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# **Ultraviolet Radiation Affects Growth and Photosynthesis in the Old Forest Lichens *Lobaria pulmonaria* and *L. virens***

Ida Karina Kann

M-LUN Teacher Education in Natural Science



## Preface

This thesis marks the end of my Master's degree of Teacher Education in Natural Sciences at the University of Life Sciences (NMBU). This thesis was written for the Department of Ecology and Natural Resource Management (INA). My time as a student in Ås has been an adventure. I have attained scientific insight and life wisdom both through lectures and friendships that I am looking forward to passing on to my future pupils.

I am very grateful for all help I have received in order to complete this thesis. Firstly, I want to sincerely thank my two supervisors – Yngvar Gauslaa and Knut Asbjørn Solhaug – for all your help during the field experiment, lab work and writing process. Knut Asbjørn: thank you for all the practical assistance during the fieldwork, and for sharing your expertise on advanced machines and your insight in photosynthetic processes. Yngvar: thank you for your inspiring interest in lichens and guidance through the writing process. In addition, I would like to thank Sara Longinotti for providing some well hidden papers on *Lobaria virens*.

Finally, I would like to express my special gratitude to my beloved housemates, friends and family: you have been a great support during the thesis work. A warm thanks goes to my parents for your constructive feedback in the writing process, and to Sara Marie Blichner and my brother Jørgen for helping me with the editing. Petter Lilleengen: track number four on the album “Led Zeppelin II”.

Ås, May 12<sup>th</sup> 2016

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Ida Karina Kann

## Abstract

Depletion of the ozone layer and human activities such as logging may increase ultraviolet (UV) radiation-exposure of forest lichen habitats. Previous studies show that some forest lichens are highly susceptible to high light exposure. Studies on the effect of UV radiation on lichen viability and growth are rare. This study assesses how UV radiation affects growth, melanic pigment formation and photosynthesis in the old forest lichens *Lobaria pulmonaria* and *L. virens*. The lichens were exposed to different radiation treatments by screening specific wavelength ranges of natural solar radiation. Lichen thalli were placed under three different screens in a fully exposed site in Ås, southeastern Norway, for four weeks during early autumn. Three radiation treatments were used: 1. Photosynthetic active radiation (PAR), 2. PAR + UV-A and 3. PAR + UV-A + UV-B.

UV exposure reduced relative growth rates (RGR) in *Lobaria pulmonaria* and *L. virens*, compared to the PAR treated thalli. In *L. pulmonaria*, UV exposure reduced relative thallus area growth rate (RT<sub>AGR</sub>) more than the RGR reduction. All treatment groups of both species experienced an increase in specific thallus mass (STM). Both UV-A and UV-B induced melanic pigment synthesis in *L. pulmonaria*, evidenced by a darker colour, lower mean reflectance and higher browning reflectance index (BRI) compared to the PAR treated thalli. The highest pigment production occurred in UV-B-exposed *L. pulmonaria*, in which no indication of photoinhibition was detected. PAR treated *L. pulmonaria* had significantly lower photosynthetic CO<sub>2</sub> uptake at high irradiances compared to the control, but no significant differences were found between the UV treated thalli and the control. There was no evidence that UV exposure induced melanic pigment production in *L. virens*. All treatment groups of *L. virens* experienced photoinhibition. These results indicate that *L. pulmonaria* has the ability to acclimate to high light conditions, if additionally exposed to UV radiation and sufficiently hydrated. No clear evidence indicated high light-acclimation in *L. virens*. The high growth rates observed in *L. pulmonaria* and *L. virens* show that growth is a highly useful parameter in short-term experiments assessing the ecophysiology of lichens.

## Abbreviations

A: Area

BRI: Browning reflectance index

Chl: Chlorophyll

DM: Dry mass

ETR: Electron transport rate

ETR<sub>app</sub>: Apparent electron transport rate

F<sub>v</sub>': Variable fluorescence in illuminated sample

F<sub>v</sub>: Variable fluorescence in dark-adapted sample

F<sub>t</sub>: Fluorescence intensity at time t

F<sub>m</sub>': Maximum fluorescence yield in illuminated sample

F<sub>o</sub>': Minimum fluorescence yield in illuminated sample

F<sub>m</sub>: Maximum fluorescence yield in the dark-adapted sample

F<sub>o</sub>: Minimum fluorescence yield in the dark adapted sample

F<sub>v</sub>/F<sub>m</sub>: Maximum quantum yield of PSII photochemistry in dark-adapted sample

F<sub>v</sub>'/F<sub>m</sub>': Quantum yield of PSII photochemistry in illuminated sample

IRGA: Infrared gas analyser

NIR: Near infrared

PSII: Photosystem II

QY: Quantum yield

PAR: Photosynthetic active radiation

RGR: Relative growth rate

RH: Relative humidity

RT<sub>AGR</sub>: Relative thallus area growth rate

ROS: Reactive oxygen species

SE: Standard error

STM: Specific thallus mass

UV: Ultraviolet

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# 1 Introduction

Effects of ultraviolet (UV) radiation (200-400 nm) on living organisms have been given increasing attention during the past three decades due to anthropogenic depletion of the protective ozone layer (Holzinger & Lutz 2006; Paul & Gwynn-Jones 2003). The biologically relevant solar UV radiation reaching the biosphere is in the UV-A (315-400 nm) and UV-B (280-315 nm) wavelength ranges, whereas all UV-C (200-280 nm) radiation is absorbed by the ozone layer (Holzinger & Lutz 2006). The implementation of the Montreal protocol in 1989 has led to reduced amounts of chlorofluorocarbons, the main drivers of ozone depletion, in the atmosphere (United Nations Environment Programme Environmental Effects Assessment 2016). Today the total amount of ozone in the stratosphere increases, thus reducing the level of UV-B radiation reaching the ground (United Nations Environment Programme Environmental Effects Assessment 2016).

Nevertheless, it is still important to understand how organisms respond to UV, because the ozone layer thickness varies locally, and depletion at high latitudes continues to occur during winter and spring (Bais et al. 2015). To illustrate this: the largest ozone loss ever observed in the Arctic, was recorded during spring 2011, which increased UV-B radiation substantially in Arctic and sub-Arctic areas (Bais et al. 2015; Manney et al. 2011). Apart from the ozone layer thickness, surface UV depends on other atmospheric phenomena such as cloud cover and amount and type of aerosols (Bais et al. 2015). Cloud cover, depending on cloud type and density, can have as great effect on transmittance of UV radiation as the ozone layer (Paul & Gwynn-Jones 2003). Interactions between atmospheric chemistry, climate changes and anthropogenic emissions are complex, which makes projections of future surface UV radiation uncertain (Bais et al. 2015). Moreover, the ozone depletion varies both seasonally and on an annual basis (Bais et al. 2015). This makes UV acclimation responses an interesting study field. Surface UV-A is affected by clouds, surface reflectivity and aerosols (Bais et al. 2015), but not by depletion of the ozone layer (Paul & Gwynn-Jones 2003).

Studies over the past decades have shown that UV exposure can be damaging to plants, but also function as an important environmental signal. Many studies have focused on the detrimental effects of UV radiation: for example, high UV-B levels can have detrimental effects on photosynthesizing organisms, including accumulation of reactive oxygen species (ROS) and DNA damage (Hideg et al. 2013; Mazza et al. 1999). Also UV-A can have detrimental effects on plants, and has been shown to increase photoinhibition (Kreuse et al. 1999) and damage to photosystem II (Turcsanyi & Vass 2000). There has recently been a shift in the focus on how UV-B radiation affects organisms, and the role of UV radiation as a key environmental signal has received increased attention in the past two decades (Paul & Gwynn-Jones 2003; Robson et al. 2015). UV radiation exposure can induce protective mechanisms in photosynthesizing organisms, such as pigment accumulation (Jenkins 2009), antioxidant defence activation (Hideg et al. 2013) and increased expression of genes involved in DNA repair mechanisms (United Nations Environment Programme Environmental Effects Assessment 2016). UV-B radiation can change plant morphology, typically making the plant structure more compact (Robson et al. 2015). Examples of such morphological changes are thicker leaves and shorter petioles and stems.

Studies on how UV radiation affects biomass accumulation in plants show varying results and depend on both experimental method and species. For example has reduced growth as response to UV-B exposure been observed in barley (Mazza et al. 1999) and sorghum (Kataria & Guruprasad 2012), whereas growth of rice (Dai et al. 1997) and northern red oak (Bassman & Robberecht 2006) was unaffected by UV-B exposure. Other photosynthesizing organisms also show varying response in growth as response to UV-B radiation. UV-B can have adverse effects on growth of macroalgae (Schmidt et al. 2010; Schmidt et al. 2012), whereas growth of benthic diatoms is not influenced by UV-B radiation (Wulff et al. 2008). Even ecotypes may differ in their growth response to UV radiation (e.g. in common sea-buckthorn (Yang et al. 2008)). Experiments in UV research differ, which can bias the results (Holzinger & Lutz 2006; Xu & Sullivan 2010). Experimental setups differ, for example with respect to the study period length, whether or not the organisms are adapted to UV, in ratios of UV and PAR, and in the use of enhancement of versus screening from UV radiation (Holzinger & Lutz 2006).



Lichens are symbiotic associations between a heterotrophic mycobiont (a fungus) and one or more autotrophic photobionts (algae and/or cyanobacteria). Lichens dominate large areas of terrestrial ecosystems, especially habitats characterized by extreme climate conditions, such as high light including high UV levels and extreme temperatures. Lichens play important roles in ecosystem functioning and biodiversity (Cornelissen et al. 2001). *Lobaria pulmonaria* (L.) Hoffm. and *Lobaria virens* (With.) J.R. Laundon are old forest foliose (= leaf-like) lichens, and are both indicators of ecological continuity in forests (Rose 1974). In Norway, *L. virens* occurs mainly along the coast, whereas *L. pulmonaria* in addition occurs in inland forests and further north than *L. virens* (Fig. 1). *Lobaria pulmonaria* and *L. virens* are members of the *Lobaria* genus. *Lobaria* is used for naming the epiphyte community *Lobarion*, dominated by bryophytes, cyano- and cephalolichens. In Europe a decline in *Lobarion* communities has been observed during the last century, linked to sulphuric acid pollution (acidification) and changes in forest management techniques (Rose 1988).

*Lobaria pulmonaria* is a widespread lichen species, occurring in Europe, Macaronesia, North America, Asia and Africa (Rose & Purvis 2009), and has become a model species in lichen studies (Otalora et al. 2015; Walser et al. 2001). *Lobaria virens* is restricted geographically to Europe, including Macaronesia (Rose & Purvis 2009), and Africa (including Madagascar) (Rambold 2015). *Lobaria virens* is extremely threatened in many of its habitats (Boardman 1977; Fischer & Killmann 2008; Scheidegger et al. 2002), thus the scientific use of it has been restricted, which is the reason why we know so little about its ecophysiology. One exception is a light susceptibility study by Gauslaa and Solhaug (1996), where *L. virens* was the most susceptible species among all the studied species to high light exposure. *Lobaria pulmonaria* has also been shown to be susceptible to excessive light (Gauslaa & Solhaug 1999; McEvoy et al. 2007). Lichens are poikilohydric organisms, which enable them to tolerate desiccation. However, photo-damage accumulation can be a severe threat to dry thalli exposed to high light conditions for longer periods (Gauslaa et al. 2012). Both *L. pulmonaria* and *L. virens* has been shown to be intolerant to extended dry periods (Gauslaa & Solhaug 1996; Wolseley & James 2000).

