The Vegetation and Hydrology of Groundwater Dependent Ecosystems on the Bogong High Plains, Victoria

Submitted by

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B.Sc. in Environmental Management and Ecology with Honours

A thesis submitted for fulfilment of the requirements for the degree of Doctor of Philosophy

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May, 2020

Statement of Authorship

This thesis includes work by the author that has been published or accepted for publication as described in the text. Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis accepted for the award of any other degree or diploma. No other person's work has been used without due acknowledgement in the main text of the thesis. This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

Victoria Ann McCartney

07 May 2020

Acknowledgements

This work has been made possible by my supervisors, Ewen Silvester and John Morgan. They are to be congratulated on persevering with a student who sometimes was thinking on an entirely different level. Maybe you should both get together with my husband whom I am sure would be able to relate. Ewen, thank you for taking me on at the beginning, I know it must have been frustrating as a chemist to relate to a student who is more botanically motivated and loves botanical art to boot. I hope that there are not too many grey hairs. John, thank you as well, you inspire me to keep going and extend myself so much more than I thought that I was capable of. It is because of you both that I am able to submit this thesis so THANK YOU.

I would also like to thank past lecturers: Susan, Phil, Peter, Dennis and Martin who have kept in contact and been interested in this journey. Also, thanks to the PhD cohort of students that have been an invaluable sounding board and emotional backup. There have been comings and goings but Julia (for proof-reading this thesis), Annaleise (my procrastination and knitting advisor), Lorena (my office buddy), the friends that I have found along the way in undergraduate studies through to PhD and everyone else - thank you to you all.

To my children and grandchildren who have had a mother and grandma who has been lost in her own bryophyte world for the past 8 years – I'm back!! Hopefully I have

inspired you to look beyond what you think that you are capable of. Last but definitely not least, I have no words to express my appreciation to my husband Rex. You have encouraged me through this entire journey of becoming a mature aged student who found a love of science. Words are not enough. With a wife who was frequently absent walking the Bogong High Plains – you stepped up on the home front and encouraged me to keep going. On the bright side you have become an environmentalist without even knowing it! Thank you.

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Abstract

Groundwater ecosystems on the Bogong High Plains, Victoria, were studied in relation to their vegetation and local hydrology. Groundwater sources occurred across a range of altitudes (1667 – 1854 m) independent of aspect, and were hydrologically connected to (and upstream of) peatlands. Groundwater source pool vegetation was dominated by bryophytes, with the aquatic bryophyte *Blindia robusta* the most common species, occurring in association with *Bartramia subsymmetrica* (previously known as *Bartramia bogongia*) in some pools. The groundwater was deficient in major ions, with ionic concentrations broadly similar to rainwater apart from elevated levels of CO_2. Based on the observed distribution in the field, *Blindia robusta* appears to be entirely dependent on the permanent aquatic environment provided by groundwater for its survival. Laboratory experiments confirmed that *Blindia robusta* was intolerant to desiccation, as measured by photosystem II flourescence measurements, and was associated with significant cell wall shrinkage. The absence of cross-linked poly-phenolic molecules in *Blindia robusta* suggests that the sensitivity of these species to drying is due to an inability to maintain structural integrity in a drying environment. Five permanent groundwater sources in the Watchbed Creek catchment were monitored seasonally over a period of 2 yrs for CFC-11 and CFC-12 levels to determine apparent water age, which varied from 10 – 38 yrs. Seasonal patterns in groundwater age showed younger ages in winter/spring for any given year and suggests that snowpack is relatively more important for groundwater recharge. Further interpretation of water age is limited by the relatively short study period and potentially complicated by the mixing of old (regional) and more recent (local) groundwaters. Predicted decreased snowpack for the Australian Alps, associated with a warming climate trend, is likely to result in decreased groundwater recharge. Under this scenario, *Blindia robusta* can be expected

to receive diminished groundwater flows and retreat from the isolated pools where it

currently occurs.

Chapter 1

Groundwater-Dependent Ecosystems

1.1   What is the structure of groundwater-dependent ecosystems and where/why do they occur?

Streams and rivers rely on precipitation (rain and snow) being held in groundwater reserves and released over time to maintain a continuous flow (Winter *et al*. 1998), as well as the seasonal terrestrial runoff of water from rainfall and snowmelt. When rain falls and snow melts, it infiltrates into spaces that lead into confined or unconfined aquifers and results in groundwater reserves that eventually exit through rock fissures or gaps in the rocks (Winter *et al.* 1998). When the water table intersects the land surface, a wetland or spring develops (Winter *et al.* 1998) with the biological communities associated with these systems generally known as groundwater-dependent ecosystems (GDEs).

Numerous GDEs have been identified, with many classification systems proposed (Hatton and Evans, 1998; Colvin *et al*. 2007; Kløve *et al.* 2011). In essence, GDEs can be described as ecosystems that exist within groundwater and are dependent on either surface or subsurface supply of groundwater and/or where part or whole of the water demand is supplied by groundwater (Eamus *et al*. 2006; Rydin and Jeglum, 2006; Orellana *et al*. 2012). In the event that groundwater ceases to flow, and the ecosystem functioning becomes impaired leading to basic alterations of the structure of the ecosystem itself, then that system is considered groundwater-dependent (Orellana *et al*. 2012).

Four main GDEs are typically identified – marsh, swamp, fen and bog (National Wetlands Working Group, 1997). Marshes have standing or slowly moving water and are either permanently or seasonally flooded (Rydin and Jeglum, 2006). Swamps are forested (i.e. thicketed wetlands) which have minerogenous water that may be provided from watercourses, the underlying soil or from lateral groundwater flow (Rydin and Jeglum, 2006). Fens have a water table at, or just below, the soil surface and usually have a slow internal drainage/seepage system (Rydin and Jeglum, 2006). Bogs are peatlands with the surface of the bogs above the surrounding terrain, or isolated from laterally moving mineral waters, are nutrient-poor and are strongly acidic (Rydin and Jeglum, 2006). Elsewhere, bog-poor fen-rich systems have been identified, with vegetation variation driven by variation in pH (bog, pH 3.5-4.2; poor fen, pH 4-5.55; intermediate and moderately rich fen, pH 5-7; and extremely rich fen, pH 6.8-8) and base cation richness (Rydin and Jeglum, 2006). Typically, the two most important influences for variation between marshes, swamps, fens and bogs are moisture (water)-aeration and pH-base cation (Rydin and Jeglum, 2006).

The age of the water that directly supplies GDEs can relate to geological processes that formed the groundwater systems which, in turn, supply the GDEs with permanent water. These systems can be old (palaeowaters) which were recharged centuries and sometimes millennia before modern times under different climatic or hydrological conditions to the present (Clark and Fritz, 1997; Currell *et al.* 2012), or they can contain water of a much younger age. The age of the water that directly recharges GDEs can be examined and identified using radiocarbon dating, stable isotope analysis and sometimes noble gasses or, in younger-aged water (1940-1996), chlorofluorocarbon dating.

1.1.2 What are the essential features of GDEs?

GDEs contain complex ecosystems which interact with surrounding surface systems affecting both groundwater and surface water quality, in ways that are not yet fully understood (Gibert *et al.* 1994; Boulton, 2000). What is important is that water is fundamental to species distributions and ecological function in GDEs (Murray *et al.* 2003). If the water supply declines, the ecosystem becomes functionally impaired, leading to essential alterations to the structure of the ecosystem (Murray *et al.* 2003; Orellana *et al.* 2012). The importance of groundwater availability, however, is not the only key determinant of GDEs. The timing (e.g. wet-dry season, extreme droughts) and location of break of slope and springs are other factors to be considered (Murray *et al.* 2003).

In the desert areas of Australia, there are well-documented GDEs that are referred to as 'spring mounds' that are associated with the Great Artesian Basin (Bayly, 1999; Harris, 2002, Pemberton, 2005; Prescott and Habermehl, 2008). These are found where there are outflows from spring vents intercepting the ground and formed by accumulation of peat and other materials (Fensham *et al.* 2004). In South Australia, desert springs are found surrounded by sand ridges and stony deserts (Harris, 2002). The arid zone freshwater biota habitats within Australia are particularly vulnerable to change because of their separation with significant tracts of isolated territory with very modest and irregular hydrological connectivity (Davis *et al.* 2013).

The arid spring mounds have been critical for the safe movement of indigenous people between large areas of desert environments, before and after European settlement (Bayly, 1999; Harris, 2002; Prescott and Habermehl, 2008). The relatively poor condition of the spring water and meagre plant and animal food sources surrounding them encouraged indigenous people to move regularly and travel to other

spring mounds whenever rainfall events permitted (Harris, 2002). Arid spring mounds have also played a vital role in Australian European pastoral history (Harris, 2002; Prescott and Habermehl, 2008). The spring mounds supplied potable water through inhospitable desert country allowing more reliable movement of stock and people (Harris, 2002; Prescott and Habermehl, 2008).

1.2 What is the function (internal dynamic) of these GDEs?

1.2.1 Spatial isolation effects of GDEs

All GDEs are vulnerable to climate change because of their physical fragmentation and isolation within landscapes (Woodward *et al.* 2010). The consequence of this is that many species associated with GDEs will have inadequate capacity to disperse as global atmospheric temperatures increase (Woodward *et al.* 2010).

GDEs are habitat to many specialist species (Fensham and Fairfax, 2003; Jolly *et al.* 2008). Isolated populations of species in some GDEs can result in strong genetic differentiation between populations, especially those more geographically isolated (Lienert *et al.* 2002). When considering the isolation of GDEs, the connectivity of the GDE systems in relation to groundwater needs to be considered (Leibowitz, 2003). The distribution of spatially isolated species, population genetics and community structure of GDEs is entirely dependent on the way that water enters and leaves these wetlands (Leibowitz, 2003). Consequently, water affects any GDE function that depends on it as a vector (Leibowitz, 2003).

GDEs are also habitat islands in which plants from separate GDEs may be genetically differentiated (Hooftman *et al.* 2003). Size and spatial isolation are important predictors for species richness, with wetland size, isolation and habitat all

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influencing colonisation success (Lehtinen and Galatowitsch, 2000). In small habitats, it is likely that genetic drift has resulted in a lowered genetic variation, inbreeding and, as a consequence, a reduction in plant fitness (Ellstrand and Elam, 1993). This not only affects rare species but also common, habitat-specific species (Hooftman *et al*. 2003).

Leibowitz (2003) suggests that isolated GDEs are often small and their loss may be expected to have a minimal effect on α-diversity (local measure of the average species diversity in a habitat or specific area) and contribution to β-diversity (the diversity of species between two habitats or regions). The magnitude of functional loss, however, is not necessarily proportional to size; GDEs often have a high species richness and biodiversity (Leibowitz, 2003) with specialised, endemic and rare taxa (Hinterlang and Leischewski, 1996; Botosaneanu, 1998). Life cycles, feeding strategies and extreme sensitivity to abiotic factors might explain why these species and others are restricted to GDEs (e.g. water mites; Di Sabatino *et al.* 2003). The continuous groundwater inflow in GDEs helps to support diverse biota with a high proportion of habitat specialists (Lehosmaa *et al*. 2017). GDEs contain endemic species as a result of their physical isolation, and may contribute to almost one third of regional freshwater biodiversity (Di Sabatino *et al*. 2003) so that a loss of these systems may have a disproportionate effect on the regional biodiversity (Di Sabatino *et al*. 2003; Leibowitz, 2003).

1.2.2 Importance of GDEs to moss

Water availability is critical to the distribution of aquatic mosses (Vanderpoorten and Goffinet, 2009). In bog and fen habitats, water can be utilised by moss through capillary action from the nearby water table, or in the case of aquatic species of moss, partially or totally submerged within the water itself (Vanderpoorten

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and Goffinet, 2009). Mosses provide shelter for micro-organisms, with aquatic moss being able to reduce water velocity and act as filters for water-borne particles (Vanderpoorten and Goffinet, 2009). Moss are important as egg-laying sites and they are also a productive nursery for small larvae and an important refuge for macroinvertebrates (Vanderpoorten and Goffinet, 2009). As a result, macroinvertebrate densities are higher in bryophyte mats than what is found on stream/riverbeds (Vanderpoorten and Goffinet, 2009).

Wet habitats in open situations with a constant supply of water enable moss to reach a high level of light saturation (Vanderpoorten and Goffinet, 2009). Photosynthetic apparatus for some species of moss appears to have evolved depending on water availability (Vanderpoorten and Goffinet, 2009). Aquatic moss that develop entirely below the water surface, or within the water itself, only tolerate minor temperature fluctuations and therefore have a limited available habitat (Glime and Vitt, 1984).

Alpine ecosystems containing aquatic/semi-aquatic moss are directly linked to permanency of water with some semi-terrestrial mosses, such as some *Sphagnum* spp., adapted and resilient to occasional short periods of desiccation (Suren, 1993; Suren, 1996; Yazaki *et al.* 2006; Price & Whittington, 2010). Some *Sphagnum* spp. found in hummocks in peatlands have high water-holding capacities, are able to metabolise for longer and recover faster upon rehydration than other species (Hájek and Beckett, 2008).

Springs or GDEs are ecotones between groundwater and surface water, as well as between terrestrial and aquatic ecosystems (Cantonati *et al.* 2012). Habitat specialists such as aquatic bryophytes have a very limited dispersal capacity and suffer disproportional losses from reduced connectivity and are therefore at a greater risk of local extinction (Lehosmaa *et al.* 2017). Spore production, patch size and isolation

contribute to dispersal success of aquatic bryophytes (Stream Bryophyte Group 1999). Lönnell *et al*. (2012) suggests that dispersal of spores larger than 20 μm is possible for up to 600 m during a season, with spores either falling close to the mother colony or uplifted to higher altitudes and dispersed to greater distances. This dispersal of spores may be possible but with aquatic bryophytes, the spores need to land in permanent water for them to be viable.

1.3   How will GDEs change over time (climate change)?

1.3.1   GDEs in Australia

GDEs are widely distributed in Australia, across a range of biomes and climate regions. One form of GDEs in Australia are peatlands. Peatlands range from button grass moorlands in Tasmania, *Sphagnum* bogs, sedge fens, coastal peatlands often dominated by shrubs to tropical *Melaleuca* swamp forests of northern Australia (Whinam and Hope, 2005). Other GDEs found in Australia are *Sphagnum* peatlands, which are extremely restricted, occurring in parts of Tasmania (Whinam *et al*. 1989), Victoria, New South Wales and the ACT (Clarke and Martin, 1999). The Australian Alps, where many *Sphagnum* peatlands are found, covers an area of approximately 0.3% of the total land mass of Australia (Costin, 1989). While some Australian peatland types have been studied (e.g. Costin, 1955; Whinam and Hope, 2005; Grover and Baldock, 2010), other GDEs types have been poorly recognised (Silvester, 2009). Alpine GDEs that are in immediate association with groundwater sources are the focus of this thesis.

1.3.2 Climate Change

Greenhouse gas emissions are a major cause of recent increases in global mean temperature (approximately 0.5 $^{\circ}$C – 0.6 °C since the start of the 20th century) (Easterling *et al.* 2000; IPCC, 2007a). Conservative scenarios for future climate changes can predict that by the year 2100 further increases in mean temperatures (approximately 2-4 °C globally) with significant drying in some regions (Christiansen *et al.* 2007; Seager *et al*. 2007). Increases in frequency and severity of extreme droughts, temperature extremes, and heat waves will also likely become more common (Nicholls, 1996; IPCC, 2007b).

The predicted changes in precipitation due to climate change will likely result in a shift in both seasonal and mean annual groundwater levels, depending upon changes in the magnitude, timing and distribution of rainfall (Liu, 2011) and snow melt (Jyrkama and Sykes, 2007; Kløve *et al*. 2014). Earlier snow melts can reduce already low summer flows (Okkonen and Kløve, 2011) and the amount of available groundwater (Ludwig and Moench, 2009). An increase in the average minimum temperature and a decrease in precipitation is forecast for the Australian alpine and subalpine areas resulting in reduced snow cover by this year compared to 1990 records (Hennessy *et al.* 2008). With changes in recharge due to climate change, we may see decreased summer groundwater levels, intensity and timing of snow melt and increased precipitation, all causing serious problems to freshwater biota (Kløve *et al.* 2014).

Decreased rainfall will lead to longer and more frequent droughts resulting in declines in groundwater. This then facilitates more intense and more frequent fires, in areas where historically fires were infrequent with longer periods between them (Horwitz *et al*. 2008; Baird and Burgin, 2016). The Australian Alps has experienced an unprecedented sequence of fires with vast areas impacted by fires in 2003, 2006-2007

and with more localised fires in 2013 and 2019/2020 (Zylstra, 2006; Williams *et al*. 2008; Mitchell *et al*. 2015).

Along with increased fire activity, the Australian Alps are facing problems with feral horses and deer (Nimmo and Miller, 2007). These pest species are known to have adverse environmental impacts on vegetation and groundwater by trampling and compaction of soil (Nimmo and Miller, 2007). The outcome of their disturbances is that they commonly favour recolonization by weed species (Cole, 1978; Rogers, 1991; Whinam *et al*. 1994).

1.4 This study

Silvester (2009) reported the existence of previously unrecognised GDE-type in the Watchbed Creek catchment of the Bogong High Plains, Victorian Alps. This system was small and restricted to the area immediately downslope of a water source. This raised many questions about the existence of similar GDEs in the broader region, as well as the species composition and ecosystem function.

This thesis surveyed the location of groundwater and groundwater source pools on the Bogong High Plains, Victoria. I start by describing patterns in the landscape (Chapter 2). The aim for Chapter 2 was to find out how many of these GDEs were in the catchments on the Bogong High Plains and where were they located? What was the vegetation surrounding and within these GDEs? What habitats do the GDEs support?

After the initial survey, it was found that an aquatic moss *Blindia robusta* was present in all permanent GDEs. The focus of this thesis then moved to *Blindia robusta* as an indicator species for GDEs. I describe previously unknown growth forms of *Blindia robusta* (Chapter 3) and examine areas within this aquatic moss where most of the photosynthesis processes take place.

Blindia robusta is an important GDEs species on the Bogong High Plains. It is restricted in distribution, with very small populations in permanent groundwater source pools. The important questions that then arose were: How well will *Blindia robusta* respond to a changing climate? Is water permanence critical to these systems? Experiments were designed to see how desiccation would affect *Blindia robusta* in Chapter 4.

If *Blindia robusta* did not respond well to desiccation, then it became important to try to find out how long the groundwater could maintain flows in the face of climate change and how vulnerable is the survival of GDEs on the Bogong High Plains? The aims for Chapter 5 were to see if snowpack was important for groundwater recharge on the Bogong High Plains and to determine the likely timescale over which changes might be expected to occur.

1.5   References

Baird IRC, Burgin S. (2016) Conservation of a groundwater-dependent mire-dwelling dragonfly: implication of multiple threatening processes. *Journal of Insect Conservation* **20**: 165-178.

Bayly IAE (1999) Review of how indigenous people managed for water in desert regions of Australia. *Journal of the Royal Society of Western Australia* **82**, 17-25

Botosaneanu L (Ed) (1998) Studies in Crenobiology. The Biology of Springs and Springbrooks. (Backhuys: Leiden)

Boulton AJ (2000) River ecosystem health Down Under: assessing ecological condition in riverine groundwater zones in Australia. *Ecosystem Health* **6**, 108-118.

Cantonati M, Fűreder L, Gerecke R, Jűttner I, Cox E (2012) Crenic habitats, hotspots for freshwater biodiversity conservation: toward an understanding of their ecology. *Freshwater Science* **31**, 463-480.

Christensen JH, Hewitson B, Busuioc A, Chen A, Gao X, Held I, Jones R, Kolli RK, Kwon WT, Laprise R, Magaña Rueda V, Mearns L, Menéndez CG, Räisänen J, Rinke A, Sarr A, Whetton P (2007) Regional climate projections. In Solomon S. et al. (Eds.), Climate Change 2007: The Physical Science Basis. Contributions of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. (Cambridge University Press: Cambridge).

Clark I, Fritz P. (1997) Environmental Isotopes in Hydrogeology. (Lewis Publishing: New York).

Clarke PJ, Martin ARH (1999) Sphagnum peatlands of Kosciusko National Park in relation to altitude, time and disturbance. *Australian Journal of Botany* **47,** 519-536.

Cole DN. (1978) Estimating the susceptibility of wild-land vegetation to trailside alteration. *Journal of Applied Ecology* **15**, 281-286.

Colvin C, Le Maitre D, Saayman I, Hughes S (2007) An introduction to aquifer dependent ecosystems in South Africa. Technical Report TT 301/07, Water Resources Commission, Pretoria, South Africa.

Costin AB (1955) Alpine soils in Australia with reference to conditions in Europe and New Zealand. *European Journal of Soil Science* **6,** 35-50.

Costin AB (1989) The Alps in global perspective. In: *The Scientific Significance of the Australian Alps* (ed. R. Good), pp. 7-20. Australian Alps National Parks Liaison Committee, Canberra.

Currell MJ, Han D, Chen Z, Cartwright I (2012) Sustainability of groundwater usage in northern China: dependence on palaeowaters and effects on water quality, quantity and ecosystem health. *Hydrological Processes* **26**: 4050-4066.

Davis J, Pavlova A, Thompson R, Sunnucks P (2013) Evolutionary refugia and ecological refuges: key concepts. *Global Change Biology* **19**, 1970–1984.

Di Sabotino A, Cicolani B, Gerecke R (2003) Biodiversity and distribution of water mites (Acari, Hydrachnidia) in spring habitats. *Freshwater Biology* **48**, 2163-2173.

Eamus D, Hatton TJ, Cook PG, Colvin C (2006) Ecohydrology: Vegetation function, water and resource management. (CSIRO, Melbourne.)

Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO (2000) Climate extremes: Observations, Modelling, and impacts. *Science's Compass* **289**, 2068-2074.

Ellstrand NC, Elam DC (1993) Population genetic consequences of small population size: implications for plant conservation. *Annual Reviews of Ecology and Systematics* **24:**214-242.

Fensham RJ, Fairfax RJ (2003) Spring wetlands of the Great Artesian Basin, Queensland, Australia. *Wetland Ecology and Management* **11**, 343-362.

Fensham RJ, Fairfax RJ, Pocknee D, Kelley J (2004) Vegetation patterns of permanent spring wetlands of arid Australia. *Australian Journal of Botany* **52**, 719-728.

Gibert J, Danielopol DL, Stanford JA (1994) 'Groundwater Ecology'. (Academic Press: London).

Glime JM, Vitt DH (1984) The physiological adaptations of aquatic musci. *Lindenbergia*, **10**:41-52.

Grover SPP, Baldock JA (2010) Carbon decomposition processes in a peat from the Australian Alps. *European Journal of Soil Science* **61**,217-230.

Hájek T, Beckett RP (2008) Effect of Water Content Components on Desiccation and Recovery in *Sphagnum* Mosses. *Annals of Botany* **101(1)**: 165–173.

Harris C (2002) Culture and geography: South Australia's mound springs as trade and communication routes. *Historic Environment* **16(2)**, 8-11.

Hatton T, Evans R (1998) Dependence of Australian ecosystems on groundwater. *Water*, **25(5)**, 40-43.

Hennessy KJ, Whetton PH, Walsh K, Smith IN, Bathols JM, Hutchinson M, Sharples J (2008) Climate change effects on snow conditions in mainland Australia and adaptation at ski resorts through snowmaking. *Climate Resources* **35(3)**, 255-270.

Hinterlang D, Lischewski D (Eds) (1996) Proceedings 1st Symposium on Spring ecology and Conservation, Műnster 1995. *Crunoecia* **5**, 1-140.

Hooftman DAP, van Kleunen M, Diemer M (2003) Effects of habitat fragmentation on the fitness of two common wetland species, *Carex davalliana* and *Succisa pratensis*. *Population Ecology* **134**: 350-359.

Horwitz P, Bradshaw D, Hopper S, Davies P, Froend R, Bradshaw F (2008) Hydrological change escalates risk of ecosystem stress in Australia's threatened biodiversity hotspot. *Journal of the Royal Society of Western Australia* **91**: 1-11.

IPCC, 2007a. Climate change 2007: the physical science basis. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor S, Miler HL (Eds) Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. (Cambridge University Press: Cambridge). pp. 996.

IPCC 2007b. Climate change 2007: impacts, adaptation and vulnerability. Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (eds) Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. (Cambridge University Press: Cambridge).pp. 976.

