Climatic specialisation: an explanation for the range size and distribution of bryophytes on a tropical island elevational gradient

By

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Contents

Abstract

Are species physiologically specialised to the specific climatic conditions in their habitats and can this explain their distributions? This thesis addresses this question using a very specific system: bryophytes on the elevational gradient of Piton des Neiges, Réunion Island. Bryophytes are expected to be specialised to fairly narrow conditions of drought, temperature and insolation, which restrict their geographic range. However, little is known about the mechanisms that connect bryophytes with climatic factors. In this thesis I test the idea of ecophysiological specialisation using reciprocal transplant experiments, along with direct laboratory measurements of species' responses to desiccation and temperature. In these experiments I use species restricted to low, mid or high elevation, as well as widespread species found along most of the gradient. The transplant experiment revealed a trend of upslope survival of restricted species, with species from all sites performing best at their elevation of origin and the site above, and badly at lower elevations. Despite macroclimate being found as an important factor shaping bryophyte range size and distribution, the effect of microhabitat could not be ignored. This was especially true for the widespread species, which showed a strong effect of microhabitat placement in the transplant experiment. Desiccation tolerance was found to increase with elevation in range-restricted species, but widespread species showed little difference in their sensitivity to desiccation, regardless of elevation of origin. Range-restricted species from low elevation were more sensitive to low temperatures and had higher optimum temperatures for photosynthesis than mid- and high-elevation species. Widespread species had narrower ranges of temperature tolerances than range-restricted species, and did not differ in their response to temperature, regardless of elevation of origin. The results of these experiments corresponded well with the climatic conditions that these species habitually experience – with extremes at the gradient peripheries and intermediate conditions in between. This thesis showed that specialisation to both macro- and microclimatic conditions can be attributed as a main driver of bryophyte range size and distribution on the elevational gradient of Piton des Neiges, Réunion Island. This research adds to the body of knowledge on the physiological responses of tropical bryophytes, which is important for species' distribution modelling. Furthermore, it provides insight into the factors that shape bryophyte distribution, critical for biodiversity management under climate change scenarios.

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CHAPTER 1

General Introduction and Thesis Structure

General Introduction

Background

The question of why some species are widely distributed and others range-restricted has long fascinated ecologists and biogeographers (Calosi et al., 2008). However, there is far less literature focusing on individual species' range sizes and distributions than on the related topic of species' richness distributions (Gaston, 1996). Despite a recent increase in interest and data availability, the factors affecting range size and distribution of species remain poorly understood. There is an increasing necessity to understand these factors in order to predict how individual species are likely to respond to climate change (Calosi et al., 2008). However, little is known about the mechanisms that connect plants with climatic factors (Woodward, 1987). Elevational gradients are indispensable in the study of species' responses to climate, as there is a large variation in climate within short geographic distances (MacArthur & Wilson, 1967). Variations in the physiological response of different species to the changing climatic conditions with elevation may explain differences in their distribution along elevational gradients (e.g. Wagner et al., 2013; Wagner et al., 2014a; Stam et al., 2017). In this thesis I investigate variation in responses of tropical bryophytes to various features of climate and whether it is correlated with variation in their elevational distributions. I explore the factors affecting species' range sizes and distributions using a very specific system: bryophytes on the elevational gradient of Piton des Neiges, Réunion Island.

Species richness patterns

Species distributions along latitudinal and elevational gradients are well studied and remain a topic of great interest (Lomolino, 2001). The pattern of species richness along latitudinal gradients is generally one where richness decreases monotonically towards the poles. The pattern of species richness along elevational gradients was long thought to mirror this, because both gradients span a transition from warm to cold climatic conditions (Rahbek, 1995). However, Rahbek (1995; 2005) showed that this view was the result of an overemphasis on a few studies. In fact, three main patterns have emerged for different taxa in different areas: a uniform decline in richness with elevation (e.g. dwarf shrubs in Northernmost Fennoscandia; Bruun et al., 2006), a uniform increase in richness with elevation (e.g. bacteria in Yunnan province, China; Wang et al., 2010), and a unimodal pattern where richness peaks at an intermediate position on the gradient (e.g. bryophytes on Réunion Island; Ah-Peng et al., 2012). This unimodal pattern is by far the most commonly reported, characterising a very wide range of organisms and localities (e.g. Nor, 2001; Vetaas & Grytnes, 2002; Bhattarai & Vetaas, 2003; Bhattarai et al., 2004; Rahbek, 2005; Grytnes & Beaman, 2006; Grau et al., 2007). Studies have often linked this unimodal pattern to climate (e.g. Gradstein & Pócs, 1989; Wolf, 1993; Acebey et al., 2003; Grau et al., 2007), and elevational gradients are a useful context in which to study the relationship between species distribution and climate, as there is a large variation in climate over small geographical distances (MacArthur & Wilson, 1967).

Factors influencing plant distribution

Although distribution patterns have often been strongly linked to climate (e.g. Hocker, 1956; Woodward, 1987; Gaston, 2003; Sexton et al., 2009), there are, in fact, four main classes of factors that are thought to influence the geographic distribution of plant species: i) abiotic factors (e.g. climate, soil, altitude), ii) biotic interactions (e.g. competition, predation, pollination), iii) dispersal ability (e.g. barriers to dispersal and the actual dispersal ability of the organism), and iv) the adaptive capacity of the organism (Soberón & Peterson, 2005). The interactions between these factors are perhaps most important in determining species' distributions and affect organisms differently at different scales (Soberón & Peterson, 2005). Among these, however, it is climate that is repeatedly highlighted as having a strong influence on the distribution of plant species (Hocker, 1956; Woodward, 1987; Gaston, 2003; Sexton et al., 2009). First, this is because species' range limits often coincide with specific climatic conditions and, second, because species' distributions often shift with climatic changes (Gaston, 2003).

For bryophytes, climate may be an even greater determinant of species distributions. Current knowledge suggests that biotic interactions may be nearly non-existent or negligible in bryophytes (Richards, 1984; Grime et al., 1990; Slack, 1990; Gignac, 1992) and their tiny, wind-dispersed spores remove many of the constraints on dispersal faced by seed plants (He et al., 2016). Furthermore, their poikilohydric nature means they are closely linked to local climatic conditions, and there is evidence to suggest that microhabitat plays an important role in bryophyte distribution, sometimes transcending large-scale climatic conditions (Forman, 1964; Hedderson & Brassard, 1990; Cleavitt, 2004). Bryophytes' small size allows them to occupy favourable microsites surrounded by otherwise hostile conditions. Nevertheless, within microhabitats, it is microclimate that is emphasised as having the largest effect on species distributions (Hedderson & Brassard, 1990).

The ability to tolerate desiccation influences the habitats in which plant species can survive (Proctor et al., 2007). Relatively few tracheophyte species can tolerate desiccation, but this is a common trait in bryophytes (Oliver at al., 2000). Desiccation tolerance is connected to withstanding other extreme environmental conditions such as high temperature and light. Some desiccation-tolerant bryophytes have been shown to tolerate temperatures of up to 100 °C (Glime & Carr 1974; Nörr, 1974), and extreme heat tolerance is often greater when plants are desiccated (Clausen, 1964; Meyer & Santarius, 1998). Other bryophyte species have shown the ability to endure very cold conditions, possibly due to the fact that the most highly damaging aspect of freezing is cell desiccation (Clausen, 1964; Alpert, 2000). In addition, species that can tolerate drying seem to tolerate UV-B radiation better than those that can't (Takács et al., 1999). These factors allow some bryophytes to inhabit areas that are unavailable to most vascular plants e.g. young lava flows (Ah-Peng et al., 2007).

Widespread and range-restricted species on elevational gradients

The factors affecting species' distributions also regulate their range sizes. Most taxa tend to be range-restricted, while few are widespread (Gaston, 1998; Gaston, 2003). This holds true for my group of interest: bryophytes on the elevational gradient of Piton des Neiges, Réunion Island (Ah-Peng et al., 2012). While there are a few widespread species that occur along most of the gradient, most species show a high degree of range-restriction and occur within smaller ranges, at low, mid or high elevation. The high proportion of range-restricted bryophytes at mid-elevation is largely responsible for the richness peak seen there (Ah-Peng et al., 2012).

The Climate Variability Hypothesis (CVH) predicts that species with larger range sizes also have larger climatic tolerances (Stevens, 1989; Stevens, 1992). Similarly, Brown (1984) hypothesised that the breadth of a species' environmental tolerance (fundamental niche breadth) determines its geographic range. Therefore, widespread species are expected to be more physiologically plastic than range-restricted species (Pohlman et al., 2005; Calosi et al., 2007; Calosi et al., 2010; see Bozinovic et al., 2011). Thus, there should be an identifiable difference between the physiological responses of widespread and range-restricted species (Brattstrom, 1968; Stanley & Parsons, 1984).

This seems intuitively attractive, but there have been few studies directly testing these hypotheses, especially for plant species. Furthermore, most studies have focused on latitudinal gradients, usually comparing single restricted and widespread species that are often unrelated (Gaston & Chown, 1999; Thompson et al., 1999; Gaston & Spicer, 2001; Calosi et al., 2008). In order to get a better understanding of the influence of ecophysiology on species' range sizes and distributions it is important for studies to compare greater numbers of closely related species. Furthermore, there is a need for knowledge on whether individuals have the capacity to adapt to new conditions (Bozinovic et al., 2011), as it is difficult to ascertain whether physiological tolerances are the cause of species' distributions or whether they are simply a result of local adaptation (Gaston, 2003). These data are of particular urgency if we are to understand the possible effects of anthropogenic climate change on ranges of widespread and restricted species (Gaston, 2003).

Implications for climate change mitigation

An objective of this study is to offer insight into the drivers of bryophyte distribution, which will improve our understanding of what shapes bryophyte range sizes. Little is known about the physiology of tropical bryophytes (Wagner et al., 2014b) and how this is affected by climate. Understanding the effect climate has on bryophyte distribution on isolated tropical islands has important implications for mitigation in an area vulnerable to island effects and mountaintop extinctions caused by climate change (Kazakis et al., 2007; Kelly & Goulden, 2008; Lenoir et al., 2008; Petit, 2008; Engler et al., 2009; Pouteau et al., 2018). It is important to include information on bryophytes' physiological tolerances and acclimatisation ability in distribution modelling in order to get an accurate picture of how distributions will change in a changing climate (Chown et al., 2004; Helmuth et al., 2005). Biodiversity management will benefit from knowledge of how bryophytes may respond to predicted changes in climate (Bader et al., 2013), which is especially important for range-restricted species in these systems. This information is not only essential to bryophytes, but to all the species that depend on them. Bryophytes can be used as early indicators of climate change due to their environmental sensitivity and this can be used as an early-warning system for other species (Gignac, 2011).

Study area

Réunion Island (21°00′S; 55°39′E) is part of the Mascarene Archipelago, which also includes Mauritius and Rodrigues, in the Western Indian Ocean. Réunion was largely formed by the dormant Piton des Neiges volcano (3 069 m), and has an area of 2 512 km^2, making it the largest and highest of the Mascarene Islands (Ah-Peng et al., 2014). The island has a tropical climate, with a summer rainfall period from November to April, and a cooler, drier period from June to September. Moist trade winds that act on the eastern side of Réunion provide the island with most of its rainfall. This eastern side has a higher mean annual precipitation, ranging from 1 500 mm yr^{-1} to over 8 000 mm yr^{-1} at higher altitudes. At 2 000 m a.s.l., there is a temperature inversion that causes the humid oceanic air to form clouds mainly between 1 400 m a.s.l. and 1 600 m a.s.l., where there is around 12 000 mm of precipitation per year (Barcelo, 1996). The mean annual temperature ranges from 24 °C at sea level to 12 °C at about 2 000 m a.s.l. (Barcelo, 1996; Ah-Peng et al., 2012). There is a temperature decrease of 0.7 - 0.8 °C every 100 m, resulting in a steep temperature gradient with altitude (Barcelo, 1996; Ah-Peng et al., 2014).

Réunion Island has remarkably well-preserved biodiversity (Strasberg et al., 2005; Ah-Peng et al., 2007) and is part of one of 35 global biodiversity hotspots (Mittermeier et al., 2005). The island is home to 831 bryophyte species – 504 mosses, 322 liverworts, and 5 hornwort species (Ah-Peng et al., 2014), and has the richest bryophyte flora in the Mascarene Archipelago. The topography of the island makes urbanisation and agriculture unviable on a large proportion of the land, thus 32 % of Réunion’s indigenous vegetation is well conserved (Ah-Peng et al., 2007). Since 2007, 42 % of the island has been under national park protection (Ah-Peng et al., 2014).

Study sites

The Piton des Neiges gradient is the longest bioclimatic gradient in the Western Indian Ocean islands, ranging from 0 m a.s.l. to 3 069 m a.s.l. (Wilding et al., 2012). For this study the gradient was divided into three elevational bands: low elevation (200 - 800 m a.s.l.), mid elevation (800 - 1 700 m a.s.l.) and high elevation (1 700 - 2 200 m a.s.l.). The sites fell within three distinct vegetation types: tropical lowland forest at low elevation (0 - 800 m a.s.l.), tropical montane cloud forest at mid elevation (800 - 1 900 m a.s.l.), and alpine shrubland at high elevation (1 900 - 3 000 m a.s.l.) (Wilding et al., 2012).

The species sampled in this study were grouped into six main categories depending on the range and elevation at which they are found on the Piton des Neiges gradient: range-restricted species from low-, mid- and high-elevation sites, and widespread from low-, mid- and high-elevation sites.

Chlorophyll fluorescence

In the following three chapters I make use of chlorophyll fluorescence as a technique for quantifying photosynthetic response to manipulations in climatic variables. Chlorophyll fluorescence is a key technique in plant ecophysiological studies and has become widely used for a range of measurements as it is quick and non-invasive (e.g. Csintalan et al., 1999; Proctor, 2003; García et al., 2016). Chlorophyll fluorescence has proved particularly useful in bryophyte studies as measurements are not dependent on the quantity of material, so small size is not an issue (Proctor & Bates, 2018). Chlorophyll fluorescence has shown particular utility in bryophyte desiccation studies (e.g. Csintalan et al., 1999; Proctor, 2001; Proctor, 2003; Bader et al., 2013) but has also shown value when measuring responses to other environmental variables e.g. temperature (e.g. Meyer & Santarius, 1998; Jägerbrand et al., 2014; Jägerbrand & Kudo, 2016; Taylor et al., 2017) and light (e.g. Tobias & Niinemets, 2010; Proctor & Bates, 2018).

The chlorophyll fluorescence technique is based on the principle that light energy absorbed by chlorophyll molecules can undergo one of three processes: i) drive photosynthesis (photochemistry), ii) be dissipated as heat, or iii) be re-emitted as light of a longer wavelength (chlorophyll fluorescence). These processes work in competition such that an increase in one results in a decrease in the others. Therefore, by controlling illumination while measuring the yield of chlorophyll fluorescence it is possible to distinguish between these processes to gain information on the photosynthetic efficiency of the plant (for review see Maxwell & Johnson, 2000).

Understanding the state of the reaction centres of photosystem II (PSII) is important for understanding chlorophyll fluorescence analysis. If all reaction centres are closed, more light energy will be re-emitted (chlorophyll fluorescence), whereas if a large proportion are open and more light energy is used in photochemistry, less will be re-emitted. External conditions (e.g. temperature) affect the metabolic state of the plant, determining what proportion of reaction centres is closed. Closure of reaction centres leads to a decline in the quantum efficiency of PSII (Murchie & Lawson, 2013). When a plant is moved from dark to light, for the first second of illumination there is a peak in fluorescence yield due to a reduction of electron acceptors downstream of PSII that cannot accept another electron until the first one is passed on. During this time the reaction centres are closed. This is known as the Kautsky effect (Maxwell & Johnson, 2000). After a short time, the fluorescence yield starts to decline. This is called fluorescence quenching and results from two processes: i) an increase in the rate of electron transport away from PSII (photochemical quenching) and ii) an increase in heat dissipation (non-photochemical quenching) (Maxwell & Johnson, 2000).

The two most useful parameters providing key insight into the functioning of PSII are the maximum quantum yield of PSII (F_v/F_m), which gives an indication of plant stress, survival and recovery, and quantum yield of PSII (Φ_{PSII}), which gives an indication of the actual photosynthesis at any given moment.

In practice, these parameters are measured by comparing fluorescence in the dark, under ambient light, and under saturating light. In order to isolate the proportion of light energy used in photochemistry, it is necessary to get a value of chlorophyll fluorescence in the presence of one or other quenching parameter. This is done by “switching off” photochemistry by flashing a high-intensity, short-duration light that closes all PSII reaction centres, reducing photochemical quenching to zero. As long as the duration of the flash is short enough, there is no increase in non-photochemical quenching. By comparing this value to the steady state value of fluorescence in the light and the yield of fluorescence in the absence of photosynthetic light, we can get an estimation of photochemical quenching and the efficiency of PSII (Maxwell & Johnson 2000).

Chlorophyll fluorescence has been used extensively in bryophyte studies, with F_v/F_m being the most commonly measured parameter. F_v/F_m is usually between 0.75 and 0.84 in healthy bryophytes (e.g. Csintalan et al., 1999; Bader et al., 2013; Proctor & Bates, 2018) with lower values indicating plant stress (Maxwell & Johnson 2000).

F_v/F_m is calculated using the following equation:

$$F_v/F_m = (F_m - F_o) / F_m \qquad (1)$$

Where F_o is minimal fluorescence and F_m is maximum fluorescence. The first expression of the equation ($F_m - F_o$) gives the variable fluorescence (F_v). F_m and F_o are measured on dark-adapted plants so that all reaction centres of PSII are open. F_o is measured in the absence of photosynthetic light when all reaction centres are open. A saturating flash is then applied and F_m is measured when all reaction centres are closed.

Φ_{PSII} is calculated using the following equation:

$$\Phi_{PSII} = (F'_m - F_t) / F'_m \qquad (2)$$

Where F'_m is the maximum fluorescence in the light and F_t is the steady state value of fluorescence. A light to drive photosynthesis is switched on, and after a time a saturating flash is applied, allowing maximum fluorescence in the light (F'_m) to be measured. The fluorescence level measured directly before the saturating flash is the steady state value of fluorescence (F_t).

Thesis Objectives

In this thesis I investigate the extent to which physiological responses to various aspects of climate explain the distribution of bryophyte species on the elevational gradient of Piton des Neiges, Réunion Island. In order to address this I:

1. Investigate the degree to which bryophyte species are able to survive outside of their natural elevational range.
2. Determine whether range-restricted and widespread bryophytes from low, mid and high elevation differ in their tolerance to desiccation.
3. Investigate variation in responses of range-restricted and widespread bryophytes from low, mid and high elevation to a range of temperatures.
4. Assess whether bryophyte range size and distribution is correlated with species' tolerance to desiccation and response to temperature.

Thesis Structure

This thesis includes three chapters presented in paper format and a general conclusion. Each chapter's discussion builds on the findings in chapter 2, and the general conclusion aims to summarise and integrate these discussions.

Chapter 2: In this chapter species' responses to transplantation were recorded. This chapter documented whether widespread and range-restricted species are specialised to the climatic conditions of their elevation of origin or whether they can survive conditions elsewhere on the Piton des Neiges gradient.

Chapter 3: This chapter documented species' responses to dehydration, and subsequent rehydration following one and seven weeks of desiccation. This chapter recorded whether desiccation tolerance depended on elevation of origin for both widespread and range-restricted species, and whether desiccation tolerance is a possible explanation for the distributional trends observed in Chapter 2.

Chapter 4: In this chapter temperature response curves of photosynthesis were measured for widespread and range-restricted species from the Piton des Neiges gradient. Species' responses to temperature were compared in order to determine whether they are specialised to the temperature conditions of their elevation of origin and whether this could explain the distributional trends observed in Chapter 2.

Chapter 5: This chapter summarises the findings of the previous three chapters, placing them in context of one another. It contains the implications and limitations of the study as a whole and makes recommendations for future research.

CHAPTER 2

Are bryophytes on a tropical montane gradient elevationally specialised? A reciprocal transplant study

Introduction

There are very few bryophyte species that can be found in all climatic conditions, and most are specialised to fairly narrow conditions of drought, temperature and insolation, which restrict their geographic range (Proctor, 2000). A pertinent explanation of species' distributions on tropical elevational gradients is the inability of montane species to tolerate lowland conditions. Richards (1984) proposed that the unsuitability of lowland conditions for bryophytes is a result of the combination of high relative humidity and temperature, and low light levels. This leads to the inability of montane species to maintain a positive carbon balance in the lowlands due to high rates of dark respiration leading to respiratory carbon losses at night (high moisture and night-time temperatures) that cannot be balanced by carbon gains during the day (low light intensities).

Few studies have tested this empirically and, as yet, there is no unequivocal evidence for this explanation. However, most laboratory and field studies on the topic have demonstrated the negative effects (e.g. lower growth rate) of the warmer, humid conditions of the lowlands on montane bryophytes (e.g. Frahm, 1987; Zotz et al., 1997; Song et al., 2012; Wagner at al., 2014a; Stam et al., 2017; see Zotz, 1999). Yet, Wagner et al. (2013) established that lowland species are specialised to the high temperatures in the lowlands, and showed that lowland and montane species did not differ in their ratio of dark respiration and net photosynthesis at temperatures in their elevation of origin.

Zotz (1999), however, suggests that there is a shortcoming in Richards' (1984) explanation, as montane bryophytes may have the ability to acclimatise to lowland conditions. To address this issue he recommended *in situ* transplant experiments. For example, Wagner et al. (2014a) conducted a transplant experiment in Panama and found that bryophytes transplanted to areas of lower elevation had a low survival rate. However, a few samples of most transplanted species did survive illustrating that there is a possibility of acclimatisation and survival in the lowlands.

Stam et al. (2017) illustrated habitat preference in a transplant experiment in Kenyan cloud forests, but did not examine acclimatisation potential. They found that bryophytes from the upper sites maintained high growth rates when auto-transplanted to their site of origin. However bryophytes transplanted from higher to lower sites had negative growth rates, which they attributed to the detrimental combination of relatively high humidity, low light levels and high temperatures for montane species in the lower forests. Their results suggest that epiphytic bryophytes are specialised to the climatic conditions of their habitats.

Transplant experiments are valuable in species distribution studies as they provide a useful way of quantifying species' responses to different habitats and climatic variables (Stam et al., 2017). Many studies in temperate and boreal regions have used transplant experiments to measure bryophyte responses to various environmental variables (e.g. Forman, 1964; Frego & Carleton, 1995; Rosso et al., 2001; Hedderson & Longton, 2008; Song et al., 2012). However, bryophyte transplant experiments in tropical areas are comparatively rare (e.g. Wagner et al., 2014a; Stam et al., 2017).

To test whether bryophytes along the Piton des Neiges gradient, are specialised to the climatic conditions in their elevation of origin, I conducted reciprocal transplants of species with varying elevational range sizes and distributions. I expected range-restricted bryophytes to be locally specialised to the climatic conditions of their elevation of origin, but widespread species to perform equally well at all elevations regardless of elevation of origin. Specifically, I hypothesised that range-restricted species from mid- and high-elevation would have low survival rates in the lowlands. In addition, I expected that low-elevation species would be specialised to the hot, wet conditions of the lowlands.

Methods

Sample collection

Thirteen bryophyte species were chosen from the Piton des Neiges gradient (Table 1):

1. three species restricted to low elevation,
2. three species restricted to mid elevation,
3. four species restricted to high elevation, and
4. three species with distributions spanning the entire gradient or at least two of the three elevational sites.

Sampling was carried out over three weeks starting in mid-July 2017. In general, up to 30 replicate samples of each range-restricted species were taken from their respective sites and 30 replicate samples of each widespread species were taken from each site. Fewer samples were taken from species of conservation concern and those difficult to find. Sample size varied between species, as a sample was considered suitable when the size and growth form were representative of that species. Care was taken to maintain the structural integrity of each sample. Samples were taken from separate clumps to increase the chances of sampling different genotypes within the population. Where possible, samples were taken along with the substrate on which they were growing, to reduce disturbance. Samples were then placed in re-sealable plastic bags to prevent desiccation and transported to the Réunion lab in an ice chest with insulated freezer blocks to keep samples cool.

Table 1: Site, range and number of bryophytes sampled and transplanted along the Piton des Neiges gradient.