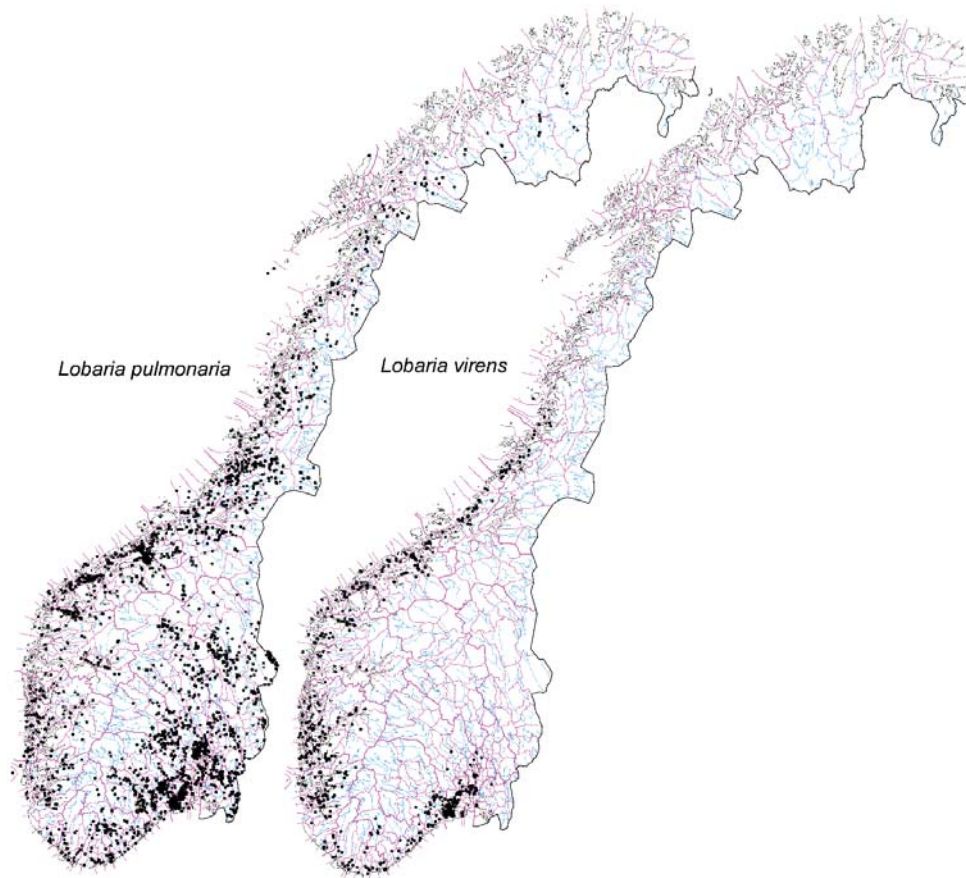


Figure 1: Distribution of *Lobaria pulmonaria* and *Lobaria virens* in Norway according to the Norwegian Lichen Database (Timdal 2016). Black dots show species observations.

Canopy openness can be an important factor for UV microclimate in forests, because canopy can reduce the UV-B transmittance below by up to 99 % (Paul & Gwynn-Jones 2003). By increasing the canopy openness, forestry operations such as logging and thinning can have a major impact on both PAR and UV levels in forests. Because *L. virens* and *L. pulmonaria* are susceptible to high light, changes in canopy openness caused by climate change, ozone depletion and human activities will presumably have adverse effects on lichen populations of these species. Hence, we need to increase our knowledge on lichen response to different environmental factors.

Most studies assessing lichen response to UV radiation have focused on formation of sun-screening compounds (Gauslaa & Solhaug 2001; Gauslaa & Solhaug 2004; Kovacik et al. 2011; Nybakken & Julkunen-Tiitto 2006; Singh et al. 2012; Solhaug et al. 2003). The photobiont partner of *L. pulmonaria* is highly susceptible to high light (Gauslaa

& Solhaug 1996; Gauslaa & Solhaug 2001). To protect their photobiont, many mycobiont partners of lichens produce secondary compounds that function as sunscreen (Solhaug et al. 2003). Such secondary compounds decrease the cortical transmittance and thus shade the photobiont layer (Gauslaa & Solhaug 2001; Solhaug et al. 2003). The mycobiont of *L. pulmonaria* produces melanic pigments in the upper cortical layers when exposed to UV-B radiation, while UV-A radiation induces much less melanic pigments (Solhaug et al. 2003). Melanic pigments absorb wavelengths in the UV-A and UV-B spectra, but also in the photosynthetic active radiation (PAR; 400-700 nm) spectrum (Gauslaa & Solhaug 2001). It is not known whether *L. virens* produce sun-absorbing pigments, but a brown colour has been observed in *L. virens* thalli in open habitats (Y. Gauslaa, personal communication). Larsson et al. (2009) showed that synthesis of radiation-protective compounds was highly responsive to UV-B exposure, with significant differences between radiation treatments in pigment production developing within 15 days.

Lichen growth has successfully been used as a response parameter to different environmental factors in both growth chamber experiments (Bidussi et al. 2013; Larsson et al. 2009) and transplantation studies (Bidussi & Gauslaa 2015; Gauslaa et al. 2006). Few have studied lichen growth as response to UV radiation, the experimental setup differ, and the results are inconsistent.

This study aims to investigate how UV radiation affects growth and photosynthesis in *L. pulmonaria* and *L. virens*, and how melanic pigments affect these parameters. Screens are used to exclude UV-A and UV-B wavelength ranges to separate the effect of each wavelength range on the study species, because induction of melanic pigments depends on wavelength range (Solhaug et al. 2003). Melanic pigments are predicted to reduce QY of CO<sub>2</sub> uptake and photoinhibition. An increase in weight relative to area is expected in all radiation treatments, and I hypothesize that lichens respond to UV by an additional increase in thickness. The shade-adapted thalli of *L. virens* and *L. pulmonaria* are expected to increase their chl *a/b* ratio and decrease their chl content. The study also aims to investigate the potential of using growth as response parameter in studies assessing lichen ecophysiology.

## 2 Material and methods

### 2.1 Lichen material

Thalli of *L. pulmonaria* (L.) Hoffm. and *L. virens* (With.) J.R. Laundon (Fig. 2) were collected June 27, 2015 from old open broadleaved oak forests, located in Langangen, Porsgrunn, Telemark (59° 06 43 N, 9° 50 05 E, 140 m above sea level). The lichens were found on a *Quercus* stem about five meters above ground. They were air-dried in the lab at room temperature and put in a freezer (-18 °C).



Figure 2: Hydrated experimental thalli of *Lobaria virens* (left) and *Lobaria pulmonaria* (right).

### 2.2 Field experiment

The lichen material was taken out of the freezer September 1, 2015, and air-dried in the laboratory for 24 hours (~20 °C). Debris and bryophytes were removed from the lichens, and they were cut into pieces referred to as ‘thalli’ in the following text (see two examples in Fig. 2). In total, 164 thalli, 82 of each species, were randomly selected and numbered. Measurements of growth, colour and chl *a* fluorescence were undertaken. Twenty-three air-dry thalli of each species were again put in the freezer to be used as controls (18 thalli) and for measuring correction factors for dry mass (DM; 5 thalli).

Fifty-four thalli of each species were sewn onto plastic grids fastened on frames. Nine frame-holders, each containing six thalli of each species, were placed outside on a short fully exposed site (Fig. 3) in Ås, Akershus, southeastern Norway (59° 30' N, 10°47'8 E, 100 m above sea level) on September 3, 2015.





*Figure 3: Field experiment set-up in Ås, Akershus, southeastern Norway.*

*Photo: Ida Karina Kann*

The frame-holders were placed under three different screens (transmittance spectra given in Fig. 3 with three replications of each screen):

1. Polycarbonate screen, screening radiation below 400 nm (transmits PAR)
2. Polyester screen, screening radiation below 315 nm (transmits PAR + UV-A)
3. Acryl screen, screening radiation below 250 nm (transmits PAR + UV-A + UV-B)

Each of the three screen types was approximately 1x1 m. The screens were placed 10 cm above the frames with attached lichen transplants to allow natural air circulation and thus avoid excessive heating. The thalli were hydrated with deionized water morning and evening, except during rainy days. At September 30, 2015, all thalli were harvested and brought to the lab for measurements.

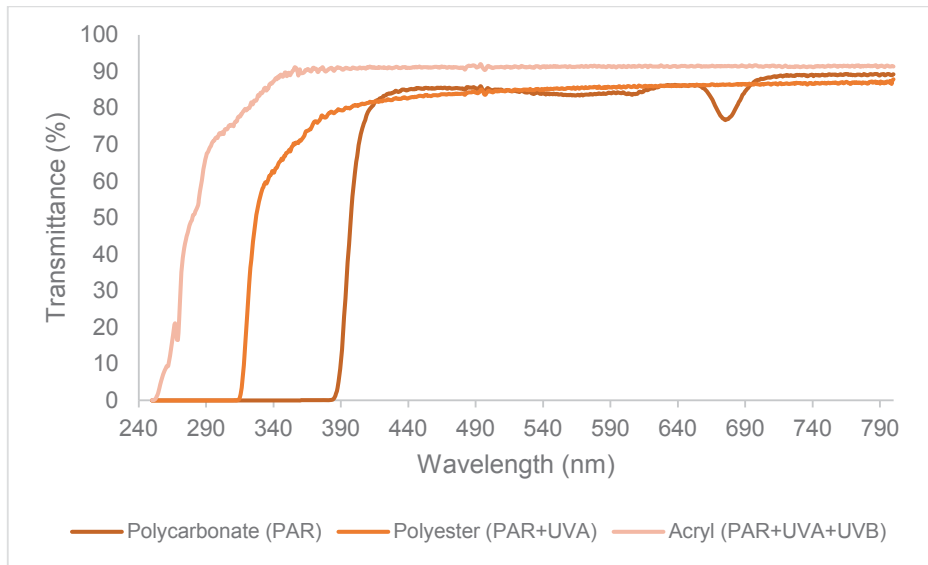


Figure 4: Mean ( $n=3$ ) transmittance spectra of polycarbonate, polyester and acryl screens used to modify the spectral composition of the natural solar radiation during the field experiment.

During the field experiment, weather data (temperature, relative humidity (RH) and irradiance in the PAR spectre) was recorded every min using a Hobo H21-002 Micro Station Data Logger (Onset Computer Corporation, Bourne, MA, USA). The UV radiation (295-385 nm) was recorded every 10 min using an Eppey Ultraviolet radiometer (The Eppey Laboratory, Inc., Newport, Rhode Island USA) (UV data received from Signe Kroken, Department of Mathematical sciences and Technology, NMBU).