Jolly ID, McEwan KL, Holland KL (2008) A review of groundwater-surface water interactions in arid/semi-arid wetlands and the consequences of salinity for wetland areas 1. *Ecohydrology* **1**, 43-58.

Jyrkama IM, Sykes JF (2007) The impact of climate change on spatially varying groundwater recharge in the grand river watershed. *Journal of Hydrology* **338 (3-4)**, 237-250.

Kløve B, Ala-aho P, Bertrand G, Boukalova Z, Erturk A, Goldscheider N, Ilmonen J, Karakaya N, Kupfersberger H, Kvoerner J, Lundberg A, Mileusnić M, Moszczynska A, Muotka T, Preda E, Rossi P, Siergieiev D, Sîmek J, Wachniew P, Angheluta V, Widerlund A (2011) Groundwater dependent ecosystems. Part I: Hydroecological status and trends. *Environmental Science and Policy* **14(7)**, 770-781.

Kløve B, Ala-Aho P, Bertrand G, Gurdak JJ, Kupfersberger H, Kværner J, Muotka T, Mykrä, Preda E, Rossi P, Uvo CB, Velasco E, Pulido-Velazquez M (2014) Climate change impacts on groundwater and dependent ecosystems. *Journal of Hydrology* **518**, 250-266.

Lehosmaa K, Jyväsjärvi J, Virtanen R, Ilmonen J, Saastamoinen J, Muotka T (2017) Anthropogenic habitat disturbance induces a major biodiversity change in habitat specialist bryophytes of boreal springs. *Biological Conservation* **215**, 169-178.

Lehtinen RM, Galatowitsch SM (2000) Colonisation of restored wetlands by amphibians in Minnesota. *American Midland Naturalist* **145**: 388-396.

Leibowitz SG (2003) Isolated wetlands and their functions: an ecological perspective. *Wetlands* **23(3)**: 517-531.

Lienert J, Fischer M, Schneller J, Diemer M (2002) Isozyme variability of the wetland specialist *Swertia perennis* (Gentianaceae) in relation to habitat size, isolation, and plant fitness. *American Journal of Botany* **89(5)**, 801-811.

Liu HH (2011) Impact of climate change on groundwater recharge in dry areas: an ecohydrology approach. *Journal of Hydrology* **407**, 175-183.

Lönnell N, Hylander K, Jonsson BG, Sundberg S (2012) The fate of the missing spores – patterns of realized dispersal beyond the closest vicinity of a sporulating moss. *Plos One* **7(7)**, 1-7.

Ludwig F, Moench M (2009) The impacts of climate change on water. In: Ludwig F, Kabat P, Schaik HV, van der Valk M (Eds.) Climate Change Adaptation in the Water Sector. (Earthscan Publishing: London)

Mitchell M, Lockwood M, Moore S, Clement S (2015) Scenario analysis for biodiversity conservation: A social-ecological system approach in the Australian Alps. *Journal of Environmental Management* **150**, 69-80.

Murray BR, Zeppel MJB, Hose GC, Eamus D. (2003) Groundwater-dependent ecosystems in Australia: it's more than just water for rivers. *Ecological Management & Restoration* **4,** 110-113.

National Wetlands Working Group (1997). The Canadian wetland classification system, 2nd edn. (University of Waterloo; Canada).

Nicholls N (1996) Long-term climate monitoring and extreme events. In: Karl, T.R. (Ed.), Long-term Climate Monitoring by the Global Climate Observing System. Kluwer, Dordrecht, p. 518.

Nimmo DG, Miller KK (2007) Ecological and human dimensions of management of feral horses in Australia: a review. *Wildlife Research* **34**, 408-417.

Okkonen J, Kløve B. (2011) A sequential modelling approach to assess groundwater-surface water resources in a snow dominated region of Finland. *Journal of Hydrology* **441 (1-2)**, 91-107.

Orellana F, Verma P, Loheide II SP, Daly E. (2012) Monitoring and modelling water-vegetation interactions in groundwater-dependent ecosystems. *Reviews of Geophysics* **50, RG3003:** 1-24.

Pemberton M (2005) Australian peatlands: a brief consideration of their origin, distribution, natural values and threats. *Journal of the Royal Society of Western Australia* **88**, 81-89.

Prescott JR, Habermehl MA (2008) Luminescence dating of spring mound deposits in the southwestern Great Artesian Basin, north South Australia. *Australian Journal of Earth Sciences* **55**, 167-181.

Price JS, Whittington PN. 2010. Water flow in Sphagnum hummocks: Mesocosm measurements and modelling. *Journal of Hydrology*. 381: 333-340.

Rogers GM. (1991) Kaimanawa feral horses and their environmental impacts. *New Zealand Journal of Ecology* **15**, 49-64.

Rydin H, Jeglum J (2006) The Biology of Peatlands. (Oxford University Press; Oxford).

Seager R, Ting M, Held I, Kushnir Y, Lu J, Vecchi G, Huang HP, Harnik N, Leetmaa A, Lau NC, Li C, Velez J, Naik N (2007) Model projections of an imminent transition to a more arid climate in southwestern North America. *Science* **316**, 1181-1184.

Silvester E (2009) Ionic regulation in an alpine peatland in the Bogong High Plains, Victoria, Australia. *Environmental Chemistry* **6**, 424–431

Stream Bryophyte Group (1999) Roles of bryophytes in stream ecosystems. *Journal of North American Benthological Society* **18**, 151-184.

Suren AM (1993) Bryophytes and associated invertebrates in first-order alpine streams of Arthur's Pass, New Zealand. *New Zealand Journal of Marine and Freshwater Research*. 27-4: 479-494.

Suren AM (1996) Bryophyte distribution patterns in relation to macro-, meso-, and micro-scale variables in South Island, New Zealand streams. *New Zealand Journal of Marine and Freshwater Research*. 30: 501-523.

Vanderpoorten A, Goffinet A (2009) Introduction to Bryophytes. (Cambridge University Press: Cambridge).

Whinam J, Eberhard S, Kirkpatrick JB, Moscal A (1989) Ecology and Conservation of Tasmanian *Sphagnum Peatlands*, Tasmanian Conservation Trust.

Whinam J, Cannell EJ, Kirkpatrick JB, Comfort M (1994) Studies on the potential impact of recreational horse riding on some alpine environments of the central plateau, Tasmania. *Journal of Environmental Management* **40**, 103-117.

Whinam J, Hope GS (eds) (2005) The Peatlands of the Australasian Region. In Moorbuch Steiner MG (ed). (Museum of Upper Austria, Linz/Dornach).

Williams RJ, Wahren C, Tolsma AD, Sanecki GM, Papst WA, Myers BA, McDougall KL, Heinze DA, Green K (2008) Large fires in Australian alpine landscapes: their part in the historical fire regime and their impacts on alpine diversity. *International Journal of Wildland Fire* **17(6)**, 793-808.

Winter TC, Harvey JW, Franke OL, Alley WM (1998) Ground water and surface water – a single resource. *United States geological survey circular* **1129**, Denver, CO.

Woodward G, Perkins DM, Brown LE (2010) Climate change and freshwater ecosystems:
impacts across multiple levels of organization. Philosophical Transactions of the Royal Society B **365**, 2093–2106.

Yazaki T, Urano S, Yabe K (2006) Water balance and water movement in unsaturated zones of *Sphagnum* hummocks in Fuhrengawa Mire, Hokkaido, Japan. *Journal of Hydrology*. 319: 312-327.

Zylstra P (2006) Fire History of the Australian Alps: Prehistory to 2003. Australian Alps Liaison Committee, Department of the Environment and Heritage, Canberra. Accessed 21 August 2019 from: https://theaustralianalps.wordpress.com/the-alps-partnership/publications-and-research/fire-history-of-the-australian-alps-prehistory-to-2003.

Chapter 2

Physical and chemical drivers of vegetation in groundwater source pools on the Bogong High Plains, Victoria

Authorship statement

This chapter appears as published in the *Australian Journal of Botany* except for formatting changes such as chapter numbering of headings and figures. The full citation is as follows:

McCartney VA, Silvester E, Morgan JW, Suter PJ (2013) Physical and chemical drivers of vegetation in groundwater-source pools on the Bogong High Plains, Victoria. *Australian Journal of Botany* **61**, 566-573.

Author contribution: Field survey work and water sampling was carried out by myself with some help from PS. I conducted anion and cation experiments under the guidance of ES. I plotted data with guidance from ES and JM. The manuscript was prepared for publication by myself with editing advice from ES and JM.

Physical and chemical drivers of vegetation in groundwater source pools on the Bogong High Plains, Victoria

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Abstract

The wetland communities intimately associated with groundwater sources in the Australian alps are poorly documented compared to the broader (and more obvious) alpine peatlands. In this work we report on the vegetation observed immediately around such sources and the characteristics of the supplying groundwater in order to understand the likely factors controlling the observed vegetation assemblage. Thirty-two groundwater sources were identified across three catchments on the Bogong High Plains, Victoria (Australia) and the vegetation associated with these sources surveyed. Groundwater sources occurred across a range of altitudes (1667 – 1854 m), independent of aspect, and were hydrologically connected to (upstream of) peatlands. Localised mounding adjacent to the groundwater sources resulted in the formation of pools ('groundwater source pools'). The vegetation within the pools was dominated by bryophytes, with the aquatic bryophyte *Blindia robusta* the most common species. The groundwater was deficient in major ions, and similar to rainwater apart from elevated levels of CO_2 accumulated in the groundwater recharge process. The high CO_2 levels, combined with the near-constant temperature conditions provided by sustained groundwater flow, are thought to be likely drivers for the high abundance of *Blindia robusta*. While the relative contributions of rain and snow to aquifer recharge are not fully understood for the Australian alps, these ecosystems are likely vulnerable to the changes in precipitation regime that are predicted under climate change scenarios.

Additional keywords: groundwater dependent ecosystems, alpine, bryophytes, *Blindia robusta.*

2.1 Introduction

Groundwater reserves result from rain and snow that infiltrates into spaces in fissured rocks as either unconfined or confined aquifers (Winter *et al.* 1998). When the aquifer water table intersects the land surface, the discharge of groundwater results in wetlands or springs (Winter *et al.* 1998). Springs with a flow rate allowing for mound accumulation, or wall accumulation resulting in small weirs, occur in a wide range of environments (Hancock and Humphreys 2005; Humphreys 2006; Keppel *et al.* 2011). The biological communities associated with these systems are generally known as groundwater-dependent ecosystems (GDEs), receiving seasonal or constant flows of water (Murray *et al.* 2003)

Groundwater sources frequently support wetland communities (Bragg 2002; Boulton 2005; Shannon and Morgan 2007; Granath *et al.* 2010). In the Australian alps, the wetlands that form around groundwater discharges are typically peat-accumulating and dominated by *Sphagnum* species (Costin 1955; Clarke and Martin 1999; Wahren *et al.* 1999; Whinam *et al.* 2003; Shannon and Morgan 2007). The floristic composition of *Sphagnum*-dominated wetlands and peatlands in the Australian alps has been well-studied (Clarke and Martin 1999; Whinam *et al.* 2003; Shannon and Morgan 2007). The hydrochemical properties of these systems, however, have received much less attention. Silvester (2009) found that the chemical composition of alpine groundwater sources in the Bogong High Plains was similar to rainwater, but more acidic due to CO_2 supersaturation. The conditions experienced by vegetation in close proximity to these groundwater sources are therefore likely quite different from those experienced at greater distance from the sources. To our knowledge, there have been no studies of the vegetation that occurs in close proximity to groundwater sources in the Australian alps.

The present study aimed to identify the location of groundwater source pools across the Bogong High Plains, and to describe the composition of the vegetation in these pools. We also recorded physical environment and chemical composition of groundwater supplying these pools to identify the key drivers of groundwater pool vegetation.

2.2 Materials and methods

2.2.1 Study area

The Bogong High Plains (BHP) is an elevated plateau within the Great Dividing Range, located in north-east Victoria, Australia, and contained within the Alpine National Park. The BHP occupy an area of approximately 120 km^2, with elevations ranging between 1650 and 1850 m. Mean annual rainfall is 2448 mm, mean maximum temperature is 9.3 ^{o}C and mean minimum temperature is 2.5 ^{o}C (Rocky Valley meteorological recording station, Bureau of Meteorology, *unpublished data*). The majority of precipitation falls as snow, with the major snowfalls between the months of June and October (Williams 1987).

The geology of the Bogong High Plains has an origin pre-dating the Oligocene, with O'Sullivan and Orr *et al*. (1999) suggesting that denuding of the present Bogong High Plains occurred during the Cretaceous. This was followed by the folding and metamorphosis of Ordovician and Silurian sediments, with intrusive basaltic plutons dating to the late Silurian to early Devonian (Costin 1955; Morand 1990; O'Sullivan *et al*. 1999). Gneiss and schists are the major rock types of the Bogong High Plains (Costin 1955; Morand 1990).

Three areas were selected for this study encompassing the catchments of Whiterock Creek (WR), Heathy Spur (HS) (part of the Watchbed Creek catchment) and Cope Creek (CC) (Figure 2.1). These catchments provide flow to either the Kiewa (HS, CC) or Mitta (WR) rivers and form part of the headwaters of the Murray-Darling Basin.

Fig.2.1 Map of the Bogong High Plains showing the three catchments with locations of groundwater sources.

2.2.2 Groundwater locations and vegetation

Potential groundwater sources were identified from the locations of stream drainage lines on topographical maps of the BHP. Candidate sites were investigated in the field during the period December 2011 to January 2012 for vegetation reliant upon constant moisture, or by following the sound of running water to the groundwater spring (groundwater source). The survey is not necessarily a comprehensive record of all sites in these catchments as it is possible that some sites were not located. Many groundwater sources investigated had an associated pool system; in this work, the term 'groundwater source pool' is taken to mean the first pool of water to accumulate downhill from the groundwater spring. Typically, the groundwater source and the source pool were very close to each other (i.e. within 1-2 m).

Altitude and aspect were recorded for each site. The length and width of each groundwater source pool were measured, as was the depth at several points within the pool to find the deepest point. Vegetation within the pools was sampled using a 25 cm $\times$ 25 cm quadrat. Five randomly placed quadrats were used to sample bryophyte cover in pools that were less than 1 m^2 in area, and ten randomly placed quadrats were used in larger pools. All vegetation was identified to species level and assigned a percent cover. The percentage of open water was also recorded for each pool. Voucher specimens with groundwater source pool location and date of collection are permanently stored at La Trobe University Albury-Wodonga campus.

2.2.3 Water sampling and water analysis

Physico-chemical parameters were recorded for each groundwater source and groundwater source pool at 5 cm depth. Physical parameters recorded were: pH (using a Metrohm 821 Mobile pH meter and Metrohm 'Aquatrode' pH electrode), dissolved

oxygen (using a HACH 40Q meter and Luminescent Dissolved Oxygen (LDO) probe) and water temperature. pH calibration was performed in the field, immediately prior to measurement. Filtered (0.45 μm, cellulose nitrate membranes) water samples (50 ml) were collected in triplicate from each groundwater source and groundwater source pool during the initial survey. Samples were transported on ice and then frozen prior to analysis.

Water samples were analysed in the laboratory for base cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) and acid anions (Cl^-, NO_3^- and SO_4^{2-}) by ion chromatography, using standard methods described by Silvester (2009). Carbonate alkalinity (HCO_3^- and CO_3^{2-}) in the water samples was measured by the fixed pCO_2 pH method, using a gas mixture containing 419 ppm CO_2 in nitrogen (Silvester, 2009).

2.2.4 Data Analysis

Identified groundwater source pools were re-visited several times over the 2011-12 summer to determine the 'permanency' of groundwater flows. These sites were also re-visited during the 2012-13 summer at the end of a particularly low spring-summer seasonal rainfall of 683 mm (compared to long term average spring-summer rainfall of 1028 mm). The assessment of groundwater source permanency in both 2011-12 and 2012-13 summers is shown in Table 3.1; the 2011-12 assessment is used in the data analysis as this was the period over which the vegetation surveys were conducted. To determine whether pool position (altitude, aspect and area) varied, we compared differences in these field site attributes using chi square tests of independence. Vegetation compositional differences within the groundwater source pools was analysed by multi-dimensional scaling (MDS) ordination, using both percent cover and presence-absence data in the PRIMER program. A chi-square test was used to examine the independence of *Blindia robusta* occurrence and groundwater source pool permanency.

Differences in water physical properties between catchments were analysed by one way ANOVA using log-transformed data. Scatter plots (means ± 2 SD) are used here to describe the temperature, pH and depths of the groundwater sources and groundwater source pools. In the case of pH, means and standard deviations were calculated from free H^+ concentrations. Ionic data for groundwater sources and pools were visualised graphically using Piper and ternary plots, generated with the GSS module of Geochemist Workbench (v8). One-way ANOVAs were used to analyse the difference in the ionic data between catchments.

2.3 Results

2.3.1 Groundwater source distribution

Thirty-two groundwater sources were identified across the three catchments (Figure 2.1). Groundwater source pools were found in all catchments and occupied various landscape positions in relation to aspect and altitude (Table 2.1). In total, there were 31 pools found and 22 groundwater sources were classified as permanent in the 2011-12 summer (Table 2.1); the remaining 9 groundwater sources were classified as temporary.

Mean groundwater source pool depth did not vary significantly between catchments (p=0.174), with depths ranging from 3 to 43 cm (Figure 2.2a; Table S2.1; Supplementary material). Pool areas did not vary significantly with altitude across all three catchments (p=0.363); however, the mean area of the pools varied between catchments. HS catchment had a mean pool area of 22.7 m^2, WR catchment had a mean pool area of 33.0 m^2 and CC catchment had a mean pool area of 6.4 m^2 (Table 2.2). Open water was found in groundwater source pools in all three catchments. HS had five pools with open water (mean: 8% of pool area); WR had six pools with open water (10%); CC had two sites with open water (41%) (Table 2.2).

Table 2.1. Groundwater source pools sites. HS= Heathy Spur, WR = Whiterock Creek, CC= Cope Creek. Shown are: the number of groundwater sources located, the number of sources with associated pools, the assessed permanency of these pools (2011-12 and 2012-13 summers) and the location (altitude and aspect) of the groundwater sources.

Catchment	Total Sources	Sources with pools	Permanent Pools (2011-12)	Permanent Pools (2012-13)	Mean Altitude (m)	Northerly Aspect	Southerly Aspect	Easterly Aspect	Westerly Aspect
HS	11	10	9	9	1750	0	6	1	3
WR	10	10	9	7	1810	8	0	0	0
CC	11	11	4	3	1710	1	3	0	0
Totals	32	31	22	19		9	9	1	3

Table 2.2. Moss species found in the permanent groundwater pools (based on 2011-12 assessment; see Table 1) across the three catchments. Shown are: the number of sites in each catchment where specific bryophyte species were found with % cover. Also shown are the % open water and mean pool area in each of the catchments. HS= Heathy Spur, WR= Whiterock Creek, CC= Cope Creek.

Catchment	Permanent Pools (2011-12)	*Blindia robusta*		*Polytrichum commune*		*Warnstorfia fluitans*		*Bartramia bogongia*		*Sphagnum cristatum*		Open water		Mean pool area
		No. sites	% cover	No. sites	% cover	No. sites	% cover	No. sites	% cover	No. sites	% cover	No. sites	% cover	(m^2)
HS	9	9	50	4	14	1	7	2	7	1	.02	5	8	22.7
WR	9	9	59	4	12	1	3	1	.5	0	0	6	10	33.0
CC	4	2	31	0	0	1	.2	0	0	0	0	3	41	6.4
Totals	22	20		8		3		3		1		14		

2.3.2 Vegetation growing in the groundwater source pools

Across all catchments, there was a total of 20 plant species found growing in the groundwater source pools with seven species of moss, three liverwort species and 10 vascular species (Table 2.3). The five most common species were all mosses, accounting for >70% of vegetation cover in pools (Table 2.3). The vascular plants *Schoenus calyptratus, Richea continentis, Poa costiniana, Empodisma minus, Isolepsis crassiuscula* and *Carex gaudichaudiana* were highly represented but only accounted for 8% of cover in pools (Table 2.3). MDS ordination did not separate the groundwater source pool vegetation by catchment (see Figures S2.7 and S2.8; Supplementary material, Table S2.3).

All catchments had *Blindia robusta* (found at a total 20 sites) and *Warnstorfia fluitans* (found at a total of 3 sites). *Blindia robusta* had the highest mean cover of any species in pools (53%). The moss *Polytrichum commune* had a mean cover of 9%, but was only found in HS and WR catchments (Tables 2.2 and 2.3). Significantly, *Blindia robusta* was present in 91% of the permanent groundwater source pools and absent in all of the temporary pools ($\chi^2 = 16.38$; p<0.05).

Table 2.3. Plant species found in the groundwater source pools across all three catchments. Shown are the total percentage cover of each species and the percentage of sites at which they were found. HS = Heathy Spur, WR = Whiterock Creek, CC = Cope Creek.

Species	Vascular plant/ Bryophyte	% coverage	% sites
Blindia robusta	Moss	53	66
Polytrichum commune	Moss	9	33
Warnstorfia fluitans	Moss	4	12
Bartramia bogongia	Moss	4	12
Pyrrhobryum mnioides	Moss	3	4
Schoenus calyptratus	Vascular	3	25
Jungermannia orbiculata	Liverwort	1	4
Riccardia sp.	Liverwort	1	8
Empodisma minus	Vascular	1	17
Richea continentis	Vascular	1	29
Isolepsis crassiuscula	Vascular	1	17
Poa costiniana	Vascular	1	21
Breutelia affinis	Moss	<1	4
Sphagnum cristatum	Moss	<1	4
Isotachis montana	Liverwort	<1	4
Acaena novea-zelandiae	Vascular	<1	4
Craspedia lamicola	Vascular	<1	4
Pratia surrepens	Vascular	<1	4
Carex gaudichaudiana	Vascular	<1	12
Viola betonicifolia	Vascular	<1	4

2.3.3 Groundwater and source pool water physico-chemical characteristics

Water temperatures ranged from 4.9°C to 14.5°C (mean 6°C) for groundwater sources, and from 5.3°C to 14.5°C (mean 9°C) for groundwater source pools across all three catchments (Figure 2.2b). Mean pH values ranged between 5.24 and 5.33 for groundwater sources and between 5.32 and 5.41 for groundwater source pools (Figure 2.4c). Within each catchment, the mean pH for the groundwater sources was consistently lower than the mean pH for the corresponding source pool.

The concentrations of the major ions in groundwater sources and groundwater source pools were generally very low and are similar to the levels observed in rainwater (Berner and Berner, 1987; Table S2.4; Supplementary material). The ionic composition of groundwater collected across all sites is shown in Figure 2.3 in the form of a Piper diagram. This representation enables a visual comparison of the base cation and acid anion dominance in the water samples; it provides no information about the concentrations of the ions in the water samples. The base cation composition was very similar across the three catchments, with a strong sodium dominance and with the balance of the charge provided by calcium and magnesium in near equal amounts. The anion composition varied more strongly between the three catchments, with a higher proportion of the negative charge provided by chloride in HS compared to WR and CC. Groundwater from the Bogong High Plains has been previously shown to have significant levels of nitrate (NO_3^-; Silvester, 2009), and given that sulfate is a minor component of the anion chemistry, a more informative representation of the anion chemistry is provided by a ternary diagram for: chloride-bicarbonate-nitrate (Figure 2.4). This shows that HS water differs from WR and CC due to higher nitrate and lower bicarbonate contributions to the negative charge. This difference in the ionic composition of groundwaters across the three catchments is confirmed by PCA (Figure S2.9; Supplementary material).

Fig 2.2. Physical measurements recorded for groundwater sources and source pools in Heathy Spur (HS), Whiterock Creek (WR) and Cope Creek (CC) catchments (S=source; P=pool). Shown are: (a) mean values for groundwater source pool depths; (b) mean values for groundwater source and pool temperatures; (c) mean values for groundwater source and pool pH (●). Also shown in (c) are the mean pH values for groundwater source samples after equilibration with atmospheric levels of CO_2 (○). All error bars are ±2 s.d.

Fig. 2.3. Piper plot for groundwater samples collected from Heathy Spur (green), Whiterock Creek (red) and Cope Creek (blue) catchments. Each plot shows the percentage contribution to positive (cationic) or negative (anionic) charge, totalling to 100% in each case.