Species	Range	Donor site	No. samples taken	Recipient site	No. samples received
Ectropothecium chenagonii Renauld & Cardot	Restricted	Low	29	Low	9
				Mid	10
				High	10
Leucoloma longifolium (Brid.) Wijk & Margad.	Restricted	Low	27	Low	8
				Mid	9
				High	10
Porotrichum elongatum (Welw. & Duby) A.Gepp	Restricted	Low	29	Low	9
				Mid	10
				High	10
Mastigophora diclados (Brid. ex F.Weber) Nees	Widespread	Low	26	Low	8
				Mid	9
				High	9
Pyrrhobryum spiniforme (Hedw.) Mitt.	Widespread	Low	30	Low	10
				Mid	10
				High	10
Atrichum androgynum (Müll. Hal.) A. Jaeger	Restricted	Mid	30	Low	10
				Mid	10
				High	10
Macromitrium serpens (Bruch ex Hook. & Grev.) Brid.	Restricted	Mid	14	Low	4
				Mid	5
				High	5
Plagiochila terebrans Nees & Mont. ex Lindenb.	Restricted	Mid	18	Low	6
				Mid	6
				High	6
Mastigophora diclados (Brid. ex F. Weber) Nees	Widespread	Mid	30	Low	10
				Mid	11
				High	9
Pyrrhobryum spiniforme (Hedw.) Mitt.	Widespread	Mid	29	Low	11
				Mid	9
				High	9

Schlotheimia badiella Besch.	Widespread	Mid	30	Low	10
				Mid	10
				High	10
Hypnum cupressiforme Hedw.	Restricted	High	30	Low	10
				Mid	10
				High	10
Leptodontium stellatifolium (Hampe) Broth.	Restricted	High	27	Low	9
				Mid	8
				High	10
Racomitrium membranaceum (Mitt.) Paris	Restricted	High	29	Low	10
				Mid	8
				High	11
Ulota fulva Brid.	Restricted	High	19	Low	7
				Mid	6
				High	6
Mastigophora diclados (Brid. ex F.Weber) Nees	Widespread	High	29	Low	10
				Mid	10
				High	9
Pyrrhobryum spiniforme (Hedw.) Mitt.	Widespread	High	15	Low	5
				Mid	5
				High	5
Schlotheimia badiella Besch.	Widespread	High	29	Low	10
				Mid	10
				High	9

Sample preparation and transplantation

In the lab, non-target bryophyte species were removed from each sample in order to create monospecific colonies. They were kept in a Strader phytotron at 21 °C, 97 % relative humidity, with low light conditions (< 100 µmol m^{-2} s^{-1}) as per the recommended growing conditions of Budke et al. (2013) and conditions that are regularly experienced along the gradient. Samples were kept fully hydrated by regularly spraying with deionised water. In order to weigh samples at the same water content before and after transplant, three days prior to weighing, samples were left to acclimatise to the phytotron conditions without further hydration.

After weighing, the samples of each species from each donor site were randomly divided into three groups: a control group to be auto-transplanted to the donor site, and two treatment groups to be transplanted at the two other recipient sites. Samples were then categorised according to the height at which each sample was collected (ground, 0.5 - 2 m, > 2 m). Samples were randomly assigned to a group of 5-8 samples in the same height category. Each group was then placed in stretchy plastic net bags (diameter approximately 15 mm) that were compartmentalised by winding wire on either side of each sample (Plate 1). Less than 5 % of samples were attached to plastic mesh grids using plastic net and monofilament fishing line (Plate 1). The latter method, however, proved to be too time-consuming which is why the stretchy plastic net bags were favoured. Due to the small number of samples on grids, this had no significant effect on the findings. Each sample was labelled, using metal tags secured to the mesh, to identify each sample after transplant. Groups containing epiphytic species were secured at the appropriate height to trees at the recipient sites using nylon string. Groups containing terricolous species were pegged in place on the ground using wooden skewers. This was done to control for microhabitat and substrate. Each transplanted grid was marked, its coordinates recorded and left for one year.

Plate 1: Top left- transplant group on mesh grid at mid elevation (> 2 m). Top right- transplant group in plastic nets at high elevation (0.5 - 2 m). Bottom- transplant group in plastic nets at low elevation (ground).

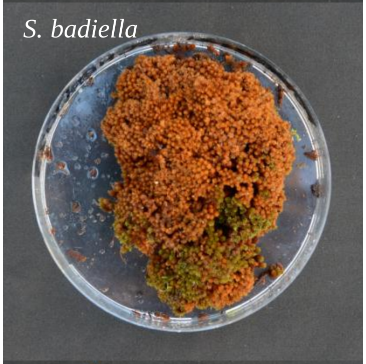
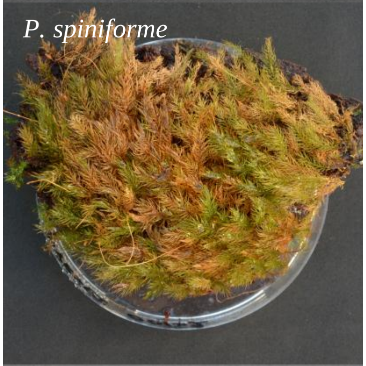
Post-transplant processing

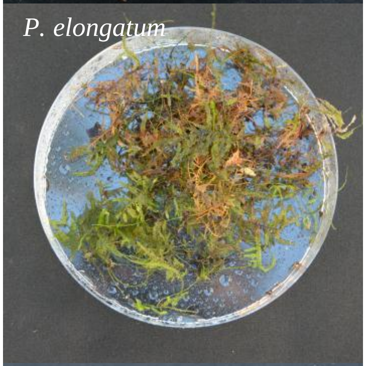
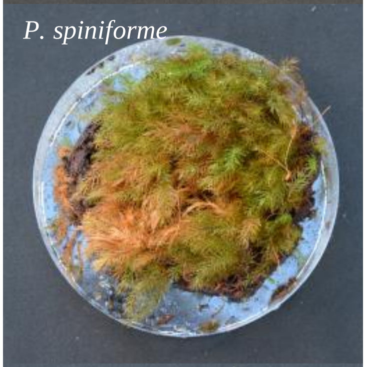
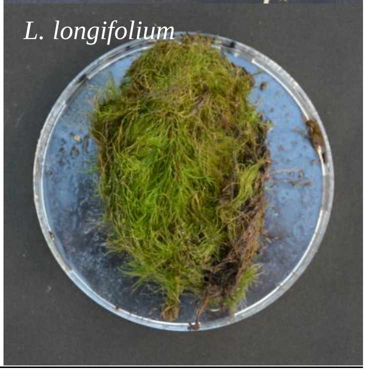
After 12 months, all of the transplants were recovered and brought to the lab.

Initially, I intended to use biomass and survival to estimate transplant performance. The samples were to be weighed before and after transplant and the change in biomass used to determine the effect of local conditions on the samples. However, after a year, many samples had accumulated organic matter and soil, and much of the original substrate had disintegrated to the point that it was not possible to gain an accurate measure of change in biomass. Therefore, I chose to focus on the survival aspect of the study. For each sample, survival and viability were assessed as per Hazell and Gustafsson (1999), Cleavitt (2004) and Song et al., (2012). Survival was determined using chlorophyll fluorescence (F_v/F_m), and viability was assessed in 10 categories (Table 2) according to the percentage of plant material that survived transplantation.

Once returned to the lab, samples were kept fully hydrated in the same phytotron set to 21 °C, 97 % relative humidity, with low light conditions (< 100 μmol m^{-2} s^{-1}), so as to store them in the same conditions before and after transplant. Samples were kept hydrated, by spraying with deionised water, during storage and assessment so that measurements could be taken at optimum health. Each sample was removed from the mesh, placed in a labelled petri dish and assessed the day after collection. One hour before measurements, lights were switched off and samples were dark adapted. Chlorophyll fluorescence was measured using a modulated chlorophyll fluorometer (MINI-PAM Portable Chlorophyll Fluorometer; H. Walz, Effeltrich, Germany) under light conditions below 4 μmol m^{-2} s^{-1} as per García et al. (2016). F_v/F_m measurements were taken over the whole area of each sample in order to assess what percentage of the plant material survived ($F_v/F_m > 0.100$). F_v/F_m was recorded for the three healthiest areas of each sample. Viability was then estimated for each sample based on these measurements of plant stress (F_v/F_m) and the percentage of plant material that survived. Visible new growth was taken into account when assessing viability and those samples with clear tissue loss were rated accordingly.

Table 2: Photographic examples illustrating the criteria for assessing viability of bryophyte samples post-transplantation.

Viability Rating	Live tissue (%)	Mean maximum F_v/F_m	Sample
1	0-10	0.000	 *R. membranaceum*
2	10-20	0.779	 *S. badiella*
3	20-30	0.625	 *P. spiniforme*
4	30-40	0.789	 *S. badiella*

5	40-50	0.720	
6	50-60	0.768	
7	60-70	0.768	
8	70-80	0.743	
9	80-90	0.730	

10	90-100	0.772	 *A. androgynum*

Data analysis

Statistical analyses were performed in R version 3.5.0 (R Core Team, 2018) using the viability rating from each sample.

Ectropothecium chenagonii and *Mastigophora diclados* exhibited a strong negative transplantation effect – almost all transplants died, including those at the donor (control) sites – and were removed from further analysis.

To determine whether species’ survival differed depending on recipient site, Kruskal-Wallis and multiple comparisons tests (“pgirmess” package in R; Giraudoux, P., 2018) were conducted for each species from each donor site, as the data were not normally distributed. However, because of the small sample size, one-way ANOVAs and Tukey's HSD (Honest Significant Difference) tests were also run. Due to the robust nature of the one-way ANOVA to departures from normality (Kutner et al., 2005), this test was chosen to represent the data, as it has higher discriminatory power. I have however included the results of the Kruskal-Wallis test, in an attempt to disentangle power effects from violations of assumptions of parametric tests. Restricted and widespread species were separated and then grouped by donor site. The same analyses were repeated in order to determine whether the average survival rate of species from a specific donor site differed depending on recipient site.

Results

Range-restricted species

Low-elevation specialists:

Of the three species, one (*E. chenagonii*) was sensitive to transplantation with only two samples surviving at the low-elevation (control) site. It was, thus, excluded from further analysis. Both *Leucoloma longifolium* and *P. elongatum* performed better at the low-elevation (control) site than the high-elevation site, with intermediate performance at the mid-elevation site ($F_{2,24} = 9.091$, $p < 0.01$) ($F_{2,26} = 5.221$, $p < 0.05$). *L. longifolium* had a mean of 6.63 (± SE 0.71) at low elevation, 7.56 (± SE 0.87) at mid elevation and 3.10 (± SE 0.80) at high elevation. *Porotrichum elongatum* had a mean of 4.33 (± SE 0.97) at low elevation, 2.20 (± SE 0.49) at mid elevation and 1.60 (± SE 0.27) at high elevation (Figure 1).

Mid-elevation specialists:

Two of the three species performed well at their site of origin, of which *Atrichum androgynum* performed significantly better at the control site (mid elevation) than either of the treatment sites ($F_{2,27} = 15.15$, $p < 0.001$). There was no statistically significant difference between performance at each site for *Macromitrium serpens* ($F_{2,11} = 2.292$, $p > 0.05$) and *Plagiochila terebrans* ($F_{2,15} = 2.935$, $p > 0.05$). However, both showed a trend of performing better at the mid- (control) and high-elevation sites and poorly at the low-elevation site. *A. androgynum* had a mean of 5.20 (± SE 1.01) at low elevation, 9.30 (± SE 0.33) at mid elevation and 3.70 (± SE 0.73) at high elevation. *M. serpens* had a mean of 3.25 (± SE 1.65) at low elevation, 7.40 (± SE 1.47) at mid elevation and 7.00 (± SE 1.26) at high elevation. *P. terebrans* had a mean of 2.33 (± SE 0.42) at low elevation, 4.00 (± SE 1.41) at mid elevation and 6.00 (± SE 1.13) at high elevation (Figure 1).

High-elevation specialists:

Of the four species, three performed significantly better at the control (high-elevation) site than the treatment sites; *Leptodontium stellatifolium* ($F_{2,24} = 34.14$, $p < 0.001$), *Racomitrium membranaceum* ($F_{2,26} = 15.24$, $p < 0.001$), *U. fulva* ($F_{2,16} = 14.28$, $p < 0.001$). *Hypnum cupressiforme* had a mean of 3.60 (± SE 0.67) at low elevation, 5.60 (± SE 1.15) at mid elevation and 2.30 (± SE 0.21) at high elevation. *L. stellatifolium* had a mean of 1.33 (± SE 0.33) at low elevation, 3.63 (± SE 1.21) at mid elevation and 9.00 (± SE 0.39) at high elevation. *R. membranaceum* had a mean of 1.10 (± SE 0.1) at low elevation, 3.25 (± SE 1.37) at mid elevation and 7.64 (± SE 0.92) at high elevation. *Ulota fulva* had a mean of 1.14 (± SE 0.14) at low elevation, 4.17 (± SE 1.01) at mid elevation and 7.67 (± SE 1.23) at high elevation (Figure 1).

Widespread species

Pyrrhobryum spiniforme:

There was no statistically significant difference between the performance of low-elevation *P. spiniforme* at the low- (control) and mid-elevation sites, but performance was significantly worse at high-elevation with only two samples surviving transplantation ($F_{2,27} = 14.13$, $p < 0.001$). There was no statistically significant difference between the performance of mid-elevation *P. spiniforme* at the mid- (control) and low-elevation sites, but performance was significantly lower at high-elevation ($F_{2,26} = 6.846$, $p < 0.01$). High-elevation *P. spiniforme* performed significantly worse at the high-elevation (control) site than the low-elevation site, with intermediate performance at the mid-elevation site. ($F_{2,12} = 5.414$, $p < 0.05$). Low-elevation *P. spiniforme* had a mean of 7.10 (± SE 0.87) at low elevation, 6.60 (± SE 1.18) at mid elevation and 1.30 (± SE 0.21) at high elevation. Mid-elevation *P. spiniforme* had a mean of 7.36 (± SE 0.82) at low elevation, 6.67 (± SE 1.05) at mid elevation and 3.11 (± SE 0.68) at high elevation. High-elevation *P. spiniforme* had a mean of 6.60 (± SE 1.03) at low elevation, 4.40 (± SE 1.54) at mid elevation and 1.60 (± SE 0.24) at high elevation (Figure 2).

Schlotheimia badiella:

There was no significant difference in the performance of mid-elevation *S. badiella* between each site ($F_{2,27} = 2.919$, $p > 0.05$). There was no significant difference in the performance of high-elevation *S. badiella* at the high- (control) and mid-elevation sites, but performance was significantly worse at the low-elevation site ($F_{2,26} = 9.907$, $p < 0.001$). However, for both mid- and high-elevation *S. badiella*, there was a trend of increasing performance with elevation. Mid-elevation *S. badiella* had a mean of 5.20 (± SE 1.11) at low elevation, 7.60 (± SE 1.23) at mid elevation and 8.60 (± SE 0.62) at high elevation. High-elevation *S. badiella* had a mean of 4.20 (± SE 0.81) at low elevation, 7.50 (± SE 1.19) at mid elevation and 9.67 (± SE 0.24) at high elevation (Figure 2).

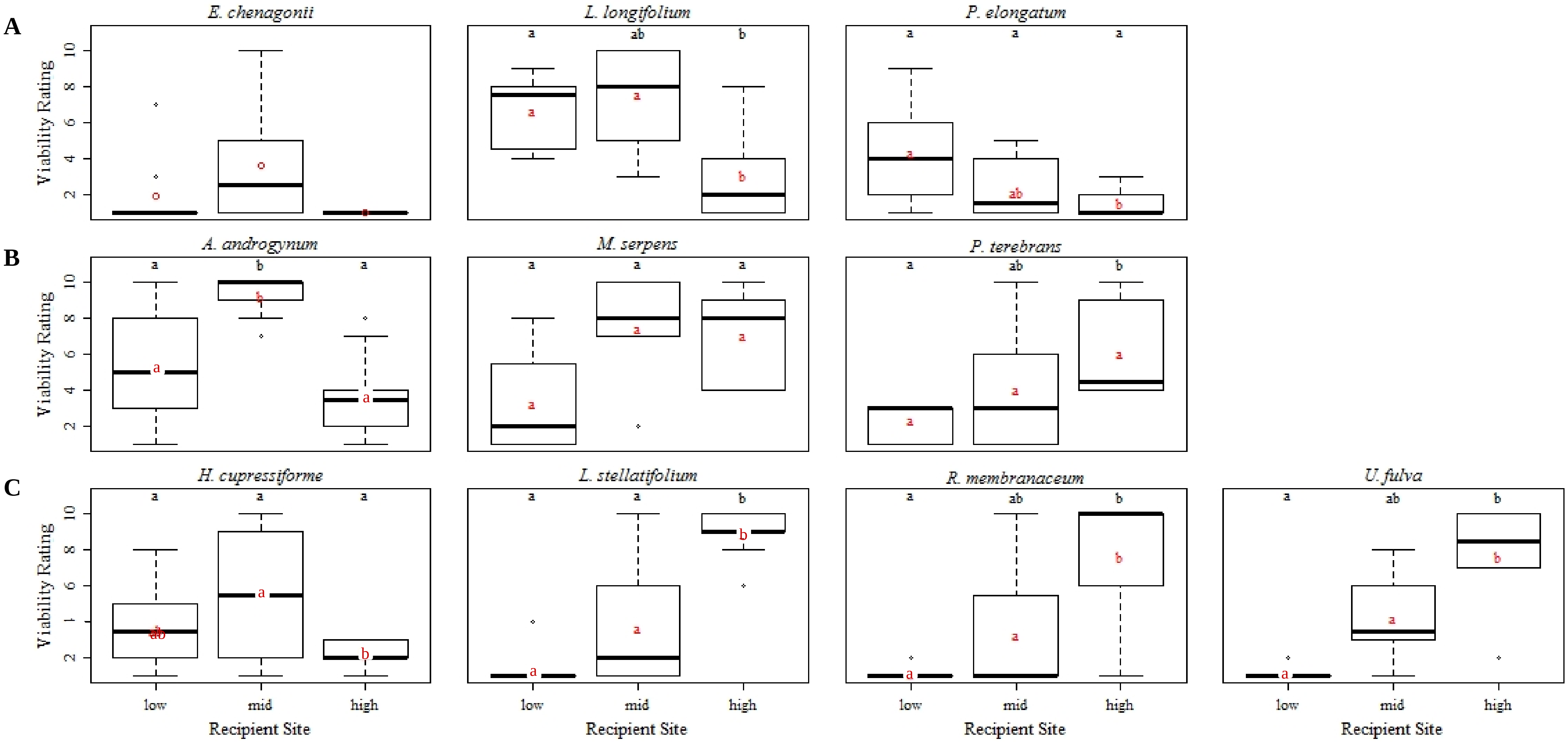


Figure 1: Viability rating of each range-restricted species from low- (A), mid- (B) and high-elevation (C) donor sites following transplantation to the three recipient sites (low, mid and high elevation). Red circles indicate means of the data in species that were not included in statistical analyses. Different letters in red indicate means of the data and represent results of ANOVA indicating significant differences between recipient sites ($p < 0.05$). Different letters in black at the top of each figure represent results of the Kruskal-Wallis tests indicating significant differences between recipient sites ($p < 0.05$).

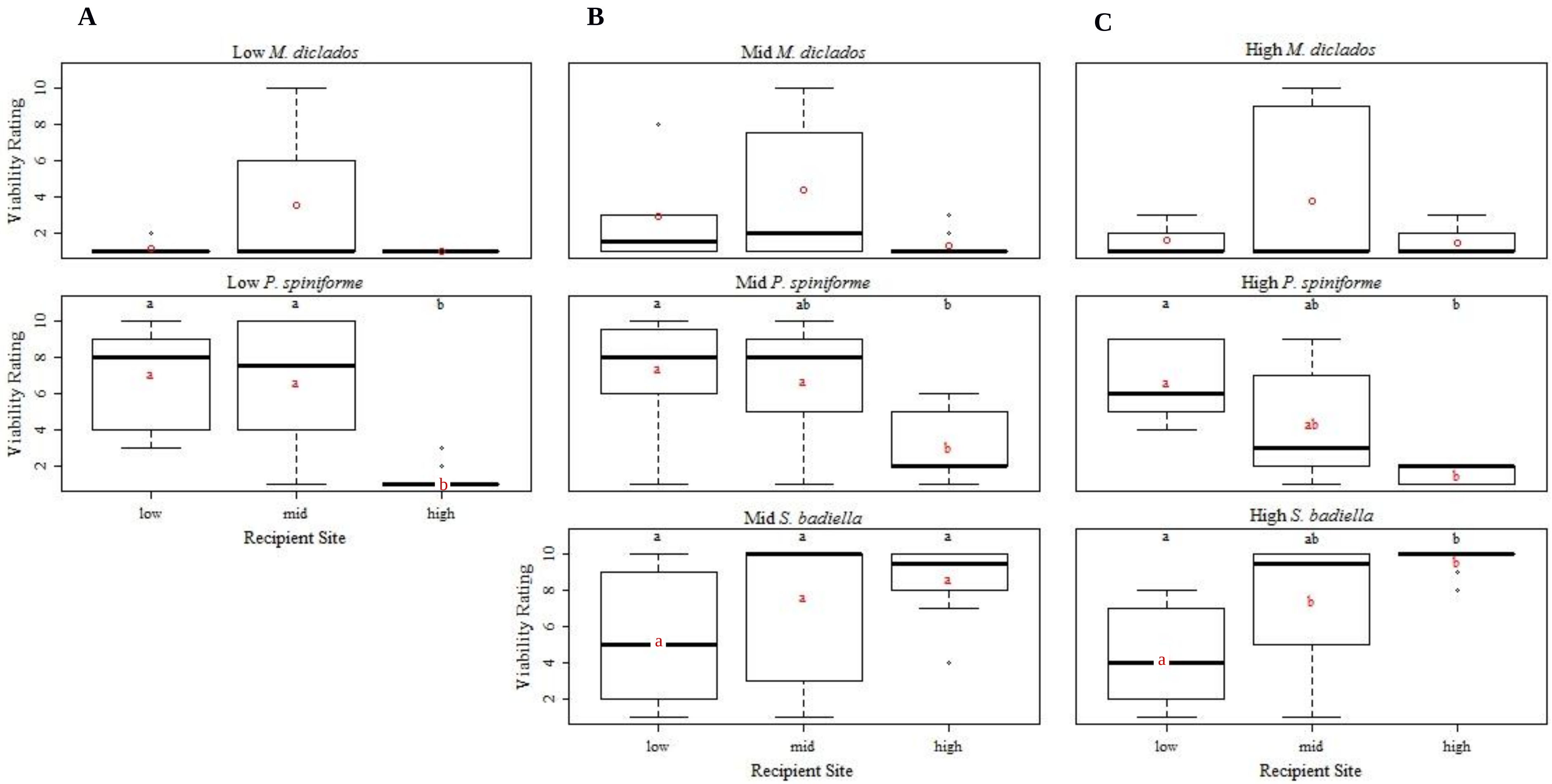


Figure 2: Viability rating of each widespread species from low- (A), mid- (B) and high-elevation (C) donor sites following transplantation to the three recipient sites (low, mid and high elevation). Red circles indicate means of the data in species that were not included in statistical analyses. Different letters in red indicate means of the data and represent results of ANOVA indicating significant differences between recipient sites ($p < 0.05$). Different letters in black at the top of each figure represent results of the Kruskal-Wallis tests indicating significant differences between recipient sites ($p < 0.05$).

Donor sites

Restricted species from low elevation performed significantly better at the low-elevation site (mean = 4.19 ± SE = 0.59) than at the high-elevation site (mean = 1.90 ± SE = 0.32), with intermediate performance at the mid-elevation site (mean = 4.34 ± SE = 0.62) ($F_{2,53}$ = 6.436, $p < 0.01$).

Restricted species from mid elevation performed significantly better at the mid-elevation site (mean = 7.33 ± SE = 0.72) than at the low-elevation site (mean = 3.95 ± SE = 0.62), with intermediate performance at the high-elevation site (mean = 5.14 ± SE = 0.62) ($F_{2,59}$ = 6.6, $p < 0.01$).

Restricted species from high elevation performed significantly better at the high-elevation site (mean = 6.57 ± SE = 0.56) than at the low- (mean = 1.86 ± SE = 0.27) and mid-elevation sites (mean = 4.25 ± SE = 0.61) ($F_{2,102}$ = 23.55, $p < 0.001$).

There was no significant difference in the performance of widespread species from mid elevation at each site. Widespread species from mid elevation performed equally well at the low- (mean = 5.23 ± SE = 0.62), mid- (mean = 6.13 ± SE = 0.70) and high-elevation sites (mean = 4.50 ± SE = 0.68) ($F_{2,41}$ = 1.053, $p > 0.05$).

There was no significant difference in the performance of widespread species from high elevation at each site. Widespread species from high elevation performed equally well at the low- (mean = 3.64 ± SE = 0.54), mid- (mean = 5.40 ± SE = 0.80) and high-elevation sites (mean = 4.70 ± SE = 0.86) ($F_{2,56}$ = 0.592, $p > 0.05$).

After removing *M. diclados* from the analysis, *P. spiniforme* was the only widespread species from the low-elevation donor site. There was no significant difference in *P. spiniforme's* performance at the low- and mid-elevation sites, but performance was significantly worse at the high-elevation site ($F_{2,27}$ = 14.13, $p < 0.001$).

The overall trend was that range-restricted species from mid and high elevation had low survival rates at low elevation, with high-elevation species also performing badly at mid elevation. Range-restricted species tended to perform best in their site of origin, with low- and mid-elevation species also having high survival rates at the site above. Widespread species from mid and high elevation performed equally well at all sites (Figure 3).

