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Growth and vitality of transplanted individuals of the critically endangered cyanolichen *Erioderma pedicellatum* 

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# Abstract

Lichens are a diverse group of species that make up one of the most successful symbiotic partnerships on Earth, yet increasing pressure from human-driven climate and area change is a significant threat to their biodiversity. The critically endangered cyanolichen *Erioderma pedicellatum* is currently confined to a small location in Rendalen, Norway, as its prehistoric occurrences in Trøndelag have disappeared. This study aimed at assessing the growth and vitality of transplanted individuals of *E. pedicellatum* in an area where it historically existed, and thus to what extent transplantation experiments have the potential to be a conservation method for this species in Norway.

54 individuals of *E. pedicellatum* were transplanted to the Gartlandselva nature reserve, where they spent 172 days from late May to early November of 2023. Transplantation success was analyzed through parameters such as relative growth rate in weight (RGR), relative growth rate in area (RTaGR), and specific thallus mass (STM). In addition, parameters of vitality were analyzed through measurements of photosystem II efficiency in dark- and light-adapted states ( $F_{\nu}/F_m$  and ETR<sub>app</sub>, respectively). Environmental factors at the transplantation site such as light transmittance, soil and bark pH, position in the terrain, vegetation composition, and diameter at breast height of the host trees, were also registered.

The results indicate that transplantation indeed has the potential to be a conservation strategy for this species in Norway. Many of the transplants had positive RGR (n = 23), even more had positive RTaGR (n = 36), and most of them had reduced STM, indicating that they grew thinner post-transplantation, as an adaptation to shade. The transplants that experienced positive RGR were associated with higher light levels and higher soil pH. The parameters of vitality remained relatively stable, except for a slight increase in  $F_v/F_m$ , likely as a response to lower light conditions. These findings not only supplement the ecophysiological knowledge about this species in Norway but also demonstrates that transplantation experiments have potential to conserve this species. Future research should aim at conducting long-term transplantation experiments, that includes many seasons as well as thorough measurements of the environmental conditions at the transplantation site, to evaluate if this species is able to establish viable populations over time.

# Abbreviations, units, and symbols

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# Units and symbols

A	Area	MJ	Megajoule
ADM	Air-dry mass	μmol	Micromol
AMSL	Above mean sea level	∆t	Time interval
DBH	Diameter at breast height		
DM	Complete dry mass		
ETR <sub>app</sub>	Apparent electron transport rate		
EIV	Ellenberg indicator value		
$F_{v}/F_{m}$	Maximum PSII yield		
HSD	Honest significant difference		
PAR	Photosynthetic active radiation		
PSII	Photosystem II		
RH	Relative humidity		
RGR	Relative growth rate in weight		
WHCblotting	Internal water holding capacity		
WHC <sub>shaking</sub>	External water holding capacity		
WMshaking	Wett mass after shaking thallus		
WMblotting	Wet mass after blotting thallus with paper		
RTaGR	Relative growth rate in area		
STM	Specific thallus mass		
WHC	Water holding capacity		

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## **1. Introduction**

## 1.1 Motivation

The partnership that is found throughout most ecosystems on Earth – lichens – was among the earliest symbiosis to colonize terrestrial habitats (Chen et al., 2000). Lichens are considered one of the most successful symbioses on our planet, partly because they display great intraspecific diversity of ecological niches (Asplund & Wardle, 2017). They can namely exist on many different substrates, ranging from trees, rocks, bare soil, and even more anthropogenic structures such as old cars, plastic fences, and house roofs (Holien & Tønsberg, 2008). The partnership consists of a filamentous fungus, which is termed the mycobiont, and a microalgae or cyanobacteria (or both), which are termed the photobionts (Lutzoni & Miadlikowska, 2009). Most often, the mycobiont creates a plantlike, vegetative body which is called a thallus (pl.: thalli), in which the photobiont lives (Holien & Tønsberg, 2008). Lichens are thus not a single organism, but a composite one, nonetheless, it will henceforth be referred to as a species for comprehensibility reasons. This intricate symbiosis has likely existed through many different eras, as lichen-like fossils have been dated back to 600 million years ago (Yuan et al., 2005). They have therefore been subject to major shifts in planetary conditions ranging from freezing glacial periods to steaming- and carbon dioxide (CO<sub>2</sub>) rich environments. However, the current rate of human-induced changes to both climate and area usage might pose a challenge to the prospective longevity of this symbiosis.

For the past centuries, human drivers have led to a significant and rapid decline in biodiversity (IPBES, 2019), with lichens being no exception. Out of all the lichens evaluated by Artsdatabanken, 23% of the Norwegian mainland species were deemed red-listed and thus threatened by extinction (Haugan et al., 2021). Lichens are important for biodiversity and ecosystem function because they, among other things, contribute to the production of oxygen through photosynthesis, they can be food and nesting material for other species and constitute a link in the flow of water and nutrients as well as in soil processes (Asplund & Wardle, 2017; Mallen-Cooper & Cornwell, 2020). Lichens are also important from an anthropocentric view, as they can function as bio-indicators for toxic pollutants (Lutzoni & Miadlikowska, 2009), continuity of old-growth forests (Jönsson et al., 2017) and climate change (Cornelissen et al., 2001 in Gauslaa, 2014). In other words, they contribute to a range of ecosystem services and functions that facilitate not only biological but also human well-being and quality of life. To effectively address the challenges posed by climate and ecosystem change, it is crucial to understand the adaptability and ecology of this complex symbiosis so that the proper conservation efforts and methodologies can be implemented.

#### 1.2 Background

There is uncertainty as to whether lichens can adapt parallelly to the changes in the environment as some other species do. Prominent examples of the effects of environmental change include range shifts of plants and animals, as their distribution patterns have changed persistently with predicted climate change (Loss et al., 2011). These trends are often demonstrated by northward and altitudinal migration patterns. In lichens, such geographical range shifts are currently projected to be lagging behind the current pace of climate- and area change (Elis, 2019). Lichens are particularly vulnerable to environmental changes due to their slow growth and thus slow dispersal rates, in addition to their sensitivity to pollutants (Chen et al., 2000; Maass & Yetman, 2002). However, the growth rate, dispersal rate, and sensitivity to chemicals differ from one lichen to another.

Many of the responses observed in lichens stemming from climatic and environmental change seem to be very species and/or habitat-specific (Stanton et al., 2023). An increasing number of studies have identified dispersal limitations as one of the primary reasons for the developmental inhibition of old-forest lichen (Jönsson et al., 2017; Lidén et al., 2004; Sillett et al., 2000; Smith, 2014). In comparison to many other groups of organisms, species of lichens typically have a narrow or more specialized ecological niche due to the essence of being in a symbiosis – in which both the mycobiont's and the photobiont's physiological requirements are to be met (Resl et al., 2018). As mentioned above, the continuation of a species is to some degree dependent on geographic relocations of distributions and/or adaptation to warming patterns. Certain lichens may lack the ability to provide such rapid responses, and alternative tools for conservation are therefore perhaps needed.

One method for species conservation that has been subject to discussion in the ecological and conservational community is transplantation experiments. Transplantation, or translocation (which will be treated synonymously in this study), has a long and sturdy history in ecology (Mallen-Cooper & Cornwell, 2020). Transplantation experiments can be defined as moving individuals of species from their original habitat and relocating them to a new area, and their responses to that new environment are consequently assessed (Nooten & Hughes, 2017). Transplantations can be divided into different classes depending on the interests or intentions

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of the research. Some ecologists use transplantation to differentiate between habitat factors and genetic factors (Mallen-Cooper & Cornwell, 2020), some use it to predict responses to future climate change (Nooten & Hughes, 2017), and others to assist migration/colonization (Loss et al., 2011). No matter the intention of the transplantation, there are some ethical aspects that need elaboration and clarification.

Transplantation experiments give rise to controversy first and foremost because of their manipulative nature (Loss et al., 2011). Moving a species from one area to another may disrupt the natural course of species migration. In addition, one cannot fully predict how the species will influence the new ecosystem that it is transplanted into. Indeed, in a time where alien/invasive species are among the main drivers of global biodiversity decline (IPBES, 2019), exercising precaution during such experiments is of dire importance. However, lichens have displayed very low invasive capacities, partly due to their preference to establish themselves in areas where other species cannot (Chen et al., 2019). Additionally, lichens that have a low geographical range are unlikely to pose a threat due to their prevailing inability to disperse in current conditions.

One lichen that is critically endangered both in Norway and on a global scale, namely *Erioderma pedicellatum*, might be a good example of a species that could benefit from alternative conservation efforts such as transplantation. The first specimen of this species was collected by William G. Farlow on Campobello Island in Canada in 1902, a collection which was later described by Auguste-Marie Hue in 1911 (Jørgensen, 2000). At the time, Hue classified the species into *Pannaria*, a genus with shell- and leaflike thalli. Several decades later, a Swedish lichenologist by the name of Sten Ahlner made three discoveries of a similar lichen in Grong, Norway, in 1938-39 (Jørgensen, 1972). Upon further taxonomic evaluation by Jørgensen (1972), the species from Canada and Norway were found to be identical, and thus its finalized classification was *E. pedicellatum*. In Norwegian, it was given the name "Trønderlav" due to the location of its discovery. Ever since Ahlner's discoveries in Norway, *E. pedicellatum* continued to be found in different areas throughout Canada, Alaska, Scandinavia, and Russia (Stehn et al., 2013; Tønsberg et al., 1996). In 1991 and 1994, two more localities were identified in Grong and Overhalla, both in Trøndelag (Tønsberg et al., 1996).

Despite the discoveries, the species has experienced a rapid decline both globally and in Norway. The main attributes of its decline have been linked to logging activities, acid rain,

and overall habitat fragmentation (Stehn et al., 2013). In Norway, the findings by Ahlner were later clear-cut and the other discoveries disappeared due to logging or random extinction (Tønsberg et al., 1996). Sweden's population in Värmland used to be quite substantial but was destroyed by nearby logging due to desiccation effects on its microhabitat (Tønsberg et al., 1996). It was therefore believed to be extinct from the European peninsula (Maass & Yetman, 2002). Recently, however, a remarkable population was found at Tegningfallet in Renndalen municipality by Reiso & Hofton (2006, as cited in Nilsson et al., 2022). Knowledge about this species and its associated ecology in Norway has been minimal up until the works of a former master's student, Alexander Nilsson, gave insights into many important ecophysiological properties of *E. pedicellatum* in 2021 (Nilsson, 2021).

Understanding a species' ecological niche is crucial when implementing management actions to increase its ecological fitness, especially for those that are critically endangered, such as E. pedicellatum. This species seems to require a rather special set of environmental properties. The findings from Nilsson (2021) show that the Norwegian phenotype thrives in high light levels and high relative humidity (RH). Temperature-wise it favors a cool climate, like the North American phenotype (Maass & Yetman, 2002; Nilsson, 2021). When it comes to atmospheric pollution, studies conducted in Canada have shown that cyanolichen, such as E. pedicellatum, is very negatively affected by acid rain (Maass & Yetman, 2002). Acid rain affects cyanolichen by making its substrates more acidic, which can deviate from their optimal pH levels. Furthermore, their nitrogen-fixing enzymes are intolerant to the presence of sulfur dioxide (SO<sup>2</sup>) (Maass & Yetman, 2002). This is true for many species within the Lobarion lichen community in which E. pedicellatum belongs, as shown by Gauslaa (1995). Even though Norway has been on the receiving end of the winds containing industrial pollution from other European countries, both its current population at Tegningfallet and its previous locations in Trøndelag have historically had low levels of SO<sup>2</sup> in its precipitation (Nilsson, 2021; Tønsberg et al., 1996). The findings by Nilsson (2021) coupled with the fact that E. pedicellatum has previously lived in Trøndelag might be a good indication for pursuing a transplantation experiment in Trøndelag. Its Norwegian name is Trønderlav, after all.

An increased understanding of the habitat requirements and the ecophysiological properties of the Norwegian phenotype of *E. pedicellatum* are now available, but knowledge about its viability when undergoing transplantation is still lacking. Since the ecology and biology of the species are now better understood, transplantation is a relatively low-risk operation, in which

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the benefits exceed the disadvantageous outcomes (Smith, 2014). The timeframe of a master's thesis is, however, limited, and it is not feasible to assess whether the transplantation experiment will lead to the long-term establishment of individuals at the transplantation site. Despite this, some interesting aspects can be evaluated even in a shorter period.

#### **1.3 Research question and hypotheses**

Analyzing the growth and vitality of the transplanted individuals before and after the experiment can provide important insights into the phenotypic plasticity and acclimatization abilities of this species. Growth will be analyzed by looking at changes in weight, area, and hydration traits, whereas vitality will be analyzed through changes in parameters for photosynthetic yield. The lichens will be placed on host trees along different environmental gradients in a nature reserve in Trøndelag, namely Gartlandselva, an area that is close to the historic findings of this species. By placing them in different environmental gradients, it is possible to evaluate whether the changes in growth and vitality can be linked to a given environmental condition. These gradients will consist of differing levels of light, bark and soil pH, position in the local ravines, and diameter at breast height (DBH) of the host tree. As mentioned above, Nilsson (2021) found that E. pedicellatum is dependent on high light levels at Tegningfallet. Regarding pH levels, a previous study has shown that lichens classified into the Lobarion community tend to prefer a substrate with high pH (Gauslaa & Holien, 1998). This was further demonstrated by Nilsson (2021), who found that the spruce trees at Tegningfallet in which *E. pedicellatum* was growing had a higher bark pH than usual for spruce. Based on these findings, this thesis work will attempt to validate three hypotheses:

- Lichens placed in higher light levels will experience higher growth rates and sustained vitality
- 2. Lichens attached to spruce trees with a high bark pH will experience higher growth rates and sustained vitality
- 3. Lichens placed in locations with high soil pH will experience higher growth rates and sustained vitality

Results demonstrating growth and sustained vitality will perhaps improve the potential for doing similar transplantation experiments in the future. Negative results (e.g. reduced biomass and reduced vitality) might strengthen the argument for conservation and protection of this species and/or its habitat (assuming that the proper environmental and physiological conditions at the transplantation site are met). The objective of the study is therefore to test the growth and vitality of transplanted individuals of *E. pedicellatum* in an area where it historically existed. Through these findings, the thesis work aims to answer the following research question: *to what extent do transplantation experiments have the potential to be a conservation method for* E. pedicellatum *in Norway*? This will hopefully lead to a better understanding of the adaptability and ecophysiology of this species, and the findings in this study will be useful for management actions regarding this species' prospective survival. Furthermore, it can prove useful for studies conducting similar transplantation experiments to other lichens with comparable ecological requirements.

### 1.4 Thesis outline

The rest of the thesis will be outlined in different sections. Section 2 will specify the materials and methods employed by this research. Section 3 presents the results, whereas Section 4 discusses those findings in the context of the research aims, their implications, and recommendations for future research. Lastly, Section 5 concludes the thesis. Additionally, data on macrolichen richness, as well as outputs from simple regression models are included for the reader's convenience in the Appendix.

# 2. Materials and methods

This section presents the materials and method used in this study, all of which are centered around an experimental case study, wherein the location of the study species is manipulated. To present a knowledge basis that allows for the testing of the study's effects, this section includes descriptions of the lichen material, study location, transplantation design, various measurements related to growth and vitality, environmental data measured in situ, additional climate data, and statistical analysis methods.