Fig. 2.4. Ternary diagram (HCO_3^--Cl^--NO_3^-) for groundwater samples collected from Heathy Spur (green), Whiterock Creek (red) and Cope Creek (blue) catchments. Data points correspond to the percentage contribution to negative ionic charge, totalling to 100%.

2.4 Discussion

2.4.1 Groundwater dependent ecosystems on the Bogong High Plains

The Bogong High Plains have been exposed to erosional processes since the Silurian-Devonian period, approximately 400 million years ago (Morand 1990). Metamorphic processes, together with uplifting and fracturing, have enabled the formation of many

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small aquifers (Taylor *et al*. 2004). These aquifers provide sustained flow during periods of low rainfall and are likely critical to the viability of alpine aquatic ecosystems. In this study, groundwater sources and associated pool systems were found across a range of altitudes and aspects suggesting that, within the sub-alpine altitudinal range, geological features controlling the locations of groundwater springs were more important factors than landscape position.

The vegetation found in groundwater source pools was dominated by bryophytes, with *Blindia robusta* by far the most common and abundant species. *Sphagnum cristatum,* a common peat-forming plant in alpine wetlands, was rarely observed in groundwater source pools. While a range of other bryophytes and vascular plants were found in groundwater source pools, we found no difference in community composition between the three catchments, suggesting that the same wetland species generally inhabit the groundwater source pools across the BHP. Groundwater source (and hence groundwater source pool) permanency varied across the three catchments studied. In the CC catchment, most pools were classified as temporary, whereas the majority of pools in the WR and HS catchments were classified as permanent. This difference in permanency likely influences the intra-annual persistence of vegetation in the pools, and may account for the variations we observed in bryophyte contribution to pool cover across the three catchments.

Water analyses from the three catchments indicate that the ionic concentrations in the groundwater sources are very low, and similar to that found in rainwater or snow (Silvester, 2009). The composition of groundwater is controlled by regolith and underlying geology, and the amount of time that the water spends in the aquifer (Eamus *et al*. 2006; Rydin and Jeglum 2009). The low ionic concentrations in these groundwaters are consistent with a low reactivity rock such as the granites/granodiorite found on the Bogong High Plains. All three catchments were similar in their ionic composition except

for some minor differences in the relative contributions of nitrate and bicarbonate to the anion charge. The similarity of the groundwater composition across these catchments suggests that the major ion composition is unlikely to be a factor in any observed differences in bryophyte distribution on the Bogong High Plains.

`Groundwater frequently has elevated levels of CO_2 compared to those found in water equilibrated with the atmosphere (i.e. they are supersaturated in CO_2). CO_2 supersaturation results from the mineralisation of organic carbon contained in water that infiltrates into aquifers. The higher levels of CO_2 are maintained until the groundwater is exposed to the atmosphere (Stumm and Morgan 1981). A consequence of elevated CO_2 levels in groundwater is lower pH, due to the increased concentrations of carbonic acid. This effect is evident in the groundwater samples collected from HS, WR and CC where equilibration with atmospheric levels of CO_2 (approximately 400 ppm) increases the groundwater pH by more than one pH unit (see Figure 2.2c). Within the groundwater source pools the pH is very close to that of the associated groundwater source, indicating that elevated CO_2 concentrations are also present in the pools.

2.4.2 Factors controlling *Blindia robusta* distribution

The key factors controlling the distribution of bryophytes (particularly *Blindia robusta*) in groundwater source pools appear to be physical and/or chemical, including: constant water supply, near-constant water temperature, low ionic composition water, and elevated levels of CO_2. The factors particularly unique to groundwater source pools, when compared to peatlands more generally, are the near-constant temperature and the elevated levels of CO_2. Downstream of these pools, water temperatures are more likely to be influenced by atmospheric temperatures and experience both diurnal as well as seasonal variations. Similarly, CO_2 out-gassing to the atmosphere means that elevated CO_2 conditions are only found in close proximity to groundwater sources. Low ionic

concentrations, on the other hand, are common to the entire peatland (Silvester, 2009), with the only significant difference between groundwater and peatland drainage water being the removal of nitrate within peatlands (Silvester, 2009). Groundwater source pools can therefore experience higher nitrate levels than the downstream peatland vegetation.

It is possible that *Blindia robusta* dominates groundwater source pools by taking advantage of the elevated CO_2 in the groundwater before it equilibrates with the atmosphere. This would be consistent with the observation that *Blindia robusta* is a submerged aquatic species; its physical environment is therefore one of near-constant temperature, and high availability of CO_2. The effective absence of *Sphagnum cristatum* from groundwater source pools indicates that either it is unable to cope with high-flow regimes commonly observed in these pools (Wahren *et al*. 2001), or that it is unsuited to water containing elevated CO_2. *Sphagnum* is highly competitive for habitat and able to colonize areas by establishing on other plants (Wahren *et al*. 2001). It has the ability to efficiently access water for survival by transporting water to the capitula, holding water in its capillary system and can create its own habitat (Ryden *et al*. 2006). The absence of *Sphagnum* from groundwater source pools strongly suggests that the physical and/or chemical environment is not suited to its growth.

The apparent absence of *Blindia robusta* from temporary groundwater source pools suggests a reliance on constant water supply. To our knowledge, very little is known about the sensitivity of *Blindia robusta* to drying and desiccation, its ability to recover through spore germination after re-wetting, or the timescales over which recovery might occur.

2.4.3 Similarity to other groundwater dependent ecosystems

Springs with a flow rate allowing for mound accumulation or wall accumulation resulting in small weirs occur in a wide range of environments (Hancock *et al*. 2005; Humphreys 2006; Keppel *et al*. 2011). The arid zone, for example, has permanent spring fed wetlands resulting in the formation of vegetation mounds (Fensham *et al* 2004). The groundwater source pools on the BHP appear to be the result of either soil slumping and then vegetation accumulation, or slow blocking of flow by *Blindia robusta* resulting in the formation of weir walls. The spring mounds of the arid region are isolated specialised habitats with reduced species richness due to succession, resulting in domination by single species such as *Phragmites australis* (Fensham *et al* 2004). The dominance of *Blindia robusta* in groundwater source pools on the Bogong High Plains may be the result of similar succession processes, although the long term trajectories of these systems is yet unknown.

2.4.4 Climate change implications

In mountain areas with winter snowfall, the highest percentage of annual streamflow occurs in the spring months when the snow is melting (Rydin and Jeglum 2006). During summer months, streamflows are reduced and more reliant upon groundwater and/or precipitation events (Rydin and Jeglum 2006). The vegetation observed in this study in groundwater source pools appears to be highly reliant upon groundwater flow and any process that impacts on the reliability of this flow is likely to adversely affect the viability of these dependent bryophyte species. Climate change projections for the Australian Alps indicate decreased winter snowpack (Green and Pickering, 2009), and therefore decreased groundwater recharge. A switch from permanent groundwater supply to temporary (seasonal) is likely to affect the viability of the bryophyte species currently growing in groundwater source pools due to (i) less reliable water supply, (ii) stronger diurnal and

seasonal temperature fluctuations, and (iii) competition from other species able to take advantage of the changed conditions. Our future work will investigate the response of *Blindia robusta* to drying and desiccation, providing information about the likely resilience of these communities to future changes in groundwater source permanency.

2.5 References

Berner EK, Berner RA. (1997) 'Global Water Cycle; Geochemistry and Environment.' (Prentice-Hall: New Jersey)

Boulton AJ. (2005) Chances and challenges in the conservation of groundwaters and their dependent ecosystems. *Aquatic Conservation: Marine and Freshwater Ecosystems* **15**(4), 319-323.

Bragg OM. (2002) Hydrology of peat-forming wetlands in Scotland. *The Science of The Total Environment* **294**(1-2), 11-129.

Climate statistics for Australian locations. (Bureau of Meteorology) Available at http://www.bom.gov.au/climate/averages/tables/cw_083084.shtml [Verified 7 August 2012]

Chapman MG, Underwood AJ. (1999) Ecological patterns in multivariate assemblages: information and interpretation of negative values in ANOSIM tests. *Marine Ecology Progress Series* **180**, 257-265.

Clarke PJ, Martin ARH. (1999) *Sphagnum* peatlands of Kosciusko National Park in relation to altitude, time and disturbance. *Australian Journal of Botany* **47**, 519-536.

Clarke KR, Warwick RM. (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. (Primer-E Ltd: Plymouth)

Costin AB. (1955) Alpine soils in Australia with reference to conditions in Europe and New Zealand. *European Journal of Soil Science* **6**(1), 35-50.

Eamus D, Hatton T, Cook P, Colvin C. (2006) 'Ecohydrology Vegetation Function, Water and Resource Management.' (CSIRO Publishing: Collingwood)

Fensham, R.J, Fairfax, R.J, Pocknee, D, Kelley J. (2004) Vegetation patterns in permanent spring wetlands in arid Australia. Australian Journal of Botany **52**(6), 719-728

Granath G, Strengbom J, Rydin H. (2010) Rapid ecosystem shifts in peatlands: linking plant physiology and succession. *Ecology* **91**(10), 3047-3056.

Green K, Pickering CM. (2009) The decline of snowpatches in the Snowy Mountains of Australia: Importance of climate warming, variable snow, and wind. *Arctic, Antarctic, and Alpine Research* **41,** 212-218.

Hancock PJ, Boulton AJ, Humphreys WF. (2005) Aquifers and hyporheic zones: Towards an ecological understanding of groundwater. *Hydrogeology Journal* **13**(1), 98-111.

Humphreys WF. (2006) Aquifers: the ultimate groundwater-dependent ecosystems. *Australian Journal of Botany* **54**(2), 115-132.

Keppel MN, Clarke JDA, Halihan T, Love AJ, Werner AD. (2011) Mound springs in the arid Lake Eyre South region of South Australia: A new depositional tufa model and its controls. *Sedimentary Geology* **240**, 55-70.

McDougall KL. (2007) Grazing and fire in two subalpine peatlands. *Australian Journal of Botany* **55**, 42-47.

Morand VJ. (1990) Low-pressure regional metamorphism in the Omeo Metamorphic Complex, Victoria, Australia. *Journal of Metamorphic Geology* **8**, 1-12.

Murray BR, Zeppel MJB, Hose GC, Eamus D. (2003) Groundwater-dependent ecosystems in Australia: It's more than just water for rivers. *Ecological Management & Restoration* **4**(2), 110-113.

Nagy L, Grabherr G. (2009) 'The biology of alpine habitats.' (Oxford University Press: Oxford)

O'Sullivan PB, Orr M, O'Sullivan AJ, Gleadow AJW. (1999) Episodic Late Palaeozoic to Cenozoic denudation of the southeastern highlands of Australia: evidence from the Bogong High Plains, Victoria. *Australian Journal of Earth Sciences* **46**, 199-216.

Rydin H, Gunnarsson U, Sundberg S. (2006) The role of *Sphagnum* in peatland development and persistence. *Ecological Studies* **188,** 47-65.

Rydin H, Jeglum J. (2009) The biology of peatlands. Oxford University Press, Oxford.

Shannon JM, Morgan JW. (2007) Floristic variation in *Sphagnum*-dominated peatland communities of the Central Highlands, Victoria. *Cunninghamia* **10**(1), 59-76.

Silvester E. (2009) Ionic regulation in an alpine peatland in the Bogong High Plains, Victoria, Australia. *Environmental Chemistry* **6**, 424-431.

Stumm W, Morgan JJ. (1981) Aquatic chemistry. In 'An Introduction Emphasizing Chemical Equilibria in Natural Waters. In 'The Regulation of the Chemical Composition of Natural Waters'. 2nd Ed. pp 534-538. (John Wiley & Sons, Inc: USA)

Taylor DH, Morand VJ, Cayley RA, Wohlt KE, Simons BA, Meagher S. (2004) *Falls Creek 1:50,000 geological map.* Geological Survey of Victoria.

Terradas J, Penuelas J, Lloret F. (2009) The fluctuation niche in plants. *International Journal of Ecology* **2009,** Article ID 959702 1-5.

Wahren C-HA, Williams RJ, Papst WA. (1999) Alpine and subalpine wetland vegetation on the Bogong High Plains, south-eastern Australia. *Australian Journal of Botany* **47**, 165-188.

Wahren C-HA, Williams RJ, Papst WA. (2001) Vegetation change and ecological processes in alpine and subalpine *Sphagnum* bogs of the Bogong High Plains, Victoria, Australia. *Arctic, Antartic, and Alpine Research* **33**(3), 357-368.

Western A, Siriwardena L, Lawrence R, Rutherford I. (2008) Sponges or wicks? What is the role of bogs in hydrological response on the Bogong High Plains? *Proceedings of Water Down Under 2008, 14-17 April* (Eds M. Lambert, T. Daniell, M. Leonard) 2008, pp 1060-1071 (Engineers Australia, Adelaide).

Whinam J, Chilcott NM, Morgan JW. (2003) Floristic composition and environmental relationships of *Sphagnum*-dominated communities in Victoria. *Cunninghamia* **8**(2), 162-174.

Williams RJ. (1987) Patterns of air temperature and accumulation of snow in subalpine heathlands and grasslands on the Bogong High Plains, Victoria. *Australian Journal of Ecology* **12**, 153-163.

Winter TC, Harvey JW, Franke OL, Alley WM. (1998) Ground Water and Surface Water – A Single Resource, United States Geological Survey. *Circular* **1139**, Denver, Colorado.

2.6 Supplementary Material

Fig. S2.1.   Location photograph of the top of the Whiterock Creek (WR) catchment.

Fig. S2.2. Photograph of part of the Watchbed Creek (HS) catchment.

Fig. S2.3. Photograph of part of the Cope Creek (CC) catchment near Pretty Valley pondage.

Fig. S2.4. Photograph of *Blindia robusta* (dark green aquatic moss) with *Bartramia subsymmetrica* (lime green semi-aquatic moss) in a typical groundwater source pool at Heathy Spur (HS) catchment.

Fig. S2.5. A typical groundwater source pool found in the Whiterock Creek (WR) catchment. This pool had *Blindia robusta* and *Bartramia subsymmetrica* present in the pool. *Empodisma minus, Richea continentis, Poa costiniana* and *Baeckea gunniana* are shown in this photograph. All of these species are commonly found in wetland areas in the Bogong High Plains.

Fig. S2.6. A typical groundwater source pool found in the Cope Creek (CC) catchment. The groundwater source pools usually consisted of very fine sediment and little to no vegetation.

Fig. S2.7. MDS ordination of Heathy Spur (HS), Whiterock Creek(WR) and Cope Creek (CC) catchments. Two dimensional configuration based on (%) cover data (Minimum stress = 0.01)

Fig. S2.8. MDS ordination of Heathy Spur, Whiterock Creek and Cope Creek catchments. Two dimensional configuration based on presence/absence data (Minimum stress = 0.01).

Fig. S2.9. Principal component analysis (PCA) for anion and cation levels in the groundwater source water comparing all catchments. This figure shows that all catchments are almost the same.

Fig. S2.10. Principal component analysis (PCA) for chloride, nitrate and bicarbonate levels in the groundwater source pools comparing Heathy Spur (HS), Whiterock Creek (WR) and Cope Creek (CC) catchments.

Table S2.1. Depths for the permanent groundwater-source pools at Heathy Spur,

Whiterock Creek and Cope Creek. HS= Heathy Spur; WR= Whiterock Creek; CC=

Cope Creek.

Site	Depth-cm
HSGW-1	27
HSGW-2	20
HSGW-3	8
HSGW-4	18
HSGW-5	14
HSGW-6	3
HSGW-7	24
HSGW-8	16
HSGW-11	26
WRGW-1	23
WRGW-3	18
WRGW-4	12
WRGW-5	13
WRGW-6	19
WRGW-7	23
WRGW-8	23
WRGW-9	20
WRGW-10	43
CCGW-3	29
CCGW-4	12
CCGW-7	4
CCGW-8	4

Table S2.2. Site descriptions for the groundwater sources at Heathy Spur, Whiterock

Creek and Cope Creek. HS= Heathy Spur; WR= Whiterock Creek; CC= Cope Creek.

Site	Length m	Width m	Alt	Aspect	Depth cm	Catchment
HSGW-1	1.5	1.8	1737	South	27	HS
HSGW-2	3.5	2.3	1759	South	20	HS
HSGW-3	7.3	4.2	1761	South	8	HS
HSGW-4	6.9	6.3	1743	East	18	HS
HSGW-5	2.1	2	1737	West	14	HS
HSGW-6	10.8	6.8	1748	WSW	3	HS
HSGW-7	2.5	2.2	1760	WSW	24	HS
HSGW-8	9.3	2.5	1747	SW	16	HS
HSGW-10	8.2	4.2	1763	South	25	HS
HSGW-11	1.7	1	1737	SW	26	HS
WRGW-1	5.1	4.5	1790	North	23	WR
WRGW-2	0.9	1.3	1758	North	5	WR
WRGW-3	0.4	0.4	1792	North	18	WR
WRGW-4	1.2	1.5	1803	North	12	WR
WRGW-5	1.7	2	1775	North	13	WR
WRGW-6	16	12	1787	North	19	WR
WRGW-7	1.4	2.2	1798	North	23	WR
WRGW-8	5.5	1.8	1854	NW	23	WR
WRGW-9	4.3	2.1	1847	NW	20	WR
WRGW-10	6.9	3.2	1780	North	43	WR
CCGW-1	1.7	0.6	1722	South	12	CC
CCGW-2	4.1	1.8	1720	SE	8	CC
CCGW-3	2.2	1.1	1737	SE	29	CC
CCGW-4	2.9	7.2	1667	SE	12	CC
CCGW-5	0.6	0.5	1680	SE	6	CC
CCGW-6	2	0.7	1691	SE	16	CC
CCGW-7	0.98	0.6	1680	SE	4	CC
CCGW-8	2	0.9	1758	North	4	CC
CCGW-9	0.33	0.24	1767	East	15	CC
CCGW-10	1.1	0.7	1733	SE	10	CC
CCGW-11	0.46	0.3	1736	SE	8	CC

Table S2.3. Mean cover (%) of vegetation species present in groundwater source pools in three catchments across the Bogong High Plains.

Species	Average %
Blindia robusta	53.00
Polytrichum commune	8.90
Warnstorfia fluitans	4.20
Bartramia bogongia	3.60
Pyrrhobryum mnioides	3.00
Schoenus calyptratus	3.00
Poa costiniana	1.30
Isolepsis crassiuscula	1.00
Richea continentis	1.00
Jungermannia orbiculata	0.90
Empodisma minus	0.70
Riccardia ssp.	0.50
Carex gaudichaudiana	0.50
Craspedia ssp.	0.40
Pratia surrepens	0.40
Acaena ssp.	0.20
Isotachis montana	0.10
Viola betonicifolia	0.08
Breutelia affinis	0.04
Sphagnum cristatum	0.01

Table S2.4. Mean and ± 2 Standard Deviation results for anions and cations in the groundwater sources at Heathy Spur, Whiterock Creek and Cope Creek. HS= Heathy Spur; WR= Whiterock Creek; CC= Cope Creek.

Anion and cation analyses for groundwater at Heathy Spur, Whiterock Creek and Cope Creek

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Chloride ppm	HS	35	.541	.095	.016	.508	.573	.449	.735
	WR	25	.488	.073	.015	.457	.518	.332	.679
	CC	25	.477	.097	.019	.437	.517	.323	.756
	Allsites	85	.510	.093	.010	.486	.526	.323	.756
Nitrate ppm	HS	35	.789	.645	.109	.567	1.011	.108	2.138
	WR	25	.274	.070	.014	.245	.303	.132	.373
	CC	25	.369	.162	.032	.302	.436	.153	.691
	Allsites	85	.514	.482	.052	.410	.618	.108	2.138
Sulphate ppm	HS	35	.201	.043	.007	.187	.216	.127	.265
	WR	25	.257	.036	.007	.242	.272	.178	.338
	CC	25	.253	.133	.027	.198	.308	.144	.846
	Allsites	85	.233	.083	.009	.215	.251	.127	.846

Table S2.4 Cont/. Mean and ± 2 Standard Deviation results for anions and cations in the groundwater sources at Heathy Spur, Whiterock Creek and Cope Creek. HS= Heathy Spur; WR= Whiterock Creek; CC= Cope Creek.

Anion and cation analyses for groundwater at Heathy Spur, Whiterock Creek and Cope Creek

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Sodium ppm	HS	35	.550	.099	.017	.516	.584	.410	.735
	WR	25	.687	.161	.032	.620	.753	.396	1.076
	CC	25	.685	.194	.039	.605	.765	.436	1.266
	Allsites	85	.630	.163	.018	.595	.665	.396	1.266

Table S2.4 (Cont.). Mean and ± 2 Standard Deviation results for anions and cations in the groundwater sources at Heathy Spur, Whiterock

Creek and Cope Creek. HS= Heathy Spur; WR= Whiterock Creek; CC= Cope Creek continued.

Anion and cation analyses for groundwater at Heathy Spur, Whiterock Creek and Cope Creek

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Calcium ppm	HS	35	.266	.109	.019	.229	.304	.169	.534
	WR	25	.259	.047	.009	.240	.278	.165	.335
	CC	25	.254	.084	.017	.219	.288	.162	.468
	All sites	85	.260	.087	.009	.242	.279	.162	.534
Magnesium ppm	HS	35	.126	.052	.009	.108	.144	.070	.269
	WR	25	.119	.013	.003	.113	.124	.098	.143
	CC	25	.130	.020	.004	.122	.138	.089	.163
	All sites	85	.125	.036	.004	.117	.133	.070	.269
HCO_3^- ppm	HS	35	.939	.368	.062	.813	1.066	.407	1.832
	WR	25	1.770	.536	.107	1.548	1.991	1.010	3.067
	CC	25	1.738	.642	.128	1.473	2.003	.918	3.261
	All sites	85	1.418	.646	.070	1.279	1.558	.407	3.261

Appendix:

Physical measurements for groundwater and groundwater source pools including CO_2 and H_2CO_3 (aq).

Physical parameters for aquatic environments were recorded during the surveys and are reported in Table 2.1. One of the observations in this work was that the pH of the groundwater increased after equilibration with the atmosphere. The explanation for this is as follows:

CO_2 in the groundwater will be in the form of dissolved CO_2 (aqueous) and carbonic acid (H_2CO_3) in dynamic equilibria (equations 1 and 2). Levels of dissolved CO_2 in groundwater can be well above atmospheric equilibration due to entrainment of high CO_2 containing water during re-charge. As the concentrations of the CO_2 increase the pH will go down (more acidic; equation 3). When the groundwater with excess CO_2 meets the atmosphere, the CO_2 will equilibrate with atmospheric levels (efflux) leading to an increase in the pH. The top part of the *Blindia robusta* plant is exposed to the atmosphere therefore it may be utilising atmospheric CO_2 like terrestrial plants or it may be taking some of the carbonic acid available in the groundwater, this needs future investigation.

$$CO_{2\ (gas)} \rightleftharpoons CO_{2\ (aq)} \qquad\qquad\qquad (1)$$

$$CO_{2\ (aq)} + H_2O \rightleftharpoons H_2CO_{3\ (aq)} \qquad\qquad (2)$$

$$H_2CO_3\ (aq) \rightleftharpoons HCO_3^-\ (aq) + H^+\ (aq) \qquad (3)$$

Chapter 3

Blindia robusta – an aquatic mossball in Australian alpine groundwater source pools

Introduction

3.1   *Blindia robusta*

Mossballs are an unusual growth habit of mosses (Frahm, 2001). It is thought that tightly compacted balls of moss can form due to storm or high wind activity. Such growth forms have not been observed previously amongst mosses in Australia. During distribution surveys for *Blindia robusta*, a recognisable - but poorly studied alpine moss that grows in groundwater source pools on the Bogong High Plains, Victoria (Meagher and Fuhrer, 2003; Chapter 2)- was observed; this species can possess a mossball growth form. In this short Note, we describe the anatomy and growth of *Blindia robusta.*

The aims of this study were to (i) examine the growth forms of *Blindia robusta* in the field, (ii) examine new leaves, growth tips and old leaves for carbohydrates, protein and pectin in relation to photosynthesis and (iii) to see if *Blindia robusta* contained phenolic compounds in relation to cell wall structure.

