Short- and long-term freezing effects in a coastal (*Lobaria virens*) versus a widespread lichen (*L. pulmonaria*)

Knut Asbjørn Solhaug, Dipa Paul Chowdhury, Yngvar Gauslaa*

Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway

ARTICLE INFO

ABSTRACT

Lichens are considered freezing tolerant, although few species have been tested. Growth, a robust measure of fitness integrating processes in all partners of a lichen thallus, has not yet been used as a viability measure after freezing. We compared relative growth rates (RGR) after freezing with short-term viability measures of photo- and mycobiont functions in the coastal *Lobaria virens* and the widespread *L. pulmonaria* to test the hypothesis that low temperature shapes the coastal distribution of *L. virens*. Hydrated thalli from sympatric populations were subjected to freezing at -10, -20 and $-40\,°C$ for 5 h. The rate of cooling and subsequent warming was $5\,°C$ h^{-1}. Short-term viability measures of photobiont (maximal photosystem II efficiency, effective PSII yield) and mycobiont viability (conductivity index), as well as subsequent RGR, were assessed. The exotherms showed that *L. virens* froze at $-3\,°C$; *L. pulmonaria*, at $-4\,°C$. Freezing significantly impaired short-term viability measures of both photo- and mycobiont, particularly in the coastal species. *Lobaria pulmonaria* grew 2.1 times faster than *L. virens*, but the short-term damage after one freezing event did not affect the long-term RGR in any species. Thereby, short-term responses were impaired by freezing, long-term responses were not. While the lacking RGR-responses to freezing suggest that freezing tolerance does not shape the coastal distribution of *L. virens*, the significant reported adverse short-term effects in *L. virens* may be aggravated by repeated freezing-thawing cycles in cold winters. In such a perspective, repeated freezing may eventually lead to reduced long-term fitness in *L. virens*.

1. Introduction

Lichens are symbiotic associations between a heterotroph mycobiont and one or more autotroph photobiont(s) (green algae and/or cyanobacteria). Being poikilohydric organisms, most lichens tolerate desiccation [11,24], some for long periods [23]. The freezing resistance of poikilohydric organisms partly depends on their desiccation tolerance of cytoplasm during extracellular freezing. Therefore, lichenized fungi and their photobionts are also often freezing resistant [15,33]; many studied species can be frozen to $-78\,°C$ or even $-196\,°C$ without damage [20,21,34].

Whereas many lichens are dominant in cold climates e.g. [29] and grow faster at extreme alpine ridges without insolating snow cover than in more sheltered positions [4], others are restricted to coastal sites [17] with little or mild frost for only short periods. For plants, oceanic distribution patterns are often associated with low frost tolerance, whereas oceanic lichens are assumed to depend more on humidity factors than on temperature [5,26]. Lichens assessed by e.g. Kappen and Lange [21] tolerated lower temperatures than those occurring in

their natural habitats, implying that frost does not limit the worldwide distribution of these lichens. Yet, a few species were more frost susceptible than others, like the coastal *Roccella* species [20,21,32]. Because the scarce data on freezing hardiness of lichens does not allow universal conclusions, there is a need for more studies.

Methodological innovations after the important freezing study of Kappen and Lange [21], like chlorophyll fluorescence techniques e.g. [14] have extended the ways in which lichen survival and subsequent performance can be assessed. Furthermore, simple protocols for assessing membrane damage [38] and growth rates [3,12] have become established as tools for studying lichens. Thereby, we can now assess both short-term effects of frost on each lichen biont and long-term effects on the entire lichen symbiosis in term of growth.