## 2.3 Growth parameters

Area (A) and dry mass (DM) of the thalli were measured before and after the field experiment. All thalli were weighed on a scale (Sartorius ED,  $\pm 0.1$  mg) to find their air-dried mass (mg). To correct for the difference in air humidity during the weighing before and after the field experiment, five additional thalli of each species were weighed, dried at  $70^{\circ}\text{C}$  for 24 h, and then weighed immediately after the oven drying. The mean change in mass of the five thalli during oven drying was used to calculate the DM of the remaining 72 thalli. After weighing, the thalli were sprayed with deionized water, and their A ( $\text{cm}^2$ ) were measured using a Licor leaf area meter (LI3100, Lincoln, Nebraska).

Relative growth rate (RGR) and relative thallus area growth rate (RT<sub>A</sub>GR) was calculated using the following formulas:

$$\text{RGR} = (\ln(\text{DM}_{\text{end}} - \text{DM}_{\text{start}})) \cdot 1000 / \Delta t \text{ mg g}^{-1} \text{ day}^{-1}$$

$$\text{RT}_{\text{A}}\text{GR} = (\ln(A_{\text{end}} - A_{\text{start}})) \cdot 100 / \Delta t \text{ mm}^2 \text{ cm}^{-2} \text{ day}^{-1}$$

$\Delta t$  is the number of days between the two weight measurements. In this experiment,  $\Delta t$  was 27 days. Specific thallus mass (STM) was calculated as  $\text{STM} = \text{DM}/A$ . Change in STM was calculated before and after the field experiment as:

$$\Delta \text{STM} = (\text{STM}_{\text{end}} - \text{STM}_{\text{start}}) \cdot 100 / \text{STM}_{\text{start}}$$

## 2.4 Measurements of chlorophyll a fluorescence

Fluorescence techniques are widespread methods used in studies assessing photosynthetic performance and stress in photosynthesising organisms (Baker 2008). When chlorophylls in a leaf (or other photosynthesising organs) absorb light energy, there are three possible pathways for the energy: it can be used to drive photosynthesis, dissipate as heat or be re-emitted as light (fluorescence) (Maxwell & Johnson 2000). An often used viability parameter is the maximum quantum yield (QY) of PSII ( $F_v/F_m$ ) in dark-adapted photosynthesizing organisms. Photoinhibition is defined as light-induced reduction in photosynthetic capacity, and is mainly linked to PS II damage (Aro et al. 1993). Depression of  $F_v/F_m$  values indicates chronic photoinhibition (Maxwell & Johnson 2000).

Before the field experiment, all thalli were kept hydrated in low light ( $\sim 10 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) at 15 °C for 24 h. Afterwards, they were dark-adapted for 15 min before the maximal yield of photosystem II ( $F_v/F_m$ ) of each thalli was recorded with a PAM-2000 fluorometer (Walz, Effeltrich, Germany). The thalli were exposed to a saturating flash with a fibre optics, and the  $F_v/F_m$  was calculated as  $(F_m - F_o)/F_m$ , where  $F_m$  and  $F_o$  are maximal and minimal fluorescence yield in the dark adapted state, respectively. After the lichen transplants were harvested the 30<sup>th</sup> September 2015, they were first hydrated with deionized water, and kept in low light ( $\sim 10 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) and 15 °C for 24 h to

recover from photoinhibition. They were then dark-adapted for 15 min before the  $F_v/F_m$  of each thallus was recorded with the PAM-2000 fluorometer.

After the measurements with the PAM-2000, the thalli were hydrated and put in a room at low light and low temperature ( $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and  $15^\circ\text{C}$ ) for 24 h. Then the thalli were dark-adapted for 15 min before their light response curves were recorded with an IMAGING-PAM fluorometer (MAXI version, Walz, Germany) with a red light (650 nm) LED lamp. The software used was ImagingWin software (Heinz Walz, Effeltrich, Germany). Photosynthetic light was increased in steps (0, 1, 21, 56, 111, 186, 281, 336,  $398 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) at 40 s intervals. In the end of each light step, the effective QY of photosystem II ( $F_v'/F_m'$ ) was measured with a saturating flash. The  $F_v'/F_m'$  was calculated as  $(F_m' - F_t)/F_m'$ , where  $F_t$  is the fluorescence value at time  $t$  and  $F_m'$  is the maximal fluorescence measured in illuminated sample during the saturating flash. The apparent electron transport rate ( $\text{ETR}_{\text{app}}$ ) was calculated using the formula (Solhaug et al. 2010):

$$\text{ETR}_{\text{app}} = [\text{PAR irradiance level}] \cdot \text{QY}_{\text{PSII}} \cdot 0.5$$

$\text{QY}_{\text{PSII}}$  is the  $F_v'/F_m'$  value. The 0.5 term of the formula is based on the assumption that 50 % of the absorbed photons is used by photosystem II (Baker 2008). The  $\text{ETR}_{\text{app}}$  calculations does not take into account the proportion of incident light not absorbed by the photobiont in the lichens. Because cortical pigments screen the light by either reflection or absorption, less light than incident light will reach the photobiont layer. The calculated  $\text{ETR}_{\text{app}}$  will thus be higher than the actual ETR in thalli containing sun-screening pigments (Solhaug et al. 2010).

## 2.5 Measurements of $\text{CO}_2$ uptake

$\text{CO}_2$  uptake was measured February 2016 for thalli that had been stored at  $-18^\circ\text{C}$  since end of experiment. The  $\text{CO}_2$  uptake was measured on 40 thalli of each species, 10 from each treatment including the controls. Prior to the  $\text{CO}_2$  uptake measurements, eight thalli (one from each treatment + one control from both species) were taken out of the freezer



and fully hydrated. The thalli were put in low light ( $\sim 10 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) and 15 °C for approximately 16-24 h.

CO<sub>2</sub> uptake (in  $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ ) was measured with a CIRAS-1 infrared gas analyser (IRGA) (PP-Systems, Hoddesdon, UK) with the following settings: 400 ( $\pm 10$ ) ppm CO<sub>2</sub>, 20 °C, H<sub>2</sub>O: 100 (no H<sub>2</sub>O removal), flow rate 200 ml per min. The light source was a LED lamp (Model SL-3500, Photon System Instruments, Brno, Czech Republic). Each thallus was placed in a cuvette and exposed to  $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  for about five min to activate their photosynthesis. Then the thalli were exposed to steps of 0, 50, 100, 200, 400 and 600  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ . The CO<sub>2</sub> uptake was recorded from the IRGA when the readings stabilized, about five minutes after each time the light was changed. The photosynthetic QY was calculated as the slope of the linear part (from 0 to 100  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) of the light response curves calculated with CO<sub>2</sub> uptake as response. QY of a process is defined by Baker (2008) as “number of molecules undergoing the process decided by the number of photons absorbed by the system”.

## 2.6 Colour measurements

Before and after the field experiment, photographs of 72 thalli of each species were taken in dry state because differences in colour are more visible in dry state. Melanic pigments cannot easily be extracted from lichens (McEvoy et al. 2007). Recordings of the reflectance spectra were therefore used as an indirect method to quantify melanic pigments in the radiation treated thalli of *L. pulmonaria* and *L. virens*. Prior to the reflectance measurements, thalli were flattened under light pressure, and then dried in 20 °C for 24 h. To measure the reflectance spectra of the radiation treated thalli, a spectrometer (model SD2000, OceanOptics, Eerbeek, the Netherlands), connected to an integrating sphere (ISP-50-REFL OceanOptics) with a 400- $\mu\text{m}$ -thick fibre, was used. Each thallus was placed on the top of the sphere. A halogen lamp (DH2000, OceanOptics) illuminated the thallus with a 600  $\mu\text{m}$  optical fibre connected to the sphere. The calculation of percentage reflectance was based on a dark spectrum and a reference spectrum from a white reference tile (WS-2, OceanOptics). The browning reflectance index (BRI) was calculated from the reflectance spectrum from each thallus using the

formula from Chivkunova et al. (2001); also used on lichen material by McEvoy et al. (2007):

$$BRI = (1/R_{550} - 1/R_{700})/R_{750}$$

$R_{550}$ ,  $R_{700}$  and  $R_{750}$  are the reflectance percentage (in decimals) at wavelengths 550 nm, 700 nm and 750 nm, respectively. In addition, the mean reflectance at each wavelength within each treatment and species was calculated.

## 2.7 Chlorophyll measurement

The thalli used for gas exchange analyses were also used to measure chlorophylls (chl). After the gas exchange measurements, the thalli were put in a freezer for about two weeks. The thalli were then taken out of the freezer and hydrated. Two discs with a diameter of 6 mm were cut from each thallus. They were then put in an Eppendorf tube together with 1.5 ml of dimethyl sulfoxide. The Eppendorf tubes were kept in a VWR Ultrasonic cleaner at 60 °C for 30 min. The absorbance at wavelengths 649, 665 and 750 nm of each solution was measured using a UV 2001 spectrophotometer (Shimadzu, Japan). To correct for impurities, absorbance at 750 nm was subtracted from the absorbance at 649 nm ( $A_{649}$ ) and 665 nm ( $A_{665}$ ). Then the chl content of each thalli was calculated using these formulas (Wellburn 1994):

$$\text{Chl } a = 12.19 \cdot A_{665} - 3.45 \cdot A_{649}$$

$$\text{Chl } b = 21.99 \cdot A_{649} - 5.32 \cdot A_{665}$$

## 2.8 Statistical analyses

The statistical analyses were run in Minitab 16 (Minitab Inc., State Collage, PA, USA). One-way ANOVAs were used to analyse differences between the treatment groups in RGR,  $RT_{AGR}$ ,  $\Delta STM$ , BRI, QY of CO<sub>2</sub> uptake, the mean of the CO<sub>2</sub> uptake at irradiances 400 and 600  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , mean reflectance, change in  $F_v/F_m$ , the mean of  $ETR_{app}$  at irradiances 281, 336 and 398 and chlorophyll content. The BRI-data was log10-transformed to satisfy the ANOVA requirements. Tukey's method (significance level =

0.05) was used for comparison between groups in ANOVA. To analyse the differences in  $F_v/F_m$  before and after the field experiment, paired t-tests were undertaken. 2-sample t-tests were used to analyse the differences in chl  $a+b$ , QY,  $STM_{start}$  RGR,  $RT_{AGR}$  and  $\Delta STM$  between the two species. When requirements for ANOVA and t-test were not satisfied, and data-transformation was not sufficient to satisfy requirements, non-parametric tests, Kruskal-Wallis and Mann-Whitey tests, respectively, were undertaken. Significance level in all tests was set to 0.05. In text, figures and tables, means of  $\pm 1$  standard error (SE) are given.

### 3 Results

#### 3.1 Climatic factors

Most days during the field experiment were partly sunny with maximum daily PAR levels around  $1100 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ ; the highest value recorded was  $1593 \mu\text{mol photons m}^{-2}\text{s}^{-1}$  (Fig. 5). During daytime, the average irradiance level was  $357 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ . Daytime maximum temperature never exceeded  $24^\circ\text{C}$ , whereas temperature dropped substantially during nights, often to less than  $8^\circ\text{C}$ , which caused the RH to rise close to 100 % (Fig. 5). Dewfall was extensive in clear nights (personal observations). The mean UV level during daytime was  $6.55 \text{ W m}^{-2}$  (Fig. 5), and the highest level measured during the field experiment was  $31.5 \text{ W m}^{-2}$ .

