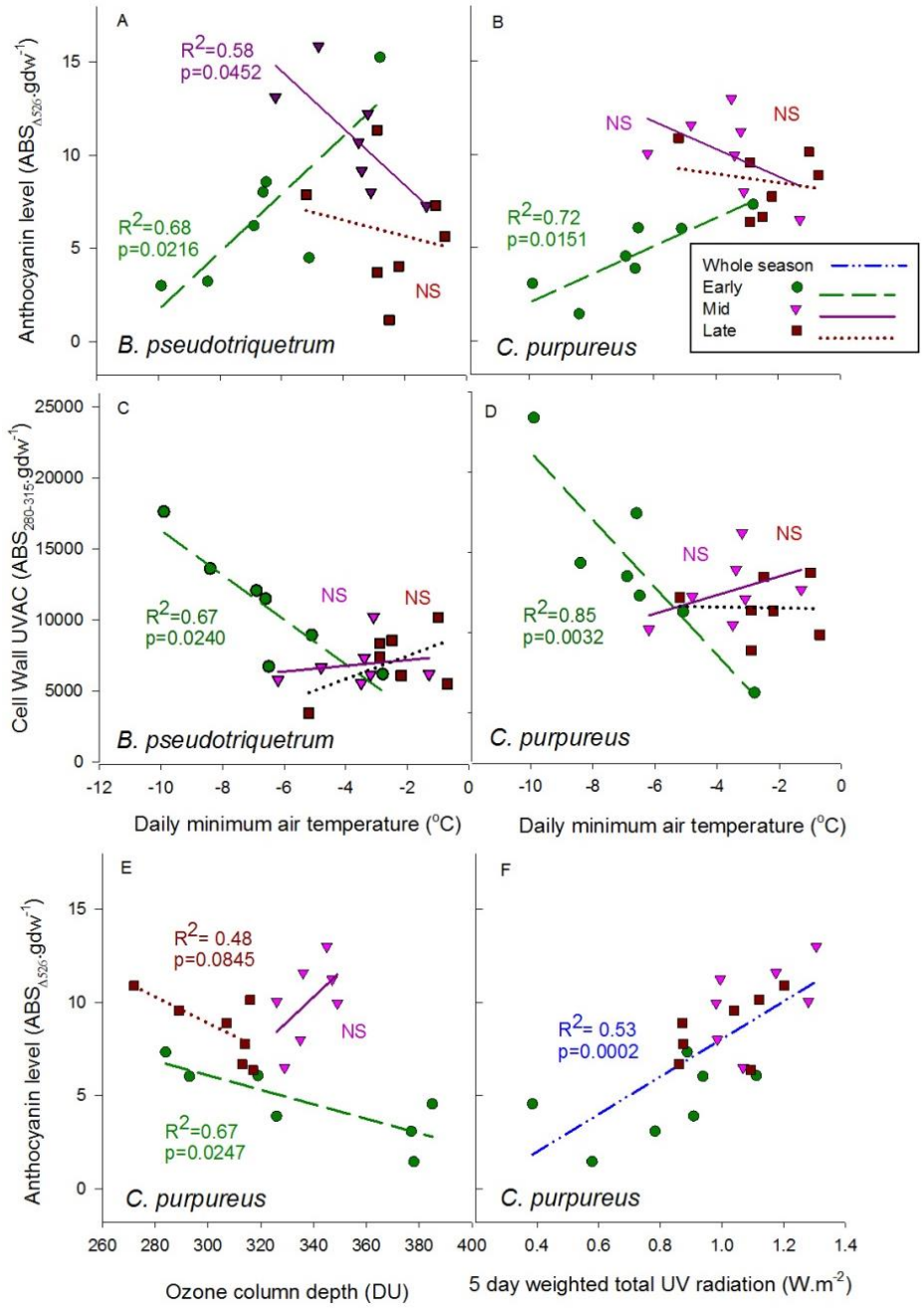


Figure 5.8: ANCOVA results for anthocyanin concentrations (A,B,E,F) and cell wall UVAC (C,D) in early, mid and late season (dates as per Table 5.2). Correlations with daily minimum air temperature in (A) *B. pseudotriquetrum* (early; Anth = 17.21+1.55*ATMIN, n=7 and late; Anth=5.34-1.53*ATMIN, n=7), in (B) *C. purpureus* (early; Anth = 9.61+0.75*ATMIN, n=7), in (C) *B. pseudotriquetrum* (early; WUVAC = 552-1570*ATMIN, n=7) and in (D) *C. purpureus* (early; $\log_{10}$WUVAC = 3.65-0.07*ATMIN, n=7) and in *C. purpureus* between (E) ozone column depth (early : Anth = 17.7-0.04*OZ, n=7) and (late; Anth = 29.9-0.07*OZ, n=7) and (F) five day weighted UV radiation over the whole season (Anth = -2.06+10.08 *wUVR5, n=21). Statistics are cited for significant linear regressions ($p<0.10$), non-significant regression are labelled NS. Data are daily means (n=3) except for sample 1 where n=1.

5.3.3 Relationships between pigments

Anthocyanin concentrations had an inverse linear association with WUVAC in *B. pseudotriquetrum* (Table 5.4; Figure 5.9 A). *Ceratodon purpureus* had a similar trend with a weaker, insignificant association between anthocyanin concentrations and WUVACs but there was no relationship between these pigments in *S. antarctici* (Table 5.4; Figure 5.9 B,C).

Table 5.4**:** Relationships between anthocyanins ($A_{\Delta 526}$ gdw^{-1}) and cell wall UV absorbing compounds (WUVAC; $A_{280-315}$ gdw^{-1}) showing significant results from three way ANCOVAs where the main effects were WUVAC, collection date (early, mid, late as per Table 5.2) and species; *Bryum pseudotriquetrum* (B), *Ceratodon purpureus* (C) and *Schistidium antarctici* (S).

Pigments	R^2	Effects Df ($_{2,1,2}$)	F value	P value
Anthocyanins and WUVAC	0.57	WUVAC	4.1100	**0.0486***
		Collection date (E,M,L)	2.0153	0.1451
		Species (B,C,S)	0.4379	0.6481
		Collection date*WUVAC	0.8378	0.4393
		Sp*WUVAC	3.3640	**0.0435***
		Collection date*Sp	0.9120	0.4652
		Collection date*Sp*WUVAC	1.5359	0.2080

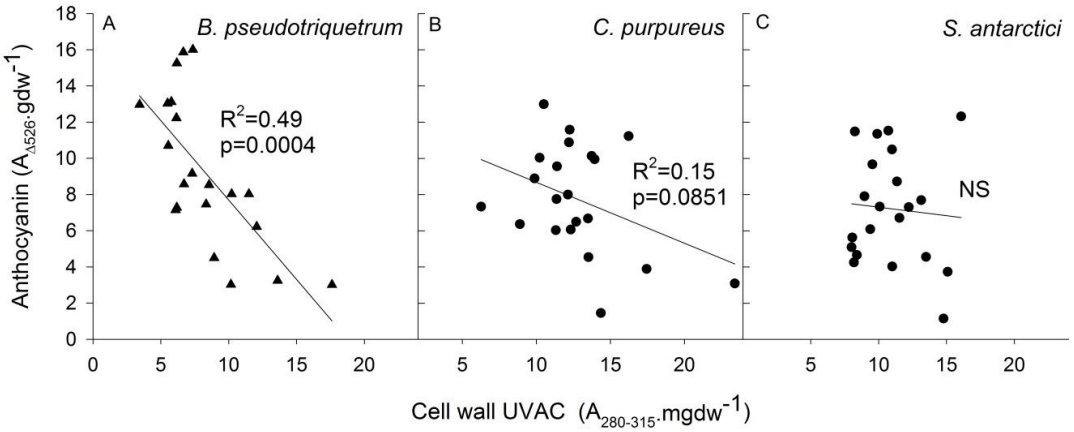


Figure 5.9**:** Relationship between anthocyanins and cell wall UVAC concentrations in (A) *Bryum pseudotriquetrum* (Anthocyanins = 16.5 - 0. 9*WUVAC,), (B) *Ceratodon purpureus* (Anth = 12.0 - 0.3*WUVAC) and (C) *Schistidium antarctici* (non-significant; NS) n=21. Data are daily means (n=3) except for sample 1 where n=1.

5.4 Discussion

The major environmental variables driving changes in moss UVACs are air temperature and solar radiation. Interestingly temperature was a stronger determinant than light in this season, possibly due to it being a smaller than average ozone hole. Despite the low UV-BR, relationships were found between anthocyanin concentrations and temperature and light, particularly UV-BR wavelengths. Cell wall and intracellular UVAC are unaffected by light, but WUVACs are reduced in warmer temperatures, probably as new growth occurs. Air temperature had the strongest influence on the pigments in this study, especially WUVACs and anthocyanins. Probably this is due to the influence of air temperature on the availability of melt water; an ATMIN of ~ -4 °C appears critical for pigment accumulation and may reflect overnight freeze thaw events. Liquid water is considered the most important factor determining metabolic activity in Antarctic ecosystems (Kennedy 1993a; Schlensog et al. 2013; Convey et al. 2014). The instantaneous measure of water availability (WC) does not indicate the phase state of water prior to sampling and thus may be less informative. Additionally, water was rarely limiting throughout the season due to the anomalous and widespread melt.

Cell wall UVACs did not accumulate in UV-BR. These compounds decreased from high early season values as temperature increased, and this may be due to modifications of the cell wall during active growth in warmer temperatures (Figure 5.4; Figure 5.7). Anthocyanin accumulation is inversely proportional to WUVACs, suggesting anthocyanins may have a protective function during growth. Pigments changed most in early season, presumably due to the major shift from winter dormancy to summer activity. The three species have different photoprotective strategies, with differences in the abundance and location of UVAC (cell wall and intracellular).

5.4.1 Environmental drivers

5.4.1.1 UV Absorbing Compounds

Light levels had no associations with iUVACs, WUVACs nor total UVACs in this season. This is unexpected as meta-analysis finds increased iUVACs are the most common plant response to elevated UV-BR (Newsham and Robinson 2009). This lack of response could be due to the atypically low ozone depletion in 2002/03 (Varotsos 2004), maintaining UV-BR levels below the threshold for UVAC induction. Previously, in a comparative Windmill Islands study, *B. pseudotriquetrum* did accumulate iUVAC in response to UV-BR while *S. antarctici* and *C. purpureus* did not (Dunn and Robinson 2006). This capacity for UV-BR induction of iUVAC in *B. pseudotriquetrum* has been confirmed by other researchers (Nunez-Olivera et al. 2010; Fabon et al. 2012a) and the much higher variation in iUVAC in this species (Figure 5.5) may be driven by differences in the UV-BR exposure at sampling microsites.

Changes in WUVACs in response to UV-BR enhancement has been reported for vascular plants in the field (Semerdjieva et al. 2003). In a laboratory UV-BR challenge (1.21 W m^{-2}), WUVACs increased in a leafy liverwort (Fabon et al. 2010) but not in the mosses *B. pseudotriquetrum* and *Fontinalis antipyretica* (Fabon et al. 2012a). Hespanhol and co-workers (2014) surveyed 23 species of alpine mosses and while bulk WUVAC concentrations were consistent, *p*-coumaric acid isolated from the cell wall of *Andreaea spp.* increased with altitude (and higher UV-BR). Different compounds may vary in their response to UV-BR and bulk extractions may not distinguish relative changes in compounds (Jaakala and Hohtola 2010). Further investigation, including characterisation of individual compounds, will be required to determine if specific WUVACs are UV-BR responsive in some bryophyte species and not others (as is the case with iUVAC) or if WUVAC accumulation is related to other factors. The UV dose response and relative timing of the UV signal and response, will need to be resolved before these compounds can be accurately used as fine temporal scale biomonitors (Bornman et al. 2015).

In this study, WUVAC decreased as air temperature warmed in both *C. purpureus* and *B. pseudotriquetrum*. This may be due to active growth in warm temperatures. Compounds secreted to the cell wall, such as flavonoids and hydroxycinnamic acids, are likely to remain for the life of the leaf. Therefore reductions in WUVAC should only be observed as new cell walls are formed during active growth and existing compounds are initially diluted. While leaf age has not been reported as a factor for WUVAC accumulation in bryophytes, WUVAC increased with leaf age in Norway Spruce (Strack et al. 1988). This study reports a transient intracellular accumulation of the flavonol glycoside kaempferol-3-O-glycoside, during leaf development, and prior to secretion to the cell wall. Furthermore, photoprotective epicuticular wax in the vascular CAM plant *Cotyledon orbiculata* accumulates only at leaf maturation, with reduced UV screening from wax in expanding leaves and a simultaneous transient increase in anthocyanins (Robinson et al. 1993; Barker et al. 1997). Decreased WUVACs during cell division and expansion in juvenile moss leaflets may similarly reduce UV-BR screening potential.

Active growth increases the proportion of expanding leaves in the sample and may reduce the overall WUVAC concentration. In mosses, new leaves usually grow from the apical cell (Figure 5.10). Mitosis is followed by cytokinesis, cell expansion, and then maturation. At cytokinesis, the cross links between cell wall fibres are broken to facilitate cell expansion. During expansion, new cell walls are created as additional cellulose is synthesised *in situ*. As the cell matures, compounds designated for the cell wall are produced in the cytosol. This includes major cell wall components such as pectin and hemicellose and minor components, for example, the UVACs vanillin, ferrulic acid and *p*-coumaric acid. The latter two compounds have recently been detected in cell walls of *C. purpureus* (Waterman 2015) and are possible candidates for accumulation in response to environmental stresses, such as UV-BR.

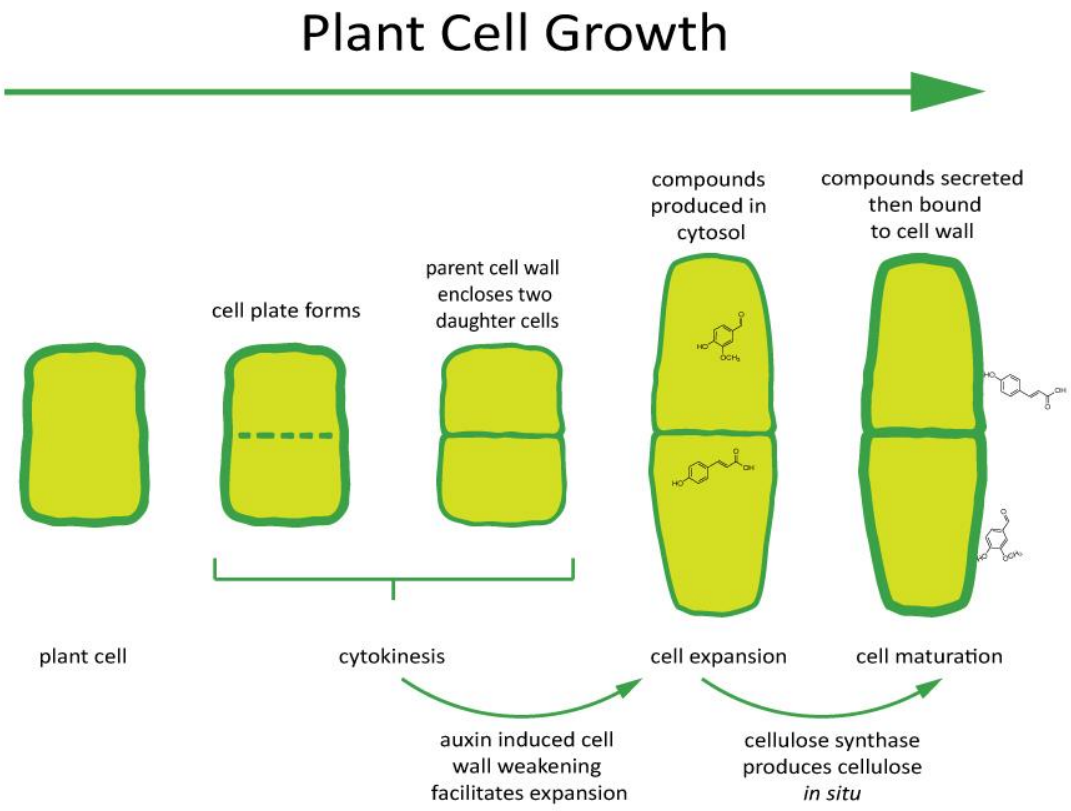


Figure 5.10: Putative changes in plant cell wall UV absorbing compounds during cell division and expansion in actively growing cells. See text for details.

Higher concentrations of WUVAC, at the start of the season, could result from the previous season's growth having been induced under higher UV-BR levels. Or high WUVAC concentrations may simply relate to cell maturity. Future studies should consider cell age as well as the prior UV-BR history of samples. Cell wall UVACs may appear to be constitutively high, if samples are at maximal levels at the start of an experiment (Martinez-Abaigar et al. 2009; Fabon et al. 2012a). Since UV-BR levels in this season were low, and I could not collect samples at the very end of the season, I cannot conclude whether WUVACs require elevated UV-BR for production in mature cells.

5.4.1.2 Anthocyanins

Anthocyanins accumulated with increasing air temperature in both *C. purpureus* and *B. pseudotriquetrum* and additionally on exposure to UV-BR wavelengths in

C. purpureus (Figure 5.8 A,B). It is important to note that *C. purpureus* has lower chlorophyll concentrations than the other species (Table 6.2; Figure 6.4) so whilst all species had similar anthocyanin concentrations; *C. purpureus* will therefore have higher anthocyanins on a chlorophyll basis. This species was also the only species where high anthocyanin concentrations were significantly associated with reduced requirement for dissipating excess light through Z (see synthesis; Figure 7.3). Anthocyanins may have a particular role in UV-BR protection in this species which frequently has red anthocyanic colouration in Antarctica (Post 1990).

All species had similar anthocyanin concentrations which was unexpected since only C. *purpureus* has characteristic anthocyanic colouration. Anthocyanin concentrations peaked mid-season at the summer solstice for all species, coinciding with maximal day length and solar elevation (Figure 5.7A). After the solstice, concentrations reduced non-significantly, but it is likely that anthocyanin levels would have decreased further if sampling had continued later in the season (Figure 5.3). After snow emergence the Antarctic liverwort *C. varians,* displayed a similar pattern of anthocyanin increase, combined with chlorophyll decrease both attributed to UV-BR (Newsham et al. 2005). This mid-season peak was not evident in an earlier study of the same three species in the Windmill Islands (Dunn and Robinson 2006). Possibly, anthocyanins may have already accumulated in the higher ozone depletion and later start for sample collection. However, in that study anthocyanins responded to ozone column depth and UV-B/Total solar radiation in multiple regression models of *C. purpureus* and *B. pseudotriquetrum* respectively (Dunn and Robinson, 2006).

In this study, anthocyanin accumulation was driven by solar radiation, only in *C. purpureus*. This relationship was only significant for UV radiation weighted to UV-B wavelengths and not for unweighted UV radiation (Table 5.3; Figure 5.8 F). This suggests it is driven by UV-BR wavelengths, rather than high PAR generally. UV-BR induction is also indicated by the correlation between anthocyanin concentrations and ozone column depth in early and mid season (Figure 5.8 E). Accumulation of

anthocyanins in response to UV-BR occurs in other Antarctic bryophytes (Newsham et al. 2005; Snell et al. 2009) and is well documented for vascular plants (Gould and Lister 2006 and references therein). Anthocyanins may thus have a role in protection of some bryophytes against damage from UV-BR.

Anthocyanin accumulation in *B. pseudotriquetrum* and *C. purpureus* was also driven by warmth (Figure 5.8 A,B), in contrast WUVACs decreased as air temperatures increased (Figure 5.8 C,D). There are several possible explanations for this. The capacity for production of these pigments may increase in good growing conditions (Belnap et al. 2008). This is supported by the increase in anthocyanin concentrations as ETR increases in mid season (see synthesis; Figure 7.2). Alternatively, solar radiation may be driving both anthocyanin accumulation and warming temperatures, so the latter is a confounding factor. Or, anthocyanins may accumulate transiently during periods of active growth. Anthocyanins have previously been suggested to screen visible light to protect the immature photosynthetic apparatus during leaf development (Robinson and Russell 1998; Steyn et al. 2002), and I suggest this may be particularly important if WUVACs are also reduced during leaf development (see above).

Supporting the latter argument, the degree of correlation between WUVACs and anthocyanin concentrations relates to the relative growth rates of the three species (Clarke et al. 2012). During the early part of the season, rising air temperatures drive opposite responses in the accumulation of WUVACs and anthocyanins. This correlation is significant, but only in *B. pseudotriquetrum*. Intriguing, as this species has the lowest WUVAC concentrations. However, it grows faster than the other two species (Clarke et al. 2012; Bramley-Alves 2015). In *C. purpureus,* which has intermediate growth rates there is a non-significant trend of anthocyanin increase and WUVAC decrease, and no trend in the slow growing *S. antarctici* (Figure 5.9). Rapid growth appears to strengthen the relationship between WUVACs and anthocyanins.

Juvenile moss leaflets may transiently increase anthocyanins as interim protection during the expansion and maturation process. This occurs in some vascular species (e.g. Lee et al. 1987; Tuohy and Choinski 1990; Barker et al. 1997; Ranjan et al. 2014). The role of anthocyanins in developing leaves is debated, but they may protect against excess light, as the photosynthetic and photoprotective capacity develops (Gould and Lister 2006; Hughes et al. 2007; Liu et al. 2009). Anthocyanin protection may be even more crucial if UV-BR screening from WUVAC is also undeveloped. To my knowledge, no studies have simultaneously investigated the accumulation of WUVACs and anthocyanins. To test this hypothesis a broad range of species that possess anthocyanic juvenile leaves should be examined.

Several studies report anthocyanin or anthocyanin like pigments to be associated histologically with the cell wall (Post and Vesk 1992; Hooijmaijers and Gould 2007; Snell et al. 2009; Newsham 2010). This is the optimal location for screening UV-BR, but an antioxidant or immunological role is not discounted in the complex matrix of the cell wall. In two New Zealand liverworts, these pigments could not be extracted from the cell wall (Hooijmaijers and Gould 2007), but in other species of liverworts from Antarctica, anthocyanins have been extracted by acidified methanol extractions (Post and Vesk 1992; Snell et al. 2009; Newsham 2010). This extraction process generally does not extract UVAC tightly bound to the cell wall (Clarke and Robinson 2008). These anthocyanins may be loosely bound to the cell wall, or are possibly extracted from the cytosol where they accumulate prior to secretion to the cell wall, such as occurs in cell wall UVACs (Figure 5.10; Strack et al. 1988). Further research to identify individual anthocyanins and characterise the properties and cellular location will be required to understand the role of anthocyanins in Antarctic moss. Unlike anthocyanins concentrations, which were similar for all species, UVACs varied in abundance and location.

Bryum pseudotriquetrum had the highest UV-BR screening potential (total UVAC =WUVAC+ iUVAC) and *S. antarctici* the lowest, with *C. purpureus* intermediate (Table 5.2; Figure 5.6), broadly consistent with the degree of DNA damage under

UV-BR (Chapter 4). In contrast, Clarke et al. (2008) found similarly high total UVAC in *B. pseudotriquetrum* and *C. purpureus*. This is likely due to the smaller sample size in that study, combined with the high variability in iUVAC concentrations in *B. pseudotriquetrum* across the season.

5.5 Conclusions

Anthocyanins are induced by UV-BR only in *C. purpureus*, however all species appear to utilise this form of protection, which peaks in mid-summer. WUVACs decrease with warmth and may reduce in actively growing cells and this factor should be considered in future experiments. Anthocyanins and WUVACs may play complementary protective roles, with a stronger relationship between anthocyanin and WUVAC concentrations in faster growing species. Since this season had atypical ozone behaviour and comparatively low UV-BR, it is unclear if WUVACs are regulated by UV-BR or constitutive.

The next chapter explores seasonal changes in photosynthesis and related pigments located within the chloroplast. Photosynthesis can be reduced by UV-BR and excess light but also provides the metabolic resources required for synthesis of protective compounds. Differences between species in optimal conditions for photosynthesis may lead to changes in community structure under anthropogenic climate change.

6 Photosynthesis and climate change in Antarctic moss: potential winners and losers.

6.1 Introduction

Atmospheric pollution has led to substantial changes in Antarctic terrestrial ecosystems (Turner et al. 2014b; Robinson and Erickson 2015). Global warming, ozone depletion and their interaction, are associated with increased incident UV radiation, temperature, windspeed and altered precipitation patterns in continental Antarctica (Larsen et al. 2014; Williamson et al. 2014; Bornman et al. 2015; Robinson and Erickson 2015). Since the early 1980s, the mean noontime UV index has increased by 40% above Antarctica, which is attributed to ozone depletion. This should reverse by 2100 as ozone recovery proceeds from the mid 21st century (Andrady et al. 2015).

This recovery is due to swift and effective international agreement to reduce emissions of ozone depleting substances via the Montreal Protocol (1987; Williamson et al. 2014). Unfortunately, international consensus for reductions in greenhouse gas emissions has not been achieved. Current warming scenarios predict 1–4 ^{o}C increase in global mean surface temperatures by the end of the 21st century (Field et al. 2014). The intergovernmental panel on climate change recommends urgent international action to reduce greenhouse gases, but failing that, an improved scientific understanding of these changes (Larsen et al. 2014).

In the Windmill Islands, an abundance of moribund moss with encrusting lichen indicates a successional change in community structure (Melick and Seppelt 1997; Wasley et al. 2012). A regional drying trend has been identified (Hodgson et al. 2006; Clarke et al. 2012; Bramley-Alves 2015) and its impact may be exacerbated by other climate factors, such as increased UV-BR or temperature. Intra-specific differences in the photosynthetic response to environmental factors may also lead to changes in species abundance within the bryophyte community (Wasley et al. 2012). Such changes in the abundance of particular moss species may be useful indicators of climatic change, but only if the environmental drivers of such changes

are understood. Long term monitoring of the health and composition of Windmill Island's plant communities forms Australia's State of the Environment Indicator 72 (http://aadc-maps.aad.gov.au/aadc/soe/dis-play_indicator.cfm?soe_id=72).

The inherent seasonal variability in photosynthesis and associated pigments is an important context for understanding the impact of a shifting climate on moss health. The capacity to maintain and optimise photosynthesis as environmental conditions vary is vital for plant health. However, the optimal environmental conditions for photosynthesis in Antarctic mosses are not known. The following section reviews the influence of water, temperature and light on photosynthesis in Antarctic mosses. The implications of differing techniques for measuring photosynthesis are also discussed.

Water is considered the prime driver of productivity in poikilohydric Antarctic vegetation. All three study species are desiccation tolerant (as reviewed in Proctor et al. 2007) but vary in relative desiccation tolerance (See Section 2.3; Robinson et al. 2000; Wasley et al. 2006b).

Generally, Antarctic plants from wetter habitats have increased productivity, growth, net photosynthesis, chlorophyll concentrations and broader temperature optima for photosynthesis (Kappen et al. 1989; Melick et al. 1994; Fowbert 1996). Photosynthesis drops sharply to zero as mosses desiccate (Wasley et al. 2006b). However, too much water can slow CO_2 diffusion into the moss leaflet and also reduce photosynthesis (see Section 1.3; Proctor et al. 1992). Of the study species, *Schistidium antarctici* and *Bryum pseudotriquetrum* are more tolerant of submergence than *Ceratodon purpureus* (Table 2.1; Wasley et al. 2006b). However, the optimal photosynthetic water contents are unclear. Maximum photosynthetic efficiency (dark adapted Fv/Fm) measured by chlorophyll fluorescence remains high at a broad range of water contents in these species (Robinson et al. 2000). For example, in *B. pseudotriquetrum* maximal Fv/Fm occurred between 4–11 gH_20 gdw^{-1} but only declined by 5% at 14 gH_20 gdw^{-1}. However, prolonged water saturation causes 40–85% reductions in effective quantum yield (Fv/Fm measured

in the light) in these moss species (Table 2.1; Rice and Cornelissen 2014). At the other end of the spectrum, complete desiccation can protect the photosynthetic apparatus against the damaging light components of sunlight (Chapter 4; Heber et al. 2006a).