# 2.1 Lichen material

The samples of *E. pedicellatum* were gathered in early May of 2023 at Tegningfallet – a canyon with a 25m tall waterfall located in Rendalen municipality, Norway. The location is of high importance and uniqueness as it contains the only known viable population of this endangered lichen in Norway (Nilsson et al., 2022). The samples were exclusively collected from dead spruce branches that had already fallen to the ground, to avoid disrupting its population due to its scarce and threatened status.

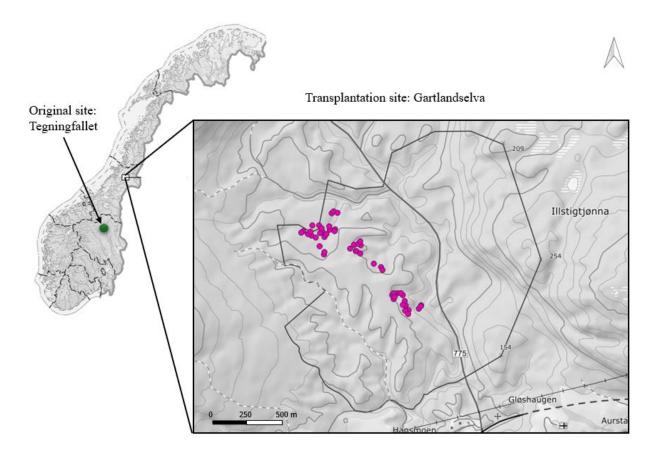
The days following collection were spent in the laboratory preparing the thalli for transplantation. The samples were cleaned and residue from twigs, moss, and other lichens was removed from the thalli. One small spruce branch could contain up to several thalli of *E. pedicellatum*, and determining individual thalli was therefore challenging. Nonetheless, a total of 54 thalli of various sizes were selected in the end (Figure 1). Afterward, the thalli underwent various measurements, which will be described in detail in Sections 2.4 and 2.5.



Figure 1. Selection of E. pedicellatum from the laboratory. Photo: Vivian Y. Bolkan

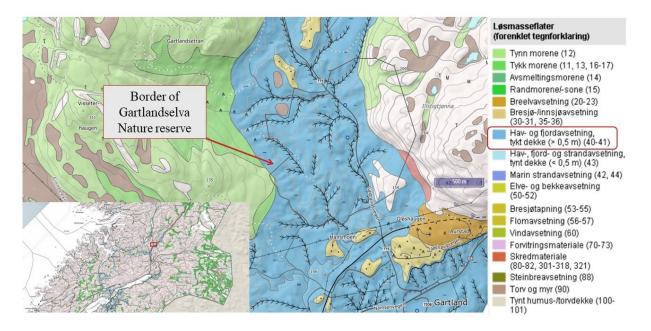
#### 2.2 Study area

The transplantation was performed in the northwest part of the Gartlandselva nature reserve, located in Grong municipality in the county of Trøndelag, Norway (Figure 2). According to the Köppen-Geiger climate classification, the area mainly experiences a continental subarctic climate (Beck et al., 2018). However, due to its relative proximity to the ocean and the prevailing westerly winds, it is also influenced by an oceanic climate to some degree. The climate in this area is therefore both relatively cool and humid (Prestø & Holien, 2001). The region receives a substantial amount of precipitation, both in terms of frequency and quantity with an average of 1250 mm per year, in reference to the normal period in 1960-1990 (Rolstad et al., 2001). The climate at its original site is slightly continental, and the average precipitation is 500 mm annually (Nilsson, 2021), which is much lower than the levels in Gartlandselva. However, as mentioned above, the lichens at Tegningfallet are located near a waterfall, which provides additional hydration.



**Figure 2**. Map of Norway together with a map section of Gartlandselva. Marking (green dot) of the original site Tegningfallet, and the transplantation site Gartlandselva (rectangle) with the location of each transplant (magenta dots), were added. The map was generated with the free and open source QGIS desktop 3.22.4 (QGIS.org, 2024) 22<sup>nd</sup> of March and later assembled in Microsoft PowerPoint.

The marine limit in Grong is approximately 180 m above mean sea level (AMSL) (Rekstad, 1923 in Prestø & Holien, 2001). The topography within the entire reserve varies, but the area used for transplantation was generally around 90 m AMSL and thus below the marine limit. The landscape in the reserve is strongly characterized by ravines, caused by running water on marine and/or fjord sediments over time, as well as tectonic uplift (NGU, n.d.). In the original location, Tegningfallet, the loose masses consist of glaciofluvial sediments (NGU, 2021). Assessing the different influences of loose masses on epiphytic communities is, however, beyond the scope of this study. The deposits at the transplantation site have a thick clay cover, more than 0.5 m deep (Figure 3). There are several brooks within these ravines, as well as one larger stream running through the middle of the reserve that eventually ends up in the Namsen River. The ravine landscape with its marine deposits leads to a very fertile soil that generates optimal growing conditions for a rich and wide variety of species (Romundset et al., 2023).



**Figure 3.** Map of loose masses in the study area, and the nature reserve's location within northern Trøndelag (NGU, 2021). The Norwegian legend describes color codes for loose masses, and the blue area as a thick (> 0.5 m) clay cover from marine and fjord sediments. A textbox and an arrow were added in Microsoft PowerPoint to highlight the nature reserve.

Due to its high precipitation levels and rich occurrence of epiphytic species, the area is defined as a boreal rainforest wherein spruce is the dominating tree species (Direktoratet for naturforvaltning, 1997). The vegetation floor is mixed; some areas are mostly covered by bryophytes, whereas others are dominated by ferns, shrubs, and/or grasses (Figure 4). Norway

spruce (*Picea abies*) is by far the most abundant tree species, but there are also patchy occurrences of birch (*Betula pubescens*), aspen (*Populus tremula*), and alder (*Alnus incana*), which aligns well with the typical vegetative community assembly in boreal rainforest described by Holien & Tønsberg (1996). The age of the spruce forest in the study area is relatively old, where a significant share of the trees are 110-170 years old, as of the end of the last century (Storaunet et al., 1998).





Figure 4. The landscape within Gartlandselva nature reserve, May 2023. Photo: Vivian Y. Bolkan

The study area has been subjected to logging multiple times throughout the last century, with varying degrees of extraction (Storaunet et al., 1998). Timber extraction has been an important element for economic development in Scandinavian boreal forests (Lidén et al., 2004), and all low-land areas within northern Trøndelag have consequently been managed for either agriculture or forestry (Rolstad et al., 2001). Although there are no nearby control areas to compare with, there seems to be no correlation between the past century's logging activities and the current distribution of epiphytes (Storaunet et al, 1998). The area has been under protection by law as a nature reserve since 2001; however, administrative authorities can permit logging in previously logged sites within the area (Forskrift om Gartlandselva

natureservat, Grong, 2001, § VI, 3). The foundation for protection is that Gartlandselva is one of the few remaining areas with boreal rainforests of considerable size – hosting many threatened species.

# 2.3 Transplantation design

Much of the transplantation design, particularly regarding the techniques used for attaching thalli on host trees, is based upon the methods used in Gauslaa & Goward (2012). In the laboratory, each of the 54 thalli was carefully sewn with polyester thread onto fine, plastic netting. Afterward, they were placed in envelopes with their respective numbers, and transported up to Gartlandselva nature reserve. Before placing the plastic nets with the thalli on host trees, the nature reserve was examined on a three-hour walk in the ravine landscape to find appropriate locations. The aim was to find Norway spruce trees whose foliage had partially or fully decreased. To obtain variance regarding environmental factors, the selection of host trees was based upon different degrees of shade, positioning in the ravine terrain (bottom, hill, top), and size of the stem. Then, randomly chosen transplants were attached to spruce branches roughly 1.5-2 m above ground with the help of cable ties (Figure 5), where they spent a total of 172 days from late May to early November of 2023.



**Figure 5**. The transplantation design with different thalli sewn on plastic nets in Gartlandselva nature reserve. Photo: Vivian Y. Bolkan

#### 2.4 Growth measurements

Before transplantation, all 54 samples of *E. pedicellatum* were weighed at different moisture levels in the laboratory. Firstly, the thalli were weighed in a state of being dripping wet (WM<sub>shaking</sub>), by spraying the thallus multiple times with deionized water and then gently shaking it. Then, they were weighed after being gently blotted with paper to remove excessive water (WM<sub>blotting</sub>). Lastly, the thalli together with five control thalli were kept in room temperature for 24 hours and their air-dry mass (ADM) was measured. The controls were later weighed after being dried in an oven for 24 hours at 70 degrees Celsius. The change in moisture from air-dry to completely dry in the controls was then used to estimate the complete dry mass (DM) of the other 54 thalli.

An estimate of thallus area was analyzed through high-resolution photographs taken with a 12-megapixel Pentax model K-5 II SLR camera with a 70 mm Sigma macro lens. Samples were grouped on top of millimeter (mm) paper with a glass plane gently pressed on top to get them more flattened out and then photographed. With the help of the software ImageJ, thallus size (A) was measured by estimating thalli size of the lichens in the photographs, where the known real distance was derived from the mm paper.

The growth parameter was calculated as relative growth rate in weight (RGR) and relative growth rate in area (RTaGR). The formula used for weight was (RGR = 1000 ×  $ln(DM_{end}/DM_{start}) / \Delta t$  in mg g<sup>-1</sup> day<sup>-1</sup>), whereas the formula for area was (RTaGR = 10000 ×  $ln(A_{end}/A_{start}) / \Delta t$  in cm<sup>2</sup> m<sup>-2</sup> day<sup>-1</sup>), where  $\Delta t$  is 172 days from the start of the experiment to the end (cf. Evans. 1972, as cited in Gauslaa & Goward, 2012). The growth parameters were further categorized into positive and negative growth to look for correlations between growth and the environmental variables in the absence of potential fragmentation.

Specific thallus mass (STM) was calculated with the following equation: STM = DM / A, which is the lichen equivalent of specific leaf area used for vascular plants (Gauslaa, 2014). STM gives an estimate of thallus thickness, which is typically higher in sun-exposed lichens and thinner in more shade-exposed lichens (Gauslaa, 2014).

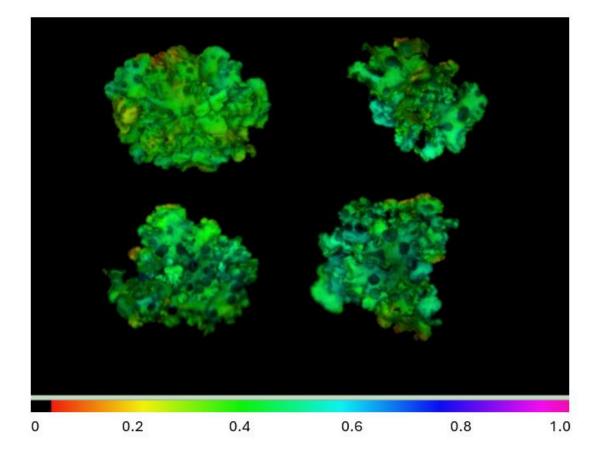
# 2.4.1 Water holding capacity

For each thallus, WHC was calculated before and after transplantation. The mass measurements at different moisture levels can be used with STM to calculate WHC of the thalli before and after transplantation with the formula: WHC =  $WM_{botting/shaking}$  - STM. This formula gives an estimate of water saturation of each thallus and the duration of the physiologically active period following hydration events (Gauslaa, 2014). A distinction has been made between WHC<sub>shaking</sub> and WHC<sub>blotting</sub>, where the former corresponds to WHC<sub>total</sub>, and the latter corresponds to WHC<sub>internal</sub>. Furthermore, external WHC was obtained by the formula: WHC<sub>external</sub> = WHC<sub>total</sub> - WHC<sub>internal</sub>, consistent with previous methods used to calculate lichen hydration traits (Gauslaa, 2014; Hovind et al., 2020). Henceforth, unless otherwise specified, the WHC in this study will refer to internal WHC.

#### 2.5 Vitality measurements

Development of  $F_{\nu}/F_m$  and ETR<sub>app</sub> in the lichens' photosynthetic apparatus was used to analyze vitality of *E. pedicellatum* in this study.  $F_{\nu}/F_m$  is a common measurement of plant stress (Johnstone et al., 2013), but also applicable to other photosynthesizing organisms such as cyanobacteria. Imaging chlorophyll fluorescence was measured with an Imaging-PAM MAXI-version fluorometer on all samples before and after transplantation (Figure 6). Measuring the yield of chlorophyll fluorescence is a useful tool for understanding potential changes in the efficiency of photosystem II (PSII) (Maxwell & Johnson, 2000). Before performing the fluorescence measurement, the thalli were kept moist and non-stressed in a dark environment. This was done to obtain a reference point of maximum PSII yield ( $F_{\nu}/F_m$ ) when in a dark-adapted state (Maxwell & Johnson, 2000).

After acclimatization to the dark for 24 hours, the lichens were subsequently put under a red LED Imaging-PAM M-series chlorophyll fluorometer red version (Heinz Walz GmbH, Effeltrich, Germany) which generated a series of 19 increasing light levels from 0 to 801 photosynthetic active radiation (PAR) lasting 10 seconds each. At each light level a saturating light pulse (1 s at 3000 mmol m<sup>-2</sup> s<sup>-1</sup>) was given to determine the operating efficiency of PSII at each light level ( $\Phi$ PSII). The equation ETR<sub>app</sub> =  $\Phi$ PSII × PAR × 0.5 (where 0.5 is the assumed equal distribution of photons between PSII and PSI) was used to estimate apparent electron transport rate (ETR<sub>app</sub>), as adopted from the methodology in Solhaug et al., (2021). In contrast to  $F_{\nu}/F_m$ , ETR<sub>app</sub> is a measurement of apparent electron transport rate through PSII in a light-adapted state, that allows for determining photosynthetic capacity at different radiation levels and  $\Phi$ PSII (Schreiber, 2004).



**Figure 6.** Image of four lichens during the fluorometer scanning (in  $F_v/F_m$  mode) extracted from Imaging-PAM.  $F_v/F_m$  values are illustrated by false color (wavelengths undetectable by the human eye), as indicated by the bar at the bottom.

## 2.6 Environmental measurements from the study area

#### 2.6.1 Light measurements

Light measurements were taken in situ on a cloudy day at the beginning of July 2023. The equipment used was a SpectraPen mini (Photon Systems Instruments, Brno Czech Republic) directly above each lichen. At the exact same time, a LI-190R quantum sensor with a LI250A Light meter was used roughly 1.5-2 km away from the study site out in an open field as a reference point together with an assistant, allowing the measurements to be taken simultaneously. Both instruments measure photosynthetic active light (400-700 nm) in micromol (µmol) photons m<sup>-2</sup> s<sup>-1</sup>. By doing so, one can obtain the percentage of light transmittance beside the lichen compared to the transmittance levels in a field where there are no shading effects from trees and such (Phinney et al., 2020).

#### 2.6.2 Vegetation analysis and diameter at breast height

Within a 2-meter radius from the host tree, all species of vascular plants were recorded. The vegetation was registered in the middle of the growing season, namely in July of 2023. Some plants may peak before or after this period and could therefore have been unrecognizable at the time of registration. To identify the plant communities' environmental preferences at each location, the plants were later categorized into the system of Ellenberg Indicator Values (EIV). In particular, the plants' preferences for soil or water acidity/pH (termed "reaction") were identified with the data set "Ellenberg-type indicator values for European vascular plant species" derived from the article with the same name (Tichý et al., 2022). Each plant species was given a score and the sum of all the plants' scores at each location were given an average reaction value on a nine-degree scale (starting at highly acidic and ending at alkaline).