3.2   Establishment and growth of *Blindia robusta*

On the Bogong High Plains, *Blindia robusta* is restricted to growing in a habitat with permanent flowing water. It is the only moss on the Bogong High Plains that is known to be aquatic and is almost always present in groundwater source pools (Chapter

2; McCartney *et al.* 2013). *Blindia robusta* has leaves consisting of a very narrow base which gradually narrow to a long complete, long slender needle, or awn-like point (Bednarek-Ochyra, 2014). The plants are slender and form dense mats (Bednarek-Ochyra, 2014). Field work observations for Chapter 2 identified that growth first begins as a ball-like structure, with plants attaching themselves to small pieces of gravel or rock surfaces below the water line. As the moss balls increase in size (at an undetermined rate), the structures float up to the top of the water column looking like balls. They then smooth out as a bubbly mat followed by the formation of a smoother surfaced, free-floating mat. These observations were documented at many locations. Figure 3.1 illustrates this process in different stages.

(a)   (b)   (c)   (d)   (e)

Fig. 3.1 (a) Ball-like growth of *Blindia robusta* in the bottom of a groundwater source pool. The *Blindia robusta* stems are attached to pieces of rock and gravel beneath the water in a ball-like growth form (refer to enlarged photograph in Figure S3.1). (b) Ball-like growth with some of the ball-like structures still submerged and others exposed above the surface. (c) Ball-like growths floating on the surface. (d) Ball-like growths forming an extended mat. (e) *Blindia robusta* flattening out and floating as a continuous mat.

Ball-like growths of *Blindia robusta* have not been previously documented. Free-floating mats of *Blindia robusta* have not been observed to have rhizoids, the slender filaments that grow on the stem or thallus of other bryophytes. Multiple rhizoids are typically a feature used by mosses to attach or anchor themselves to substrates. It was observed to take two years for groups of mossballs to form a flat mat in one of the groundwater source pools. The whole process of starting out as submerged mossballs until *Blindia robusta* is growing as a flat mat has been observed in the field but the factors that control this process are unknown.

3.3   Fourier-transform infrared (FTIR)

The composition of the cell wall and cell contents of *Blindia robusta* was investigated. Describing the cell wall composition of plants is important because cell walls give plants shape and support, and such information aids our understanding of why plants occupy different habitats (Bowes and Mauseth, 2008). Methods (described more fully in Chapter 4) were used for Fourier-transform infrared (FTIR) microscopy to determine the dominant (bio) molecular composition of cell wall material and cell contents in *Blindia robusta*. We use paraffin embedded samples sectioned to 8 micron thickness and mounted onto poly-L-lysine coated CaF_2 slides (0.5 mm $\times$ 22 mm; Crystan, UK) (Heraud *et al*., 2007). Infrared (IR) maps were acquired from two sections: (i) a section at the water line that captured both old leaves (OL) that originate from the stem below the water line as well as the growth tip (GT) (Figure 3.1) and (ii) a section of new leaves (NL) (Figure 3.1) above the water line.

We used a Hyperion 3000 IR microscope (Bruker Optik GmbH, Ettlingen, Germany) equipped with a focal planar array (FPA) imaging detector coupled to a Vertex 70 FTIR spectrometer (Bruker Optik GmbH) (Chapter 4, methods). IR images

were collected in transmission mode using a 15× objective and condenser (0.4 numerical aperture) (see Chapter 4, methods). FPA maps consisted of multiple 'blocks', each containing 4096 data points (64 × 64-element array), with 2 × 2-pixel binning to give a spatial resolution of approximately 10 µm (Chapter 4, methods). For each mapped area, 64 scans were co-added, referenced against a background on the calcium fluoride slide free of leaf and stem material (Chapter 4, methods). Spectra were acquired over the range 3900 – 900 cm^{-1} at a spectral resolution of 6 cm^{-1}, using a Blackman-Harris three-term apodisation function. *Opus 6.5* software (Bruker Optik gmbH) was used to control the instrument (Chapter 4, methods). Spectral heat maps were generated for four wavenumber ranges: (i) 3000 – 2800 cm^{-1} (C-H stretch), (ii)1705 – 1570 cm^{-1} (Amide I/pectin), (iii) 1530 – 1480 cm^{-1} (phenolic) and (iv) 1180 – 950 cm^{-1} (carbohydrates); all band assignments from Heraud *et al.* (2007).

It is clear from the Fourier-transform infrared (FTIR) images (Fig. 3.2 (d)) that *Blindia robusta* has a high concentration of carbohydrates within the new leaves at the top of the plant where most of the photosynthesis is likely taking place; however, it was not possible to distinguish between metabolic (soluble, storage) carbohydrates and the structural ones used in cell walls using this method. The new leaf (NL) in *Blindia robusta* is part of the plant above the water column and therefore more exposed to light, thus enabling higher photosynthesis to occur. The C-H maps give a general description of the density of organic material, with higher distribution shown in NL and costa of OL (Figure 3.2). Figure 3.2 (a) shows C-H which gives some measure of all organic material within *Blindia robusta*.

Plants contain pectins in the plant primary cell walls within the middle lamella (Voragen *et al.* 2009). Pectins also form part of the secondary cell wall (Voragen *et al.* 2009). Pectin is deposited in the initial stages of development during cell enlargement/expansion (Voragen *et al.* 2009). It is thought that pectins have multiple

functions in plant growth and development (Ridley *et al*. 2001). These include helping to control the porosity of primary cell walls and are a major component of the middle lamella and are therefore one of the major plant cell wall components (Bowes and Mauseth, 2008; Voragen *et al.,* 2009). FTIR maps do not allow pectins and proteins (as mapped by the Amide I band) to be discriminated as the IR bands are very close together. FTIR maps showing high Amide I/pectin can therefore represent high concentrations of one or both these components.

Amide I/pectins are present at high concentrations in the new leaf (NL), consistent with expected high levels of chlorophyll (and the associated accessory proteins) found in new leaves (Bowes and Mauseth, 2008). Elevated protein levels are also expected in areas of new cell formation/division (Bowes and Mauseth, 2008). The new leaves show these high levels of proteins and carbohydrates where maximum photosynthesis is happening within the *Blindia robusta*. Figure 3.2 (b) also shows stronger concentrations of Amide I/pectin present in GT, as well as in the costa of OL. The costa contains specialized cells including stereids (stiffener cells that support the leaves and stems and occur in bundles), guide cells (large vacuolate, thin-walled cells stretching across the costa), hydroids (cells that conduct water and transport products of photosynthesis) and leptoids (specialised cells that that conduct dissolved energy-rich products of photosynthesis) (Malcolm and Malcolm 2006). The costa area of *Blindia robusta* with its' specialised cell structures also shows high levels of proteins and carbohydrates as a result of photosynthesis within the plant.   It would appear from the FTIR images that *Blindia robusta* does not contain high concentrations of phenolic compounds (Figure 3.2 (c)) to help strengthen cell wall structure.

Fig. 3.2. FPA-FTIR maps of sections of a *Blindia robusta* plant. These images show C-H stretch (a), amide-I/pectin (b), phenolics (c) and carbohydrate bands (d) for new leaves that are exposed to direct sunlight (NL), growth tip where the leaves are still forming close to the stem apex, and situated close to the water surface (GT) and an old leaf within the water column and fully formed and open not showing stem attachment (OL). Heat map scales showing colours pink (high) to blue (low) are a visual representation of the relative concentrations of carbohydrates, amide-I/pectin and phenolics that are present in the cells of *Blindia robusta*.

Fig. 3.3. The region of the moss that is exposed to light and therefore where you would expect high photosynthetic activity. This correlates with the 'chemistry' of the plant where we would expect high amounts of protein (associated with chlorophyll) – or Amide I/pectin as shown for NL in Figure 3.2.

3.4   Structure of the leaf and capsules of *Blindia robusta*

The stems, leaves and capsules of *Blindia robusta* were examined under the microscope and drawn to scale. Illustrations were drawn using an Olympus SZ30 microscope with an attached drawing arm. The external structures of the sporophyte (stem and capsule) were drawn using a fresh sample. The stem and leaf cell structures of *Blindia robusta* were drawn using samples stained with toluidine blue and mounted on slides.

The cell structure of the leaves and costa (Figure 3.4) aids in understanding how *Blindia robusta* photosynthesises in the FTIR sections where the spectra of the leaf and costa of *Blindia robusta* are shown (Figure 3.2). The costa, which is the part of the leaf where transport occurs, is also where there are high levels of both Amide I/pectins and carbohydrates. It contains specialized cells such as hydroids (cells that conduct water and transport products of photosynthesis) and leptoids (specialised cells that conduct dissolved energy-rich products of photosynthesis) (Malcolm and Malcolm 2006). The

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stems of *Blindia robusta* aids in supporting the plants within the water as free-floating mats and the cell structure of the stem is illustrated in Figure 3.4(b). Figure 3.4(a) shows the seta with the capsule and the calyptra in the process of being separated. The calyptra was manually removed to enable the internal structures of the capsule to be drawn. Figure 3.4(b) shows the internal cell structure of the *Blindia robusta* stem with leaves starting to form on the outer margins. Figure 3.4(c) shows the capsule with the 16 peristome (teeth) intact; the number of peristomes within the capsule are used to key out *Blindia* species, along with long apical arranate leaves and exerted, somewhat stout, rigid seta (Bartlett and Vitt, 2011). Figure 3.4(d) is a drawing of the cross section of a *Blindia robusta* leaf showing the cell structure of the whole leaf section including the costa.

Fig. 3.4. *Blindia robusta* showing capsules and cell structures. Shown are: (a) the seta

of *Blindia robusta* with a capsule with calyptra in the process of falling off, (b) a cross

section drawing of the cell structures of a *Blindia robusta* stem, (c) a capsule showing

the peristomes in place with spores still to be released and (d) a cross section of leaf

cells of *Blindia robusta*.

3.5   Summary

Blindia robusta is known to grow in the permanent, flowing, groundwater source pools on the Bogong High Plains, Victoria (Chapter 2). The growth forms of *Blindia robusta* are unique; the moss starts fully submerged until moss balls eventually float to the surface and all join up to form a large, flat, floating mat. High levels of carbohydrates and Amide I/pectins were observed in the region of the floating mats that are exposed to sunlight. This change in growth structure would increase access to sunlight. By forming a mat with growth tips facing up, *Blindia robusta* can maximise energy gain.

3.5   References:

Bednarek-Ochyra H. 2014. A major range extension of *Blindia robusta* (Seligeriaceae, Bryophyta) based on the type of *Ditrichum tenuinerve*. *Telopea Journal of Plant Systematics* **17:** 323-328.

Bowes BG, Mauseth JD. (2008) *Plant Structure. A Colour Guide Second Edition*. CSIRO Publishing Collingwood: Australia.

Frahm J-P. (2001) Aquatic mossballs *Blindia inundata* of Patagonia. *The Bryologist* **104**, 503

Heraud P, Caine S, Sanson G, Gleadow R, Wood BR, McNaughton D. 2007. Focal plane array infrared imaging: a new way to analyse leaf tissue. *New Phytologist* 173: 216-225.

McCartney VA, Silvester E, Morgan JW, Suter PJ. (2013) Physical and chemical drivers of vegetation in groundwater-source pools on the Bogong High Plains, Victoria. *Australian Journal of Botany* **61,** 566-573.

Malcolm B, Malcolm N (2006) Mosses and other Bryophytes, an illustrated glossary (2nd edition) (Micro-optic Press: New Zealand).

Meagher D, Fuhrer B. (2003) *A Field Guide to the Mosses and Allied Plants of Southern Australia.* Flora of Australia Supplementary Series Number 20. (The Australian BiologicalResources Study and The Field Naturalists Club of Victoria: Canberra)

Ridley BL, O'Neill MA, Mohnen D. (2001) Pectins: structure, biosynthesis, and

    oligogalacturonide-related signaling. *Phytochemistry* **57,** 929-967.

Voragen AGJ, Coenen G-J, Verhoef RP, Schols HA. (2009) Pectin, a versatile

    polysaccharide present in plant cell walls. *Structural Chemistry* **20,** 263-275.

3.6 Supplementary Material

Fig. S3.1. Enlarged photograph of submerged *Blindia robusta* mossballs in a

groundwater source pool.

Chapter 4

Desiccation tolerance in the groundwater dependent moss *Blindia robusta*.

Authorship statement

This following chapter is in preparation for submission to the Annals of Botany.

Author contribution: I collected the *Blindia robusta* and *Sphagnum cristatum* samples for the desiccation experiments and performed the desiccation experiments myself with advice from ES and JM. I prepared *Blindia robusta* samples for histological purposes with advice from ES. The FTIR mapping was conducted by myself under the guidance of ES. Live cell FTIR were conducted by ES. Bulk composition analyses were collected and prepared by myself. I analysed the data for the desiccation experiment, FTIR and bulk composition analysis with advice from ES and JM. I prepared the manuscript for publication, with editing advice from both ES and JM.

Abstract

We predicted that groundwater-dependent species such as *Blindia robusta* have low tolerance to drying relative to species at the margins of water bodies. Here, we investigate the desiccation tolerance of *Blindia robusta*. Cell wall chemical composition and nutrient analyses of *Blindia robusta* were also characterised and compared to the wetland moss *Sphagnum cristatum*.

Blindia robusta was highly intolerant to desiccation, or the combination of desiccation and higher temperatures over short periods of time. There were significant differences in cell wall composition of *Sphagnum cristatum* and *Blindia robusta.* Cross-linked phenolic compounds, while clear in the cell walls of *Sphagnum cristatum*, are effectively absent in *Blindia robusta*; these compounds provide structural support to the cell walls.

Blindia robusta appears entirely dependent on continuous moisture provided by groundwater for its survival. Climate change predictions indicate there will be significant changes to precipitation and temperature in the Australian alpine environment. Groundwater pools have been observed to be drying out in the initial field survey and follow up data collection surveys. *Blindia robusta* can be expected to retreat from marginal groundwater pools with climate change.

Keywords: *Blindia robusta, Sphagnum cristatum*, desiccation, phenolics, climate change, synchrotron.

4.1 Introduction

The distribution of mosses in alpine ecosystems are likely linked to differences in hydrology (i.e. permanency of water), with clear niche separation likely reflecting differences in desiccation and inundation tolerance (Suren, 1993; Suren, 1996; Yazaki *et al.* 2006; Price & Whittington, 2010). In the Australian alps, two key mosses are *Sphagnum cristatum*, found in peatlands but not permanent water, and *Blindia robusta*, a groundwater-dependent species (Chapter 2). On the Bogong High Plains, *Blindia robusta* is restricted to permanently-flowing groundwater pools whereas *Sphagnum cristatum* occurs outside of water bodies dominating boggy areas and is maintained by a high water-table in the peat soils it creates (Chapter 2).

Climate change is expected to alter precipitation patterns in alpine areas, with predicted higher temperatures causing a higher proportion of winter precipitation to fall as rain (Knowles *et al.* 2006) resulting in a reduction of the area of snow cover. Predictions indicate that only 4% of the current area of snow cover will continue by 2070 (Whetton, 1998). Higher temperatures and lower precipitation (including winter snow) over the next 50 years (Whetton, 1998; Green and Pickering, 2009) will most probably contribute to more variable groundwater flow and, ultimately, the frequency with which groundwater pools dry out. Studies of tolerance to desiccation suggests that some mosses have the capacity to recover (rehydrate) after desiccation; it is the duration of desiccation that will determine the capacity to rehydrate (Hájek and Beckett 2008). Moisture is vital to plant cells to enable internal turgor (hydrostatic) pressure for cell expansion and provides rigidity to the plant (Alberts *et al.* 1994). The internal pressure is due to osmotic swelling of the protoplast which is resisted within the mechanical strength of the cell wall (Alberts *et al.* 1994).

Hájek and Beckett (2008) exposed *Sphagnum* to a dehydration period of 25 hrs and a rehydration period of four days and concluded that *Sphagnum* can rehydrate under

these conditions. They concluded that it could be viewed as a 'drought tolerator' because of the ability to store water in dead hyaline cells and that turgor only starts dropping at low water contents. Such rehydration capacity has not been studied for groundwater-dependent species; it may be hypothesised that they would have a lower rehydration capacity due to different cell wall traits with less moisture holding capabilities due to living in permanently moist environments.

Desiccation tolerance among bryophytes varies (Glime, 1971; Bewley, 1979; Oliver *et al*. 2005; Proctor *et al*. 2007), with some species capable of maintaining water by capillary action and water storage capability in the hyaline cells e.g. *Sphagnum*, (Hájek and Beckett, 2008; McCartner and Price, 2012). *Fontinalis antipyretica*, an aquatic bryophyte found in Mediterranean rivers, has been known to survive intermittent desiccation (Cruz de Carvalho *et al*. 2011). However, desiccation tolerance in mosses has rarely been measured, nor have the cell wall traits that may contribute to desiccation been described.

The aims of this study were to determine how *Blindia robusta* respond to desiccation, compare Fourier-transform infrared (FTIR) mapping of *Blindia robusta* to the semi-aquatic *Sphagnum cristatum,* and to compare nutrient analyses of *Blindia robusta* to *Sphagnum cristatum.*

4.2   Materials and Methods

4.2.1   Site description and sample collection

This study was conducted with specimens collected from the Bogong High Plains (BHP), Victoria Australia, an elevated plateau contained within the Alpine National Park. Mean annual precipitation is 2449 mm, with much of it falling as snow in the winter months (Williams 1987). Mean maximum temperature is 9.4 $^{\circ}$C and mean

minimum temperature is 2.6 °C (Rocky Valley Meteorological recording station, Bureau of Meteorology, 2016).

In Chapter 2, I previously identified groundwater source sites in catchments on the BHP containing both *Blindia robusta* and *Sphagnum cristatum*: Heathy Spur (HS) and Whiterock Creek (WR). Three locations in each (HSGW1, HSGW8, HSGW11 WRGW1, WRGW8, WRGW9) were chosen to collect plant materials. These locations have been described previously in Chapter 2.

Single stems of *Blindia robusta* were used for the desiccation experiments. It is acknowledged that the drying rate of mats of *B. robusta* would likely be slower than single stems. However, the use of mats would have required a lot more material and this species is very limited on the Bogong High Plains. The response of single stems in the mats would also be dependent upon where they were located within the mat which would still result in measuring the response of single stems.

The whole specimens of *B. robusta* and *S. cristatum* plants were collected in the field in November 2014, placed in ziplock bags, and then transported at 5 °C to the laboratory. Samples for histological examination were stored at 5 °C and used within 7 days of collection; samples for bulk chemical analysis were frozen until required. *B. robusta* samples for desiccation experiments were stored in the dark at 5 °C prior to their use and were kept in groundwater collected from the groundwater-source pools from where they were originally located. Desiccation experiments were conducted within 2 weeks of collection; after this time, the viability of the material decreased (as determined by measurement of photosystem II efficiency).

4.2.2   Measurement desiccation tolerance using photosystem II efficiency

To determine desiccation tolerance in *B. robusta*, we used dark-adapted F_v/F_m (ratio of the rate of photochemical activity and the total rate of absorbed energy

dissipation) photosynthesis efficiency rate as a measure of plant condition (Lovelock *et al.* 1995; Robinson *et al.* 2000). We used the time taken to reach 0.3 F_v/F_m (the point of negligible quantum yield because the photosynthetic apparatus cannot perform photochemical processes in the protein complex PSII) as the dependent variable to assess sensitivity of *B. robusta* to desiccation. This follows studies where (a) observations found F_v/F_m <0.3 signified the point at which *Sphagnum* did not revive upon rehydration (Hájek and Vicherová, 2014) and (b) no efficient photochemical reactions occurred in bryophytes when F_v/F_m was <0.3 (Liepina and Ievinsh, 2013). F_v/F_m measurements were recorded using a Handy-PEA (*Hansatech*, Kings Lynn UK). To test desiccation tolerance, *B. robusta* stems were cut to a length of 3 cm from the apex and patted dry with absorbent paper. Single apical shoots were placed onto a petri dish (*n* = 5 shoots per treatment) without water and placed in a Controlled Temperature Cabinet (CT cabinet) at one of six constant temperatures (5 ℃, 10 ℃, 15 ℃, 20 ℃, 25 ℃, 30 ℃). Initial measurements were taken before treatment (0 minutes) and at five-minute intervals, between 0 and 45 minutes, at which time each stem was measured for Photosystem II efficiency (F_v/F_m). It is acknowledged that slow drying/hardening of the samples is recommended (Hájek and Beckett 2008) but due the short-term viability of the specimens, it was decided to adopt this drying and then rehydrating procedure.

Blindia robusta* responded rapidly to dehydration followed by rehydration experiments. This experiment was to determine whether the moss could be restored to pre-dehydration photosystem II (PSII) photochemistry efficiency. For this experiment, two different dehydration conditions were used: (i) 10 minutes at 5 °C and (ii) 60 minutes at 5 °C. Rehydration was also carried out at 5 ℃ using groundwater from the groundwater source pools, with the photosystem II efficiency (F_v/F_m) measured every five minutes for 60 minutes after re-immersion in water.

4.2.3   Optical microscopy

To describe the structure of stems and leaves of the groundwater moss *B. robusta* and the wetland moss *S. cristatum*, nine samples were collected in the field in November 2014 (randomly collected samples from three locations in each of the three moss mats/beds) and immediately preserved in formaldehyde in glass vials. The samples were treated in the laboratory by alcohol dehydration and paraffin embedding to prepare them for transverse sectioning. Paraffin was removed by 3× washing in a xylene bath.

To visually record the effects of desiccation in leaf cell structures in *B. robusta*, fresh and desiccated (30 minutes) samples were mounted on slides using a standard oil immersion technique. A fluorescence microscope (Zeiss axioskop HBO-50) with a 100× objective was used with a 16× digital camera (Canon) for the photographs.

4.2.4   Fourier-transform infrared (FTIR) mapping

Fourier-transform infrared (FTIR) is a technique used to obtain a spatially resolved infrared spectrum of a solid material. FTIR was used to determine the dominant (bio)molecular composition of cell wall material in moss tissue. This analysis used the same paraffin embedded samples used in optical microscopy, but the sections were cut to 8 µm thickness and mounted onto poly-L-lysine coated CaF_2 slides (0.5 mm × 22 mm; Crystan, UK) (Heraud *et al*., 2007). Infrared maps were acquired both from stem and leaf sections of *B. robusta* as well as stem sections of *S. cristatum* for comparison. Leaves of *S. cristatum* were too small to be imaged by this technique with a diffraction limit of 2 µm.

The instrument used for this study was a Hyperion 3000 IR microscope (Bruker Optik GmbH, Ettlingen, Germany) equipped with a focal planar array (FPA) imaging

detector, coupled to a Vertex 70 FTIR spectrometer (Bruker Optik GmbH). IR images were collected in transmission mode using a 15× objective and condenser (0.4 numerical aperture). FPA maps consisted of multiple 'blocks', each containing 4096 data points (64 × 64-element array), with 2 × 2-pixel binning to give a spatial resolution of approximately 10 µm. For each mapped area, 64 scans were co-added, referenced against a background on the calcium fluoride slide free of leaf and stem material. Spectra were acquired over the range 3900 – 900 cm^{-1} at a spectral resolution of 6 cm^{-1}, using a Blackman-Harris three-term apodisation function. *Opus 6.5* software (Bruker Optik gmbH) was used to control the instrument.

4.2.5   Synchrotron FTIR microspectroscopy on 'live' cell material

In order to understand the interaction of *B. robusta* cell wall material with its aqueous environment in more detail, experiments were conducted on 'live' tissue using synchrotron light source FTIR, following procedures developed by Silvester *et al.* (2018). *B. robusta* and *S. cristatum* specimens were embedded in Optimal Cutting Temperature (OCT) compound (Tissue-Tek) and sectioned at 10 µm thickness with a cryostat microtome at -19 ℃ (Leica CM1850). In this work, we focused on stem sections that were taken within 10 mm from the top of the gametophyte. The sections were stored as a suspension in site water at 5 ℃ until required and used within 48 hrs; after that time, new sections were cut. Immediately prior to experimental work, a subsample of suspended sections was washed with the selected medium by centrifugation (5 mins; 1500 g; 70% volume decant ×3).