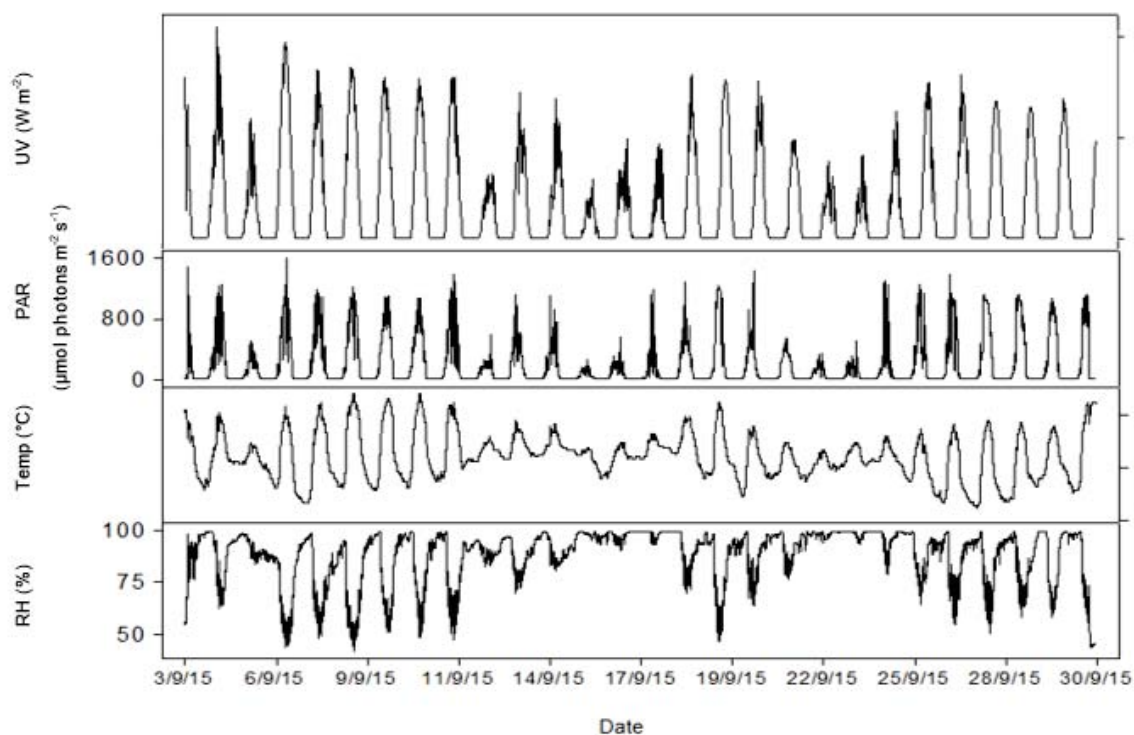


Figure 5: UV level (295-385 nm), relative humidity (RH), air temperature on ground level and photosynthetic active radiation (PAR) recorded every min at the transplantation site at Ås, south-eastern Norway during the field experiment (3-30 September 2015). The UV level was recorded on a nearby meteorological station.

### 3.2 Lichen growth rates

The mean relative growth rate (RGR) of all radiation treated thalli of *L. pulmonaria* was  $3.89 \pm 0.33 \text{ mg g}^{-1} \text{ day}^{-1}$ , significantly higher ( $P < 0.001$ ) than the mean RGR ( $1.84 \pm 0.26 \text{ mg g}^{-1} \text{ day}^{-1}$ ) of *L. virens* (mean  $\pm 1$  SE;  $n=108$ ). Also the mean relative thallus area growth rate (RT<sub>AGR</sub>) of all radiation treated thalli of *L. pulmonaria*,  $0.29 \pm 0.04 \text{ mm}^2 \text{ cm}^{-2} \text{ day}^{-1}$ , was significantly higher ( $P < 0.001$ ) than the mean RT<sub>AGR</sub> of *L. virens* ( $-0.01 \pm 0.07 \text{ mm}^2 \text{ cm}^{-2} \text{ day}^{-1}$ ) (mean  $\pm 1$  SE;  $n=107$ ). The mean change in specific thallus mass ( $\Delta\text{STM}$ ) of all radiation treated *L. virens* thalli was  $5.99 \pm 2.20 \%$ , substantially higher than the mean  $\Delta\text{STM}$  of *L. pulmonaria* ( $2.68 \pm 0.91 \%$ ). However, this difference was not significant ( $P=0.116$ ).

Both UV-A and UV-B radiation highly significantly reduced growth in *L. pulmonaria* (Fig. 6). Both RGR and RT<sub>AGR</sub> for thalli exposed to UV radiation were lower than for those screened from UV radiation ( $P < 0.001$ ; Fig. 6). In fact, UV exposure reduced RT<sub>AGR</sub> more than RGR in *L. pulmonaria* thalli (Fig. 6): RGR was reduced by 39 % in thalli exposed to UV-A in addition to PAR only, and the RT<sub>AGR</sub> was reduced by 51 %. In *L. pulmonaria* thalli exposed to UV-B, RGR was reduced by 44 % and RT<sub>AGR</sub> by 68 % compared to the thalli exposed to PAR only. *Lobaria virens* thalli exposed to UV radiation had significantly lower RGR than those exposed to PAR only ( $P < 0.05$ ; Fig. 6). The RGR was reduced by 26 % in the *L. virens* thalli exposed to PAR + UV-A, and by 24 % in the thalli in addition exposed to UV-B, compared with thalli exposed to PAR only. However, this species did not exhibit significant differences in RT<sub>AGR</sub> ( $P=0.475$ ) between treatment groups (Fig. 6).

STM measured before the field experiment was significantly higher (23 %) in *L. virens* thalli compared to *L. pulmonaria* thalli ( $P < 0.001$ ). Both species experienced an increase in STM as response to all radiation treatments. For *L. pulmonaria*, there was a non-significant ( $P=0.135$ ) increase in STM with expanding wavelength range from just PAR to PAR + UV-A and PAR + UV-A + UV-B (Fig. 6). *Lobaria virens* exposed to PAR only showed high  $\Delta\text{STM}$ , but due to the high within-group variation, the difference between treatment groups was not significant (Fig. 6).

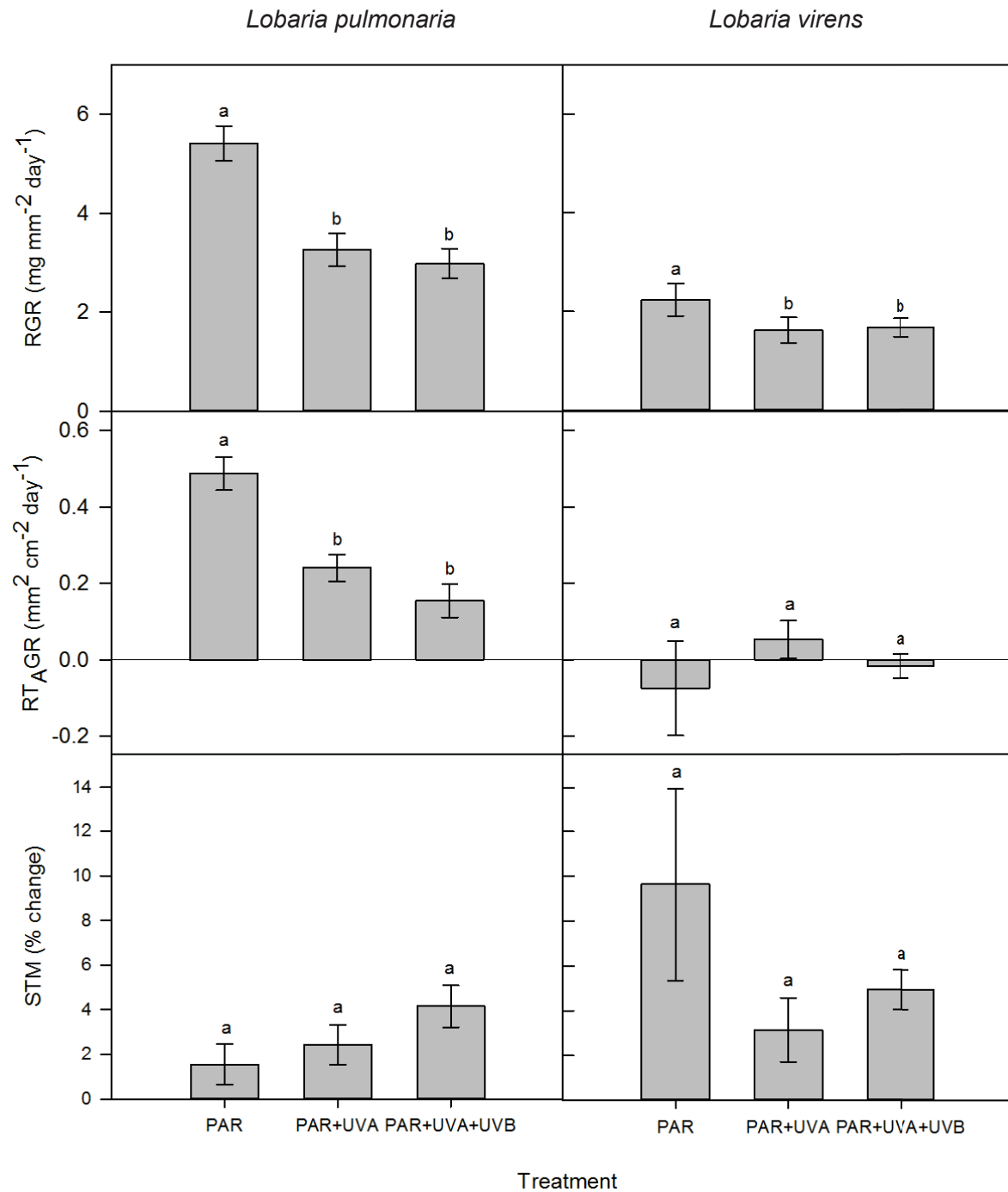


Figure 6: UV radiation effect on RGR, RT<sub>A</sub>GR and change in STM for *L. pulmonaria* and *L. virens*. Columns show the mean of each treatment group, error bars show  $\pm 1$  SE. Groups that do not share a letter are significantly different ( $P < 0.05$ ; ANOVA for *L. pulmonaria* and Kruskal-Wallis for *L. virens*).  $N=18$  for each treatment and species, except for calculations of RT<sub>A</sub>GR and STM for *L. pulmonaria* exposed to PAR + UV-A + UV-B, in which  $n=17$ .

### 3.3 Photosynthetic CO<sub>2</sub> uptake

The light response curves of all the radiation treated *L. pulmonaria* thalli were lower compared to the control thalli, whereas the radiation treatment had no effect on the CO<sub>2</sub> uptake as response to light in *L. virens* (Fig. 7). Both species reached light saturation at approximately 400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . In *L. pulmonaria*, the CO<sub>2</sub> uptake differed between treatment groups: all radiation treated thalli showed lower QY of CO<sub>2</sub> uptake than the control thalli (Tab. 1), but only the difference between the thalli exposed to UV-B and the control was significant ( $P<0.05$ ). The mean of the measured CO<sub>2</sub> uptake of *L. pulmonaria* at 400 and 600  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  was lower in all treatment groups compared to the control, but only the thalli exposed to PAR treatment differed significantly from the control ( $P<0.05$ ; Tab. 2). There were no significant differences in QY of CO<sub>2</sub> uptake (Tab. 1) and CO<sub>2</sub> uptake at irradiances of 400 and 600 between the *L. virens* treatment groups (Tab. 2). The total QY of CO<sub>2</sub> uptake of all treated thalli including control thalli pooled together was significantly higher in *L. pulmonaria* thalli than in *L. virens* ( $P<0.01$ ).