In the ecologically important nitrogen-fixing old forest lichen genus *Lobaria* (s. lat.), the widespread *L. pulmonaria* is common in Norway, extending far north, to the timberline, and into inland sites with minimum temperatures below $-40\,°C$, whereas *L. virens* is a southern coastal lowland species (Fig. 1), restricted to areas with mild winters without hard frost [30]. The common *L. pulmonaria* grows in most sites

---

* Corresponding author.
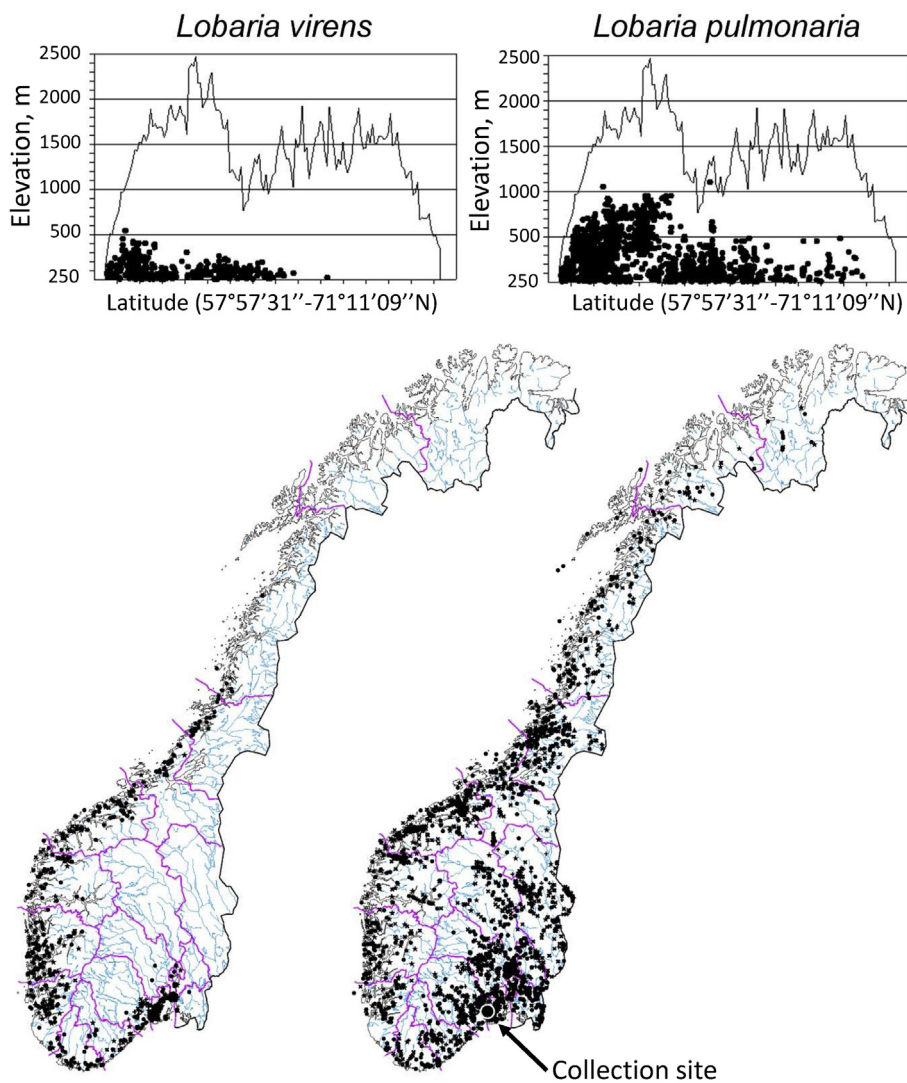 *E-mail address:* yngvar.gauslaa@nmbu.no (Y. Gauslaa).

Fig. 1. Occurrences of *Lobaria virens* and *L. pulmonaria* in Norway plotted as latitude-elevation diagrams (top) and maps (bottom). In the two upper diagrams, the curve shows the highest position of the land surface at each latitude. The maps and the diagrams were based on the NLD-database at http://nhm2.uio.no/lav/web/index.html (Natural History Museum, University of Oslo; accessed 24 October 2017). The two categories of lines represent borders between administrative units versus rivers. Filled circles; Herbarium records with precise coordinates, determination not doubtful, stars: Herbarium records, precise coordinates lacking, symbol placed in centre of municipality x: Data from field note database, whereas + represent Data from field investigation database (see the web page). The collection site for both species is shown by the large black dot with an arrow in the map showing the distribution of *L. pulmonaria*.

where *L. virens* is present. However, *L. virens* is often restricted to more sheltered positions on the trunk closer to the ground [2]. Also in Britain, *L. virens* is strongly associated with a high level of oceanity (Amman's index of hydrothermy), whereas *L. pulmonaria* is not [7]. Worldwide, *L. virens* is only known in Western Europe and Macaronesia. So far, there are no data on frost tolerance in *L. virens*, whereas the ubiquitous *L. pulmonaria* is highly frost resistant [21].