All macrolichens that were within a 0.5-meter radius from the transplanted thallus were also registered in situ. At the end of the transplantation period, in November 2023, the twigs in which the thalli were placed onto were collected and brought back to the laboratory. Each sample of twigs was thoroughly examined under a stereomicroscope and macrolichens were again accounted for but this time with a closer look. All macrolichen species at each location were registered by combining the results from the laboratory together with the registrations from the field site. An overview of all the makrolichen species can be found in Table 1 in the Appendix.

DBH of the host trees was calculated by measuring their circumference approximately 1.5 m above ground with a soft measuring tape. Later, the circumference was divided by  $\pi$  (or 3.1416 to be exact) to obtain DBH.

# 2.6.3 pH measurements

pH was measured at all the 54 transplantation sites, both from the soil and from the branch segment that the plastic nettings were placed onto. Soil samples were gathered in early July of 2023, wherein 1 dl of soil was dug up from 4 opposing sides of the host tree. Note that the soil sample for thallus #17 was gathered in November because its host tree could not be located during the field work in July. The samples were dried out in the sun and later debris and organic material (bryophytes in particular) were removed from the samples. Then, the soil was grinded in a hammer mill with a xx mm sieve for pH analysis. 10 mL of soil from each sample were measured and weighed and put in 50 mL Eppendorf tubes together with 25 mL

15

of water. The tubes were shaken for 10 seconds by hand and 3 seconds with a vortex mixer and then left at room temperature for 24 hours. The next day, a pH meter was calibrated with buffer solutions of pH 4 and 7, before the pH of the soil samples was registered.

pH levels of the branches were measured in November, together with soil sample #17. Approximately 6 cm of the branches were cut off into segments, most often from right beneath where the plastic nettings had been. Then the segments were placed into 15 mL Eppendorf tubes together with 7 mL of 25 mM potassium chloride (KCI) solution. The tubes were then turned upside down and back 15 times before being placed in a shaking machine for 15 minutes, a process which was repeated 4 times and lasted for approximately one hour. In this way, H+ in the branch is exchanged with K+ from the KCI and the pH level of the branch can effectively be measured. The methods applied for pH measurements of both the soil and the branches are based upon the methods used by Gauslaa & Goward (2012).

#### 2.7 Additional climate data

Data on temperature, precipitation, and RH from Gartland weather station (SN73550) were downloaded and retrieved from the Norwegian Meteorological Institute (Hanssen-Bauer, 2017). Gartland weather station lies approximately 1 km in aerial distance from the nearest lichen transplant and 1.6 km from the furthest lichen transplant. The temperature data consists of daily minimum, average and maximum values (in °C), whereas precipitation was recorded in mL per day. RH consisted of maximum RH (%) and minimum RH (%) per day. RH refers to how much water vapor is in the air compared to the amount of vapor the air could theoretically hold at the given temperature (EPA, 2013). Data on global solar radiation (Watt m<sup>2</sup> per hour, later converted to MJ m<sup>2</sup> per day) was retrieved from Overhalla – Skogmo weather station, which lies 17.3 km in aerial distance from the study area (the closest station that had such measurements). The selected timeframe for all the data was the entire transplantation period: 23<sup>rd</sup> of May to 11<sup>th</sup> of November 2023, i.e. 172 days.

#### 2.8 Statistical analyses

All statistical analyses were conducted in R Statistical Software (version: 4.3.2) and Rstudio (R Core Team, 2021). The data was downloaded through the readxl R package (Wickham & Bryan, 2023). For data wrangling and sorting purposes, the tidyr R package (Wickham et al., 2024) and the dplyr R package (Wickham et al. 2023) were employed. Functions from the

MASS R package (Venables & Ripley, 2002) were used to evaluate and compare different linear models. Most graphs and figures (except for Figures 14, 15, 20, 24 and 26A:D) were generated via the ggplot2 R package (Wickham, 2016). The exceptions listed above were created in Microsoft Excel (Microsoft Corporation, 2018). Figure 26A:D consists of four graphs that were first created in Excel and then later merged in Microsoft PowerPoint (Microsoft Corporation, 2018) for aesthetical and comprehensible purposes.

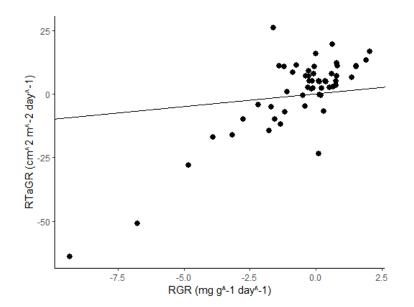
A best subset regression model was applied for all the relevant predictor variables and their potential influence on positive RGR, which is demonstrated in Figure 25. Linear regression analysis was the most frequently used statistical analysis to explore the patterns, trends, and correlations in the data. The following figures includes linear regression models: 12AB, 16, 18AB, 21AB, 22, 24 and 25. One-way ANOVA followed by Tukey's Honest Significant Difference (HSD) was used when assessing significance of differences between groups with categorical variables that followed normal distribution (which was the case for Figure 19). When the variables did not follow normal distribution, paired Wilcoxon signed-rank test was applied to test whether two groups were statistically different from one another. This was the case for the figures 10, 11 and 13AB.

# 3. Results

This section displays the results from the experiments and measurements described in the previous section. Specifically, measurements pertaining to the success of the transplantation are presented and processed. Most attention will be given to positive RGR in the analysis, as it excludes the possible fragmented thalli and because it was the main dependent variable that could be linked to the environmental parameters.

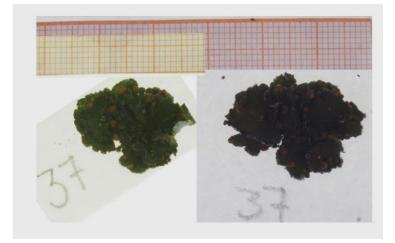
## 3.1 Growth parameters

RTaGR for the thalli varied, but most of the thalli had increased A (n = 36) and fewer thalli had decreased A (n = 18). The highest RTaGR measured was 26 cm<sup>2</sup> m<sup>-2</sup> day<sup>-1</sup>, which in total was an increase of 57% compared to the start of the experiment. The lowest RTaGR was -64 cm<sup>2</sup> m<sup>-2</sup> day<sup>-1</sup>, which translates to a 67% decrease in area from start to end. In terms of the RGR for weight, the pattern was opposite, where a lesser part of the thalli had increased DM (n = 23) and a greater part of them had decreased DM (n = 31). Here, the highest RGR was 2.06 mg g<sup>-1</sup> day<sup>-1</sup>, which gives a total increase in DM of 42%. The lowest RGR measured was -9.32 mg g<sup>-1</sup> day<sup>-1</sup>, which gives a total decrease in DM of -80%. Figure 7 plots RTaGR versus RGR for each thallus. It is the same individual, #24, that experienced the greatest loss in both size and weight, which can be spotted as the lowest point to the left of the figure.



**Figure 7.** Scatter plot of relative growth rate in weight (RGR) in mg  $g^{-1}$  day<sup>-1</sup> and relative growth rate in area (RTaGR) in cm<sup>2</sup> m<sup>-2</sup> day<sup>-1</sup>. An identity line crossing through 0 and 100 (solid, black) was added.

Photographs of the thalli before and after transplantation give a visual representation of changes in area from start to end. When averaging area change and weight change, the highest increase observed was for thallus #37 (Figure 8).



**Figure 8.** Lichen #37 before (left) and after (right) transplantation. The only editing performed in the assembly of these photos are rotation of the left photo to match the placement of the lichen to the right, moving the mm paper from the bottom to the top and cropping out other thalli for an easier, visual comparison.

The thallus that decreased the most in area and weight was #24, which shows a clear sign of fragmentation (Figure 9). Only the top left part of the thallus remained after transplantation, as can be seen by comparing the lobes and overall structure of the thallus.

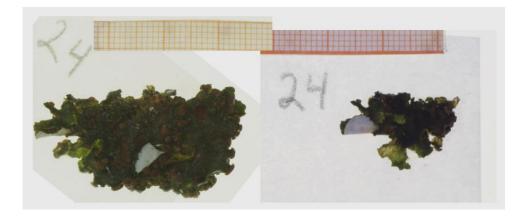


Figure 9. Lichen #24 before (left) and after (right) transplantation. Same editing disclosure as in Figure 8.

Mean STM before transplantation was  $22 \pm 20$  mg per cm<sup>2</sup> and  $19 \pm 20$  mg per cm<sup>2</sup> after transplantation. STM, the proxy for thallus thickness, was therefore reduced on average (Figure 10). A Wilcoxon signed-rank test revealed that the median difference between the paired observations (STM before and after transplantation) was statistically different from zero. In other words, there was a clear statistical difference in STM between the two time periods.

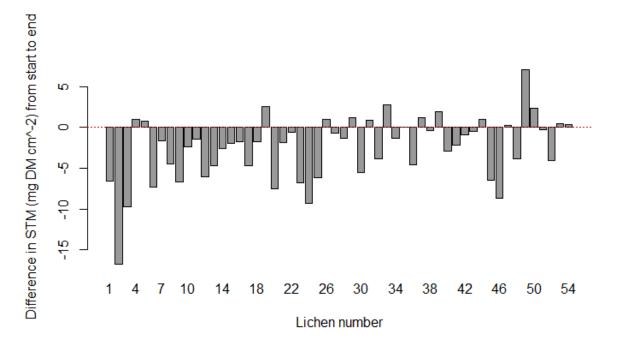
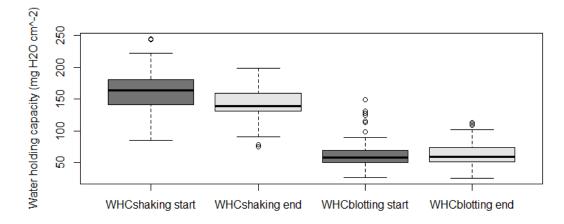


Figure 10. Difference in specific thallus mass (STM) in mg DM cm<sup>-2</sup> from the start of the experiment to the end. (Wilcoxon test: w = 1830, p < 0.05). The horizontal dotted line (red) was added to demonstrate no difference (at 0).

## 3.1.2 Water holding capacity

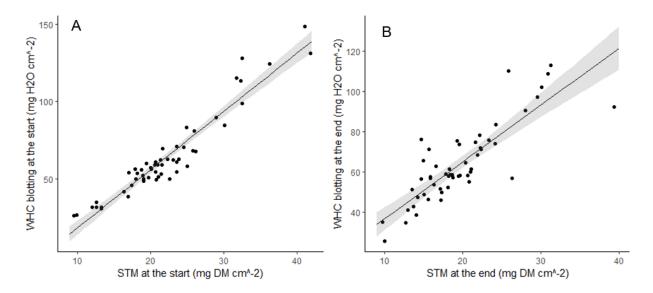
The average WHC<sub>shaking</sub> across all thalli decreased on throughout the experiment and the standard deviation (SD) was greater at the start compared to the end (Figure 11). Mean WHC<sub>shaking</sub> pre-transplantation was  $162 \pm 35 \text{ mg H}_2\text{O cm}^{-2}$  and  $144 \pm 28 \text{ mg H}_2\text{O cm}^{-2}$  post transplantation. A Wilcoxon signed rank test revealed that the median difference between WHC<sub>shaking</sub> at the start and WHC<sub>shaking</sub> at the end was statistically different from zero. Average WHC<sub>blotting</sub> was the same before and after the experiment, specifically 63 mg H<sub>2</sub>O cm<sup>-2</sup>. However, the dispersal from the mean was greater at the start ( $\pm$  27) compared to the end ( $\pm$  19). The highest registered WHC<sub>blotting</sub> at the start was 149 mg H<sub>2</sub>O cm<sup>-2</sup>, whereas the highest at the end was 113 mg H<sub>2</sub>O cm<sup>-2</sup>, both measurements were obtained from the same individual.

The Wilcoxon test showed that thalli's change in WHC<sub>blotting</sub> from start and end was not statistically different from zero.



**Figure 11**. Box plot showing the distribution of WHC<sub>shaking</sub> and WHC<sub>blotting</sub> (in mg H<sub>2</sub>O cm<sup>-2</sup>) at the start compared to the end of the experiment (Wilcoxon test; WHC<sub>shaking</sub>: w = 1217,  $p = 4.48e^{-05}$ , WHC<sub>blotting</sub>: w = 689, p = 0.6481)

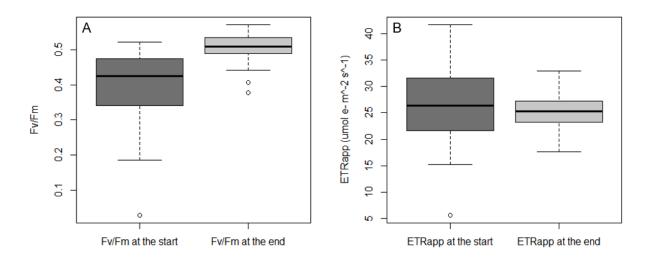
There was a highly significant relationship between STM (mg DM cm<sup>-2</sup>) and WHC<sub>blotting</sub> both at the start of the experiment and at the end (p < 0.001) (Figure 12AB). The difference between the observed and predicted values in the regression analysis was lower at the start; 8.16 residual standard error compared to the end 10.51 residual standard error (both on 52 degrees of freedom). In other words, the deviation from the regression line was greater at the end compared to the start in the relationship between STM and WHC.



**Figure 12**. (A) Simple linear regression for the relationship between STM (mg DM cm<sup>-2</sup>) at the start and WHC<sub>blotting</sub> at the start (Adj.  $R^2 = 0.91$ ,  $p < 2e^{-16}$ ). (B) Simple linear regression for the relationship between STM (mg DM cm<sup>-2</sup>) at the end and WHC<sub>blotting</sub> at the end (Adj.  $R^2 = 0.7$ ,  $p = 1.69e^{-15}$ ). Solid regression lines (black) and 95% CI intervals (light grey area) were added.

#### 3.2 Vitality parameters

One of the parameters for vitality,  $F_{\nu}/F_m$ , changed throughout the transplantation (Figure 13A). The mean  $F_{\nu}/F_m$  at the start was 0.398 ± 0.013, with a minimum of 0.028 and a maximum of 0.521. The minimum was an outlier; it had a value much lower than the rest and the next lowest one, which was 0.185. Using the interquartile range (IQR) method and a lower bound of 0.160, it deviated significantly from the typical range of the other observations. After spotting the outlier, the tests were re-run multiple times in Imaging-PAM MAXI version, but the same number was retrieved each time. It did, however, not have a significant effect on the mean and overall results and was thus not removed from the data. After transplantation, the mean  $F_{\nu}/F_m$  was 0.507 ± 0.005, with a minimum of 0.377 and maximum of 0.571. In terms of the other vitality parameter, ETR<sub>app</sub>, there was not a big change in mean before and after transplantation, with values of 27.25 ± 0.97 and 25.27 ± 0.42, respectively (Figure 13B). The range of values for both  $F_{\nu}/F_m$  and ETR<sub>app</sub> were greater before transplantation compared to after.