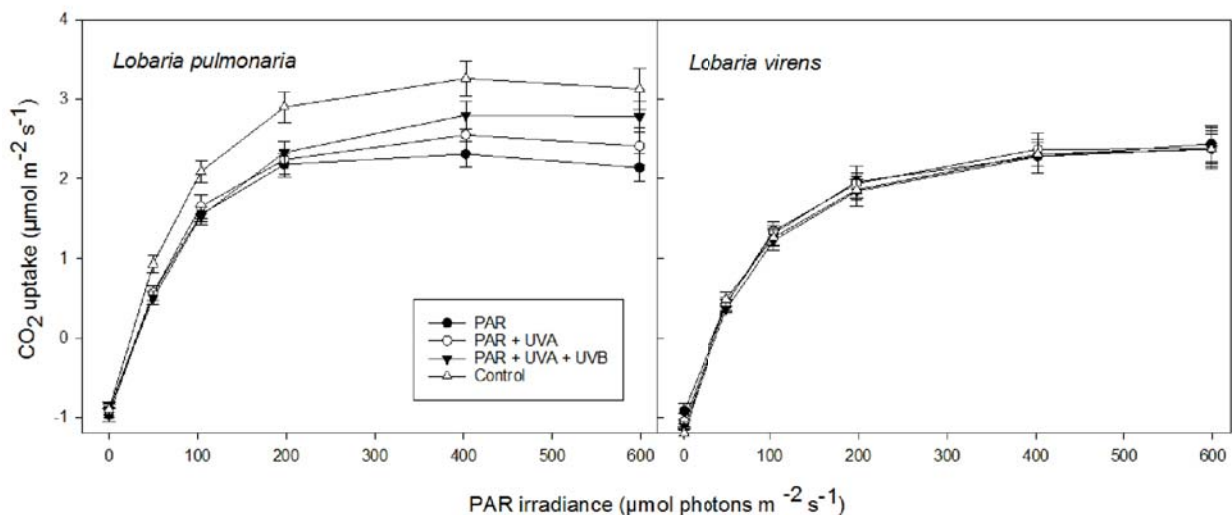


Figure 7: The effect of different radiation treatments on CO<sub>2</sub> uptake as response to increasing irradiance in *L. pulmonaria* and *L. virens*. Plotted curves show means of CO<sub>2</sub> uptake  $\pm 1$  SE as response to light for each treatment group.  $N=10$  for each treatment and species.

Table 1: Effect of different radiation treatments on quantum yield (QY) in *L. pulmonaria* and *L. virens*. Table show mean QY ( $\pm 1$  SE) based on measurements of CO<sub>2</sub> uptake (slope from irradiance 0 to 100). Groups that do not share a letter are significantly different ( $P < 0.05$ ; ANOVA for *L. pulmonaria* and Kruskal-Wallis for *L. virens*).  $N=10$  for each treatment and species.

	<i>Lobaria pulmonaria</i>	<i>Lobaria virens</i>
Control	0.0287 ( $\pm 0.0013$ ) <sup>a</sup>	0.0236 ( $\pm 0.0007$ ) <sup>a</sup>
PAR	0.0242 ( $\pm 0.0008$ ) <sup>ab</sup>	0.0214 ( $\pm 0.0018$ ) <sup>a</sup>
PAR + UV-A	0.0246 ( $\pm 0.0014$ ) <sup>ab</sup>	0.0229 ( $\pm 0.0011$ ) <sup>a</sup>
PAR + UV-A + UV-B	0.0233 ( $\pm 0.0012$ ) <sup>b</sup>	0.0223 ( $\pm 0.0014$ ) <sup>a</sup>

Table 2: Effect of different radiation treatments on the mean of the CO<sub>2</sub> uptake (in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) ( $\pm 1$  SE) at 400 and 600  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in *L. pulmonaria* and *L. virens*. Groups that do not share a letter are significantly different ( $P < 0.05$ ; ANOVA).  $N=10$  for each treatment and species.

	<i>Lobaria pulmonaria</i>	<i>Lobaria virens</i>
Control	3.195 ( $\pm 0.237$ ) <sup>a</sup>	2.340 ( $\pm 0.156$ ) <sup>a</sup>
PAR	2.225 ( $\pm 0.169$ ) <sup>b</sup>	2.360 ( $\pm 0.214$ ) <sup>a</sup>
PAR + UV-A	2.480 ( $\pm 0.237$ ) <sup>ab</sup>	2.375 ( $\pm 0.233$ ) <sup>a</sup>
PAR + UV-A + UV-B	2.790 ( $\pm 0.188$ ) <sup>ab</sup>	2.330 ( $\pm 0.209$ ) <sup>a</sup>

### 3.4 Changes in colour

In *L. pulmonaria*, the colour of thalli exposed to UV-B was darker than those exposed to PAR and PAR + UV-A, and there was also a difference in colour between the PAR and the PAR + UV-A treatment groups, visible to the naked eye (Fig. 10) and evidenced by the reflectance spectra (Fig. 9). There was a highly significant difference in mean reflectance between all *L. pulmonaria* treatment groups in the PAR spectrum ( $P < 0.001$ ; Fig. 8A). In the near infrared (NIR) wavelength range spectrum, the difference between thalli exposed to just PAR versus those experiencing one of the UV treatments was highly significant ( $P < 0.001$ ; Fig. 8C). In addition, the browning reflectance index (BRI) indicated an increasingly darker colour with expanding wavelength range from just PAR to



PAR+UV-A and PAR+UV-A+UV-B (Tab. 3); the mean BRI differed highly significantly between all three treatment groups ( $P<0.001$ ).

By contrast, there was no visible difference in colour between the treatment groups of *L. virens* (Fig. 11). However, there was a small difference in the mean reflectance in the thalli exposed to UV-B compared to the two other treatment groups. This difference was not significant in the PAR spectrum ( $P=0.076$ ; Fig. 8B), but in the NIR spectrum there was a significant difference between the thalli exposed to PAR only and the thalli exposed to UV-B ( $P<0.05$ ; Fig. 8D and Fig. 9). The BRI was slightly higher in the *L. virens* thalli exposed to UV-B compared to thalli exposed to the other radiation treatments, but the difference between the three treatments was not significant ( $P=0.168$ ; Tab. 3).

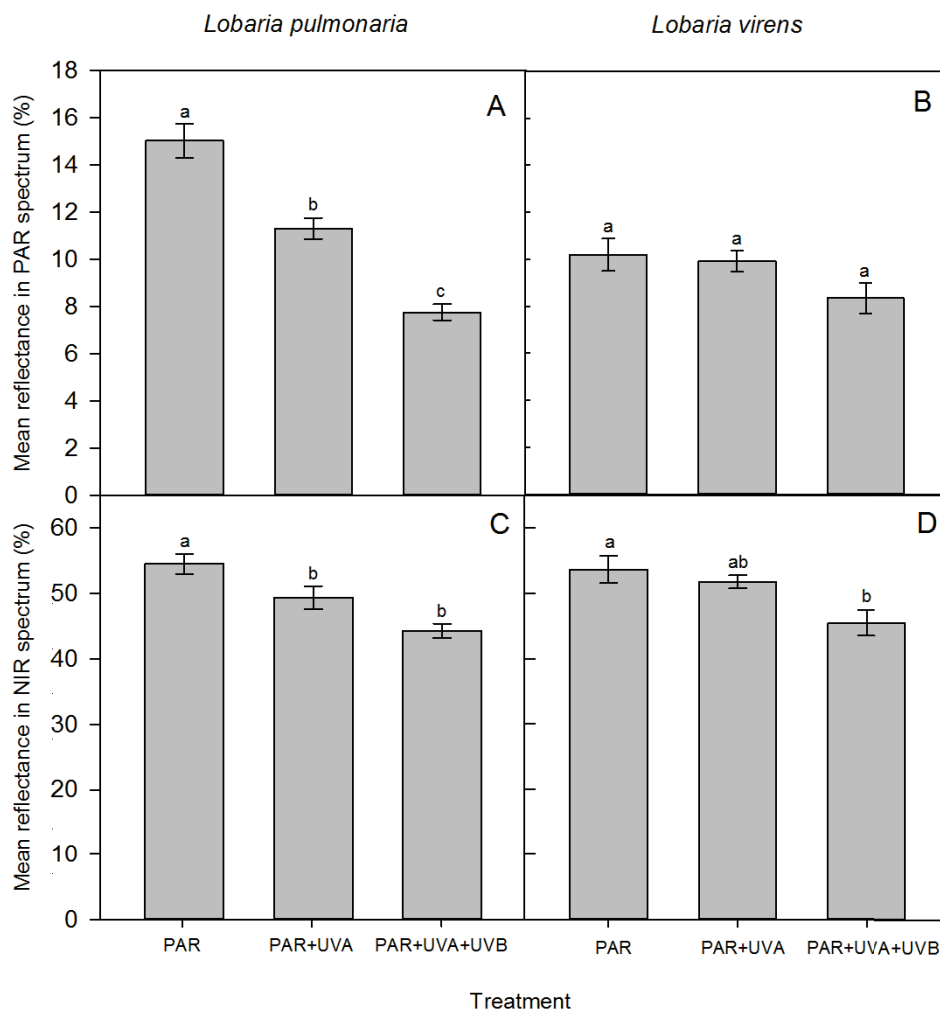


Figure 8: UV effect on reflectance from thalli of *L. pulmonaria* and *L. virens*. Columns show mean reflectance from thalli in dry state in the visible range (PAR; 400-700 nm) (figs. A and B) and in the near infrared range (NIR; 700-1000 nm) (figs. C and D). Error bars show  $\pm 1$  SE. Groups that do not share a letter are significantly different ( $P<0.05$ ; ANOVA for *L. pulmonaria* in both spectra and for *L. virens* in PAR spectrum, and Kruskal-Wallis for *L. virens* in the NIR spectrum).  $N=18$  for each treatment and species.

Table 3: Mean browning reflectance index (BRI) ( $\pm 1$  SE) of *L. pulmonaria* and *L. virens* exposed to different radiation treatments. Groups that do not share a letter are significantly different ( $P < 0.05$ ; ANOVA for *L. pulmonaria* and Kruskal-Wallis for *L. virens*.)  $N=18$  for each treatment and species.