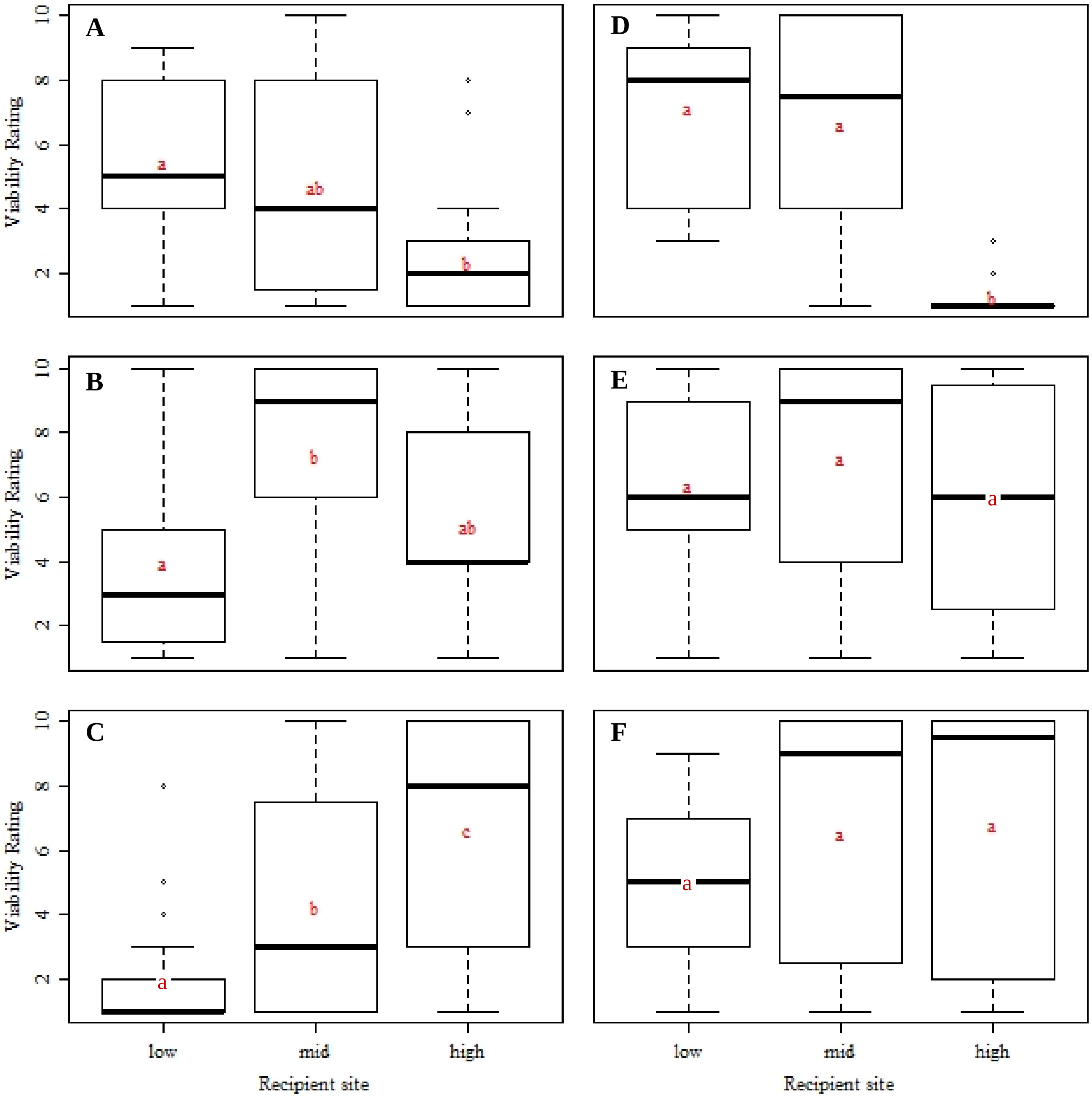


Figure 3: Average viability rating of species from each donor site (A- low-elevation restricted, B- mid-elevation restricted, C- high-elevation restricted, D- low-elevation widespread, E- mid-elevation widespread, F- high-elevation widespread) following transplantation to the three elevational sites (low, mid and high elevation). Different letters indicate means of the data and represent results of ANOVA indicating significant differences between recipient sites ($p < 0.05$).

Discussion

Range-restricted species

The reciprocal transplant experiment showed a definite effect of elevational location on performance of range-restricted species from all donor sites. In general, this seems to have an effect of limiting the distribution of mid- and high-elevation species downslope. This result was similar to that of Wagner et al. (2014a) and Stam et al. (2017), who found low growth and survival rates in tropical bryophytes transplanted to lower elevations. Stam et al. (2017) attributed the low survival rate to the unfavourable conditions of the lowlands caused by high temperatures, high relative humidity and low light levels. In the current study, all mid- and high-elevation species did significantly worse at low elevation and most samples had very low survival and viability ratings at low elevation. These results support the idea that the wetter, warmer climatic conditions of low-elevation are unfavourable for both mid- and high-elevation species.

Low-elevation species performed best at their site of origin and at mid elevation, but had very low survival rates at high elevation. This illustrates that low-elevation species are able to tolerate the intermediate conditions at mid elevation, but are not able to survive the very different (colder and dryer) conditions at high elevation. Bryophytes tend to be specialised to the conditions of humidity habitually experienced (Proctor 2001), therefore, the low survival rates of low-elevation species at high elevation is probably due to an inability to tolerate the much dryer conditions at high elevation. Furthermore, Wagner et al. (2013) and Lösch et al. (1994) found that low-elevation species have higher optimum temperatures for photosynthesis than those from higher elevations. This indicates that low-elevation species may not be photosynthesising at full capacity at colder temperatures. The combined effect of suboptimal photosynthetic rates at lower temperatures and frequent periods of inactivity when desiccated may lead to low carbon gains, and eventual death, of low-elevation species at high elevation.

Acclimatisation

There may be potential for species to acclimatise to new climatic conditions (Zotz, 1999). In order to get a complete picture of species' responses to climate it is important to have information on their acclimatisation potential (Jägerbrand et al., 2014), as it may be the case that physiological tolerances are not the cause of species' range limits, but are rather the result of local adaptation where other limits to distribution are imposed e.g. dispersal or competition

(Gaston, 2003). Like Wagner et al. (2014a), the current study found that some samples of all transplanted species did survive at all treatment sites, with the exception of *E. chenagonii* at high-elevation. Wagner et al. (2014a) attributed this to the ability of some individuals in the population to acclimatise to new climatic conditions. It should be noted, however, that many of the samples in the current study still had very low viability ratings and would probably not survive another year, or even the next wet season.

Nevertheless, if at least some individuals have the ability to acclimatise to new conditions and survive at other elevations, why do they not occur there? For example, it may be establishment or propagation limitations, rather than adult survival, in other climatic conditions that restrict species to certain elevational ranges (Forman, 1964; Wagner et al., 2014a). Bryophytes in different life history stages have varying requirements for growth, developing at different times in the annual seasonal cycle (Forman, 1964; Glime, 2007), responding to cues such as temperature and photoperiod (Newton, 1972) and requiring different levels of temperature, humidity, pH and light (Forman, 1964). This highlights the role that microsites may play in all stages of bryophyte development. To illustrate, Cleavitt (2004) found that microsite had a larger effect than elevation on both the establishment of apical fragments and adult survival of *Mnium arizonicum* in a transplant experiment.

Microhabitat

Although it is clear that macroclimate plays a large role in species' distributions, the effect of microhabitat cannot be ruled out, at least in some species. For instance, *E. chenagonii* was sensitive to transplantation with very few samples surviving even at the control site. It is unlikely that this was due to the disturbance of removal from the field and handling, as in a separate experiment I have recorded growth in this species after two months in the lab. Therefore, it was probably due to microhabitat conditions that were not met upon transplantation. *E. chenagonii* was abundant at its low-elevation donor site, and although the low-elevation recipient site had similar macroclimatic conditions to the donor site, no *E. chenagonii* was seen in this area (personal observation). This may be due to differences in microclimate or lack of exposed rocks for substrate.

Furthermore, the reason for *P. spiniforme* transplanting well at low and mid elevations, but badly at high elevation, was probably also due to microhabitat effects. Although *P. spiniforme* is found at high elevation, it is not widespread in all habitats. It is restricted to shady, forested areas, and the transplant site was an open, shrubby canopy where *P. spiniforme* is not usually found. Similarly, *H. cupressiforme* showed an irregular response to transplantation, performing poorly at the control (high-elevation) site, which may also be due to the fact that although it occurs at high elevation, it is usually found on wet, decaying wood in more shaded areas, while the transplant site was an open canopy with live wood. *H. cupressiforme* did, however, perform fairly well at the treatment sites, perhaps due to the fact that it is not a true high-elevation specialist, and can be found at lower elevations in other locations. For example, this species is found on Table Mountain, South Africa, at elevations much lower than it is recorded on Réunion (Hedderson, personal communication 2018, October).

In addition, *M. diclados* was sensitive to transplantation and even the controls did not have high survival rates. This may be due to the fact that *M. diclados* is a very sensitive species and disruptions to its structural integrity may disrupt its water relations. Therefore, maintaining colony architecture may be crucial for the survival of this species. Nevertheless, some samples of *M. diclados* from all sites did survive at mid elevation. *M. diclados* may be more sensitive to microhabitat placement than the other species and the intermediate temperature and humidity conditions of mid elevation may provide more suitable microhabitat spaces for *M. diclados.* *S. badiella* also showed an effect of microhabitat placement, having lower survival rates at low elevation. *S. badiella* is a mid-canopy species that is usually exposed to a lot of light. The low-elevation transplant site had a much fuller canopy than this species is accustomed to, probably reducing its rate of survival at this site.

Hedderson and Brassard (1990) have highlighted the importance of microclimate in bryophyte distributions. Due to their small size, bryophytes have the ability to inhabit favourable microsites surrounded by an otherwise hostile matrix. The species in this study were chosen because they are abundant in their elevation of origin, but some of these species can be found in isolated populations or solitary shoots at other elevations. A pertinent example is *Calyptrochaeta asplenioides*, a mid-elevation specialist, which has been observed as isolated shoots on especially cool, wet slopes at low elevation (personal observation). This may be the reason that some samples of all but one species survived transplantation to all elevations, as they may have been placed in favourable microhabitats surrounded by mostly unfavourable conditions.

Widespread species

Although the results of the ANOVA showed that, on average, widespread species performed equally well at all elevations, individual species showed idiosyncratic responses to transplantation. The individual responses of widespread species indicate that microhabitat is very important for these species. The Climate Variability Hypothesis predicts that widespread species should have broader environmental tolerances, allowing them to occupy a wide range of habitats (Stevens, 1989; Stevens, 1992). However, the results of this study suggest that microhabitat is more important than macroclimate in these species. Therefore, widespread species don't necessarily have broader climatic tolerances, but rather occupy similar microsites in different habitats. Therefore, contrary to Brown's (1984) hypothesis, species' fundamental niche breadths do not always determine their geographic range. Furthermore, despite findings from other taxa (Pohlman et al., 2005; Calosi et al., 2007; Calosi et al., 2010; see Bozinovic et al., 2011), the ability of bryophytes to occupy microsites may allow widespread species to be even be less physiologically plastic than range-restricted species, occupying microhabitats with a very narrow range of climatic conditions that happen to be available in a wide range of habitats.

Conclusion

In conclusion, range-restricted species performed best at their elevation of origin, with species from all other sites performing badly at lower elevations, but low- and mid-elevation species performing well at the site above. This is consistent with some level of climatic specialisation, with conditions in the elevational band above also proving favourable, or at least tolerable. Despite the general macroclimatic explanation for these patterns, microhabitat and microclimate play a definite role, at least in some species. Furthermore, as some individuals of almost all species were able to survive conditions along the entire gradient, species may have the ability to acclimatise to new conditions, although it might be the case that they were just placed in favourable microsites. Similar to the results reported by Cleavitt (2004), microsite appeared to have a larger effect than elevation on widespread species. This result provides an interesting alternative to the CVH and Brown's hypothesis, at least in bryophytes and other species closely linked to microclimatic conditions.

To test the continuing survival of these species at other elevations, further research should consider longer-term transplant experiments. Réunion Island is subject to cyclones (Garnier & Desarthe, 2013) that modify bryophyte habitats and may affect the success of transplants. With cyclones in mind, the samples in this study were placed in sheltered areas and well-attached to the substrate, so harsh weather did not affect them. Studies that extend over multiple wet seasons should, thus, regard the potential effects of cyclones when considering transplant placement.

This chapter aimed to test whether species are specialised to the climatic conditions of their elevation of origin or whether they have the capacity to survive conditions at other elevations. The following two chapters will test species' physiological responses to humidity and temperature in order to provide insight into the pattern of transplant survival seen in this chapter.

CHAPTER 3

Variations in desiccation tolerance as an explanation for bryophyte range size and distribution along the Piton des Neiges gradient, Réunion Island

Introduction

The poikilohydric nature of bryophytes means that they are strongly linked to local and microclimatic conditions and, in particular, to the availability of water in the environment. Hosokawa et al. (1964) showed a direct correlation between the photosynthetic rate and relative humidity for several epiphytic bryophytes, and numerous studies have demonstrated a link between bryophyte distributions and environmental water availability (Alpert & Oechel, 1985; Gradstein & Pócs, 1989; Wolf, 1993; Acebey et al., 2003; Grau et al., 2007).

Frequently coupled with poikilohydry is the ability to tolerate dehydration to low cell water content and to recover from this with limited physiological damage. Most bryophyte species can tolerate vegetative desiccation, but this is a rare trait in vascular plants (Oliver et al., 2000). Species that are sensitive to drying experience cell death, due to loss of cellular membrane integrity, when dehydrated (Oliver & Bewly, 1984). Bryophytes prevent cell death during dehydration by means of protective mechanisms when drying and reparative mechanisms during rehydration (Bewly & Krochko, 1982). Desiccation tolerance is a trait more common in bryophytes than any other group of land plants (Alpert, 2000) and most bryophytes can regain full metabolic function minutes or hours after rehydration (e.g. Csintalan et al., 1999, León-Vargas et al., 2006, Pardow & Lakatos, 2012).

Bryophytes require some degree of desiccation tolerance in any habitat that does not have a continuous supply of moisture. Thus, they are as diverse in their ability to tolerate desiccation as the vast range of habitats they occupy (Proctor et al., 2007). Species that inhabit wet, shady habitats generally have the lowest tolerance to desiccation, while extreme tolerance is found in species inhabiting arid areas, which must endure both excessive dryness and intense UV radiation. Even aquatic species can handle some level of drying when water levels are low (Proctor et al., 2007).

According to Proctor (2001), desiccation tolerance in bryophytes generally mirrors their environmental moisture availability (Clausen, 1952; Ochi, 1952; Höfler, 1954; Abel, 1956). This has been illustrated in a number of studies. To mention a few, Lee and Stewart (1971) found intra-specific differences in the response to, and recovery from, desiccation in a number of bryophyte species from wet and dry habitats. Alpert and Oechel (1985) found experimental evidence that *G. laevigata* (Brid.) Brid. had water relations specifically adapted to xeric habitats and suggested that lack of water availability limits bryophyte distribution in xeric environments. Additionally, Rundel and Lange (1980) ascribed the relative scarcity of bryophytes in deserts to the inability of some species to efficiently use atmospheric water vapour, allowing them to tolerate desiccation for extended time periods, reducing time available for photosynthesis. Many studies have compared desiccation tolerance of xeric, mesic and hydric species using differences in photosynthetic efficiency during drying, ability to recover after periods of desiccation, and other methods. They identified differences in their water relations, with xeric species being more desiccation tolerant and specially adapted to erratic water supply (Di Nola et al., 1983; Seel et al., 1992; Oliver et al., 1993; Davey, 1997; Deltoro et al., 1998; Csintalan et al., 1999; Robinson et al., 2000).

Furthermore, a study of three Antarctic mosses indicated that their tolerance to desiccation matched the moisture conditions within their distributions. The study used the relationship between photosynthetic efficiency and water content during drying, water content when fully hydrated, as well as the rate of drying to assess the differences in response to desiccation and the plasticity of response. They found that the species limited to wet environments exhibited the lowest desiccation tolerance; the species common at the driest sites showed the highest capacity to tolerate desiccation; and the species that showed intermediate tolerance and greatest plasticity, had a widespread distribution and were found at both extremes (Robinson et al., 2000).

Most literature on elevational patterns of bryophyte richness attributes their distribution to environmental water availability (e.g. Gradstein & Pócs, 1989; Wolf, 1993; Acebey et al., 2003; Grau et al., 2007), but there is a paucity of studies testing this empirically and very few studies focus on tropical bryophytes (e.g Bader et al., 2013; Wagner et al., 2013) and, to the best of my knowledge, there are none focusing on southern hemisphere bryophytes. While most of the literature on the topic supports the hypothesis that bryophyte distributions are limited by environmental humidity, the study most similar to my own research does not. This study by Bader et al. (2013) made a comparison of desiccation tolerance between lowland and montane species in Panama. They found that both lowland and montane bryophytes could tolerate dry periods that far exceed periods of drought experienced in their habitats. However, Alpert (2000) suggests that the ability to tolerate such excessive periods of drought is a trait that may be functionally linked to other traits of desiccation tolerance that are directly selected for. Therefore, Alpert (2000) maintains that the length of drought tolerated is not necessarily the best criterion for measuring desiccation tolerance.

Here, I aim to test whether differences in desiccation tolerance are correlated with the elevational distribution of bryophytes along the Piton des Neiges gradient, Réunion Island. I tracked photosynthetic efficiency, using chlorophyll fluorescence, in dehydration and subsequent rehydration experiments in species with varying elevational range sizes and distributions. I hypothesised that tolerance to desiccation would increase with elevation for range-restricted species, with low- and mid-elevation species having low tolerances to desiccation, and high-elevation species having high desiccation tolerance. I expected that widespread species would not show local specialisation in their tolerance to desiccation. I predicted that there would be a reduction in recovery of all species after seven weeks of dehydration, compared with one week, with the results after one week being a more realistic indication of desiccation tolerance, as these species probably never experience seven weeks of desiccation in the field. Consistent with this assumption, Alpert (2000) argues that exposing bryophytes to excessively long dry periods is not a good measure of specific desiccation tolerance.

Methods

Climate data

Climate data were collected by UMR PVBMT lab.

Readings were taken 1 m above the ground, at elevational intervals of 200 m a.s.l. from 350 m a.s.l. to 2 950 m a.s.l. using MadgeTech data loggers (RHTemp1000, Warner, USA). Temperature and relative humidity were recorded at hourly intervals between June 2011 and May 2012.

Sampling

Fieldwork was carried out in June 2016. Within each elevational band, between four and nine replicate samples of several widespread and several range-restricted bryophyte species were collected (Table 3). Sample size varied between species, as a sample was considered suitable when the size and growth form were representative of that species. Care was taken to maintain the structural integrity of each sample. Samples were taken from separate clumps to increase the chances of sampling different genotypes within the population. Samples were placed in re-sealable plastic bags to prevent desiccation and transported to the Réunion lab in an ice chest.

Table 3: Site, range and number of bryophyte samples collected along the Piton des Neiges gradient

Species	Range	Site	No. samples collected
Ectropothecium chenagonii Renauld & Cardot	Restricted	Low	6
Leucoloma longifolium (Brid.) Wijk & Margad.	Restricted	Low	5
Porotrichum elongatum (Welw. & Duby) A.Gepp	Restricted	Low	6
Mastigophora diclados (Brid. ex F.Weber) Nees	Widespread	Low	6
Pyrrhobryum spiniforme (Hedw.) Mitt.	Widespread	Low	5
Atrichum androgynum (Müll. Hal.) A. Jaeger	Restricted	Mid	6
Calyptrochaeta asplenioides (Brid.) Crosby	Restricted	Mid	6
Macromitrium serpens (Bruch ex Hook. & Grev.) Brid.	Restricted	Mid	4
Dicranoloma billardierei (Brid. ex Anon.) Paris	Widespread	Mid	8
Holomitrium borbonicum Hampe ex Besch.	Widespread	Mid	9
Mastigophora diclados (Brid. ex F.Weber) Nees	Widespread	Mid	6
Pyrrhobryum spiniforme (Hedw.) Mitt.	Widespread	Mid	6
Schlotheimia badiella Besch.	Widespread	Mid	6
Leptodontium stellatifolium (Hampe) Broth.	Restricted	High	5
Racomitrium membranaceum (Mitt.) Paris	Restricted	High	5
Ulota fulva Brid.	Restricted	High	5
Dicranoloma billardierei (Brid. ex Anon.) Paris	Widespread	High	5
Holomitrium borbonicum Hampe ex Besch.	Widespread	High	5
Mastigophora diclados (Brid. ex F.Weber) Nees	Widespread	High	5
Pyrrhobryum spiniforme (Hedw.) Mitt.	Widespread	High	5
Schlotheimia badiella Besch.	Widespread	High	5

Chlorophyll fluorescence

For both the dehydration and rehydration experiments chlorophyll fluorescence was measured using a modulated chlorophyll fluorometer (PAM-2100 Portable Chlorophyll Fluorometer; H. Walz, Effeltrich, Germany). All samples were dark adapted prior to measuring chlorophyll fluorescence, and measurements were taken under low light conditions (< 8 µmol m^{-2} s^{-1}), as per García et al. (2016).

Dehydration experiment

On the day of collection all samples were placed in labelled petri dishes and the following measurements carried out under ambient laboratory conditions. The laboratory was not climate controlled and tracked ambient relative humidity (± 65%). Samples were fully hydrated, by spraying with deionised water, and the initial fresh weight of each sample was measured (Precisa, XB620M). Samples were purposely not dehardened so as not to erase their field history.

The three healthiest areas of each sample were chosen. One chlorophyll fluorescence reading was taken on each of these three areas every two hours. The three chlorophyll fluorescence readings and a weight reading were taken for each sample at two-hour intervals until the samples were fully dehydrated (no change in mass over successive weighing periods) and no longer photosynthesising ($F_v/F_m < 0.010$). With the aim of examining the integrated response of the samples to desiccation, the rate of drying was not controlled. All species were kept in the same environment, so variations in drying rate should be as a result of architectural variations between the species. At the end of the experiment the dehydrated samples were placed in labelled paper packets and stored under ambient laboratory conditions.

Rehydration experiment

One week after dehydration, samples were halved. One of these subsamples from each sample was placed in a labelled petri dish and rehydrated by spraying with de-ionised water until fully hydrated. The laboratory was not climate controlled and tracked ambient relative humidity (± 65%). One chlorophyll-fluorescence reading was taken on each of the two healthiest areas of each sample five minutes, one hour and 24 hours after rehydration. In between measurements, the samples were placed inside re-sealable plastic packets to prevent desiccation.

The other subsamples were transported in paper packets to the lab in Cape Town, where the above process was repeated seven weeks after initial dehydration.

Data analyses

Atmospheric water potential (Ψ_w, in MPA) was calculated from the climate data using the following equation:

$$\Psi_w=(RT/V_w)\ \ln(RH / 100) \qquad (3)$$

Where R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T the absolute temperature (K), V_w the partial molar volume of water (18 cm^3 mol^{-1}), and RH the relative humidity (%).
Days where the maximum daily relative humidity fell below 95 % were considered dry days (León-Vargas et al., 2006). This was chosen as a conservative estimate of a dry day, as with water potential well below -2 MPa, the water potential of the air is so low that bryophytes would not be able to gain moisture from it (Taiz et al., 2015).

Sigmoidal curves of the form:

$$y=a/(1 + e^{-b\,(x-c)}) \qquad (4)$$

were fit to examine the relationship between photosynthetic efficiency (F_v/F_m) and water content (g H_2O g^{-1} dry weight) for each species. Coefficient *c* (x-value of the sigmoid's midpoint) was estimated for each curve using the "nls" (nonlinear least squares) function in R (R Core Team, 2018). Coefficient *c* was used in order to compare species' sensitivity to desiccation where a larger *c* indicates higher sensitivity to desiccation (reaching half photosynthetic efficiency at a higher water content).

To determine whether sensitivity to desiccation differed depending on species' sites of origin coefficient *c* was compared using one-way ANOVAs and Tukey's HSD (Honest Significant Difference) tests and two-sample t-tests where data were normally distributed. Where the data were not normally distributed Kruskal-Wallis tests and multiple comparison tests ("pgirmess" package in R; Giraudoux, P., 2018) were used.

Percent total recovery was calculated for each sample using the average value of chlorophyll fluorescence (F_v/F_m) at 24 hours and the maximum (initial, fully hydrated) chlorophyll fluorescence value obtained for that sample. To determine whether percent total recovery (24 hours after rehydration) differed, depending on the species' site of origin, Kruskal-Wallis and multiple comparisons tests ("pgirmess" package in R; Giraudoux, P., 2018) and Mann-Whitney U tests ("exactRankTests" package in R; Hothorn & Hornik, 2017) were used where the data were not normally distributed. Two-sample t-tests were used where the data were normally distributed.