**Figure 13**. (A) Comparison of maximum PSII yield  $(F_v/F_m)$  at the start (dark grey) of the experiment versus the end (light grey) (Wilcoxon test; w = 255, p < 0.0001). (B) Comparison of electron transport rate (ETR<sub>app</sub>) at the start (dark grey) of the experiment versus the end (light grey) (Wilcoxon test; w = 1647, p = 0.2466).

During the first six light treatments (0 to 81 PAR), the average  $ETR_{app}$  was higher posttransplantation than pre-transplantation (Figure 14). But as the light levels increased from treatment 7 and onward (> 100 PAR) the average  $ETR_{app}$  was higher at the start of the experiment compared to the end. Overall, the  $ETR_{app}$  at all light levels combined was slightly higher at the start, but not significantly. Average SD in  $\text{ETR}_{app}$  at each light level was 0.26 at the start and 0.48 at the end.

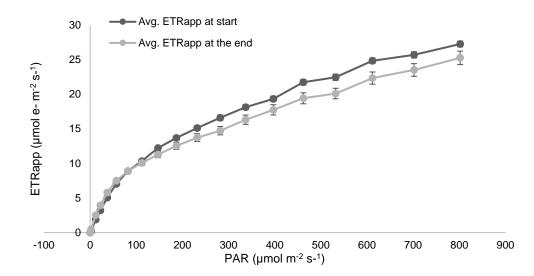
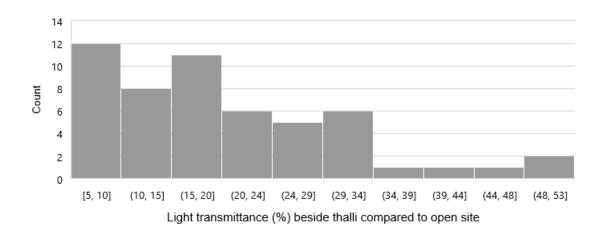


Figure 14. Light response curve for the average ETRapp at each light level for all the thalli before and after transplantation. SD lines were added.

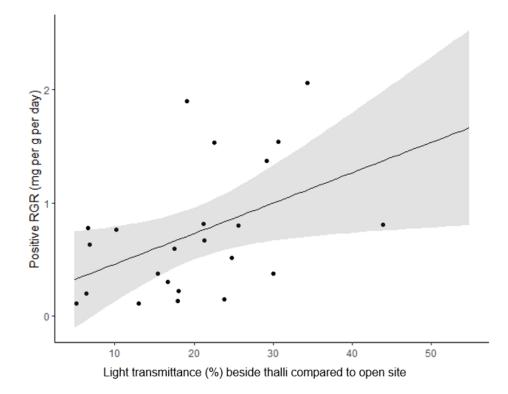
#### 3.3 Light

The average light level for the thalli at the transplantation site was  $102 \pm 10.25 \mu$ mol, whilst the average recorded light out in the open was  $495 \pm 19.85 \mu$ mol. After converting to percentage, the average light transmittance beside the thalli compared to the open site was therefore approximately  $20\% \pm 1.55$  with a median of 18% (Figure 15).



**Figure 15**. Histogram of the distribution of light transmittance beside the thalli compared to open site (in %). The x-axis was divided into 10 classes with intervals of fives.

There was not a statistically significant relationship between general RGR and light (p = 0.1090, Adj.  $R^2 = 0.03$ ) (Figure 1, Appendix), and the separated categories for positives and negatives was thus applied. For thalli that experienced positive RGR, there was a statistically significant relationship (p < 0.05) between RGR and light levels (Figure 16). However, due to the low r-squared value (Adj.  $R^2 = 0.17$ ), the association is very weak, meaning that light might only be a small contributing factor to the increase in thallus weight for the thalli that experienced increased weight. A simple linear regression between negative RGR and light was also tested, but it was not statistically significant (Figure 2, Appendix).



**Figure 16**. Relationship between light and positive RGR (n = 23, Adj.  $R^2 = 0.1683$ , p = 0.0295. A regression line (solid, black) and a 95% confidence interval (light-grey area) were added.

# 3.4 Soil and bark pH

The pH of the soil was generally less acidic than the bark pH of the Norway spruce host trees. The mean soil pH was  $5.04 \pm 0.59$ , which is slightly acidic. The minimum pH registered in the soil was 4.01 (moderately acidic) whereas the maximum was 6.08 (mildly acidic). The bark pH had an average of  $4.43 \pm 0.28$ . The minimum bark pH was 3.86 (moderately acidic), with a maximum of 5.4 (mildly acidic).

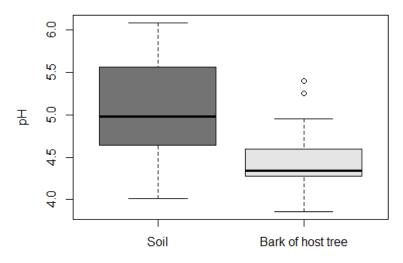
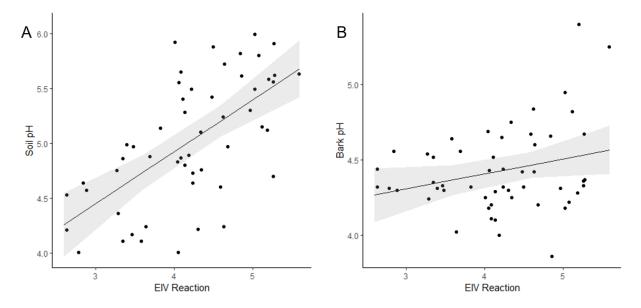


Figure 17. Box plot of the distribution of soil pH (dark grey) and bark pH of the host trees (light grey).

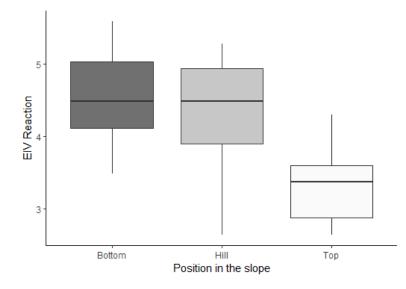
#### 3.5 Vegetation and diameter at breast height

The plant communities and their associated EIV for soil reaction correlated with soil pH (Figure 18A). Plants such as *Vaccinium myrtillus* (bilberry), and *Linnea borealis* (twinflower) were typical species that decreased the EIV value, whereas *Crepis paludosa* (marsh hawk's beard), and *Alnus incana* (grey alder) increased the EIV value. The relationship between the locations' EIV and the host tree's bark pH was also significant (Figure 18B). However, the R<sup>2</sup> value was very low which indicates that the independent variable (bark pH) was not explaining much of the variation detected in the dependent variable (EIV).



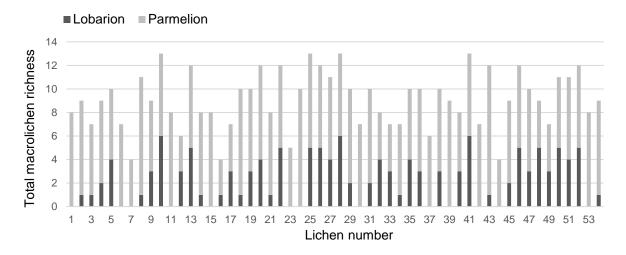
**Figure 18.** (A) Relationship between Ellenberg indicator value (EIV) and soil pH (Adj.  $R^2 = 0.39$ , p < 0.0001). (B) Relationship between EIV and bark pH (Adj.  $R^2 = 0.06$ , p <0.05). Regression lines (solid, black) and 95% confidence intervals (light-grey areas) were added.

Mean EIV reaction score for bottom  $(4.55 \pm 0.55)$  and hill  $(4.34 \pm 0.75)$  was rather similar, whereas the top category  $(3.35 \pm 0.51)$  had a much lower score (Figure 19). One-way ANOVA with a follow up of Tukey's HSD test revealed that the EIV for reaction differed significantly between top-bottom (p < 0.0001) and top-hill (p < 0.001).



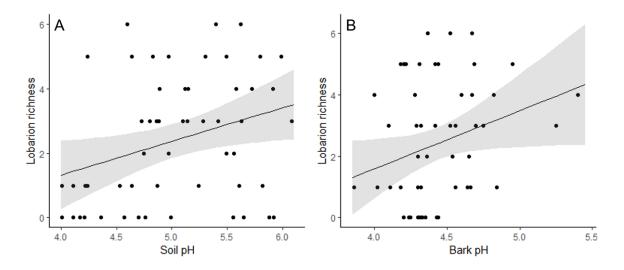
**Figure 19.** Ellenberg indicator value (EIV) as a response to the gradient of position in the slope; bottom (n=23), hill (n = 19) and top (n = 12). (Tukey's HSD test; Hill-Bottom p = 0.532, Top-Bottom p < 0.0001, Top-Hill p < 0.001).

Macrolichen richness was on average  $9 \pm 2$  species for all the thalli locations (Figure 20). Species of the Parmelion community had a higher abundance than species of the Lobarion community.



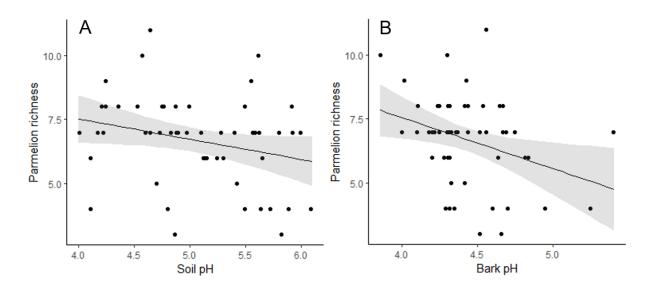
**Figure 20.** Distribution of macrolichen richness where the data is stacked with species belonging to either the Lobarion or the Parmelion community of lichens.

The richness of Lobarion significantly correlated with soil pH (p < 0.05, Adj.  $R^2 = 0.056$ ) (Figure 21A). Additionally, Lobarion richness correlated with bark pH (p < 0.05, Adj.  $R^2 = 0.078$ ) (Figure 21B). In other words, as the amount of Lobarion species at the location increased, so did the pH of the soil and the pH of the bark. Both regression models had low  $R^2$  values, suggesting weak associations.



**Figure 21.** (A) Relationship between Lobarion richness and soil pH (p < 0.05, Adj.  $R^2 = 0.078$ ). (B) Relationship between Lobarion richness and bark pH (p < 0.05, Adj.  $R^2 = 0.056$ ). Regression lines (solid, black) and 95% confidence intervals (light-grey areas) were added.

There was a statistically significant relationship between Parmelion richness and soil pH (p < 0.05, Adj.  $R^2 = 0.07$ ) (Figure 22A). There was also a significant relationship between Parmelion richness and bark pH (p < 0.05, Adj.  $R^2 = 0.09$ ) (Figure 22B). In contrast to the Lobarion community, the relationship between Parmelion and pH was negative, signifying that as the amount of Parmelion species increased, the pH of the soil and the bark got lower. However, both linear regressions had low  $R^2$  values ( $\leq 0.09$ ), indicating that the associations were weak.



**Figure 22.** (A) Relationship between Parmelion richness and soil pH (p = 0.0229,  $R^2 = 0.07$ ). (B) Relationship between Parmelion richness and bark pH (p = 0.0154, Adj.  $R^2 = 0.09$ ). Regression lines (solid, black) and 95% confidence intervals (light-grey areas) were added.

The DBH of the host trees was on average 15.76 cm  $\pm$  11.45 and a median of 12 (Figure 23). The samples thus consisted mostly of small-to medium-sized Norway spruce, although some of them were quite large, with the greatest being 61 cm in diameter.

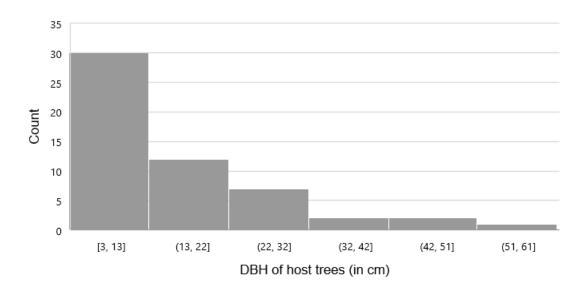


Figure 23. Histogram of diameter at breast height (DBH) distribution (in cm) of the thalli host trees. The x-axis was divided into 6 classes with intervals of 10.

There was a statistically significant relationship between Lobarion richness and DBH of the host tree (p < 0.01) (Figure 24). In other words, the wider the host tree was, the more species from the Lobarion community were present, according to the linear model. However, a low R<sup>2</sup> value indicates that the association was weak, and that DBH was thus not an important driver for the Lobarion richness at each location.

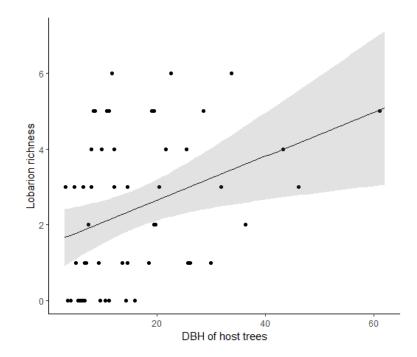


Figure 24. Relationship between Lobarion richness and diameter at breast height (DBH) of host trees (p = 0.008, Adj.  $R^2 = 0.1094$ )

#### 3.6 Positive RGR tested against all relevant variables

The best subset regression model revealed that the top predictor variables for supporting the explanatory variable positive RGR were  $ETR_{app}$  at the start (p < 0.001), light (p < 0.001), pH of the soil (p < 0.05) and EIV (p < 0.05).  $ETR_{app}$ (start) and EIV had a negative relationship with positive RGR, meaning that as  $ETR_{app}$ (start) and EIV increased, RGR decreased. On the other hand, light and soil pH had a positive relationship, where both had higher values as positive RGR increased (Figure 25). These four top predictor variables explain just under 70% of the variation seen in the development of positive RGR, and the relationship between them can thus be considered strong.

```
Call:
lm(formula = logRGRpos ~ ETRappstart + Light + pHsoil + EIV,
    data = yngmod)
Residuals:
     Min
               1Q
                    Median
                                 3Q
                                         Max
-0.93697 -0.18883
                   0.00491 0.25429
                                    1.05330
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.03323
                        1.03823
                                -0.032 0.974819
ETRappstart -0.08842
                        0.01644
                                -5.377 4.14e-05 ***
Light
             0.04911
                        0.01136
                                  4.321 0.000411 ***
pHsoil
                                  2.742 0.013411 *
             0.61657
                        0.22490
EIV
            -0.52826
                        0.19031
                                -2.776 0.012465 *
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.5052 on 18 degrees of freedom
  (31 observations deleted due to missingness)
Multiple R-squared: 0.7504,
                                Adjusted R-squared: 0.6949
F-statistic: 13.53 on 4 and 18 DF, p-value: 2.915e-05
```

**Figure 25.** Output from multiple linear regression model in R, where logarithmic values of positive RGR were tested against the predictors; ETR<sub>app</sub> at the start, light, pH of the soil and Ellenberg indicator value (EIV).

# 4. Discussion

This study aimed to assess the growth and vitality of transplanted individuals of *E*. *pedicellatum* in an area where it historically existed, and thus to what extent transplantation experiments have the potential to be a conservation method this species in Norway. Transplantation is being considered due to the threatened status of *E*. *pedicellatum* populations in Norway, and the associated need for alternative conservation methods.