Stem sections were mounted in a liquid flow cell (Bioptechs FCS3; Butler, USA) with 1 mm CaF$_2$ windows separated by either a 6 µm Mylar$^®$ or a 7.6 µm Kapton$^®$ gasket. Solutions from an external reservoir were passed through the liquid cell

using a peristaltic pump at a flow rate of 80 µL min^{-1}, corresponding to a liquid-cell exchange rate of 60 min^{-1} (calculated flow-cell volume = 1.28 µL). The dead volume of the delivery line was 650 µL, corresponding to an 8 min delay between the reservoir and the liquid cell. The external reservoir was a 20–90 mL titration vessel (Metrohm 6.1415.220) with a 5-port lid (Metrohm 6.1414.010) equipped with an 'Aquatrode' pH electrode (Metrohm 6.0253.100) and connected to a Metrohm 718 STAT Titrino (burette). Whenever the solution conditions were changed in the reservoir, a minimum of 30 min was allowed to elapse (to flush the delivery line and allow equilibration of the tissue) before IR spectra were recorded.

Experiments with *B. robusta* and *S. cristatum* sections were conducted in deuterated water (D$_2$O) with 60 µM NaCl; the concentration of NaCl approximately equal to that in the groundwater supplying this peatland (Silvester, 2009). All solutions included 1 mM 2-(N-morpholino) ethanesulfonic acid (MES; pK_a = 6.15) as a pH stabiliser and were vacuum degassed prior to use to minimise bubble formation in the liquid cell. Note that in D$_2$O, the pH electrode senses predominantly D$^+$ (rather than H$^+$) and returns a value that is termed 'pH*' when the electrode calibration is performed in normal water (Krężel and Bal, 2004). Sections were initially equilibrated with the D$_2$O medium without pH* adjustment (pH* 5.7) and the IR spectrum recorded. The pH* of the solution was then adjusted to ~2.5 by the addition of HCl. This was done to protonate the carboxylic acids (i.e. convert all carboxylic acids to their undissociated form (COOH)), allowing identification of other bands (e.g. Amide I & II, aromatic bands). The sections were then titrated to ~ pH* 8 by the addition of NaOH. IR spectra were recorded under both acidic and basic conditions.

The liquid flow cell containing the stem sections was placed on the motorised sample stage of the microscope, inside an N$_2$-purged chamber. Infrared spectra were acquired from three separate locations within the stem from at least two *B. robusta* and

S. cristatum stems. Spectra were collected using the 36× objective of a Hyperion 2000 IR microscope, coupled to a Vertex 80v IR spectrometer with a photovoltaic liquid-nitrogen cooled mercury-cadmium-telluride detector system (Bruker Optik GmbH, Ettlingen, Germany). The microscope was connected to the Infrared Microspectroscopy Beamline at the Australian Synchrotron (Clayton, Australia) and the system controlled using Bruker Opus 6.5 software. The knife-edge aperture was set to 20 μm with spectral acquisition conditions: 3500–700 cm^{-1}; 4 cm^{-1} resolution; 256 scans co-added. Background spectra were taken from a point in the liquid cell free of stem tissue. Apodisation was performed using the Happ-Genzel function.

In this work, we focus on the spectral region 1800–1400 cm^{-1}; these contain IR bands of carboxylic and phenolic groups as well as the amide bands of proteins (Amide I and II). Assignment of IR bands (Table 4.1) is based on published studies of plant tissue (Sene *et al.*, 1994; Heraud *et al.*, 2007; Kerr *et al.*, 2013; Bağcıoğlu *et al.*, 2015) and pure reference materials (Silverstein *et al.*, 1974; Tian *et al.*, 2000).

4.2.6 Bulk composition analyses

Mineral nutrients were analysed for both *Blindia robusta* and *Sphagnum cristatum* to determine differences in calcium and magnesium which are used for cell wall stabilisation, photosynthesis and movement of chloroplasts (Haupt and Weisenseel, 1976; Grundon et al. 1997). To determine macro- and micro-nutrient content in both *B. robusta* and *S. cristatum*, frozen moss samples were first defrosted, rinsed with deionised water (18 MΩ. cm) to remove foreign matter, dried at 70 ºC for 24 hrs and then finely ground. Carbon, nitrogen and sulfur contents were measured by combustion using a LECO CNS2000 analyser; remaining major (Na, K, Ca, Mg and P) and minor (Cu, Zn, Mn, Fe, B, Mo, Co, Si) elements were measured by ICP-OES on nitric acid

digests. All measurements were performed by Environmental Analysis Laboratory, Southern Cross University, Lismore and are reported here on a dry weight basis.

Some loss of K^+ would have occurred due to injured tissues in the freeze/defrost then rinse procedures (Arora, 2018). The methods used were not ideal but with time and distance constraints, and samples that were contaminated by other plant material, this was the only method available at the time.

4.2.7   Data Analysis

Differences in nutrient contents were analysed by principal components analysis (PCA; PRIMER; Clark and Warwick, 2001) using moss species, catchment and combined catchment and moss species as factors. Ratios of calcium to magnesium were analysed using Principle Component Analysis (PCA) to illustrate the differences with Ca:Mg ratios between *B. robusta* and *S. cristatum*. A Multidimensional Scaling (MDS) plot was used with the factors: catchment and species of moss for Ca:Mg. A PCA plot for all micronutrients using the factors: catchment and species of moss was explored for micronutrients excluding Fe and Mn which were particularly elevated in some (*Sphagnum*) samples and silicon amounts which are considered unreliable due to potential contamination from silica (sand). A two-way ANOVA using the variables copper, zinc, boron, molybdenum and cobalt were analysed to see if there were any interactions for catchment $\times$ species.

Data analyses for the FTIR maps of leaf and stems of *B. robusta* and *S. cristatum* were implemented with *Opus 7.0* software using the *Chem Imaging* module with band assignments from Heraud *et al*., 2007, Kerr *et al.,* 2013, and Silverstein *et al*. 1962. A standard set of wavenumber ranges were integrated, including: (i) 2980–2800 cm^{-1} (v(C-H) of most organic molecules); (ii) 1705–1570 cm^{-1} (including v(C=O) of proteins (commonly known as Amide 1) and v_{as}(COO$^-$) of carboxylate groups); (iii) 1525–1480

cm^{-1} (aromatic groups, including phenolics); (iv) 1180–950 cm^{-1} (ν(C-O-C) of carbohydrates). The absorbance at each wavenumber range was visualised as a heat map, with the minimum absorbance set to zero and the maximum to a consistent value across all samples, allowing direct comparison of tissue concentrations.

4.3   Results

4.3.1   Desiccation and rehydration of *Blindia robusta*

The desiccation experiment confirmed that *B. robusta* is very sensitive to desiccation; leaves exposed to 5 ℃ for ~19 mins were no longer photosynthetically active and classified as dead (Figure 4.1). Increasing temperatures caused more rapid mortality. At 30 ℃, *Blindia* plants were classified as dead within just 11 mins (Figure 4.1).

Blindia robusta stems that were desiccated for 10 mins at 5 ℃, then rehydrated at 5 ℃, appeared to recover to viable F_v/F_m efficient photochemical rates within 60 mins (Figure 4.2). The *B. robusta* samples that were desiccated at 5 ℃ for 50 mins, then rehydrated for 60 mins at 5 ℃, did not recover efficient photochemical rates (Figure 4.2) indicating that *B. robusta* cannot recover to efficient photosynthetic activity from the damage sustained in the longer (50 mins) dehydration.

Fig. 4.1. Response of maximum PSII (F_v/F_m) during dehydration of *Blindia robusta* samples at increasing temperatures. Shown are the response times for samples have no efficient photochemical reaction (F_v/F_m=0.3) at increasing temperatures. All error bars are ± 2SE (*n*=5).

Fig. 4.2. Response of maximum PSII (F_v/F_m) (F_v/F_m) for fresh samples and during re-hydration of previously dehydrated *Blinda robusta* samples. Shown are the rehydration responses for samples dried for 10 mins (●) and 50 mins (o) at 5 ℃. All error bars are ± 2 SE (*n*=5).

4.3.3 FTIR microspectroscopy

FTIR microspectroscopy maps of *B. robusta* stem and leaf and *S. cristatum* stem tissue are shown in Figure 4.3. The heat maps for both *B. robusta* and *S. cristatum* mirror the tissue density of the samples, with higher concentrations in the high-density cortex and costa (*B. robusta*) and prosenchyma (*S. cristatum*). The biggest difference between the two mosses is the apparent absence of phenolic groups in *B. robusta* – which by comparison are high throughout the prosenchyma (supporting cells) of *S. cristatum*.

Selected single point spectra are shown for stem and leaf tissue from *B. robusta* and stem tissue from *S. cristatum* (Figure 4.3(b); also shown in Figure 4.3(b) are the integration ranges corresponding to the maps shown in Figure 4.3(a)). Comparison of the spectra captured by the Amide I/pectin integration window reveals that there are strong differences between the stem/leaf of *B. robusta* compared to the stem of *S. cristatum*. Comparison of the aromatic stretch of phenolic groups between *B. robusta* and *S. cristatum* confirms the information shown in the FTIR maps for these samples (Figure 4.3(a)) with no detectable peak in the case of *B. robusta*, contrasted with a strong peak for *S. cristatum*. This suggests the absence of these chemical groups in the cell walls of *B. robusta* (both stem and leaf).

Fig. 4.3. (a) FPA-FTIR maps for stem and leaf sections of *Blindia robusta* and stem for *Sphagnum cristatum*. Images show spatial distributions of (i) C-H stretch (CH_2, CH_3 and all alkyl groups), (ii) amide-I/pectin, (iii) phenolic groups and (iv) carbohydrate groups in tissue sections of *B. robusta* and *S. cristatum*. (b) single point spectra for *B. robusta* leaf and stem as well as *S. cristatum* stem showing the wavenumber range and peaks for: amide-I/pectin (1705-1525 cm^{-1}), phenolics (1525-1480 cm^{-1}) and carbohydrates (1180-950 cm^{-1}).

4.3.4 Synchrotron FTIR microspectroscopy of live *Blindia robusta*

Acidification of *B. robusta* (from pH 5.71 to pH 2.5) is expected to result in the protonation of carboxylic groups. Consistent with this, a decreased absorbance at 1608 cm^{-1} and 1426 cm^{-1} was observed, as well as loss of the spectra shoulders at 1588 cm^{-1} and 1466 cm^{-1} (Figure 4.5(a), Table 4.1). The only increase is at 1729 cm^{-1}, corresponding to that expected for protonated carboxylic groups (Figure 4.5(a)). The bands at 1655 cm^{-1} (Amide I – alpha helix), 1633 cm^{-1} (Amide I – beta sheet, alkene groups or structural water) and 1543 cm^{-1} (Amide II) also become more prominent at pH 2.5 due to the decreased absorbance at 1608 cm^{-1} (Figure 4.5(a)).

Table 4.1. Infrared absorption bands corresponding to spectra shown in Figure 4.5(a) and 4.5(b).

Wavenumber (cm^{-1})	Band assignment
1729	Ester, protonated carboxylic acid
1655	Amide I (proteins; alpha helix)
1633	Amide I (proteins; beta-sheet) / Alkene group / structural water
1608	Deprotonated carboxylic acid (pectins; asymmetric stretch)
1606–1604	Aromatic (phenolic)
1588	Complexed carboxylic group
1543	Amide II (of proteins)
1519–1512	Aromatic (phenolic)
1466	Complexed carboxylic group
1426	Deprotonated carboxylic group (symmetric stretch)

When the pH is changed from pH 2.5 to pH 7.85 (Figure 4.5(a)), the carboxylic groups are deprotonated with absorbances at 1608 cm^{-1} and 1426 cm^{-1} increasing and the band 1729 cm^{-1}, corresponding to protonated carboxylic groups, decreasing. The shoulders at 1588 cm^{-1} and 1466 cm^{-1} that were initially present at pH 5.71 do not reappear and the Amide I and Amide II bands also become more obscured.

Figure 4.4(b) shows a comparison of *Blindia robusta* and *Sphagnum cristatum* at pH 2.5 (the *B. robusta* spectrum is the same as in Figure 4.4(a); the *S. cristatum* spectrum is from Silvester *et al.* (2018). Similarly to the Amide bands described above, protonation of the carboxylic groups allows the aromatic (phenolic) bands at 1606–1604 cm^{-1} and

1519–1512 cm^{-1} to be revealed and more easily compared. The results confirm those observed in Figure 4.3, showing that the aromatic groups are much weaker in *B. robusta* than in *S. cristatum*.

Legend (top of panel a): Protein (red), Complexed carboxylic (orange), Carboxylic (green), Phenolic / aromatic (blue)

(a) Spectra labeled pH 5.71, pH 2.50, pH 7.85

Wavenumber markers: 1729, 1655, 1633, 1608, 1588, 1543, 1519, 1466, 1426

(b) Spectra labeled Sphagnum and Blindia

Peak labels: 1606, 1512, 1604, 1519

Wavenumber (cm^{-1}) axis: 1800, 1700, 1600, 1500, 1400, 1300

Absorbance (y-axis)

Fig. 4.4 (a) FTIR absorption spectra for *Blindia robusta* at pH 5.71, pH 2.5 and pH 7.85. (b) FTIR absorption spectra bands for both *Blindia robusta* and *Sphagnum cristatum* at pH 2.5.

4.3.5   Desiccated *Blindia robusta* cells

Desiccated cells of *Blindia robusta* were examined and photographed for physical changes. Figure 4.5 (a) and 4.5 (b) are photomicrographs showing the dorsal view of cell structures in a *Blindia robusta* leaf. Figure 4.5 (a) shows a fresh *Blindia robusta* leaf with chloroplasts occupying most of the cell volume and with thick cell walls. Figure 4.5 (b) shows the cell walls thinning or shrinking but still maintaining the rigidity of the cell wall structure as the drying process occurs. The chloroplasts within the cells are visually not occupying most of the cell volume with more vacant spaces within the cells.

(a)   (b)

Fig. 4.5. Photomicrographs of upper leaf side of *Blindia robusta* show thick cell walls and chloroplasts occupying most of the cell volume. (a) Chloroplasts in a fresh *Blindia*

robusta leaf removed from water and immediately mounted on a slide. (b) After air drying at 21°C for 30 mins, the *Blindia robusta* leaf shows evidence of cell wall shrinkage and obvious spaces between chloroplasts.

4.3.6   Elemental analysis

Elemental concentrations of both *B. robusta* and *S. cristatum* are shown in Table 4.2. Principal Components Analysis (PCA) on macronutrients shows separation between moss types, driven by higher levels of phosphorus and nitrogen in *B. robusta* and higher levels of sodium and magnesium in *S. cristatum*, across both catchments (Figure S4.3). Based on the assumption that all nitrogen is part of amino acids, the N content can be converted to protein (factor 6.25) yielding 6.16% for *B. robusta* and 4.21% for *S. cristatum*.

Both calcium and magnesium levels are lower in *B. robusta* (0.18% and 0.03% respectively) than in *S. cristatum* (0.28% and 0.11%) (Table 5.1, Table S4.1). Despite the lower concentrations of these cations, the Ca:Mg ratio is substantially higher in *B. robusta* (2.54:1) compared to *S. cristatum* (1.5:1) (on a molar basis) across both catchments. The results show that the average Ca:Mg ratio of approximately 1.5:1 applies for *S. cristatum* in both catchments but for *B. robusta*, the average ratio is 2.1:1 in HS catchment compared to 3:1 in WR catchment (Table S4.1). PCA and MDS analysis also confirm the differences between Ca:Mg ratios for moss type, but not catchment (Figures 4.6 and S4.4). A One-Way MANOVA was also performed confirming these results with a statistically significant difference in moss type based on calcium and magnesium levels ($F_{(4, 92)} = 5.462$, $p < 0.001$; Wilk's $\Lambda = 0.653$, partial $\eta^2 = .20$). There was no significant difference between catchments (p=0.108) (Table S4.3; Figure 4.6).

Fig. 4.6. PCA showing calcium and magnesium separation for the moss species *Blindia robusta* and *Sphagnum cristatum*.

Iron and manganese levels were higher in *S. cristatum* samples than in *B. robusta* samples while copper, boron and cobalt levels were higher in *B. robusta* samples (Table 4.2). PCA analyses for all micronutrients measured in *B. robusta* and *S. cristatum* across the two catchments (Figure S4.5) found separation between moss types is strongly driven by Fe and Mn, with both elements high in many *S. cristatum* samples, and also some *B. robusta* samples (Table S4.1).

Due to the strong dominance of Fe and Mn in the separation between samples, and the potential for soil-driven contamination of these elements, the PCA was repeated with Fe and Mn removed. PCA analysis of the reduced micronutrient set indicated separation occurring between mosses but not between catchment (Figure S4.6). There was a statistically significant difference between the species of moss and levels of copper ($p < 0.005$) (Table S4.4), zinc ($p < 0.005$) (Table S4.4) and boron ($p < 0.005$)

(Table S4.4). There was no significant difference in mean amounts of copper (p=0.220) or zinc (p=0.139) between catchments; however, there was a significant difference in the amounts of boron (p=0.042) between catchments. The amounts of molybdenum were not significantly different between moss (p=0.325) or catchment (p=0.325) (Table S4.4), nor for cobalt between catchment (p=0.309) (Table S4.4).

Table 4.2. Mean (±1SD) elemental contents for *Blindia robusta* (*n*=18) and *Sphagnum cristatum* (*n*=18) samples collected from Heathy Spur (HS) and Whiterock Creek (WR) catchments.

Macronutrients (%)

Moss	N	Protein	P	K	S	C	Ca	Mg	Ca:Mg (molar)	Na
Blindia robusta	0.99±0.16	6.16 ±0.71	0.15 ±0.02	0.39 ±0.06	0.09 ±0.01	41.3 ±0.7	0.15 ±0.05	0.04 ±0.01	2.20	<0.01
Sphagnum cristatum	0.66 ±0.09	4.11 ±0.56	0.03 ±0.01	0.38 ±0.11	0.08 ±0.01	41.9 ±0.8	0.28 ±0.12	0.11 ±0.03	1.49	0.04 ±0.02

Micronutrients (mg/kg)

Moss	Cu	Zn	Mn	Fe	B	Mo	Co	Si
Blindia robusta	5.7 ±1.4	10.1 ±2.4	150 ±60	232 ±139	2.96 ±0.94	<0.2	0.90 ±0.39	443 ±70
Sphagnum cristatum	2.6 ±0.3	15.7 ±4.2	696 ±561	447 ±438	1.31 ±1.38	<0.2	0.36 ±0.29	545 ±139

4.4 Discussion

4.4.1 Desiccation

In this study, we examined the desiccation tolerance of the groundwater-dependent moss *Blindia robusta* and compared its cell wall composition to the semi-aquatic wetland moss *Sphagnum cristatum*. We relate these responses of desiccation tolerance to cell tissue characteristics using Fourier-transform infrared (FTIR) microspectroscopy and using bulk composition analyses. We found that *B. robusta* rapidly dies after drying, even at low temperatures (5 °C). The extreme sensitivity of *B. robusta* to desiccation is in stark contrast to *Sphagnum* which takes much longer, in some cases days, to desiccate and subsequently die (Liepina and Ievinsh, 2013; Hájek and Vicherova, 2014). Sensitivity to desiccation is so extreme in *B. robusta* that it does not recover photosynthetic activity after 50 mins of dehydration and 60 mins of rehydration, even at 5 °C.

The distribution of bryophytes strongly reflects their tolerance and preferences for moisture. Niche partitioning at local scales occurs in response to topography and site wetness (Suren, 1993; Suren, 1996; Gajewski *et al.* 2001; Geffert *et al.* 2013; Roland *et al.* 2017) and is assumed to be due to trait differentiation relating to desiccation tolerance. It is likely that if the groundwater pools on which *B. robusta* rely were to periodically dry out, local extinction of the pool population could occur.

Semi-aquatic mosses such as *Sphagnum cristatum* survive in locations where they are partly in water and partly out of it, but usually always moist. Water is transported along the plant internally by capillary action. This internal capillary water is important physiologically, in relation to the length of time that the shoots

remain turgid (plump, swollen or inflated), thereby allowing photosynthesis, respiration and growth (Goffinet and Shaw, 2008). On the other hand, obligate aquatic mosses (hydrophytes) have less tolerance to desiccation, as seen in these experiments for *Blindia robusta*. Aquatic and terrestrial mosses can experience membrane damage during desiccation with rehydration resulting in loss of nutrients (Brown and Buck, 1979).

Sphagnum cristatum appears to be much more tolerant to drying. The separation of hyaline cells within *Sphagnum* is a continuous process, forming from elongation, secondary-wall formation, pore formation and cytoplasmic degeneration (Kremer and Drinnan, 2004). Hyaline cells transport water by capillary action to maintain moisture to the capitula (Hájek and Beckett, 2008; McCartner and Price, 2012). This would explain their ability to survive in semi-terrestrial ecosystems, disconnected from direct groundwater feed (McCartney *et al.* 2013).

4.4.2 Synchrotron FTIR microspectroscopy

Vascular plants use lignin to aid structural support (Bowes and Mauseth 2008) and have been researched more extensively than mosses in relation to cell structure. Plant cell walls contain cellulose with 30 or more chains aligned to form microfibrils (Bowes and Mauseth 2008). These microfibrils are held together by hemicelluloses tied together by hydrogen bonds (Bowes and Mauseth 2008). The cell wall matrix contains a pectin network surrounding the cellulose-hemicellulose network (Bowes and Mauseth 2008). The bonding of hemicelluloses to adjacent cellulose microfibrils is carried out by calcium ions cross-linking negatively charged pectin molecules, bonding between hemicellulose molecules and covalent hemicellulose-pectin bonds (Bowes and Mauseth 2008). Cosgrove (2005) reported

that hemicelluloses are cellulose-binding polysaccharides that use cellulose to form a strong hardy system for cell structure in plants. Cosgrove (2005) also reported that plants use Ca^{2+} to crosslink with carboxyl groups. The carbohydrate bands shown for both mosses (Figure 4.3) exhibit features commonly observed in plant tissue, with *B. robusta* leaf bands at 1040 cm^{-1} (Heraud *et al*. 2007; Kerr *et al*. 2013), *B. robusta* stem bands at 1010 cm^{-1} (Heraud *et al*. 2007; Kerr *et al*. 2013) and *S. cristatum* bands at 1010 cm^{-1} (Heraud *et al*. 2007; Kerr *et al*. 2013).

FTIR results for *S. cristatum* indicate the main carboxylate peak occurs at 1600 cm^{-1}, typical of the carboxylic group of pectin-type molecules (Heraud *et al*. 2007) whereas for *B. robusta* multiple peaks occur in this wavenumber region at 1633 (Fig. 4.4), 1608 (Fig. 4.4) and 1572 cm^{-1} (Fig. 4.4). The band at 1608 cm^{-1} is consistent with that expected for carboxylate groups of pectin and this assignment is further supported by the loss of this band at pH 2.5 (Figure 4.4(a)). The band at 1627 cm^{-1} may also be due to carboxylic groups of pectin molecules, but also corresponds to that of (structural) water (Popescu *et al.* 2007) as well as Amide I of beta sheet proteins and alkene (C=C) groups (Figure 4.4(a)). The band at 1572 cm^{-1} is more typical of metal complexed carboxylic groups, but could also be due to the presence of α,β-unsaturated or aryl carboxylic groups. This band disappears at low pH, consistent with that expected for a carboxylic acid, but does not reappear at high pH. For this reason, metal complexation is more likely than unsaturated or aryl carboxylates because in the latter case the 1572 cm^{-1} band should re-appear; in the liquid cell configuration, as any complexed metal released from the moss tissue at low pH will be flushed from the cell. While the exact identification of this metal-carboxylate cannot be determined from FTIR spectroscopy alone, it is most likely

calcium complexed pectins, based on the importance of this metal ion in cell walls (discussed below).