To determine whether the percent total recovery (24 hours after rehydration) differed for each species depending on time dehydrated Mann-Whitney U tests ("exactRankTests" package in R; Hothorn & Hornik, 2017) were conducted, where the data were not normally distributed, and two-sample t-tests were conducted where the data were normally distributed. Data were analysed using R version 3.5.0 (R Core Team, 2018).

Results

Climate Data

There was a trend of high relative humidity along the Piton des Neiges gradient, with a large proportion of days having above 95 % relative humidity, with 100 % relative humidity occurring at a high frequency. This trend was also seen in the minimum daily relative humidity, meaning that the relative humidity remained 100 % throughout the day (Figure 4).

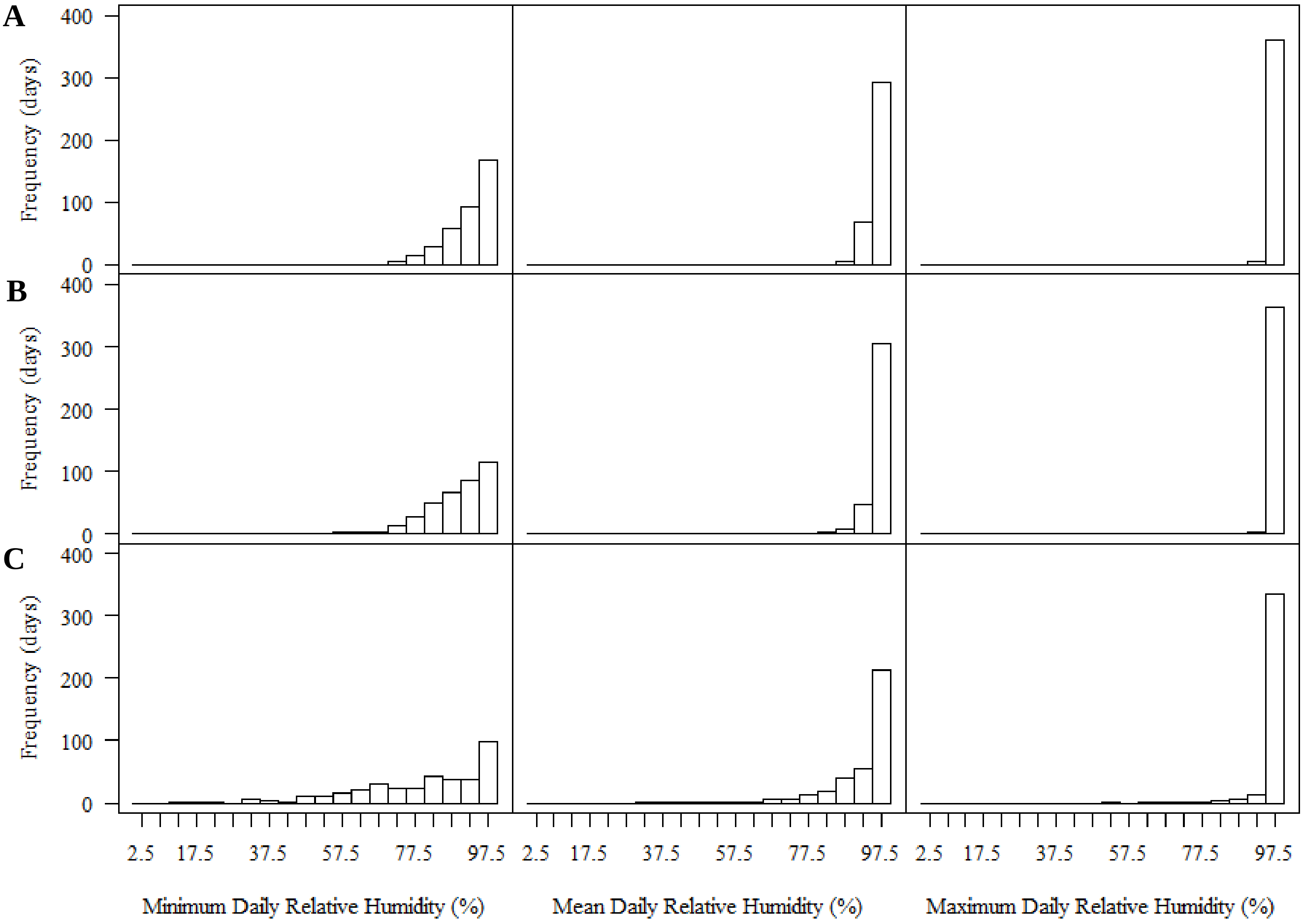


Figure 4: Minimum, mean and maximum daily relative humidity (%) from June 2011 to May 2012 at low- (A), mid- (B) and high-elevation (C) sites along the Piton des Neiges gradient.

Over the recorded year, there were only three dry days at the low-elevation site and one dry day at the mid-elevation site. The high-elevation site had 29 dry days, with a cluster of dry days occurring from October to December. The low- and mid-elevation sites also had fewer days where daily mean and minimum relative humidity values were below 95 % (Figure 5).

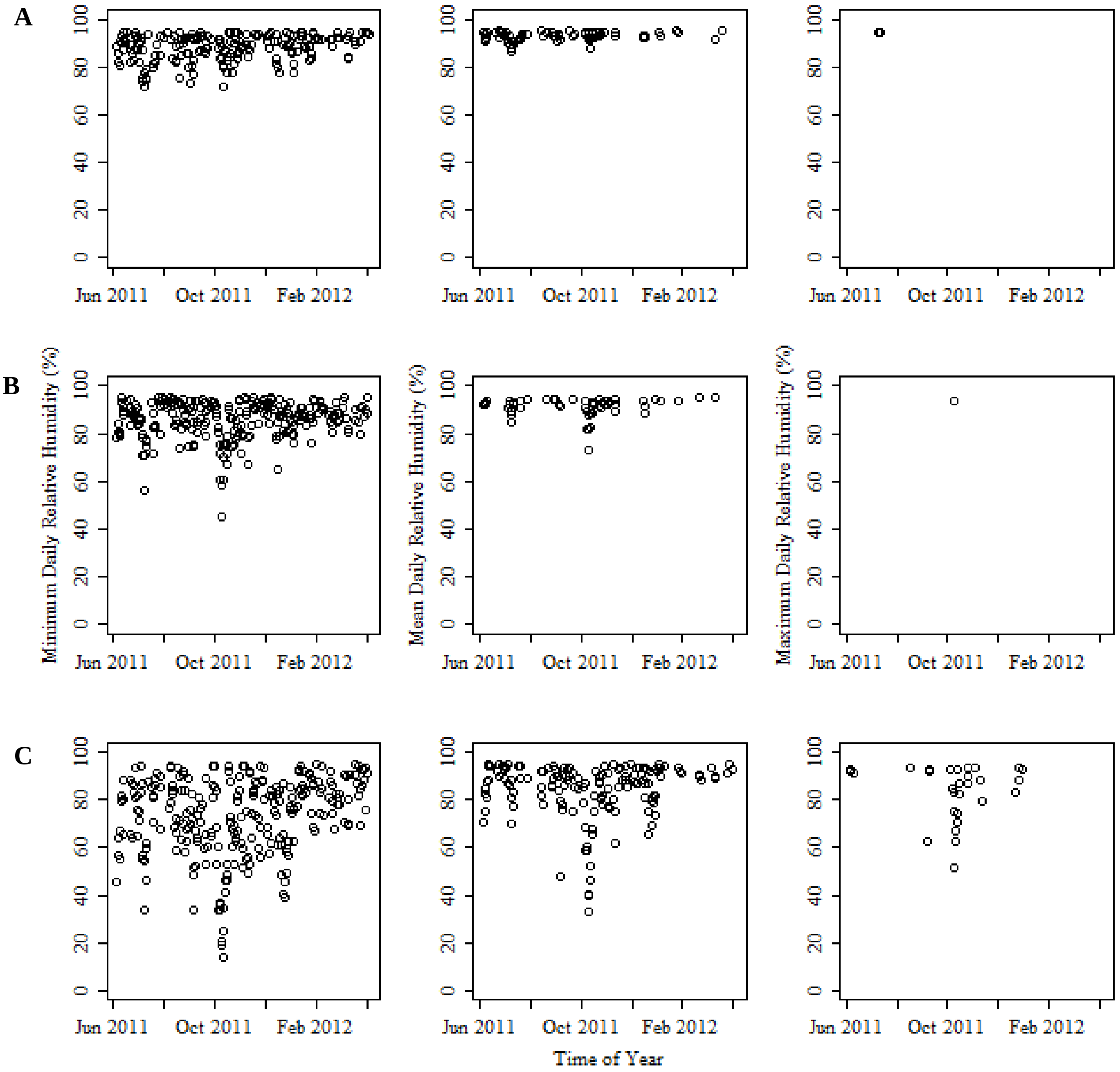


Figure 5: Days where the minimum and mean daily relative humidity fell below 95 % and dry days (maximum daily relative humidity < 95 %) from June 2011 to May 2012 at low- (A), mid- (B) and high-elevation (C) sites along the Piton des Neiges gradient.

The low- elevation site had two dry spells lasting 1 and 2 days, and the mid-elevation site had one dry spell lasting 1 day. The high-elevation site had more dry spells than the other two sites, and was the only site that experienced a dry spell lasting longer than 1 week (Figure 6).

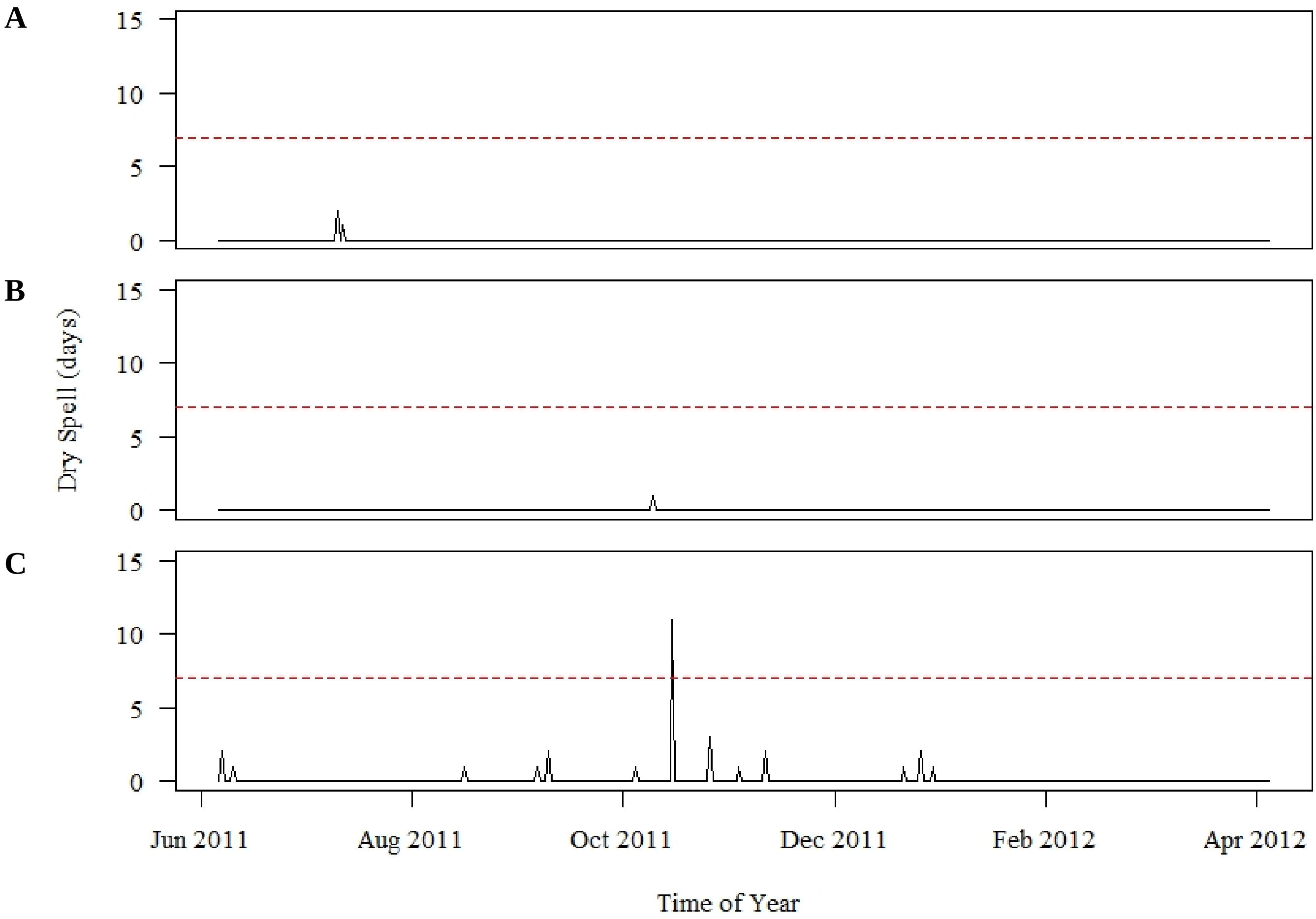


Figure 6: Dry spells (consecutive dry days) from June 2011 to May 2012 at low- (A), mid- (B) and high-elevation (C) sites along the Piton des Neiges gradient. The red line marks seven days.

Dehydration Experiment

Range-restricted species

Low-elevation species lost 50 % of their function at an average of 1.21 g H_2O g^{-1} dry weight (± SE 0.12 g H_2O g^{-1} dry weight). Two of the three low-elevation species lost 50 % of their function at relatively high water contents: *Leucoloma longifolium* at 1.67 g H_2O g^{-1} dry weight (± SE 0.10 g H_2O g^{-1} dry weight) and *Ectropothecium chenagonii* at 1.30 g H_2O g^{-1} dry weight

(± SE 0.21 g H_2O g^{-1} dry weight). *Porotrichum elongatum* had a dry-down response more similar to that of mid- and high-elevation species and lost 50 % function at 0.75 g H_2O g^{-1} dry weight (± SE 0.09 g H_2O g^{-1} dry weight).

Mid-elevation species lost 50 % of their function at an average of 1.14 g H_2O g^{-1} dry weight (± SE 0.17 g H_2O g^{-1} dry weight). Two of the three mid-elevation species lost 50 % function at relatively low water contents with *Macromitrium serpens* losing 50 % function at 0.84 g H_2O g^{-1} dry weight (± SE 0.14 g H_2O g^{-1} dry weight) and *Atrichum androgynum* at 0.60 g H_2O g^{-1} dry weight (± SE 0.05 g H_2O g^{-1} dry weight). *Calyptrochaeta asplenioides* was the most sensitive to water loss and lost 50 % function at the highest water content of all species (1.88 ± 0.20 g H_2O g^{-1} dry weight).

High-elevation species lost 50 % of their function at an average of 0.62 g H_2O g^{-1} dry weight (± SE 0.08 g H_2O g^{-1} dry weight). All three high-elevation species lost 50 % of their function at similarly low water contents with *Ulota fulva* losing 50 % function at 0.73 g H_2O g^{-1} dry weight (± SE 0.10 g H_2O g^{-1} dry weight), *Leptodontium stellatifolium* at 0.69 g H_2O g^{-1} dry weight (± SE 0.19 g H_2O g^{-1} dry weight) and *Racomitrium membranaceum* at 0.43 g H_2O g^{-1} dry weight (± SE 0.11 g H_2O g^{-1} dry weight).

These results show a decreasing trend in coefficient *c* with elevation, indicating that low-elevation species lost half of their function at higher water contents than high-elevation species. However, there were some species-specific responses that did not follow the trend, e.g. *P. elongatum* and *C. asplenioides*. However, with so few species from each elevational site it is difficult to make broad comparisons across sites, especially with the idiosyncratic behaviour of individual species. Regardless of these species-level idiosyncrasies, the results of the Kruskal-Wallis test support the visual trend, where coefficient *c* was significantly higher for low-elevation species than high-elevation species, with mid-elevation species having an intermediate response (K = 12.245, df = 2, $p < 0.01$) (Figure 8; Table 4).

Table 4: Differences in mean (± SE) coefficient *c* (the x-value of the sigmoid's midpoint) for the dry-down curves of range-restricted species at the three sites. Results of Kruskal-Wallis test comparing differences between sites ($p < 0.05$) are indicated by superscript letters.

Site	Coefficient *c*
Low-elevation species	1.21^{a} (± 0.12)
Mid-elevation species	1.14^{ab} (± 0.17)
High-elevation species	0.62^{b} (± 0.08)

Widespread species

Mastigophora diclados:

Low-elevation samples of *M. diclados* lost 50 % of their function at a significantly higher water content than high-elevation samples, with mid-elevation samples having an intermediate response (K= 7.3399, df = 2, $p < 0.05$): Low-elevation samples lost 50% function at 1.06 H_2O g^{-1} dry weight (± SE 0.12 g H_2O g^{-1} dry weight), mid-elevation samples at 0.90 g H_2O g^{-1} dry weight (± SE 0.10 g H_2O g^{-1} dry weight) and high-elevation samples at 0.60 g H_2O g^{-1} dry weight (± SE 0.04 g H_2O g^{-1} dry weight).

Pyrrhobryum spiniforme:

There was a slight trend of coefficient *c* decreasing with elevation, however the differences between elevations were not significant ($F_{2,12} = 0.974$, $p > 0.05$): Low-elevation samples lost half of their function at 1.10 g H_2O g^{-1} dry weight (±SE 0.22 g H_2O g^{-1} dry weight), mid-elevation samples at 0.91 g H_2O g^{-1} dry weight (±SE 0.20 g H_2O g^{-1} dry weight) and high-elevation samples at 0.75 g H_2O g^{-1} dry weight (±SE 0.06 g H_2O g^{-1} dry weight).

Dicranoloma billardierei:

Mid-elevation samples of *D. billardierei* lost 50 % of their function at a significantly higher water contents than high-elevation samples (t = -2.3001, df = 10, $p < 0.05$): Mid-elevation samples lost 50% function at 1.30 g H_2O g^{-1} dry weight (± SE 0.17 g H_2O g^{-1} dry weight) and high-elevation samples at 0.67 g H_2O g^{-1} dry weight (± SE 0.19 g H_2O g^{-1} dry weight).

Holomitrium borbonicum:

There was no significant difference in the water content at which mid- and high-elevation *H. borbonicum* lost 50 % of their function ($t = 0.2012$, $df = 12$, $p > 0.05$): Mid-elevation samples lost 50 % of their function at 0.84 g H_2O g^{-1} dry weight (± SE 0.07 g H_2O g^{-1} dry weight) and high-elevation samples at 0.86 g H_2O g^{-1} dry weight (± SE 0.08 g H_2O g^{-1} dry weight).

Schlotheimia badiella:

There was no significant difference in the water content at which mid- and high-elevation *S. badiella* lost 50 % of their function ($t = 0.60717$, $df = 3.0552$, $p > 0.05$): Mid-elevation samples lost 50 % of their function at 0.61 g H_2O g^{-1} dry weight (± SE 0.04 g H_2O g^{-1} dry weight) and high-elevation samples at 0.84 g H_2O g^{-1} dry weight (± SE 0.37 g H_2O g^{-1} dry weight).

Three of the five widespread species showed a slight decrease in coefficient c with elevation, with two being significant. However, the small sample size of low-elevation species poses an issue with regard to how significant this trend is. Overall, widespread species showed very similar dry-down responses regardless of elevation of origin (Figure 10).

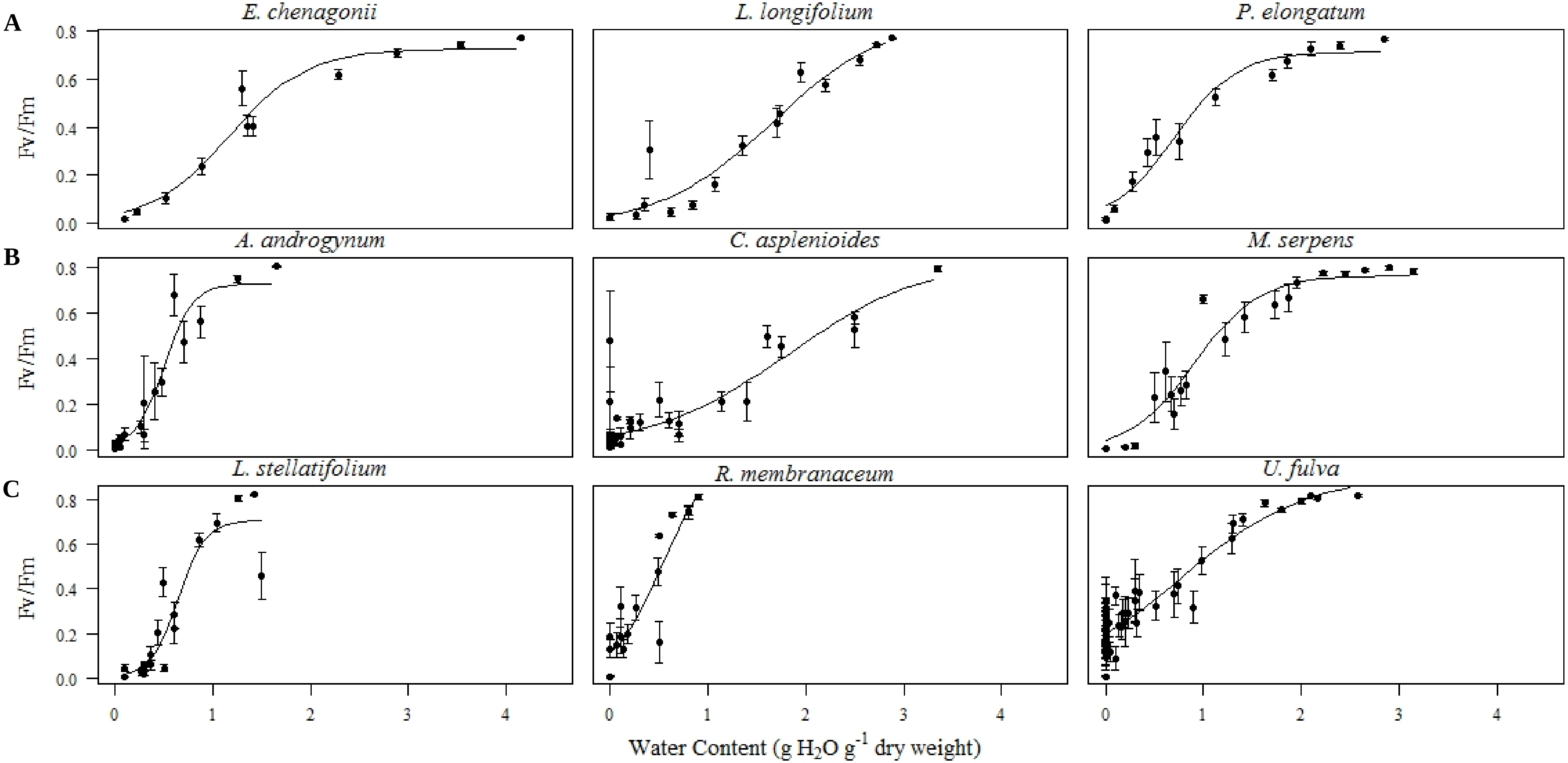


Figure 7: Dry-down curves showing the relationship between photosynthetic efficiency (F_v/F_m) and water content (g H_2O g^{-1} dry weight) for range-restricted bryophyte species sampled at low- (A), mid- (B) and high-elevation (C) sites along the Piton des Neiges gradient (mean ± SE).