Water has a buffering effect on moss temperature with a reduced diel range in wet environments (Melick and Seppelt 1994; Lewis Smith 1999). Absorption of solar radiation increases moss turf temperature (MT) above ambient air temperature (Bramley-Alves et al. 2014). Moss surface temperatures have been recorded as high as 42.8 ^{o}C (Lewis Smith 1988c), and whilst rare, extreme heat events may occur more frequently in the future (Meehl et al. 2012). In East Antarctica, summer field moss turf temperatures (MT) were above 0 ^{o}C for 80%, and above 10 ^{o}C for 25% of the 56 days of measurement, whilst air temperature remained below 0 ^{o}C for 61% of this time (Lewis Smith 1999). Generally, high temperatures occur for short periods of high insolation at noon, but extreme temperatures can cause lasting damage.

Temperature increases may improve productivity for moss communities in East Antarctica. However photosynthesis in Antarctic bryophytes appears well suited to cold temperatures. Positive rates of carbon gain are maintained, even at 0 ^{o}C and below (Kappen et al. 1989; Green et al. 2000; Nakatsubo 2002; Pannewitz et al. 2005). Low temperatures will limit metabolic activity and also increase the risk of tissue damage during freezing events (Lovelock et al. 1995a; Lovelock et al. 1995b). However, high temperatures can also limit carbon gain through photoinhibition, or due to different temperature response dynamics for respiration and photosynthesis (Takahashi and Badger 2011; Way and Yamori 2014).

In the maritime Antarctic, warmer temperatures attributed to global warming are associated with an expansion of vascular plant communities into areas of ice retreat (Convey and Smith 2006). This may also occur in East Antarctica. However, variation between species in the capacity to optimise and maintain photosynthesis in higher temperatures may affect community composition. For example, as growth cabinet

temperatures were increased from field conditions, the temperature optima of the Antarctic Maritime mosses *Polytrichum alpestre* increased from 5–10 ^{o}C to 15 ^{o}C, whilst *Drepanocladus uncinatus* remained at 15 ^{o}C throughout the experiment (Collins 1977). However, while optimal temperatures for photosynthesis in Antarctic mosses are considerably above ambient air temperature, there is wide variation in reported temperature optima for photosynthesis (Table 6.1).

Mid summer photosynthetically active radiation (PAR) is potentially damaging to Antarctic mosses. Polar species are most active in the intense sunlight that drives the summer melt. This is in contrast to other ecosystems, where mosses are frequently shade dwelling and precipitation dependent, becoming active after rain in diffuse light. In these mosses, metabolism ceases due to desiccation as sunlight intensifies (Marschall and Proctor 2004). In a two month summer period in Victoria Land, Antarctica the PAR reached 2000 µmol quanta m^{-2} s^{-1}, on ~67% of days (Pannewitz et al. 2005). This compares with an equatorial PAR of 2209 µmol quanta m^{-2} s^{-1}, during the equinox at solar noon (Ritchie 2010). Antarctic mosses have evolved under these conditions of potentially damaging strong sunlight.

Antarctic mosses appear to acclimate to light as vascular plants do. In full sunlight plants shift from investing in light harvesting pigments to photosynthetic processing (with increased photosynthetic enzymes eg. RuBisCO; Demmig-Adams 1998; Matsubara et al. 2012; Esteban et al. 2015). This is concomitant with increased photoprotection. Examples of this are increases in protective pigments within the chloroplast, or in non-assimilatory electron transport (eg. photorespiration; Demmig-Adams 1998; Matsubara et al. 2012; Esteban et al. 2015). Chloroplastic pigment concentrations can rapidly change (<24 hours) through synthesis or photo-oxidation destruction in abrupt high light (Newsham et al. 2002; Matsubara et al. 2012).

The undulating microtopography of Antarctic moss turves leads to considerable variation in solar exposure, so mosses are acclimated to a range of different

intensities from full shade (between rocks) to full sun (see Figure 5.1). The photosynthetic light response is different in moss from sun and shade habitats (Proctor and Smirnoff 2011). In the Antarctic moss species *C. purpureus,* chlorophyll concentrations decreased, while carotenoid concentrations and thylakoid stacking increased with solar exposure, as is typical for sun acclimation. However, bryophytes show less variation in the chla/b ratio between sun and shade samples (Post 1990; Post and Vesk 1992; Robinson et al. 2005). These pigments also vary between sites and seasonally, presumably in response to climatic conditions but the exact drivers of these changes are unknown (Lovelock and Robinson 2002; Robinson et al. 2005; Snell et al. 2007; Schroeter et al. 2012).

For at least two of my study species, photosynthetic temperature optima vary between years, sites and measurement techniques. For example, gas phase CO_2 measurements report temperature optima of 6.6 °C in *Ceratodon purpureus* and 12 °C in *Bryum pseudotriquetrum* (Pannewitz et al. 2005). However, aqueous phase O_2 evolution and chlorophyll fluorescence measurements of electron transport rates (ETRs) report higher optima (>20 °C) for both species (Lewis Smith 1999; McKinley 2012). Currently, comparisons between studies are hampered by differences in methodology and there is a lack of comprehensive research with reliable replication.

6.1.1 Different techniques for estimating photosynthesis

Measurements by gas exchange (O_2 or CO_2) and chlorophyll fluorescence give conflicting results for photosynthetic temperature responses in Antarctic mosses (Table 6.1). Higher photosynthetic temperature optima are reported for O_2 evolution and ETR measurements than for CO_2 uptake. The reported CO_2 uptake studies are also conducted in natural sunlight (containing UV-BR), but artificial light sources have been used for O_2 evolution and ETR studies. Differences may thus arise from the choice of measuring technique, and/ or from altered conditions between the laboratory and field. In the field elevated CO_2 from microbial respiration within moss turves may increase photosynthetic productivity. This is not

accounted for in most measurements. Additionally, full spectrum solar radiation may increase both photoinhibition and non-assimilatory electron transport, compared to artificial light sources.

Table 6.1: Photosynthetic temperature optima for photosynthesis, measured using gas exchange (CO_2 uptake or O_2 evolution) or chlorophyll fluorescence (ETR) in various Antarctic moss species. The light source during measurement is reported as sun+ (supplemented sunlight), sun (sunlight and filters) or artificial (usually LED or halogen sources). Table adapted from Bramley-Alves et al. (2014).

Species	Optima (ºC)	Method	Light source	Reference
Bryum subrotundifolium	13.7	CO_2	sun+	Pannewitz et al. (2005)
Bryum pseudotriquetrum	10–12	CO_2	sun+	Pannewitz et al. (2005),Ino (1990)
	≥20	O_2 †	artificial	Lewis Smith (1999)
	20	ETR*	artificial	McKinley (2012)
Bryum argenteum	15	CO_2	sun	Green et al.(2000)
	≥20	O_2 †	artificial	Lewis Smith (1999)
	25	O_2 **	artificial	Rastofer (1970)
Ceratodon purpureus	6.6	CO_2	sun+	Pannewitz et al. (2005)
	≥20	O_2 †	artificial	Lewis Smith (1999)
	20	ETR	artificial	McKinley (2012)
Schistidium antarctici	5–10	CO_2	sun	Kappen et al. (1989)
	20	ETR*	artificial	McKinley (2012)
Cephaloziella varians	≥20	O_2 **	artificial	Newsham (2010)
Sanionia uncinata	5–15	CO_2	sun	Nakatsubo (2002)

*ETR measured with mini-PAM with leaf clip attachment. **Aqueous phase †gas phase (5% CO_2)

Photoinhibition occurred in the continental Antarctic moss *Schistidium antarctici* in high temperatures under ambient sunlight (Kappen et al. 1989) but not in artificial light. This highlights the importance of outdoor measurements in the full solar spectrum (including UV-BR). Photosynthetic response curves can be attained in both gas exchange and fluorescence measurements under natural sunlight using filters. However this is logistically complex, particularly in Antarctica, and relies on clear sky days.

The constraint of working in Antarctic conditions typically limits the duration and extent of research. This makes it difficult to assess variability in photosynthesis and related pigments over the whole season. To determine the photosynthetic temperature response, optimal water and light conditions should be used. However, the time consuming nature of gas exchange measurements makes the optimal conditions hard to determine with sufficient replication for accuracy (Convey 1994).

In sub-optimal conditions, the peak photosynthetic rate in the temperature response curve will be limited by other factors eg. water, light or CO_2 concentrations. Nakatsubo (2002) presents a temperature response curve for net photosynthesis in the moss *Sanionia uncinata* from King George Island, Antarctica. This sample was identified as having particularly low photosynthetic rates and CO_2 uptake is clearly limited by other factors (Figure 6.1). For comparison, gross photosynthesis is calculated from the data in Figure 6.1 (net photosynthesis + dark respiration) and photosynthetic ETRs measured at a higher light level are shown (Ashcroft et al. 2015).

Gas exchange measurements seal the sample in a chamber and necessarily alter at least some field conditions, eg. light, water, temperature and CO_2 levels (eg. Pannewitz et al. 2005). Additionally, the photosynthetically active green tips are excised from the moss substrate to avoid microbial respiration (eg. Lewis Smith 1999). The higher photosynthetic temperature optima reported from O_2 evolution (Figure 6.1) may be due to the use of saturating available CO_2 in these studies. Aqueous phase O_2 evolution uses sodium bicarbonate solution, and other studies used 5% CO_2 (Lewis Smith 1999; Newsham 2010; Rastorfer 1970). This likely leads to higher photosynthetic rates compared to CO_2 uptake measurements, which are usually in ambient air with limiting CO_2 (350–380 ppm).

Such high CO_2 concentrations may, however be biologically relevant. Tarnawksi and co-workers (1992) found CO_2 concentration (ppm) in *Schistidium antarctici*

increased seasonally from 333±21 (mean±se, n=18) in November to 4229±747 in January. This only occurred in wet sites. This increase is attributed to temperature dependent respiration from heterotrophic microbial communities within moss substrata (Tarnawski et al. 1992). This likely has a strong impact on photosynthesis. In Antarctic *B. pseudotriquetrum* raising the $C0_2$ concentration to 2000 ppm more than doubled the rates of CO_2 uptake at 20 ^oC (Pannewitz et al. 2005), while in *S. antarctici* photosynthesis saturated at ~ 3000 ppm at 20 ^oC (Tarnawski et al. 1992). Thick boundary layers above moss turves may contribute to a strong CO_2 concentration gradient especially on low wind days.

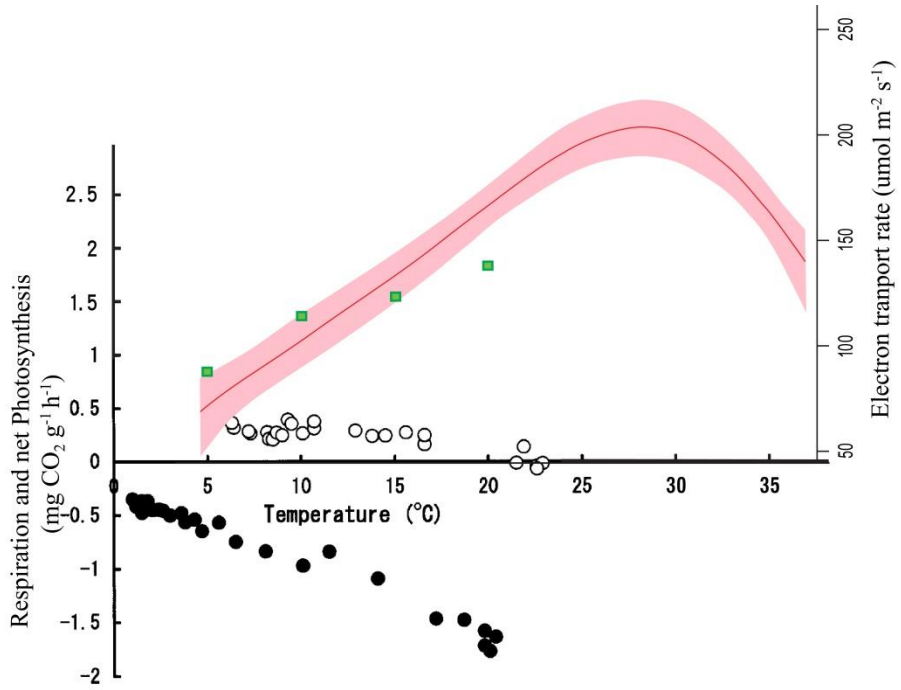


Figure 6.1: Different methods of measuring photosynthesis generate different temperature responses in *Sanionia uncinata* from King George Island. Gas exchange measurements of CO_2 show net photosynthesis (open circles) and respiration rates (closed circles) at 600 ± 50 µmol m^{-2} s^{-1} PPFD (both reproduced from Nakatsubo 2002). These were used to estimate gross photosynthesis (green squares). Photosynthetic electron transport rates (pink line) with 95% confidence limits (pink shading) measured at 1000 µmol photons m^{-2} s^{-1} PPFD in the laboratory at King George Island using a mini-PAM (Ashcroft et al. 2015). NB. The y axes represent different units and their relationship is not known.

As an alternative to gas exchange, chlorophyll fluorescence provides a rapid and noninvasive estimate of electron transport rates through Photosystem II (ETRs;

Maxwell and Johnson 2000). In contrast to gas exchange, chlorophyll fluorescence is typically measured using whole moss plugs, including substrata (or *in situ*). This may retain microbial CO_2, without confounding with the measurement of photosynthetic rates (Wasley et al. 2006b). Photosynthetic ETRs correlate with gas phase O_2 evolution, and both measures include some non-assimilatory electron transport (Genty et al. 1989; Takahashi and Badger 2011). This alters the basis of comparisons with photosynthetic CO_2 uptake but does not invalidate them.

Photosynthetic electron transport generated from water splitting at Photosystem II (forming O_2) provides ATP which drives carbon assimilation through the Calvin-Benson cycle. Additionally, non-assimilatory ETRs can dissipate excess light energy under conditions which limit CO_2 uptake by RuBisCO (e.g. low or high temperatures; Robinson and Waterman 2014). This protects the photosynthetic apparatus by reducing the production of reactive oxygen species. Such alternative electron sinks include photorespiration, the Mehler reaction and cyclic electron flow (Takahashi and Badger 2011). Non-assimilatory ETRs manifest as a lack of correlation between CO_2 uptake (which saturates at low light) and ETRs (saturates at higher light or does not saturate).

In high light measurements of ETRs and O_2 evolution it is unclear what proportion of electrons proceed to carbon fixation or to other non-assimilatory processes. In Antarctic mosses, high non-assimilatory ETRs are suggested. Electron transport rates did not saturate with increasing light in *Hennediella heimii* (sunlight; <2000 µmol quanta m^{-2} s^{-1}) or *Bryum argenteum* (<2000 µmol quanta m^{-2} s^{-1}) (Pannewitz et al. 2003; Schroeter et al. 2012). However, in the study species *C. purpureus*, *B. pseudotriquetrum* and *S. antarctici,* laboratory measurements found the chlorophyll fluorescence parameter ETRmax saturated between 1200 and 1500 µmol quanta m^{-2} s^{-1}, (halogen light source; McKinley 2012). These conflicting results may be due to real differences between these separate species, or to altered conditions between the laboratory and field, such as the light source and CO_2

availability. These differences could also be generated by differences between sun and shade grown samples which respond differently to high light.

The capacity for non-assimilatory electron transport increases with exposure to high light (Takahashi and Badger 2011). In European mosses, Proctor and Smirnoff (2011) report non-assimilatory ETRs only in sun adapted moss species. Carbon dioxide uptake in both sun and shade species saturated at low light levels (< 500 µmol m^{-2} s^{-1}), as did ETR in *Plagiomnium undulatum*, usually found in deep shade. However, in *Schistidium apocarpum,* which typically grows in high solar exposure, chlorophyll fluorescence measurements of ETR did not saturate in high light (< 1000 µmol m^{-2} s^{-1}). When the experiment was repeated in CO_2 free atmosphere, *S. apocarpum* maintained ETRs, using O_2 as the only electron sink. The authors propose a significant role for oxygen photoreduction, suggesting the Mehler-peroxidase reaction. Thus the prior growing conditions of the sample may influence the capacity for protective, non assimilatory electron transport. This emphasises the importance of adequate replication and particular attention to the growing conditions of samples used.

Adding to this complexity, some gas exchange studies suggest Antarctic moss may continue to fix carbon at higher light intensities. Pannewitz and co-workers (2005) found CO_2 uptake failed to saturate (< 1300 µmol quanta m^{-2} s^{-1}) in three continental Antarctic mosses. These plants are adapted to function in high light, and it is possible, especially in elevated ambient CO_2, that Antarctic mosses continue CO_2 uptake at higher light levels than reported elsewhere.

6.1.2 Carotenoids

Protective carotenoids, including the VAZ cycle (violaxanthin + antheraxanthin + zeaxanthin) and β-carotene, commonly increase in response to high photosynthetically active radiation (PAR; Esteban et al. 2015), and in Antarctic plants to elevated UV-BR (Ruhland and Day 2001; Newsham et al. 2002; Newsham

2003; Robinson et al. 2005). They are thus useful in comparing the response of different species to climatic stressors.

The role of xanthophylls in protecting chloroplasts from excess light has received much attention (reviewed in Ruban and Johnson 2010; Jahns and Holzwarth 2012), particularly the role of zeaxanthin (Z) in non-photochemical quenching (NPQ; see section 1.4.2.1). Xanthophyll cycle pigments and β-carotene can also function as antioxidants by directly quenching excited chlorophyll or scavenging free radicals formed by high light and have important roles in membrane stability (Beddard et al. 1977; Havaux et al. 2007; Jahns and Holzwarth 2012; Fanciullino et al. 2014).

The VAZ pool size, as well as Z concentrations increase either in high light or with other environmental stressors that exacerbate light damage to photosystem II (PSII) (Takahashi and Murata 2008). Antarctic mosses have a large pool of VAZ cycle pigments and thus resemble sun plants (Demmig-Adams and Adams 1992b; Dunn 2000; Lovelock and Robinson 2002). In high light Z can form within 5-15 minutes, but can only dissipate light as heat when high light creates a pH gradient (ΔpH) across the thylakoid membrane. Conversion of Z back to Violaxanthin (V) is a slower process, occurring overnight in darkness in most ecosystems (Demmig-Adams et al. 2012). Cold temperatures and desiccation events affect Z accumulation (see Section 1.4.2.1). The percentage of VAZ as Z is usually high in Antarctic mosses (Lovelock et al. 1995b; Lovelock and Robinson 2002). In field samples of *S. antarctici* 50% of the VAZ pool was present as A+Z even after 24 hours in low light in the laboratory (Lovelock et al. 1995a).

Zeaxanthin dependent non-photochemical quenching (reversible photoinhibition) occurs in Antarctic bryophytes during freeze thaw cycles and in high light at both low and high temperatures (Adamson et al. 1988; Kappen et al. 1989; Lovelock et al. 1995a; Lovelock et al. 1995b). It is unclear if Z is retained for long periods or to what extent photosynthesis is down-regulated throughout the growing season. Differences in environmental drivers of Z accumulation may indicate differences

between the three species in stress tolerance or in exposure to ambient conditions within each species' habitat niche.

This study aims to characterise seasonal changes in photosynthesis and chloroplastic pigments. Additionally the optimal light, water and temperature conditions for photosynthesis in the field are compared in the three study species (see Section 2.3). This will facilitate predictions of which species will thrive under future global warming scenarios, and inform the use of these species as climate indicators in long term monitoring.

6.2 Methods

Sample collection is described in Section 3.2.1 and 5.2.1.

6.2.1 Chlorophyll fluorescence field measurements

The effective quantum yield parameter (ϕPSII) was used to calculate ETRs. The following equation from Genty *et.al* (1989) was used, which multiplies the efficiency of light use (ϕPSII) with the amount of light available to PSII. This is assumed to be 0.84 * 0.5, representing the amount of absorbed light and 0.5 for each photosystem (PSII and PSI).

$$ETR = \phi PSII*0.5*0.84*PAR$$

Absorbance may vary between species but this is difficult to measure. A MINI-PAM (Walz, Effeltrich, Germany) with leaf clip (model 2030B) measured ϕPSII and ambient PAR directly adjacent to the sample. Blocking solar radiation to dark adapt samples in the field affected moss turf temperature and was deemed unsuitable. For the assessment of optimal temperature and water content for photosynthesis ϕPSII was more informative than ETRs, whilst the relationship between ETR and PAR is more intuitive so both measurements are reported.

6.2.2 Chlorophyll and carotenoid analysis

Pigments were extracted in acetone and quantified by High Pressure Liquid chromatography (HPLC; Shimadzu Scientific Instruments, Australia) as in Lovelock and Robinson (2002) with the following modifications. Following the initial

extraction in 100% acetone, moss cell debris were re-extracted twice in 0.5 mL 80% acetone (20 minutes on ice in the dark) to ensure complete extraction of pigments (Dunn et al. 2004). The extract was made up to 2 or 3 mL with 100% acetone and filtered with a 0.45 μm syringe. Samples were stored at –20°C for up to 48 hours before analysis. The HPLC protocol used a different ratio for the HPLC Solvent A (acetonitrile: methanol: 0.1M TRIS HCl buffer, pH 8.0; 72:8:3), and the linear gradient between Solvent A and B was run for an extra 30 seconds.

Total chlorophyll (Tchl: chlorophyll a + chlorophyll b) is reported on a fresh weight basis. Dry weights were not used as correcting pigment water contents introduced error. However all analyses were also run for Tchl gdw^{-1} with similar trends but more unexplained error. Other pigments are expressed as ratios: chlorophyll a/b ratio (chla/b), xanthophyll pool (VAZ/Tchl) and β-carotene (β-carotene/Tchl) and the xanthophyll conversion state is expressed as the amount of Z relative to the VAZ pool (Z/VAZ). Zeaxanthin was also calculated on a per chlorophyll basis (Z/Tchl ratio) but since it was highly correlated with Z/VAZ, (ρ=0.81 n=188 $p<0.0001$ using data from all species) the latter is reported. This correlation makes it unlikely that the interpretation of photoprotective status based on Z/VAZ is confounded by changes in the size of the VAZ pool.

6.2.3 Statistical analysis

Photosynthetic rates (ϕPSII and ETR) rapidly responded to the instantaneous variables PAR, WC and MT collected with each sample so the full data set (n=64) was analysed (ETR and ϕPSII; n=64 per species). Pigment concentrations responded over a longer time frame (days) to daily climatic data, collected at nearby Casey station, so daily mean pigment data was used (n=21, within each species). Data are analysed and presented accordingly with different sample sizes, and exclusions to achieve balance in the factorial analyses.

Pigment concentrations were analysed for differences between species, environmental drivers and relationships between pigments as described in Section

5.2.4. Where daily light levels (wUVR1) were more influential to pigment concentrations these presented instead of 5 day mean light levels (wUVR5).

6.2.3.1 Environmental drivers of photosynthesis

Photosynthesis (ϕPSII and ETRs) was compared between species using ANOVAS (see Section 5.2.4.1). Environmental drivers of photosynthesis were analysed using Generalized Additive Models (GAMs) with a Gaussian distribution and no link function. Since PAR is a factor used to calculate ETR, the majority of the variability in the GAMs was attributed to PAR. This confounded the effects of the other variables on ETR. This model is only partially shown, to facilitate the interpretation of the light response of the effective quantum yield (ϕPSII; full model in Appendix B).

GAMs are related to Generalized Linear Models but allow flexibility in the shape of non-linear effects. The response variable is estimated as the sum of unknown smoothed functions for each predictor variable. All GAMS had the form:

$$y_i = \beta_0 + s_1(PAR_i, 2) + s_2(TWC_i, 2) + s_3(MT_i, 2) + s_4(AT10_i, 2) + \varepsilon_i$$

where the response variable is denoted as y_i, the intercept as β_0 and s_{1-4} are smooth functions of the predictor variables. Second order thin plate smoothing splines were used for each covariate, as preliminary analysis found overfitting occurred with higher order splines. The predictor variables were selected *a priori* as photosynthesis in bryophytes is known to have strong, rapidly responsive relationships with PAR, WC, and MT (Rice and Cornelissen 2014). These three variables represent the microhabitat at each sampling site. A macrohabitat variable, the ten day running mean air temperature (AT10, calculated from 3 hourly meteorological observations; n=80) was also included. This represents seasonal change and reflects cold overnight temperatures unlike the MTs which were measured at solar noon. Whilst AT10 is likely to influence photosynthesis directly, it is also strongly associated with other climatic variables with collinearity between variables increasing with longer time scales.

Goodness of fit was assessed as the proportion of deviance explained by the model (Dev^2). The stability of the models, including the location of optima, was judged by constructing models from 10 randomly generated data sets, each using 90% of the data, in addition to the model from the full data set (eg. Ashcroft et al. 2014). The relative importance of the covariate to the model was inferred from the range of predicted values in its partial response curves. All GAMs were analysed in R statistical version 3.1.0 (R Core Team 2014) using the packages gam (Hastie 2013) and faraway (Faraway 2014) with R codes and generous assistance provided by M. Ashcroft(Ashcroft et al. 2014). R software and packages are available at http://cran.r-project.org/.

6.2.3.2 Photoprotective strategies- pigment signatures

To visualise differences in photoprotective strategies between the three study species, a non-metric multidimensional scaling ordination plot was generated from eight photosynthetic and photoprotective pigments. Intracellular UV absorbing compounds (iUVACs), cell wall UV absorbing compounds (WUVACs), and anthocyanins, from Chapter 5 were combined with the ratio of zeaxanthin to xanthophyll pool (Z/VAZ) the VAZ pool to Total chlorophyll ratio, (VAZ/Tchl), chlorophyll a/b ratio (chla/b), Total chlorophyll (Tchl) and β-carotene to total chlorophyll ratio (β-carotene /Tchl) from this chapter. The overlay vector graphing function illustrates the five environmental variables with the highest correlations to the MDS plot (Spearman's ranks $\rho>0.2$).

A two factor permutational multivariate ANOVA (PERMANOVA), with *post hoc* pairwise comparisons was performed. The factors were collection date, species and included the interaction term (see Section 5.2.4.1). Type III partial sums of squares with 9999 permutations of residuals under a reduced model, were used. Prior inspection of draftsman's plots found β-carotene/Tchl and Total carotenoids (gdw^{-1}) were correlated ($\rho>0.70$), so β-carotene/Tchl was used in subsequent analysis.

6.2.3.3 Environmental drivers of pigment signatures

To test the best environmental predictors of pigments within each species the DISTLM routine was applied (McArdle and Anderson 2001). This applies distance based redundancy analysis to investigate linear relationships between the pigment cloud and environmental variables using adjusted R^2 and 9999 permutation of residuals under a reduced model. The marginal tests report the contribution of each individual variable to the pigment cloud.

Analyses were conducted within the Primer-E v 6.1.13 computer program (Clarke and Gorley 2006) with the add-on package PERMANOVA+ v 1.0.3 software (Anderson et al. 2008) with all matrices derived from Euclidean distances of normalised data.

6.3 Results

6.3.1 Variation in photosynthetic parameters and pigments across the season and between moss species

6.3.1.1 Photosynthesis

Both electron transport rates (ETR) and effective quantum yield (ϕPSII) are reported as proxies for photosynthetic rates. Variation in photosynthetic electron transport rates (ETRs) was similar for all species (Table 6.2; Figure 6.2). Low ETRs in early season doubled to peak in mid-season, with intermediate rates in late season (Tukey's HSD $p<0.05$; Table 6.2; Figure 6.6 A). Effective quantum yield (ϕPSII) increased throughout the season producing late season values 30% higher than in early season (Tukey's HSD; $p<0.05$;Table 6.2; Figure 6.3; Figure 6.6 B). Higher values in late season occurred in the lower incident PAR levels at this time (Figure 2.2). In *S. antarctici* ϕPSII was 10% lower than in *B. pseudotriquetrum* with *C. purpureus* intermediate (Tukey's HSD; $p<0.05$;Table 6.2; Figure 6.3).