A range of phenolic compounds have been identified in cell fluids and the cell walls of *Sphagnum*, some of which are involved in forming a poly-phenolic network, giving the much-needed strength for the large water-holding hyaline cells (Verhoeven and Liefveld, 1997). The phenolic compounds may also provide a defence against herbivores and diseases (Verhoeven and Liefveld, 1997). When the pH of both plants was lowered to pH 2.5, it became apparent that *B. robusta* had a very weak peak for aromatic phenolics. This result was shown specifically at the aromatic peak at 1510-1520 cm^{-1}. Zhong *et al*. (2000) reported that the ratio of the two bands (1512 cm^{-1} and 1604 cm^{-1}) represent the degree of crosslinking between phenolic groups. This would imply that there is less crosslinking of phenolic groups in *B. robusta* compared to more intense highly crosslinked 'lignin-like' structures in *S. cristatum*. The distinctive band at 1633 cm^{-1} is present in *B. robusta* but is not present in *S. cristatum.* This band may be assigned to alkene (C=C) groups and would be consistent with phenolic groups that have not polymerised in *B. robusta* and the lower degree of cross-linking. This lack of polymerisation in *B. robusta* in the phenolic groups suggests that the cell wall structures are not as stable. Like other true aquatic plants, *B.robusta* appears to need less structural support, likely because it is supported within the water itself. These experiments suggest that differences in the sensitivity of these mosses to desiccation likely reflects differences in cell and stem traits.

This study illustrates that there is a significant difference in phenolic composition between *B. robusta* and *S. cristatum*, with *B. robusta* having a much less obvious poly-phenolic network (Fig. 4.4(a) and (b)) and possibly using high

levels of calcium-complexed pectins as a cell wall structure component for mechanical strength. Conversely, there is no direct evidence for metal complexed carboxylic groups in the spectra for *S. cristatum* (absence of absorption bands in the $1600–1570$ cm^{-1} region), but presumably these groups are present. All plants have metal complexed carboxylic groups as part of their cell adhesion mechanism; for *B. robusta,* these groups appear to be more strongly evident in the infrared spectra.

4.4.3 Micro and macro nutrients

Both calcium and magnesium levels were higher in *S. cristatum* than in *B. robusta*. However, the Ca:Mg ratio is elevated in *B. robusta* compared to *S. cristatum* and is also higher than in the groundwater in which it grows. In vascular plants, calcium is transported to the cell walls where it is utilised by the carboxylate groups of cell wall pectins (Haynes, 1980).

Cosgrove (2005) states that with Ca^{2+} mediated crosslinking (the egg carton theory) with carboxyl groups through ionic and coordinate bonds the homogalacturonan forms "stiff gels". Based on both the observation of a distinctive IR absorption band corresponding to a metal-carboxylate complex, and the relatively higher Ca content, it appears likely that *B. robusta* is using Ca^{2+} for this cell crosslinking. Hirschi (2004) suggests that while magnesium is present in chlorophyll, it is indicated that calcium is largely absent from the cytoplasm but is important as a stabiliser of cell membranes and cell walls in bryophytes. Calcium carboxylates contribute to vascular plant structural integrity and it is very likely that this is what is happening in *B. robusta.* The groundwater supplying the peatlands in these catchments has been surveyed extensively (Chapter 2), showing a generally uniform Ca:Mg ratio of 1.2:1. This ratio remains effectively constant in peatland

drainage water, under all flow conditions (Silvester, 2009), and is very similar to that observed in *S. cristatum* in this study. The pH of the groundwater is very low (Chapter 2), and the accumulation of calcium by *B. robusta* suggests a very strong affinity for this metal, while *Sphagnum* has a high cation exchange and is limited to acidic conditions to avoid excessive binding of Ca^{2+} (Hájek and Adamec, 2009). This assumes that groundwater is the only source of calcium; other sources such as precipitation and dust deposition may also contribute to calcium uptake.

MDS analysis of nutrients on both *B. robusta* and *S. cristatum* show that there is a difference in the micronutrient composition. Potassium levels were similar both for *B. robusta* and *S. cristatum*, at 0.39% and 0.38%, respectively. Potassium is required for plant enzyme activation, stomatal activity (water use), photosynthesis (the electrical charge balance at the site of ATP production is maintained by K^+), transport of sugars, water and nutrient transport, protein synthesis and starch synthesis (Prajapati and Modi, 2012).

The results from this study suggest that *S. cristatum* contains higher amounts of iron and manganese; these cations have roles in a variety of biochemical processes (Grundon *et al*. 1997) including lignin building for walls and phenolic compounds for resistance to herbivores and disease (Romheld and Marschner, 1991; Verhoeven and Liefveld, 1997). *Blindia robusta* contains relatively higher levels of copper and boron, implicated in lignin synthesis, terminal oxidation in redox reactions, cell elasticity and in the formation and composition of cell walls (Grundon *et al*., 1997). *Blindia robusta* may be using the accumulated cobalt to aid as a natural fungicide (Grundon *et al*., 1997).

4.4.3 Distribution of moss in the landscape

Blindia robusta is considered to be a nearly pan-south-temperate species in the Australasian area (Vitt, 1974; Vitt, 1979; Ochyra *et al.* 2002). It has also been found in the subantarctic Macquarie Island (Seppelt, 2004), and the Auckland and Campbell Islands (Vitt, 1974; Vitt 1979). Records indicate that it has also been found in subantarctic South Georgia (Ochyra *et al.* 2002) and Tristan da Cunha, on three islands in the South Atlantic Ocean in the southern cool-temperate zone (Bednarek-Ochyra, 2014).

A semi-aquatic moss such as *Sphagnum* can survive habitats where they are in contact with moisture but not submerged, enabling capillary action to function effectively. Capillary water is fundamental to *Sphagnum* surviving in relation to the length of time the shoots remain turgid and allowing photosynthesis, respiration and growth (Goffinet and Shaw, 2008). *Blindia robusta*, on the other hand, appears to have no tolerance to desiccation. This is consistent with other aquatic mosses that have been shown to suffer cell membrane damage during desiccation, with rehydration resulting in loss of nutrients (Brown and Buck, 1979). The distribution of *B. robusta* can therefore be explained by its requirement for a permanent water supply to avoid desiccation. *B. robusta* lives in the groundwater source pools, a very acidic environment which would make the Ca less available. To survive in this environment would require an extremely high affinity for Ca, consistent with the results presented here.

The polymerised phenolics in bryophytes perform a similar role as lignin in vascular plants, providing structural integrity (reinforces cell walls), hydrophobicity (stops the cell walls collapsing under water pressure) and resistance to other stresses (Verhoeven and Liefveld, 1997). The lower amounts of these molecules and

decreased crosslinking would make the cell walls of *B. robusta* more porous, facilitating water uptake from surroundings, but also more vulnerable to water loss. This would explain why *B. robusta* is so vulnerable to desiccation and lives in the groundwater source pools.

4.5   Conclusions

Climate change in the Australian Alps will have potentially big impacts on species reliant on moisture such as mosses. Higher temperatures and lower precipitation (including winter snow) over the coming century (Green and Pickering, 2009) will likely contribute to more variable groundwater flow and, ultimately, the frequency with which groundwater pools dry out. This will have substantial effects on the landscape persistence of *B. robusta*, a species with virtually no tolerance for desiccation. Desiccation adversely affects the photosynthetic viability of *B. robusta* which, based on the results presented here, cannot be recovered upon rehydration. Re-colonisation of these habitats will depend on the availability and viability of spores; very little is known of the dispersal and colonisation potential of *B. robusta*. Identifying source populations and improved understanding of dispersal mechanisms would give greater insight into the likely fate of this moss under a changing climate. Given *B. robusta* is restricted to a few catchments on the Australian Alps, protection of all source water pools is paramount to species persistence.

Acknowledgements

4.6 References

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. 1994. Molecular Biology of the Cell. 3rd Edition. (Garland Publishing, Inc. New York & London) pgs 1001-1002.

Arora R (2018) Mechanism of freeze-thaw injury and recovery: A cool retrospective and warming up to new ideas. *Plant Science* **270**: 301-313.

Bağcıoğlu M, Zimmermann B, Sandt C, KohlerA. 2015. Vibrational microspectroscopy enables chemical characterization of single pollen grains as well as comparative analysis of plant species based on pollen ultrastructure. *Planta* **242(5):** 1237–1250.

Bednarek-Ochyra H (2014) A major range extension of *Blindia robusta* (Seligeriaceae, Bryophyta) based on the type *Ditrichium tenuinerve*. Telopea, *Journal of Plant Systematics* **17**, 323-328.

Bewley, JD. 1979. Physiological aspects of desiccation tolerance. *Annual Review of Plant Physiology* 30:195-238.

Bowes BG, Mauseth JD. 2008. Plant Structure – A Colour Guide. 2nd edn. CSIRO Publishing, Collingwood.

Brown DH, Buck GW (1979) Desiccation effects and cation distribution in bryophytes. *New Phytologist* **82:** 115-125.

Bureau of Meteorology. 2016`. 'Climate statistics for Australian locations.' Available at http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_nccObsCode=38&p_display_type=dataFile&p_startYear=&p_c=&p_stn_num=083084 [Verified 26 October 2016]

Clarke KR, Warwick RM. 2001 Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. Primer-E Ltd, Plymouth.

Cosgrove DJ. 2005. Growth of the plant cell wall. *Nature Reviews* 6: 850 – 861

Cruz de Carvalho R, Branquinho C, Marques da Silva J. (2011) Physiological consequences of desiccation in the aquatic bryophyte *Fontinalis antipyretica*. *Planta* **234(1):** 195-205.

Gajewski K, Viau A, Sawada M, Atkinson D, Wilson S. 2001. Sphagnum peatland distribution in North America and Eurasia during the past 21,000 years. *Global Biogeochemical Cycles* 15(2):297-310.

Geffert JL, Frahm J-P, Barthlott W, Mutke J. 2013. Global moss diversity: spatial

and taxonomic patterns of species richness. *Journal of Bryology* 35(1):1-11.

Glime JM. 1971. Response to two species of *Fontinalis* to field isolation from

stream water. *The Bryologist* 74 (3): 383-386.

Goffinet B, Shaw A.J. 2009. Bryophyte Biology. 2nd Edition. (Cambridge University:

Melbourne) pgs 237-268

Green K, Pickering CM. 2009. The decline of snowpatches in the Snowy Mountains of

Australia; importance of climate warming, variable snow, and wind. *Arctic,*

Antarctic, and Alpine Research 41, 212-218.

Grundon NJ, Robson AD, Lambert MJ, Snowball K. 1997.   Nutrient Deficiency and

Toxicity Symptoms. In: Reuter DJ, Robinson JB, eds. *Plant Analysis an*

Interpretation Manual. Collingwood: Australia: CSIRO Publishing, 37-51.

Hájek T, Beckett RP. 2008. Effect of Water Content Components on Desiccation and

Recovery in *Sphagnum* Mosses. *Annals of Botany* 101: 165-173.

Hájek T, Adamec L (2009) Mineral nutrient economy in competing species of

Sphagnum moss. *Ecological Research* **24:** 291-302.

Hájek T, Vicherová E. 2014. Desiccation tolerance of *Sphagnum* revisited: a puzzle resolved. *Plant Biology* 16: 765-773.

Haynes RJ. 1980. Ion exchange properties of roots and ionic interactions within the root apoplasm: their role in ion accumulation by plants. *Botanical Reviews* 46: 75-99

Heraud P, Caine S, Sanson G, Gleadow R, Wood BR, McNaughton D. 2007. Focal plane array infrared imaging: a new way to analyse leaf tissue. *New Phytologist* 173: 216-225.

Hirschi KD. 2004. The calcium conundrum:both versatile nutrient and specific signal. *Plant Physiology* 136: 2438-2442.

Kerr JL, Baldwin DS, Tobin MJ, Puskar L, Kappen P, Rees GN, Silvester E. 2013. High spatial resolution infrared micro-spectroscopy reveals the mechanism of leaf lignin decomposition by aquatic fungi. *Plos One* 8(4): 1-10.

Knowles N, Dettinger MD, Cayan DR (2006) Trends in snowfall versus rainfall in the Western United States. *Journal of Climate* **19:** 4545 – 4559.

Kremer C, Drinnan AN. (2004) Secondary walls in hyaline cells of Sphagnum. *Australian Journal of Botany* **52**: 243-256.

Kręzel A, Bal W. 2004. A formula for correlating pKa values determined in D_2O and H_2O. *Journal of Inorganic Biochemistry* 98(1): 161–166.

Liepina L, Ievinsh G. 2013. Potential for fast chlorophyll a fluorescence measurement in bryophyte ecophysiology. *Estonian Journal of Ecology* 62(2): 137-149.

Lovelock CE, Osmond CB, Seppelt RD. 1995. Photoinhibition in the Antarctic moss (*Grimmia antarctici* Card. When exposed to cycles of freezing and thawing. *Plant, Cell and Environment* 18: 1395-1402.

Max J, Chapados C (2009) Isotopic effects in liquid water by infrared spectroscopy. III. H_2O and D_2O spectra from 6000to0cm -1. *Journal of Chemical Physics* **131 (18)**: 184505-1 – 184505-13.

McCarter CPR, Price JS. 2012. Ecohydrology of Sphagnum moss hummocks: mechanisms of capitula water supply and simulated effects of evaporation. *Ecohydrology* 7 (1): 33-44.

McCartney VA, Silvester E, Morgan JW, Suter PJ. 2013. Physical and chemical drivers of vegetation in groundwater source pools on the Bogong High Plains, Victoria. *Australian Journal of Botany* 61: 566–573.

Ochyra R, Bednarek-Ochyra H, Lewis-Smith RI (2002) New and rare moss species from subantarctic South Georgia. *Nova Hedwigia* **74**, 121-147.

Oliver MJ, Velten J, Mishler BD. 2005. Desiccation tolerance in bryophytes: A reflection of the primitive strategy for plant survival in dehydrating habitats? Annual Meeting of the Society for Integrative and Comparative Biology 45:788-799.

Popescu C-M, Popescu M-C, Singurel G, Vasile C, Argyropoulos DS, Willfor S. 2007. Spectral Characterisation of Eucalyptus Wood. *Applied Spectroscopy* 61(11): 1168 – 1177.

Prajapati K, Modi HA (2012) The importance of potassium in plant growth – a review. *Indian Journal of Plant Sciences* **1**: 177-186

Price JS, Whittington PN. 2010. Water flow in Sphagnum hummocks: Mesocosm measurements and modelling. *Journal of Hydrology*. 381: 333-340.

Proctor MCF, Ligrone R, Duckett JG. 2007. Desiccation tolerance of the moss *Polytrichum formosum*: Physiological and fine-structural changes during desiccation and recovery. *Annals of Botany* 99:75-93.

Robinson SA, Wasley, Popp JM, Lovelock CE. 2000. Desiccation tolerance of three moss species from continental Antarctica. *Australian Journal of Plant Physiology* 27:379-388.

Roland CA, Stehn SE, Schmidt JH. 2017 Species richness of multiple functional groups peaks in alpine tundra in subarctic Alaska. *Ecosphere* 8(6):1-28.

Romheld V and Marschner H. 1991 Function of Micronutrients in Plants. Madison:

   USA: Soil Society of America Book Series.

Séné CFB, McCann MA, Wilson RH, Grinter R. 1994. Fourier-transform raman and

   Fourier-transform infrared spectroscopy – An investigation of five higher plant

   cell walls and their components. *Plant Physiology* 106: 1623-1631.

Seppelt RD (2004) The moss flora of Macquarie Island. (Kingston, Tasmania,

   Australian Antarctic Division).

Silverstein R, Bassler G, Morrill T. 1974. *Spectrometric identification of organic*

   compounds, 3rd edn. New York, USA: John Wiley and Sons Inc.

Silvester E. 2009. Ionic regulation in an alpine peatland in the Bogong High Plains,

   Victoria, Australia. *Environmental Chemistry*, **6:**424–431

Silvester E, Klein AR, Whitworth KL, Puskar L, Tobin MJ. 2018. Synchrotron infrared

   microspectroscopy reveals the response of Sphagnum cell wall material to its

   aqueous chemical environment. *Environmental Chemistry* **15:** 513-521

Suren AM. 1993. Bryophytes and associated invertebrates in first-order alpine streams

   of Arthur's Pass, New Zealand. *New Zealand Journal of Marine and Freshwater*

   Research. 27-4: 479-494.

Suren AM. 1996. Bryophyte distribution patterns in relation to macro-, meso-, and micro-scale variables in South Island, New Zealand streams. *New Zealand Journal of Marine and Freshwater Research*. 30: 501-523.

Tian W, Yang L-M, Xu Y-Z, Weng S-F, Wu J-G. 2000. Sugar interaction with metal ions. FT-IR study on the structure of crystalline galactaric acid and its K^+, NH_4^+, Ca^{2+}, Ba^{2+}, and La^{3+} complexes. *Carbohydrate Research* 324(1): 45–52.

Verhoeven JTA, Liefveld WM. 1997. The ecological significance of organochemical compounds in *Sphagnum*. *Acta Botanical Netherlands* 46(2): 117-130.

Vitt DH (1974) A key and synopsis of the mosses of Campbell Island, New Zealand. *New Zealand Journal of Botany* **12**, 185-210.

Vitt DH (1979) The moss flora of the Auckland Islands, New Zealand, with a consideration of habitats, origins, and adaptations. *Canadian Journal of Botany* **57**, 2226-2263.

Whetton P (1998) Climate change impacts on the spatial extent of snow-cover in the Australian Alps. In: Green K (ed) Snow a natural history: uncertain future. Australian Alps Liaison Committee, Canberra/Surry Beatty and Sons, Sydney, Australia.

Williams RJ. 1987. Patterns of air temperature and accumulation of snow in

    subalpine heathlands and grasslands on the Bogong High Plains, Victoria.

    Australian Journal of Ecology 12: 153-163

Yazaki T, Urano S, Yabe K. 2006. Water balance and water movement in

    unsaturated zones of Sphagnum hummocks in Fuhrengawa Mire, Hokkaido,

    Japan. *Journal of Hydrology*. 319: 312-327.

Zhong R, Morrison III WH, Himmelsbach DS, Poole II FL, Ye Z-H. 2000.

    Essential role of caffeoyl Coenzyme A O-Methyltransferase in lignin

    biosynthesis in woody poplar plants. *Plant Physiology* 124:563-577.

S4.1   Supplementary Material

Fig. S4.1. Slides showing leaf formation and separation from a stem of *Blindia robusta*.

Stem starting to form.

Offshoot stem still forming.

Stem formation and separation almost complete.

Stem and leaf separation almost complete.

Stems still held together by one cell.

Stems separated

Fig. S4.2. Stem formation off main *Blindia robusta* stem.

Fig. S4.3. PCA analysis of macronutrients within catchments and between Blindia robusta and Sphagnum cristatum plants. The macronutrients analysed were: nitrogen, phosphorous, potassium, sulfur, carbon, calcium, magnesium and sodium.

Fig. S4.4. PCA plot illustrating Ca:Mg levels between species of moss growing in two catchments Heathy Spur and Whiterock Creek (HS and WR).

Fig. S4.5. PCA analysis of micronutrients within *Blindia robusta* and *Sphagnum cristatum* plants and HS and WR catchments. The micronutrients analysed were: copper, zinc, manganese, iron, boron, molybdenum, cobalt and silicon.

Fig. S4.6 PCA analysis of micronutrients between *Blindia robusta* and *Sphagnum cristatum* plants and catchments. The micronutrients analysed were: copper, zinc, boron, molybdenum, and cobalt. Iron and manganese were excluded.

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Table S4.1. Concentrations of macronutrients and micronutrients found in *Blindia robusta* and *Sphagnum cristatum* at groundwater – source pools at Heathy Spur and Whiterock Creek. Average data for all locations.

Macronutrients % Heathy Spur

Site	N	P	K	S	C	Ca	Mg	Na	Crude Protein %	Moss
HSGW1	1.03	0.14	0.37	0.09	41.1	0.11	0.03	<0.01	6.4	*Blindia*
HSGW1S	0.67	0.04	0.36	0.07	42.1	0.23	0.12	0.05	4.2	*Sphagnum*
HSGW8	0.96	0.16	0.43	0.10	40.5	0.12	0.04	<0.01	5.8	*Blindia*
HSGW8S	0.59	0.04	0.26	0.07	42.6	0.28	0.07	0.03	3.7	*Sphagnum*
HSGW11	0.79	0.16	0.46	0.07	41.1	0.15	0.03	<0.01	4.9	*Blindia*
HSGW11S	0.55	0.03	0.34	0.07	42.0	0.25	0.12	0.03	3.4	*Sphagnum*

Table S4.1 cont. Concentrations of macronutrients and micronutrients found in *Blindia robusta* and *Sphagnum cristatum* at groundwater – source pools at Heathy Spur and Whiterock Creek. Average data for all locations.

Micronutrients mg/kg Heathy Spur

Site	Cu	Zn	Mn	Fe	B	Mo	Co	Si	Moss
HSGW1	7	14	163	304	3.1	<0.2	1.0	447	*Blindia*
HSGW1S	2	22	90	323	<1	<0.2	0.2	571	*Sphagnum*
HSGW8	6	10	153	433	3	<0.2	1.1	475	*Blindia*
HSGW8S	2	13	224	417	1	<0.2	0.3	667	*Sphagnum*
HSGW11	5	8	152	118	2	<0.2	0.9	425	*Blindia*
HSGW11S	1	15	402	308	<1	<0.2	0.2	686	*Sphagnum*

Table S4.1. Concentrations of macronutrients and micronutrients found in *Blindia robusta* and *Sphagnum cristatum* at groundwater – source pools at Heathy Spur and Whiterock Creek. Average data for all locations.

Macronutrients % Whiterock Creek

Site	N	P	K	S	C	Ca	Mg	Na	Crude Protein %	Moss
WRGW1	1.02	0.13	0.35	0.09	40.9	0. 20	0.03	<0.01	6.4	*Blindia*
WRGW1S	0.72	0.03	0.39	0.08	40.57	0.37	0.15	0.06	4.5	*Sphagnum*
WRGW8	1.12	0.16	0.40	0.10	42.16	0.20	0.05	<0.01	7.0	*Blindia*
WRGW8S	0.76	0.04	0.55	0.09	42.4	0.16	0.10	0.03	4.8	*Sphagnum*
WRGW9	1.0	0.12	0.32	0.10	42.1	0.18	0.04	<0.01	6.2	*Blindia*
WRGW9S	0.65	0.02	0.44	0.08	42.0	0.40	0.08	0.02	4.1	*Sphagnum*

Table S4.1 cont. Concentrations of macronutrients and micronutrients found in *Blindia robusta*

and *Sphagnum cristatum* at groundwater – source pools at Heathy Spur and Whiterock Creek.

Average data for all locations.

Micronutrients mg/kg Whiterock Creek

Site	Cu	Zn	Mn	Fe	B	Mo	Co	Si	Moss
WRGW1	7	10	224	229	3	<0.2	1.4	502	*Blindia*
WRGW1S	5	17	487	1274	4	<0.02	0.8	501	*Sphagnum*
WRGW8	4	10	107	151	4	<0.2	0.8	466	*Blindia*
WRGW8S	3	15	1430	182	<1	<0.2	0.1	435	*Sphagnum*
WRGW9	5	8	105	132	2	<0.2	0.7	347	*Blindia*
WRGW9S	2	12	1227	177	1	<0.2	<0.1	443	*Sphagnum*

Table S4.2. Ratio of Ca:Mg found in *Blindia, Sphagnum* and catchment groundwater at

Heathy Spur (HS) and Whiterock Creek (WR).

Sample	Ca	Mg
HS Blindia	2.079	1
HS Sphagnum	1.42	1
HS Water	1.2	1
WR Blindia	3	1
WR Sphagnum	1.68	1
WR Water	1.43	1

Table S4.3. One-Way MANOVA explaining the statistical significance of Ca:Mg in moss but that catchments indicate no difference.

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[d]	
Intercept	Pillai's Trace	.743	66.633[b]	2.000	46.000	.000	.743	133.265	1.000	
	Wilks' Lambda	.257	66.633[b]	2.000	46.000	.000	.743	133.265	1.000	
	Hotelling's Trace	2.897	66.633[b]	2.000	46.000	.000	.743	133.265	1.000	
	Roy's Largest Root	2.897	66.633[b]	2.000	46.000	.000	.743	133.265	1.000	
Moss	Pillai's Trace	.350	4.990	4.000	94.000	.001	.175	19.958	.954	
	Wilks' Lambda	.653	5.462[b]	4.000	92.000	.001	.192	21.848	.970	
	Hotelling's Trace	.526	5.921	4.000	90.000	.000	.208	23.684	.980	
	Roy's Largest Root	.517	12.139[c]	2.000	47.000	.000	.341	24.279	.993	
Catchment	Pillai's Trace	.092	2.341[b]	2.000	46.000	.108	.092	4.681	.450	
	Wilks' Lambda	.908	2.341[b]	2.000	46.000	.108	.092	4.681	.450	
	Hotelling's Trace	.102	2.341[b]	2.000	46.000	.108	.092	4.681	.450	
	Roy's Largest Root	.102	2.341[b]	2.000	46.000	.108	.092	4.681	.450	
Moss * Catchment	Pillai's Trace	.022	.258	4.000	94.000	.904	.011	1.034	.104	
	Wilks' Lambda	.978	.254[b]	4.000	92.000	.906	.011	1.017	.103	
	Hotelling's Trace	.022	.250	4.000	90.000	.909	.011	1.000	.102	
	Roy's Largest Root	.022	.514[c]	2.000	47.000	.601	.021	1.028	.130	

a. Design: Intercept + Moss + Catchment + Moss * Catchment

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

d. Computed using alpha = .05

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Table S4.4 Two-Way ANOVA test between copper, zinc, boron, molybdenum and

cobalt in moss and between catchment.