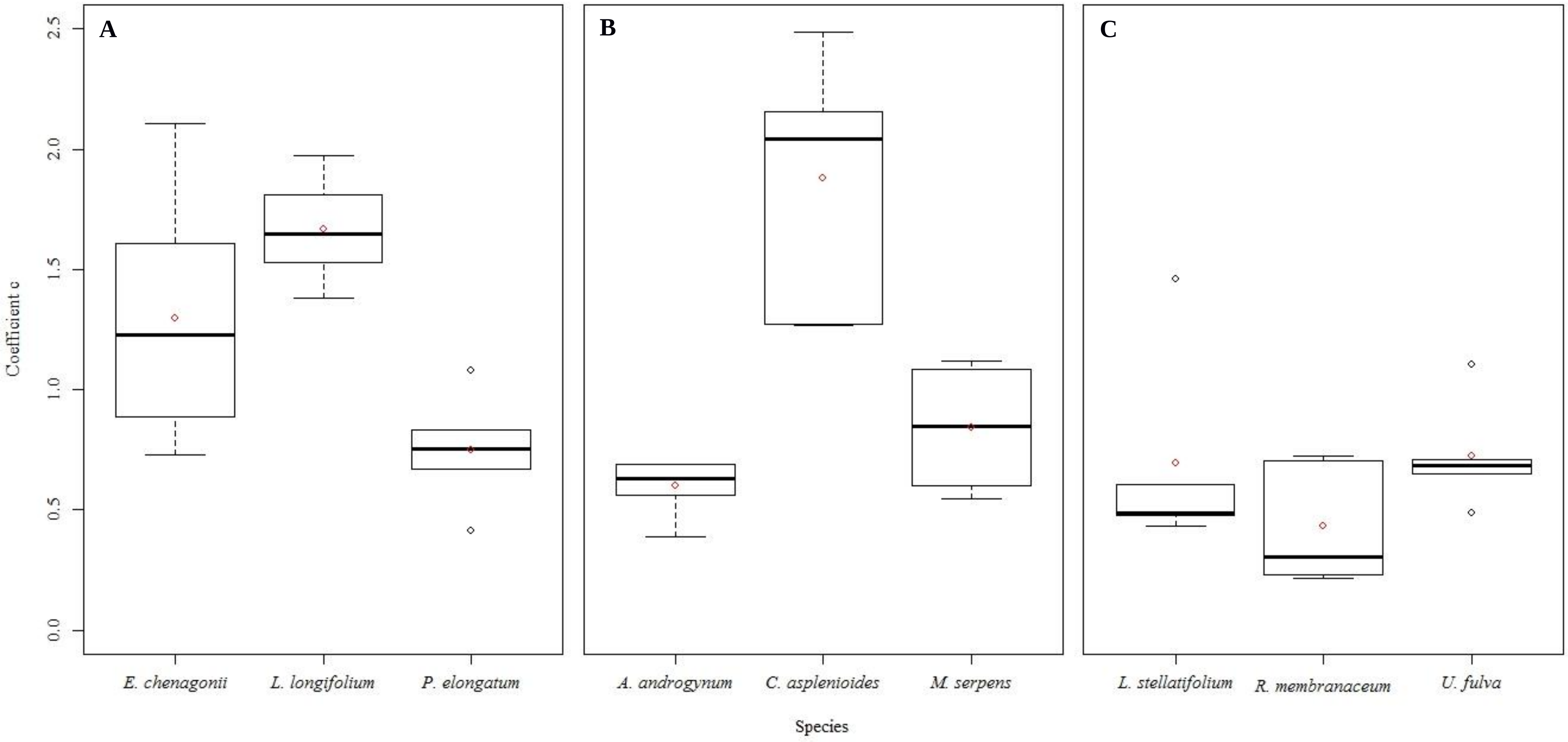


Figure 8: Coefficient *c* extracted from the dry-down curves of each sample of range-restricted species from low- (A), mid- (B) and high-elevation (C) sites along the Piton des Neiges gradient. Red circles indicate means of the data.

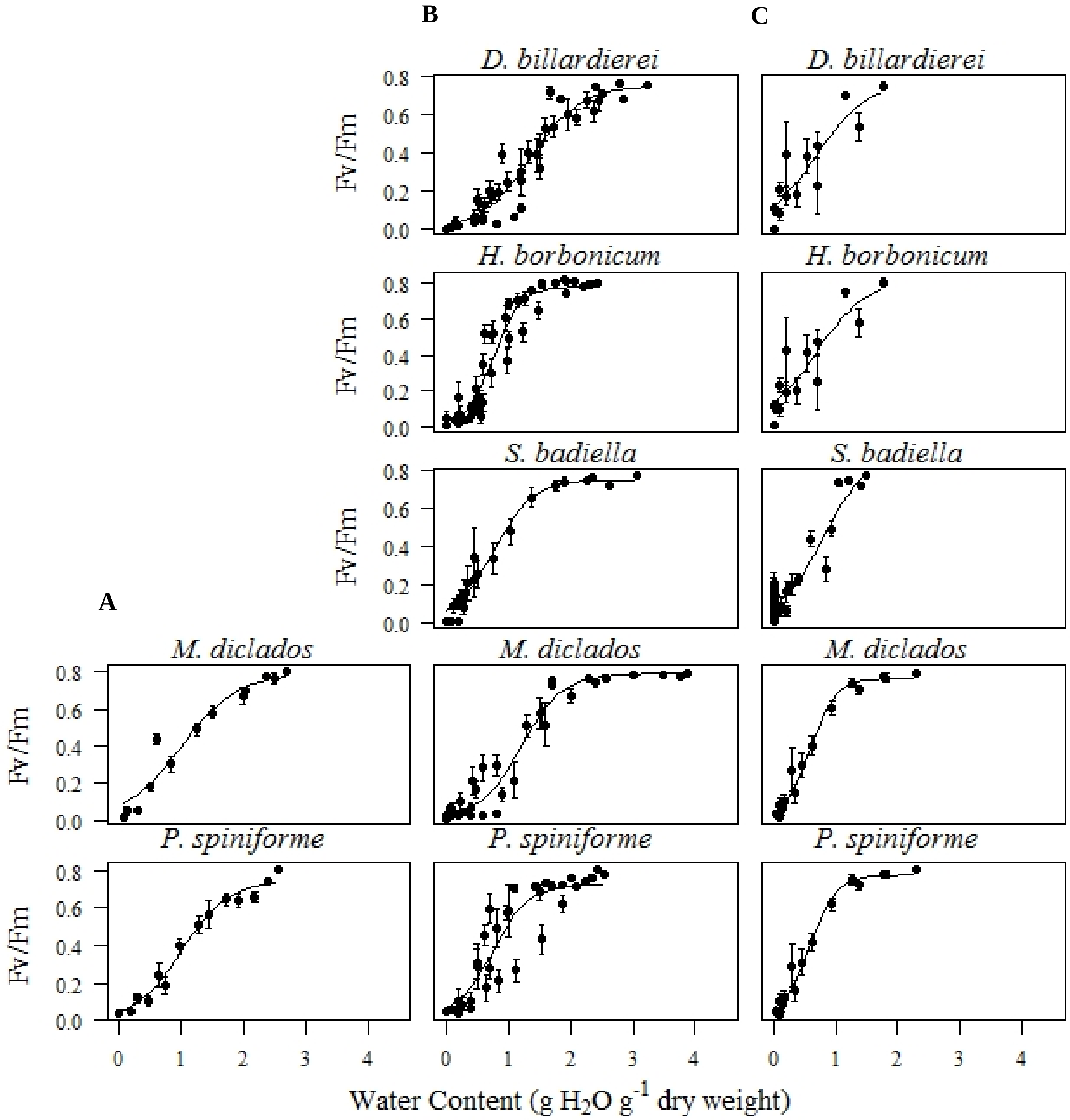


Figure 9: Dry-down curves showing the relationship between photosynthetic efficiency (F_v/F_m) and water content (g H_2O g^{-1} dry weight) for widespread bryophyte species sampled at low- (A), mid- (B) and high-elevation (C) sites along the Piton des Neiges gradient (mean ± SE).

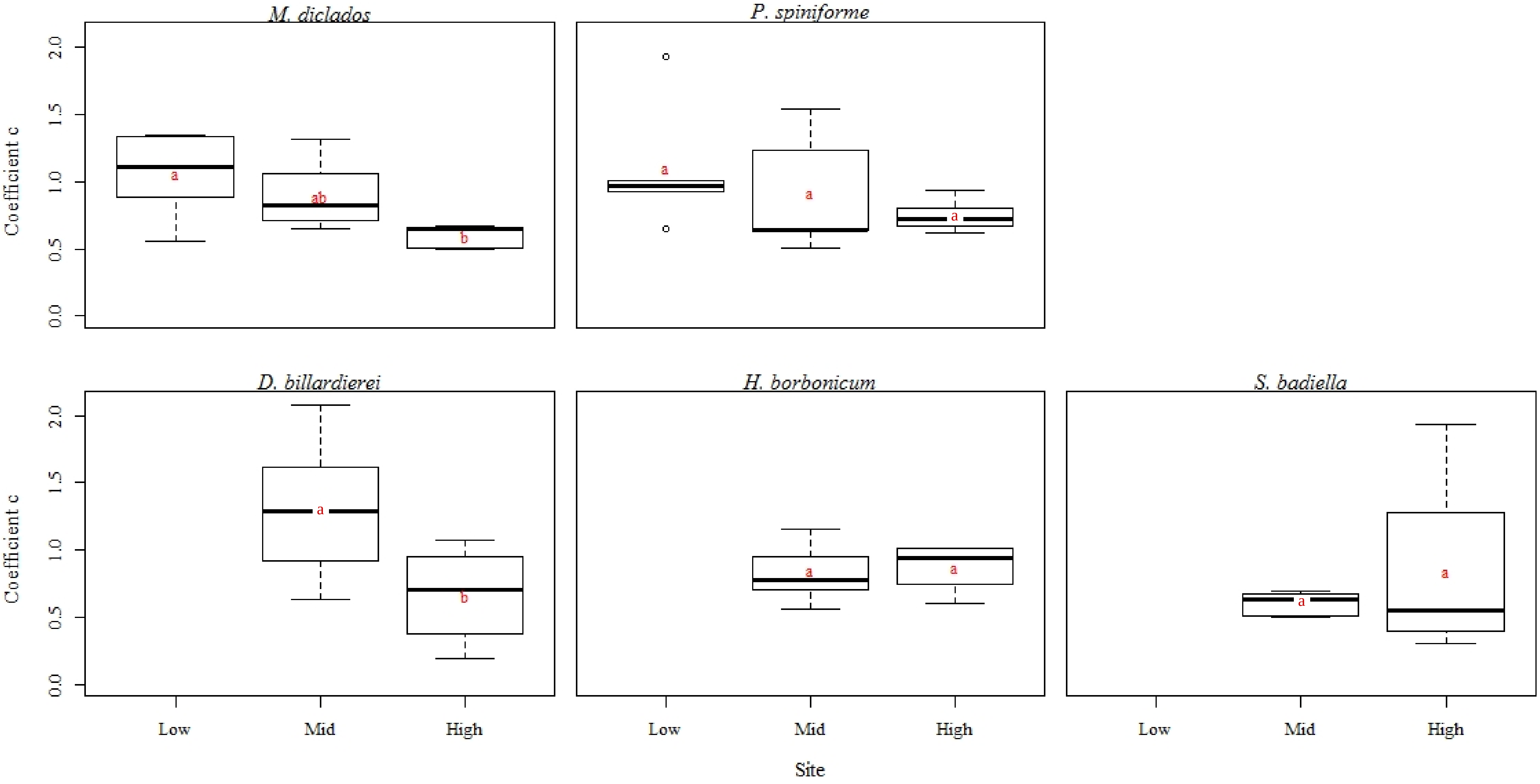


Figure 10: Coefficient *c* extracted from the dry-down curves of each sample of widespread species from low-, mid- and high elevation sites along the Piton des Neiges gradient. Different letters indicate means of the data and represent results of ANOVA, Kruskal-Wallis and t-tests indicating significant differences between sites ($p < 0.05$).

Rehydration Experiment

Range-restricted species

One Week:

Low-elevation species generally exhibited low levels of recovery, with an average of 22.70 % (± SE 5.35 %) total recovery 24 hours after rehydration. This differed widely across the three species: *E. chenagonii* recovered 3.37 % (± SE 1.88 %), *L. longifolium* 19.60 % (± SE 4.94 %) and *P. elongatum* 44.60 % (± SE 8.20 %).

Two of the three mid-elevation species showed an intermediate response, recovering more function than the low-elevation species, but less than the high-elevation species: *A. androgynum* recovered 53.37 % (± SE 12.36 %) and *M. serpens* recovered 89.19 % (± SE 1.48 %). As in the dehydration experiment, *C. asplenioides* had a response inconsistent with the other two mid-elevation species, recovering only 1.47 % (± SE 0.67 %).

High-elevation species recovered consistently high levels of function, with an average of 95.71 % (± SE 1.25 %) total recovery 24 hours after rehydration. *U. fulva* recovered 94.63 % (± SE 1.68 %), *R. membranaceum* 95.92 % (± SE 1.52 %) and *L. stellatifolium* 96.58 % (± SE 2.95 %).

Evident from these results is a trend of increasing recovery with elevation, which is supported by the results of the Kruskal-Wallis test indicating that low- and mid-elevation species recovered significantly less function than high-elevation species ($K = 29.425$, $df = 2$, $p < 0.001$). However, due to the small sample size of species from each elevational site, it is unclear whether the very different response of *C. asplenioides* is simply an outlier, or whether similar responses would emerge in other species if more species were included in the analysis. Therefore, the ANOVA result should also be considered: low-elevation species recovered significantly less function than high-elevation species, with mid-elevation species recovering intermediate levels of function ($F_{2,45} = 31.1$, $p < 0.001$). Nevertheless, there is a clear difference between the levels of recovery between low- and high-elevation species following one week of dehydration (Figure 11, Table 5).

Seven Weeks:

Following seven weeks of dehydration all three low elevation species exhibited very low levels of function, with an average of 3.79 % (± SE 1.80 %) total recovery 24 hours after rehydration. *L. longifolium* recovered 0.38 % (± SE 0.04 %), *E. chenagonii* 0.50 % (± SE 0.10 %) and *P. elongatum* 9.93 % (± SE 4.22 %).

Two of the three mid-elevation species recovered similarly low levels of function: *C. asplenioides* recovered 1.40 % (± SE 0.41 %) and *A. androgynum* 4.18 % (± SE 2.61 %). *M. serpens* recovered intermediate levels of function with a mean of 50.47 % (± SE 10.30 %). The mean total recovery of mid-elevation species was 14.71 % (± SE 5.89 %).

High-elevation species recovered high levels of function, with an average of 76.53 % (± SE 3.64 %) total recovery 24 hours after rehydration. *U. fulva* recovered 60.03 % (± SE 1.69 %), *L. stellatifolium* 80.78 % (± SE 4.91 %) and *R. membranaceum* 88.77 % (± SE 1.17 %).

As with the one-week rehydration experiment there is a clear difference between the recovery of low- and high-elevation species following seven weeks of desiccation, which is supported by the results of the Kruskal-Wallis test indicating that low- and mid-elevation species recovered significantly less function than high-elevation species (K = 29.95, df = 2, $p < 0.001$). Low- and high-elevation species had fairly consistent responses to rehydration. Mid-elevation species, on average, recovered low levels of function, but individual species had very different responses and a bigger sample size is needed to gain more information about the general response of mid-elevation species (Figure 12; Table 5).

Table 5: Differences in mean (± SE) percent total recovery of range-restricted species at the three sites following one and seven weeks of desiccation. Results of Kruskal-Wallis test comparing differences between sites ($p < 0.05$) are indicated by superscript letters.

Differences in total recovery between sites		
Site	One-week rehydration	Seven-week rehydration
Low-elevation species	22.70^{a} (± 5.35) %	3.79^{A} (± 1.80) %
Mid-elevation species	42.87^{a} (± 10.03) %	14.71^{A} (± 5.89) %
High-elevation species	95.71^{b} (± 1.25) %	76.53^{B} (± 3.64) %

All range-restricted species recovered less function after seven weeks than after one week of desiccation, with seven of the nine having significantly lower recovery (Table 6). Significant differences did not occur in *E. chenagonii* and *C. asplenioides*, which recovered almost no function, even after only one week of desiccation.

Table 6: Differences in mean (± SE) percent total recovery of range-restricted species following one and seven weeks of desiccation. Results of Mann-Whitney U and two-sample t-test comparing differences between the one-week and seven-week rehydration experiment ($p < 0.05$) are indicated by superscript letters.

Site	Species	Total recovery (1 week)	Total recovery (7 weeks)	Test statistics
Low	*Ectropothecium chenagonii*	3.37^{a} (± 1.88) %	0.50^{a} (± 0.10) %	W = 21, $p > 0.05$
Low	*Leucoloma longifolium*	19.60^{a} (± 4.94) %	0.38^{b} (± 0.04) %	t = 3.8902, df = 4.0006, $p < 0.05$
Low	*Porotrichum elongatum*	44.60^{a} (± 8.20) %	9.93^{b} (± 4.22) %	t = 3.761, df = 10, $p < 0.01$
Mid	*Atrichum androgynum*	53.37^{a} (± 12.36) %	4.18^{b} (± 2.61) %	W = 35, p = 0.01
Mid	*Calyptrochaeta asplenioides*	1.47^{a} (± 0.67 %)	1.40^{a} (± 0.41) %	W = 13.5, $p > 0.05$
Mid	*Macromitrium serpens*	89.19^{a} (± 1.48) %	50.47^{b} (± 10.30) %	t = 3.7224, df = 3.1244, $p < 0.05$
High	*Leptodontium stellatifolium*	96.58^{a} (± 2.95) %	80.78^{b} (± 4.91) %	t = 2.6704, df = 8, $p < 0.05$
High	*Racomitrium membranaceum*	95.92^{a} (± 1.52) %	88.77^{b} (± 1.17) %	t = 3.3169, df = 8, $p < 0.05$
High	*Ulota fulva*	94.63^{a} (± 1.68) %	60.03^{b} (± 1.69) %	t = 14.527, df = 8, $p < 0.001$

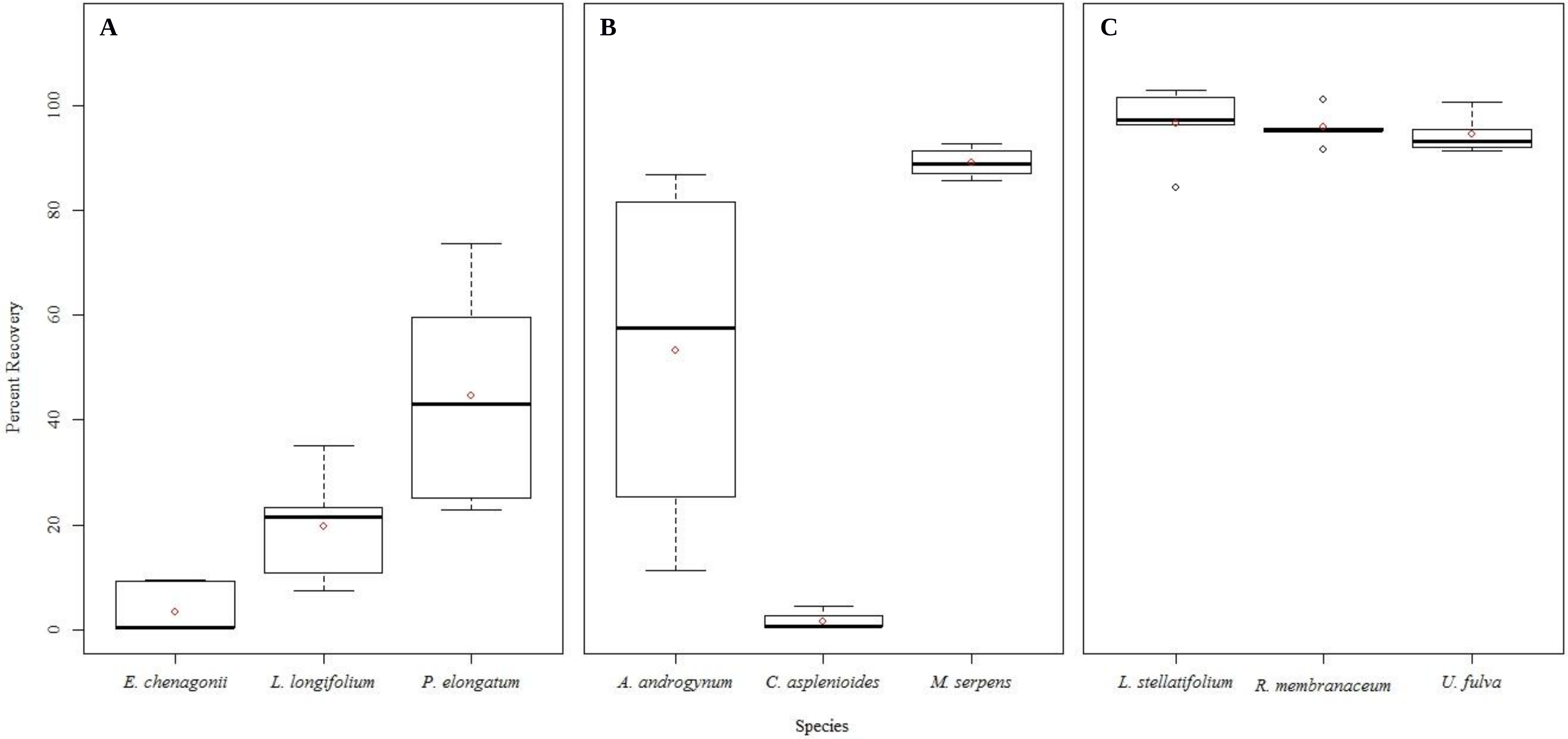


Figure 11: Percent total recovery of photosynthetic efficiency (F_v/F_m) of samples of each range-restricted species from low- (A), mid- (B) and high-elevation sites (C) following one week of dehydration. Red circles indicate means of the data.

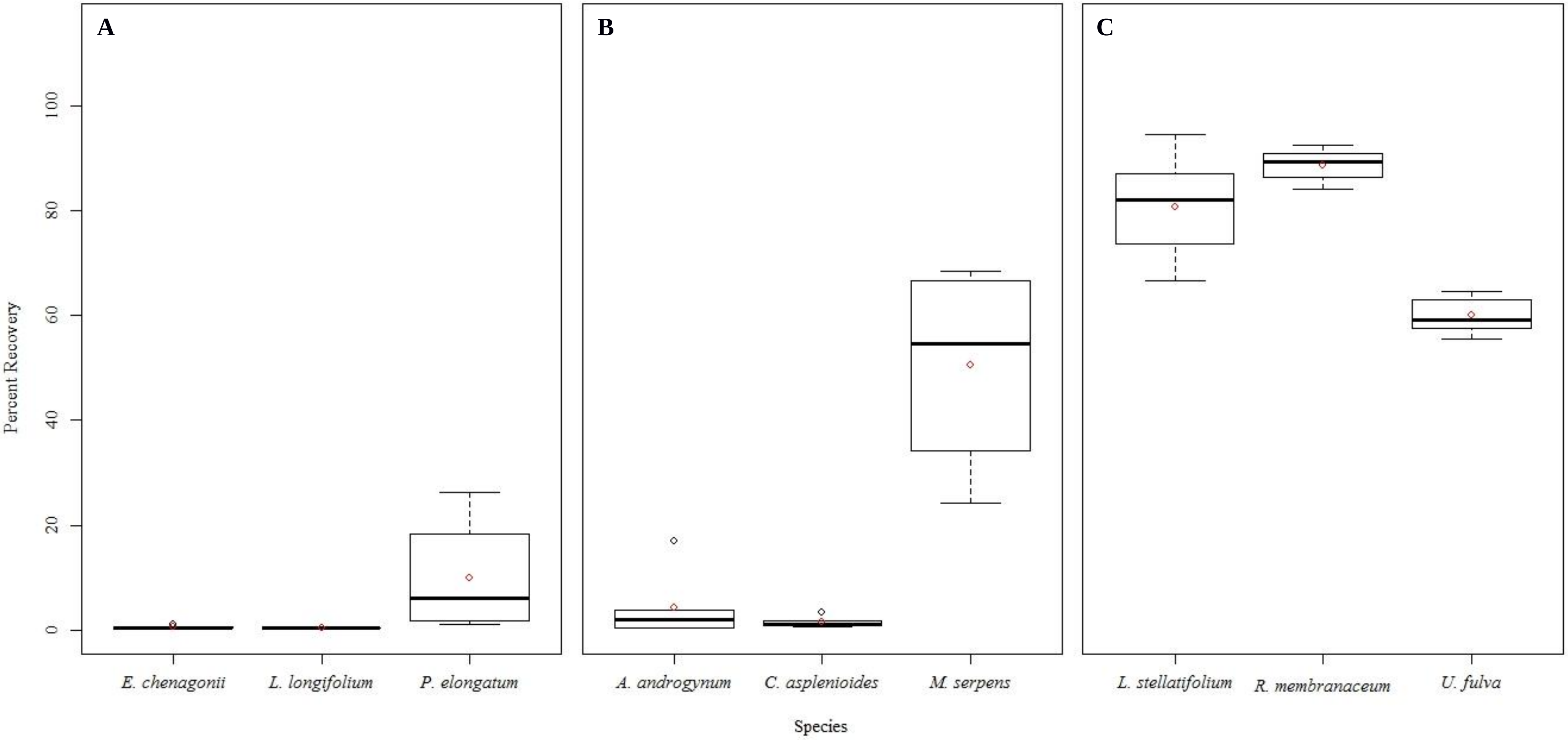


Figure 12: Percent total recovery of photosynthetic efficiency (F_v/F_m) of samples of each range-restricted species from low- (A), mid- (B) and high-elevation sites (C) following seven week of dehydration. Red circles indicate means of the data.

Widespread species

One Week:

Mastigophora diclados:

There was a small increase in recovery of *M. diclados* with elevation following one week of dehydration, but the differences between sites were not significant (K = 4.9033, df = 2, p > 0.05): Low-elevation samples recovered 77.64 % (± SE 6.97 %), mid-elevation samples recovered 78.03 % (± SE 7.57 %) and high-elevation samples recovered 93.13 % (± SE 3.26 %).

Pyrrhobryum spiniforme:

Low-elevation samples of *P. spiniforme* recovered significantly less function than mid- and high-elevation samples after one week of dehydration (K = 9.7059, df = 2, p < 0.01): Low-elevation samples recovered 9.13 % (± SE 6.70 %), mid-elevation samples recovered 85.07 % (± SE 1.42 %) and high-elevation samples recovered 87.67 % (± SE 4.51 %).