Table 6.2: Results of two-way ANOVA for electron transport rates (ETR; µmol e m^{-2} s^{-1}), effective quantum yield (ϕPSII; power 2 transformed), total chlorophyll (nmol gfw^{-1}) and chlorophyll a/b ratio (nmol $nmol^{-1}$) with species (*Bryum pseudotriquetrum*, *Ceratodon purpureus*, *Schistidium antarctici,*) and collection date (early -9 November to 2 December 2002, mid -5 December 2002 to 4 January 2003 and late season-7 January to 1 February 2003) as the main effects. n=63 for chlorophyll pigments and n=183 for ETR and yield.

Pigments	Factors	D.F.	F Value	P value	Tukeys HSD p<0.05
ETR (µmol e m^{-2} s^{-1})	Species (B,C,S)	2	0.0981	0.9066	M>E
	Collection date (E,M,L)	2	29.7935	**<.0001***	
	Collection date*sp	4	0.7151	0.5827	
	Error	174			
Effective yield (ϕPSII)	Species (B,C,S)	2	3.9805	**0.0204***	B>S
	Collection date (E,M,L)	2	79.9314	**<.0001***	C=B,S
	Collection date*sp	4	2.2312	0.0676	L>M>E
	Error	174			
Total chlorophyll (nmol gfw^{-1})	Species (B,C,S)	2	12.0671	**<.0001***	B,S >C
	Collection date (E,M,L)	2	5.0077	**0.0101***	E>ML
	Collection date*sp	4	0.7820	0.5419	
	Error	54			
Chlorophyll a/b ratio (nmol $nmol^{-1}$)	Species (B,C,S)	2	4.3955	**0.0170***	B>S
	Collection date (E,M,L)	2	0.4190	0.6598	C=B,S
	Collection date*sp	4	1.5638	0.1972	
	Error	54			

*significant at $p < 0.05$

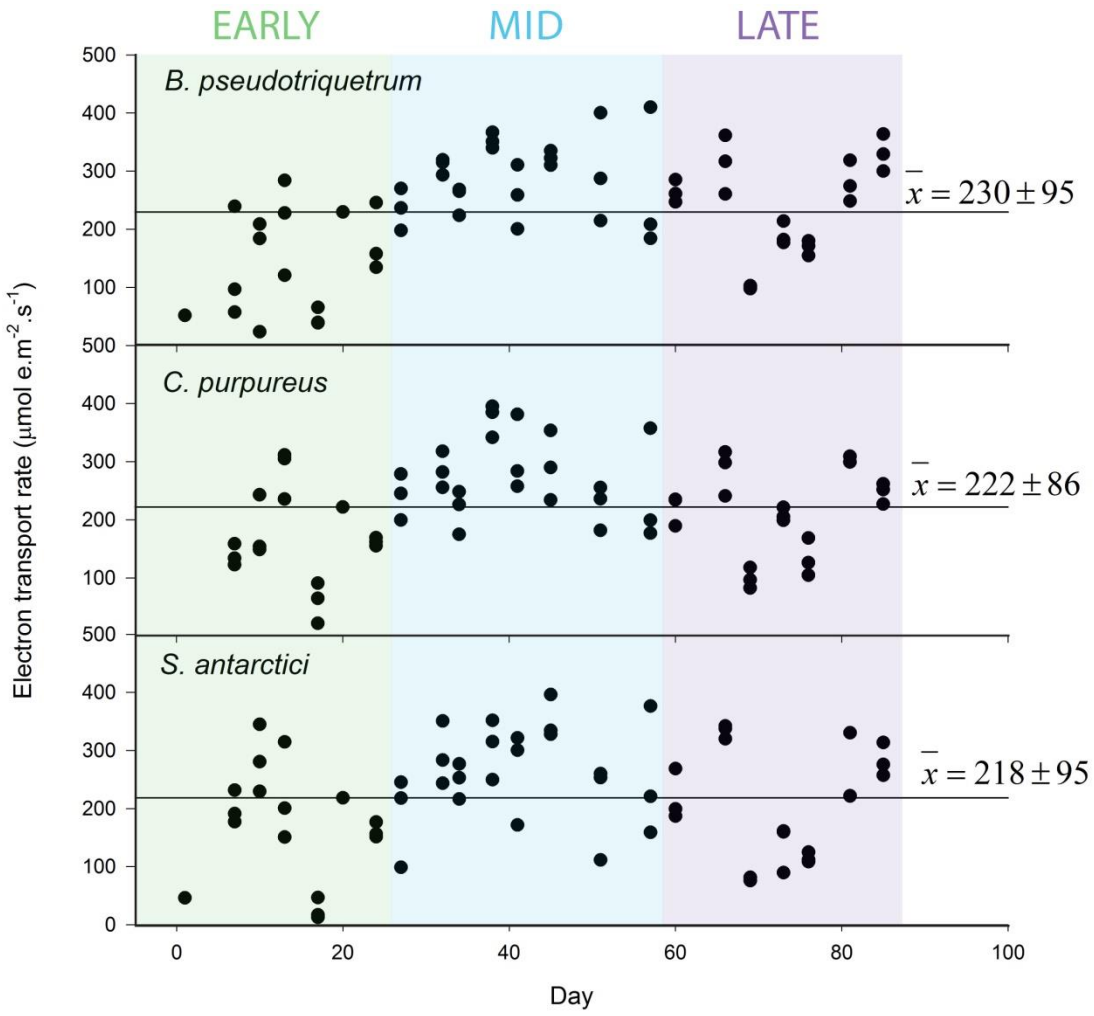


Figure 6.2: Variation in electron transport rate (µmol e m^{-2} s^{-1}) measured in the field in three Antarctic moss species *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* from 9 November 2002 to 1 February 2003. Shading indicates early, mid and late season, dates as per Table 6.2. Reference lines show season mean and standard deviation for each species (n= 64). Day 0 = 8 November.

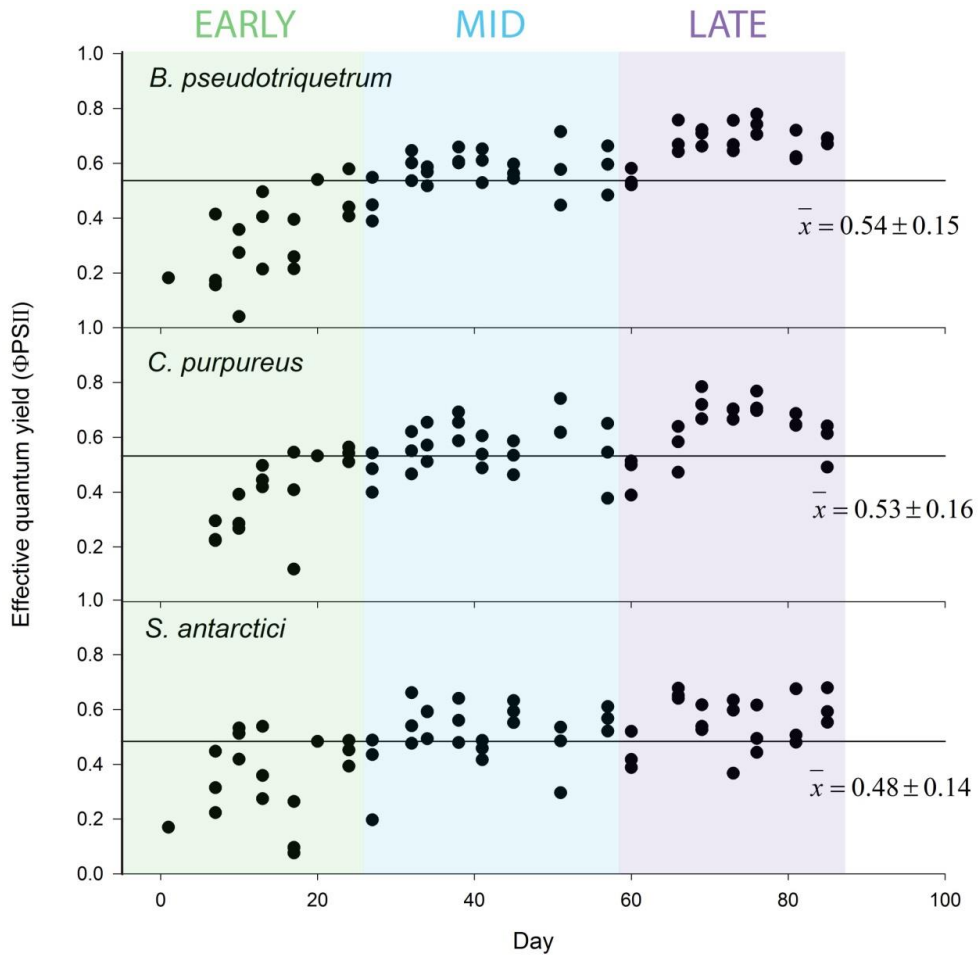


Figure 6.3: Variation in effective quantum yield (ϕPSII) in the three moss species *Bryum pseudotriquetrum, Ceratodon purpureus* and *Schistidium antarctici*. Shading indicates early, mid and late season, dates as per Table 6.2. Reference lines show season mean and standard deviation for each species (n= 64). Significant differences between species shown in Table 6.2. Day 0 = 8 November.

Variation in daily mean Tchl values was high, especially at the season's start (Figure 6.4). Total chlorophyll concentrations were ~40% lower in *C. purpureus* than in the other two species (Tukey's HSD; $p<0.05$; Table 6.2; Figure 6.4). At the beginning of the season Tchl concentrations were more than double those found in mid and late season samples (Tukey's HSD; $p<0.05$; Table 6.2; Figure 6.6 C). The chla/b ratio was 10% higher in *B. pseudotriquetrum* than *S. antarctici,* with *C. purpureus* intermediate but similar to both other species (Tukey's HSD $p<0.05$; Table 6.2; Figure 6.5). The chla/b ratio had a non-significant trend to low values in mid season (Figure 6.6).

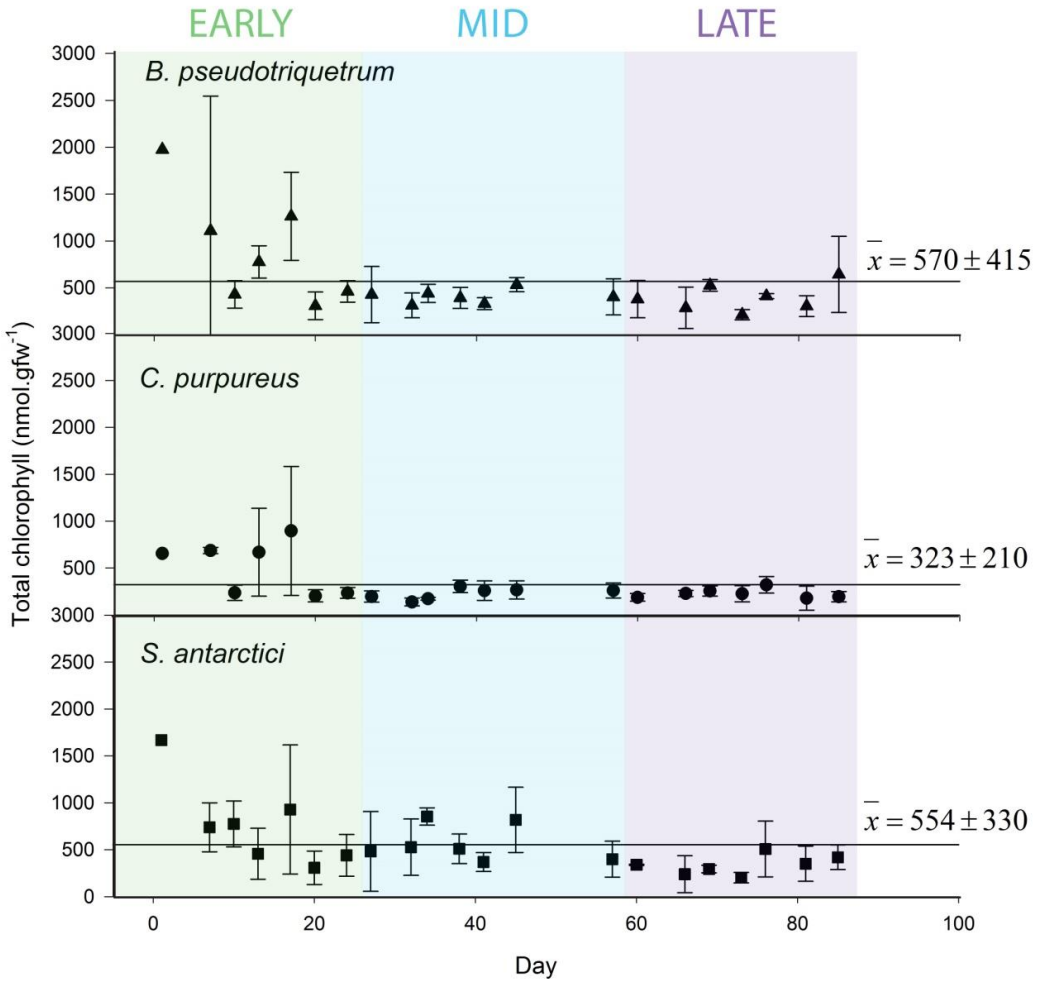


Figure 6.4: Variation in daily mean total chlorophyll in the three moss species *Bryum pseudotriquetrum, Ceratodon purpureus* and *Schistidium antarctici* between 9 November 2002 and 1 February 2003. Error bars denote standard deviation (n=3 except day 1 n=1). Shading indicates early, mid and late season, dates as per Table 6.2. Reference lines show season mean and standard deviation for each species (n= 21). Significant differences between species shown in Table 6.2. Day 0 = 8 November.

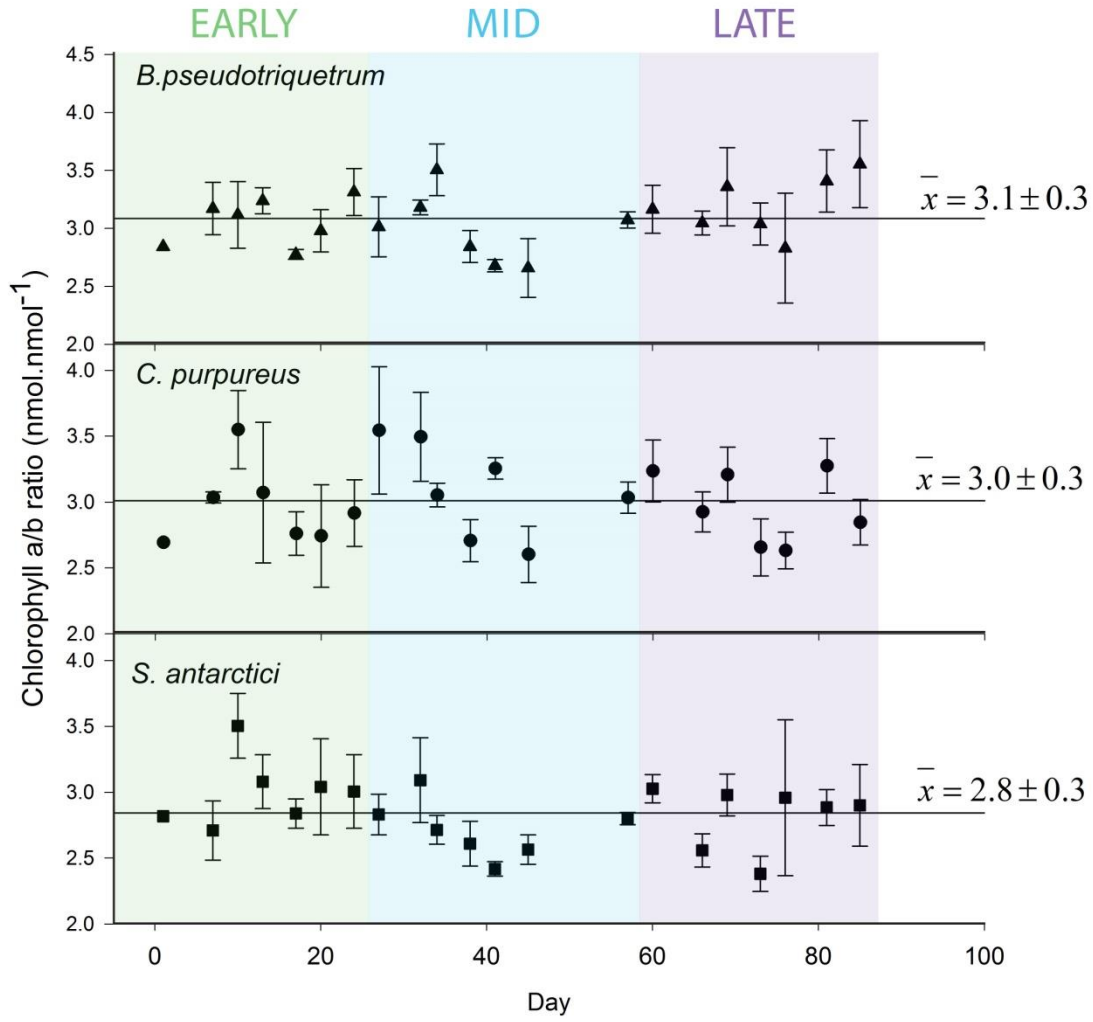


Figure 6.5: Variation in daily mean chlorophyll a/b ratios in the three moss species *Bryum pseudotriquetrum, Ceratodon purpureus* and *Schistidium antarctici* from 9 November 2002 to 1 February 2003. Error bars denote standard deviation (n=3 except day 1 n=1). Shading indicates early, mid and late season, dates as per Table 6.2. Reference lines show season mean and standard deviation for each species (n= 21). Significant differences between species shown in Table 6.2. Day 0 = 8 November.

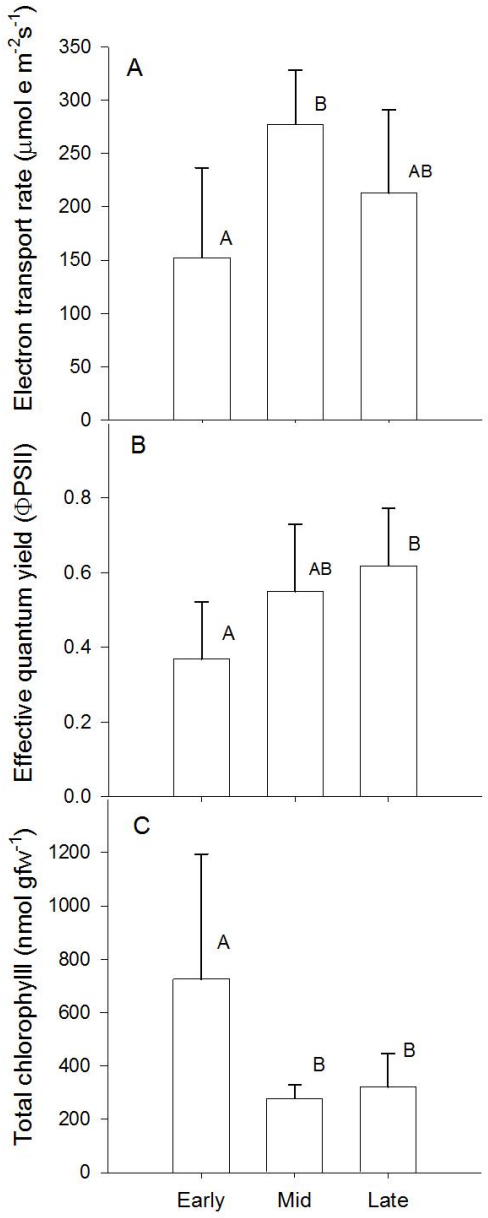


Figure 6.6: Seasonal variation in (A) photosynthetic electron transport rates (n=64 per bar), (B) effective quantum yield (n=64 per bar) and (C) total chlorophyll (n=21 per bar) for all three moss species in early, mid and late season (as per Table 6.2). Letters indicate significant differences when adjusted for multiple comparisons (Tukey's HSD $p<0.05$). Data are mean ± standard deviation.

6.3.1.2 Photoprotective carotenoids

Seasonal variation in carotenoid concentration is shown in figures 6.7–9. Carotenoids include the antioxidant carotenoid *β*-carotene/Tchl, VAZ/Tchl and Z/VAZ. The latter indicates the capacity and extent of NPQ. The VAZ pool was 10–20% higher in *C. purpureus* and *S. antarctici* than in *B. pseudotriquetrum* (Tukey's HSD $p<0.05$; Table 6.3: Figure 6.7).

Variation over the season in the requirement for NPQ, determined as increased Z/VAZ, had similar trends for all species; however differences were not significant in *S. antarctici* due to high variability, with particular samples apparently highly stressed (Table 6.3; Figure 6.8; Figure 6.10). All species had an equally high need for NPQ in early season with ~50% of the VAZ pool present as Z (Tukey's HSD $p<0.05$; Figure 6.8; Figure 6.10). In *B. pseudotriquetrum* and *C. purpureus* Z/VAZ values reduced in mid season by 50% and 40% respectively, and remained low in late season (Tukey's HSD $p<0.05$; Figure 6.8; Figure 6.10). There were no significant differences between the three species in terms of Z/VAZ within any particular collection period.

β-carotene/Tchl concentrations were one and a half times higher in *S. antarctici* than in the other two species but varied little across the season (Tukey's HSD $p<0.05$; Table 6.3; Figure 6.9).

Table 6.3: Results of two-way ANOVA for the protective carotenoids: daily mean VAZ pool (VAZ/TChl; nmol nmol^{-1}), zeaxanthin/Total xanthophyll ratio (Z/VAZ; nmol nmol^{-1}) and *β*-carotene concentrations (*β*-carotene /Tchl; nmol nmol^{-1}) for each moss species (*Bryum pseudotriquetrum, Ceratodon purpureus, Schistidium antarctici*) over collection date (early, mid and late season; dates as per Table 6.2). n=63

Pigment	Factors	DF	F values	p value	Tukey's HSD p<0.05
VAZ/Tchl (nmol nmol^{-1})	Species (B,C,S)	2	10.9935	**<0.0001***	C,S>B
	Collection date (E,M,L)	2	0.3965	0.6746	
	Collection date*species	4	2.1627	0.0856	
	Error	54			
Z/VAZ (nmol nmol^{-1})	Species (B,C,S)	2	2.1727	0.1237	E<M,L
	Collection date (E,M,L)	2	28.2747	**<.0001***	
	Collection date*species	4	1.8812	0.1270	
	Error	54			
β-carotene/ Tchl (nmol nmol^{-1})	Species (B,C,S)	2	12.7240	**<0.0001***	S>B,C
	Collection date (E,M,L)	2	2.1043	0.1318	
	Collection date*species	4	1.1153	0.3589	
	Error	54			

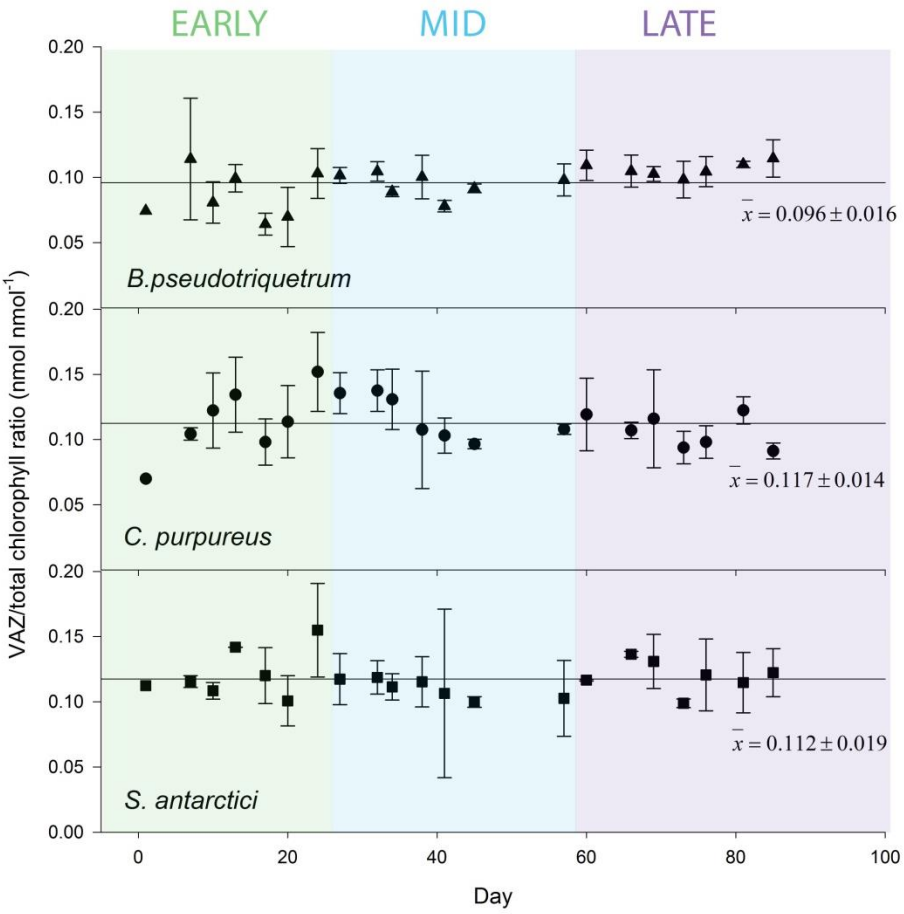


Figure 6.7: Variation in daily mean VAZ pool/T chlorophyll ratios for the three moss species *Bryum pseudotriquetrum, Ceratodon purpureus* and *Schistidium antarctici* between 9 November 2002 and 1 February 2003. Error bars denote standard deviation (n=3 except day 1 n=1). Shading represents early, mid and late season, dates as per Table 6.2. Reference lines show season mean and standard deviation for each species (n= 21). Significant differences between species are shown in Table 6.3. Day 0 = 8 November.