We aimed to compare viability measures subsequent to freezing in *L. pulmonaria* and *L. virens*. As short-term measures, we assessed photobiont responses using chlorophyll fluorescence tools and mycobiont responses by using a conductivity index. Furthermore, we assessed long-term effects by measuring growth rates because we consider growth to be one of the most robust and reliable measures of lichen fitness. Based on the contrasting distribution patterns of the two species (Fig. 1) and the previously reported low freezing tolerance in a few coastal lichens [20,21,32], we hypothesize that the coastal *L. virens* is more susceptible to severe freezing damage than the ubiquitous *L. pulmonaria*. Based on a high level of resilience in lichen thalli in general e.g. [9], we also hypothesize that short-term effects of freezing within a one-day timescale after freezing are stronger than long-term effects over a few weeks.

2. Materials and methods

2.1. Lichen material

The cephalolichens *Lobaria pulmonaria* and *L. virens* were collected from many trunks of oak trees at their optimal heights above the ground (5 and 1 m, respectively) in old oak forests at Langangen, SE Norway (59°06′43″ N, 9°50′05″ E, 150 m a.s.l.) in July 2017. The collection site of both species, shown on the map of *L. pulmonaria* (Fig. 1), is located near the south-eastern distribution border of *L. virens* in Norway where winters are colder than in the more oceanic sites west- and northwards along the western coast [30]. The two lichens share the same green-algal *Dictyochloropsis* photobiont genotype [6]. In the lab, we removed debris, tree bark and bryophytes from the lichens. For each species, we cut the thalli into equally-sized pieces, 100 in total, and randomized them for each species separately. For the growth experiment, we measured air-dry mass after 24 h drying at 20 °C for each thallus before and after the experiment. Afterwards, five additional thalli were further oven-dried 24 h at 70 °C to measure DM. We used the oven-dried/air-dried mass ratio for these thalli to calculate oven-dry mass (DM) for the experimental thalli. All experiments and measurements were completed within 3 weeks after collection.

2.2. Freezing test

The freezing tests were run in three separate programmable freezing chambers (Weiss Umwelttechnik, Reiskirchen, Germany) at the Centre for Plant Research in Controlled Climate (SKP, Norway). The freezing test program was initiated by exposing moist thalli to 5 °C. We used moist thalli because thalli are often wet at the onset of freezing periods. The chamber temperature was gradually lowered by 5 °C h^{-1} until the test temperatures of -10, -20 and -40 °C were reached. All freezing temperatures, applied for 5 h at the target temperature, are ecologically relevant for inland sites of *L. pulmonaria*, but not for *L. virens*. Afterwards, temperature was increased by 5 °C h^{-1} until 5 °C. We fastened thin thermocouples type E to thalli in the freezing chamber cooled to -40 °C using one control thermocouple to measure the air in the chamber. The thallus and air temperatures were logged every 15 s with an eight channel OctTemp 2000 Data Logger (MadgeTech Inc., Contoocook, New Hampshire, USA). During the freezing treatment, the lichens were kept dark.

2.3. Evaluation of short-term effects

For studying F_V/F_M, serving as a short-term viability measure we used 25 randomly selected thalli from the total pool of 100 thalli of each species as controls. They had not been subjected to freezing. Likewise, three additional batches, each of 25 randomly selected thalli, were exposed to three respective freezing temperatures ($n = 25$). After the freezing test, we placed all thalli of each species in a controlled temperature room at 15 °C under dim light (≈ 5 µmol photons m^{-2} s^{-1}) for 14 h to optimize detection of more lasting damage [36]. Thalli were then dark-adapted for at least 15 min before measurement of maximal photosystem II efficiency (F_V/F_M) with a PAM-2000 fluorometer (Walz, Effeltrich, Germany).