After being transplanted to Gartlandselva nature reserve for 172 days in 2023, the results indicate that the transplants of *E. pedicellatum* have generally tolerated the transplantation. Interestingly, more than half (n = 36) of the transplants increased in area, and more than a third (n = 23) increased in weight (Figure 7). The vitality parameters remained unchanged or only decreased slightly in  $F_v/F_m$  for some of the thalli, indicating healthy individuals. There was support for hypothesis #1 *Lichens placed in higher light levels will experience higher growth rates and sustained vitality* and hypothesis 3# *Lichens attached to spruce trees with a high soil pH will experience higher growth rates and sustained vitality*. However, this study did not support hypothesis #2 *Lichens placed in locations with high bark pH will experience higher growth rates and sustained vitality*.

This section presents an interpretation of these findings and discusses the factors that may have influenced these outcomes. It will start with a discussion about the growth parameters, followed by the development of the vitality parameters. Then, there will be a broad discussion about the climatic conditions near the study area, as well as growth and vitality and their linkages to the environmental measurements taken in situ. Lastly, there will be sections about the limitations of this study and recommendations for future research.

#### 4.1 Growth and specific thallus mass

STM, the proxy for thallus thickness, got significantly lower with a 13% decrease on average for all the thalli throughout the experiment (p < 0.05), as demonstrated by the skewed development in RTaGR and RGR (Figure 7). In other words, most of the thalli grew thinner after being in Gartlandselva. Considering that Gauslaa (2014) found that lichens tend to acclimatize their STM in new environments, these findings give reason to believe that the individuals of *E. pedicellatum* have indeed acclimatized to the environmental conditions at Gartlandselva. There are several drivers for changes in STM, but the main determiner is exposure to solar radiation (Asplund et al., 2012). Previous transplantation experiments have

demonstrated that low light conditions led to decreased STM of lichen transplants (Gauslaa et al., 2006; Gauslaa et al., 2008). The linkages to light in this thesis work will be further discussed in Section 4.4.1.

## 4.1.1 Water holding capacity

Hydration traits for *E. pedicellatum* altogether did not change or only changed slightly throughout the experiment. Hydration traits are important to study in lichens, partially due to their poikilohydric nature, i.e. their inability to store water long-term and thus dependency on the surrounding environment and its provisioning of hydration (Mallen-Cooper & Cornwell, 2020). WHC<sub>blotting</sub>, the internal water holding capacity, had the same values at the start of the experiment and at the end. The only change occurring for this measurement was the decrease in variation at the end.

WHC<sub>shaking</sub>, the external water holding capacity, was on average slightly reduced throughout the experiment. In other words, the ability of *E. pedicellatum* to withhold water on its cortex was slightly lowered. Previous studies have found that certain species of lichen can acclimatize their WHC to different environmental conditions, such as changes in topography and thus temperature and precipitation (Dominguez et al., 2022). The decrease in WHC<sub>shaking</sub> did not correlate with any of the other environmental variables in this study's data set. It is, however, more likely that the small changes in WHC<sub>shaking</sub> could be caused by inconsistent handling during the shaking measurements before and after transplantation. It can prove challenging to standardize and be consistent during manual shaking of a lichen thallus.

There was a strong correlation between STM and WHC<sub>blotting</sub>, both at the start and at the end of the transplantation (Figure 12AB). The variables demonstrate a 1:1 relationship, which is consistent with previous studies (Gauslaa, 2014; Nilsson, 2021), which have shown that as a thallus gets thicker it can withhold more water, and vice versa. Furthermore, STM is the main driver of WHC<sub>blotting</sub>, because STM reflects the anatomical structure of the thallus, i.e. thickness, which is directly related to the amount of water the lichen is able to hold per area unit (Dominquez et al., 2022). Although both strongly and significantly correlated, the association of WHC<sub>blotting</sub> and STM at the end was a bit weaker compared to the start. As mentioned above, STM decreased significantly, whereas WHC<sub>blotting</sub> did not. There may be a time lag in the acclimatization between STM and hydration traits, although the true nature of such a dynamic requires further research to be more thoroughly demonstrated.

#### 4.2 Vitality

Similarly to the growth parameters, the measurements of vitality also indicate that the thalli tolerated the transplantation well. As mentioned in Section 2.5, changes to these parameters can be linked to stress, and the most common type of stress is photoinhibition caused by excessive light (Schreiber, 2004). The range of values for both  $\text{ETR}_{app}$  and  $F_{\nu}/F_m$  were greater at the start of the experiment than at the end. This may be attributed to the fact that the lichen material was gathered from the ground during collection at Tegningfallet. After transplantation, despite differing light conditions, the thalli were at least up in the trees and thus presumably receiving more similar conditions than at Tegningfallet.

 $ETR_{app}$  did not change significantly throughout the experiment; the mean values were the same at the start and at the end. The light response curve showed an overall slight decrease in  $ETR_{app}$  at the end compared to the start (Figure 14). The range of the values was greater at the start of the experiment. The results of the measurements of  $ETR_{app}$  demonstrated that the PSII yield measured in a light-adapted state remained constant, and thus undamaged from the experiment. Surprisingly,  $ETR_{app}$  at the start of the experiment had a highly significant (p < 0.0001) and negatively associated correlation with positive RGR, in the best subset regression model (Figure 25). Meaning that higher  $ETR_{app}$  values at the start yielded lower positive RGR after transplantation. This relationship was somewhat surprising, and the opposite would be more expected, i.e. that "healthier" lichens would have had higher growth rates. Replicating the study with a larger sample size could explore this relationship more in-depth and demonstrate whether it is grounded in real events or simply due to random occurrences in this study.

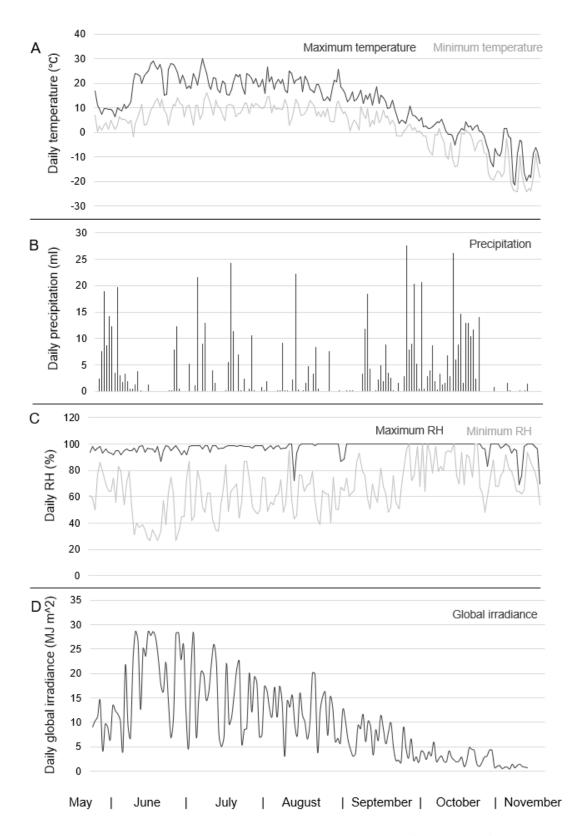
 $F_{\nu}/F_m$  increased to an average of 0.507 after the experiment, compared to the starting values of 0.398 (p < 0.0001). The opposite, decreased  $F_{\nu}/F_m$ , is well-known for being caused by excessive light and indicates damage to photosystem II (Schreiber, 2004; Khan et al., 2020). Similarly, higher  $F_{\nu}/F_m$  values can be connected to adaptations to shade/lower light conditions. A study on Sweet pepper plants found that  $F_{\nu}/F_m$  was always higher under lowlight conditions than high-light conditions (Sui et al., 2012). These results thus indicate that the light levels in Gartlandselva were lower compared to Tegningfallet and that the transplants were more exposed to shade in the study area. However, if the recorded  $F_{\nu}/F_m$  values at the end were at a threshold where they might negatively affect the efficiency of photosystem II in a dark-adapted state, is currently not known. In Canada,  $F_{\nu}/F_m$  measurements of four cyanolichens in different habitats were in the range of 0.510 – 0.594 (Gauslaa et al., 2012),

which is close to the end measurements of the transplants in this study. It should be noted, however, that there can be intraspecific variations in the efficiency of photosystem II of cyanolichens. Interestingly, the change in  $F_{\nu}/F_m$  could also occur as a result of differences in the seasonal timing of the measurements. It has been shown that Bryophytes, which share the same poikilohydric features as lichens (albeit belonging to a different taxonomic kingdom), demonstrated seasonal variations in  $F_{\nu}/F_m$  due to the temporal adherences of environmental stress (Ruchika et al., 2020). Therefore, given that the increase in  $F_{\nu}/F_m$  was small, and that this might be due to seasonal variations, the vitality of the transplants was deemed sustained, and in a healthy state after transplantation.

#### 4.3 Climatic conditions near the transplantation site

It is interesting to evaluate whether the climatic conditions near the transplantation site have been favorable for *E. pedicellatum*. It should still be noted that the data stems from open weather stations and not directly from the study area. The light transmittance recorded from the study area does not account for the level of irradiance when the lichens are exposed to the sun. Even at sites with very low transmittance (e.g. 5% for thallus #34), there can be times during the day when the sun directly hits the lichen. Factors such as canopy dynamics and openings in the upper vegetation layer can yield different total sun exposure than what the in situ measurements can demonstrate. On the other hand, weather stations can provide data on average global radiation, and the nearest weather station that had public data on irradiance was Overhalla – Skogmo.

There was a clear trend that the most irradiance occurred in the summer months when Norway has the longest day and receives the most concentrated sunlight – as one might expect (Figure 26D). Out of this global irradiance, only radiation of wavelengths between 0.4 and 0.7  $\mu$ m (also known as PAR) are effectively utilizable in photosynthesis for autotrophs (Tsubo & Walker, 2004). Calculations for converting between MJ to  $\mu$ mol to estimate PAR are accessible; however, the factors constituting those conversions are generally imprecise. Furthermore, due to the uncertainties regarding canopy dynamics for each thallus, calculations of PAR will entail too many uncertainties, and PAR will thus not be estimated for each transplant.



**Figure 26**. (A-C) Climate data from Gartland weather station in the period 23<sup>rd</sup> of May to 11<sup>th</sup> of November 2023 (Hanssen-Bauer, 2017). (A) Daily minimum and maximum temperature (°C) (average temperature was left out in the graph due to overlapping with minimum and maximum). (B) Daily precipitation (mL). (C) Daily maximum and minimum RH (%). (D) Global irradiance (MJ m<sup>2</sup> per day) from Overhalla – Skogmo weather station. All figures (A:D) have the same timeframe.

The global radiation levels do seem to satisfy the light conditions required by *E. pedicellatum* overall; however, the relationship between light and access to water can affect the growing conditions for this species. Limited light in periods of hydration can limit lichen growth in forests. Similarly, sufficient light during hydration periods strongly promotes growth (Gauslaa et al., 2012). The optimal growing conditions at its original site (Tegningfallet) had a rare combination of light and adequate access to water (Nilsson, 2021). At Gartland weather station, there were a total of 111 days out of 172 that received precipitation and it rained an average of 3.64 mL every day (Figure 26B). At the start of the transplantation, the conditions in the Gartlandselva nature reserve were generally wet upon inspection. The figure also shows that there was substantial precipitation in the beginning (late May and early June). After this period, the precipitation levels were reduced with only some sporadic days of heavy rainfall throughout the summer months. Most of the rainfall was centered around early autumn in September/October and the trends were parallel with the fluctuations in RH.

Before discussing the data on RH, it is important to note that RH varies greatly within local scales (Laurence et al., 2002). For example, forests tend to be more humid in the summer months compared to open sites, partially due to increased evapotranspiration at higher temperatures (Alfieri et al., 2018). Furthermore, the multiple brooks and the larger stream in this reserve can also influence evaporation rates. The stream had a relatively high water level during all the visual inspections during fieldwork, which were in different climatic phases, namely May, July, and November. The data on RH must therefore be used with caution as the RH at the weather station compared to the transplantation site can differ from each other, i.e. the RH was likely higher than the measurements from the weather station. Albeit this difference, the RH fluctuations likely follow the same trends in both locations, with maxima and minima occurring in the same periods.

Daily minimum RH was at its lowest in the summer months, with some days experiencing RH in the lower 30% (Figure 26C). The average minimum RH for the entire transplantation period was 65.3%. Maximum RH is almost at a constant high, with an average of 97.35%. It was particularly high at the end of summer towards autumn, wherein only 9 out of 81 days did not experience 100% maximum RH in the period from the 7<sup>th</sup> of August until the 26<sup>th</sup> of October. During the summer months with high global radiation and low precipitation levels, other forms of hydration could have been important for the transplants. Cyanolichens like *E. pedicellatum* were previously thought to be dependent on either rain or dew to activate their photosynthesis, and thus incapable of utilizing humid air as a hydration source (Gauslaa,

2014). However, recent studies have found that high RH (> 95%) over time, can slowly but steadily contribute to the photosynthetic activation in cyanolichens (Schlensog et al., 2000 in Nilsson, 2021). It is therefore likely that humid air could have contributed to photosynthetic activation of the transplants, particularly in the summer months when global radiation was at its highest and there was an absence of precipitation.

The temperature levels during the transplantation period in Gartland were optimal for the ecological niche of *E. pedicellatum*. In his artificial temperature experiments, Nilsson (2021) found that the RGR and net photosynthesis of E. pedicellatum were significantly suppressed when exposed to temperature treatments exceeding 25 °C. Within the transplantation period, there were a total of 15 days that reached a maximum temperature of 25 °C or warmer in Gartland, meaning that on some days the carbon uptake in photosynthesis was reduced. This could have affected the RGR of the lichens negatively, particularly if the high temperatures persisted over several days. This was the case between the 16<sup>th</sup> to 22<sup>nd</sup> of June when there was a full week of maximum temperatures between 25 and 29.2 °C (Figure 26A), with precipitation towards the end of this period (Figure 25B). However, E. pedicellatum has been shown to have a relatively high desiccation tolerance (i.e. they can endure longer dry periods), in combination with both low RH and low precipitation (Nilsson, 2021). On average, the temperature was  $9.84 \pm 0.5$  °C for the entire period in Gartlandselva, which corresponds relatively well to the findings of Nilsson (2021), where the average temperature at Tegningfallet was 9.21 from April to October. With the temperature data from 2023 coupled with the fact that the species is known for having a boreal distribution (Maass & Yetman, 2002), there is no reason to believe that the temperature conditions would be unfavorable at the transplantation site.

When summarizing the insight provided by data on global radiation, precipitation, RH, and temperature, the overall impression is that the climatic conditions were relatively optimal for the species (at the weather station). Despite lower precipitation levels in the summer months, when temperatures and global radiation were high, humid air has likely contributed to hydration for the lichen transplants. It is important to keep in mind that the climatic measurements were from weather stations in open areas, and not directly from the study site. This is particularly important when analyzing RH and light, which differentiates a lot depending on local factors. Despite deeming the climatic conditions in the region favorable, the local environmental conditions measured in situ might explain more of the variation seen in the results regarding growth and vitality.