Dicranoloma billardierei:

Following one week of desiccation, mid-elevation samples of *D. billardierei* recovered 70.37 % (± SE 9.48 %) function that was significantly lower than the 102.72 % (± SE 5.79 %) recovered by high-elevation samples (W = 35, p < 0.05).

Holomitrium borbonicum:

There was no significant difference in the total recovery of mid- (93.90 ± SE 1.21 %) and high-elevation (96.34 ± SE 2.61 %) samples of *H. borbonicum* (W = 26, p > 0.05).

Schlotheimia badiella:

There was no significant difference in the total recovery of mid- (89.14 ± SE 4.25 %) and high-elevation (96.09 ± SE 1.54 %) samples of *S. badiella* (W = 25, p-value = 0.05).

As in the dehydration experiment, the same three species showed a slight increase in recovery with elevation, with two being significant. However, the small sample size of low-elevation species poses an issue with regard to how significant this trend is. Overall, following one week of desiccation, widespread species showed similar responses regardless of elevation of origin (Figure 13).

Seven Weeks:

Mastigophora diclados:

No samples of *M. diclados* recovered any measurable function after seven weeks of desiccation.

Pyrrhobryum spiniforme:

Low-elevation samples of *P. spiniforme* recovered significantly less function than mid and- high-elevation samples after seven weeks of dehydration (K = 5.496, df = 2, $p < 0.05$): Low-elevation samples recovered 0.28 % (± SE 0.07 %), mid-elevation samples recovered 33.68 % (± SE 11.72 %) and high-elevation samples recovered 44.26 % (± SE 10.43 %) 24 hours after rehydration.

Dicranoloma billardierei:

Following seven weeks of desiccation, mid-elevation *D. billardierei* samples recovered 30.55 % (± SE 9.28 %) function that was significantly lower than the 89.76 % (± SE 6.19 %) recovered by high elevation samples (t = 4.6074, df = 11, $p < 0.001$).

Holomitrium borbonicum:

Mid-elevation *H. borbonicum* samples recovered 75.41 % (± SE 5.37 %) function that was significantly lower than the 88.70 % (± SE 3.13 %) recovered by high-elevation samples (W = 38, p-value < 0.05).

Schlotheimia badiella:

Mid-elevation *S. badiella* samples recovered 33.42 % (± SE 7.00 %) function that was significantly lower than the 66.92 % (± SE 3.31 %) recovered by high-elevation samples (t = 4.0406, df = 12, $p < 0.01$).

Following 7 weeks of desiccation, separation started to emerge between each elevation, with low-elevation samples recovering almost no function, mid-elevation samples recovering some intermediate level of function and high-elevation species recovering generally high levels of function. Again, a larger sample size of species from low-elevation is needed to make more robust observations about the recovery of low-elevation species following seven weeks of desiccation (Figure 14).

All widespread species recovered less function after seven weeks than after one week of desiccation, with nine of the twelve having significantly lower recovery (Table 7). Significant differences did not occur in *D. billardierei* and *H. borbonicum* from high elevation, which recovered lower, but still relatively high, function following seven weeks of desiccation. *P. spiniforme* from low elevation did not recover significantly less function after seven weeks, as it recovered almost no function, even after only one week of desiccation.

Table 7: Differences in mean (± SE) percent total recovery of widespread species following one and seven weeks of desiccation. Results of Mann-Whitney U and two-sample t-test comparing differences between the one-week and seven-week rehydration experiment ($p < 0.05$) are indicated by superscript letters.

Site	Species	Total recovery (1 week)	Total recovery (7 weeks)	Test statistics
Low	*Mastigophora diclados*	77.64^{a} (± 6.97) %	0^{b} %	W = 36, $p < 0.01$
Mid	*Mastigophora diclados*	78.03^{a} (± 7.57) %	0^{b} %	W = 36, $p < 0.01$
High	*Mastigophora diclados*	93.13^{a} (± 3.26) %	0^{b} %	W = 25, $p < 0.01$
Low	*Pyrrhobryum spiniforme*	9.13^{a} (± 6.70) %	0.28^{a} (± 0.07) %	W = 15.5, $p > 0.05$
Mid	*Pyrrhobryum spiniforme*	85.07^{a} (± 1.42) %	33.68^{b} (± SE 11.72) %	t = 4.3536, df = 5.1462, $p < 0.01$
High	*Pyrrhobryum spiniforme*	87.67^{a} (± 4.51) %	44.26^{b} (± SE 10.43) %	t = 3.8207, df = 8, $p < 0.01$
Mid	*Dicranoloma billardierei*	70.37^{a} (± 9.48) %	30.55^{b} (± SE 9.28) %	W = 57, $p < 0.01$
High	*Dicranoloma billardierei*	102.72^{a} (± 5.79) %	89.76^{a} (± SE 6.19) %	t = 1.529, df = 8, $p > 0.05$
Mid	*Holomitrium borbonicum*	93.90^{a} (± 1.21) %	75.41^{b} (± SE 5.37) %	W = 81, $p < 0.001$
High	*Holomitrium borbonicum*	96.34^{a} (± 2.61) %	88.70^{a} (± SE 3.13) %	W = 20, $p > 0.05$
Mid	*Schlotheimia badiella*	89.14^{a} (± 4.25) %	33.42^{b} (± SE 7.00) %	W = 36, $p < 0.01$
High	*Schlotheimia badiella*	96.09^{a} (± 1.54) %	66.92^{b} (± SE 3.31) %	t = 7.9876, df = 8, $p < 0.001$

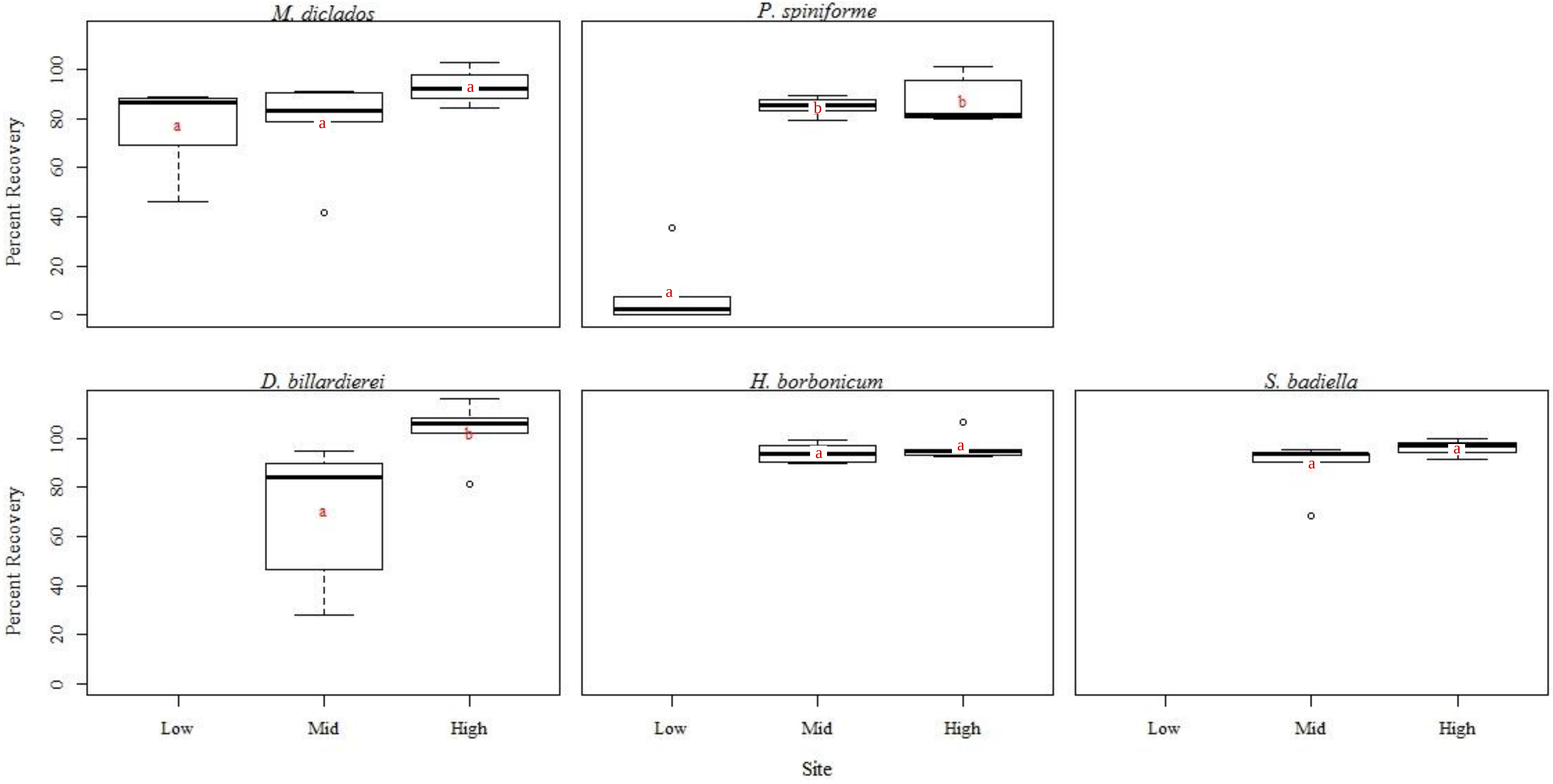


Figure 13: Percent total recovery of photosynthetic efficiency (F_v/F_m) of samples of each widespread species following one week of dehydration. Different letters indicate means of the data and represent results of Kruskal-Wallis and Mann-Whitney U tests indicating significant differences between sites ($p < 0.05$).

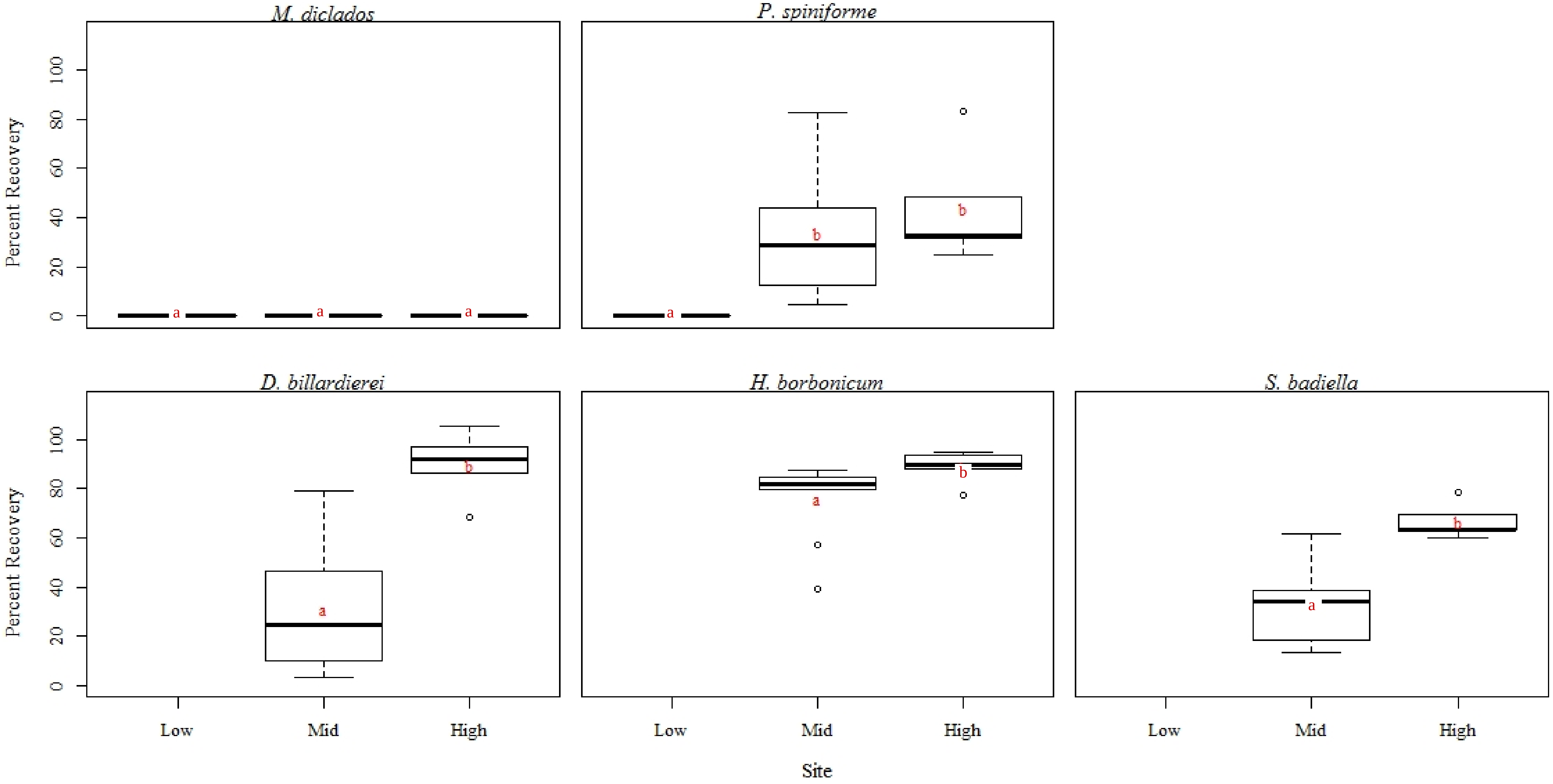


Figure 14: Percent total recovery of photosynthetic efficiency (F_v/F_m) of samples of each widespread species following seven week of dehydration. Different letters indicate means of the data and represent results of Kruskal-Wallis, Mann-Whitney U and t-tests indicating significant differences between sites ($p < 0.05$).

Discussion

Range-restricted species

The dehydration and rehydration experiments showed that tolerance to desiccation increased from low to high elevation in range-restricted species. During dehydration, low-elevation species reduced to half photosynthetic efficiency at water contents significantly higher than those of the high-elevation species, with mid-elevation species having intermediate responses. High-elevation species were, thus, able to maintain almost full photosynthetic efficiency, while dehydrating, at water contents where the low-elevation species were only photosynthesising at half capacity (Figure 7). Furthermore, on average, mid-elevation species recovered almost double the function of low-elevation species after one week of desiccation, with high-elevation species recovering more than double that of mid-elevation species. Low-elevation species recovered significantly less function than the high-elevation species after one week of desiccation, with mid-elevation recovering some intermediate level of function. Following seven weeks of desiccation, both low- and mid-elevation species recovered significantly less function than high-elevation species.

These results support the notion that bryophyte desiccation tolerance is expected to reflect moisture conditions regularly experienced (Proctor, 2001; Proctor et al., 2007), and is in accordance the findings of Robinson et al. (2000) among others (Brown & Buck, 1979; Di Nola et al., 1983; Seel et al., 1992; Oliver et al., 1993; Davey, 1997; Deltoro et al., 1998; Csintalan et al., 1999). Species' desiccation tolerance corresponds with the pattern of water availability seen along the gradient, demonstrating that range-restricted species are specialised to the conditions of relative humidity in their elevation of origin. During the recorded year, the high-elevation site experienced the driest conditions with the lowest relative humidity, the most dry days and the most and longest dry spells, whereas the low- and mid-elevation sites experienced almost no dry days or dry spells (Figures 4, 5 and 6). Due to the temperature inversion causing cloud formation between 1 400 m a.s.l. and 1 600 m a.s.l. (Barcelo, 1996; Ah-Peng et al., 2007), and the comparatively high rainfall at low and mid elevation (12 000 mm p.a.) (Barcelo, 1996), this pattern of dry day occurrence along the Piton des Neiges gradient was largely unsurprising.

Throughout the recorded year, the low- and mid-elevation sites did not experience any week-long dry spells. The high-elevation site had many more dry spells, but only one lasting longer than a week, and none lasting as long as seven weeks (Figure 6). Desiccation tolerance in bryophytes generally mirrors their environmental moisture availability (Proctor, 2001;

Proctor et al., 2007). Thus, the high-elevation species were expected to regain full photosynthetic efficiency when rehydrated after a week of desiccation, and all species were expected to do worse after seven weeks of desiccation. As predicted, the extent of recovery of species from all sites was lower after seven weeks than after one week of desiccation. However, many species still recovered high levels of function after periods of desiccation far exceeding those experienced in their natural habitats. This is similar to the results of Bader et al.'s (2013) study, which found that both lowland and montane bryophytes in Panama survived dry periods lasting much longer the periods of drought naturally experienced. Bader et al. (2013), therefore, concluded that species' tolerance of long dry periods does not explain their elevational distribution. However, in the current study, there is a clear trend of increasing desiccation tolerance with elevation, matching water availability along the gradient. Although species may only very occasionally experience dry periods lasting much longer than a week, survival of these conditions is still necessary for the long-term persistence of a species (Bader et al., 2013). Furthermore, Alpert (2000) maintains that the duration of drought tolerated is often functionally linked to other traits of desiccation tolerance.

Stark (2017) explains the importance of prehydration in recovery from desiccation, although few studies have tested this in bryophytes. Nevertheless, the utilisation of atmospheric water vapour is increasingly thought to be essential in maintaining a positive carbon balance in tropical bryophytes (Pardow & Lakatos, 2012). Due to the notable increase in ambient humidity in the hours before a storm (Stark, 2017), the high levels of humidity in tropical forests are likely to increase recovery from desiccation when liquid water becomes available (Pardow and Lakatos, 2012). Future studies should, therefore, consider the effects of prehydration on rate and extent of recovery.

Widespread species

The small sample size of low-elevation species posed an issue with regard to how significant the trends in the data are, and different patterns may have arisen if all measured species spanned the entire elevational gradient. Therefore, considering the difficulty of comparing the small number of widespread species (especially at low elevation), the following will discuss the overarching trends in the data.

Widespread species from all elevations showed similar responses to dehydration. They also recovered similar levels of function upon rehydration after one week of desiccation, regardless of elevation of origin. These results show that widespread species aren't all uniformly acclimatised to the climatic conditions of their elevation of origin. This is probably because many species are not affected by macroclimatic conditions at different sites, as they occupy microsites with similar microclimatic conditions at each elevation (Chapter 2).

While widespread species generally recovered high levels of function after a week of desiccation, most widespread species did not recover as much function as range-restricted species from high elevation. As all the sampled widespread species occur at high elevation, it would be expected that they should have similar levels of desiccation tolerance to high-elevation specialists. This provides further evidence against the Climate Variability Hypothesis (Stevens, 1989; Stevens, 1992) and Brown's (1984) hypothesis, showing that widespread species do not always have larger environmental tolerances.

As expected, all widespread species recovered less function after seven weeks of desiccation than after one week. In both the dehydration and one-week rehydration experiments some species showed a slight trend of increasing desiccation tolerance with elevation. However, following seven weeks of desiccation, significant differences started to emerge between each elevation, with the trend of increasing desiccation tolerance with elevation in all species except *M. diclados* where all samples died. This suggests that there is some low level of acclimatisation to local climatic conditions, despite the fact that seven weeks far exceeds the longest dry periods experienced along the Piton des Neiges gradient. Alpert (2000) upholds that survival of excessive dry periods is not the best criterion for directly measuring drought tolerance. Nevertheless, the differences in recovery may become important as prolonged dry spells become more frequent under scenarios of climate change (Solomon et al., 2007). However, this is unlikely to have detrimental effects on the species (with the exception of *M. diclados*) as low- and mid-elevation populations would either acclimatise to dryer conditions or those populations that can tolerate long dry periods would be expected to disperse downslope.

Microhabitat and growth form

Although the results of the desiccation experiments for range-restricted species correspond with the macroclimatic conditions experienced along the gradient, microhabitat definitely plays a large role in species' tolerances to desiccation (Hosokawa et al., 1964; Pardow & Lakatos, 2012). It may also be the case that each elevation has more of one specific type of microhabitat, with specific conditions, leading to broad differences between species across elevational sites.

Whereas range-restricted species from high elevation generally had very similar desiccation responses, species-level differences in response to, and recovery from, desiccation were common among low- and mid-elevation species. This can largely be explained by growth form and microhabitat. For example, *C. asplenioides* was particularly sensitive to desiccation, losing function quickly when dehydrated and recovering almost no function upon rehydration. This is perhaps due to the fact that *C. asplenioides* rarely experiences desiccation in the field (Proctor, 2001; Proctor et al., 2007) as it sits flat on the soil, usually in seepages (personal observation).

P. elongatum was somewhat more desiccation tolerant than the other two low-elevation species, behaving more like mid- and high-elevation species. That may be because *P. elongatum* is prone to desiccating in the field (Proctor, 2001; Proctor et al., 2007) owing to its growth form of individual shoots and exposed, epiphytic habit on tree trunks (Bader et al., 2013). Epiphytic habitats in the tropics are sometimes considered xeric, as the substrate does not hold water and high temperatures lead to fast drying (Bader et al., 2013). *M. serpens* recovered more function than the other two mid-elevation species, especially following seven weeks of desiccation. The higher desiccation tolerance of *M. serpens* can be attributed to its epiphytic habit in the low canopy (Hosokawa et al., 1964). *M. serpens* is accustomed to drying, whereas the other two mid-elevation species are terricolous species accustomed to generally wetter conditions. This is consistent with the findings of Pardow and Lakatos (2012) who established that the desiccation tolerance of tropical bryophytes increases with height above ground.

Among the widespread species, *P. spiniforme* from low elevation showed an anomalous rehydration response, recovering almost no function. Interestingly, the low-elevation populations of *P. spiniforme* on Réunion have occasionally been recognised as a separate taxon at varietal (*P. spiniforme* var. *brevifolium* (Besch.) Manuel) or even species level (Manuel, 1980). The drastically different response in recovery from desiccation is of considerable interest in this context and warrants investigation into the taxonomic status of the populations.

The longer and more intense dry spells predicted under climate-change scenarios (Solomon et al., 2007) may be detrimental to species sensitive to desiccation (Pardow & Lakatos, 2012). The loss of wetter microhabitat spaces may lead to higher rates of extinction in those species reliant on wet microsites (Pardow & Lakatos, 2012). However, species may possess the ability to acclimatise to gradual changes in precipitation. This was illustrated by the survival of some samples of most transplanted species in the dry conditions of high elevation. At least some individuals in the population may have the ability to acclimatise to dryer conditions over the short timeframe of a year (Chapter 2).

The influence of desiccation on range-restricted bryophyte distribution

Desiccation tolerance in range-restricted species may partly explain the pattern of survival seen in the reciprocal transplant experiment (Chapter 2). The low desiccation tolerance of range-restricted species from low elevation provides an explanation as to why they were unable to survive the dry conditions of high elevation. However, mid elevation has conditions of relative humidity similar to that of low elevation (Figures 4, 5 and 6), which may have facilitated the high survival rates of low-elevation species at mid elevation. Despite these similar conditions of relative humidity, mid-elevation species were somewhat more desiccation tolerant than low-elevation species. The greater ability of mid-elevation species to tolerate drying may have allowed them to survive transplantation to high elevation. However, it should be noted that the desiccation-sensitive *C. asplenioides* was not included in the transplant experiment (due to difficulties in locating enough samples).

However, this does not give much insight into why range-restricted species from mid- and high-elevation had low survival rates at lower elevations. The interaction between temperature and humidity is thought to be an important factor in bryophyte distribution (Glime, 2007; He et al., 2016). Therefore, in order to gain a more complete picture, the following chapter focuses on species' responses to temperature.

CHAPTER 4

Variations in temperature response as an explanation for bryophyte range size and distribution along the Piton des Neiges gradient, Réunion Island

Introduction

Temperature is one of the major factors shaping bryophyte distribution (Glime, 2007; He et al., 2016). Although there is generally a smaller seasonal range of temperatures in the tropics than in other regions, the carbon losses to respiration at high temperatures can limit bryophyte ranges (Glime, 2007). Furthermore, there is thought to be a large number of thermal specialists among tropical species adapted to the narrow range of temperatures experienced between seasons in the tropics (Janzen, 1967). As a consequence, tropical species tend to be elevationally specialised (Ghalambor et al., 2006; Colwell et al., 2008), with narrow elevational ranges (Laurance et al., 2011), as there is a large variation in temperature with elevation (Barcelo, 1996). For example, Wagner et al. (2013) and Lösch et al. (1994) found that tropical bryophytes tend to be physiologically well adapted to the mean daytime temperatures in their elevation of origin. Wagner et al. (2013) found this to be true of bryophytes worldwide, from a wide range of ecosystems, whose temperature optima of photosynthesis were found to closely match those prevailing in their habitats, an adaptation that could restrict species to certain elevational ranges (Furness & Grime, 1982; Wagner et al., 2013). However, there is some evidence to suggest that bryophytes have the ability to acclimatise to the temperature conditions of new habitats (Hicklenton & Oechel, 1976; Wagner et al., 2014a), which is important for the survival and growth of populations, especially in a changing climate (Jägerbrand & Kudo, 2016). However, little is known on the topic of thermal acclimation in bryophytes (Wagner et al., 2013; Wagner et al., 2014a; Jägerbrand et al., 2014).