Dependent Variable: Copper mg/kg

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Moss	90.250	1	90.250	60.447	.000
Catchment	2.250	1	2.250	1.507	.229
Moss * Catchment	10.028	1	10.028	6.716	.014

a. R Squared = .682 (Adjusted R Squared = .652)

Zinc mg/kg

Moss	283.361	1	283.361	24.492	.000
Catchment	26.694	1	26.694	2.307	.139
Moss * Catchment	.028	1	.028	.002	.961

a. R Squared = .456 (Adjusted R Squared = .405)

Boron mg/kg

Moss	24.668	1	24.668	19.794	.000
Catchment	5.601	1	5.601	4.494	.042
Moss * Catchment	2.151	1	2.151	1.726	.198

a. R Squared = .448 (Adjusted R Squared = .397)

Molybdenum mg/kg

Moss	.001	1	.001	1.000	.325
Catchment	.001	1	.001	1.000	.325
Moss * Catchment	.001	1	.001	1.000	.325

a. R Squared = .086 (Adjusted R Squared = .000)

Cobalt mg/kg

Moss	3.300	1	3.300	28.837	.000
Catchment	.123	1	.123	1.070	.309
Moss * Catchment	.202	1	.202	1.769	.193

a. R Squared = .497 (Adjusted R Squared = .450)

Chapter 5

Groundwater age on the Bogong High Plains, Victoria

Authorship statement

This chapter has been prepared for as a manuscript to be submitted for publication. However, wherever I reference work presented in Chapter 2 (which has been published as McCartney et al. 2013), I cite it as "Chapter 2"

Author contribution: Water samples were collected by Victoria McCartney in summer, autumn and spring and by Ewen Silvester in winter. Victoria McCartney prepared the manuscript for publication with editing from supervisors Ewen Silvester and John Morgan.

Abstract

Groundwater sources are a critical hydrological component of alpine wetlands in the Australian Alps; the age and recharge of water is important in understanding the vulnerability of these systems to a changing climate. Five permanent groundwater sources on the Bogong High Plains (Victoria, Australia) were monitored seasonally for chlorofluorocarbons to quantify the apparent recharge year and water age. The apparent groundwater age of these sources varied, ranging from 15-38 years old. Despite the limited time period of this study, there is evidence that snow-pack is an important component to groundwater recharge, with younger water generally observed after snowmelt. The interpretation of water age is possibly complicated by the mixing of old (regional water) and more recent water ages (local water) within the groundwater system. The relatively young age of the water indicates that groundwater flows are likely to respond on decadal timescales to changing precipitation amounts. In turn, this suggests that the groundwater-dependent ecosystems associated with these sources may be vulnerable to changing precipitation patterns.

5.1   Introduction

Groundwater is vital to alpine wetlands, with studies in the Australian Alps showing that groundwater discharges are typically associated with peat-accumulating wetlands (peatlands) (Costin, 1955; Clarke and Martin, 1999; Wahren *et al.* 1999; Whinam *et al*. 2003; Shannon and Morgan, 2007). Not all groundwater sources that sustain alpine wetlands are permanent.   Chapter 2 has shown that permanent groundwater sources sustain mats of the localised aquatic moss *Blindia robusta*. Chapter 2 also described wetland vegetation for both permanent and ephemeral groundwater sources. There are no known studies of groundwater age on the Bogong High Plains. Through measurement of groundwater age, this chapter will try to identify the processes that sustain permanent groundwater sources on the Bogong High Plains.

Groundwater typically occurs below the land surface as a direct result of precipitation that permeates through to the water table. Jasechko *et al.* (2014) suggests that in arid and temperate climates, groundwater recharge is consistently higher in winter than in summer due to lower evapotranspiration. In colder climates where snowpack can accumulate, snow melt is likely an important contributor to groundwater recharge (Jasechko *et al*. 2014). For example, a seasonal pattern linking precipitation, evapotranspiration, base-flow and runoff has been found for the Bogong High Plains (Western *et al*. 2008). For the Australian Alps, Fiddes *et al.* (2015) has used past snow records to predict that the future occurrence of snowfall will likely be reduced with likely impacts on stream flows.

The Watchbed Creek area on the Bogong High Plains, Victoria, has permanent groundwater sources; these have been described in Chapter 2 and are shown in Figure 5.1. This area contains granite that is highly fractured due to uplifting and faulting (Morand, 1990; Taylor *et al*. 2004). Rainfall and melting snowpack permeate through soil and rocks and become groundwater.

Fig. 5.1. Map of study area showing groundwater collection sites in the Watchbed Creek catchment (in red). Hollands Knob and Mount Nelse are shown in relation to the groundwater collection sites.

Watchbed Creek has an aquifer system where the water travels near vertical fracture joints and exits as permanent groundwater sources maintaining local stream flows in dry periods (Fussell, 2014). Fussell (2014) noted well-developed foliation layers in the Cobungra Granite that was consistently orientated vertically. This foliation would allow for infiltration of surface water to join up with local and regional groundwater (Figure 5.1). Fussell (2014) also noted the existence of low angle joint planes. These may act as conduits for groundwater and result in the migration of groundwater to the surface at lower elevations. Fussell (2014) proposed two models for groundwater flowing into the Watchbed Creek catchment (Figure 5.2, 5.3). Figure 5.2 illustrates water infiltrating through faults and fracture joints in the Hollands Knob area and travelling in intermediate and regional groundwater flows and exiting as permanent groundwater sources. Figure 5.3 demonstrates how water can be captured and contained as groundwater through faulting and fracture joints at Mount Nelse. Bedding planes allow the water to be transported both vertically and horizontally. This water is then transported regionally and locally, eventually exiting into the Watchbed Creek area.

In recent times human activities have released a range of synthetic chemicals into the atmosphere that dissolve in precipitation and become part of groundwater. One such class of chemicals are chlorofluorocarbons (CFCs) that were produced commercially at the beginning of the 1930s for use in refrigeration. CFC-12 (dichlorodifluoromethane, CF_2Cl_2), produced from 1930, and CFC-11 (trichlorodifluoromethane, $CFCl_3$), produced from 1936, were used as insulation and packing materials, blowing agents in foam, propellants in aerosol cans and as solvents (Gamlen $et\ al.,$ 1986; Midgely and Fisher, 1993). Restrictions were placed on the release of chlorofluorocarbons in the 1980s, with atmospheric levels of chlorofluorocarbons plateauing in the 1990s (Walker $et\ al.$ 2000). CFC molecules

equilibrate with the soil water at the time of recharging which imprints the atmospheric

concentration permanently into the water table (CSIRO, 2019). Release of

chlorofluorocarbons into the atmosphere and subsequent absorption into the Earth's

hydrological cycle has directly followed their production (Gamlen *et al.,* 1986; Midgely

and Fisher, 1993) making chlorofluorocarbons a useful and cost effective way to

establish the age of relatively young groundwater (Plummer and Busenberg, 2000;

Walker *et al.,* 2000; Bockgard *et al.* 2004; Bullister and Wisegarver, 2008; Labasque *et al.* 2014).

Fig. 5.2. Demonstrating a model of how local and regional groundwater could mix before emerging at the surface (Fussell, 2014).

Fig. 5.3. A model of regional and local groundwater flow showing bedding planes with joint angle planes within the vicinity of Mount Nelse and Watchbed Creek (Fussell, 2014).

Silvester (2009) found a previously unknown groundwater supply in the Watchbed Creek catchment, known in this study as WBGW1. From the work described in this thesis, we now know that this groundwater source is one of many found in the Watchbed Creek catchment, one of many catchments on the Bogong High Plains, and described in Chapter 2. Determining the age of the water supplying these systems is important to understanding how climate change will impact them.

In this study, I ask the following questions:

- How old is the groundwater of the Watchbed Creek catchment?
- Is there a link between precipitation type and amount with groundwater age on the Watchbed Creek catchment?
- How vulnerable are the bryophyte pools to changing precipitation associated with climate change?

5.2   Methods and Materials

5.2.1   Site description

The Bogong High Plains (BHP) is an elevated plateau on the Great Dividing Range, located within the Alpine National Park in north-eastern Victoria, Australia. The BHP occupies an area of approximately 120 km^2 with elevations ranging from 1650 to 1850 m. Mean annual precipitation is 2449 mm, with snow being the major form of precipitation during the winter months (Williams 1987). Snow is declining by 23 cm per decade for maximum snow depth, and 21 cm per decade for snow accumulation (Fiddes *et al.* 2014). Mean maximum temperature is 9.4 ^{o}C and mean minimum temperature is 2.6 ^{o}C (Rocky Valley Meteorological recording station, Bureau of Meteorology (BOM) 2017). The results of the CFC-11 and CFC-12 analyses were

compared to precipitation records from the BOM Rocky Valley weather station data; this station is the closest monitoring station to the Watchbed Creek catchment.

5.2.2 Sample collection

The Watchbed Creek catchment was chosen to investigate apparent water age using CFC-11(trichlorofluoromethane) and CFC-12 (dichlorodifluoromethane). The physical and chemical properties of permanent groundwater sources in this catchment have been previously documented (Chapter 2). Five permanent groundwater sources were chosen on the Watchbed Creek catchment (WBGW1, WBGW2, WBGW7, WBGW9 and WBGW11. Initially it was decided to take a water sample from WBGW1 and test the suitability of CFC measurement for water age determination. The chlorofluorocarbon testing of this sample was found to fit within a usable water age range, based on CSIRO guidelines developed from Cape Grim data (Figure 5.4 and Figure 5.5). Based on this result, water samples from each of the five groundwater sources were collected at approximately 3 monthly intervals over a period of 2 years.

Not all sites could be accessed on every sampling occasion. The sites WBGW2 and WBGW7 could not be accessed in spring 2012. Groundwater samples from WBGW9 could not be obtained due to very low water flows in the summer of 2013. The groundwater source for WBGW9 was not sampled in the winter of 2013.

5.2.3 Water collection for CFC analysis

Triplicate water samples were collected from each of the five permanent groundwater sites following CSIRO Isotope Analysis Service, Adelaide, South Australia guidelines. Water was collected by inserting a 5 m long, 7mm diameter nylon hose into the groundwater flow path. The air in the hose was then evacuated and water flow allowed for a few minutes before filling commenced into a 2 L water container.

Glass screw capped bottles (200 mL) were put into the 2 L water container and allowed to fill and overflow for a few minutes. The lid was placed onto the 200 mL bottle underwater so that there was no contamination from the atmosphere. The bottles were then dried and labelled. A final examination was carried out to make sure that there were no large air bubbles within the 200 mL bottle. The lids were tightened using a pair of multigrips and samples stored on ice for transportation back to La Trobe University, Wodonga, Australia. Samples were then refrigerated until packaged and sent overnight to CSIRO Isotope Analysis Service, Adelaide, South Australia. Averages of two of the replicates were used for apparent water ages and cross-referenced with Cape Grim data to determine the apparent water charge year (discussed below). The third sample was only measured if the difference between the two measurements was larger than the analytical error of the method.

5.2.4 CSIRO Cape Grim Data

Cape Grim is situated in north-west Tasmania, Australia and is a Baseline Air Pollution Station in the World Meteorological Organization-Global Atmosphere Watch (WMO-GAW) network (Commonwealth Scientific and Industrial Research Organisation, 2019). This baseline air is characteristic of a large area of the Southern Hemisphere, unaffected by regional pollution sources (there are no nearby cities or industry that would influence air quality) (Commonwealth Scientific and Industrial Research Organisation, 2019). The calculation of an apparent recharge year is based on the atmospheric levels of CFC compounds, by reference to the levels measured at Cape Grim (Tasmania, Australia).

Cape Grim data shows that both CFC-11 (Figure 5.4) and CFC-12 (Figure 5.5) have peaked at their maximum values and are now declining in the atmosphere. The maximum amounts of CFC-11 peaked in the year 1993 at a concentration of 263 pptv

(parts per trillion by volume) (Figure 5.4). The maximum amounts of CFC-12 peaked in the year 2003 at a concentration 547 pptv (Figure 5.5). The recent decline in CFC levels in the atmosphere makes it impossible to apply this aging technique to more recently recharged groundwaters.

Fig. 5.4. Plot of CFC-11 atmospheric concentrations using Cape Grim records. ●
1930-1996 data and ●1978-2009 data are shown with CFC-11 amounts plateauing
in the year 1993 at 263 pptv.

Fig. 5.5. Plot of CFC-12 atmospheric concentrations using Cape Grim records. ⬤ 1930-1996 data and ⬤1978-2009 data are shown with CFC-12 amounts plateauing in the year 2003 at 547 pptv.

5.3   Results

5.3.1   Snow and rainfall

Snowfall occurs falls between the months of May and October but amounts and timing varies between years (Fig. 5.6). Precipitation varies from year to year for all months; Figure 5.6 shows precipitation over the study period. Compared to the long-term average, February 2011 had extremely high rainfall while the precipitation in the winter of 2011 was generally lower than 2012 and 2013 (Figure 5.6). In 2012, a longer snow season was maintained due to precipitation that fell as snow. Higher precipitation was observed in July and August 2013 compared to either 2011 or 2012. (Figure 5.6).

During the study period, the cumulative snow depths in 2011 were lower compared to the long-term average; maximum snow depth occurred earlier than the long-term average. The 2012 snow season had snow depths that were higher than in 2011 and 2013 and were also higher that the long-term average. The 2013 snow season was close to an average snow depth and time of peak snowpack.

Fig. 5.6. **(a)** Cumulative daily snow depths (shown in grey) at Rocky Valley (top), with long-term average daily depths shown in red. **(b)** The precipitation (snowfall and rainfall combined) at Rocky Valley measured monthly over the years of January 2011 to December 2013, with long-term average monthly measurements shown in red.

5.3.2 Groundwater sites and water age

Groundwater ages ranged from 38 years ((WBGW7; CFC-11; Spring 2013)) to 13 years ((WBGW1; CFC-11; Spring 2012)). Each site is described in more detail below, with groundwater age data shown in Figure 5.7 (WBGW1, WBGW2, WBGW11) and Figure 5.8 (WBGW7, WBGW9). Also shown with these data are the cumulative precipitation data for the 3-month period preceding each sampling event, included to explore possible links between precipitation amount and groundwater age. Over the period Spring 2011 to Spring 2012, 3-month precipitation ranged from 600 to 800 mm. A substantial decrease in precipitation was observed for summer 2012/13 and autumn 2013 (281 mm and 357 mm, respectively) followed by substantially higher precipitation in Winter 2013 (985 mm) and Spring 2013 (862 mm). Summer 2013/4 had 410 mm of precipitation.

Groundwater sampled at WBGW1 varied in age from 14 yrs to 36 yrs using CFC-11 data (23 yrs to 37 yrs using CFC-12 data) (Figure 5.7). The youngest water was observed in spring 2012 while the oldest water was observed in summer 2013 (Figure 5.7). The oldest age observed in summer 2013 corresponded to a drier than average preceding three-month period (Figure 5.6b; Figure 5.7). A general pattern of older water was observed across the three summer periods with CFC-12 tracer; however, the CFC-11 analysis shows a younger water for the 2012/13 summer (Figure 5.7).

Groundwater sampled at WBGW2 varied in age from 21 yrs (most recent using CFC-11) data to 36 yrs (oldest using CFC-11 data) (24 yrs to 35 yrs using CFC-12 data) (Figure 5.7). The youngest water was observed in autumn 2013 while the oldest water was observed in spring 2013 (Figure 5.7). Unlike WBGW1 a general pattern of older water was not observed across the summer periods with CFC-12 tracer (Figure 5.7).

Groundwater sampled at WBGW11 varied in age from 22 yrs (most recent using CFC-11) data to 33 yrs (oldest using CFC-11 data) (24 yrs to 33 yrs using CFC-12 data)

(Figure 5.7). The youngest water was observed in spring 2012 while the oldest water was observed in summer 2013 (Figure 5.7). The oldest age was observed in summer 2013. A general pattern of older water was observed across the three summer periods with CFC-12 tracer (Figure 5.7).

Groundwater sampled at WBGW7 varied in age from 20 yrs (most recent) to 38 yrs (oldest) using CFC-11 data (23 yrs to 36 yrs using CFC-12 data) (Figure 5.8). The youngest water was observed in winter 2012 and again in autumn 2013, while the oldest water was observed in spring 2013 (Figure 5.8). A general pattern of older water was observed across the three summer periods with CFC-12 tracer with the same trends observed in the CFC-11 analysis (Figure 5.8).

Groundwater sampled at WBGW9 varied in age from 22 yrs (most recent CFC-11) to 35 yrs (oldest) using CFC-11 data (24 yrs to 36 yrs using CFC-12 data) (Figure 5.8). The youngest water was observed in winter 2012 while the oldest water was observed in summer 2013 (Figure 5.8). The oldest age observed in summer 2013 corresponded to a drier than average preceding three-month period (Figure 5.6b; Figure 5.8). The results are similar to other sites with older water observed in the summer period of 2013 with CFC-12 tracer, with a slightly younger age using the CFC-11 analysis (Figure 5.8). Long-term trends cannot be detected for WBGW9 because of missing data.

Fig. 5.7. Groundwater age data using CFC-11 and CFC-12 at WBGW1, WBGW2 and WBGW11, collected seasonally. These groundwater sources are all found in the western area of Watchbed Creek catchment. Also shown are the cumulative precipitation amounts for the 3-month period preceding each sampling event. Red lines show approximate year boundaries.
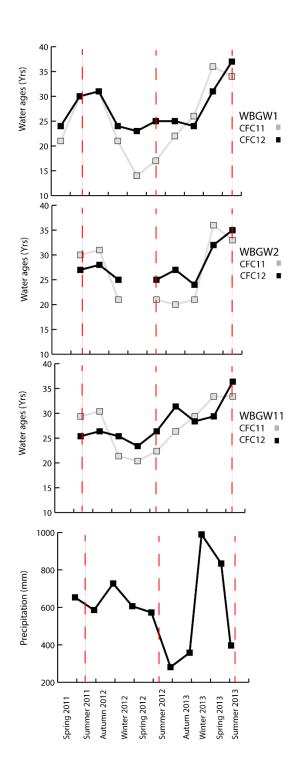
Fig. 5.8. Groundwater age data using CFC-11 and CFC-12 at WBGW7 and WBGW9,
collected seasonally. These groundwater sources are all found in the eastern area of
Watchbed Creek catchment. Also shown are the cumulative precipitation amounts for
the 3-month period preceding each sampling event. Red lines show the year boundaries.

5.4 Discussion

5.4.1   CFC water age and groundwater recharge

Theories on how groundwater is sustained have been suggested by Troth (1963) and, in the case of the Bogong High Plains, by Fussell (2014) (Figure 5.1 and Figure 5.2). Troth (1963) proposed a model of how water that enters the water table follows flow paths on local, intermediate and broader regional levels. According to this model, variations in groundwater flows would occur according to recharge regime, as well as the composition and structure of the saturated substrate and topography. On the Bogong High Plains, the groundwater recharge systems proposed by Troth (1963), Western et al. (2008) and Karis (2016) suggests that a rapid infiltration of local water mixes with older regional groundwater, resulting in a younger apparent age when the supply of groundwater is highest, i.e. during snow melt/thaw (August to November). During summer, an older water age would be expected when the more constant contribution of the intermediate flow would be dominant.

The pattern predicted for older water age in summer and autumn was generally observed in data for 2012 and 2013. The pattern predicted for younger water ages in winter and spring was also generally observed in data for 2012 and 2013. These patterns are consistent with the idea that snowmelt (recent recharge) makes a larger contribution to groundwater flow in winter/spring while regional groundwater is more dominant in summer/autumn. Moreover, even though a limited data set has been collected for this study, the CFC data indicates that the youngest water was observed after the highest snowpack in the study period (winter 2012). On the other hand, the highest rainfall year of 2013 (and particularly the winter and spring seasons) did not result in younger water ages. This suggests that snowpack, and by inference snowmelt, is relatively more important to groundwater recharge on the Bogong High Plains than

total precipitation. To obtain a more robust understanding of the link between precipitation patterns and groundwater age would require many more years of CFC data collection. The pattern observed in water ages for this study is consistent with the hypothesis that snowmelt is relatively more important than total precipitation for groundwater recharge on the Bogong High Plains.

The calculation of an apparent recharge year is based on the atmospheric levels of CFC compounds, as measured at Cape Grim (Tasmania, Australia). However, other factors can contribute to uncertainty in the apparent water ages. For example, microbial degradation can lead to an old apparent water age bias with chlorofluorocarbon analyses, particularly for CFC-11 (Oster *et al.* 1996). Oster *et al.* (1996) reported that in some anoxic environments, the degradation of CFC-11 is ten-fold faster than CFC-12. Some of the results from this study show CFC-11 as being older than CFC-12, e.g. at WBGW1, WBGW2 and WBGW11. However, it not clear if this is due to microbial degradation or some other process.

5.4.2   Bogong High Plains Hydrology

Western *et al.* (2008) found that on the Bogong High Plains there was a seasonal pattern linking precipitation, evapotranspiration, base-flow and runoff. Summer rainfall produced a noticeable spike in runoff but the store of water in the snowpack made the largest contribution to runoff that occurred from August to November (Western *et al.* 2008). Karis (2016) found that following a summer rainfall event, a spike in runoff occurred but a considerable portion of the total amount of precipitation in the catchment did not report to stream flows, even after several weeks. This water, amounting to 62% of the rainfall, was presumably lost by evapotranspiration or recharged to groundwater reserves at lower elevation through regional groundwater.

This study suggests that snowpack (snow melt) is extremely important for the groundwater component that sustains stream flow over dry periods for the Watchbed Creek catchment. Expected changes in snow fall and snowpack in the Australian Alps (Fiddes *et al*. 2015) from climate change will likely be reflected in changing (decreasing) groundwater recharge occurring over decadal timescales. Any changes in recharge will likely impact on both local as well as regional groundwater reserves, leading to a general trend of increasing groundwater age. These changes, combined with increased frequency of high intensity rainfall events, will likely change the hydrology of these alpine aquatic ecosystems. For example, the groundwater supply to the groundwater source pools will likely decrease, resulting in mortality of dependent flora such as *Blindia robusta* so as to make these ecosystems increasingly reliant on recolonization mechanisms. Similarly, the broader peatland systems that are also dependent upon groundwater might be expected to decline in condition and the associated streams become ephemeral.

5.5   Conclusion

Groundwater collected from the Watchbed Creek catchment was found to vary in age over the range 15-38 years, with relatively younger water observed in winter/spring compared to summer/autumn for any given year. This suggests that snow-melt (and by inference the accumulated snow pack) is more important for groundwater recharge than total precipitation. Predicted changes in snow pack from climate change may therefore result in decreased recharge and ultimately change these groundwater sources from permanent to ephemeral flows. This range of water ages observed in this study likely originates from the mixing of older intermediate and regional groundwater reserves with more recently recharged local (younger) groundwater. A more complete understanding of the groundwater recharge mechanisms will require multi-year data,

allowing possible correlation of snow-pack extent with water age and groundwater flow

to be more fully explored. Such studies will be necessary to fully understand these

groundwater systems and the implications of climate change on their flows.

Acknowledgements

5.6   References

Bockgard N, Rodhe A, Olsson KA. (2004) Accuracy of CFC groundwater dating in a

   crystalline bedrock aquifer: Data from a site in southern Sweden. *Hydrogeology*

   Journal **12,** 171-183.

Bullister JL, Wisegarver DP. (2008) The shipboard analysis of trace levels of sulfar

   hexafluoride, chlorofluorocarbon-11 and chlorofluorocarbon-12 in seawater. *Deep-*

   Sea Research **1:55,** 1063-1074.