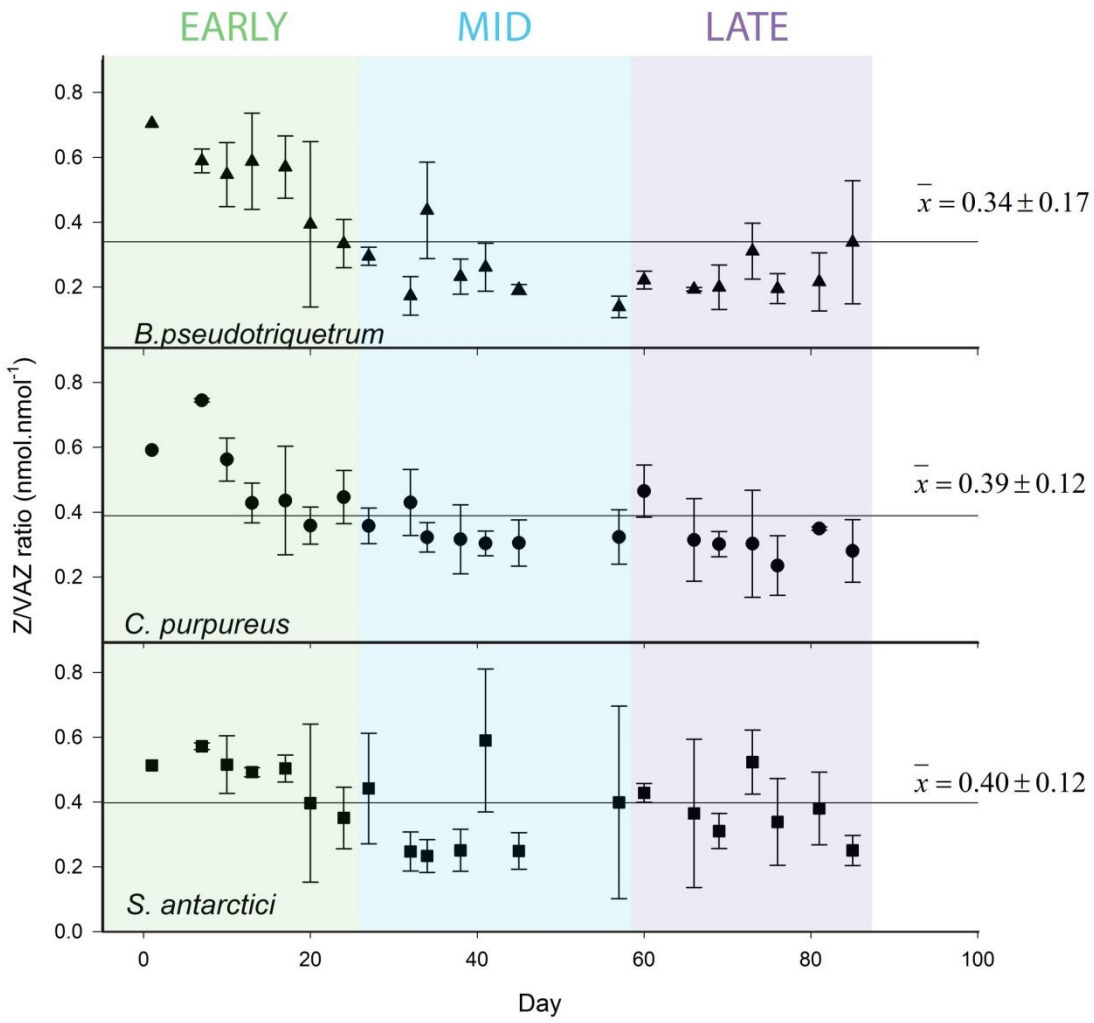


Figure 6.8: Variation in mean proportion of xanthophyll cycle present as zeaxanthin (Z/VAZ) in the three moss species, *Bryum pseudotriquetrum, Ceratodon purpureus* and *Schistidium antarctici,* between 9 November 2002 and 1 February 2003. Shading denotes early, mid and late season, dates as per Table 6.2. Error bars denote standard deviation (n=3 except day 1 n=1). Reference lines show season mean and standard deviation for each species (n= 21). Day 0 = 8 November.

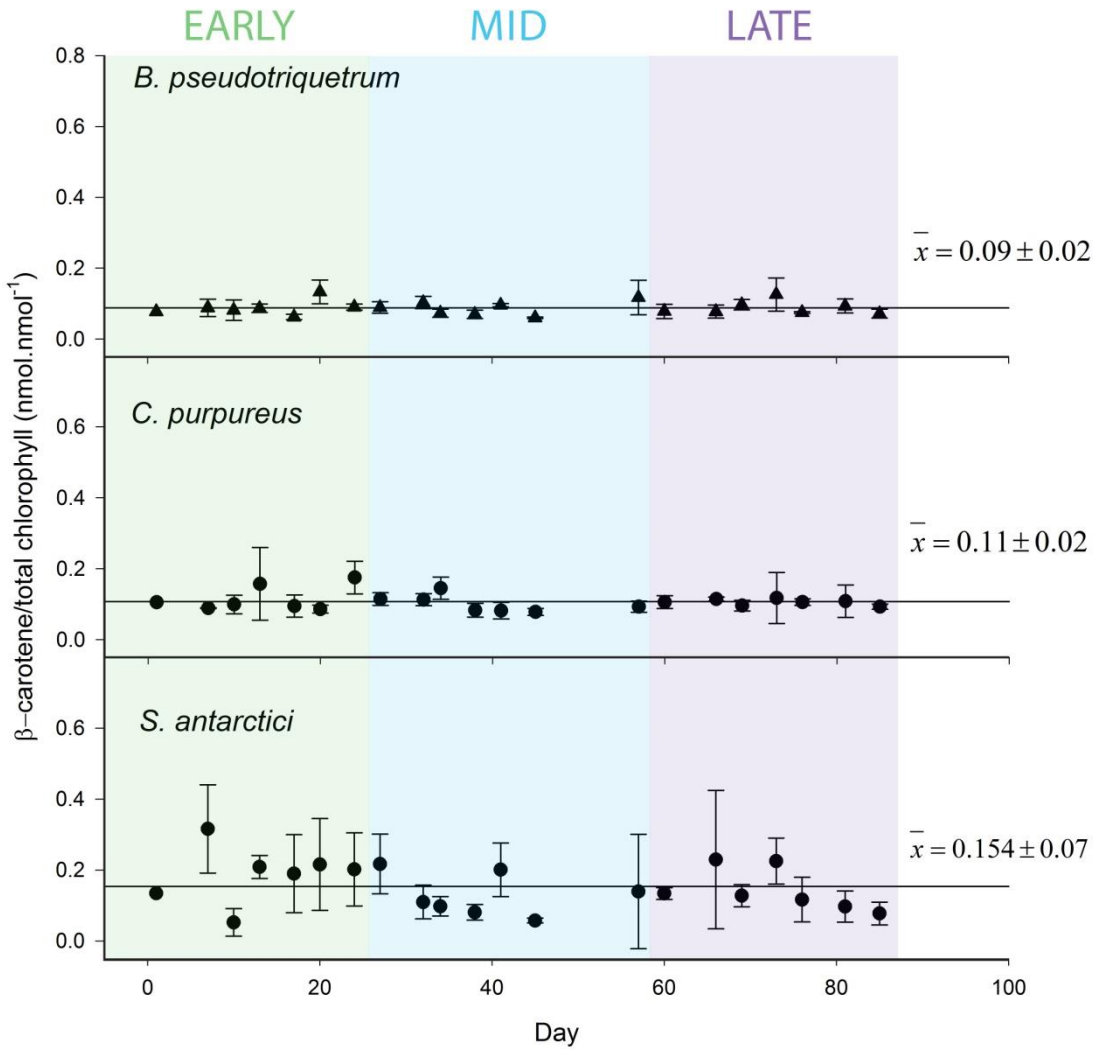


Figure 6.9: Variation in daily mean β-carotene to total chlorophyll ratios in the three moss species *Bryum pseudotriquetrum, Ceratodon purpureus* and *Schistidium antarctici* between 9 November 2002 and 1 February 2003. Error bars denote standard deviation (n=3 except day 1 n=1). Shading shows early, mid and late season, dates as per Table 6.2. Reference lines show season mean and standard deviation for each species (n= 21). Significant differences between species shown in Table 6.3. Day 0 = 8 November.

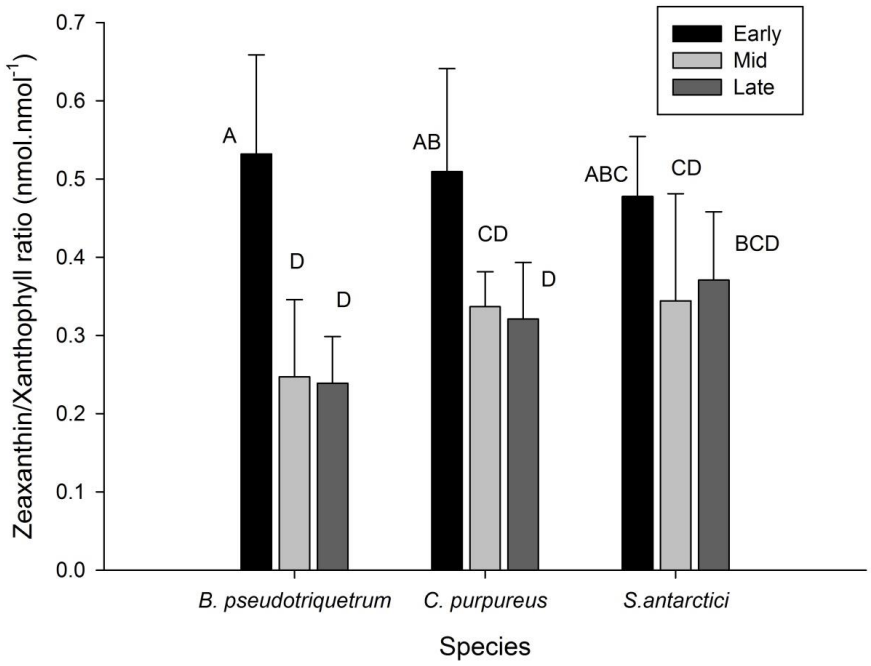


Figure 6.10: Variation in the mean proportion of xanthophyll cycle present as zeaxanthin (Z/VAZ) in *Bryum. pseudotriquetrum, Ceratodon purpureus and Schistidium antarctici* in early (black), mid (light grey) and late season (mid grey),dates as per Table 6.2. Bars with different letters indicate significant differences when adjusted for multiple comparisons (Tukey's HSD $p<0.05$). Data are mean ± standard deviation (n=7).

6.3.2 Potential environmental drivers

6.3.2.1 Photosynthetic parameters

The influence of environmental variables on photosynthetic efficiency (ϕPSII) were analysed using Generalized Additive Models (GAMs; Figure 6.11). Models produced better fits for *B. pseudotriquetrum* (D^2=0.81) and *C. purpureus* (D^2=0.72) than for *S. antarctici* (D^2=0.58; Figure 6.11: A5, B5, C5). The predicted values of ϕPSII are quite high since apart from the variable of interest, the three other environmental variables were set to a constant near the maximum ϕPSII. There is variation between the 11 models generated from this dataset for the predicted values for ϕPSII, but the characteristics of the curves, such as light saturation points and Pmax are broadly consistent (Figure 6.11). These models were stable over the most frequent parts of the environmental data but not at the extreme ranges, which had limited samples.

The relationship between PAR and ϕPSII varied between species (Figure 6.11: A1, B1, C1). In *B. pseudotriquetrum*, ϕPSII decreased with the slope becoming steeper after 1400 µmol.m^{-2}.s^{-1}. In *C. purpureus,* ϕPSII decreased linearly with PAR until ~1200 µmol quanta m^{-2} s^{-1} then levelled off. For *S. antarctici* ϕPSII increased at low light to peak at ~1200 µmol quanta m^{-2} s^{-1} before a slight decline.

All species had broad photosynthetic optima for water content (Figure 6.11: A2, B2, C2) which were similar in *B. pseudotriquetrum* (4.0-7.0 gH_20 gdw^{-1}) and *S. antarctici* (4.5-6.5 gH_20 gdw^{-1}). In *C. purpureus* WC was much lower with only four samples above 4.5 gH_20 gdw^{-1} and a low, narrow optimum of (2-4 gH_20 gdw^{-1}). Considerable model instability occurs at high water contents (>6 gH_20 gdw^{-1}) in all species.

In all species ϕPSII increased linearly with moss turf temperature from zero until ~10 ^oC (Figure 6.11: A3, B3, C3). *B. pseudotriquetrum* and *C. purpureus* then saturate with a suggestion of a slight decline at temperatures >20 ^oC in *C. purpureus.* In *B. pseudotriquetrum* model instability occurred above ~10 ^oC. In *S. antarctici* the optimal temperature for ϕPSII was between 8 and 12 ^oC, with a steep decline at higher temperatures.

The models included 10 day mean air temperature. This variable strongly correlates with the collection date and 10 day mean light levels so also represent seasonal change. Notably this variable was the most important influence on ϕPSII for all three species, indicated by a larger range of predicted ϕPSII. In *B. pseudotriquetrum* and *C. purpureus* ϕPSII increases steeply between -5 ^oC and ~-2 ^oC, then saturates. In *S. antarctici* ϕPSII continues to rise with increasing temperature.

Electron transport rates increase with PAR with no evidence of saturation in *C. purpureus,* little in *B. pseudotriquetrum* but some in *S. antarctici* between 1200 and 1500 µmol e m^{-2} s^{-1} (see Figure 6.12, Appendix B).

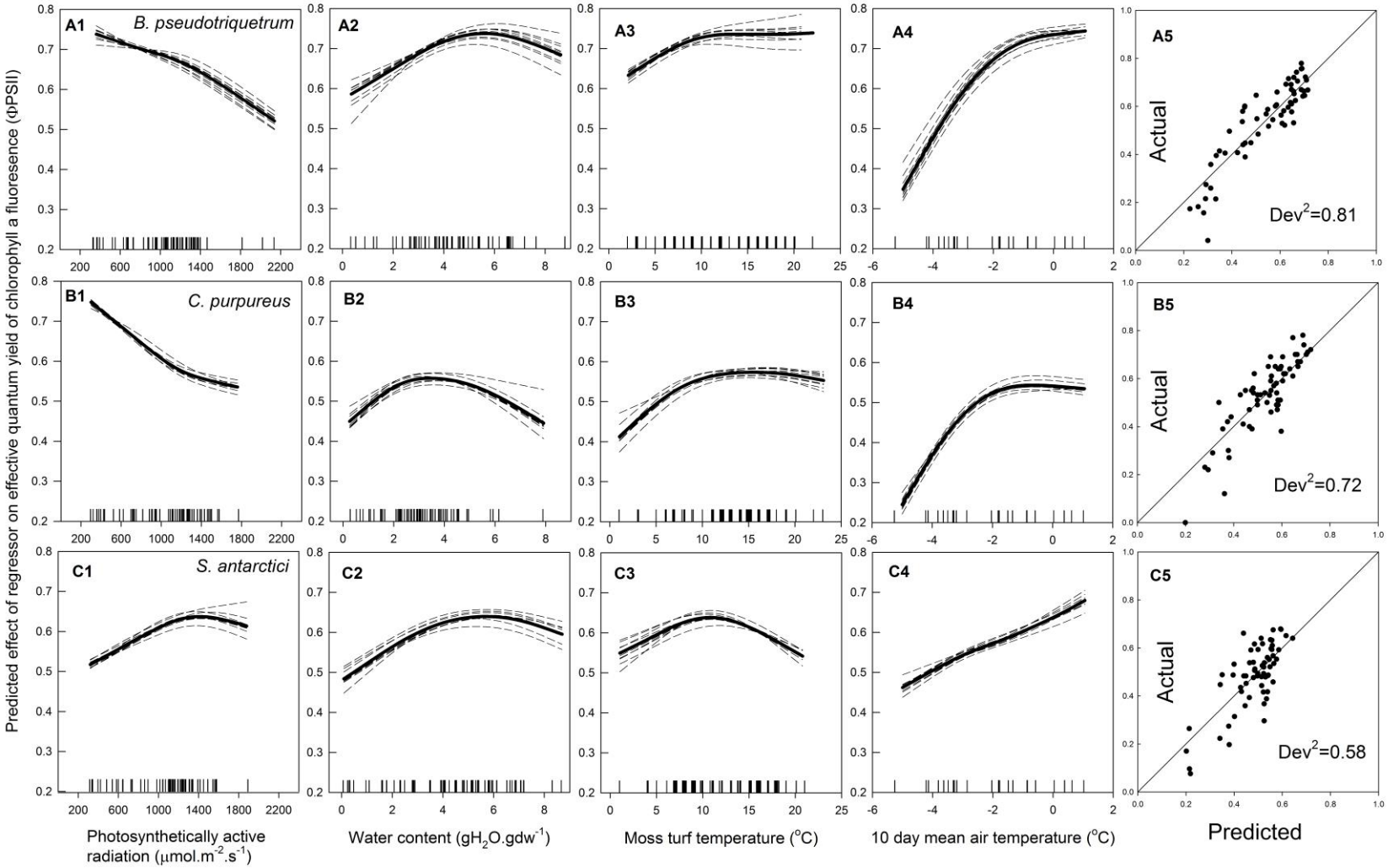


Figure 6.11: Effective quantum yield (ϕPSII) as predicted from generalized additive models (GAMS) in *Bryum pseudotriquetrum* (A1-A4), *Ceratodon purpureus* (B1-B4) and *Schitidium antarctici* (C1-C4). Partial plots of each variable holding other variables constant near the maxima for ϕPSII; (A1, B1, C1) photosynthetically active radiation (PAR), (A2, B2, C2) water content, (A3, B3, C3) turf moss temperature and (A4, B4, C4) 10 day mean air temperature. The GAM from all data points is shown (solid line) with 10 models each created using 90% of the data (dashed lines). The rugplot on the x-axis shows the number and distribution of the observations. Actual versus predicted effective yield and the goodness of fit (D^2) are shown (A5, B5, C5). n=64

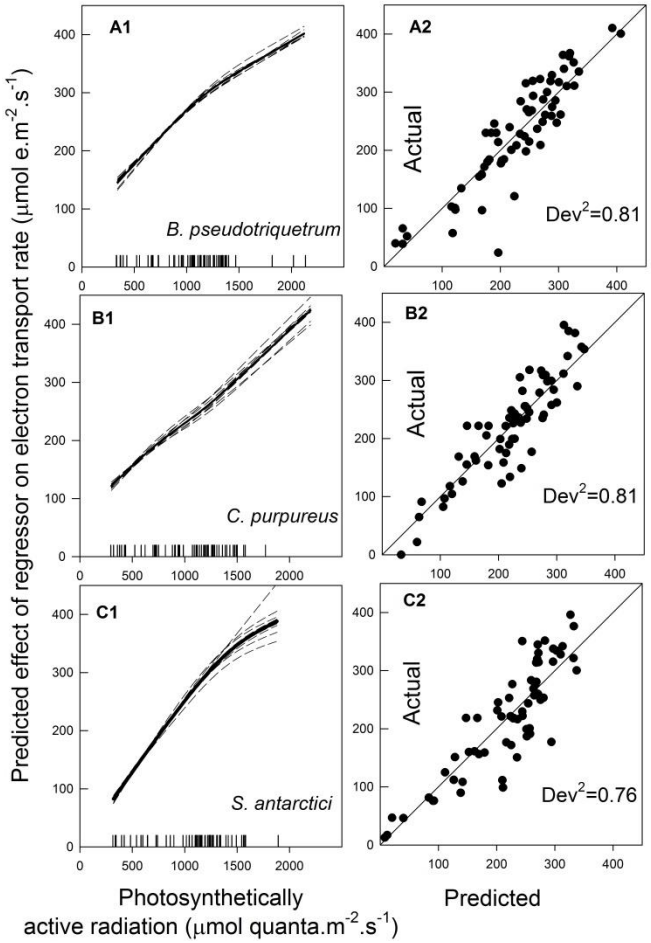


Figure 6.12: Electron transport rates (ETR) predicted from generalized additive models (GAMS) in *Bryum pseudotriquetrum* (A1-A2), *Ceratodon purpureus* (B1-B2) and *Schistidium antarctici* (C1-C2). See Figure 6.11 legend for details. Full model in Appendix B.

6.3.2.2 Environmental drivers of chlorophyll concentration

Total chlorophyll concentration in *S. antarctici* decreased with increasing daily light but only in early season with no associations with these variables later in the season (Table 6.4; Figure 6.13 A). Total chlorophyll concentration in *C. purpureus* decreased as relative humidity increased in early and mid season (Table 6.4; Figure 6.13 B).

The relationship between the chla/b ratio and light in *B. pseudotriquetrum* was also dependent on the collection date of the season, decreasing as 5 day light levels increased but only in mid season (Table 6.2; Figure 6.14).

Table 6.4: Significant ANCOVA results for total chlorophyll and chlorophyll a/b ratio in *Bryum pseudotriquetrum* (B), *Ceratodon purpureus* (C) and *Schistidium antarctici* in response to environmental variables and collection date (early mid, and late; dates as per Table 6.2; n=21).

Pigments	Climatic variables	Sp	R^2	Effects	F value	P value
Total chlorophyll (nmol gfw^{-1})	Daily wUVR (W m^{-2})	S	0.64	Collection date (E,M,L)	1.549	0.2446
				wUVR	1.946	0.1833
				Collection date*wUVR	4.479	**0.0298***
	Relative humidity (%)	C	0.74	Collection date (E,M,L)	3.388	0.0611
				RH	5.487	**0.0334***
				Collection date*RH	4.146	**0.0369***
Chlorophyll a/b ratio (nmol $nmol^{-1}$)	5 day mean wUVR5 (W m^{-2})	B	0.49	Collection date (E,M,L)	0.316	0.7342
				wUVR5	0.858	0.3689
				Collection date*wUVR5	5.347	**0.0177***

* Significant at p=0.05

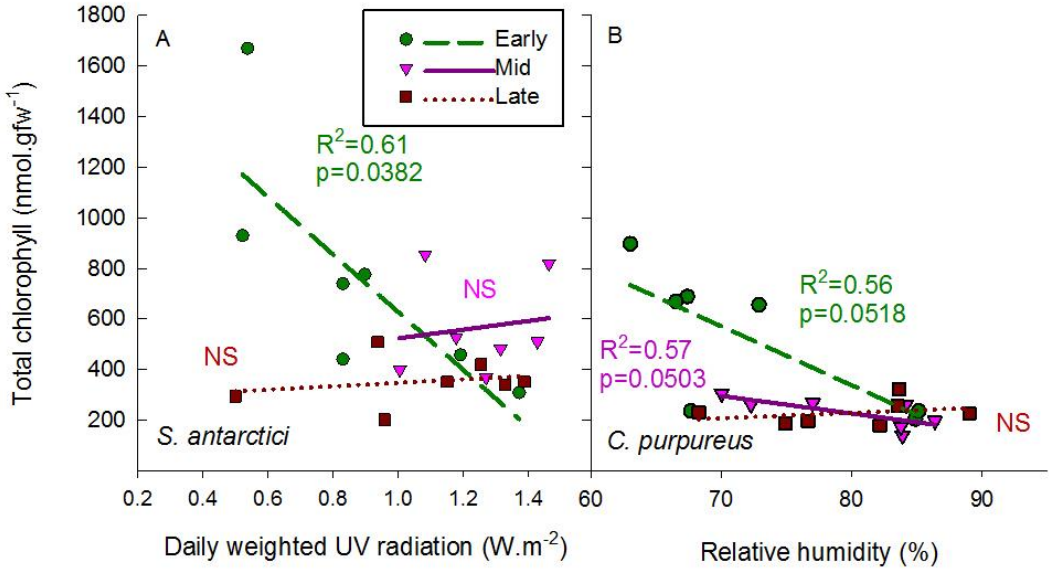


Figure 6.13: Significant correlations between daily mean total chlorophyll concentration and (A) light in *Schistidium antarctici* in early season (Tchl = 1766 - 1140*wUVR1), and (B) relative humidity in *Ceratodon purpureus* in early season (Tchl = 2202 - 23*RH) and mid season (Tchl = 780 - 7*RH). Statistics are cited for significant linear regressions within early, mid and late season (dates as per Table 6.2; n=7). Data are daily means (n=3) except for sample 1 where n=1.

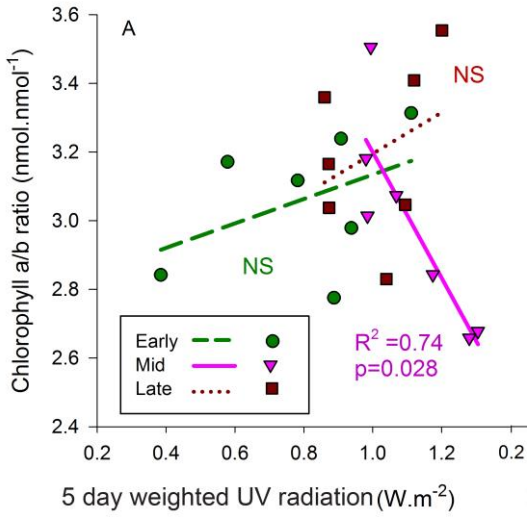


Figure 6.14: Significant correlations in *Bryum pseudotriquetrum* between daily mean chlorophyll a/b ratio and 5 day weighted total UV radiation in mid season (chla/b = 5.03 - 1.83*wUVR5). Statistics are cited for significant linear regressions within early, mid and late season (dates as per Table 6.2; n=7). Data are daily means (n=3) except for sample 1 where n=1.

6.3.2.3 Environmental drivers of carotenoid pigment concentrations

Environmental drivers of carotenoid accumulation varied between species and were usually dependent on the time of the season. Carotenoid accumulation in *S. antarctici* was uncoupled from the measured environmental variables.

The proportion of the VAZ pool present as Z (Z/VAZ) responded to different climatic variables in the other two species and these relationships changed over the season. In *B. pseudotriquetrum* Z/VAZ decreased with RH humidity, but only in the early part of the season (Table 6.5; Figure 6.15 A). In *C. purpureus,* there was a nonsignificant trend to decreasing Z/VAZ with warmer temperatures, but only in early season when temperatures were mostly below ~-4 ^{o}C. This relationship dissipated in the warmer temperatures of mid season to become a weak positive relationship in late season (Table 6.5; Figure 6.15 B). In the same species, The Z/VAZ ratio decreased with wUVR5 over the whole season (Table 6.5; Figure 6.15 C).

Table 6.5: Significant ANCOVA results for daily mean Z/VAZ ratio within the moss species: (B) *Bryum pseudotriquetrum* and (C) *Ceratodon purpureus.* Factors are collection date (early, mid and late season as per Table 6.2) and environmental variables. ANCOVA results where collection date was the only significant effect are not shown, (n=21) * Significant at p=0.05.

Pigments	Climatic variable	Sp	R^2	Effects	F value	P value
Z/VAZ ($nmol.nmol^{-1}$)	RH mean (%)	B	0.79	Collection date (E,M,L)	15.7758	**0.0002***
				Rhmean	0.0630	0.8053
				Collection date*Rhmean	4.2569	**0.0343***
Z/VAZ ($nmol.nmol^{-1}$)	ATMIN (ºC)	C	0.70	Collection date (E,M,L)	3.1521	0.0720
				ATMIN	0.0134	0.9095
				Collection date*ATMIN	4.2766	**0.0339***
Z/VAZ ($nmol.nmol^{-1}$)	wUVR5 ($W.m^{-2}$)	C	0.73	Collection date (E,M,L)	3.4569	0.0582
				wTUV5	6.3331	**0.0237***
				Collection date*wTUV5	0.3540	0.7076

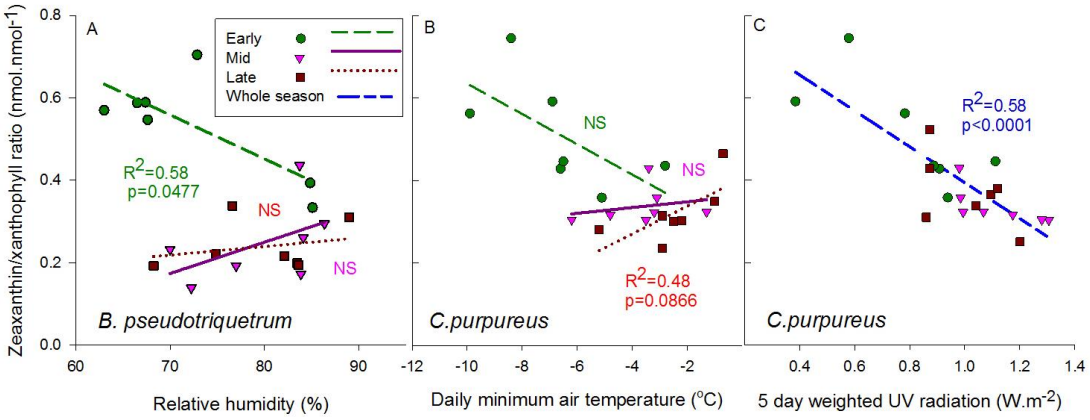


Figure 6.15: Correlations between the ratio of zeaxanthin to xanthophyll cycle pigment (Z/VAZ) and (A) relative humidity in *B. pseudotriquetrum* in early season (Z/VAZ = 1.30 - 0.01*RH), (B) daily minimum air temperature in *C. purpureus* in late season (Z/VAZ = 0.40 + 0.03*ATMIN) and (C) 5 day weighted UV radiation in *C. purpureus* over the whole season (Z/VAZ = 0.80 - 0.43*wUVR5). Statistics are cited for significant linear regressions in early mid or late season (dates as per Table 6.2, n=7) or for the whole season (n=21). Data are daily means (n=3) except for sample 1 (n=1).