Wagner et al. (2014a) showed that tropical bryophytes transplanted to lower elevations with higher temperatures had low success rates compared to those transplanted in their site of origin. However, some did survive, which demonstrates that there is the possibility of acclimatisation to different temperature conditions.

Jägerbrand et al. (2014) compared temperature responses of samples of *Pleurozium schreberi* (Willd. ex Brid.) Mitt. from eight different elevations on the gradient of Mt. Oakan, Japan, using chlorophyll fluorescence, among other techniques. They found site-specific differences between the populations, i.e. response to increased temperature depended on elevation of origin.

According to He et al. (2016) and others (Frahm, 1990; Glime, 2007) tropical bryophytes have a narrow range of temperature optima around 25 - 26 °C (Frahm, 1987; Wagner et al., 2013). Temperatures on either side of this optimum are expected to lead to rapid declines in photosynthesis. High temperatures seem to be especially damaging, particularly when hydrated (Clausen, 1964; He et al., 2016), with tissue injury occurring at temperatures above 40 °C (Larcher, 2003). Because bryophytes are poikilohydric, they are sensitive to water losses as humidity declines at elevated temperatures. Temperatures above 30 °C generally lead to desiccation in the upper portions of the shoots, therefore limiting photosynthesis (He et al., 2016). Furthermore, Frahm (1987) demonstrated that the combination of low light levels and temperatures at 30 °C and higher led to death of tropical montane bryophytes within 24 hours.

Elevational gradients provide an easy way to compare populations naturally adapted to different temperature conditions (Jägerbrand et al., 2014). Therefore, in this chapter I aimed to test whether differences in habitual temperature conditions can be correlated with the elevational distribution of bryophytes along the Piton des Neiges gradient, Réunion Island.
Chlorophyll fluorescence has been demonstrated as a useful tool in bryophyte desiccation research (e.g. Csintalan et al., 1999; Proctor, 2003; Bader et al., 2013), tracking bryophyte light response curves (e.g. Tobias & Niinemets, 2010; Proctor & Bates, 2018), as well as in studies of temperature stress in vascular plants (e.g. Kitao et al., 2000). However, relatively few studies have utilised this useful, non-invasive method to measure bryophyte temperature response (e.g. Meyer & Santarius, 1998; Jägerbrand et al., 2014; Jägerbrand & Kudo, 2016; Taylor et al., 2017). Hence, I tracked photosynthetic efficiency (Φ_{PSII} and F_v/F_m) using chlorophyll fluorescence, over an ecologically relevant acclimation period, in species with varying elevational range sizes under different temperature conditions. I hypothesised that species' acclimated temperature optima for photosynthesis would decrease with increasing elevation. I expected low-elevation species to better tolerate high temperatures and high-elevation species to better tolerate low temperatures. I predicted that widespread species would have a similar response to temperature, regardless of elevation of origin.

Methods

Climate data

See Chapter 3 methods.

Sample collection

Sample collection was done at all three sites on 9 July 2018. The thirteen species chosen for the reciprocal transplants were also used in this experiment (Table 8). Five replicates of each range-restricted species were sampled from their respective sites and five replicates of each widespread species were sampled from each site. Sample size varied between species, as a sample was considered suitable when the size and growth form were representative of that species. Care was taken to maintain the structural integrity of each sample. Samples were taken from separate clumps to increase the chances of sampling different genotypes within the population. Each sample was placed in a re-sealable plastic bag to prevent desiccation and transported to the Réunion lab in an ice chest with insulated freezer blocks to keep samples cool. Samples were stored in the ice chest overnight to keep them cool and prevent temperature fluctuation.

Table 8: Location, range and number of bryophyte samples collected along the Piton des Neiges gradient.

Species	Range	Site	No. samples collected
Ectropothecium chenagonii Renauld & Cardot	Restricted	Low	5
Leucoloma longifolium (Brid.) Wijk & Margad.	Restricted	Low	5
Porotrichum elongatum (Welw. & Duby) A.Gepp	Restricted	Low	5
Mastigophora diclados (Brid. ex F.Weber) Nees	Widespread	Low	5
Pyrrhobryum spiniforme (Hedw.) Mitt.	Widespread	Low	5
Schlotheimia badiella Besch.	Widespread	Low	5
Atrichum androgynum (Müll. Hal.) A. Jaeger	Restricted	Mid	5
Macromitrium serpens (Bruch ex Hook. & Grev.) Brid.	Restricted	Mid	5
Plagiochila terebrans Nees & Mont. ex Lindenb.	Restricted	Mid	5
Mastigophora diclados (Brid. ex F.Weber) Nees	Widespread	Mid	5
Pyrrhobryum spiniforme (Hedw.) Mitt.	Widespread	Mid	5
Schlotheimia badiella Besch.	Widespread	Mid	5
Hypnum cupressiforme Hedw.	Restricted	High	5
Leptodontium stellatifolium (Hampe) Broth.	Restricted	High	5
Racomitrium membranaceum (Mitt.) Paris	Restricted	High	5
Ulota fulva Brid.	Restricted	High	5
Mastigophora diclados (Brid. ex F.Weber) Nees	Widespread	High	5
Pyrrhobryum spiniforme (Hedw.) Mitt.	Widespread	High	5
Schlotheimia badiella Besch.	Widespread	High	5

Chlorophyll-fluorescence

Temperature response curves were measured using chlorophyll fluorescence. Two parameters of chlorophyll fluorescence were measured using a modulated chlorophyll fluorometer (MINI-PAM Portable Chlorophyll Fluorometer; H. Walz, Effeltrich, Germany): maximum quantum yield of PSII (F_v/F_m) and quantum yield of PSII (Φ_{PSII}).

Temperature response

The methods below aimed to test the acclimated response of samples to changes in temperature over a realistic timeframe in order to inform on ecologically relevant responses to temperature. The samples were placed in labelled petri dishes, sprayed with de-ionised water and transferred to a Sanyo Versatile Environmental Test Chamber (MLR 350) at 12:00 the day after collection, where they were left to acclimate to the first temperature point (12 °C). Photosynthetic response to temperature was measured on fully hydrated samples at high humidity at eight different temperature points. The air temperature in the climate chamber was changed at 12:00 daily in the following order: 12, 15, 18, 20, 23, 25, 30, 35, 40 °C. Hydrated samples were left overnight to acclimate to the new temperature conditions to ensure equilibrium of physiological processes. Each morning at 08:00, samples were sprayed with de-ionised water. An hour later, light-adapted measurements (Φ_{PSII}) were taken at a PAR of around 60 µmol m^{-2} s^{-1}. Following this, the lights were switched off and the samples fully hydrated by spraying with de-ionised water. An hour later, dark-adapted measurements (F_v/F_m) were taken in light conditions below 4 µmol m^{-2} s^{-1}. For each measurement, individual samples were briefly removed from the climate chamber.

Data analysis

Statistical analyses were performed in R version 3.5.0 (R Core Team, 2018).

One sample of *Pyrrhobryum spiniforme* from mid elevation was removed from the analysis as it was not healthy and the aim of the study was to measure optimum response curves, rather than variation in the population.

Parabolic curves of the form:

$$y = ax^2 + bx + c \qquad (5)$$

were fit to the data of quantum yield of PSII (Φ_{PSII}) and maximum quantum yield of PSII (F_v/F_m) against temperature (°C) for each species. This model provided a good fit to the data of each species (Figures 16, 17, 18 and 19). Coefficient *a* (curvature) was estimated for each curve using the “lm” (linear model) function. Coefficient *a* was used in order to compare species’ envelopes of temperature tolerance, where a larger *a* indicated higher sensitivity to temperature.

The optimum temperature for photosynthesis (x-coordinate of the vertex) was extracted from each curve using the equation:

$$T_{opt} = -b / 2a \tag{6}$$

The x-coordinates were then substituted into the quadratic equation of each curve to find maximum photosynthetic efficiency ($\Phi_{PSIImax}$ and F_v/F_{mmax}). The same was done to find Φ_{PSII} and F_v/F_m at 12 °C and 40 °C in order to calculate percentage loss of function at 12 °C (PLF_{12}) and 40 °C (PLF_{40}) °C using the following equations:

$$PLF_{12\ or\ 40} = (\Phi_{PSIImax} - \Phi_{PSII12\ or\ 40}) / \Phi_{PSIImax} * 100 \tag{7}$$

$$PLF_{12\ or\ 40} = (F_v/F_{mmax} - F_v/F_{m12\ or\ 40}) / F_v/F_{mmax} * 100 \tag{8}$$

The envelope of optimum temperature for photosynthesis (temperature where Φ_{PSII} and $F_v/F_m \geq 90$ % of $\Phi_{PSIImax}$ and F_v/F_{mmax}) was calculated by using the “abline” function in R version 3.5.0 (R Core Team, 2018) to draw a straight line through the curves at 90 % and finding the x-coordinates of the intersection points ($T_{opt(90\%)}$). The same was done at 80 % to find the temperatures at 20 % loss of function ($T_{20\%LF}$).

In order to determine whether there is a significant difference between the temperature responses of species at each elevation one-way ANOVAs and Tukey's HSD (Honest Significant Difference) tests were run on each parameter. Kruskal-Wallis and multiple comparison tests were run where the data were non-normal (“pgirmess” package in R; Giraudoux, P., 2018). In order to compare coefficient *a* between range-restricted and widespread species, a two-sample t-test was used where the data were normally distributed and a Mann-Whitney U test (“exactRankTests” package in R; Hothorn & Hornik, 2017) was used where the data were not normally distributed.

Results

Climate Data

There was a trend of decreasing temperature from low to high elevation on the gradient of Piton des Neiges. The average minimum, mean and maximum temperatures at low elevation were 16.4 °C (± SE 0.08 °C), 18.5 °C (± SE 0.07 °C) and 21.0 °C (± SE 0.07 °C), respectively. The average minimum, mean and maximum temperatures at mid elevation were 10.8 °C (± SE 0.08 °C), 14.4 °C (± SE 0.07 °C) and 18.6 °C (± SE 0.10 °C), respectively. The average minimum, mean and maximum temperatures at high elevation were 7.9 °C (± SE 0.09 °C), 12.0 °C (± SE 0.08 °C) and 17.8 °C (± SE 0.13 °C), respectively (Figure 15).

The annual range in temperature increased from low to high elevation, with higher elevations having more extreme events (lower minimum and higher maximum temperatures). Low elevation experienced a minimum of 8.7 °C and a maximum of 28.4 °C. Mid elevation experienced a minimum of 1.5 °C and a maximum of 31.3 °C. High elevation experienced a minimum of -0.4 °C and a maximum of 33.2 °C (Figure 15).

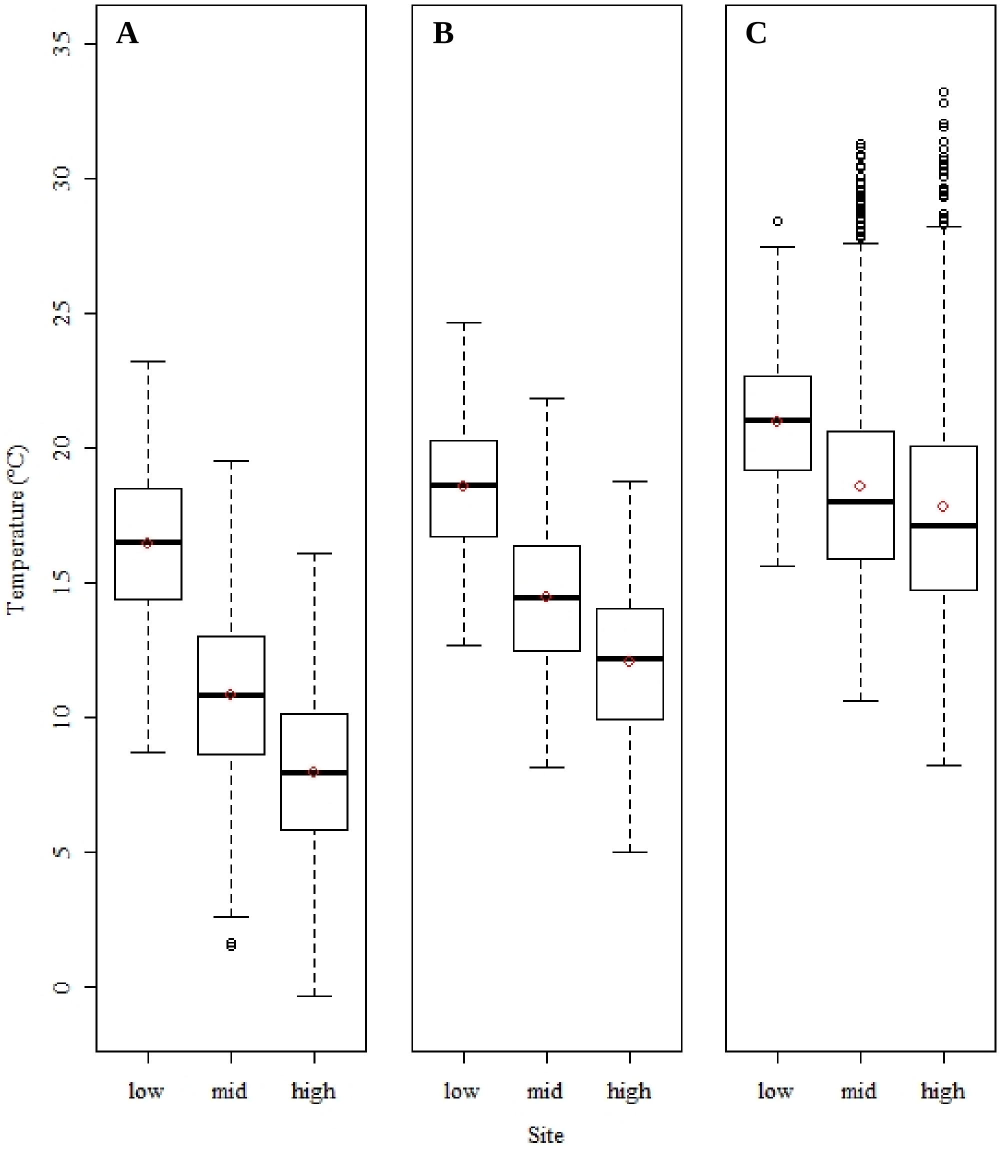


Figure 15: Minimum (A), mean (B) and maximum (C) daily temperature (°C) from June 2011 to May 2012 at low-, mid- and high-elevation sites along the Piton des Neiges gradient. Red circles indicate means of the data.

Temperature Response

The mean optimum range of temperatures for F_v/F_m ($F_v/F_m \geq 90$ % of maximum F_v/F_m) spanned 15.4 °C (± SE 0.45 °C).

The mean optimum range of temperatures for Φ_{PSII} ($\Phi_{PSII} \geq 90$ % of maximum Φ_{PSII}) spanned 13.5 °C (± SE 0.43 °C).

The mean upper temperature at which species lose 20 % of their function is 32.5 °C (± SE 0.57 °C) and 32.3 °C (± SE 0.45 °C) for F_v/F_m and Φ_{PSII} respectively.

There was no difference in coefficient *a* (temperature sensitivity) between widespread and range-restricted species for F_v/F_m ($t = -1.3817$, $df = 11.034$, $p > 0.05$). Widespread species had a significantly higher coefficient *a* than range-restricted species for Φ_{PSII} ($W = 20$, $p < 0.05$), meaning that the photosynthetic function of widespread species was more sensitive to changes in temperature than range-restricted species.

Widespread species

There was no significant difference in any of the parameters between each site for either F_v/F_m (Table 10) or Φ_{PSII} (Table 12).

Mastigophora diclados had the narrowest range of temperature optima for Φ_{PSII} spanning 11.4 °C at low and high elevation, and 11.3 °C at mid elevation.

Range-restricted species

F_v/F_m:

There was no significant difference in any of the parameters between each site for F_v/F_m (Table 9).

Φ_{PSII}:

Low-elevation species had an optimum temperature of 24.3 °C (± SE 0.11 °C), which was significantly higher than the 21.2 °C (± SE 0.58 °C) of the high-elevation species ($K = 6.7455$, $df = 2$, $p < 0.05$). Mid-elevation species had an optimum temperature of 22.5 °C (± SE 0.82 °C), which was not significantly different from low- or high-elevation species. High-elevation species lost 14.8 % (± SE 2.63 %) function at 12 °C, which was significantly less than the 35.4 % (± SE 4.97 %) lost by low elevation species ($F_{2,7} = 7.972$, $p < 0.05$). Mid-elevation species lost 24.7 % (± SE 3.88 %) of their function at 12 °C, which was not significantly different from low- or high-elevation species (Table 11).

There was no significant difference in the sensitivity of the curves (coefficient *a*) or the loss of function at 40 °C between each site for Φ_{PSII}.

Porotrichum elongatum had the narrowest range of temperature optima for Φ_{PSII} of any range-restricted species spanning 11.6 °C from 18.5 °C to 30.1 °C. *Leptodontium stellatifolium* had the widest range of temperature optima for Φ_{PSII} spanning about 20 °C from below 12.0 °C to 32.0 °C.

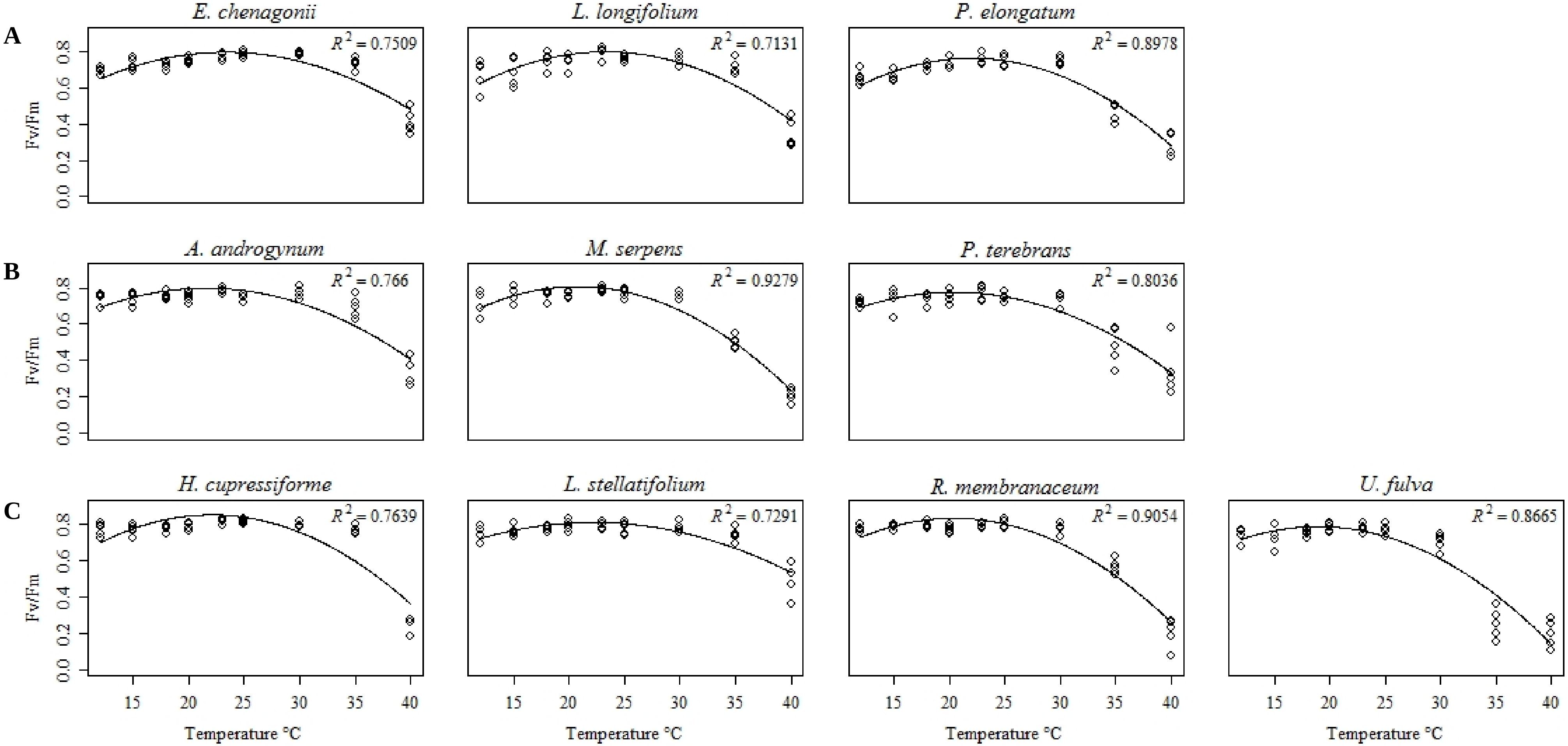


Figure 16: Temperature (°C) response curves of F_v/F_m for range-restricted bryophyte species sampled at low- (A), mid- (B) and high-elevation (C) sites along the Piton des Neiges gradient.

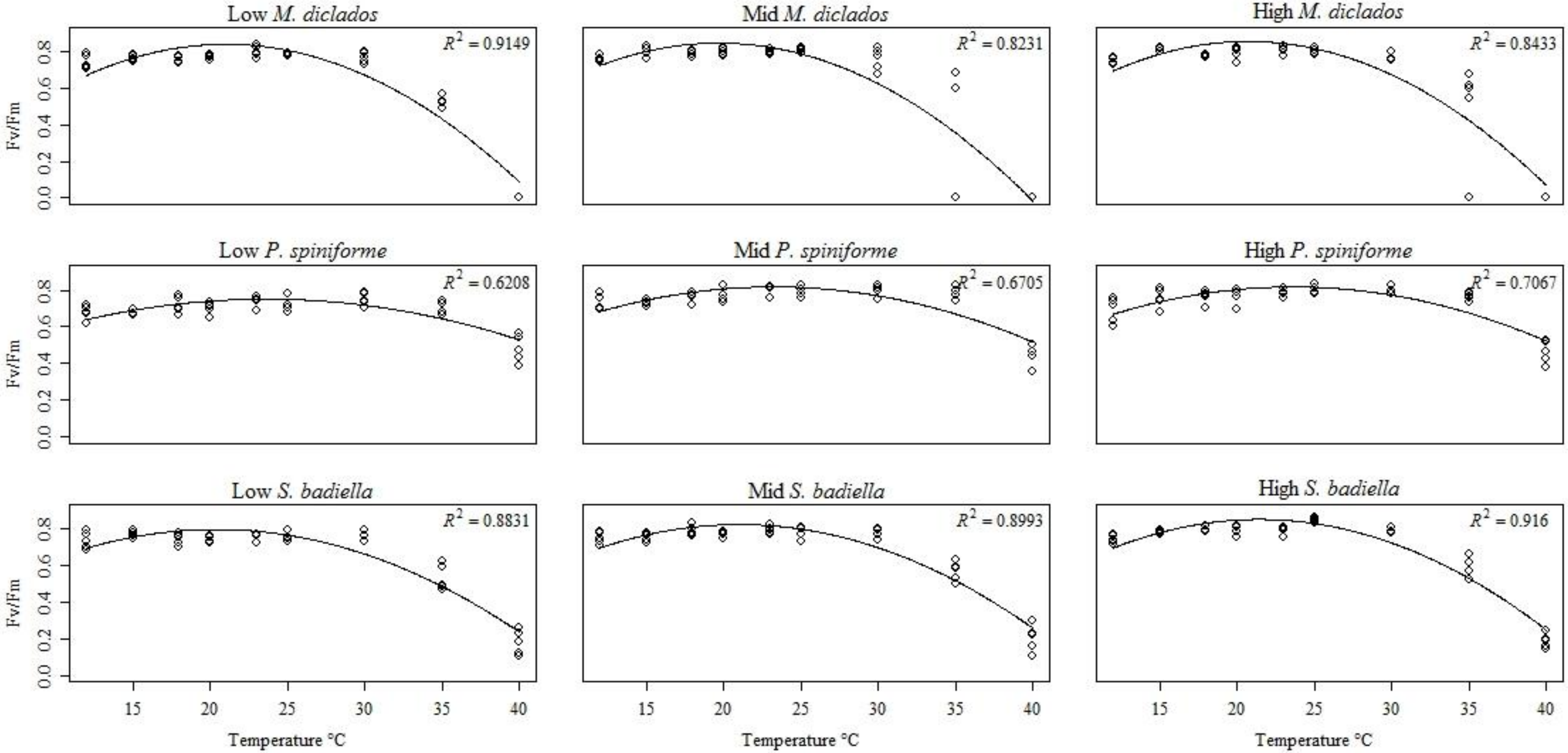


Figure 17: Temperature (°C) response curves of F_v/F_m for widespread bryophyte species sampled at low-, mid- and high-elevation sites along the Piton des Neiges gradient.

Table 9: Parameters extracted from temperature response curves of photosynthesis (F_v/F_m) of range-restricted bryophyte species from low-, mid- and high-elevation sites along the Piton des Neiges gradient.