### 4.4 Growth and vitality in relation to environmental conditions in situ

# 4.4.1 Light

The diffuse light levels beside the transplants were generally low with an average of 102  $\mu$ mol, compared to 495  $\mu$ mol out in the open (Figure 15). The average light transmittance was thus around 20% compared to an open site. At Tegningfallet, Nilsson (2021) found that *E. pedicellatum* required strong light during the growing season and that some days even exceeded 2000  $\mu$ mol when clear skies were assumed. Furthermore, another study has found that deficient light was inhibiting other epiphytic species in their transplantation experiment (Gauslaa et al., 2007). As mentioned in Section 4.1, insufficient light can cause lichens to become thinner and thus have a low STM. Acclimatizing by becoming thinner is a mechanism to maximize the efficiency of light absorption in the photobiont layer. Most of the transplants grew thinner, which further supports the findings that the light levels were too low, as previously shown by the slight increase in  $F_v/F_m$ .

There was a statistically significant relationship between positive RGR and light transmittance (Figure 16), signifying that higher light levels yielded higher RGR (p < 0.05). This confirms the hypothesis that the lichens placed in areas with higher light levels would experience the most growth. However, the sample size was small (n = 23) and the R<sup>2</sup> value was very low (0.1683). Nevertheless, light was a highly significant (p < 0.001) explanatory variable for positive RGR in the best subset regression model (Figure 25). Based on these findings, hypothesis #1, stating that *lichens placed in higher light levels will experience higher growth rates and sustained vitality*, can be confirmed. Such a relationship could also suggest that the negative RGR values would have had the lowest light levels, but this was not the case statistically. The fact that positive RGR significantly correlated with light, whereas negative RGR did not, further supports that fragmentation has occurred.

## 4.4.2 Soil and bark pH

Soil pH was a strong predictor for both vegetation (EIV) (p < 0.0001) and macrolichen composition (p < 0.05) in this study. The average soil pH for the locations was 5.0 (Figure 17). The species dominating the locations with low soil pH were typical plants that thrive in acidic soils, such as Bilberry and Twinflower. Similarly, the Lobarion-dominated locations with relatively high soil pH. These findings support the preciseness of the measurements of soil pH in this study. Soil pH also came out as a significant predictor of positive RGR in the multiple regression analysis (p < 0.05), signifying that higher soil pH yielded higher RGR (when excluding fragmented thalli). Soil pH was thus an important explanatory element in much of the variation seen in the positive RGR variable. Hypothesis #3, *lichens attached to spruce trees with a high soil pH will have higher rates of growth and sustained vitality*, can thus be confirmed. However, measurements of bark pH were more challenging to link to any of the parameters related to growth and vitality.

Different tree species have different bark pH. The chemical properties, or more importantly, the amount of calcium (Ca) within the bark, is an important trait of the bark that is closely linked to its pH level (Holien & Tønsberg, 2008). Norway spruce is a tree species that typically has low amounts of calcium and thus low pH, but there exists interspecific variation. Factors such as being close to the spray zone of a waterfall or in the drip zone of other trees can increase the bark pH of spruce (Gauslaa & Goward, 2012). Indeed, Nilsson (2021) found that spruces dominated by *E. pedicellatum*, which received moisture carried by water droplets from the waterfall, had a relatively high pH. Only a small portion of the trees in this study had a relatively high bark pH (> 4.75, n = 6). The average bark pH for all the trees was 4.43 (Figure 17), which is moderately acidic and lower than the average for the E. pedicellatumdominated trees at Tegningfallet, which was 4.98 (Nilsson, 2021). It therefore seems that the selected trees in this study generally had a pH that was below the optimum level previously demonstrated for the Norwegian phenotype. Hypothesis #2, stating that lichens attached to spruce trees with a high bark pH will have higher rates of growth and sustained vitality, thus has to be discarded. In terms of other environmental variables, the EIV reaction values had a statistically significant correlation with bark pH; however, the association was weak (Adj. R<sup>2</sup> = 0.06), likely owing to the relatively low bark pH amongst all the samples.

## 4.4.3 Vegetation, position, and diameter at breast height

The locations at the top of the ravines differed significantly (p < 0.001) in terms of the EIV reaction for soil compared to the bottom and hill locations (Figure 19). As expected, the top parts of the ravine gradients were on average more acidic with a score of 3.4, whereas bottom and hill had a mean reaction score of 4.5. As mentioned in Section 3.5, the top parts of the ravine mostly consisted of plants like Bilberry and Twinflower. These are species that prefer acidic soils and therefore lowered the EIV scores. Similarly, the hill and bottom parts of the ravine had high occurrences of species like Marsh Hawk's Beard and Grey alder, which thrive in more neutral soils and thus increased the EIV scores. The EIV reaction for soil was a

significant predictor (p < 0.05) in the best subset regression model for positive RGR (Figure 24). However, the relationship between EIV and positive RGR was negative, meaning that as the average EIV score got lower, the transplants with positive RGR got higher RGR values. This relationship was unexpected, and not easily explained by the prior research conducted for this study. In addition, given that EIV correlated significantly with soil pH, it was surprising that EIV had a negative relationship with positive RGR, whereas soil pH had a positive relationship with positive RGR. This might stem from differences in sample sizes, where the simple linear regression between EIV and soil pH had 54 samples, and the best subset regression model had 23 samples. More research is thus required to identify whether this is a recurring dynamic, or simply a random relationship.

In the macrolichen analysis, there was a significant correlation between the richness of lichen communities and pH levels (Figure 21AB and 22AB). Species within the Lobarion community thus increased in quantity as the pH levels increased in the soil and the host tree's bark. Similarly, as the species within the Parmelion community increased, the pH levels decreased. The associations in the linear regressions were not strong, likely because the pH levels measured in the bark of the host trees were generally low. Finding an explanation for why the pH in the soil also had low explanatory capabilities is more challenging, given that it correlated so well with EIV. A potential reason might be that the macrolichens were recorded in occurrence/richness and not abundance, which might explain why they didn't have a strong association with the pH levels. However, there were statistical significances, and these relationships have been proven in many studies regarding the preferences of these communities. The Lobarion community is restricted to high bark pH (Gauslaa, 1995), and its abundance is also linked to the amount of Ca present in the soil, which further leads to higher soil pH (Gauslaa, 1985). In contrast, the Parmelion community grows on trees with acidic bark (Gauslaa, 1985). The findings in this study therefore support previous research on the dynamics of lichen communities and pH optima, despite the low R<sup>2</sup> values.

There was a significant correlation between Lobarion richness and DBH of the host tree (p < 0.005), meaning that as the quantity of Lobarion members increased, so did the thickness (at 1.5 m above ground) of the host tree (Figure 24). These findings give reason to believe that the growth and vitality of *E. pedicellatum* also could be linked to DBH. However, there were no correlations between RGR, RTaGR, nor their positive and negative categories, to DBH. Furthermore, the R<sup>2</sup> value was weak, which has been a recurring pattern for all the

macrolichen regressions. The low explanatory power of these regression models might be due to the sampling method for macrolichens, as mentioned above.

#### 4.5 Limitations and future recommendations

It became clear post-transplantation that fragmentation of some of the transplants had occurred throughout this study. Through examination of the photographs and analysis of the growth measurements, it was prominent that parts of certain thalli were missing/broken off. This, of course, generates noise in the data and disrupts the findings to a certain degree. It is challenging to distinguish whether the changes occurred due to the environmental conditions at the study site, or simply random, external factors. There are a lot of factors that can contribute to the fragmentation of a lichen thallus, such as poor handling during transportation, physical force from animals, such as birds or moose, or natural tear-off due to dying or weak parts within the thallus lobes. After running multiple tests, the transplants with negative RGR and RTaGR could not be attributed to any of the other variables in the dataset. Therefore, the likelihood that fragmentation caused some of the loss in DM and A is prevalent.

The attachment technique employed in this study, polyester thread and plastic nettings, has proved quite successful in another transplantation experiment (Gauslaa & Goward, 2012). Even though some fragmentation also occurred in their study, they were able to identify which lobes were missing and thus subtracted these missing parts from the start area (Gauslaa & Goward, 2012). This technique, however, was inapplicable to the transplants in this study, due to the challenges of identifying lost lobes due to their complex morphology and growth form (with a lot of overlapping lobes and dark cortex). For future research, it is very important to consider that *E. pedicellatum* is quite brittle and delicate, both in a moist and dry state, which can lead to fragmentation). This species requires careful handling/treatment during measurements and transportation, and more importantly, it requires a robust attachment technique onto host trees in the case of transplantation experiments. Perhaps future studies could identify alternative attachment techniques, more accustomed to the sensitivity of this species.

The study period was from May to November, which means that the growth and vitality parameters were measured during very different seasons. For a more representative comparison, it could be advantageous in future research to conduct these measurements at the

same time of the year (e.g. after one completed year, preferably multiple years), to minimize the effect of environmental stress related to different seasons. As mentioned in Section 4.2, bryophytes displayed seasonal variations in  $F_{\nu}/F_m$ , which potentially could be a phenomenon for the lichens in this study as well. The prolonged precipitation periods in autumn coupled with decreasing light availability due to shorter days, affect the photosynthetic regime of the lichen, and thus potentially vitality during the measurements. It is therefore recommended to perform an analysis of the growth and vitality parameters at the same time of the year to ensure more accurate comparisons.

The short timeframe of a master's thesis and thus the associated short field season might not typically accord to the necessary preparatory work regarding proper selection of study area. Some of the environmental variables had a solid range and sample size, e.g. the slope gradient with top, hill, and bottom, in addition to DBH of host trees. On the contrary, bark pH and light generally had lower values than what has previously been identified as the optimum for this study species. It could therefore have been more useful to conduct the environmental measurements before the selection of host trees. In addition, more detailed and site-specific climate measurements should accompany long-term transplantation experiments. The climate data in this thesis work was retrieved from weather stations outside the study area, meaning that generalizations had to be made as to whether the climatic conditions were optimal for the transplants. As mentioned in Section 4.3, precipitation and temperature can more accurately be compared to the study area, whereas solar radiation and RH are less applicable to areas outside the weather stations due to high local variation. Therefore, long-term light exposure measurements in situ beside the lichens, together with humidity measurements, could have yielded a better understanding of the climatic conditions for the transplants. For studies aiming at conducting long-term transplantation experiments, wherein facilitated colonization and thus the establishment of viable populations are the objectives, it is highly recommended to conduct thorough preparatory measurements of the climatic and environmental conditions at the transplantation site.

# **5.** Conclusion

Lichens, one of the oldest known symbioses on the planet, are important parts of many ecosystems. However, they are threatened by human-driven biodiversity loss, and many lichen species are critically endangered both in Norway and globally. Thus, research efforts are being made to evaluate whether transplantation can support endangered species in overcoming the threat of extinction. One example of a critically endangered species is *E. Pedicellatum*, a species whose known extent in Norway has been reduced to only one area: Tegningfallet. This thesis has thus aimed to answer the research question: *to what extent do transplantation experiments have the potential to be a conservation method for* E. pedicellatum *in Norway*? In pursuing this research question, the thesis has further presented and tested the following three hypotheses:

- Lichens placed in higher light levels will experience higher growth rates and sustained vitality
- 2. Lichens attached to spruce trees with a high bark pH will experience higher growth rates and sustained vitality
- 3. Lichens placed in locations with high soil pH will experience higher growth rates and sustained vitality

To answer the research question and test the hypotheses, an experimental case study through transplantation was designed. Close to one of the species' former known habitats, Gartlandselva in Trøndelag, was identified as a potentially well-suited place for such a transplantation experiment, and 54 lichen thalli were transplanted there from May to November 2023. Various parameters were measured before and after the experiment to evaluate the viability of such a transplantation as a means of conservation.

The parameters for growth were calculated by RGR in weight, and RTaGR in area, which were based on weight and area measurements in the laboratory, as well as the time spent at the transplantation site. STM, a proxy of thallus thickness, was also calculated from these measurements. WHC, an important estimate related to hydration traits was derived from measurements of the thallus at different moisture levels and STM. Vitality was measured through parameters of photosystem II efficiency, both in a light-adapted state (ETR<sub>app</sub>) and a dark-adapted state ( $F_{v}/F_m$ .). There were also conducted measurements of the environmental

conditions at the study site, to look for linkages between the growth and vitality parameters. Studying the development of the growth and vitality parameters, allowed for the evaluation of the extent to which a transplantation experiment could be a potential conservation method for this species in Norway. Despite noise from the fragmentation of certain samples, many transplants demonstrated promising acclimatization capabilities and perseverance. Many of the transplants experienced increased, but interestingly, most of them also grew thinner at the transplantation site, likely as a result of the lower light levels observed in Gartlandselva compared to their original site. Most of the transplants showed no signs, or only slight declines, of vitality and WHC, after spending 172 days in Gartlandselva.

The results showed that light availability and soil pH had significant effects on the positive growth of the transplants, and that vitality was sustained, in concordance with hypotheses #1 and #3. Bark pH, however, also hypothesized to yield higher growth rates and sustained vitality (hypothesis #2), could not be linked to any of the growth parameters, most likely caused by deficient bark pH values in most of the samples. One of the vitality parameters, ETR<sub>app</sub>, remained constant throughout the transplantation period, indicating that the health of photosystem II in a light-adapted state was maintained. These parameters are most often influenced by light, which this study found to be below the general optimum for this species. This may have been reflected in the slight increase in  $F_v/F_m$  (although not statistically significant), however, the increase in  $F_v/F_m$  could also be attributed to seasonal variations, and the efficiency of photosystem II in a dark-adapted state was thus deemed sustained.

For future research, it is advantageous that transplantation experiments aim at conducting experiments over longer temporal scales, with measurements of vitality and growth conducted at the same time of the year. It is also important to be aware of the delicate nature of this species, as fragmentation easily occurs. Nonetheless, this study has shown that *E. pedicellatum* is capable of acclimatizing to a new location and that transplantation experiments can be a viable alternative for conserving this species. Furthermore, thorough preparatory work aiming at precisely reflecting the environmental conditions at its original site (particularly in terms of light levels and humidity), is strongly advised for long-term transplantation experiments, wherein the establishment of viable populations is the objective.