After measurement of F_V/F_M, we randomly selected 10 control thalli and 10 thalli from each freezing temperature and species for measurement of effective PSII yield (Φ_{PSII}), which is a proxy of photosynthesis and thus another short-term photobiont viability measure. These thalli were placed at 100 µmol photons m^{-2} s^{-1} from a LED panel (Model SL-3500, Photon System Instruments, Brno, Czech Republic) with equal irradiances from blue, green and red light for 15 min before measurement of Φ_{PSII} ($n = 10$). Yield measurements were completed within 2–3 h after the F_V/F_M measurements.

After these non-destructive measurements, we measured membrane integrity by running a conductivity test. This is a short term viability test for mainly the mycobiont [31] because the mycobiont comprises the far highest biomass component in these two lichens. We placed the 10 thalli for each treatment that had been used for Φ_{PSII} measurements 2–3 h earlier in 15 ml plastic test tubes with 8 ml de-ionized water. They were shaken until the next day. Electrolyte leakage from each thallus was measured as conductivity (Cv) in the water by a portable conductivity meter (Mettler-Toledo International Ltd, Singapore). Then, the tubes with thalli were boiled at 100 °C for 15 min in a water bath to cause total rupture of cell membranes and release of all electrolytes; cooled to 25 °C and the final electrical conductivity (Cf) was measured after shaking until next day. A conductivity index indicating loss of membrane integrity [38] was calculated as: (Cv/Cf) × 100 ($n = 10$).

2.4. Evaluation of longer effects (12 days)

The remaining 15 thalli from each freezing treatment including 15 controls were subjected to a 12 d growth experiment in a Sanyo MLR-351 growth cabinet (Sanyo Electric, Osaka, Japan). The temperature was 18/12 °C (day/night; 12 h photoperiod), which is within the optimal range for *Lobaria* [3]. The relative humidity was kept high (70 ± 5% during the light period and 90 ± 5% during the night) by placing repeatedly wetted newspapers on the top and bottom shelves. Light at 200 µmol photons m^{-2} s^{-1} (from Mitsubishi/Osram FL 40SS

W/37) was used because it approximates light saturation in *L. pulmonaria*. The thalli were placed upon plastic nets on Petri dishes with wet filter paper in the bottom following Gauslaa et al. [12], and sprayed with deionized water in the beginning and the end of the photoperiod. This method ensured optimal growth by keeping thalli moist most of the time without direct contact with the wet filter paper.

After 12 days, thalli were air-dried at room temperature and weighed. Relative growth rate was computed as RGR = (ln (DM$_{end}$/DM$_{start}$))*1000/Δt (mg g^{-1}day^{-1}). Δt is the 12 d between start and end at which DM (g) was measured [8].

2.5. Statistical analyses

We analysed the data by General Linear Models (GLM) followed by Tukey multiple comparison test using Minitab ver. 16. Lichen species and temperatures were used as factors. When analysing F_V/F_M, two extreme outliers for *L. virens* with the lowest F_V/F_M (standard residuals -3.69 and -6.30) were excluded, although the removal of these outliers did not change the trends. Furthermore, the conductivity data was log-transformed. By these actions, the GLM-requirements were satisfied for all studied parameters.

3. Results

The exotherms, which refer to the heat released to the surroundings by the freezing of internal water (Fig. 2), showed that *L. virens* thalli supercooled down to -3 °C, *L. pulmonaria* to almost -4 °C. During start of freezing, the peaks of the exotherms showed that the freezing point of *L. pulmonaria* was approximately -1 °C, and slightly higher for *L. virens*. The exotherms disappeared at approximately -6 °C for *L. virens* and at -8 °C for *L. pulmonaria* (Fig. 2), indicating the respective temperatures at which most water was frozen. The area below the exotherms was lower for *L. virens* than for *L. pulmonaria*, showing that more water froze in *L. pulmonaria*. No exotherms at lower temperatures were detected.