6.3.3 Relationships between physiological variables

The relationship between Tchl and ETR was the same for all species but varied with collection date, so pooled species data within each collection date category was used for linear regression (Table 6.6; Figure 6.16). Throughout early season chlorophyll was lost but ETRs increased (Figure 6.16). Electron transport rates also decreased with increased Z/VAZ. This loose but significant relationship was consistent across species and collection date, so all data was pooled for linear regression (Table 6.6; Figure 6.17).

Table 6.6: Relationships between photosynthetic rates (ETR), total chlorophyll (Tchl) and zeaxanthin/xanthophyll pool (Z/VAZ), showing significant results from ANCOVA where pigments, collection date (early, mid, late; dates as per Table 6.2) and species (*Bryum pseudotriquetrum*, *Schistidium antarctici* and *Ceratodon purpureus*) were the main effects.

Pigments		R^2	Effects Df $_{2,1,2}$	F value	P value
ETR (µmol e.m^{-1}.s^{-1})	ETR vs log_{10}Tchl (nmol.gfw^{-1})	0.52	log_{10}Tchl Tchl	0.2578	0.6141
			collection date (E,M,L)	4.3526	**0.0187***
			Sp	0.2620	0.7707
			log_{10}Tchl *collection date	3.9519	**0.0262***
			log_{10}Tchl *Sp	0.0422	0.9587
			Sp*collection date	0.7931	0.5359
			log_{10}Tchl*collection date*Sp	0.3141	0.8670
	ETR vs Z/VAZ (nmol.gfw^{-1})	0.47	Z/VAZ	3.7336	**0.0596****
			collection date (E,M,L)	2.6823	0.0793
			Sp	0.1043	0.9012
			collection date* Z/VAZ	0.0525	0.9489
			Sp* Z/VAZ	0.1310	0.8775
			Sp*collection date	0.0506	0.9950
			Sp*collection date* Z/VAZ	0.3469	0.8448

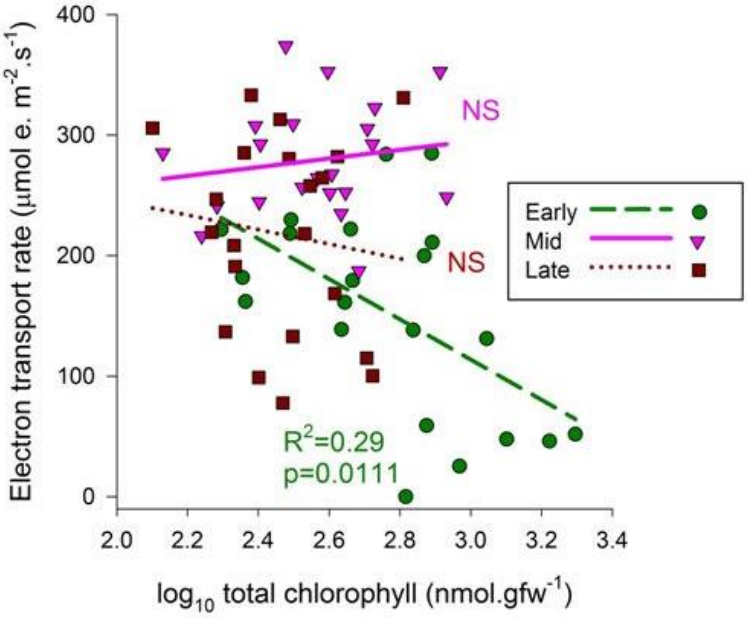


Figure 6.16: Correlations between daily mean electron transport rates (ETR) and daily mean log_{10} total chlorophyll (log_{10}Tchl) for all species within early (ETR =614 - 166*log_{10}Tchl) mid (NS) and late season (NS). Dates as per Table 6.2. n=21. Data are daily means (n=3) except for sample 1 (n=1).

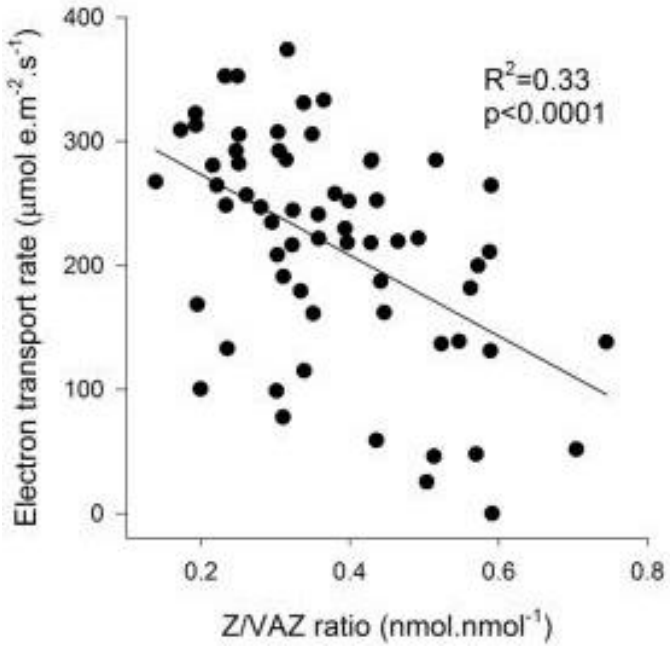


Figure 6.17: Correlations between daily mean electron transport rates and daily mean zeaxanthin to xanthophyll pool ratio (Z/VAZ) using data from all species over the season (ETR = 351-366*Z/VAZ, n=63). Dates as per Table 6.2. Data are daily means (n=3*)* except for sample 1 (n=1).

6.3.4 Pigment signatures

6.3.4.1 Variation in pigment signatures between species

Variation in photoprotective strategies between species are compared by combining UVACs from chapter 5 with chloroplastic pigments presented in this chapter (Figure 6.18). These pigment signatures varied between species (PERMANOVA: factor- Species: Pseudo$F_{2,54}$ = 12.003 p=0.001) and over time (PERMANOVA: factor- Time; Pseudo$F_{2,54}$ = 6.503 p=0.001). The interaction between time and species was *almost* significant (PERMANOVA interaction: sp*time; Pseudo$F_{4,54}$ = 1.4602 p=0.085). Pairwise comparisons showed that *C. purpureus* and *S. antarctici* were nearly similar in their pigment signatures in the early part of the season (PERMANOVA; pairwise comparison p=0.053), but otherwise all species were distinct from each other, in each time period (PERMANOVA; pairwise comparisons; p<0.05).

Most variation in pigments occurs in the early part of the season (Figure 6.18) followed by a contraction in mid and late season, with similar dispersion for all species in each time period (PERMDISP; Psuedo$F_{2,60}$=11.354 p=0.003, pairwise comparisons; E>M,L, p<0.05). Within each species, the pigment clouds were similar in mid and late season (PERMANOVA; pairwise comparisons; p=0.224-0.413), and both mid and late season differed from early season in *B. pseudotriquetrum* and *C. purpureus* (PERMANOVA; pairwise comparisons; p=0.001-0.019). Similarly, in *S. antarctici*, the pigment signatures were different in early and mid season (PERMANOVA; pairwise comparison; p=0.037), but early and late season were similar (PERMANOVA; pairwise comparison; p=0.055). The PRIMER-e overlay vector function gives a general sense of the direction of the forcing environmental variables (Figure 6.18).

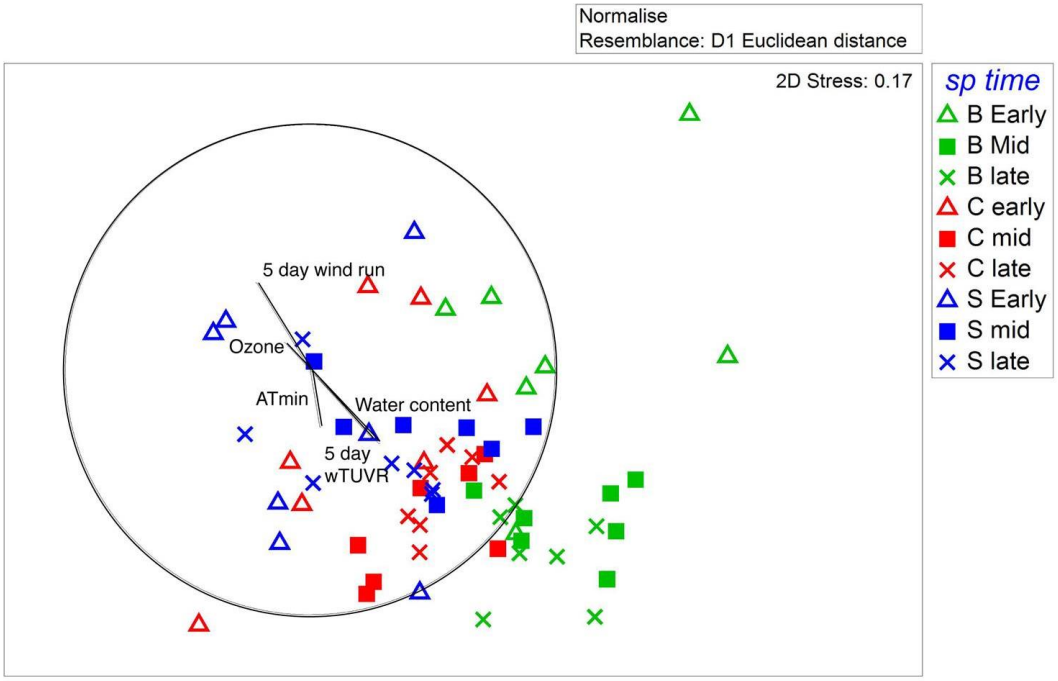


Figure 6.18: Non-metric MDS plot of 8 photoprotective and photosynthetic pigments for the three mosses: *Bryum pseudotriquetrum* (B; green symbols), *Ceratodon purpureus* (C; red symbols) and *Schistidium antarctici* (S; blue symbols), in early (triangle symbols), mid (square symbols) and late season (crossed symbols), dates as per Table 6.2. The Primer-E overlay vector function shows the five most influential environmental variables on the ordination: 5 day light levels (ρ=0.35, p=0.0001), 5 day wind run (ρ=0.31, p=0.0001), ozone column depth (ρ=0.30 p=0.0001), ATMIN (ρ=0.21, p=0.0002), water content (ρ=0.20, p=0.0001). Resemblance matrices are based on Euclidean distances of normalised data.

6.3.4.2 Environmental variables forcing pigment signatures

Marginal tests from the DISTLM routine in PRIMER report the individual linear association for each environmental variable to the pigment cloud within each species. These associations are strongest in *B. pseudotriquetrum* and weakest in *S. antarctici.* Light and wind were the only variables important to all three species, both at 5 day time scales. Light explained most variation in *B. pseudotriquetrum* (ρ=29%) and *C. purpureus* (ρ=21%), whereas WC (ρ=18%) and wind speed (ρ=15%) were most important to *S. antarctici*. Water content explained 12% of variation in *C. purpureus*, but interestingly, was not significant in *B. pseudotriquetrum,* whilst RH was (ρ=12%). Temperature did not influence pigments in *S. antarctici,* but ATMIN influenced the pigment clouds of *C. purpureus* (ρ=16%) and *B. pseudotriquetrum* (ρ=19%) with the latter species also affected by maximum air temperature (ρ=23%).

Table 6.7: DistLM marginal tests showing the association between individual environmental variables and the pigment cloud of each moss species (B*ryum pseudotriquetrum, Ceratodon purpureus* and *Schistidium antarctici*). Resemblance matrices based on Euclidean distances on normalised data with 9999 permutations. n=21 *significant at p<0.05.

DISTLM marginal tests	*B. pseudotriquetrum* Total SS(trace): 142.04					*C. purpureus* Total SS(trace): 107.42					*S. antarctici* Total SS(trace): 123.59				
Variable	SS (trace)	Pseudo-F	p	variation explained	Rank	SS (trace)	Pseudo-F	p	variation explained	Rank	SS (trace)	Pseudo-F	P	variation explained	Rank
Water content (gH_20 gdw^{-1})	10.305	1.4863	0.2	0.07	9	13.223	2.6669	**0.034***	0.12	4	22.057	4.1274	**0.008***	0.180	1
Moss temp (^oC)	18.42	2.8312	**0.036***	0.13	7	10.364	2.0289	0.088	0.10	6	8.7344	1.4449	0.195	0.07	5
Ozone depth (DU)	21.938	3.4706	**0.016***	0.15	6	18.148	3.8624	**0.008***	0.17	2	7.5489	1.236	0.317	0.06	6
Daylength	24.786	4.0164	**0.004***	0.17	5	11.173	2.2055	**0.069***	0.10	6	6.1715	0.99862	0.381	0.05	9
Max air temp (^oC)	33.252	5.8074	**0.001***	0.23	3	9.3635	1.8142	0.112	0.09	9	10.555	1.7741	0.095	0.09	4
Min air temp (^oC)	27.301	4.5209	**0.004***	0.19	4	17.125	3.6032	**0.009***	0.16	3	7.8096	1.2815	0.28	0.06	6
Relative humidity (%)	16.604	2.515	**0.064***	0.12	8	11.935	2.3748	0.054	0.11	8	4.9765	0.79713	0.566	0.04	10
TUVB1 ($W\ m^{-2}$)	5.4019	0.75114	0.534	0.04	10	6.2215	1.168	0.292	0.06	10	7.2646	1.1865	0.312	0.06	6
5 day wind run	37.65	6.8527	**0.002***	0.27	2	12.996	2.6149	**0.039***	0.12	4	18.406	3.3246	**0.007***	0.15	2
wTUV5 ($W\ m^{-2}$)	41.716	7.9005	**0.001***	0.29	1	22.62	5.0679	**0.001***	0.21	1	16.239	2.874	**0.022***	0.13	3

6.4 Discussion

This study provides comprehensive information about moss photosynthesis and associated pigments over a single Antarctic season. Studies such as these are logistically difficult and few in number, especially with adequate replication. Since three moss species were investigated it allows comparison and some predictions as to the importance of the various environmental drivers of moss physiology. Climate is hugely variable from season to season in Antarctica and this season was particularly warm in November leading to an early and extensive melt (Section 3.3.4; Figure 3.2 B). This is important since high melt and abundant water throughout mid and late season reduced the influence of water, as it was rarely limiting. Since water is a key factor in this cold desert, this probably makes it a good season to explore the influence of other environmental variables.

Photosynthetic rates and pigment accumulation were strongly seasonal and the gradual change in environmental gradients prevailed over the influence of any particular environmental stressor. Most variation was observed early in the season (Figure 6.18). This was, in part due to a larger range in the environmental variables in early season in this year, but also to emergence of moss from snow cover at the season's start. Each season as moss emerges from under snow it is simultaneously exposed to rapid environmental change. In general, for all three moss species photosynthesis (ETR and ϕPSII) increases from early to mid season and then remains high (Figure 6.2; Figure 6.3; Figure 6.6 A,B), whilst Tchl and Z/VAZ decline from early to mid season and then stay low (Figure 6.4; Figure 6.8; Figure 6.10). This is consistent with the moss transitioning from a dormant and photoprotected state (low ϕPSII and high Z/VAZ) under frozen, low light conditions (snow covered and high Tchl) to higher photosynthetic efficiency and rate as the season warms. Early season is undoubtedly the most stressful time, even in a low ozone depletion year and characterised by extremes of cold, low light, high wind, low water availability and low relative humidity (Section 3.3.1; Table 3.1; Figure 3.1; Section 2.2.1.1; Figure 2.2; Figure 2.3; Figure 2.4). Mid and late season were warmer, with very high melt and good growing conditions for mosses generally.

It is likely that similar variation would be observed at the end of the season as at the start, however, although this dataset began as mosses emerged from snow, it did not capture the last 2-4 weeks of the growing season. My prediction is that moss transitions to its frozen state exhibiting a reverse of early season change. It should be noted that snow cover may provide a time of low light and benign growing conditions at either end of the season, depending on the rapidity of the Spring melt and Autumn freeze.

Different photoprotective strategies are used by the three species (Figure 6.18) with differences in photosynthetic response to environmental variables consistent with their respective ecological niche (summarised in Table 6.8). Photosynthesis was strongly seasonal and also responded rapidly to the ephemeral variables, MT, WC and PAR. Chloroplastic pigments (chlorophylls and carotenoids) responded over longer time frames to climatic data.

Cold overnight temperatures (AT10 and ATMIN) were more influential to both photosynthesis and pigment concentrations than MTs (Figure 6.11, cf. column 3 and 4; Table 6.5; Table 6.7; Figure 6.15). The latter were measured at solar noon so likely to be close to the daily maxima, and do not reflect the freeze thaw events which often occur overnight.

Water was limiting only at the beginning of this season, nevertheless, high and low water contents reduced photosynthesis in all species (Figure 6.11, column 2). Somewhat unexpectedly, low chlorophyll concentrations occurred when photosynthetic rates were highest, and several possible explanations for this counterintuitive result are discussed.

Table 6.8: Comparison of physiological responses of *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* to environmental conditions in early, mid and late season (dates as per Table 6.2).

Response to environmental variables	*B. pseudotriquetrum*	*C. purpureus*	*S. antarctici*
Photosynthetic temperature response	Increases up to 12 ºC, then remains high	Increases up to 12 ºC, then remains high	Increases up to 12 ºC, then decreases
Photosynthetic water optima ($gH_20\ gdw^{-1}$)	4.0-7.0	2.0-4.0	4.5-6.5
Species differences and environmental drivers of pigments consistent over the season	• Highest ϕPSII B>S, C=B,S • Higher chla/b B>S, C=B,S • Lowest VAZ pool B<C,S	• Lowest Tchl; C< B,S • Z/VAZ decreases with wUVR5	• Highest β-carotene S>B,C
Early season	• Z/VAZ decreases with RH	• Tchl decreases with RH	• Tchl decreases with WC • Tchl decreases with light
Mid season	• Chla/b decreases with light	• Tchl decreases with RH	
Late season		• Z/VAZ increases with AT	

6.4.1 Photosynthesis

The season mean effective quantum yield (ϕPSII) was highest in *B. pseudotriquetrum* (Table 6.2; Figure 6.6). *Schistidium antarctici* had the lowest season mean ϕPSII and the GAMs suggest a striking difference in the response of ϕPSII to light and air temperature in this species (Figure 6.11). Other studies also report photosynthetic rates are highest in *B. pseudotriquetrum,* intermediate in *C. purpureus* and lowest in *S. antarctici* under optimal conditions (Lewis Smith 1999; McKinley 2012). However, the season mean electron transport rates (ETRs) were similar for all species; ~200 µmol e m^{-2} s^{-1}, and approximately half the maximum ETR (Table 6.2; Figure 6.2). Some caution should be exercised in species comparisons since the calculation for ETR assumes a constant leaf absorbance for each species. Similar ETRs between species could also be explained by the suboptimal field conditions (eg. median moss turf temperature = 12 ^{o}C). As temperature lowers the photosynthetic rates for all species decrease and converge (Lewis Smith 1999; McKinley 2012; Ashcroft et al. 2015).

Electron transport rates were considerably higher than previously reported for these Antarctic species (Wasley et al. 2006a; McKinley 2012), but within the range of field measurements of Antarctic moss (Pannewitz et al. 2003; Schroeter et al. 2012). High ETRs may be due to increased CO_2 uptake in the extensive melt and generally good growing conditions of this season, particularly if CO_2 was elevated by microbial communities within the moss turf (Tarnawski et al. 1992). Alternatively, the high ETR may include non-assimilatory electron transport (Proctor and Smirnoff 2011; Schroeter et al. 2012). This is discussed further in the following chapter (Section 7.3).

6.4.1.1 Environmental variables driving photosynthesis

The explanatory variables PAR, WC, MT and AT10, provided strong models for ϕPSII in *B. pseudotriquetrum* and *C. purpureus*, but not in *S. antarctici* (Figure 6.11). Possibly *S. antarctici* is buffered from ambient air temperature in its wetter habitat (Melick and Seppelt 1994; Lewis Smith 1999; Wasley et al. 2012). This is supported

by the lack of influence of temperature on the pigment signature of this species (Table 6.7).

6.4.1.1.1 Light

Electron transport rates (ETRs) generally increased with PAR up to at least 1200 µmol quanta m^{-2} s^{-1} (Figure 6.12, A1, B1, C1). Both *B. pseudotriquetrum* and *S. antarctici* show some evidence of light saturation above 1200 µmol quanta m^{-2} s^{-1} but *C. purpureus* shows little evidence of a change in rate below 2000 µmol quanta m^{-2} s^{-1}. In contrast, McKinley (2012) found saturation occurred at PAR levels between 1200 and 1500 µmol quanta m^{-2} s^{-1} for all three species measured in the laboratory. Field conditions differ to the laboratory. This lack of saturation in my field ETRs could be due to elevated CO_2 in the field facilitating higher photosynthetic rates. Alternatively, a higher component of protective non-assimilatory ETR may occur in strong sunlight (full spectrum containing UV-BR), compared to artificial PAR. Additionally, my field ETRs were all collected from full sun microsites. Sun grown mosses have an increased capacity for non-assimilatory ETRs, while ETRs saturate at lower irradiance in shade mosses (Proctor and Smirnoff 2011). Such differences in the prior growth of samples could also affect the light saturation point.

As expected, the partial curves show high ϕPSII at low PAR, which declines as PAR increases in both *C. purpureus* and *B. pseudotriquetrum*. This presumably reflects increased photochemical and nonphotochemical quenching at higher PAR. The response of ϕPSII to PAR is distinct in *S. antarctici* and with a lower ϕPSII at low light levels; this may reflect sustained NPQ in this species, consistent with a higher proportion of photoprotective pigments and lower ϕPSII (Figure 6.3; Figure 6.10). However, the model for this species has a high proportion of unexplained variation, and care should be taken with interpretation.

6.4.1.1.2 Temperature

In all species, photosynthetic efficiency increased with temperature: up to a MT of ~ 12 ^{o}C and an AT10 of ~-2 ^{o}C (Figure 6.11; column 3). In *B. pseudotriquetrum* and

C. purpureus the distinct saturation of ϕPSII at temperatures above AT10 ~-2 ^{o}C suggests cold overnight temperatures including freeze thaw events have a strong influence below this temperature (Figure 6.11; A4, B4). The Antarctic species *Bryum subrotundifolium* took 24 hours for full recovery of ϕPSII and net CO_2 uptake from the overwintering state (Schlensog et al. 2004). Mosses can recover potential quantum yield (dark adapted Fv/Fm) rapidly when rehydrated following desiccation, but the time taken varies between moss species and is influenced by prior environmental conditions and the severity and duration of the desiccation (Proctor 2010). The distinct saturation above AT10 ~-2 ^{o}C does not occur in *S. antarctici* (Figure 6.11; C4) and perhaps moss turf temperature in this species is less coupled to air temperature, due to thermal buffering in its wetter ecological niche (Bramley-Alves 2015).

All species display a strongly seasonal pattern with AT10 the strongest influence on photosynthesis (Figure 6.11; column 4). Whilst AT10 correlates with other ten day mean climatic variables, such as light, it is highly likely that ϕPSII is affected by the temperature of the previous days, particularly if this drops below freezing. Freezing temperatures of moss turf are reported between -4 ^{o}C and -8 ^{o}C in moss from the Windmill Islands (Melick and Seppelt 1992). In this season AT10 was above ~-2 ^{o}C from mid-December until the end of January, allowing some 6 weeks of potentially uninterrupted metabolism. Such prolonged opportunities for growth are also predicted from measurements of bryophyte microclimates elsewhere in continental Antarctica (Lewis Smith 1999; Green et al. 2000; Pannewitz et al. 2003).

Optimal temperatures for ETR are reported at 20 °C in my study species (McKinley 2012), which is similar to the broad temperature optima described here for *B. pseudotriquetrum* and *C. purpureus.* However, the decline in ϕPSII above ~12 °C in *S. antarctici* is unexpected. Nevertheless, McKinley (2012) also found evidence that *S. antarctic*i is more vulnerable to high temperatures, with a 60% reduction in ETRmax at 28 °C, compared with just a 30% reduction in the other two species. *Schistidium antarctici* may not reach such high temperatures as often as the other

species in the natural setting and so may be more negatively affected by heat (Figure 6.11; A3, B3, C3). This species may thus be vulnerable under increased temperatures from global warming. These results will need to be validated. Whilst this method for estimating photosynthetic temperature optima is a significant improvement on previous studies, differences in temperature response between samples due to seasonal and microclimate influences are not quantified. Factorial experiments could investigate if the photosynthetic temperature response varies with acclimation to growing conditions, including high light and water availability. Seasonal comparisons of temperature response curves in laboratory conditions could also be conducted.

6.4.1.1.3 Water

Both low and high water availability reduced photosynthetic efficiency (ϕPSII) for all species with a lower optima in *C. purpureus* (3–4 $gH_20\ gdw^{-1}$) than in either *B. pseudotriquetrum* (4.0–7.0 $gH_20\ gdw^{-1}$) or *S. antarctici* (4.5–6.5 $gH_20\ gdw^{-1}$; Figure 6.11; A2, B2, C2). This concurs with the relative desiccation tolerance and hydrological distributions of these species in the Windmill Islands (Melick and Seppelt 1997; Wasley et al. 2006b; Wasley et al. 2012). Elsewhere in Antarctica, similar optimal water contents for photosynthesis, are also reported at 4.7 and 6.7 $gH_2O\ gdw^{-1}$ for *C. purpureus* and *B. pseudotriquetrum* respectively (Lewis Smith 1999). However, another study using two samples, found a lower optimal water content of 2.30–3.35 $gH_2O\ gdw^{-1}$at 10 ^oC in *B. pseudotriquetrum* (Pannewitz et al. 2005) consistent with its reported plasticity (Wasley et al. 2006b).

The influence of water on photosynthetic efficiency was weaker than expected, probably since water was limiting only early in the season due to the high melt. However, the importance of air temperature to photosynthesis (Figure 6.11; column 3 and 4) may be largely due to its control on the availability of melt water. This is supported by the critical AT10 ~ -2 ^oC in *B. pseudotriquetrum* and *C. purpureus* (Figure 6.11; column 3 and 4). This is close to the freezing point of water. Moss tissue freezes at subzero temperatures, and latent heat of freezing uncouples MT

from air temperature in wet environments (Melick and Seppelt 1992; Bramley-Alves 2015). The influence of water availability on pigment accumulation was also weaker than anticipated.

6.4.2 Photosynthetic and protective pigments

6.4.2.1 Photoprotective strategies; pigment signatures

Photoprotective strategies were compared between species by combining UVACs from chapter 5 with the chloroplastic pigments in this chapter. The resulting pigment signatures were different in each species. However, pigments in *C. purpureus* and *S. antarctici* were most similar, particularly in early season (Figure 6.18). *Bryum pseudotriquetrum* has a higher chla/b ratio, lower VAZ pool and higher total UV screening capacity generated mostly from intracellular UVACs (Table 5.2; Table 6.3; Figure 6.18; see also SIMPER tables in Appendix C). The other two species have similar WUVAC concentrations with lower Tchl concentrations in *C. purpureus* (Table 5.2; Figure 5.4; Table 6.2; Figure 6.4). Throughout the season, *S. antarctici* maintained higher β-carotene concentrations and the lowest UV screening capacity with particularly low iUVAC (Table 6.3; Figure 6.9; Table 5.2; Figure 5.5; Figure 5.6, Appendix C). The large seasonal changes in chlorophyll, anthocyanins and Z/VAZ were similar for all species. However the environmental drivers of pigment accumulation differed between species.

The associations between environmental variables and the pigment data clouds were generally strongest in *B. pseudotriquetrum* and weakest in *S. antarctici* (Table 6.7). *Bryum pseudotriquetrum* may have the greatest capacity to alter its pigments and has previously demonstrated high phenotypic plasticity in its photosynthetic response to desiccation (Wasley et al. 2006b). By contrast, there were only three significant associations between environmental variables and the pigment cloud in *S. antarctici* (Table 6.7). This may reflect lower acclimation capacity and/or less exposure to environmental extremes in the more sheltered ecological niche of this species.