	<i>Lobaria pulmonaria</i>	<i>Lobaria virens</i>
PAR	1.814 ( $\pm 0.211$ ) <sup>a</sup>	5.75 ( $\pm 1.27$ ) <sup>a</sup>
PAR + UV-A	5.676 ( $\pm 0.874$ ) <sup>b</sup>	4.31 ( $\pm 0.43$ ) <sup>a</sup>
PAR + UV-A + UV-B	23.63 ( $\pm 3.660$ ) <sup>c</sup>	8.05 ( $\pm 1.51$ ) <sup>a</sup>

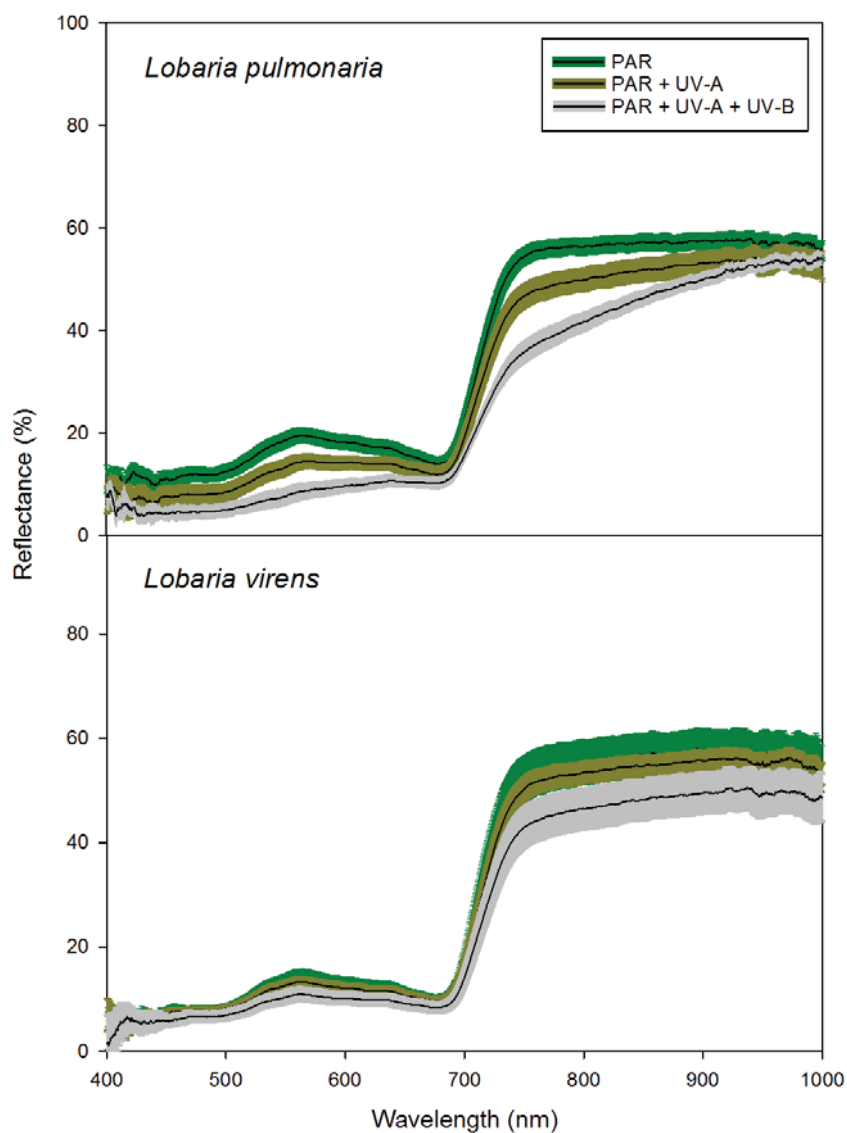


Figure 9: Mean reflectance (as percentage of total light) of air-dry *L. pulmonaria* and *L. virens* thalli, exposed to different radiation treatments, at wavelengths from 400 to 1000 nm. Coloured fields show  $\pm 95\%$  confidence intervals.  $N=18$  for each treatment and species.

PAR



PAR + UV-A

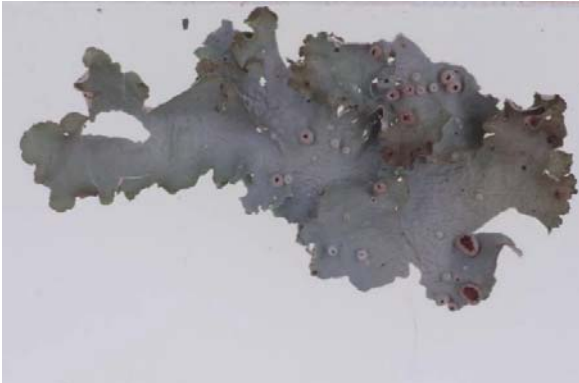


PAR + UV-A + UV-B



*Figure 10: Thalli of one representative thallus of L. pulmonaria before (left side photo) and after (right side photo) for each of the three treatments used in the field experiment. Treatment type is specified above each row of photos. Thalli exposed to UV-A in addition to PAR irradiance had developed a slightly darker colour, and thalli that had also been exposed to UV-B had developed an even darker colour.*

PAR



PAR + UV-A



PAR + UV-A + UV-B



Figure 11: Thalli of one representative thallus of *L. virens* before (left side photo) and after (right side photo) for each of the three treatments used in the field experiment. Treatment type is specified above each row of photos. There was no visible difference in colour between the treatments.

### 3.5 Chlorophyll *a* fluorescence

The measured mean QY of PSII ( $F_v/F_m$ ) at the start of the field experiment was 0.698 ( $\pm 0.005$ ) in *L. pulmonaria* and 0.664 ( $\pm 0.004$ ) in *L. virens*. All treatment groups of both species experienced a significant reduction in  $F_v/F_m$ , except for *L. pulmonaria* thalli exposed to UV-B (Fig. 12). The *L. pulmonaria* thalli which had been exposed to PAR and the thalli which had been exposed to PAR + UV-A had a small, significant reduction in  $F_v/F_m$  values after the field experiment ( $P < 0.01$  and  $P < 0.001$  respectively). The increase in  $F_v/F_m$  of UV-B treated *L. pulmonaria* thalli was not significant. The  $F_v/F_m$  value was reduced in all *L. virens* treatment groups after the field experiment ( $P < 0.001$  for all treatment groups). The reduction was slightly lower in the thalli exposed to one of the two UV treatments, but this difference was not significant ( $P = 0.206$ ). The reduction in  $F_v/F_m$  values was higher in *L. virens* compared with *L. pulmonaria* ( $P < 0.001$ ; Fig. 12).

Light response curves of QY of PSII ( $F_v'/F_m'$ ) was slightly higher for the UV-B exposed thalli compared to the thalli exposed to PAR only and PAR + UV-A of both *L. pulmonaria* and *L. virens* (Fig. 13). This was also the trend in the light response curves of apparent electron rate ( $ETR_{app}$ ) (Fig. 14): The mean  $ETR_{app}$  of the three highest irradiances was significantly higher in *L. pulmonaria* thalli exposed to UV-B compared to the two other radiation treatments ( $P < 0.01$ ; ANOVA;  $n = 18$ ). Also UV-B exposed *L. virens* thalli had a higher  $ETR_{app}$  than the two other treatment groups, but only the difference between the PAR + UV-A and thalli exposed to UV-B was significant ( $P < 0.01$ ; Kruskal-Wallis;  $n = 18$ ). Both species reached light saturation of  $ETR_{app}$  at approximately 300  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . The mean  $ETR_{app}$  of all radiation treated *L. pulmonaria* thalli at the three highest irradiances was 47 % higher than for *Lobaria virens*.

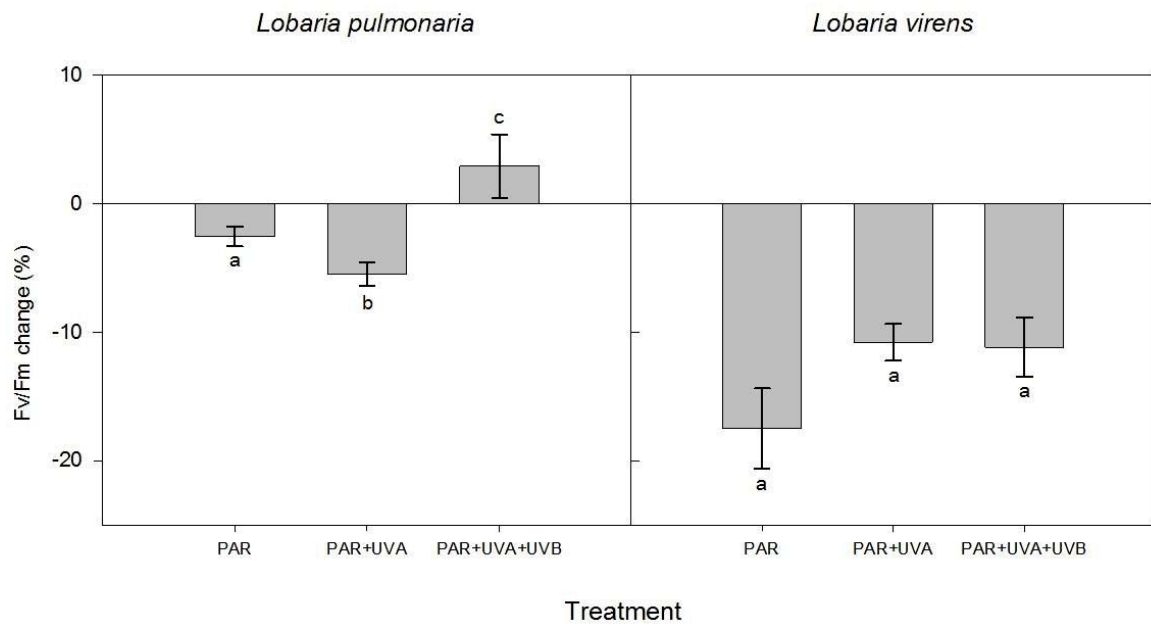


Figure 12: Effect of different light treatments on maximum yield of PSII ( $F_v/F_m$ ) in *L. pulmonaria* and *L. virens*. The measured mean quantum yield of PSII ( $F_v/F_m$ ) at the start of the field experiment was  $0.698 (\pm 0.005)$  in *L. pulmonaria* and  $0.664 (\pm 0.004)$  in *L. virens*. There was no statistical difference between treatment groups in mean  $F_v/F_m$  before the field experiment. Columns show mean change in  $F_v/F_m$  value as percentage ( $\pm 1$  SE). Groups that do not share a letter are significantly different ( $P < 0.05$ ; Kruskal-Wallis).  $N=18$  for each treatment and species.