Species	Site	T_{opt} (°C)	PLF_{12} (%)	PLF_{40} (%)	Coefficient *a*	$T_{opt\ (90\%)}$ (°C)	$T_{20\%LF}$ (°C)
Ectropothecium chenagonii	Low	23.3	18.51	39.97	1.15×10^{-3}	15.0-31.7	<12.0-35.1
Leucoloma longifolium	Low	23.3	21.62	47.57	1.36×10^{-3}	15.6-31.0	15.6-34.1
Porotrichum elongatum	Low	22.0	19.33	63.40	1.49×10^{-3}	14.8-29.1	<12.0-32.1
Atrichum androgynum	Mid	21.6	13.32	49.24	1.16×10^{-3}	13.3-29.9	<12.0-33.3
Macromitrium serpens	Mid	20.7	14.50	70.84	1.54×10^{-3}	13.5-28.0	<12.0-31.0
Plagiochila terebrans	Mid	20.4	10.52	57.31	1.16×10^{-3}	12.2-28.6	<12.0-32.0
Hypnum cupressiforme	High	22.0	18.06	57.66	1.53×10^{-3}	14.6-29.5	<12.0-32.6
Leptodontium stellatifolium	High	22.1	10.90	34.05	8.64×10^{-4}	12.4-31.8	<12.0-35.9
Racomitrium membranaceum	High	20.4	12.56	68.91	1.49×10^{-3}	12.9-27.9	<12.0-31.0
Ulota fulva	High	18.9	8.95	82.70	1.47×10^{-3}	<12.00-26.3	<12.0-29.3

Table 10: Parameters extracted from temperature response curves of photosynthesis (F_v/F_m) of widespread bryophyte species from low-, mid- and high-elevation sites along the Piton des Neiges gradient.

Species	Site	T_{opt} (°C)	PLF_{12} (%)	PLF_{40} (%)	Coefficient a	$T_{opt\ (90\%)}$ (°C)	$T_{20\%LF}$ (°C)
Mastigophora diclados	Low	21.0	20.22	89.68	$2.09\ x10^{-3}$	14.7-27.4	12.1-30.0
Mastigophora diclados	Mid	19.7	14.57	100.00	$2.09\ x10^{-3}$	13.3-26.0	<12.0-28.7
Mastigophora diclados	High	20.7	18.88	91.87	$2.12\ x10^{-3}$	14.4-27.1	<12.0-29.7
Pyrrhobryum spiniforme	Low	23.6	14.90	29.65	$8.31\ x10^{-4}$	14.1-33.2	<12.0-37.1
Pyrrhobryum spiniforme	Mid	23.2	16.48	36.75	$1.07\ x10^{-3}$	14.5-32.0	<12.0-35.6
Pyrrhobryum spiniforme	High	23.6	18.23	36.12	$1.10\ x10^{-3}$	15.0-32.3	<12.0-35.8
Schlotheimia badiella	Low	20.4	12.87	70.5	$1.46\ x10^{-3}$	13.0-27.8	<12.0-30.9
Schlotheimia badiella	Mid	21.0	15.53	68.73	$1.57\ x10^{-3}$	13.8-28.3	<12.0-31.2
Schlotheimia badiella	High	21.4	18.39	70.87	$1.76\ x10^{-3}$	14.5-28.4	<12.0-31.3

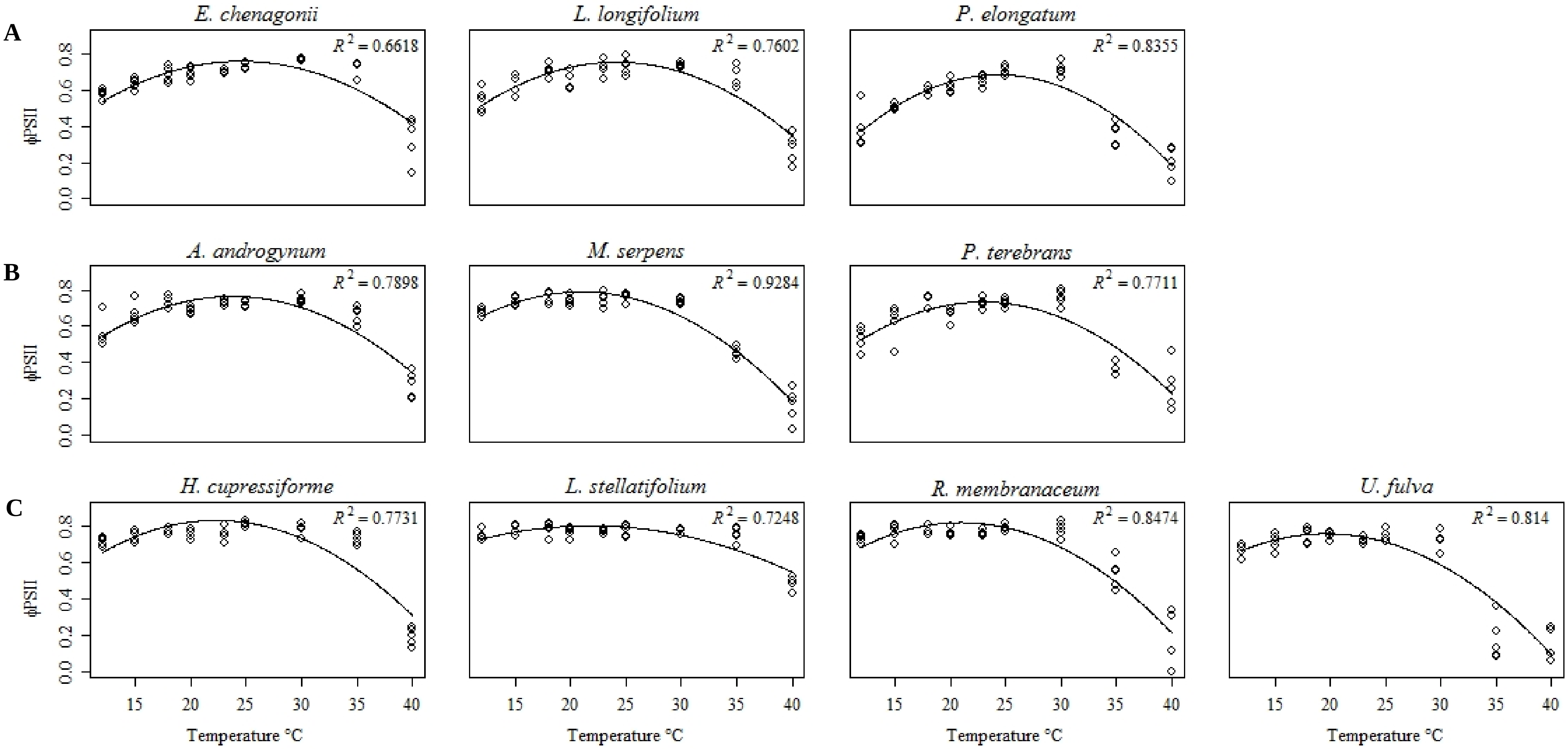


Figure 18: Temperature (°C) response curves of Φ_{PSII} for range-restricted bryophyte species sampled at low- (A), mid- (B) and high-elevation (C) sites along the Piton des Neiges gradient.

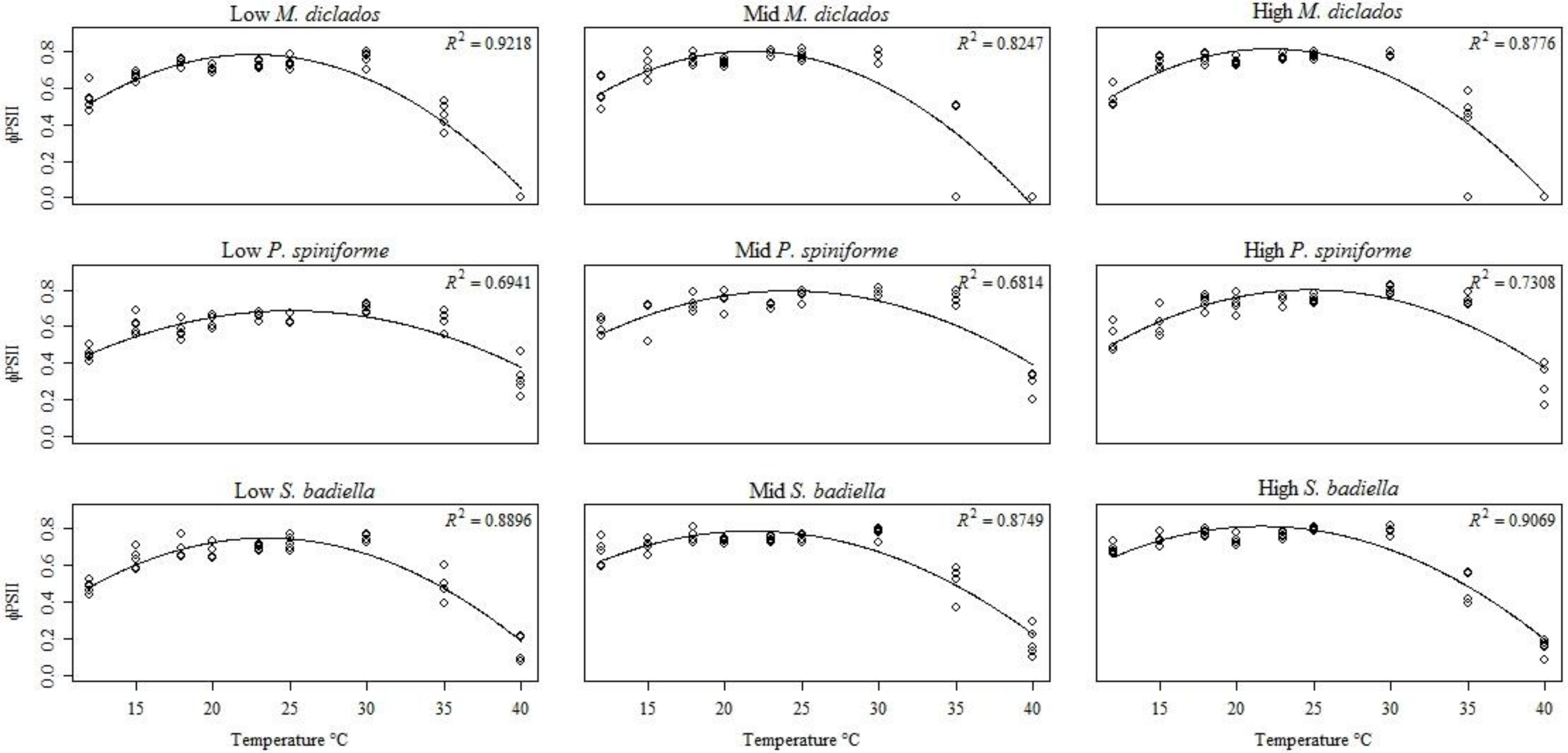


Figure 19: Temperature (°C) response curves of Φ_{PSII} for widespread bryophyte species sampled at low-, mid- and high-elevation sites along the Piton des Neiges gradient.

Table 11: Parameters extracted from temperature response curves of photosynthesis (Φ_{PSII}) of range-restricted bryophyte species from low-, mid- and high-elevation sites along the Piton des Neiges gradient.

Species	Site	T_{opt} (°C)	PLF_{12} (%)	PLF_{40} (%)	Coefficient *a*	$T_{opt\ (90\%)}$ (°C)	$T_{20\%LF}$ (°C)
Ectropothecium chenagonii	Low	24.5	29.38	44.94	1.43×10^{-3}	17.2-31.8	14.2-34.8
Leucoloma longifolium	Low	24.1	31.52	53.80	1.62×10^{-3}	17.3-31.0	14.5-33.8
Porotrichum elongatum	Low	24.3	45.23	73.48	2.05×10^{-3}	18.5-30.1	16.2-32.5
Atrichum androgynum	Mid	23.7	28.91	55.29	1.61×10^{-3}	16.8-30.7	14.0-33.5
Macromitrium serpens	Mid	21.0	16.94	76.53	1.67×10^{-3}	14.1-27.8	<12.0-30.7
Plagiochila terebrans	Mid	22.9	28.22	69.06	1.74×10^{-3}	16.4-29.4	13.7-32.1
Hypnum cupressiforme	High	22.3	21.24	62.99	1.67×10^{-3}	15.3-29.3	12.3-32.3
Leptodontium stellatifolium	High	21.7	9.08	31.88	7.69×10^{-4}	<12.0-32.0	<12.0-36.2
Racomitrium membranaceum	High	20.9	16.38	74.35	1.68×10^{-3}	14.0-27.9	<12.0-30.9
Ulota fulva	High	19.6	12.34	88.1	1.61×10^{-3}	12.8-26.5	<12.0-29.3

Table 12: Parameters extracted from temperature response curves of photosynthesis (Φ_{PSII}) of widespread bryophyte species from low-, mid- and high-elevation sites along the Piton des Neiges gradient.

Species	Site	T_{opt} (°C)	PLF_{12} (%)	PLF_{40} (%)	Coefficient *a*	$T_{opt\ (90\%)}$ (°C)	$T_{20\%LF}$ (°C)
Mastigophora diclados	Low	22.6	34.43	93.39	2.41×10^{-3}	16.9-28.3	14.5-30.6
Mastigophora diclados	Mid	21.6	28.53	100.00	2.49×10^{-3}	15.9-27.3	13.6-29.6
Mastigophora diclados	High	22.2	31.53	96.93	2.49×10^{-3}	16.4-27.9	14.1-30.3
Pyrrhobryum spiniforme	Low	25.1	34.67	45.09	1.39×10^{-3}	18.1-32.1	15.1-35.0
Pyrrhobryum spiniforme	Mid	24.1	29.74	50.77	1.61×10^{-3}	17.1-31.2	14.2-34.1
Pyrrhobryum spiniforme	High	24.7	37.08	53.27	1.83×10^{-3}	18.1-31.4	15.4-34.1
Schlotheimia badiella	Low	23.4	36.05	75.45	2.06×10^{-3}	17.4-29.5	14.9-32.0
Schlotheimia badiella	Mid	21.8	20.79	71.75	1.71×10^{-3}	15.0-28.6	12.2-31.4
Schlotheimia badiella	High	21.6	20.73	76.20	1.83×10^{-3}	14.9-28.3	12.2-31.0

Discussion

F_v/F_m gives an indication of the maximum efficiency of PSII, whereas Φ_{PSII} gives a proportion of absorbed light that is actually used in PSII photochemistry. An unstressed plant may not be photosynthesising to full capacity at any given time, depending on external conditions (Murchie & Lawson, 2013). F_v/F_m is generally almost temperature-independent at temperatures above 0 °C in bryophytes, but has been found to decrease at subzero temperatures and temperatures above 30 °C (Hájek et al., 2001). As in Hájek et al. (2001) Φ_{PSII} was more sensitive to changes in temperature than F_v/F_m, therefore, the Φ_{PSII} results will be the focus of the discussion.

Range-restricted species

Range-restricted species from different elevational ranges showed little difference in how they responded to high temperatures. Most species still maintained 80 % of their function at around 32.3 °C. This corresponds well with the climatic data, as even the highest daily maximum temperature along the gradient was never above 33.2 °C. These results demonstrate that these species have the ability to continue photosynthesising under high temperature conditions for a short period of time. This confirms that species can survive the maximum temperatures experienced at mid and high elevations (extreme temperatures that are not experienced at low elevation). However, these maxima are occasional and are not experienced long term. Therefore, mid- and high-elevation species may be able to continue photosynthesis under high temperatures, but it is long-term high temperatures that have the most damaging effects (Wagner at al., 2014a).

Wagner at al. (2014a) maintain that constant high temperatures may limit bryophyte cover in the tropical lowlands. In addition, Richards (1984) posited that high nocturnal temperatures in the lowlands may cause elevated rates of dark respiration, leading to large CO_2 losses at night that cannot be countered by carbon gains during the day, due to high rates of evaporation and low light levels. Along the Piton des Neiges gradient, low elevation experienced higher average temperatures than mid and high elevation (Figure 15B). Also the minimum (night-time) temperatures at low elevation were relatively high, with an average minimum temperature of 16.4 °C (Figure 15A). Yet Wagner et al. (2013) found that lowland bryophytes had higher temperature optima than montane species and were acclimatised to these high-temperature conditions. Similarly, the results of the current study showed that low-elevation species had higher optimum temperatures for photosynthesis. This may allow them to maintain net carbon gains under constantly warm conditions (Wagner et al., 2013). The inability of the mid- and high-elevation transplants to survive the conditions at low elevation (Chapter 2) indicates that they may not be able to acclimatise their metabolic rates to the long-term high temperatures at low elevation. These constant warm temperatures may result in net carbon loss, and eventual death, for mid- and high-elevation species (Richards, 1984). Therefore, these results illustrate that mean high temperatures may be more important in shaping bryophyte distribution than extreme high temperatures. However, data on long-term temperature response and respiration is needed to confirm this.

Low-elevation species clearly have the ability to tolerate high temperatures, even for long periods of time, but may be more sensitive to low temperatures. The low-elevation species had a greater loss of function at 12 °C than the species from other elevations. Again, this corresponds well with the climate data, as even the minimum temperatures at low elevation rarely dropped below 12 °C (Figure 15A). However, temperatures at high elevation often dropped well below 12 °C, sometimes reaching temperatures below freezing. Reduced photosynthesis at low temperatures and desiccation damage during periods of freezing (Clausen, 1964; Alpert, 2000) may partly explain why low-elevation species did not survive transplantation to high elevation (Chapter 2).

Widespread species

Widespread species from each site did not differ in any of the parameters extracted from the temperature-response curves. This suggests that widespread species are not adapted to the local temperature conditions in their elevation of origin. A likely explanation is that these species are not affected by the different macroclimatic conditions at different sites, as they occupy microsites with similar microclimatic conditions at each elevation (Chapter 2).

The photosynthetic function of widespread species was more sensitive to changes in temperature than that of range-restricted species, demonstrating that widespread species have a narrower range of temperature tolerance. This suggests that range-restricted species are accustomed to larger fluctuations in macroclimatic temperature, whereas widespread species occur within microsites with more stable temperatures. These results are contrary to the Climate Variability Hypothesis (Stevens, 1989; Stevens, 1992) and Brown’s hypothesis (1984), which both expect widespread species to have broader climatic tolerances.

High temperatures

Bryophytes are known to be sensitive to high temperatures, especially when wet (Clausen, 1964). He et al. (2016) and others (Frahm, 1990; Glime, 2007) propose that tropical bryophytes have a narrow range of temperature optima around 25 - 26 °C (Frahm, 1987, Wagner, 2013) with rapid declines in photosynthesis at temperatures on either side of the optimum. However, the reason for these declines at high temperatures is not fully understood in bryophytes (He et al., 2016). Frahm's (1987) study illustrated that high temperatures (30 °C and above), coupled with low light levels, led to mortality for tropical montane bryophytes within 24 hours. However, Wagner et al. (2013) showed that tropical bryophytes have a wide range of optima, spanning about 10 °C, including temperatures of up to 31 °C. The current study showed similar results, with species having optimum envelopes of photosynthesis spanning approximately 13.5 °C, and still maintaining about 80 % of their function at 32.3 °C when hydrated. These results show that tropical bryophytes are not as sensitive to high temperatures when hydrated as previously thought, which is supported by Larcher (2003) who maintains that, when hydrated, tissue injury only occurs at temperatures above 40 °C. However, both my and Wagner's (2013) experiments measured short-term responses to temperature, and long-term responses *ex situ* should be tested under these conditions. Furthermore, both my and Wagner's (2013) experiments did not capture the lower end of the temperature curves, which may be important for understanding species' distributions and should be the focus of further research

Conclusion

This study provides insight into the pattern of distribution observed in the reciprocal transplant experiment (Chapter 2). However, the interaction of climatic variables is probably more important in determining species' distributions than any one factor alone (Soberón & Peterson, 2005), therefore, the following chapter will discuss the combination of temperature and humidity (Chapter 3) in the context of the reciprocal transplant experiment (Chapter 2).

CHAPTER 5

General Conclusions

The results of this study show that specialisation to both macro- and microclimatic conditions can be attributed as a main driver of bryophyte range-size and distribution on the elevational gradient of Piton des Neiges, Réunion Island.

It was found that the effect of microhabitat is especially important in widespread species, which appear to occupy similar microsites at all elevational sites along the gradient. The results of the desiccation and temperature experiments lend support to this idea. These experiments showed that widespread species are not specialised to the climatic conditions of their elevations of origin, as they probably experience similar microclimatic conditions at low, mid and high elevations. Widespread species were found to have lower desiccation tolerance than high-elevation specialists and a narrower range of temperature tolerance than range-restricted species. These results contest the credibility of the Climate Variability Hypothesis (Stevens, 1989; Stevens, 1992) and Brown's (1984) hypothesis in bryophytes and other species closely linked to microclimatic conditions.

Although some range-restricted species were also dependent on specific microhabitats, there was a general trend of upslope survival of restricted species observed in the transplantation experiment. Species from all sites performed best at their site of origin and badly at lower elevations, but low- and mid-elevation species also performed well at the elevational site above their site of origin. This can be explained by the physiological responses of low-, mid- and high-elevation species to temperature and desiccation. There was a clear separation in the physiological responses of low- and high-elevation species, with mid-elevation species showing intermediate responses to temperature and desiccation. This corresponded well with the climatic conditions habitually experienced – with extremes at the gradient peripheries and intermediate conditions in between.

In summary, physiological responses to humidity and temperature can be correlated with range-restricted species' responses to transplantation along the gradient. Low-elevation species were able to tolerate the cool, wet conditions of mid elevation. However, they were not able to survive the colder, dry conditions of the high elevation site. Mid-elevation species were able to tolerate the dryer, cooler conditions of high elevation, but not the combination of high temperatures with high humidity at low elevation (Richards, 1984). High-elevation species were unable to survive downslope due to the constantly wet, warmer conditions of low and mid elevation (Richards, 1984). It should be noted that one of the defining features of high elevation is high light intensity (Barcelo, 1996), therefore, low light levels may be a limiting factor for high-elevation species at low and mid elevation. However, further research is needed to confirm whether this is a limiting factor or whether high-elevation species can acclimatise to low light levels.

Research limitations

The species chosen in this study represent a variety of growth forms and microhabitats. However, this was too broad for the scope of the study and made it difficult to make general comparisons between elevational sites. Therefore, growth form and microhabitat should have been better controlled for. It is also important to keep in mind the three-dimensional character of the forest habitat for bryophytes. Pardow and Lakatos (2012) describe the large variety of habitats along the vertical gradient within the canopy. Choosing species within the same height zone within the canopy should, thus, be considered when selecting to compare species along an elevational gradient. Furthermore, a larger sample size of species would have been beneficial to the results of this study. However, this is often difficult in physiological studies, and the time constraints did not allow for it.

Wagner et al. (2014a) explain why our knowledge on the subject of species’ environmental tolerances is still incomplete, by highlighting the difficulty in separating the effects of different climatic variables. Even coupled with *ex situ* experiments under controlled conditions, it is still difficult to determine the role played by each climatic variable and the effect they have *in situ*.

Research implications

Although the upward shift in vegetation bands with rising temperatures is estimated to be small on tropical islands, it still has the potential to do significant damage to range-restricted species on elevational gradients (Pouteau et al., 2018). This is in accordance with Song et al. (2012) who found that even small changes in climate had negative effects on transplanted epiphytes. The trend of upslope survival seen in the current study indicates that range-restricted species may already be at their thermal maxima, with low potential for survival in warmer climates. With the upward shift in climatic bands, high-elevation species are at risk of mountaintop extinctions (Kelly & Goulden, 2008; Lenoir et al., 2008; Engler et al., 2009). However, this may not be a definitive outcome, if those individuals that showed the potential for survival in the climatic conditions of lower elevations are able to maintain populations at high elevation and adapt to higher temperatures (Wagner et al., 2014a).

Very little is known about the ecophysiology of tropical bryophytes (Wagner et al., 2014b). Accordingly, this study makes important contributions to the body of knowledge on how tropical bryophytes respond to climatic variables. Estimating changes in species' distributions with predicted changes in climate, without knowledge about species' physiological tolerances and acclimatisation potentials, may lead to inaccurate predictions (Chown et al., 2004; Helmuth et al., 2005). Therefore, data such as these are imperative for accurate modelling in order to predict how species' distributions will change in a changing climate.

Where to go from here?

Knowledge on species' physiological tolerances and ability to acclimatise to new conditions is vital for understanding their current distributions, as well as predicting future distributions (Chown et al., 2004; Helmuth et al., 2005). Forman (1964) states that in order to get a complete description of species' distributions it is important to have knowledge about their physiological tolerances, as well as the environmental conditions *in situ*. He suggests growing species under controlled conditions and making use of climatic data. Forman (1964) maintains that if accurate knowledge about environmental tolerances and conditions is gained, a theoretical distribution can be predicted and this should match up precisely with actual distributions. Where they do not coincide, dispersal must be a limiting factor, and the evolutionary history should be examined. The current study provides the first insight into the physiological tolerances and possible acclimation potential of these species on Réunion Island. Further research should focus on growing these species under controlled conditions and examining a larger variety of climatic variables separately and in combination (see Forman, 1964). A more detailed analysis of climatic data, including macroclimatic conditions, is needed to gain a clearer understanding of species' distributions.

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