The five day light dose was the prime environmental driver for the pigment data clouds of *B. pseudotriquetrum* and *C. purpureus* whilst WC was most important in *S. antarctici* (Table 6.7). Air temperature was influential to the pigment clouds of *B. pseudotriquetrum* and *C. purpureus* but not in *S. antarctici* (Table 6.7). Variation between species and environmental drivers of individual chloroplastic pigments are discussed below, whilst UVACs are discussed in Chapter 5. Ozone column depth and UV-BR influenced anthocyanin accumulation in *C. purpureus* (Figure 5.8*;* E, F). However, other components of the association between ozone column depth and the combined pigment clouds may be spurious (Table 6.7). The unusual behaviour of the ozone hole led to some unusually high ozone column values (>375 DU) in early season. This co-occurred with extremes of cold, wind and low water availability. At this stressful time these high levels of protective ozone, are unlikely to be the cause of the observed changes in pigment concentrations.

6.4.2.2 Chlorophyll

High chlorophyll concentrations at the season's start rapidly reduced in the first two weeks in all species (Figure 6.4; Figure 6.6 C). Several factors could be influencing chlorophyll concentrations. There may be a minor contribution from changes in water content (See Appendix D). This could also be due to the shift from shade to sun acclimation. This occurs after mosses emerge from under protective snow cover into full sunlight. This transition involves reductions in Tchl and increases in photosynthetic enzymes as the emphasis shifts from light capture to photosynthetic processing (Demmig-Adams 1998; Matsubara et al. 2012). For example, a change from high light to low light conditions resulted in a 60% decrease in Tchl concentrations in the epiphytic bromeliad *Guzmania monostachia* (Maxwell et al. 1999). Increased quantities of photosynthetic enzymes and photosynthate (soluble sugars), could also contribute to an apparent reduction in the relative chlorophyll concentration on a mass basis. Alternatively, photoxidative destruction of chlorophyll in high light may lower chlorophyll concentrations. The frozen desiccated state may offer some protection at the season's start (Chapter 4; Heber

et al. 2006a). There is no reason why these factors could not simultaneously act to cause the observed changes in Tchl.

Sun/shade acclimation is reported in Antarctic mosses and shade acclimation may occur in response to low light levels under snow (Post 1990; Schroeter et al. 2012). Cold temperatures preclude metabolism during winter, yet moss may be metabolically active under snow at the tail ends of the summer season, as long as free water is available. In Spring overlying snow melts directly onto the moss, even under a blanket of snow. Snell and co-workers (2007) found a 10cm depth of snow melted over two weeks and suggests reactivation of photosynthesis from overwintering occurs during this time. Growth under snow has been recorded for Antarctic bryophytes, although this is rare (Longton 1988 and references therein). Substantial photosynthetic rates have been reported at MTs of 0 ^{o}C in *B. pseudotriquetrum* and *C. purpureus* (Pannewitz et al. 2005) with photosynthetic activity predicted as low as -8 ^{o}C in *S. antarctici* (Kappen et al 1989). Snow cover reduces light and protects against photoinhibition (Lovelock et al. 1995b). It provides a moist, humid microclimate, perhaps permitting some days or weeks of slow metabolic activity, during which time Tchl concentrations may increase to enhance light capture.

Decreases in Tchl concentrations may then occur as plants transition from the shade to sun acclimation state as snow melts, exposing them to full sunlight. This abrupt change in light levels from under snow to full solar exposure occurs in early season. This contributes to the stress experienced in extreme climatic variables at the beginning of the season, manifesting in high zeaxanthin concentrations (Z/VAZ; Figure 6.8; Figure 6.10).

Additionally, as photosynthetic processing is upregulated, synthesis of other cellular components, such as carbon fixing enzymes, may increase the tissue mass relative to chlorophyll. The enzyme RuBisCO can comprise up to 23-30% of leaf nitrogen and 50% of leaf soluble proteins and is rapidly catabolised and synthesised as needed

(Feller et al. 2008). In vascular species pigments can be reported on a leaf mass per area (LMA) basis, but area is difficult to estimate in the small leaflets of mosses. Some researchers have used proxies for area at the canopy and shoot scale and this is discussed further in the following chapter (Rice and Cornelissen 2014).

All three species showed similar reductions in Tchl throughout early season (Figure 6.4; Figure 6.6 C). Interestingly, decreases in chlorophyll in early season were associated with humid conditions in *C. purpureus* (Figure 6.13 B), and with increasing light in *S. antarctici* (Figure 6.13 A). This could be interpreted as acclimation to the higher light levels driving the summer melt and increasing water availability (see above). Alternatively, chlorophyll bleaching may reduce Tchl.

Chlorophyll bleaching in ambient UV-BR is reported for some Antarctic bryophytes (Newsham et al. 2005; Robinson et al. 2005). Mosses as desiccation tolerant organisms, are better protected in the dry state from photo-oxidative damage from UV-BR (see Chapter 4) and excess PAR (Heber et al. 2006a; Heber et al. 2006b; Yamakawa et al. 2012). At the start of the season, low water contents can occur in exposed moss, and these samples may be protected from photoxidative damage until they are hydrated and metabolically active. Supporting this, the chla/b ratio in *B. pseudotriquetrum* decreased with solar radiation, but only in the highest light levels in mid-season (Figure 6.14), suggesting preferential destruction of chlorophyll a by UV-BR. This species has the highest chla/b ratio of the three species and tends to appear blue green at the start of the season, progressing to a yellow green colour in mid season, which is consistent with the loss of chlorophyll a.

6.4.2.3 Electron transport rates decrease with chlorophyll

Contrary to expectations, photosynthetic rates (ETRs) increased as Tchl concentrations decreased in all species (Figure 6.16). This relationship occurred only in early season as chlorophyll concentrations were lower and more stable at other times. The highest ETRs and lowest chlorophyll concentrations both occur in mid season when solar radiation is high (Figure 6.6 A,C). There are several potential

explanations for this. A minor contribution to this relationship may arise from the increasing water content throughout early season (see Appendix D). Most likely, acclimation to high light results in reduced Tchl and increased photosynthetic enzymes and photosynthate (see above). High ETRs could also reflect higher rates of non-assimilatory electron transport in mid season due to protective processes in high light (Proctor and Smirnoff 2011). Alternatively, photosynthesis may increase due to elevated carbon dioxide produced by heterotrophic microbes associated with the moss beds (see Synthesis). This result cautions against using chlorophyll quantity as a sole measure of plant health or productivity. It also emphasises the importance of technologies such as PAM and LIFT, which provide instantaneous measures of chlorophyll activity via fluorescence (Nichol et al. 2012).

6.4.2.4 Carotenoids

Photoprotective capacity (Z/VAZ) varied most over the season and was responsive to different environmental variables in each species. Other carotenoids, such as VAZ/Tchl and β-carotene were more constant over the season but varied more between species (Table 6.5; Figure 6.7; Figure 6.8; Figure 6.9; Figure 6.10). The lower Tchl concentrations in *C. purpureus* means that the mass of all carotenoids are also lower with respect to tissue mass.

Concentrations of β-carotene were highest in *S. antarctici* concurring with Dunn (2000). This pigment was unaffected by the measured climatic variables in all species (Table 6.3; Figure 6.9). The VAZ pool was lower in *B. pseudotriquetrum* than the other two species (Table 6.3; Table 6.8; Figure 6.7). Perhaps this plant has more capacity to utilise high light in photosynthesis so is less reliant on photoprotection (Lewis Smith 1999).

In all species, the requirement for quenching excess light (determined as Z/VAZ) was twice as high at the season's start, but there were differences between species in the time taken for these high starting Z/VAZ levels to reduce (Figure 6.8; Figure 6.10;). In *B. pseudotriquetrum* stress levels (Z/VAZ) rapidly decreased between early

and mid season, compared with the other species (Figure 6.10). Lovelock and Robinson (2002) also found lower values of A+Z/VAZ conversion in this species. In *C. purpureus,* Z/VAZ reduced gradually between early and late season but in *S. antarctici* particular samples remained stressed (high Z/VAZ). This was unexplained by the measured variables and may relate to microclimate variability (see synthesis).

Different environmental factors appear to force Z accumulation in each species; *C. purpureus* was affected by temperature and light*,* and *B. pseudotriquetrum* by relative humidity*.* In early season, in *C. purpureus,* Z/VAZ decreases with increasing light, as sunlight drives warmer conditions and increased water availability. In *C. purpureus*, in late season, Z/VAZ increased with warmth suggesting heat stress at this time (Figure 6.15 B).

The large gametophytes and abundant rhizoids in *B. pseudotriquetrum* may facilitate responsiveness to RH (see Section 2.3; Figure 2.5; Lewis Smith 1988a; Ochyra et al. 2008). This is supported by the importance of RH, rather than WC to its pigment data cloud (Table 6.7). Additionally, there is a diminished relationship between $\delta^{13}C$ ratios and water availability in this species compared with the two co-occurring species (Bramley-Alves 2015). This suggests it is less affected by water inundation under field conditions. Interestingly, my data finds this species has few samples with low WC (Figure 2.4). It may have more capacity for regulating WC, perhaps using rhizoids or hydroids (Lewis Smith 1988a; Stanton et al. 2014).

Zeaxanthin conversion was generally maintained at high rates (> 20%) throughout the season suggesting sustained Z accumulation as occurs, as in some cold climate vascular plants (Demmig-Adams et al. 2012; Garcia-Plazaola et al. 2012; Verhoeven 2014). High rates of Z conversion were associated with reduced ETR in all three species (Figure 6.17). This is due to Z stabilised NPQ, which safely converts absorbed light energy to heat thus reducing photosynthetic efficiency. The relationship between ETR and Z/VAZ is somewhat weak (R^2=0.33: Figure 6.17) because Z only

actively reduces photosynthetic efficiency when strong light creates a pH gradient across the thylakoid membrane (Garcia-Plazaola et al. 2012). Retention of Z is advantageous in a predictably cold climate since NPQ can then activate within seconds, rather than with the time delay of some minutes required for enzymatic conversion of V to Z (Demmig-Adams et al. 2012).

6.5 Conclusions

Photosynthesis and related pigments are strongly seasonal in Antarctic mosses and this should be accounted for in interannual studies. Photosynthesis is rapidly responsive to the ephemeral variables WC, MT, and PAR in *B. pseudotriquetrum* and *C. purpureus,* but less so in the endemic species *S. antarctici.* The latter species requires wetter conditions, and may be most suited to colder temperatures thus could be disadvantaged in a warmer drier future. These findings are discussed in relation to the other chapters in Chapter 7.

7 Chapter 7 Synthesis

In this thesis I set out to investigate if elevated UV-BR from ozone depletion caused DNA damage in Antarctic mosses within one summer growing season. I also considered if UV damage was affected by UV absorbing compound (UVAC) accumulation, photosynthesis and other environmental factors such as water availability. The study species were two cosmopolitan species, *Bryum pseudotriquetrum*, *Ceratodon purpureus* and one endemic *Schistidium antarctici* found in the Windmill Islands region of Antarctica. During the 2002 atypical ozone behaviour led to low UV-BR over the following summer growing season. These climatic events reduced the impact of UV-BR in this season, and likely led to reductions in field DNA damage and protective UVAC concentrations.

Consequently some of the questions formulated for this thesis shifted. This season actually provided a unique opportunity to study the inherent seasonal variability in photosynthesis and UVACs without the influence of elevated UV-BR. It provides a useful baseline and may better represent the pre-ozone depletion era, facilitating the characterisation of moss physiology without additional UV-BR from ozone depletion.

The following three questions were formulated for this thesis and are reviewed below;

1. Are Antarctic mosses damaged by increased UV-BR from ozone depletion?
2. Are there differences in the effectiveness of protective strategies between co-occurring species?
3. What is the inherent variability in photosynthesis and protective pigments within one summer growing season?

7.1 Are Antarctic moss damaged by UV-BR?

This thesis demonstrates UV-BR specific DNA damage accumulates in natural sunlight under the Antarctic ozone hole in all three species of moss. Since the 2002/03 summer had atypically low UV-BR these damage levels are likely an

underestimate of the interannual average. The effectiveness of the UV protective strategies vary between species, due to differences in the abundance and location of UVACs. I also found species varied in the response of DNA damage accumulation and photosynthesis to other environmental factors such as water and temperature. These interspecific differences indicate that the response to sunlight cannot be broadly typified by classifying mosses as a functional group.

The implications of ozone depletion for the genetic integrity, growth rates and community composition for Antarctic mosses are discussed here. Additionally, the impacts of other environmental factors and the effect of the timing of the UV-BR dose on the seasonal cycle are examined.

Plants are generally reported to be highly tolerant of realistic UV-BR increases in outdoor studies. In the literature, reports of UV-BR damage to plants in natural sunlight, whether as DNA damage, or reductions in growth and photosynthesis are rare. This is because plants have sophisticated mechanisms for protecting against UV-BR. However studies from Polar and desert regions where plants exist at the limits of survival find more evidence of UV-BR impact. This is related to interactions with other environmental stressors (Montiel et al. 1999; Xiong and Day 2001; Newsham et al. 2005; Robinson et al. 2005; Albert et al. 2008; Belnap et al. 2008; Albert et al. 2011). Concurrent multiple stressors can limit the metabolic capacity for induction of repair and protective mechanisms. The Antarctic location, as well as the duration of the study explain why DNA damage was detected here but not in other studies (Lud et al. 2002). This thesis finds that the desiccated state protects the two cosmopolitan mosses from UV-BR induced DNA damage, and also that warmer and wetter conditions reduced DNA damage, but only in *B. pseudotriquetrum*. This study was comprehensive, particularly for Antarctica where logistical constraints frequently limit the duration of research.

The irradiation study found UV tolerance highest in *C. purpureus,* intermediate in *B. pseudotriquetrum* and lowest in *S. antarctici* (Chapter 4). The latter species was

also unable to reduce field DNA damage as UV-BR eased (Chapter 4). Confirming this vulnerability, our group also found morphological changes and chlorophyll bleaching under ambient UV-BR in this species (Robinson et al. 2005). The low levels of field DNA damage accumulation under ambient UV-BR (Chapter 3) are repairable so may not impact long term genetic integrity. However, repair rates may be constrained by other environmental factors.

The capacity for enzymatic repair of DNA damage appears to vary between species. Overnight repair is only suggested for *B. pseudotriquetrum* where damage correlates most strongly with daily UV-BR parameters. This was the only species with negative associations between DNA damage accumulation and water availability and air temperature, suggesting repair plays an important role in warmer, wetter conditions in this species. This implies the impact of UV-BR in this species may be most affected by factors other than incident UV-BR. Longer repair times are inferred in *C. purpureus* where the strongest associations with ambient UV-BR were at the five day time scale. No associations between DNA photoproducts and environmental variables were found in *S. antarctici*. Unlike the other two species this endemic species was unable to reduce DNA damage in the lower UV-BR levels in the latter half of the season. This sustained DNA damage reflected the sustained levels of Z accumulation (Figure 6.10). The environmental drivers of these high stress levels found in particular samples were unclear.

In the two cosmopolitan species the desiccated state protects DNA from UV-BR damage (Chapter 4). This is likely due to passive protection from high concentrations of UVACs, which increase relative to cell area as cells shrink upon drying. In *C. purpureus* the desiccated state provided near complete protection from UV-BR, with dry samples accumulating no increase in DNA damage in UV-BR compared to the UV-BR free controls (Figure 4.2). This is remarkable given that the UV-BR dose used was high: effectively double the highest irradiance received anywhere on Earth (Kinzie et al. 1998).

Since mosses are better protected from damaging UV-BR when dry this implies the timing of snow melt and hydration events will also influence UV-BR exposure and impact. Mosses are inevitably moist as overlying snow melts, so may be particularly vulnerable to UV-BR when emerging from snow cover in Spring. Currently, mosses are protected by snow during the major ozone depletion events in September and October. If global warming leads to earlier snowmelt this will increase the exposure of mosses to UV-BR. We know for example, that light driven tipping points in Antarctic shallow marine ecosystems are fuelled by changes in the timing of protective ice cover, which can exponentially increase incident sunlight (Clark et al. 2013).

Throughout the season, the level of protection provided by desiccation may also depend on the nature of the water source. Mosses relying on seasonal snow banks may become dry and hence protected from UV-BR after the snow is exhausted, receiving only intermittent water from precipitation. However, moss growing on the perimeter of melt lakes and streams, will be metabolically active in strong sunlight which increases meltwater availability.

Whilst desiccation can provide temporary protection from UV-BR in the two cosmopolitan species, long term reductions in water availability will affect survival. Desiccation and/or freeze thaw events are metabolically costly and deleterious when frequent. Moss must be hydrated and photosynthetically active for extended periods to meet minimum energy requirements for survival. The climatic drying trend identified in the Windmill Islands is likely to be the prime driver for shifts in terrestrial vegetation from moisture dependent bryophytes towards more desiccation tolerant lichens (Figure 1.3; Hodgson et al. 2006; Clarke et al. 2012; Wasley et al. 2012; Bramley-Alves et al. 2014; Bramley-Alves 2015). However, increased UV-BR may be a contributing stress.

Reductions in growth due to ozone depletion are reported from long shoots of Antarctic moss, but only *C. purpureus* has been investigated (Clarke et al. 2012).

Since this thesis demonstrates *C. purpureus* is the most UV-BR tolerant species (Chapter 4), it could be anticipated that growth rates in the other two species are even more limited by ozone depletion events. Importantly, ozone depletion increases wind speeds and lowers air temperature as well as affecting incident UV-BR (Robinson and Erickson 2015), so it is unclear which of these environmental factors is actually reducing growth (Clarke et al. 2012).

Even low and transient accumulation of DNA damage has been linked to UV-BR growth reductions in plants growing under the ozone hole (see Section 3.4; Giordano et al. 2003; Giordano et al. 2004). They are implicated in cell cycle checkpoints and reduced transcription (Callegari and Kelly 2007; Vass et al. 2013). The transient accumulation of DNA damage demonstrated under ambient UV-BR may slow growth, with the UV sensitive species *S. antarctici* particularly vulnerable (Chapter 3). In the field study, the relationship between the incident UV-BR component of natural sunlight and DNA damage accumulation in *B. pseudotriquetrum* and *C. purpureus* (Chapter 3) imply an important role for UV-BR in the reported decrease of growth in years with increased ozone depletion (Clarke et al. 2012).

This thesis has focused on the direct impacts of UV-BR on plant physiology. However recent research highlights the UV-BR impact on plants via an indirect effect on associated microbial communities (reviewed in Bornman et al. 2015). The contribution of microbial associations to the health and productivity of Antarctic mosses is poorly characterised across the continent (Cannone et al. 2012). Ultraviolet radiation has complex effects on soil microbes associated with vascular plant species, directly reducing microbial growth and altering community composition, and indirectly, via changes in plant litter (Bornman et al. 2015). Plant litter can be broken down directly via UV (photodegradation), but litter quality is also influenced by UV induced changes to plant tissue chemistry and structure. This includes changes to phenolic compounds (discussed in the following section), C:N ratios, and leaf size and thickness. It is unclear how deeply UV-BR can penetrate

into moss substrata, but environmentally driven changes in bryophyte leaf and shoot structure influence light penetration into the moss turf (Malenovsky et al. 2015).

Increased UV-BR potentially alters microbial populations in moss substrata, and may reduce available CO_2 concentrations and/or increase pathogenicity. In Antarctic moss turves, at a depth of 10 mm, CO_2 concentrations vary seasonally; rising as high as 4229 ppm in midsummer (cf ambient 350 ppm; Tarnawski et al. 1992). This is presumably due to microbial respiration and is likely to strongly influence moss productivity (Tarnawski et al. 1992). In a sample of Antarctic *B. pseudotriquetrum*, photosynthetic carbon uptake increased almost threefold as CO_2 concentrations were increased from 350 to 2000 ppm at 20 ^{o}C (Pannewitz et al. 2005). The impact of UV on microbial communities associated with Antarctic mosses warrants further investigation.

The interspecific differences in UV sensitivity (Chapter 4) may alter community composition, away from the endemic *S. antarctici* towards the two cosmopolitan species. Such changes may have already occurred, but this is difficult to assess as Antarctic terrestrial ecosystems were insufficiently characterised prior to the ozone depletion era. It is also possible that genetic shifts towards more UV-BR tolerant ecotypes have occurred, but this is also difficult to determine, especially if deleterious mutations are rapidly removed from the population. However, the regional drying trend will also affect these species in the same direction since desiccation tolerance and UV tolerance are correlated (discussed below). Variation in UV protective strategies; such as the abundance and location of UVACs is discussed in the next section.

7.2 Are there differences in the efficiency of protective strategies between co-occurring species?

This section relates interspecific differences in UV tolerance (measured as DNA damage) to differences in UVAC quantity and location. Variation in the UV protective strategies utilised by my study species are related to postulated life history strategies derived from functional leaf traits (Wright et al. 2004). The study species are categorised as “fast return” or “slow return” species inferred by combining my functional leaf trait data (chapter 5 and 6) with other published data. These strategies are placed in the context of the UV protective strategy used by each species (see Figure 7.1).

Interspecific differences in quantity of UVACs and their location in the intracellular or cell wall compartments of leaves (chapter 5) can partly explain the clear differences in UV-BR tolerance between the three study species (Chapter 4). The UV sensitivity in the endemic species *S. antarctici* is likely because it has the lowest total UV-screening capacity (cell wall + intracellular UVAC; Chapter 5), due mainly to its low intracellular UVAC concentrations. *Ceratodon purpureus* has the highest UV-BR tolerance (Chapter 4). However, it has intermediate concentrations of total UVACs (Chapter 5), but higher concentrations of cell wall UVACs compared to *B. pseudotriquetrum.* This suggests that the cell wall location confers the increased UV protection in *C. purpureus*. At the cell surface, these compounds may provide a more uniform screen for the underlying contents (Clarke and Robinson 2008). However, the concentrations of cell wall UVACs are similar in *C. purpureus* and *S. antarctici;* respectively the most and least UV-BR tolerant species (chapter 4). Particular UVACs may be more effective at UV protection, for example ρ-coumaric acid (Otero et al. 2009; Rozema et al. 2009; Ryan et al. 2009; Hespanhol et al. 2014).

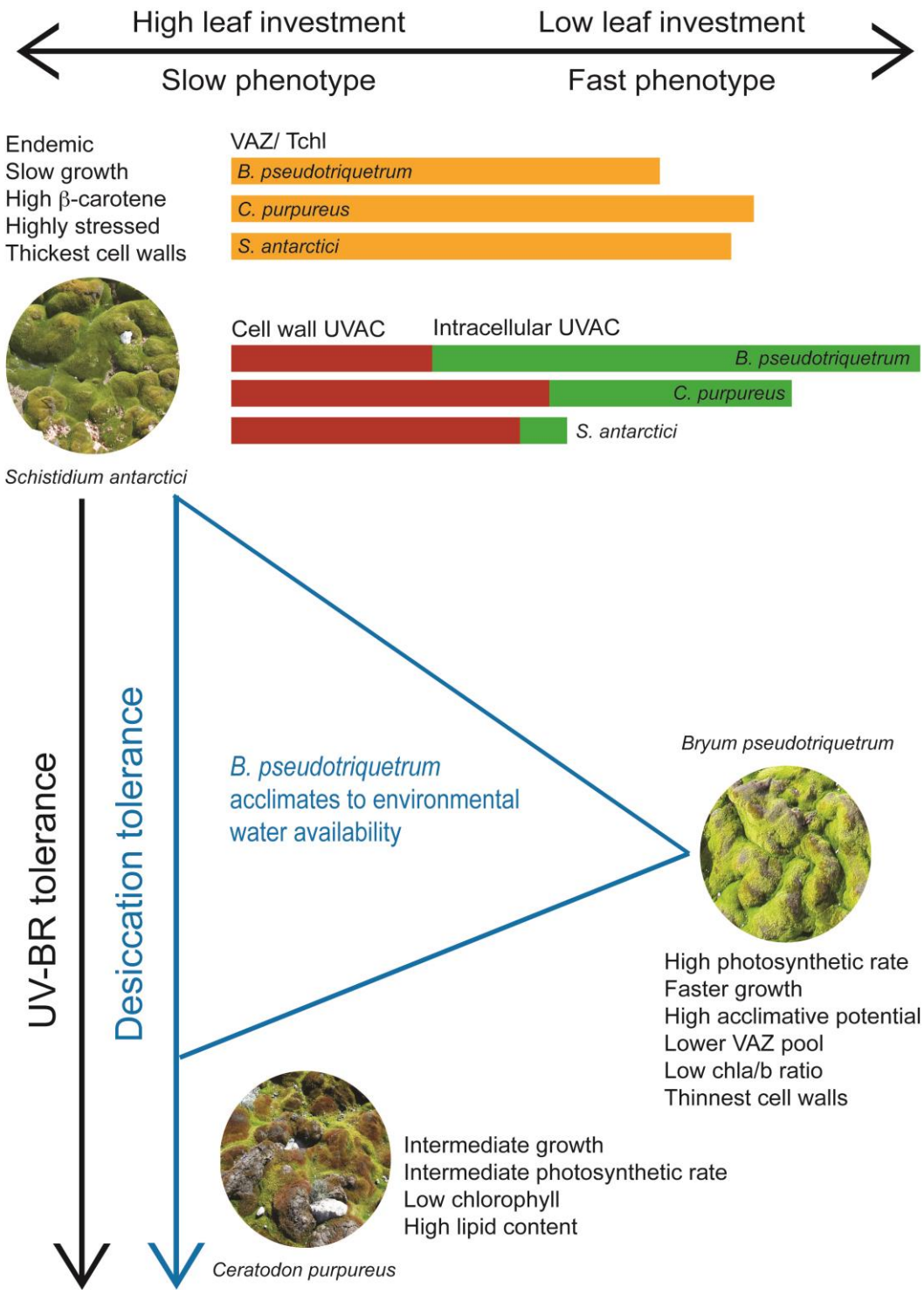


Figure 7.1: Schematic comparing the three co-occurring Antarctic moss species *Ceratodon purpureus, Schistidium antarctici* and *Bryum pseudotriquetrum*. The relative UV-BR tolerance reflects the desiccation tolerance (vertical axis). *B. pseudotriquetrum* appears to have a 'fast' phenotype, while *C. purpureus* and *S. antarctici* may be considered slow phenotypes (horizontal axis). Relative abundance and location of UVAC are shown in red and green. Relative VAZ/Tchl is shown in orange. See text for details.

The isolation and characterisation of individual compounds in the cell wall and intracellular compartments is underway (Waterman 2015), but requires larger samples than used here. This work is crucial to relating the role of these compounds to the variation in UV tolerance between Antarctic mosses, demonstrated in chapter 4. This will also facilitate the use of these compounds as proxies for historical UV-BR levels (reviewed in Bornman et al. 2015). Other factors may also influence UV tolerance; for example DNA repair rates, and the presence of other compounds which absorb UV-BR such as lipids, which occur in high concentrations in *C. purpureus* (Wasley et al. 2006b).

The location of UVAC sequestration may be a leaf trait which relates to metabolic investment and longevity in leaves, and correlates with productivity as described by the worldwide leaf economics spectrum (WLES; see Section 1.4.2.2; Wright et al. 2004). In vascular species, a higher investment in cell walls occurs in the thick, long lived leaves of slow growing species (Hikosaka 2004). In the small leaflets of bryophytes, leaf traits are substituted for more easily measured canopy level traits and these are discussed further below. Testing the applicability of the WLES in *Sphagnum* mosses, Rice et al. (2008) identified two contrasting strategies; one of maximising photosynthesis and the other investing in stress tolerance.