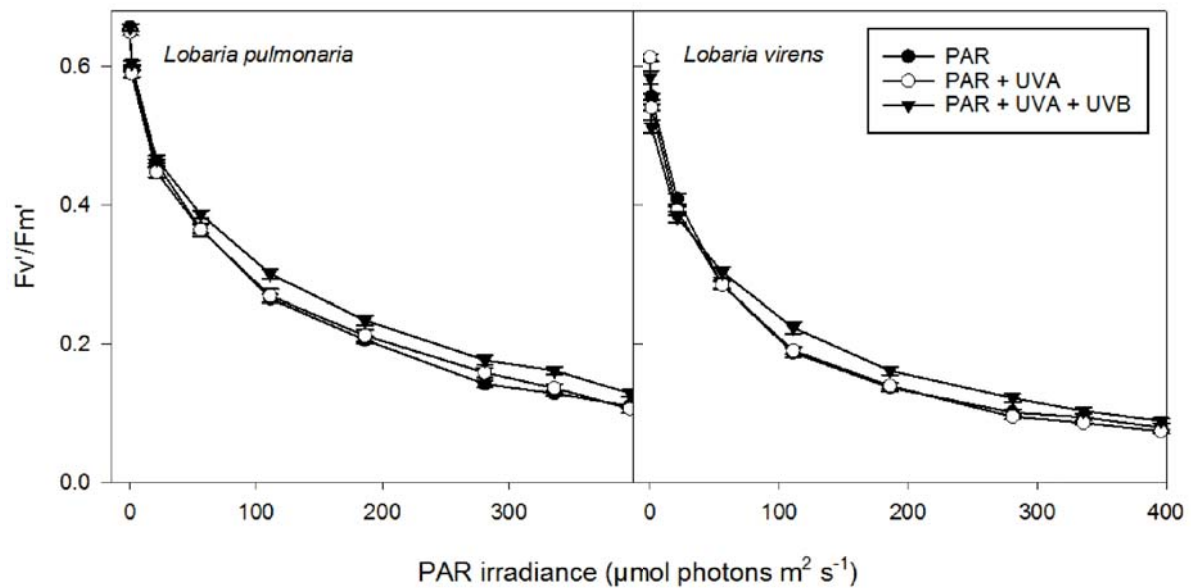


Figure 13: Effect of different radiation treatments on quantum yield of PS II ( $F_v'/F_m'$ ) with increasing irradiance in *L. pulmonaria* and *L. virens* at  $20^\circ\text{C}$ . Plotted curves show mean yield ( $\pm 1$  SE).  $N=18$  for each treatment and species.

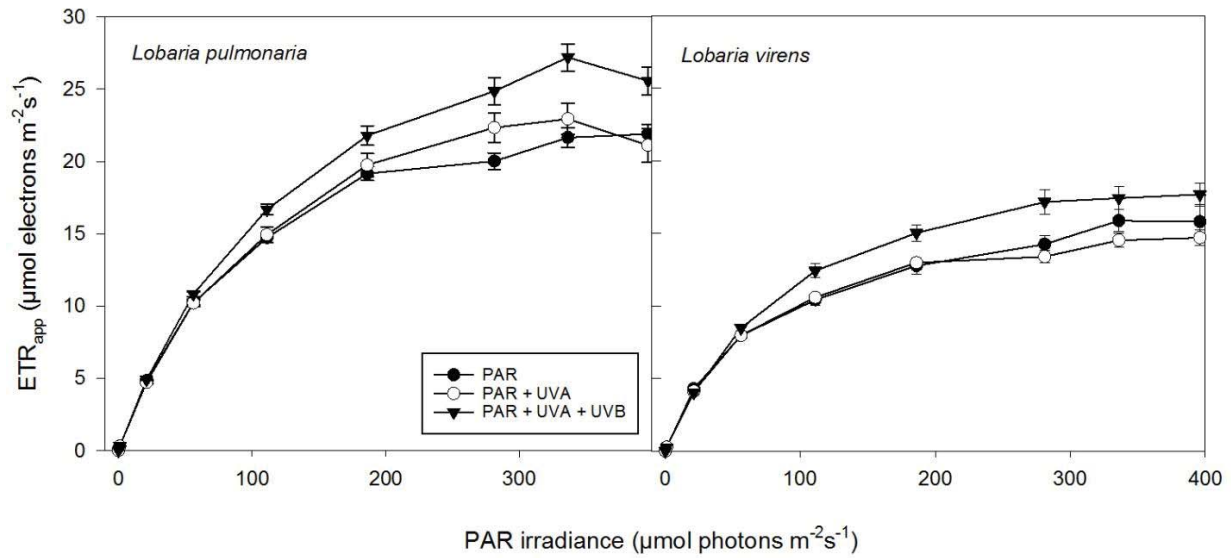


Figure 14: Effect of different radiation treatments on light response curves of apparent ETR in *L. pulmonaria* and *L. virens* at 20 °C. Plotted curves show mean yield ( $\pm 1$  SE) of each treatment.  $N=18$  for each treatment and species.

### 3.6 Chlorophyll concentrations

In *L. pulmonaria*, the chl *a/b* ratio was slightly higher in the PAR treated thalli compared with the thalli exposed to PAR + UV-A and thalli exposed to PAR + UV-A + UV-B ( $P<0.05$ , Fig. 15). *Lobaria virens* did not exhibit significant differences in chl *a/b* ratio between the treatment groups ( $P=0.107$ ). There were no significant differences in chl *a+b* content for neither *L. pulmonaria* nor *L. virens* ( $P=0.628$  and  $0.114$ , respectively; Fig. 15). The overall chl content in all treatment groups differed highly significantly between the two species ( $P<0.001$ ). The mean chl content in all *L. pulmonaria* treatment groups was  $8.31 \pm 0.21$  mg g<sup>-1</sup>, significantly lower than the mean chl content in *L. virens* ( $10.36 \pm 0.45$  mg g<sup>-1</sup>;  $P<0.001$ ).

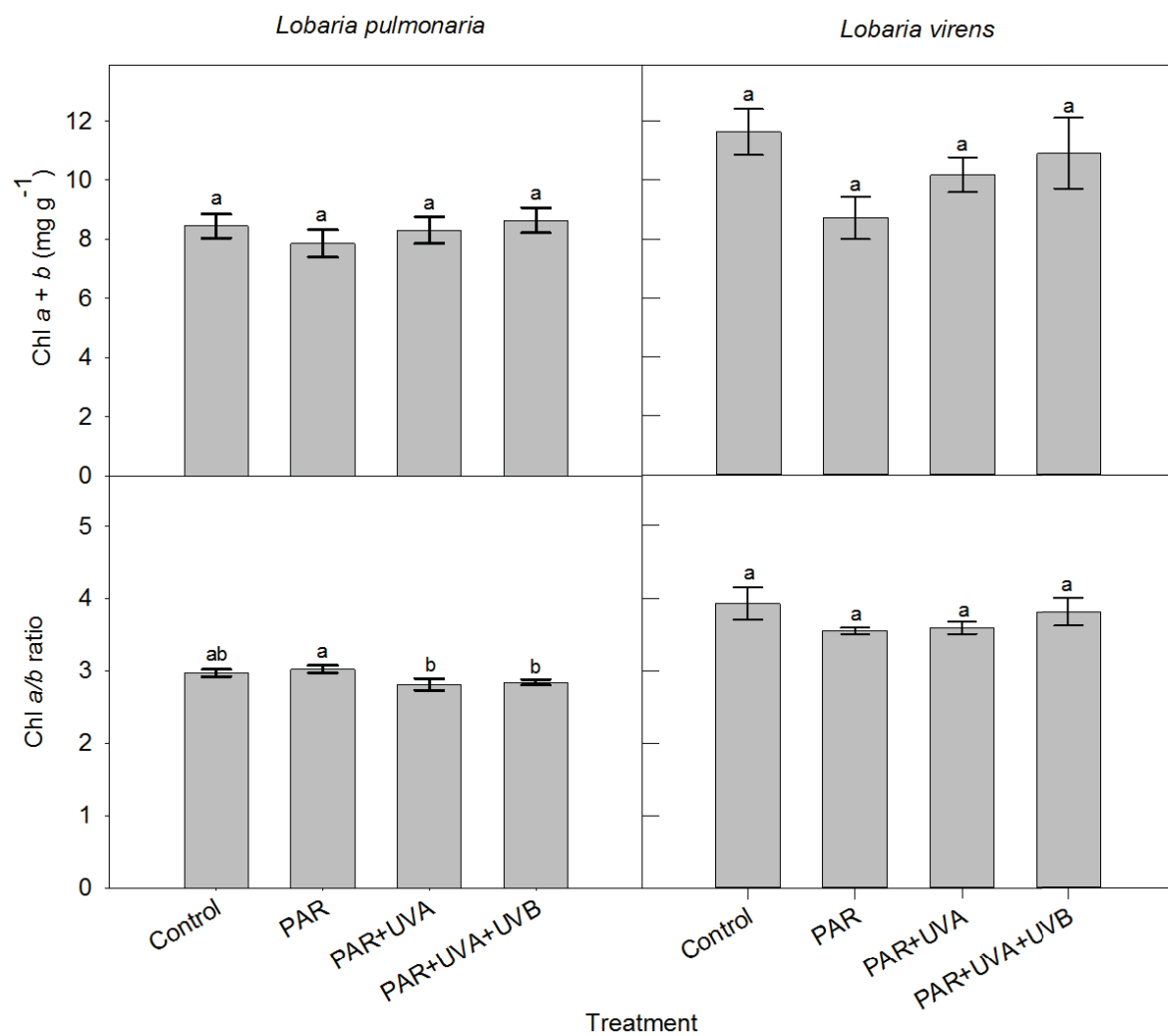


Figure 15: Effect of different light treatments on chlorophyll (chl) content a+b and chl a/b ratio in thalli of *L. pulmonaria* and *L. virens*. Columns show mean chl content, error bars show  $\pm 1$  SE for each treatment. Groups that do not share a letter are significantly different ( $P < 0.05$ ; ANOVA for chl a+b level, Kruskal-Wallis for a/b ratio).  $N=10$  for each treatment and species.



## 4 Discussion

The mean RGR in all treated *L. pulmonaria* thalli in this study ( $3.89 \text{ mg g}^{-1} \text{ day}^{-1}$ ) was higher than the highest RGR for any one single thallus of *L. pulmonaria* ( $3.77 \text{ mg g}^{-1} \text{ day}^{-1}$ ) recorded in a 100-day-transplantation period by Gauslaa et al. (2006), implying adequate growth conditions for this species during the field experiment. To my knowledge, no growth measurements have previously been performed on *L. virens*. *Lobaria virens* experienced net growth in terms of RGR within all treatment groups during the field experiment. The high growth rates of *L. pulmonaria* and the net growth of *L. virens* show that growth is a highly useful parameter in short-term field experiments assessing how environmental factors affect the lichen viability. Both growth, melanic pigment formation and recovery from high-light-damage depends on hydration (Bidussi et al. 2013; Gauslaa & Solhaug 2000; McEvoy et al. 2007). Hence, the hydration regime (the high RH measured during the field experiment, extensive dewfall most of the mornings and supplementary irrigation) may have been the most important success factor for growth in *L. pulmonaria* and *L. virens*.

### 4.1 UV-A and UV-B radiation reduce growth in *L. pulmonaria* and *L. virens*

In the present study, UV-B radiation exposure reduced both the RGR and  $\text{RT}_{\text{AGR}}$  of *L. pulmonaria* (Fig. 6). The few results on how UV radiation affects growth in *L. pulmonaria* differ. Larsson et al. (2009) found no significant effects of UV-B radiation on growth in a growth chamber experiment (duration of 15 days with a maximal UV-B exposure of  $1 \text{ W m}^{-2}$ ). However, this was not the case in a growth chamber experiment (Paul Chowdhury 2015) using the same experimental setup as Larsson et al. (2009), where growth of *L. pulmonaria* responded negatively to UV-B. The reason for the different response of *L. pulmonaria* growth to UV in (Paul Chowdhury 2015) and (Larsson et al. 2009) is not known. The effect of UV radiation on growth in *L. virens* was lower than that of *L. pulmonaria*, but there was some evidence ( $P < 0.05$ ) suggesting that both UV-A and

UV-B reduce the RGR in *L. virens* thalli compared to the thalli exposed to PAR only (Fig. 6).