Bureau of Meteorology. (2017) 'Climate statistics for Australian locations.' Available

   at http://www.bom.gov.au/climate/averages/tables/cw_083084.shtml (Verified 7

   August 2017 and 18 July 2018)

Clarke PJ, Martin ARH. (1999) *Sphagnum* peatlands of Kosciusko National Park in

   relation to altitude, time and disturbance. *Australian Journal of Botany* **47,** 519-536.

Commonwealth Scientific and Industrial Research Organisation. (2019) 'Cape Grim

   Greenhouse Data' Available at

   https://www.csiro.au/en/Research/OandA/Areas/Assessing-our-climate/Greenhouse-

   gas-data (Verified 23 April 2019).

Commonwealth Scientific and Industrial Organisation (2019) Tracing groundwater
movement using CFCs and SF_6. Available at
https://www.google.com.au/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&ved=
2ahUKEwjIpsW2qbriAhVTfisKHQg2BXcQFjABegQIABAC&url=https%3A%2F
%2Fpeople.csiro.au%2F-%2Fmedia%2FPeople-Finder%2FS%2FA%2FAxel-
Suckow%2FFact-sheet-CFC-prel.pdf&usg=AOvVaw2x40g1osdd-2tS013M-pqc
(verified 27 May 2019)

Costin AB. (1955) Alpine soils in Australia with reference to conditions in Europe and
New Zealand. *European Journal of Soil Science* **6**, 35-50.

Falls Creek Board of Management (2013) Average snow depths from 2000 to 2013.

Fussell MS. (2014) A survey of groundwater flow Heathy Spur Bogong High Plains
(unpublished)

Gamlen PH, Lane BC, Midgely PM, Steed JM. (1986) The production and release to
the atmosphere of CCl3F and CCl2F2 (Chlorofluorocarbons CFC 11 and CFC
12). *Atmospheric Environment* **20 (6):** 1077-1085.

Jasechko S, Birks SJ, Gleeson T, Wada Y, Fawcett PJ, Sharp ZD, McDonell JJ, Welker
JM. (2014) The pronounced seasonality of global groundwater recharge. *Water
Resources Research* **50 (11):** 8845-8867.

Karis T, Silvester E, Rees G. (2016) Chemical regulation of alpine headwater streams during a storm event (Bogong High Plains, Victoria, Australia) *Journal of Hydrology* **542:** 317-329.

Labasque T, Aquilina L, Vergnaud V, Hochreutenue R, Barbecot F, Casile G (2014) Inter-comparison exercises on dissolved gases for groundwater dating – (1) Goals of the exercise and site choice, validation of the sampling strategy. *Applied Geochemistry* **40:** 119-125.

McCartney VA, Silvester E, Morgan JW, Suter PJ. (2013) Physical and chemical drivers of vegetation in groundwater-source pools on the Bogong High Plains, Victoria. *Australian Journal of Botany* **61,** 566-573.

Midgley PM, Fisher DA. (1993) The production and release to the atmosphere of chlorodifluoromethane (HCFC 22). *Atmospheric Environment* **27A (14):** 2215-2223.

Morand VJ. (1990) Low-pressure regional metamorphism in the Omeo Metamorphic Complex, Victoria, Australia*. Journal of Metamorphic Geology* **8**, 1-12.

Morand VJ, Simons BA, Taylor DH, Cayley RA, Maher S, Wohlt KE, Radojkovic AM. (2005) Geological Survey of Victoria Report 125. *Geoscience Victoria*, Victorian Government Department of Primary Industries.

Oster H, Sonntag C, Munnich KO. (1996) Groundwater age dating with chlorofluorocarbons. *Water Resources Research*. **32 (10),** 2989-3001.

Plummer LN, Busenberg E. (2000) Chlorofluorocarbons, in Environmental Tracers in Subsurface Hydrology, ed. P. Cook and A. Herczeg, chap. 15, pp 441-478, Springer, New York.

Shannon JM, Morgan JW. (2007) Floristic variation in *Sphagnum*-dominated peatland communities of the Central Highlands, Victoria. *Cunninghamia* **10,** 59-76.

Taylor DH, Morand VJ, Cayley RA, Wohlt KE, Simons BA, Meagher S. (2004) "Falls Creek 1:50 000 geological map." (Geological Survey of Victoria: Melbourne)

Troth J. (1963) A theoretical analysis of groundwater flow in small drainage basins. *Journal of Geophysical Research* **68, No 16**, 4795-4812.

Wahren C-HA, Williams RJ, Papst WA. (1999) Alpine and subalpine wetland vegetation on the Bogong High Plains, south-eastern Australia. *Australian Journal of Botany* **47,** 165-188.

Walker SJ, Weiss RF, Salamuch PK. (2000) Reconstructed histories of the annual mean atmospheric mole fractions for the halocarbons CFC-11, CFC-12, CFC-113, and carbon tetrachloride. *Journal of Geophysical Research* **105:14**, 285-296.

Western A, Siriwardena L, Lawrence R, Rutherford I. (2008) Sponges or wicks? What is the role of bogs in hydrological response on the Bogong High Plains. Conference Paper. Proceedings of Water Down Under 2008, 2008, p.1060-1071 (ISBN: 0858257351).

Whinam J, Chilcott NM, Morgan JW. (2003) Floristic composition and environmental relationships of *Sphagnum*-dominated communities in Victoria. *Cunninghamia* **8,** 162-174.

Williams R.J. (1987) Patterns of air temperature and accumulation of snow in subalpine heathlands and grasslands on the Bogong High Plains, Victoria. *Australian Journal of Ecology* **12,** 153-163.

S5.1 Supplementary Material

Table S5.1. Chlorofluorocarbon concentrations from five groundwater sources on Watchbed Creek that were collected over a two-year time frame.

Site	Collection Date	CFC-11 (pptv)	CFC-12 (pptv)
WBGW1	1 Aug 2011	223	466
	19 Dec 2011	169	303
	28 Mar 2012	168	303
	30 Jul 2012	232	483
	8 Oct 2012	276	551
	17 Jan 2013	235	529
	26 Mar 2013	227	487
	4 Jul 2013	262	421
	16 Oct 2013	178	246
	16 Dec 2013	124	271
WBGW2	1 Aug 2011		
	19 Dec 2011	189	310
	28 Mar 2012	189	310
	30 Jul 2012	217	473
	8 Oct 2012		
	17 Jan 2013	232	503
	26 Mar 2013	212	509
	4 Jul 2013	244	494
	16 Oct 2013	163	245
	16 Dec 2013	137	290

Site	Collection Date	pptv CFC-11	pptv CFC-12
WBGW7	1 Aug 2011		
	19 Dec 2011	134	218
	28 Mar 2012	169	264
	30 Jul 2012	242	495
WBGW7	8 Oct 2012	241	485
	17 Jan 2013	202	428
	26 Mar 2013	234	509
	4 Jul 2013	181	357
	16 Oct 2013	126	213
	16 Dec 2013	125	288
WBGW9	1 Aug 2011		
	19 Dec 2011	189	297
	28 Mar 2012	189	279
	30 Jul 2012	232	479
	8 Oct 2012	212	454
	17 Jan 2013		
	26 Mar 2013	189	297
	4 Jul 2013		
	16 Oct 2013	170	258
	16 Dec 2013	128	278

Site	Collection Date	pptv CFC-11	pptv CFC-12
WBGW11	1 Aug 2011		
	19 Dec 2011	208	327
	28 Mar 2012	208	327
	30 Jul 2012	217	473
	8 Oct 2012	239	484
	17 Jan 2013	216	484
	26 Mar 2013	174	414
	4 Jul 2013	203	364
	16 Oct 2013	186	298
	16 Dec 2013	125	284

Chapter 6

Concluding discussion and directions for further research

The Bogong High Plains, Victoria has groundwater sources and groundwater source pools that, until this study, were largely unknown. The main aims of my research were to locate the groundwater source pools and then to describe them to gain a basic understanding of the functioning of the dependent ecosystems (groundwater dependent ecosystems; GDEs). Three water catchments were surveyed, and I described the associated vegetation surrounding and within the GDEs. Within the GDEs, the water chemistry from the groundwater sources and within the groundwater source pools was examined to determine concentrations of key nutrients supporting the vegetation and the impact of the source pools on these nutrients.

Chapter 2 examined the distribution and composition of GDEs. The GDEs were located and mapped, with 22 permanent GDEs found. GDEs all supported wetland vegetation but, importantly, were essential habitat for the aquatic moss *Blindia robusta.* *Blindia robusta* is considered to be a nearly pan-south-temperate species in the Australasian area, including the subantarctic Macquarie Island (Seppelt, 2004), and the Auckland and Campbell Islands (Vitt, 1974; Vitt 1979). It has also been recorded from subantarctic South Georgia (Ochyra *et al.* 2002) and Tristan da Cunha, on three islands in the South Atlantic Ocean in the southern cool-temperate zone (Bednarek-Ochyra, 2014). On the Bogong High Plains, this species was not found in the stream systems but was entirely dependent upon groundwater. Here, GDEs formed as pools of permanent water with free-floating mats of the aquatic moss *Blindia robusta*. The combination of high concentrations of CO_2, a near constant temperature of 6 $^{\circ}$C, and a sustained water flow are thought to be key drivers for the high abundance of *Blindia robusta*.

Chapter 3 described the growth of submerged 'mossball'-like structures of *Blindia robusta* present on pebbles and rocks on the bottom of groundwater source pools. It was observed that these structures eventually separate from the pebbles and rocks in an undetermined timeframe and float to the surface where they all eventually join and form a large mat. It was observed that a "bubbly mat" of *Blindia robusta* can take two years to form a smooth surface, free-floating mat.

The plant resources in *Blindia robusta* are concentrated in the upper most part of the plant which is exposed to light, as described in Chapter 3. This is clear from the Fourier-transform infrared (FTIR) images obtained. *Blindia robusta* has a high concentration of carbohydrates at the very top of the plant where most of the photosynthesis is likely taking place; however, it was not possible to distinguish between metabolic (soluble, storage) carbohydrates and the structural ones used in cell walls. This is also where new leaves are being formed; hence, it seems likely that the resources are supporting this function (Proctor, 1990; Waite and Sack, 2010).

The reliance of *Blindia robusta* on a permanent groundwater source, as identified in Chapter 2, was examined in more detail in Chapter 4. With climate change a real threat to the survival of *Blindia robusta*, it was decided to test *Blindia robusta* to see how this aquatic moss species would tolerate desiccation. The desiccation experiment confirmed that *Blindia robusta* is very sensitive to drying out. Leaves exposed to 5 ℃ for ~19 minutes were no longer photosynthetically active. Increasing temperature caused a more rapid decline in photosynthetic activity. At 30 ℃, *Blindia* plants were photosynthetically inactive within 11 minutes. In experiments designed to investigate whether *Blindia robusta* could recover if dehydrated and then rehydrated, it was found that leaves that were desiccated for 10 minutes at 5 ℃, then rehydrated at 5 ℃, appeared to recover to viable F_v/F_m efficient photochemical rates within 60 minutes. Conversely, samples that were desiccated at 5 ℃ for 50 minutes, then rehydrated for 60

minutes at 5 ℃, did not recover efficient photochemical rates. This indicated that *Blindia robusta* cannot recover to efficient photosynthetic activity from the damage sustained by longer exposure to dehydration. By comparison, other wetland mosses (e.g. *Sphagnum* spp.) have been reported to sometimes take days to desiccate and subsequently die (Liepina and Ievinsh, 2013; Hájek and Vicherova, 2014).

A comparison of cell wall composition using FTIR microspectroscopy showed that the cell wall composition of *Blindia robusta* and *Sphagnum cristatum* differ strongly. The crosslinked polymerised phenolic molecules in *Blindia robusta* were almost non-existent when compared to *Sphagnum cristatum*. While *Sphagnum* uses capillary action to maintain moisture, *Blindia robusta* is entirely dependent on continuous moisture/water provided by groundwater for its survival and cannot survive dehydration, in part explaining its' observed distribution in the landscape as found in Chapter 2.

The relationship or close association of *Blindia robusta* with the groundwater sources was documented (Chapter 2 and Chapter 4). The predicted effects of climate change in the alpine area will affect *Blindia robusta*, with this species shown to be sensitive to desiccation (Chapter 4). The age of the groundwater feeding into these GDEs helps to understand the permanency of these systems and the timescales over which they will respond to changing recharge. Projected decreased snowpack, together with lower than average rainfall in the Australian Alps (Hennessy *et al.* 2008; Pickering, 2011; Mitchell *et al.* 2015), will likely result in less groundwater recharge and affect the permanency of these groundwater sources. Chapter 5 investigated the age of the groundwater in the Watchbed Creek catchment to determine the vulnerability of these water sources to changing recharge and, in particular, the time scales over which changes in recharge may manifest as changes in groundwater flow. Samples were collected seasonally over a two-year period and then analysed for CFC-11

(trichlorodifluoromethane, $CFCl_3$) and CFC-12 (dichlorodifluoromethane, CF_2Cl_2) to determine the average groundwater age. Apparent groundwater ages ranged from 10-38 years with some discernible inter-annual patterns. These results suggest that groundwater flows are likely to respond to decreasing recharge on a decadal timescale and ultimately become ephemeral. A shift from groundwater permanency to ephemeral flow will very likely impact on the viability of *Blindia robusta* (based on Chapter 2 data that showed *Blindia robusta* was absent from ephemeral pools).

6.2   Future perspectives – challenges to be faced now and in the future

6.2.1   Climate change implications – alpine systems on the Bogong High Plains

This study suggests that global change drivers such as climate change, drought, fire and invasive species have the potential to impact GDEs. Climate change is threatening high mountain environments with predicted rising temperatures resulting in reduced snowpack with altered spring/summer meltwater times (Green and Pickering, 2009).

Globally, ecosystems in alpine areas are subject to severe climatic conditions with short growing seasons (Costin *et al.* 2000; Nagy *et al.* 2003).   Changes in annual snow cover patterns are likely to have broad scale impacts on alpine vegetation. The establishment, growth, reproduction and phenology of plants are directly influenced by snow cover (Körner, 1999; Arft *et al*. 1999). The length of the growing season of plants is determined by snow distribution, along with protection against extreme cold, strong winds and drought (Schaminée, 1992; Körner, 1999). While the snow cover affects the growing season, temperature affects species distribution and abundance (Körner, 1999).

Climate change scenarios lead to questions about the long-term persistence of *Blindia robusta*. Predicted changes in the precipitation regime under climate change scenarios in the Australian Alps suggest a receding snowpack with more unpredictable (and high intensity) rain events (Green and Pickering, 2009). From the work in thesis, we have a greater understanding of how rain and snow recharge aquifers on the Bogong High Plains. If, as suggested in this thesis, snow melt is a major form of recharge for the groundwater sources, a receding snowpack will likely result in groundwater sources that are currently permanent becoming ephemeral (as shown in Chapter 5). If the groundwater sources do become ephemeral, *Blindia robusta* is unlikely to persist at its current locations.

The trajectory of climate change in south-eastern Australia suggests that there will be an increase in fire frequency in the alpine areas and elsewhere (Williams *et al*. 2009; Morrison and Pickering, 2013). The change in fire frequency may initially promote *Blindia robusta* establish in groundwater source pools that have previously been covered in shrubs (where low light may limit moss survival). This scenario would allow *Blindia robusta* to dominate the groundwater source pools until other plants could then dominate this habitat, e.g. *Richea continentis* and *Empodisma minus*. Both these shrubs have been observed on the BHP to use *Blindia robusta* as an organic layer to grow upon.

Any changes in groundwater hydrology will likely affect distribution and persistence of habitats such as fens, bogs and peatlands and their dependent species (Hughes, 2011). Water quality will be affected by changes in flows to wetlands which will then impact on nutrient concentrations, sediment transport, as well as O_2 and CO_2 concentration (Hughes, 2011). The groundwater dependent ecosystems described in this thesis help to condition the chemical composition of the groundwater once it is

exposed to the atmosphere before the water reaches the peatland areas (Clements *et al.* 2016). Under climate change scenarios, it is not clear if this groundwater will in fact be able to reach the peatlands, changing these permanent flow systems into ephemeral systems. Long-term monitoring, ecophysiological and phenological studies of alpine fens, bogs and peatlands (with their dependent species) needs to be a priority. Further research is critical to understand these systems and the likely impacts that will occur from climate change, drought and increased fire regimes.

6.2.3   Climate change implications – outlook for *Blindia robusta*

This study has found that the GDEs on the Bogong High Plains, Victoria are at risk of drying out due to climate change. This work has shown that these GDEs are much more widely distributed than previously recognised. They therefore need a management plan given their likely susceptibility to climate change.   These GDEs also need to have long-term monitoring in relation to water permanence and vegetation present to understand how they are proceeding in response to climate change. It is not known if similar GDEs exist outside the study area on the Bogong Plains, or indeed any other sub-alpine regions of the Australian Alps. A comprehensive wide-ranging survey to examine whether the GDEs has a wider geographic distribution needs to be completed. This needs to happen as soon as possible before major changes occur.

The long-term permanence of the GDEs found in this study is at risk with a receding snowpack and change in precipitation patterns.   It is suggested in Chapter 5 that snowmelt is the main driver behind these systems, indicating that they will not be permanent in the foreseeable future. Understanding how these alpine groundwater sources are responding to changes in precipitation patterns will be of critical importance in future management of these ecosystems in the Australian Alps.

It was found that the groundwater source pools contained elevated levels of nitrate which may be due to local wildfires in 2003 and 2006-2007. The occurrence of *Blindia robusta* in groundwater source pools may be a response to elevated levels of nitrate from fire in the broader landscape. Monitoring of the groundwater source pools for water quality and the vegetation present needs to be conducted over a long period of time to understand how populations of *Blindia robusta* respond to fire. This idea could be further explored in laboratory experiments to investigate the growth response of *Blindia robusta* to differing nitrate levels.

Field observations of mossball growth forms of young gametophytes of *Blindia robusta*, resulting in free floating flat mats, has raised questions that need further investigation. This species may be restricted in how much water is available in the future and the availability of pool environments. The time period *Blindia robusta* needs to be submerged before forming free floating mats needs to be explored and could be answered by monitoring this species in the field and conducting laboratory experiments manipulating its growth patterns.

The fate of *Blindia robusta* in relation to climate change and dispersal is largely unknown. This thesis has not investigated the dispersal capabilities of spores or recolonization mechanisms of spores of *Blindia robusta*. When these systems dry up, it is not known how the recolonization of *Blindia robusta* spores will proceed with the return of water, or the period of time over which this occurs. Translocation of *Blindia robusta* into source pools elsewhere needs to be conducted to understand colonisation dynamics. With these systems at high risk, further experimentation on dispersal mechanisms is critical to long term persistence of this species in the Australian Alps.

The GDEs surveyed on the Bogong High Plains form part of larger peatland systems and, for this reason, have not been recognised previously as distinct ecosystems. It is vitally important that these systems have specific legislation and

management actions put in place to protect them. One such action that could assist these systems is fencing erected around GDEs to stop damage from feral animals where such damage is occurring (or likely to occur). How *Blindia robusta* withstands disturbances such as trampling from feral animals could also be explained by using this approach. Another measure would be to inform the general public of these ecosystems through education programs.   Signs at the start of the alpine walks informing people of the significance of these GDEs would be a useful starting point. The increase in large fires and the shorter intervals between fires in the alpine area suggests that we need measures put in place to protect more vulnerable areas. In the case of wildfires, a targeted defence of fire-sensitive communities could be implemented as part of an action plan for national parks. The damage to GDEs could then be minimised. The best outcome for GDEs on the Bogong High Plains would be for global action on climate change. In the absence of significant change in climate trajectory, it is likely that we will be monitoring these systems as they decline in condition.

6.3   References

Arft AM, Walker MD, Gurevitch J, Alatalo JM, Bret-Harte MS, Dale M, Diemer D, Gugerli F, Henry GHR, Jones MH, Hollister RD, Jónsdóttir IS, Laine K, Lévesque E, Marion GM, Molau U, Mølgaard P, Nordenhäll U, Raszhivin V, Robinson CH, Starr G, Stenström A, Stenström M, Totland Ø, Turner PL, Walker LJ, Webber PJ, Welker JM, Wookey PA (1999) Responses of tundra plants to experimental warming: Meta-analysis of the International Tundra Experiment. *Ecological Monographs* **69(4)**, 491-511.

Bednarek-Ochyra H (2014) A major range extension of *Blindia robusta* (Seligeriaceae, Bryophyta) based on the type *Ditrichium tenuinerve*. Telopea, *Journal of Plant Systematics* **17**, 323-328.

Clements AR, Suter PJ, Fussell M, Silvester E (2016) Macroinvertebrate communities in spring-fed alpine source pools. *Hydrobiologia* **777**:119–138

Costin AB, Gray M, Totterdell C, Wimbush D (2000) Kosciusko alpine flora (CSIRO Publishing: Collinwood, Victoria)

Green K, Pickering CM (2009) The decline of snowpatches in the Snowy Mountains of Australia: Importance of climate warming, variable snow and wind. *Arctic, Antarctic and Alpine Research* **41(2),** 212-218.

Hájek T, Vicherová E (2014) Desiccation tolerance of *Sphagnum* revisited: a puzzle resolved. *Plant Biology* **16,** 765-773.

Hennessy KJ, Whetton PH, Walsh K, Smith IN, Bathols JM, Hutchinson M, Sharples J (2008) Climate change effects on snow conditions in mainland Australia and adaptation at ski resorts through snowmaking. *Climate Resources* **35(3)**, 255-270.

Hughes L (2011) Climate change and Australia: key vulnerable regions. *Environmental Change* **11(1):** S189-S195.

Körner C (1999) Alpine plant life. Functional Plant Ecology of High Mountain Ecosystems. (Springer: Berlin)

Leipina L, Ievinsh G (2013) Potential for fast chlorophyll a fluorescence measurement in bryophyte ecophysiology. *Estonian Journal of Ecology* **62(2),** 137-149.

Mitchell M, Lockwood M, Moore S, Clement S (2015) Scenario analysis for biodiversity conservation: A social-ecological system approach in the Australian Alps. *Journal of Environmental Management* **150**, 69-80.

Morrison C, Pickering CM (2013) Perceptions of climate change impacts, adaptation and limits to adaptation in the Australian Alps: the ski-tourism industry and key stakeholders. *Journal of Sustainable Tourism* **21(2),** 173-191.

Nagy L, Grabherr G. Korner C, Thompson DBA (Eds.) 2003: Alpine Biodiversity in Europe. (Springer: Berlin)

Ochyra R, Bednarek-Ochyra H, Lewis-Smith RI (2002) New and rare moss species from subantarctic South Georgia. *Nova Hedwigia* **74**, 121-147.

Pickering C (2011) Changes in demand for tourism with climate change: a case study of visitation patterns to six ski resorts in Australia. *Journal of Sustainable Tourism* **19(6)**, 767-781.

Proctor MCF (1990) The physiological basis of bryophyte production. *Botanical Journal of the Linnean Society* **104,** 61-77.

Schaminée JHJ (1992) The vegetation of snow-beds in the Monts du Forez (Massif Central, France). *Phytocoenologia* **21**, 175-206.

Seppelt RD (2004) The moss flora of Macquarie Island. (Kingston, Tasmania, Australian Antarctic Division).

Vitt DH (1974) A key and synopsis of the mosses of Campbell Island, New Zealand. *New Zealand Journal of Botany* **12**, 185-210.

Vitt DH (1979) The moss flora of the Auckland Islands, New Zealand, with a consideration of habitats, origins, and adaptations. *Canadian Journal of Botany* **57**, 2226-2263.

Waite M, Sack L (2010) How does moss photosynthesis relate to leaf and canopy structure? Trait relationships for 10 Hawaiian species of contrasting light habitats. *New Phytologist* **185,** 156-172.

Williams RJ, Bradstock RA, Cary GJ, Enright NJ, Gill AM, Leidloff AC, Lucas C, Whelan RJ, Andersen AN, Bowman DJMS, Clarke PJ, Cook GD, Hennessy KJ, York A (2009) Interaction between climate change, fire regimes and biodiversity in Australia. A preliminary report. Australian Government CSIRO Department of Climate Change, Department of the Environment, Water, Heritage and the Arts.