Bryum pseudotriquetrum appears to have a 'fast return' phenotype compared to the other two species (sensu Wright et al. 2004). It has relatively fast growth rates driven by high photosynthetic rates and is a rapid coloniser with high acclimative potential (Lewis Smith 1999; Robinson et al. 2000; Dunn and Robinson 2006; Wasley et al. 2006b; Clarke et al. 2012; McKinley 2012). Throughout the 2002/03 season it had the highest photosynthetic efficiency (Table 6.2; Figure 6.3), a rapid and consistent decline in Z/VAZ (Figure 6.10), lower VAZ pool (Table 6.3) and a higher chla/b ratio (Table 6.2). This suggests this species has less requirement for quenching of excess light and a high capacity for processing strong sunlight (Chapter 6). Equally, *B. pseudotriquetrum* may have a greater capacity for repair of DNA damage, potentially enabled through its higher productivity. This species has lower

investment in cell walls, with thinner cell walls and lower WUVAC levels (Chapter 5; Bramley-Alves 2015).

The other two species may be 'slow return' phenotypes with slower growth and photosynthetic rates, and thicker cell walls containing more UVAC (Chapter 5) (Lewis Smith 1999; McKinley 2012; Bramley-Alves 2015). *Ceratodon purpureus* also has a heavy investment in fats and oils and low total chlorophyll (Tchl) concentrations possibly diverting metabolic resources from rapid growth to stress protection (Table 2.1; Wasley et al. 2006a). The placement of these species on a fast to slow axis of functional leaf traits requires clarification by further characterisation of chemical, structural and metabolic traits.

The low UV tolerance in the endemic species *S. antarctici* could reflect its polar evolutionary history under historically low UV-BR levels (Ziska et al. 1992). In contrast, Antarctic cryptoendolithic fungal species had high concentrations of UV absorbing compounds and were substantially more tolerant of UV-BR than species from elsewhere. Perhaps aspects of fungal adaptation to the extreme conditions also preadapts them to tolerate UV-BR (Selbmann et al. 2011).

The relative desiccation tolerance of these mosses (Wasley et al. 2006b) is a useful predictor of UV tolerance (Figure 7.1). High desiccation and UV-BR tolerance co-occur in *C. purpureus,* are intermediate in *B. pseudotriquetrum* and are lowest in *S. antarctici* (Figure 4.2; Figure 4.3). This correlation between UV and desiccation tolerance is also reported for other moss species (Seel et al. 1992a; Tákacs et al. 1999; Csintalan et al. 2001). Certain features of desiccation tolerance such as thicker leaves and increased branching and reduced plant height may also reduce light penetration into the leaf, improving UV-BR tolerance (Manetas et al. 1997; Gitz and Liu-Gitz 2003; Poulson et al. 2006).

Further characterisation of functional shoot and colony traits is an interesting area for future research. Functional leaf traits, such as used in the WLES, and their shifts along environmental gradients have potential for understanding ecosystem scale

responses to global climatic changes. In vascular species, the WLES describes correlations between productivity and other leaf traits, for example leaf thickness (Wright et al. 2004). This facilitates the prediction of plant productivity from more easily measured leaf traits. Bryophytes are major components of alpine and Polar ecosystems, so make a considerable contribution to global carbon storage. There are large ranges in productivity within taxa and functional types (eg. mosses, shrubs forbs etc.). Thus, such categories are unreliable for estimating productivity and carbon storage used for modelling ecosystem carbon fluxes (Laing et al. 2014 and references therein).

In Antarctic mosses, shoot leaf density can be estimated using spectroscopy imaging (Malenovsky et al. 2015), but its relationship to productivity requires further investigation. Other structural traits could be characterised in these Antarctic mosses, such as canopy mass per area and canopy density, which vary across light and water gradients (Waite and Sack 2010; Rice and Cornelissen 2014) and like leaf density are potentially detected by near infrared wavelengths (Malenovsky et al. 2015). Furthermore, measuring area based colony traits would allow pigment concentrations to be reported on an area basis, facilitating the interpretation of the mass based measurements (see below). Hyperspectral scans in near remote sensing from unmanned aerial vehicles or tripods, can potentially capture these bryophyte shoot and canopy attributes which relate to productivity (Malenovsky et al. 2015). Thus, rapid and non-destructive assessment of plant health and productivity may be ultimately possible.

7.3 What is the inherent variability in photosynthesis and protective pigments within one summer growing season?

This section discusses the seasonal variation in photosynthesis, UVACs and chloroplastic pigments presented in Chapters 5 and 6. Relationships between anthocyanins and both photosynthetic electron transport rates (ETRs) and zeaxanthin (Z/VAZ) concentrations are presented.

The first three weeks of the summer season were when most changes in photosynthesis and pigments occurred within the mosses (Figure 6.18). This is consistent with the moss transitioning from the overwintering, dormant state to higher photosynthetic efficiency as the season warmed. Throughout the early part of the season, photosynthesis and anthocyanin concentrations increased while Z/VAZ, WUVACs and Tchl decreased, remaining fairly stable for the remainder of the sampling period. Sampling was not continued through the later stages of the season but I predict that reverse changes would occur in photosynthesis and pigments as the season cools.

The photosynthetic electron transport rates (ETRs) reported here are somewhat higher than laboratory measurements in the same species (McKinley 2012) but correspond to other field measurements of ETR in Antarctic mosses (Pannewitz et al. 2003; Schroeter et al. 2012). Field chlorophyll fluorescence measurements of photosynthesis may be higher due to additional assimilatory and non-assimilatory contributions from elevated field CO_2 (see Section 6.1.4; Tarnawski et al. 1992) and excess light (Proctor and Smirnoff 2011), respectively. Indeed these components potentially have large and opposing impacts on bryophyte productivity. Thus it is unclear what proportion of electrons proceed to photosynthetic carbon fixation.

Laboratory conditions are different from field conditions. In artificial laboratory light, protective non-assimilatory ETRs may be reduced. The full spectrum of natural sunlight, including the UV-BR component may be an important driver of protective and dissipative electron transport (discussed in Section 1.4.1; Section 6.1.1).

Furthermore, increases in photosynthetic carbon gain due to *in situ* elevated CO_2 from microbial respiration will alter in gas exchange chambers or ETRs measured in the laboratory (discussed in Chapter 6). To accurately predict moss productivity using fluorescence, the proportion of electrons resulting in carbon fixation will need to be assessed. This could be achieved through simultaneous measurements of gas exchange and chlorophyll fluorescence. Light response curves could be attempted in ambient sunlight using filters and/or light supplementation to adjust incident light and under a range of different CO_2 concentrations. Furthermore, new technology such as the NADPH9-AA module for the Dual-PAM-100 has the potential to non-invasively provide rates of non-assimilatory ETR, such as cyclic electron flow (Schreiber and Klughammer 2009). Characterisation of the diurnal and seasonal variation in CO_2 concentrations within the moss turf is also important (Tarnawski et al. 1992).

The high ETRs in the 2002/03 season may be partly due to elevated CO_2 from high levels of microbial respiration (Tarnawski et al. 1992), amplified by the early and widespread melt. Currently little is known about the factors governing the *in situ* CO_2 concentrations in Antarctic moss turves, and seasonal measurements of CO_2 are an important next step for understanding productivity in these communities (Tarnawski et al. 1992; Cannone et al. 2012). For example, how do wind speed, temperature and water availability affect both the production of CO_2 and its gradients within moss turves? These factors influence photosynthesis directly but also affect microbial activity and related CO_2 availability. Increased windspeeds may cause both cooling and desiccation, resulting in lowered metabolism. Additionally, wind can disturb the moss boundary layer and disperse CO_2. It may be difficult to tease apart such complex interactions. Moss substrate depth and vertical gradients in temperature are simple and non-destructive measurements that may influence microbial CO_2 accumulation. These may be useful environmental factors for predicting field ETRs, in studies like mine (Chapter 6; Figure 6.12). Furthermore,

modelling the dynamics of the moss boundary layer will also facilitate understanding of CO_2 gradients above the moss turf.

This thesis demonstrates that anthocyanins accumulate in Antarctic moss as conditions warm, with the highest concentrations in mid summer (Chapter 5). However the role of such transient accumulation of anthocyanins in vegetative tissue is unclear (see Chapter 5). The relationships between anthocyanins (Chapter 5), electron transport rates and the rates of zeaxanthin conversion (Chapter 6) are reported here (for statistics see Section 5.2.4.2).

Anthocyanin concentrations increased with photosynthetic electron transport rate in all species but only in mid season (Table 7.1; Figure 7.2). Anthocyanin concentrations had an inverse relationship with the capacity for heat dissipation in photosystem II (PSII) via zeaxanthin (Z/VAZ) but this was only significant in *C. purpureus* (Table 7.1; Figure 7.3). The other two species had similar, but insignificant associations (data not shown).

Anthocyanins appear to be produced when ETRs are high (Figure 7.2). Anthocyanin concentrations and ETRs were also higher in samples from under shade cloth and from the valleys of the undulating moss microtopography in our field UV screening experiment using *S. antarctici* (Robinson et al. 2005). Shaded samples may be protected from photoinhibition and chlorophyll bleaching in full solar radiation, and thus maintain higher ETRs.

Table 7.1: Relationships between anthocyanins (Anth; $A_{\Delta526}.gdw^{-1}$) and electron transport rates (ETR; µmol e m^{-2} s^{-1}), and the ratio of zeaxanthin to xanthophyll pool (Z/VAZ; nmol $nmol^{-1}$) showing significant results from three way ANCOVAs where the main effects were pigments or ETR, collection date (early; 9 November to 2 December 2002, mid; 5 December 2002 to 4 January 2003; late; 7 January to 1 February 2003) and species: *Bryum pseudotriquetrum* (B), *Ceratodon purpureus* (C) and *Schistidium antarctici* (S). If collection date or species were the only significant effects results are not shown. Significant at p=0.05*-0.01**.

Pigments	R^2	Effects $DF_{(2,1,2)}$	F value	P value
Anth vs ETR	0.48	ETR	2.9173	**0.0945****
		Collection date (E,M,L)	2.1360	0.1299
		Sp	0.1815	0.8347
		ETR*Collection date	2.6587	**0.0810****
		ETR*Sp	0.1280	0.8802
		Collection date*Sp	2.3261	**0.0707****
		Collection date*Sp*ETR	1.7920	0.1471
Z/VAZ vs Anth	0.67	Anth	4.6448	**0.0365***
		Collection date (E,M,L)	7.6277	**0.0014***
		Species (B,C,S)	2.7637	0.0738
		Collection date* Anth	0.9309	0.4017
		Sp*Anth	2.6169	**0.0841****
		Collection date*Sp	3.4854	**0.0146***
		Collection date*Sp*Anth	1.9562	0.1175

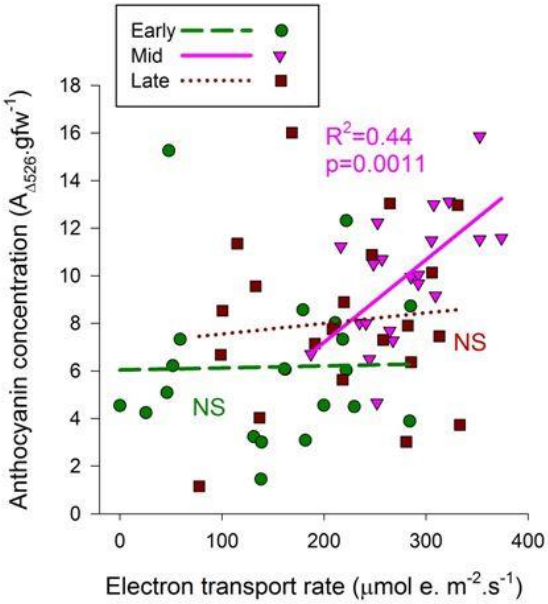


Figure 7.2: Correlations between daily mean anthocyanin concentration and daily mean electron transport rate in all three species, in mid season (Anthocyanin = 0.35+ 0.035*ETR, n=21). Dates as per Table 7.1. Non-significant (NS). Data are daily means (n=3) except for sample 1 (n=1).

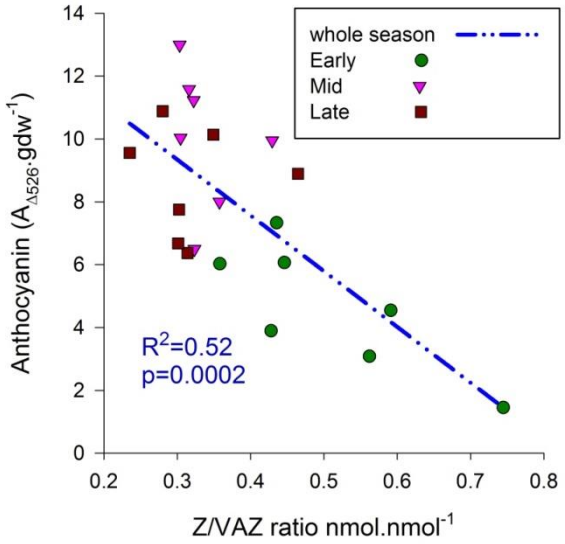


Figure 7.3: Correlations between anthocyanin concentration and Z/VAZ ratio over the whole season in *Ceratodon purpureus* (Anthocyanin = 14.7 - 17.8*Z/VAZ, n=21). Data are daily means (n=3) except for sample 1 where n=1.

There are several possible explanations for the relationship between high anthocyanin concentrations and ETRs. Anthocyanin accumulation may artifactually increase ETR by absorbing PAR before it reaches the chloroplast, but it is not possible to quantify this effect. However, it is unlikely that the increases in anthocyanins alone are responsible for the high ETRs seen in mid-season. Higher photosynthetic rates may provide adequate metabolic resources for the synthesis of these compounds. Anthocyanin synthesis requires light and is reported to increase with intracellular sugar concentrations (Steyn et al. 2002; Tognetti et al. 2013; Landi et al. 2015), both of which increase in conditions of high photosynthesis.

Anthocyanins also increased as cell wall UVAC decreased implying complementary roles for these compounds (Figure 5.10). If higher photosynthetic rates enable active growth, anthocyanins may provide protection to actively growing cells when cell wall UVACs are low (Figure 5.10). This relationship would be easier to investigate in the expanding leaves of suitable vascular species, where leaf age is easily monitored. In mosses it is difficult to isolate individual leaflets and physiological measurements combine many vertical layers of different ages within the moss canopy.

In mosses, further investigation of the relationship between cell wall UVACs and growth may assist in resolving whether cell wall UVACs are induced by UV-BR. This would assist in the use of these compounds as proxies for historical UV-BR. Future experiments may consider UV irradiation in conditions which reduce growth (for example in lower temperatures) however growth rates are difficult to reliably measure in these tiny plants (Clarke et al. 2012). Cell size may also be a useful indicator of active growth. Both the complete genome (Nordberg et al. 2014) and transcriptome is now known for *C. purpureus* (Szövényi et al. 2015). Thus transcriptome profiling could track growth and pigment production under different light conditions in growth chambers.

Only *C. purpureus* has a characteristic anthocyanic red colouration (Post 1990). This is perhaps explained by its higher anthocyanin concentrations relative to chlorophyll. This is the only species where anthocyanin concentrations inversely correlate with Z/VAZ (Figure 7.3; also see Chapter 5). So, more anthocyanins result in a lower requirement for excess light to be dissipated. This was also the only species where anthocyanin concentrations increased with UV-BR. These compounds may have a particular role in UV-BR protection in this species.

However, all three species had similar seasonal variation in anthocyanin concentrations, peaking in mid-season when photosynthetic electron transport was highest (Figure 7.2). Colour may not always relate directly to anthocyanin concentrations. Anthocyanin colour is regulated by pH and colourless tautomers can exist in the cytosol (Neill and Gould 2003). Colour can also be stabilised by glycosylation and acylation with other moieties such as flavonoids (Manetas 2006). Chloroplast movement in high light may also reduce the green reflectance from chlorophyll and enhance the red signal (Takahashi and Badger 2011). The isolation and characterisation of the individual compounds is the obvious next step in understanding their functional role. However, they can be difficult to isolate and large amounts of tissue are required (Hooijmaijers and Gould 2007).

Understanding the changes in Tchl discussed in chapter 6 could be facilitated by reporting concentrations with respect to area based measurements at the shoot or colony scale. These have been used to test the WLES in bryophytes (Rice et al. 2008; Waite and Sack 2010; Waite and Sack 2011). This could overcome the complications inherent in mass based measurements due to variation in other cellular constituents such as sugars, lipids and enzymes. There are also differences between species in the proportion of cellular to structural constituents. For example, thicker cell walls and higher concentrations of lipids in *C. purpureus* imply it has less active metabolic components (less cytosolic contents) than *B. pseudotriquetrum* on a weight basis. In vascular species pigments are often reported relative to both mass and area. Leaf mass per area is simple to estimate in the large leaves of vascular species but more difficult in the small leaflets of mosses. It would be worth investigating shoot or colony mass per area. The latter perhaps being easier to measure in these small Antarctic mosses (Rice and Cornelissen 2014).

Microsite variation remains unresolved in this study. Pigment concentrations were highly variable between samples, probably due to microtopography and the influence of surrounding rocks and distance to water sources. Small changes in moss microtopography may cause large differences in exposure to solar radiation, wind, temperature and water availability on the small scale of these tiny plants. Only unshaded microsites were sampled, yet generally weak associations occurred between pigments and the environmental data measured nearby, at Casey station. 'Instantaneous' WC, PAR and MT, measured at each microsite, were important for rapidly responsive photosynthesis but lacked strong associations with pigments, which responded over a longer time scale. New techniques are currently in development to characterise moss microsites. Stable isotope analysis can indicate long term water availability (Wasley et al. 2006b; Clarke et al. 2012; Royles et al. 2012; Bramley-Alves et al. 2015) and near remote sensing in hyperspectral or visible wavelengths could provide information on aspect, exposure and light interception (Lucieer et al. 2014; Turner et al. 2014a; Malenovsky et al. 2015). These techniques

may be fruitful in characterising differences in growth conditions between microsites.

A warmer climate may increase the extent of bryophyte cover, with improved productivity for all species, but this will be strongly dependent on concomitant changes in water availability. Increased warmth may increase available melt water, but also may exhaust snow reserves and result in drier conditions (Wasley 2004).

Differences between species in the temperature and water response for photosynthesis and related pigments suggest the two cosmopolitan species *B. pseudotriquetrum* and *C. purpureus* may benefit under future warming scenarios. Both species maintain photosynthetic efficiency (PSII) at high temperatures (Figure 6.11; A2, B2, A4). Currently*, B. pseudotriquetrum* is least abundant in the Windmill Islands, rarely forming extensive turves, and may be more vulnerable to cold, losing more soluble carbohydrates than the other two species in freeze-thaw events (Melick and Seppelt 1992). Its distribution may expand under a warming climate, if water availability is sufficient. Drier conditions are preferred by *C. purpureus* (Figure 6.11; A3, B3) and its distribution may expand if the drying trend evident in the Windmill Islands continues (Hodgson et al. 2006; Bramley-Alves 2015).

Schistidium antarctici was the only species with a similar pigment cloud in early and late season, mainly due to an unexplained and sustained requirement for quenching (Z/VAZ) observed in particular samples over the season (Figure 6.10). The sustained high concentrations of β-carotene in *S. antarctici*, may also indicate higher stress levels (Fanciullino et al. 2014). Photosynthesis in *S. antarctici* appears better suited to cold temperatures (Figure 6.11 C3), and this species is most dependent on high water availability (Wasley et al. 2006b). It has the lowest UV screening capacity (Figure 5.6) and the highest UV-BR sensitivity (Figure 4.2; Figure 4.3). Photosynthesis and pigments are generally less responsive to environmental variables, than in the other two species (Table 6.7).

The photosynthetic temperature response of *S. antarctici* requires confirmation in controlled conditions since photosynthesis was not well explained by the environmental variables used. In this species, the relatively weak response of photosynthesis to temperature, water and light, is in itself an interesting result. What then is driving photosynthetic rates in this species? In its wetter niche, this species may be buffered from temperature extremes. This endemic species currently dominates the landscape, but it may be vulnerable in a drier, hotter future.

7.4 Conclusions

All species accumulated DNA photoproducts in natural sunlight. Since this study was conducted in a year of atypically low UV-BR these may underestimate the levels found in other seasons. Tolerance to UV-BR is highest in *C. purpureus*, intermediate in *B. pseudotriquetrum* and lowest in the endemic *S. antarctici.* In *B. pseudotriquetrum,* DNA damage was negatively associated with water content and air temperature, implying enzymatic repair is important in warmer, wetter conditions.

This is also the first study, to my knowledge, that demonstrates dry moss have increased protection from UV-BR. This identifies an important role for the passive protection provided by UVACs. Avoiding UV-BR absorption using UVACs is an effective strategy for the Antarctic setting where concurrent multiple stressors reduce metabolism and the capacity for enzymatic repair of DNA damage.

Anthocyanin concentrations are only positively associated with the ambient UV-BR dose in *C. purpureus* and may have a particular role in UV-BR protection in this highly UV tolerant species. However, all species utilise this form of protection. No relationship between UV-BR dose and concentrations of UV absorbing compounds was found. This result is inconclusive since UV-BR may have remained below the threshold for induction in this season. However, concentrations of cell wall UVACs decreased in warmer conditions, possibly due to the synthesis of cells walls in

actively growing cells. This should be considered when investigating WUVAC accumulation in UV-BR. Anthocyanins and UVAC concentrations were inversely associated. These pigments may provide complementary UV protective roles, particularly during periods of active growth.

The differences in the effectiveness of protective strategies between species may lead to future changes in community composition within the bryophyte community. The endemic species *S. antarctici* is most vulnerable to elevated UV-BR, increased temperatures and lowered water availability. Since desiccation tolerance and UV tolerance are correlated both pressures will act in the same direction, favouring the expansion of the *C. purpureus* population.

APPENDIX A

Modelled missing light data

Building a model to fill missing light days using raw standard erythemal dose and light data from all days across the season. The same main effects were achieved using BIB AICc and p values.

Stepwise Fit for standard erythemal dose (SEDs) in W.m^{-2}

Stepwise Regression Control

Stopping Rule:

Prob to Enter	0.25
Prob to Leave	0.1

Direction:

19 rows not used due to excluded rows or missing values.

SSE	DFE	RMSE	RSquare	RSquare Adj	Cp	p	AICc	BIC
2.595122	77	0.1835835	0.7290	0.7079	5.607476	7	-35.78	-18.26

Current Estimates

Lock	Entered	Parameter	Estimate	nDF	SS	"F Ratio"	"Prob>F"
X	X	Intercept	2.30005412	1	0	0.000	1
	X	mxw km/hr	-0.0032816	1	0.680909	20.203	2.42e-5
	X	day #	-0.0024735	1	0.115889	3.439	0.06752
	X	AT min	0.01822602	1	0.136507	4.050	0.04766
		CC mean	0	1	0.012699	0.374	0.5428
	X	CCsd	0.04840855	1	0.100766	2.990	0.0878
	X	sunhours	0.03305124	1	1.513199	44.898	3.05e-9
	X	oz	-0.0037752	1	0.600362	17.813	6.6e-5
		wr km/hr	0	1	0.009232	0.271	0.60396

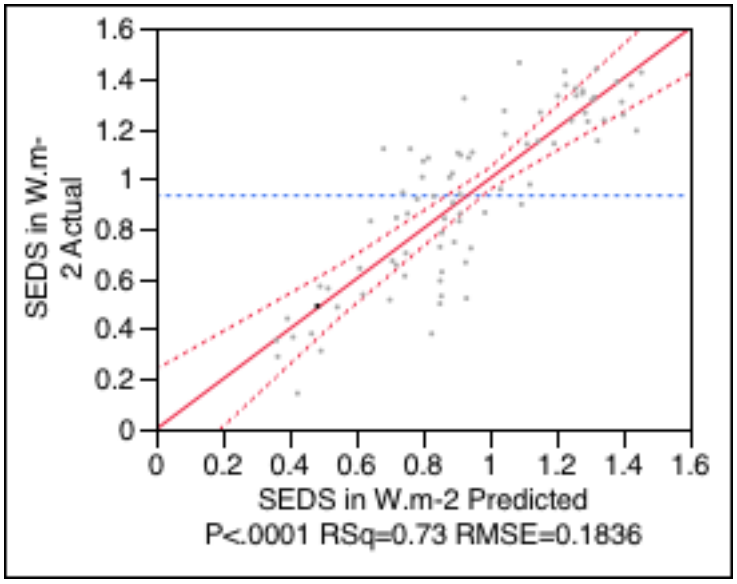
Step History

Step	Parameter	Action	"Sig Prob	Seq SS	R Square	Cp	p	AICc	BIC
1	All	Entered	.	.	0.7312	9	9	-31.366	-10.071
2	wr km/hr	Removed	0.6274	0.008152	0.7303	7.2375	8	-33.681	-14.236
3	CC mean	Removed	0.5428	0.012699	0.7290	5.6075	7	-35.782	-18.255
4	CCsd	Removed	0.0878	0.100766	0.7185	6.5432	6	-35.028	-19.486
5	AT min	Removed	0.0877	0.10337	0.7077	7.5549	5	-34.25	-20.756
6	day #	Removed	0.1928	0.061135	0.7013	7.336	4	-34.757	-23.372
7	mxw km/hr	Removed	0.0000	0.665914	0.6318	24.737	3	-19.44	-10.223
8	CCsd	Entered	0.1354	0.097526	0.6420	23.896	4	-19.532	-8.1476
9	Best	Specific	.	.	0.7013	7.336	4	-34.757	-23.372
10	All	Entered	.	.	0.7312	9	9	-31.366	-10.071
11	wr km/hr	Removed	0.6274	0.008152	0.7303	7.2375	8	-33.681	-14.236
12	CC mean	Removed	0.5428	0.012699	0.7290	5.6075	7	-35.782	-18.255

Response SEDS in W.m-2

Whole Model

Actual by Predicted Plot



Summary of Fit

RSquare	0.729024
RSquare Adj	0.707909
Root Mean Square Error	0.183583
Mean of Response	0.931641
Observations (or Sum Wgts)	84

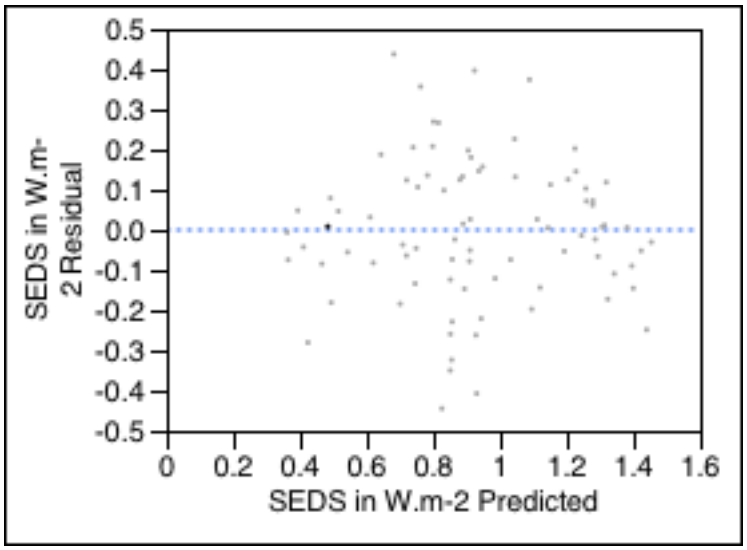
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	6	6.9818096	1.16363	34.5263
Error	77	2.5951220	0.03370	**Prob > F**
C. Total	83	9.5769316		<.0001*