An explanation for the lower growth rates in melanic thalli can be that the melanic pigment screening reduce photosynthesis under low light conditions. Because weight gain in lichens is primarily dependent on CO<sub>2</sub> assimilation (Palmqvist 2000), the reduced RGR in UV-B treated thalli of *L. pulmonaria* (Fig. 6) can partly be explained by the significantly lower QY of CO<sub>2</sub> uptake compared to the control thalli (Tab. 1). The darkest colour (Fig. 10), highest BRI (Tab. 3) and lowest reflection (Fig. 9) occurred in the UV-B-exposed *L. pulmonaria* thalli. The lower QY of CO<sub>2</sub> uptake in UV-B treated thalli was thus expected, because melanic pigments reduce the photosynthetic light-capture in the lichen by absorbing light. This causes reduced transmittance through the cortex to the photobiont layer (Gauslaa & Solhaug 2001), reducing the potential photosynthetic capacity. Lichens are only photosynthetic active when they are wet. In this study, the lichen thalli were wet mainly during the morning, when the solar irradiance is low. Hence, because melanic pigments reduced the QY of CO<sub>2</sub> uptake in *L. pulmonaria* (Tab. 1), the melanic pigment production can thus have been a disadvantage for growth.

Exposure to high UV-A levels has been shown to increase ROS formation in the lichen *Xanthoria parietina* (Kovacik et al. 2011), which can cause photoinhibition (Nishiyama et al. 2006). In my study, UV-A treated *L. pulmonaria* thalli experienced almost the same reduction in RGR and RT<sub>A</sub>GR as the UV-B treated thalli (Fig. 6). Pigment formation was higher in the UV-A treated thalli compared to thalli exposed to PAR only, but lower than in UV-B treated thalli (Figs. 8 and 10, Tab. 3). This confirms the findings of Solhaug et al. (2003), that pigment induction depends mainly on UV-B radiation, but that some pigment formation is induced by UV-A. In my study, the pigment formation in UV-A-exposed thalli was not high enough to significantly reduce the QY of CO<sub>2</sub> uptake (Tab. 1). However, the reduction in growth in UV-A treated *L. pulmonaria* thalli can be explained by the reduction in maximal QY of PSII ( $F_v/F_m$ ; Fig. 12), which indicates photoinhibition. The  $F_v/F_m$  was more reduced in UV-A treated thalli compared to the thalli exposed to PAR only. This suggests that UV-A exposure of *L. pulmonaria* causes more

photoinhibition than exposure to PAR only, and that the melanic pigment production induced by UV-A is insufficient to reduce photoinhibition.

*Lobaria virens* growth rates were significantly lower than *L. pulmonaria* growth rates, consistent with the higher susceptibility of *L. virens* to high light (Gauslaa & Solhaug 1996). Exposure to high light conditions leads to fast drying of the lichen thalli. In the dry state, lichens are more susceptible to high light, because they need water to recover from photo-damage (Gauslaa & Solhaug 2000). In addition, lichens must be hydrated to be photosynthetically active (Lange et al. 2001). Because the initial STM was higher in *L. virens* than in *L. pulmonaria* (23 % higher before the field experiment), they were dependent on more hydration to repair photo-damage and be photosynthetically active. Dewfall may not always have been sufficient to hydrate the thalli of *L. virens* during the field experiment, which can explain the significantly higher photoinhibition compared to that of *L. pulmonaria*. Hence, a higher STM can be an explanation of the low growth rates in *L. virens*. The lower growth rate was also consistent with a significantly lower QY of CO<sub>2</sub> uptake in *L. virens* compared to *L. pulmonaria* (Tab. 1).

McEvoy et al. (2007) hypothesize that the reduction in growth due to high exposure to natural light can be explained by a trade-off between production of melanic pigments and growth. Measurements have shown that melanic pigments in *L. pulmonaria* obtain a low proportion of the dry mass of the thalli (K. A. Solhaug, unpublished data). However, melanic pigments are nitrogen-rich (Matee et al. 2016, in press). Because area growth can be N-limited in *L. pulmonaria* (Gauslaa et al. 2006), the trade-off hypothesis could be a plausible explanation for reduced growth in terms of RT<sub>AGR</sub>.

## 4.2 Acclimation to high light and UV

Melanic pigments are hypothesized to reduce photoinhibition, because they reduce transmittance of PAR, UV-A and UV-B reaching the photobiont layer. UV-B dependent relaxation of  $F_v/F_m$  has been shown in *L. pulmonaria* (Larsson et al. 2009), suggested to be caused by shading pigments. Also a positive correlation between  $F_v/F_m$  and melanic pigment content in thalli of *L. pulmonaria* has been observed (McEvoy et al. 2007). However, (Gauslaa & Solhaug 2004) found no additional effect of UV-A and UV-B on

photoinhibition compared to the effect of PAR only in *L. pulmonaria*. In this study, relaxation of  $F_v/F_m$  was observed in UV-B treated *L. pulmonaria* (Fig. 12), which also exhibited the highest level of melanic pigments. In addition, the melanic pigments seem to enable *L. pulmonaria* to have a higher photosynthetic activity at high irradiances due to the screening function. The PAR treated thalli without melanic pigments had lower CO<sub>2</sub> uptake at high irradiances (400 and 600  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) compared to the control thalli, while the CO<sub>2</sub> uptake in UV-treated thalli at high irradiance did not differ from the control (Fig. 7 and Tab. 2). These data suggest that melanic pigments are important for acclimation of *L. pulmonaria* to higher light conditions.

There was no clear evidence from the photos and the reflection measurements indicating pigment production in *L. virens* (Figs. 8 and 11, Tab. 3). In addition, no differences were found in QY of CO<sub>2</sub> uptake (Tab. 1), and the light response curves of all *L. virens* treatment groups were almost identical (Fig. 7). However, some small differences could be worth highlighting regarding pigment production in *L. virens*. The mean reflectance was slightly lower in the UV-B treated *L. virens* thalli (Fig. 8), but not significantly different from the other treatment groups ( $P=0.076$ ). In addition, there was a non-significant, slightly lower photoinhibition in the UV treated thalli compared to thalli exposed to PAR only (Fig. 12). Furthermore, the  $\text{ETR}_{\text{app}}$  was significantly higher in the UV-B treated *L. virens* thalli at high irradiances compared to the thalli exposed to PAR + UV-A (Fig. 14). Because  $\text{ETR}_{\text{app}}$  will be overestimated (see chapter 2.4) in thalli containing pigments, this result is thus an indication that UV-B treated *L. virens* thalli to some degree produce pigments. The abovementioned results are weak evidences indicating that *L. virens* may be able to produce sun-screening pigments. The reason for the weak results may be that four weeks of solar exposure was not sufficient for *L. virens* to induce pigments.

As discussed above, a high STM may be disadvantageous for lichens exposed to high light conditions. However, an increase in STM is suggested to be an acclimation response of some lichens (including *L. pulmonaria*) to high light exposure (Gauslaa et al. 2006). Gauslaa and Coxon (2011) found a linear relationship between water holding capacity and STM with increasing exposure to sunlight in *L. pulmonaria*. The same study

also found a correlation between STM and cortex thickness. In this study, all treatment groups of both species experienced an increase in STM during the field experiment (Fig. 6), which therefore can be seen as a response to increased light exposure. There are two plausible explanations for this. Firstly, that the species acclimate their morphology to higher light conditions by increasing their water holding capacity, because recovery from photoinhibition is dependent on hydration (Bidussi et al. 2013). Another explanation can be that the lichens increase the thickness of their cortex to reduce the transmittance to the photobiotic layer (Gauslaa & Coxson 2011).

In addition, the  $RT_{AGR}$  of *L. pulmonaria* thalli was reduced more than RGR as response to expanding UV exposure from PAR to PAR + UV-A and PAR + UV-A + UV-B (Fig. 6). It can thus be hypothesized that the increase of STM in *L. pulmonaria* with exposure to expanding wavelength range in the UV spectrum reflects an increasing need for the thalli to have a high water holding capacity and/or improve solar radiation screening. However, the increase was not significant, thus further investigations are needed.

Chl content in *L. pulmonaria* has been shown to decrease with increasing exposure to light (Gauslaa et al. 2006; Gauslaa et al. 2007). However, no differences in chl content were found between the treatment groups in neither *L. pulmonaria* nor *L. virens* (Fig. 15). Chl *a/b* ratio is correlated with sun/shade acclimation, and typically a higher *a/b* ratio occurs in sun-acclimated organisms (Kitajima & Hogan 2003; Lindahl et al. 1995), including *L. pulmonaria* exposed to high light (Gauslaa et al. 2006). In this study, however, there were no significant differences in chl *a/b* ratio in any of the radiation-treated thalli compared to the controls in neither *L. pulmonaria* nor *L. virens*. Larsson et al. (2009) found no effect of UV-B exposure of *L. pulmonaria* on chl content and chl *a/b* ratio, and explained this by the slow response of chl content in lichens. *Lobaria pulmonaria* thalli which were placed at the same site at the same time as the field experiment, but without screens and for one more week, experienced a significant increase in chl *a/b* ratio compared to the controls (unpublished data, n=12). The results of this study confirms that chl responses in lichens are slow, and suggests that UV radiation does not affect chl content in *L. pulmonaria* and *L. virens*.

*Lobaria virens* and *L. pulmonaria* have been characterized as ecological indicators of continuity (Rose 1988), and the decline in their populations may thus be seen as indication of environmental change. Multiple environmental changes, which can have detrimental effects on lichens, have been observed during the past century, e.g. increased temperatures due to climate change. The results of this study show that UV radiation is a stressor for the two species. This suggests that increased UV-B radiation due to ozone depletion during the past decades may have contributed to additional lichen stress.

In conclusion, growth is a highly useful parameter in short-term field experiments assessing lichen response to environmental factors. UV radiation strongly influence growth in *L. pulmonaria*, and to some degree also affects growth in *L. virens*. *Lobaria pulmonaria* acclimates to high light exposure by producing melanic pigments, which reduce photoinhibition. The induction of melanic pigments depends mainly on UV-B-exposure. UV-B radiation can thus be seen as an important environmental signal for *L. pulmonaria* to acclimate to high light conditions. Melanic pigments reduce photosynthesis at low irradiances, which is suggested to be the main reason for the lower growth rates in *L. pulmonaria* as response to UV-B exposure. UV-A radiation is shown to be a stressor in *L. pulmonaria*, and without a concurrent exposure to UV-B, UV-A radiation may be a bigger threat to this species than UV-B radiation. There was no clear evidence of pigment production in *L. virens* as response to neither PAR nor UV radiation. Hence, an explanation for the brown colour observed in *L. virens* in open habitats needs further investigations. The lower growth rates and the higher photoinhibition of *L. virens* confirms that this species is more susceptible to solar exposure than *L. pulmonaria*, probably because *L. virens* has a lower capability to acclimate to high light than *L. pulmonaria*.



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Norges miljø- og biovitenskapelig universitet  
Noregs miljø- og biovitenskapelige universitet  
Norwegian University of Life Sciences

Postboks 5003  
NO-1432 Ås  
Norway