# 6. References

- Alfieri, J. G., Kustas, W. P., & Anderson, M. C. (2018). A Brief Overview of Approaches for Measuring Evapotranspiration. *Agronomy Monographs*. Doi: 0.2134/agronmonogr60.2016.0034
- Asplund, J., Sandling, A., & Wardle, D. A. (2012). Lichen Specific Thallus Mass and Secondary Compounds Change across a Retrogressive Fire-Driven Chronosequence. *PLoS ONE*, 7(11). Doi: 10.1371/journal.pone.0049081
- Asplund, J., & Wardle, D. A. (2017). How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews*, *92*(3), 1720-1738. Doi: 10.1111/brv.12305
- Beck, H., Zimmermann, N., McVicar, T., Vergopolan, N., Berg, A., & Wood, E. F. (2018).
   Present and future Köppen-Geiger climate classification maps at 1-km resolution.
   *Scientific Data*, *5*, 180214. Doi: 10.1038/sdata.2018.214
- Chen, J., Blume, H-P., & Beyer, L. (2000). Weathering of rocks induced by lichen colonization a review. *Catena*, 39(2), 121-146. Doi: 10.1016/S0341-8162(99)00085-5
- Direktoratet for naturforvaltning (1997). *Boreal regnskog i Midt-Norge* (DN-rapport 1997:2). Direktoratet for naturforvaltning. Retrieved from: https://www.miljodirektoratet.no/globalassets/publikasjoner/dirnat2/attachment/743/ka p-1---6.pdf
- Domingues, R. D., Stanton, D., Peralta, M., & Rodriguez, J. M. (2022). Water regulation dynamics of lichens as functional traits could predict future climate change scenarios in an elevational gradient from Central Argentina. *The Bryologist*, 125(3), 468-478. Doi: 10.1639/0007-2745-125.3.466
- Elis, C. J. (2019). Climate Change, Bioclimatic Models and the Risk to Lichen Diversity. *Diversity*, 11(4), 54. Doi: 10.3390/d11040054
- Environmental Protection Agency (EPA) (2013). Moisture Control Guidance for Building Design, Construction and Maintenance (EPA 402-F-13053). Retrieved from: https://www.epa.gov/sites/default/files/2014-08/documents/moisture-control.pdf
- Forskrift om Gartlandselva naturreservat, Grong (2001). Forskrift om fredning av Gartlandselva naturreservat, Grong kommune, Nord-Trøndelag. (FOR-2001-08-31-956). Lovdata. https://lovdata.no/dokument/LF/forskrift/2001-08-31-956

- Gauslaa, Y. (1985). The ecology of Lobarion pulmonariae and Parmelion Caperatae in *Quercus* Dominated Forests in South-West Norway. *The lichenologist*, 17(2), 117-140. Doi: 10.1017/S0024282985000184
- Gauslaa, Y. (1995). The *Lobarion*, an epiphytic community of ancient forests threatened by acid rain. *The Lichenologist*, 27(1), 59-76. Doi: 10.1017/D0024282995000077
- Gauslaa, Y. (2014). Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *The lichenologist*, 46(1), 1-16.
  Doi:10.1017/S0024282913000753
- Gauslaa, Y., Coxson, D. S., & Solhaug, K. A. (2012). The paradox of higher light tolerance during desiccation in rare old forest cyanolichens than in more widespread cooccurring chloro- and cephalolichens. New Phytologist, 195(4), 812-822. Doi: 10.1111/j.1469-8137.2012.04221.x
- Gauslaa, Y., & Goward, T. (2012). Relative growth rates of two epiphytic lichens, *Lobaria pulmonaria* and *Hypogymnia occidentalis*, transplanted within and outside of *Populus* dripzones. *Botany*, *90*, 954-965. Doi: 10.1139/b2012-062
- Gauslaa, Y., & Holien, H. (1998). Acidity of boreal *Picea abies*-canopy lichens and their substrates modified by local soils and airborne acidic depositions. *Flora*, 193(3), 249-257. Doi: 10.1016/S0367-2530(17)30845-9
- Gauslaa, Y., Lie, M., Solhaug, K. A., & Ohlson, M. (2006) Growth and ecophysiological acclimation of the foliose lichen Lobaria pulmonaria in forests with contrasting light climates. *Oecologia*, *147*(3), 406-416. Doi: 10.1007/s00442-005-0283-1
- Gauslaa, Y., Palmqvist, K., Solhaug, K. A., Holien, H., Hilmo, O., Nybakken, L., Myhre, L.
  C., & Ohlson, M. (2007). Growth of epiphytic old forest lichens across climatic and successional gradients. *Canadian Journal of Forest Research*, 37(10), 1832-1845.
  Doi: 10.1139/X07-048
- Gauslaa, Y., Palmqvist, K., Solhaug, K. A., Hilmo, O., Holien, H., Nybakken, L., & Ohlson,
   M. (2008). Size-dependent growth of two old-growth associated macrolichen species.
   New Phytologist, 181(3), 683-692. Doi: 10.1111/j.1469-8137.2008.02690.x
- Hanssen-Bauer, I., Førland, E. J., Haddeland, I., Hisdal, H., Lawrence, D., Mayer, S., Nesje, A., Nilsen, J. E. Ø., Sandven, S., Sandø, A. B., Sorteberg, A., & Ådlandsvik, B. (2017). *Climate in Norway 2100 A knowledge base for climate adaptation*. NCCS report no. 1/2017. Norsk klimaservicesenter. Retrieved 25-30.04.2024 from: https://seklima.met.no/observations/

- Haugan, R., Holien, H., Hovind, A. A., Ihlen, P. G., & Timdal, E. (2021) Artsgruppeomtale lav ('Lichenes'). Norsk rødliste for arter 2021. Artsdatabanken. Retrieved 8th of september 2023 from: https://artsdatabanken.no/rodlisteforarter2021/Artsgruppene/lav
- Holien, H., & Tønsberg, T. (1996). Boreal regnskog i Norge habitatet for trøndelagselementets lavarter. *Blyttia*, 54(4), 157-177. ISSN: 0006-5269. https://nhm2.uio.no/botanisk/nbf/blyttia/DR-2010C/Blyttia 54-4.pdf
- Holien, H., & Tønsberg, T. (2008). Norsk lavflora (2. utgave). Tapir akademiske forlag, Trondheim
- Hovind, A., Phinney, N., & Gauslaa, Y. (2020). Functional trade-off of hydration strategies in old forest epiphytic cephalolichens. *Fungal Biology*, 124(10). Doi: 10.1016/j.funbio.2020.07.008
- IPBES (2019). Summary for policymakers of the global assessment report on biodiversity and ecosystem service of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem services. Diaz, S., Settele, J., Brondízio, E. S., Ngo, H. T., Guèze, M., Agard, J., Arneth. A., Balvanera, P., Brauman, K. A., Butchart, S. H M., Chan, K. M. A., Garibaldi, L. A., Ichii, K., Liu, J., Subramanian, M. S., Midgley, G. F., Miloslavich, P., Molnár, Z., Obura, D., ... Zayas, C. N. IPBES secretariat, Bonn, Germany. 56 pp. doi: 10.5281/zenodo.3553579
- Johnstone, D., Moore, G., Tausz, M., & Nicolas, M., (2013). The measurement of plant vitality in landscape trees. *Arboricultural Journal*, 35(1), 18-27. Doi: 10.1080/03071375.2013.783746
- Jönsson, M. T., Ruete, A., Kellner, O., Gunnarsson, U., & Snäll, T. (2017). Will forest conservation areas protect functionally important diversity of fungi and lichens over time? *Biodiversity and Conservation*, 26(11), 2547-2567. Doi: 10.1007/s10531-015-1035-0
- Jørgensen, P. M. (1972). Erioderma pedicellatum (=E. boreale) in New Brunswick, Canada. *The Bryologist*, 75(3), 369-371. Doi: 10.2307/3241481
- Jørgensen, P. M. (2000). Survey of the Lichen Family Pannariaceae on the American Continent, North of Mexico. *The Bryologist*, 103(4), 670-704. http://www.jstor.org/stable/3244332
- Khan, N., Jemaa, E., Hamdani, S., Qu, M., Luy, M-J. A., Perveen, S., Stirbet, A., Govindjee, G., & Zhu, X-G. (2020). Natural variation in the fast phase of chlorophyll *a* fluorescence induction curve (OJIP) in a global rice minicore panel. *Photosynthesis Research*, *150*, 137-158. Doi: 10.1007/s11120-020-00794-z

- Laurence, H., Fabry, F., Dutilleul, P., Bourgeois, G., & Zawadzki, I. (2002). Estimation of the spatial pattern of surface relative humidity using ground based radar measurements and its application to disease risk assessment. *Agricultural and Forest Meteorology*, 111(3), 223-231. Doi: 10.1016/S0168-1923(02)00019-9
- Lidén, M., Pettersson, M., Bergsten, U., & Lundmark, T. (2004). Artificial dispersal of endangered epiphytic lichens: a tool for conservation in boreal forest landscapes. *Biological Conservation*, 118(4), 431-442. Doi: 10.1016/j.biocon.2003.09.026
- Loss, S., Terwilliger, L., & Peterson, A. (2011). Assisted colonization: integrating conservation strategies in the face of climate change. *Biological Conservation*, 144(1), Doi: 10.1016/j.biocon.2010.11.016
- Lutzoni, F., & Miadlikowska, J. (2009). Lichens. *Current Biology, 19*(13). R502-R503. Doi: 10.1016/j.cub.2009.04.034
- Maass W., & Yetman, D., (2002). COSEWIC assessment and status report on the boreal felt lichen *Erioderma pedicellatum* in Canada, *in* COSEWIC assessment and status report on the boreal felt lichen *Erioderma pedicellatum* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 1–50.

https://www.gov.nl.ca/ffa/files/wildlife-endangeredspecies-sr-boreal-felt-lichen-e.pdf

- Mallen-Cooper, M., & Cornwell, W. K. (2020). A systematic review of transplant experiments in lichens and bryophytes. *The Bryologist*, *123*(3), 444-454. Doi: 10.1639/0007-2745-123.3.443
- Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence a practical guide. *Journal of Experimental Botany*, 51(345), 659-668. Doi: 10.1093/jxb/51.435.659. PMID:10938857
- Microsoft Corporation (2018). *Microsoft Excel* and *Microsoft PowerPoint, versions 2403*. Retrieved from https://office.microsoft.com/excel and https://office.microsoft.com/powerpoint
- NGU (n.d.) Om løsmasser. Retrieved the 5th of September 2023 from https://www.ngu.no/om-geologi/om-losmasser
- NGU (2021). Loose masses N50/N250.

https://register.geonorge.no/register/versjoner/produktspesifikasjoner/norgesgeologiske-undersokelse/losmasser-n50-n250

 Nilsson, A. (2021). Erioderma pedicellatum: an ecophysiological study of a globally threatened pioneer lichen on thin spruce branches in old forests. [Master's Thesis, Norwegian university of life sciences]. Brage. Retrieved from: https://nmbu.brage.unit.no/nmbu-

xmlui/bitstream/handle/11250/2766062/Alexander\_Nilsson\_M.Sc.pdf?sequence=1&is Allowed=y

- Nilsson, A., Solhaug, K., & Gauslaa, Y. (2022). The globally threatened epiphytic cyanolichen Erioderma pedicellatum depends on a rare combination of habitat factors. *The lichenologist*, 54(2), 123-136. Doi: 10.1017/S00242892200007X
- Nooten, S. & Hughes, L. (2017). The power of the transplant: direct assessment of climate change impacts. *Climatic Change*, *144*(2). Doi: 10.1007/s10584-017-2037-6
- Phinney, N. H., Gauslaa, Y., Palmqvist, K., & Esseen, P-A. (2020). Macroclimate drives growth of hair lichens in boreal forest canopies. *Jorunal of Ecology*, *109*(1), 478-490. Doi: 10.1111/1365-2745.13522
- Prestø, T. & Holien, H. (2001). Management of lichens and bryophytes in boreal rainforest. NTNU. Vitensk.mus. bot. Ser. 2001-5: 1-77. Retrieved from: https://www.researchgate.net/publication/277771485\_Forvaltning\_av\_lav\_og\_moser\_i \_boreal\_regnskog
- QGIS.org (2024). QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.org
- R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
- Resl, P., Fernández-Mendoza, F., Mayrhofer, H., & Spribille, T. (2018). The evolution of fungal substrate specificity in a widespread group of crustose lichens. *Proceedings: Biological Sciences, 285*(1889), 20180640. Doi: 10.1098/rspb.2018.0640.
- Rolstad, J., Gjerde I., Storaunet, K. O., & Rolstad, E. (2001). Epiphytic lichens in Norwegian coastal spruce forest: historic logging and present forest structure. *Ecological Applications*, 11(2), 421-436. Doi: 10.2307/3060899
- Romundset, A., Bøe, L., & Høgaas, F. (2023). *Istiden la grunnlaget for nature og nordmannen (2/3)*. Norges Geologiske Undersøkelse (NGU). Retreived the 2<sup>nd</sup> of November from: https://www.ngu.no/ngu-bloggen/istiden-la-grunnlaget-naturen-og-nordmannen-23
- Ruchika, Csintalan, Z., & Péli, E. R. (2020). Seasonality and Small spatial-Scale variation of Chlorophyll a Fluorescence in Bryophyte Syntrichia ruralis [Hedw.] in Semi-Arid Sandy Grasslands, Hungary. *Plants (Basel)*, 9(1), 92. Doi: 10.3390/plants9010092

- Schreiber, U. (2004). Pulse-Amplitude-Modulation (PAM) Fluorometry and Saturation Pulse
   Method: An overview. Advances in Photosynthesis and Respiration, 19. Doi:
   10.1007/978-1-4020-3218-9 11
- Sillett, S. C., McCune, B., Peck, J. E., Rambo, T. R., & Ruchty, A. (2000). Dispersal limitations of epiphytic lichens result in species dependent on old-growth forests. *Ecological Applications*, 10(3), 789-799. Doi: 10.2307/2641045
- Smith, P. L. (2014). Lichen translocation with reference to species conservation and habitat restoration. *Symbiosis*, *62*(1), 17-28. Doi: 10.1007/s13199-014-0269-z
- Solhaug, K. A., Asplund, J., & Gauslaa, Y. (2021). Apparent electron transport rate a noninvasive proxy of photosynthetic CO2 uptake in lichens. *Planta*, 253(14) Doi: 10.1007/s00425-020-03525-9
- Stanton, D. E., Ormond, A., Koch, N. M., and Colesie, C. (2023). Lichen ecophysiology in a changing climate. *American Journal of Botany*, 110(2): e16131. Doi: 10.1002/ajb2.16131
- Stehn, S. E., Nelson, P. R., Roland, C. A. & Jones, J. R. (2013). Patterns in the occupancy and abundance of the globally rare lichen Erioderma pedicellatum in Denali National Park and Preserve, Alaska. *The Bryologist*, *116*(1), 002-014. Doi: 10.1639/0007-2745-116.1.002
- Storaunet, K. O., Rolstad, J., Gjerde, I., & Rolstad, E. (1998). Nyere skogshistorie og forekomst av utvalgte lav-arter i kystgranskog i Namdalen (Rapport fra skogforskningen - supplement 4: 1-102). Norsk institutt for skogforskning (NISK). ISBN 82-7169-857-5 ISSN 1500-323X. Retrieved from: https://nibio.brage.unit.no/nibio-xmlui/bitstream/handle/11250/2560271/Skogforsk-Rapport-Supplement-1998-04.pdf?sequence=1
- Sui, X.-l., Mao, S.-l., Wang, L.-h., Zhang, B.-x., & Zhang, Z.-x. (2012). Effect of Low Light on the Characteristics of Photosynthesis and Chlorophyll a Fluorescence During Leaf Development of Sweet Pepper. *Journal of Integrative Agriculture*, 11(10), 1633-1643. Doi: 10.1016/S2095-3119(12)60166-X
- Tichý, L., Axmanová, I., Dengler, J., Guarino, R., Jansen, F., Midolo, G., Nobis, M. P.,
  Van Meerbeek, K., Aćić, S., Attorre, F., Bergmeier, E., Biurrun, I., Bonari, G.,
  Bruelheide, H., Campos, J. A., Čarni, A., Chiarucci, A., Ćuk, M., Ćušterevska, R., ...
  Chytrý, M. (2022). Ellenberg-type indicator values for European vascular plant
  species. *Journal of Vegetation Science, 34*(1), e13168. Doi: 10.1111/jvs.13168

- Tsubo, M., & Walker, S. (2004) Relationships between photosynthetically active radiation and clearness index at Bloemfontein, South Africa. *Theoretical and Applied Climatology*, 80(1), 17-25. Doi: 10.1007/s00704-004-0080-5
- Tønsberg, T., Gauslaa Y., Haugan R., Holien, H., & Timdal, E. (1996). Threatened macrolichens of Norway – 1995. *Sommerfeltia 23*, 001-258. ISBN 82-74200-029. ISSN 0800-6865
- Venables, W. N., & Ripley, B. D. (2002). Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN: 0-387-95457-0
- Wickham, H. (2016). \_ggplot2: Elegant Graphics for Data Analysis\_. Springer-Verlag New York.
- Wickham, H., & Bryan, J. (2023) \_readxl: Read Excel Files\_. R Package version 1.4.3, https://CRAN.R-project.org/package=readxl
- Wickham, H., Francois R., Henry, L., Muller K., & Vaughan, D. (2023). \_Dplyr: A Grammar of Data Manipulation\_. R package version 1.1.3. https://CRAN.Rproject.org/package=dplyr
- Wickham, H., Vaughan D., & Girlich, M. (2024). \_tidyr: Tidy Messy Data\_. R package version 1.3.1, https://CRAN.R-project.org/package=tidyr
- Yuan, X., Xiao, S., & Taylor, T. N. (2005). Lichen-like symbiosis 600 million years ago. Science, 308(5724), 1017-1020. Doi: 10.1126/science.1111347

# Appendix

Table 1. Overview of all macrolichen species registered at each host tree within a radius of approx.0.5 m from
the transplants, classified into their respective macrolichen communities. The upper table represents lichen #1-
27, and the lower table #28-54.