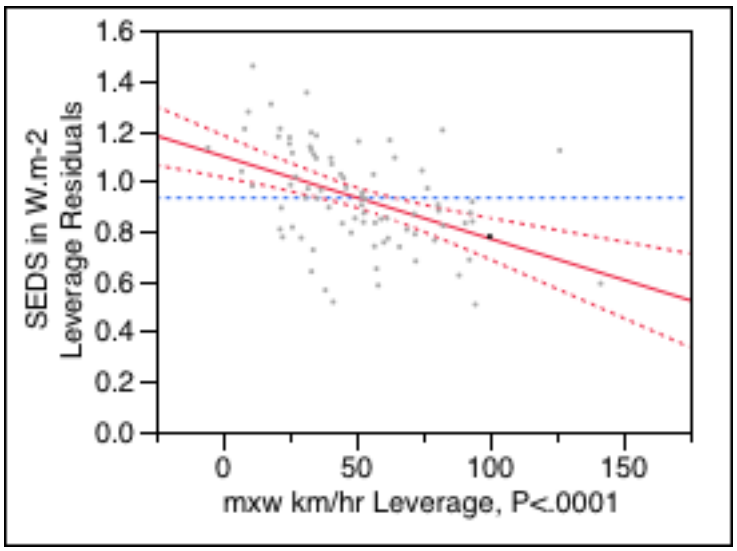
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	VIF
Intercept	2.3000541	0.347047	6.63	<.0001*	.
mxw km/hr	-0.003282	0.00073	-4.49	<.0001*	1.4181483
day #	-0.002474	0.001334	-1.85	0.0675	3.5413283
AT min	0.018226	0.009056	2.01	0.0477*	1.9184195
CCsd	0.0484085	0.027996	1.73	0.0878	1.7690065
sunhours	0.0330512	0.004933	6.70	<.0001*	1.8553798
oz	-0.003775	0.000894	-4.22	<.0001*	2.3000411

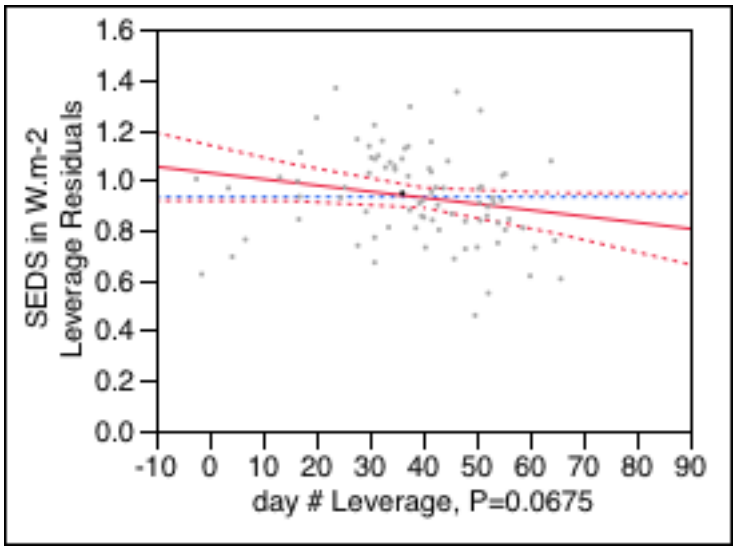
Residual by Predicted Plot



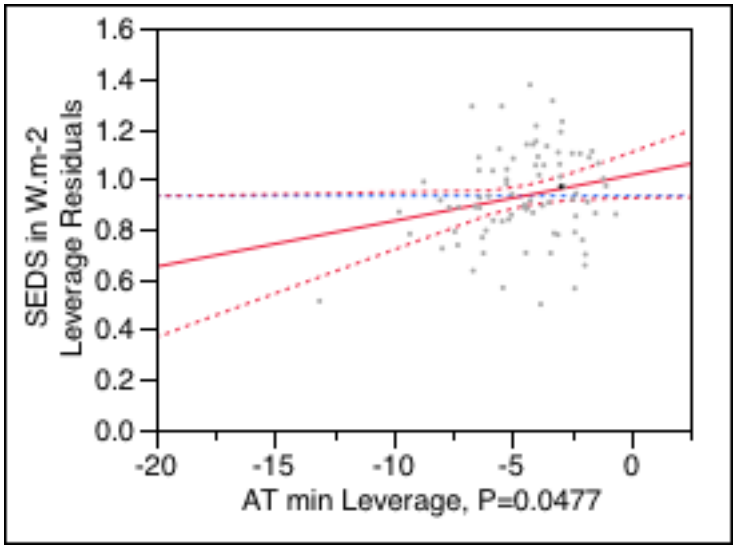
mxw km/hr
Leverage Plot



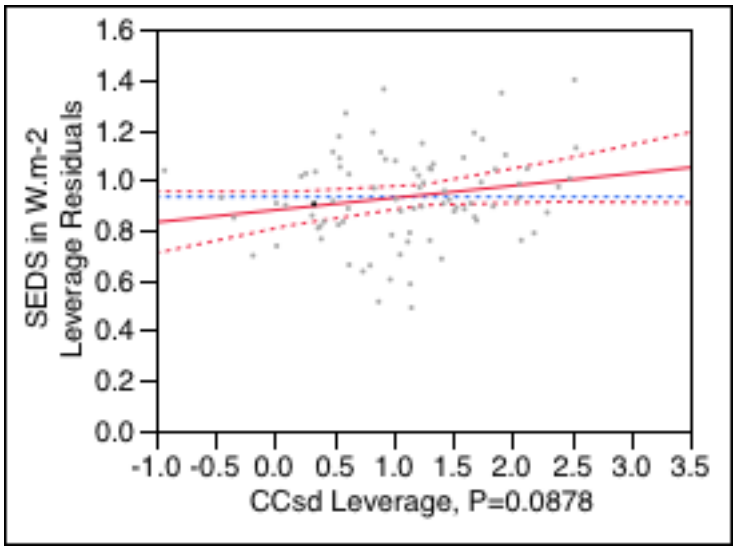
day #
Leverage Plot



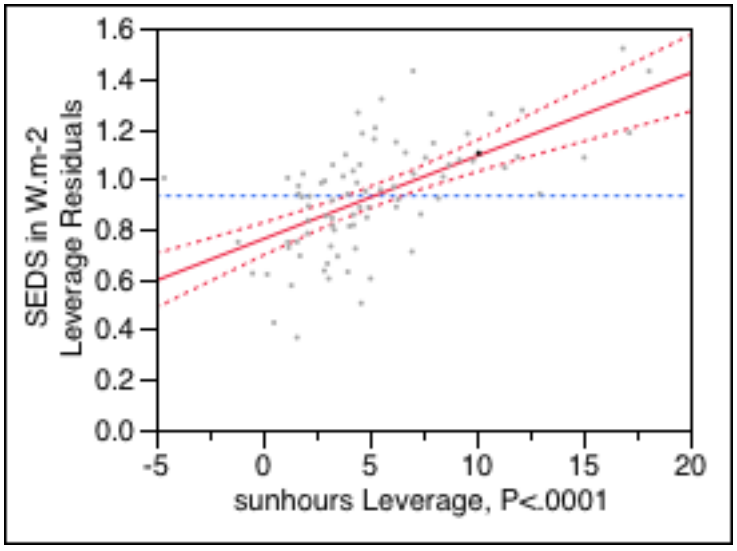
AT min
Leverage Plot



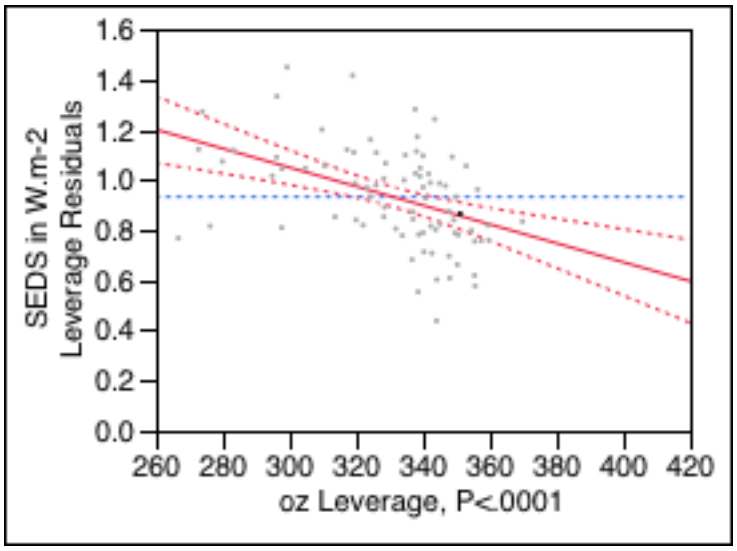
CCsd
Leverage Plot



sunhours
Leverage Plot



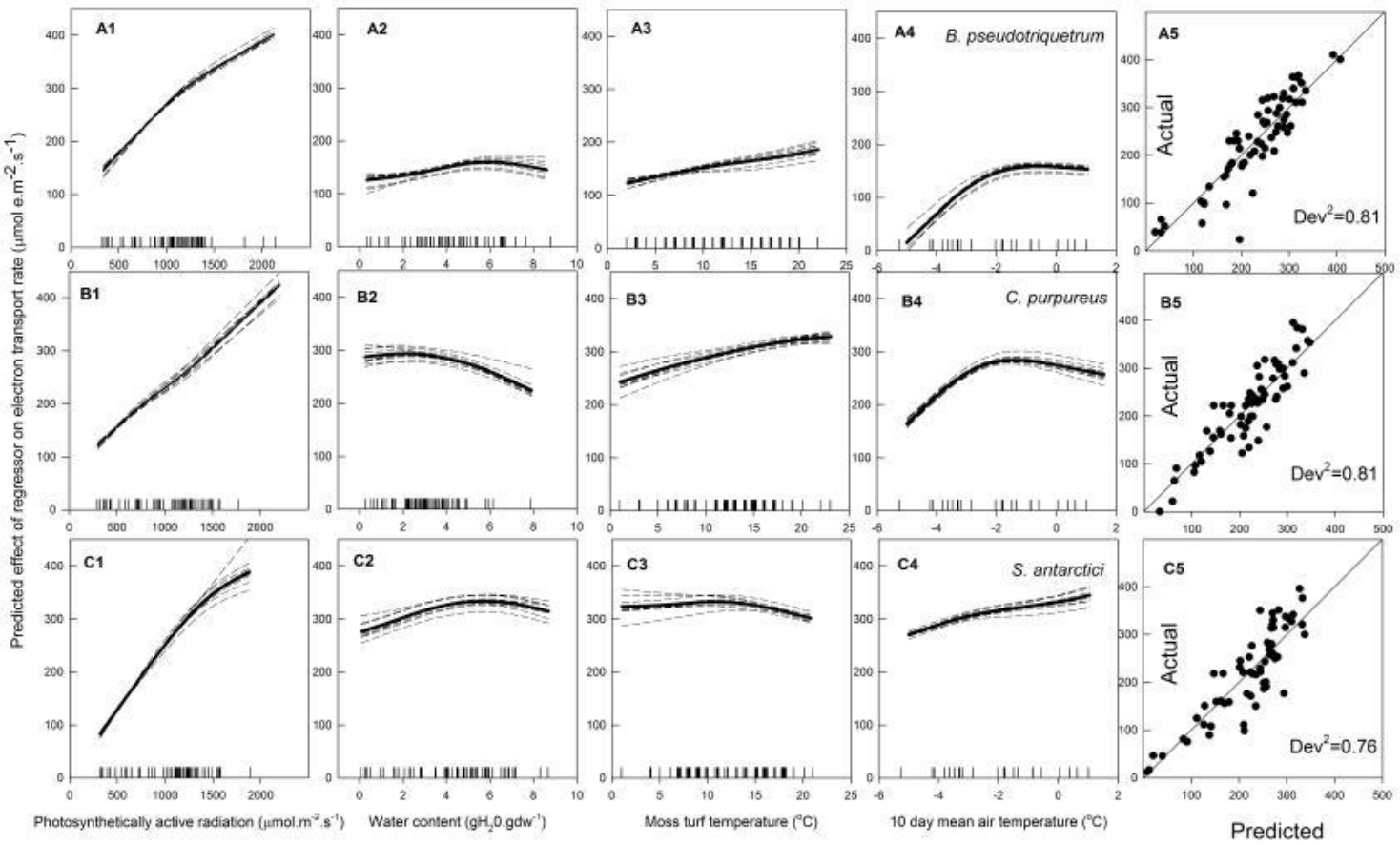
oz
Leverage Plot



Durbin-Watson

Durbin-Watson	Number of Obs.	AutoCorrelation	Prob<DW
1.4190605	84	0.2731	0.0010*

APPENDIX B



Electron transport rates predicted from GAMS

Figure 1: Electron transport rates (ETR) predicted from generalized additive models (GAMS) in *B. pseudotriquetrum* (A1-A4), *C. purpureus* (B1-B4) and *S. antarctici* (C1-C4) holding other variables constant using; (A1,B1, C1) photosynthetically active radiation (PAR), (A2, B2, C2) water content, (A3, B3, C3) turf moss temperature and (A4, B4, C4) 10 day mean air temperature. The GAM from all data points is shown (solid line) along with 10 models each created using 90% of the data (dashed lines). The rugplot on the x-axis shows the number and distribution of the observations. Actual versus predicted effective yield and the goodness of fit (Dev^2) are shown (A5, B5, C5).

APPENDIX C SIMPER TABLES

The average squared distance is the Euclidean distance between the samples in high dimensional space. This measure assesses the percent contribution from each pigment to either the dissimilarity between two groups or the similarity within a group. A two way crossed layout only considers dissimilarities across time within each species (and *vice versa*) thus removing the effects of each factor from the other. High variability leads to inconsistent contributions from pigments except where there are large differences between species in the pigment levels (eg. intracellular UVAC)

Similarity within Species (across all time groups) two way crossed

Different pigments contribute to similarity within each species (see Table 1). In *B. pseudotriquetrum* anthocyanins and total chlorophyll combine to account for 45% of these similarities. In *C. purpureus* the most important pigments were the cell wall UVAC and the chla/b and VAZ/ Tchl, accounting for 71% of the similarity. Within *S. antarctici* β-carotene/Tchl ratio was the most important, yet this pigment accounts for <5% of similarity for the other two species. Z/ VAZ made a consistent contribution of about 10% to similarity for all species. Intracellular UVAC was a low contributor for all species.

Table 1: Average similarity results within *B. pseudotriquetrum, C. purpureus and S. antarctici*. SIMPER resemblance matrix based on Euclidean distances.

Species	Pigments	Av.Value	Av.Sq. Dist	Sq.Dist /SD	Contrib %	Cum. %
B. pseudotriquetrum Average squared distance = 5.25	Anthocyanin	9.2	1.29	0.5	24.58	24.58
	Tchl	570	1.07	0.3	20.36	44.94
	Chl a/b	3.1	0.782	0.52	14.89	59.83
	WUVAC	8280	0.628	0.41	11.95	71.78
	Z/VAZ	0.34	0.518	0.45	9.87	81.65
	VAZ/ Tchl	0.10	0.468	0.4	8.92	90.57
	IUVAC	16774	0.365	0.45	6.94	97.51
	β-carot /Tchl	0.09	0.131	0.49	2.5	100
C. purpureus Average squared distance = 4.60	Chl a/b	3.01	1.18	0.53	25.6	25.6
	VAZ/Tchl	0.11	1.14	0.44	24.73	50.33
	WUVAC	12736	0.941	0.34	20.43	70.76
	Z/VAZ	0.39	0.432	0.38	9.39	80.15
	Anthocyanin	7.76	0.326	0.54	7.08	87.23
	Tchl	323	0.239	0.34	5.2	92.43
	β-carot /Tchl	0.11	0.205	0.41	4.46	96.89
	IUVAC	7808	0.143	0.49	3.11	100
S. antarctici Average squared distance = 5.51	β-carot /Tchl	0.16	1.67	0.48	30.4	30.4
	Tchl	554	0.729	0.32	13.23	43.63
	Anthocyanin	7.22	0.714	0.51	12.97	56.6
	Chl a/b	2.84	0.701	0.5	12.73	69.33
	VAZ/Tchl	0.12	0.57	0.42	10.36	79.69
	Z/VAZ	0.40	0.569	0.45	10.33	90.02
	WUVAC	10912	0.509	0.51	9.25	99.27
	IUVAC	3511	0.0404	0.43	0.73	100

Dissimilarity between each pair of species (across all time groups)

The dissimilarity between species was largest between *B. pseudotriquetrum* and *S. antarctici* at 20.54 with the highest contributions from intracellular UVAC and β-carotene/Tchl levels (see Table 2). *C. purpureus and S. antarctici* were most similar with an average squared Euclidean distance of 12.03 and the highest contribution from β-carotene. The average squared Euclidean distance between

C. purpureus and *B. pseudotriquetrum* was 15.03 with cell wall and intracellular UVAC and VAZ/Tchl ratio as the main contribution to differences. The Z/VAZ ratio was consistently low contributor to differences between species.

Table 2: Average dissimilarity results of Euclidean distance for pigments between species' pairs: *B. pseudotriquetrum* (B)*, C. purpureus* (C) *and S. antarctici* (S)*.* Pigments contributing <5% are not shown.

Between species	Pigments	Av.Value	Av.Value	Av.Sq. Dist	Sq.Dist /SD	Contrib%	Cum. %
B and C		Gp B	Gp C				
	W UVAC	8280	12736	3	0.83	19.95	19.95
Ave sq Dist = 15.03	iUVAC	16774	7808	2.64	1.09	17.55	37.49
	VAZ/Tchl	0.096	0.11	2.59	0.65	17.25	54.74
	Chl a/b	3.09	3.01	1.98	0.82	13.17	67.91
	Tchl	570	323	1.73	0.39	11.51	79.41
	Anthocyanins	9.21	7.75	1.59	0.65	10.59	90.01
B and S		Gp B	Gp S				
	IUVAC	16774	3512	5.09	1.69	24.79	24.79
Ave sq Dist = 20.54	β-carot /Tchl	0.088	0.160	3.51	0.73	17.08	41.87
	VAZ /Tchl	0.096	0.12	2.56	0.61	12.47	54.35
	Anthocyanins	9.21	7.22	2.24	0.71	10.9	65.24
	Chl a/b	3.09	2.84	2.2	0.72	10.69	75.94
	WUVAC	8280	10912	1.8	0.86	8.78	84.72
	Tchl	570	554	1.68	0.43	8.18	92.9
C and S		Gp C	Gp S				
Ave sq Dist = 12.60	β-carot /Tchl	0.11	0.160	2.71	0.68	21.52	21.52
	Chl a/b	3.01	2.84	2.29	0.74	18.2	39.73
	VAZ/Tchl	0.11	0.12	1.75	0.64	13.92	53.65
	WUVAC	12736	10912	1.63	0.53	12.94	66.59
	Tchl	323	554	1.36	0.48	10.78	77.36
	Anthocyanins	7.76	7.22	1.27	0.73	10.11	87.48
	Z/VAZ	0.39	0. 40	0.921	0.71	7.31	94.78

Examines time GROUPS (across all species' groups)

Analyses of similarities over time (across all species), found the pigments in early season were most similar with an average squared distance of 8.28 while mid and late season were < 3.5. In early season total chlorophyll, VAZ/Tot chl ratio and Cell wall UVAC contributed 55.94% of similarities (see Table 3). Total chlorophyll then drops to be the least important contributor in mid and late season. The pigments chlorophyll a/b and anthocyanins comprised 10% of similarity in early season then rose to become the most important pigment in mid (45%) and late season (50%). Additionally, Z/VAZ was unimportant in early season and late season (~8%) but rises to 14.62% in mid-season. Intracellular UVAC had a consistently low contribution all season, whilst β carotene had a consistent mid-level contribution all season.

Appendix C

Table 3: Average similarity results for pigment composition measured as average squared Euclidean distance between samples within early, (9 November to 2 December 2002), mid (5 December to 4 January 2003) and late season (7 January to 1 February 2003).

	Pigments	Av. Value	Av.Sq. Dist	Sq.Dis t/SD	Contrib%	Cum.%
Early season Ave sq. dist = 8.28	Total chl	726	1.81	0.44	21.82	21.82
	VAZ/ tot chl	0.107	1.42	0.5	17.15	38.97
	Cell wall UVAC	11989	1.4	0.46	16.97	55.94
	β-carot /tot chl	0.137	1.1	0.34	13.24	69.18
	Anthocyanins	6.17	0.83	0.41	10.03	79.21
	Chl a/b	3.01	0.788	0.47	9.52	88.73
	Z/VAZ	0.506	0.692	0.48	8.36	97.09
	IntraUVAC	9414	0.241	0.34	2.91	100
Mid season Ave sq. dist = 3.68	Pigments	Av. Value	Av.Sq. Dist	Sq.Dist/ SD	Contrib%	Cum.%
	Chl a/b	2.94	1.08	0.51	29.25	29.25
	Anthocyanins	9.95	0.574	0.53	15.58	44.83
	Z/VAZ	3.09	0.538	0.41	14.62	59.45
	β-carot /tot chl	0.106	0.488	0.38	13.24	72.69
	VAZ /tot chl	1.07	0.424	0.46	11.53	84.22
	Cell wall UVAC	9921	0.26	0.49	7.06	91.28
	Intra UVAC	9282	0.189	0.39	5.14	96.42
	Total chl	401	0.132	0.37	3.57	100
Late season Ave sq. dist = 3.41	Anthocyanins	8.07	0.927	0.45	27.24	27.24
	Chl a/b	2.99	0.797	0.56	23.41	50.65
	β-carot /tot chl	0.112	0.427	0.33	12.54	63.19
	Cell wall UVAC	10018	0.413	0.52	12.13	75.32
	VAZ /tot chl	0.111	0.333	0.47	9.79	85.11
	Z / VAZ	0.310	0.289	0.48	8.5	93.61
	Intra UVAC	9397	0.118	0.44	3.46	97.07
	Total chl	320	0.1	0.43	2.94	100

Comparisons of dissimilarity between time (across all species)

Early season was quite different to both mid and late season (16%) and the distinguishing pigments were Z/VAZ, Tchl and anthocyanins all with similar average squared Euclidean distances of 16 (see Table 4). There was less distinction between mid and late season with average squared Euclidean distance of 7.28, mainly contributed to by chlorophyll a/b ratio and anthocyanins 47.25%. Therefore, the largest changes in pigments over the season are anthocyanins, Tchl, chlorophyll a/b and Z/VAZ ratio.

The SIMPER results indicate which pigments are most important in differences between species and across the season and are further explored with univariate analyses below. β-carotene levels are distinctive in *S. antarctici* but make little contribution to seasonal changes. Z/VAZ ratio changes over the season in a similar way for all species. VAZ/ Tchl ratio is an important contributor to early season and within *C. purpureus* similarity.

Cell wall UVAC is distinctive in early season and differs between *B. pseudotriquetrum* and *C .purpureus.* Intracellular UVAC is an important contributor to differences between *B. pseudotriquetrum* and *C. purpureus* but changes little over the season. Anthocyanin levels and Tchl distinguish seasonal changes and are distinct in *B. pseudotriquetrum.* Total chlorophyll changes over the season and is particularly important in distinguishing early season and *B. pseudotriquetrum* from other groups. The chlorophyll a/b ratio is important in distinguishing mid and late season (which are otherwise quite similar), and this ratio is different between *C. purpureus* and *S. antarctici. Bryum pseudotriquetrum* and *S. antarctici* are most dissimilar.

Appendix C

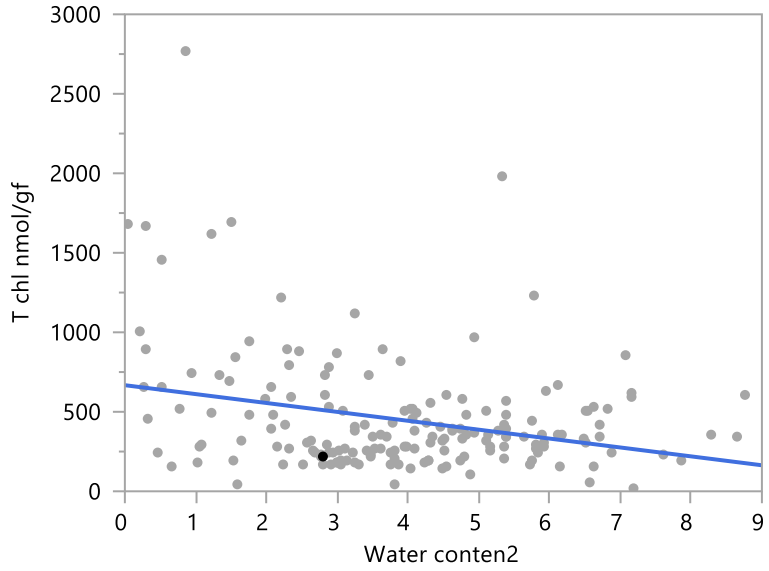
Table 4: SIMPER results of dissimilarity in time between early, (9 November to 2 December 2002), mid (5 December to 4 January 2003) and late season (7 January to 1 February 2003), excluding pigments whose contributions were <5 %. (average squared Euclidean distance). two way crossed

Compare between time	Variable	Mean	Mean	Av.Sq. Dist	Sq.Dist /SD	Contrib %	Cum. %
Early & Mid season Ave sq. dist = 16.68		Group E	Group M				
	Z/VAZ	0.506	0.309	3.33	0.94	19.95	19.95
	Tchl	726	401	2.69	0.55	16.14	36.09
	Anthoycanins	6.17	9.95	2.53	0.95	15.19	51.28
	Chl a/b	3.01	2.94	2	0.78	12.02	63.3
	β-carot /Tchl	0.137	0.106	2	0.49	11.98	75.27
	WUVAC	11989	9921	1.94	0.66	11.63	86.91
	VAZ /Tchl	0.107	0.107	1.79	0.72	10.76	97.67
Early & Late season Ave sq. dist = 16.17		Group E	Group L				
	Z/VAZ	0.506	0.310	3.18	0.91	19.7	19.7
	Tchl	726	321	3.1	0.57	19.17	38.87
	Anthocyanins	6.17	8.07	2.18	0.78	13.47	52.34
	WUVAC	11989	10018	2.13	0.66	13.16	65.5
	VAZ /Tchl	0.107	0.111	1.94	0.84	11.98	77.48
	β-carot /Tchl	0.137	0.113	1.72	0.49	10.62	88.1
	Chl a/b	3.01	2.99	1.58	0.69	9.74	97.84
Mid & Late season Ave sq. dist = 7.28		Group M	Group L				
	Chl a/b	2.94	2.99	1.88	0.82	25.86	25.86
	Anthocyanins	9.95	8.07	1.63	0.75	22.39	48.25
	VAZ /Tchl	0.107	0.111	0.98	0.75	13.46	61.71
	β-carot /Tchl	0.106	0.113	0.81	0.48	11.13	72.84
	Z/VAZ	0.309	0.310	0.727	0.68	9.99	82.83
	WUVAC	9921	10018	0.634	0.75	8.71	91.54

APPENDIX D

Total chlorophyll does decrease with turf water content but it has a poor fit for all three species. (R^2=0.08 n=180).

Bivariate linear Fit of T chl nmol/gfw^{-1} By Water content gH_2O gdw^{-1}



Linear Fit

T chl nmol/gfw = 667.38171 - 55.963809*Water content

Summary of Fit

RSquare	0.081464
RSquare Adj	0.076303
Root Mean Square Error	355.7747
Mean of Response	448.3679
Observations (or Sum Wgts)	180

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1998197	1998197	15.7866
Error	178	22530459	126576	**Prob > F**
C. Total	179	24528655		0.0001*

Appendix D

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	667.38171	61.16917	10.91	<.0001*
Water content	-55.96381	14.08521	-3.97	0.0001*

Linear Fit

T chl nmol/gfw = 667.38171 - 55.963809*Water content

Summary of Fit

RSquare	0.081464
RSquare Adj	0.076303
Root Mean Square Error	355.7747
Mean of Response	448.3679
Observations (or Sum Wgts)	180

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1998197	1998197	15.7866
Error	178	22530459	126576	**Prob > F**
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Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	667.38171	61.16917	10.91	<.0001*
Water content	-55.96381	14.08521	-3.97	0.0001*

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