					1.												-				-		-				-
Species / Lichen nr	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Alectoria sarmentosa					1				1	1					1			1	1			1		1	1		1
Bryoria	1	1	1	1	1		1	1		1	1	1	1	1		1	1	1	1	1	1	1		1	1	1	1
Hypogymnia hultenii		1	1	1	1		1	1	1		1		1	1	1		1	1	1				1	1	1	1	1
Hypogymnia physodes	1				1	1		1	1		1		1	1	1	1		1	1		1		1	1			
Hypogymnia tubulosa		1			1			1		1		1								1	1	1		1		1	
Hypogymnia vittata	1		1	1		1	1	1		1	1			1	1		1	1		1			1				
Melanohalea exasperatula																				1							1
Parmelia sulcata	1	1	1	1		1	1	1		1	1		1	1	1	1		1	1	1	1	1	1	1	1	1	1
Platismatia glauca	1	1	1	1		1		1	1	1	1		1	1	1		1	1		1	1	1	1	1	1	1	
Platismatia norvegica	1	1				1		1	1		1		1	1	1					1	1			1			
Ramalina thrausta																			1					1	1		
Tuckermanopsis chlorophylla	1	1		1		1		1										1				1			1	1	1
Usnea	1	1	1	1	1	1		1	1	1	1	1	1		1			1	1	1	1	1		1	1	1	1
Total Parmelion	8	8	6	7	6	7	4	10	6	7	8	3	7	7	8	3	4	9	7	8	7	7	5	10	8	7	7
Lobaria pulmonaria	_		-	-	-	-	-		1	1	-	-	1	-	_	_	-	_	-	_	-	-	-		_	-	
Lobaria scrobiculata		1	1	1	1			1	1	1		1	1	1		1	1		1	1		1			1	1	1
Nephroma bellum		-	-	-	1			-	-	1		1	1	-		-	1		1	1	1	1			1	1	1
Nephroma laevigatum					-					1		-	-				-		-	1	-	-			-	-	1
Nephroma parile				1	1				1	1		1	1				1		1	1		1			1	1	1
Nephroma resupinatum	-			-	1				-	-		1	1				1		-	-		-			1	-	-
Parmeliella parvula					1					1			1					1				1			1	1	
	-									1								1				1			1	1	
Pseudocyphellaria citrina		-			-			-	_	_		_	-	_	_	-	_	_	_	_					-	-	-
Total Lobarion	0	1	1	2	4	0	0	1	3	6	0	3	5	1	0	1	3	1	3	4	1	5	0	0	5	5	4
Total macrolichens:	8	9	7	9	10	7	4	11	9	13	8	6	12	8	8	4	7	10	10	12	8	12	5	10	13	12	11
Species / Lichen nr	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Alectoria sarmentosa	1	1	1								1					1			1	1	1		1		1		
Bryoria	1	1	1	1	1	1		1	1	1	1	1	1		1	1	1	1	1	1	1		1	1	1	1	1
Hypogymnia hultenii	1	1	-	1	1	1	1	1	1	1	-	1	1	1	1	1	-	1	1	1	-		1	1	1	-	-
Hypogymnia physodes	-	1		1	-	-			<u> </u>	-			-	-	-				-						-	1	1
Hypogymnia tubulosa		-					1	1	1	1		1		1		1	1			1			1				
							1	1	1	1		1		1	1	1	1	1		1		1	1				1
HVDOQVMDIQ VITTATA	1		1	1	1	1		1	1	1	1			1	1	1				1		1	1		1	1	1
Hypogymnia vittata Melanohalea exasperatula	1		1	1	1	1	1	1	1	1	1	1		1	1		1	1		1		1	1		1		1
Melanohalea exasperatula		1			1	1		1				1			1	1		1	1			1	1	1	1	1	1
Melanohalea exasperatula Parmelia sulcata	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1 1 1		1	1	1	1			1		1	1
Melanohalea exasperatula Parmelia sulcata Platismatia glauca		1	1 1	1 1	1		1	1	1			1 1 1 1	1	1	1 1 1	1 1 1 1		1	1		1	1	1	1	1	1	1 1 1
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica	1		1	1	1	1	1		1	1	1	1	1	1	1	1 1 1		1		1	1					1	1
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta	1	1	1 1 1	1 1	1		1	1	1	1	1	1 1 1 1		1 1 1	1 1 1	1 1 1 1		1 1 1	1	1	1	1		1	1	1 1 1 1 1	1 1 1
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla	1	1	1 1	1 1 1			1	1	1 1 1	1	1 1 1 1	1 1 1 1 1	1	1 1 1 1	1 1 1	1 1 1 1 1 1	1	1	1	1		1 1	1	1 1 1	1	1 1 1 1 1	1 1 1 1 1
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea	1 1 1 1 1 1	1 1 1	1 1 1 1	1 1 1	1	1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1	1 1 1 1 1 1 1 1	1	1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1	1 1 1 1 1 1 1	1	1 1 1 1	1	1 1 1 1	1	1 1 1 1	1	1 1 1 1 1 1 1	1 1 1 1 1
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea Total Parmelion	1 1 1 1 7	1	1 1 1	1 1 1			1	1	1 1 1	1	1 1 1 1	1 1 1 1 1	1	1 1 1 1 1 7	1 1 1	1 1 1 1 1 1	1	1 1 1	1 1 1 7	1	1 4	1 1	1	1 1 1 1 7	1 1 1 1 7	1 1 1 1 1	1 1 1 1 1
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea <b>Total Parmelion</b> Lobaria pulmonaria	1 1 1 1 7 1	1 1 1 8	1 1 1 1	1 1 1 1 1 8	1 4	1	1 1 1 1 6	1 1 1 6	1 1 1 1 7	1 1 1 1	1 1 1 1 1 7	1 1 1 1 1 1 1 1	1 1 5	1 1 1 1 1 7 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1	1 1 1 1 7	1 1 1 7 1	1 1 1 7	1 4 1	1 1 1 4	1	1 1 1 1 7 1	1 1 1 1 7 1	1 1 1 1 1 1 1	1 1 1 1 1 8
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea <b>Total Parmelion</b> Lobaria pulmonaria Lobaria scrobiculata	1 1 1 1 7 1 1	1 1 1	1 1 1 1	1 1 1	1 4 1	1 4 1	1 1 1 1	1 1 1 6 1	1 1 1 1 7	1 1 1 1	1 1 1 1 7 7	1 1 1 1 1 1 1 1	1 1 5 1	1 1 1 1 1 7 7 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1	1 1 1 1 1 1 1	1 1 1 7 1 1	1 1 1 7 7	1 4 1 1	1 1 1 4	1 1 6 1	1 1 1 1 7 1 1 1	1 1 1 7 1 1 1	1 1 1 1 1 1 1	1 1 1 1 1
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea <b>Total Parmelion</b> Lobaria pulmonaria Lobaria scrobiculata Nephroma bellum	1 1 1 1 7 1	1 1 1 8	1 1 1 1	1 1 1 1 1 8	1 4	1	1 1 1 1 6	1 1 1 6	1 1 1 1 1 7	1 1 1 1	1 1 1 1 1 7	1 1 1 1 1 1 1 1	1 1 5	1 1 1 1 1 7 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1	1 1 1 1 7	1 1 1 7 1 1 1 1	1 1 1 7	1 4 1	1 1 1 4	1 1 1 6 1 1	1 1 1 1 7 1	1 1 1 1 7 1	1 1 1 1 1 1 1	1 1 1 1 1 8
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea <b>Total Parmelion</b> Lobaria pulmonaria Lobaria scrobiculata Nephroma bellum Nephroma laevigatum	1 1 1 1 1 1 1 1	1 1 1 8 1	1 1 1 1	1 1 1 1 8 1	1 4 1	1 4 1 1	1 1 1 1 6	1 1 1 6 1 1	1 1 1 1 7 7	1 1 1 1	1 1 1 1 7 7	1 1 1 1 1 1 1 1	1 1 5 1 1	1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1	1 1 1 7 7	1 1 1 7 1 1	1 1 1 7 7	1 1 1 1	1 1 1 1 4 1 1 1	1 1 6 1 1 1	1 1 1 1 7 1 1 1	1 1 1 1 1 1 1 1	1 1 1 1 1 1 1	1 1 1 1 1 8
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea <b>Total Parmelion</b> Lobaria pulmonaria Lobaria scrobiculata Nephroma bellum Nephroma laevigatum Nephroma parile	1 1 1 1 7 1 1	1 1 1 8	1 1 1 1	1 1 1 1 1 8	1 4 1	1 4 1	1 1 1 1 6	1 1 1 6 1	1 1 1 1 7	1 1 1 1	1 1 1 1 7 7	1 1 1 1 1 1 1 1	1 1 5 1	1 1 1 1 1 7 7 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1	1 1 1 1 7	1 1 1 7 1 1 1 1	1 1 1 7 7	1 4 1 1	1 1 1 4	1 1 1 6 1 1	1 1 1 1 7 1 1 1	1 1 1 7 1 1 1	1 1 1 1 1 1 1	1 1 1 1 1 8
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea <b>Total Parmelion</b> Lobaria pulmonaria Lobaria scrobiculata Nephroma bellum Nephroma laevigatum Nephroma parile Nephroma resupinatum	1 1 1 1 1 1 1 1 1 1	1 1 1 8 1	1 1 1 1	1 1 1 1 8 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 4 1 1	1 1 1 1 6	1 1 1 6 1 1	1 1 1 1 7 7	1 1 1 1	1 1 1 1 7 7	1 1 1 1 1 1 1 1	1 1 5 1 1	1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1	1	1 1 1 7 7	1 1 1 1 1 1 1	1 1 1 7 7	1 1 1 1	1 1 1 1 4 1 1 1	1 1 6 1 1 1	1 1 1 1 7 1 1 1	1 1 1 1 1 1 1 1	1 1 1 1 1 1 1	1 1 1 1 1 8
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea <b>Total Parmelion</b> Lobaria pulmonaria Lobaria scrobiculata Nephroma bellum Nephroma laevigatum Nephroma parile Nephroma resupinatum Parmeliella parvula	1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 8 1	1 1 1 1	1 1 1 1 8 1	1111	1 4 1 1	1 1 1 1 6	1 1 1 6 1 1 1	1 1 1 1 7 7	1 1 1 1	1 1 1 1 7 7	1 1 1 1 1 1 1 1	1 1 5 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1	1	1 1 1 7 7	1 1 1 7 1 1 1 1	1 1 1 7 7	1 1 1 1 1 1	1 1 1 1 4 1 1 1	1 1 6 1 1 1 1 1	1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1	1 1 1 1 1 8
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea <b>Total Parmelion</b> Lobaria pulmonaria Lobaria scrobiculata Nephroma bellum Nephroma laevigatum Nephroma parile Nephroma resupinatum Parmeliella parvula Pseudocyphellaria citrina	1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 8 1 1 1	1 1 1 1 7	1 1 1 1 8 7 1 1	1 1 1 1 1 1 1 1 1	1 4 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 6 1 1 1 1 1 1	1 1 1 1 1 7 7 1 1 1 1	1 1 6	1 1 1 1 1 1 1 1 1	1 1 1 1 1 9 9	1 1 5 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 7	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 4	1 1 1 1 7 7	1 1 7 1 1 1 1 1 1 1 1	1 1 1 7 7 1 1 1	1 1 1 1 1 1 1 1 1 1	1 1 1 1 4 1 1 1	1 1 6 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 8 8 1
Melanohalea exasperatula Parmelia sulcata Platismatia glauca Platismatia norvegica Ramalina thrausta Tuckermanopsis chlorophylla Usnea <b>Total Parmelion</b> Lobaria pulmonaria Lobaria scrobiculata Nephroma bellum Nephroma laevigatum Nephroma parile Nephroma resupinatum Parmeliella parvula	1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 8 1	1 1 1 1	1 1 1 1 8 1	1 1 1 1 1 1	1 4 1 1	1 1 1 1 6	1 1 1 6 1 1 1	1 1 1 1 7 7	1 1 1 1	1 1 1 1 7 7	1 1 1 1 1 1 1 1	1 1 5 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1	1	1 1 1 7 7	1 1 1 1 1 1 1	1 1 1 7 7	1 1 1 1 1 1	1 1 1 1 4 1 1 1	1 1 6 1 1 1 1 1	1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1	1 1 1 1 1 8

```
Call:
lm(formula = RGR ~ Light, data = yngmod)
Residuals:
    Min
             1Q Median
                             3Q
                                    Max
-8.2643 -0.7240 0.5577
                         1.1407
                                 2.5004
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
                                          0.0166 *
(Intercept) -1.35557
                        0.54713
                                -2.478
Light
             0.03961
                        0.02428
                                  1.631
                                          0.1090
____
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 1.971 on 51 degrees of freedom
  (1 observation deleted due to missingness)
Multiple R-squared: 0.04958, Adjusted R-squared:
                                                     0.03094
F-statistic: 2.66 on 1 and 51 DF, p-value: 0.109
```

**Figure 1.** Output of the simple linear regression model for the relationship between RGR and Light (p = 0.1090, Adj.  $R^2 = 0.03094$ )

Call: lm(formula = RGRneg ~ Light, data = yngmod) Residuals: Min 1Q Median 30 Max -7.2628 -0.3906 0.3867 1.4480 2.0481 Coefficients: Estimate Std. Error t value Pr(>|t|)(Intercept) -2.36489 0.71827 -3.292 0.00269 \*\* Light 0.04065 1.290 0.20759 0.03151 \_\_\_\_ Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1 Residual standard error: 2.106 on 28 degrees of freedom (24 observations deleted due to missingness) Multiple R-squared: 0.0561, 0.02239 Adjusted R-squared: F-statistic: 1.664 on 1 and 28 DF, p-value: 0.2076

**Figure 2.** Output of the simple linear regression model for the relationship between negative RGR and light (p = 0.20759, Adj.  $R^2 = 0.02239$ )



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