All short-term responses, measured after 14 (F_V/F_M), 17 (Φ_{PSII}) and 24 h (conductivity index) recovery at low light and room temperature after freezing, were significantly affected by the freezing treatments (Table 1), particularly in *L. virens* (Fig. 3A–F). In *L. virens*, F_V/F_M decreased with increasing level of freezing from 0.712 ± 0.002 for the controls to 0.676 ± 0.004 for those exposed to -40 °C (Fig. 3B); Φ_{PSII}
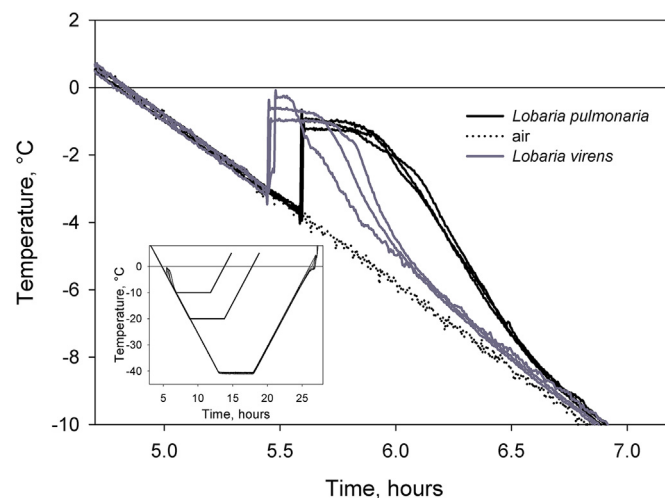

Fig. 2. Exotherms in three *Lobaria pulmonaria* and three *L. virens* thalli during an early part of the freezing experiment, measured with thin type E-thermocouples. Air temperature was measured by a thermocouple close to each thallus. The insert shows the temperatures for the entire experiment including the initial freezing and the final heating process after freezing. The -40 °C curve is based on thermocouple measurements, whereas the -10 and -20 °C curves are schematic plots of the freezing program used.

Table 1
General linear models (GLM) for maximal photosystem II efficiency (*FV/FM*), effective PSII yield (Φ_{PSII}), conductivity index, and relative growth rate (RGR) for two lichen species (*Lobaria pulmonaria*, *L. virens*) and four freezing treatments (untreated controls, -10, -20 and -40 °C). All parameter responses are shown in Fig. 3.

Parameter				
Source	*df*	*F*	*P*	r^2_{adj}
FV/FM				0.468
Species (S)	1	12.23	0.001	
Temperature (T)	3	52.71	0.000	
S x T	3	3.13	0.027	
Error	190			
Total	197			
PSII yield				0.123
Species (S)	1	0.17	0.682	
Temperature (T)	3	4.48	0.006	
S x T	3	1.50	0.223	
Error	72			
Total	79			
Conductivity[a]				0.131
Species (S)	1	2.23	0.139	
Temperature (T)	3	3.19	0.029	
S x T	3	2.35	0.079	
Error	72			
Total	79			
RGR				0.640
Species (S)	1	209.88	0.000	
Temperature (T)	3	1.60	0.193	
S x T	3	1.23	0.302	
Error	112			
Total	119			

[a] The conductivity index was log-transformed.

declined from 0.538 ± 0.007 to 0.458 ± 0.018 (Fig. 3D), whereas its conductivity index doubled from $15.1 \pm 1.8\%$ to $30.9 \pm 4.7\%$ (Fig. 3F). In *L. pulmonaria*, such trends were only significant for F_V/F_M (Fig. 3A).

Unlike F_V/F_M, Φ_{PSII} and conductivity index, the longer term response represented by RGR during the subsequent growth experiment did not change with temperature in any species (Fig. 3G–H; Table 1). However, the RGR in *L. virens* averaged across all treatments was much lower $(4.86 \pm 0.24 \text{ mg g}^{-1} \text{d}^{-1})$ than in *L. pulmonaria* $(10.5 \pm 0.31 \text{ mg g}^{-1} \text{d}^{-1}; n = 60)$.

4. Discussion

By lowering the freezing temperature, short-term viability measures (F_V/F_M, Φ_{PSII}, conductivity index) were impaired, particularly in *L. virens*. The reduction in F_V/F_M and Φ_{PSII} shows that the photobiont was temporarily affected by freezing, whereas the increased conductivity index is likely due to temporary mycobiont damage [38]. Such responses are consistent with the earlier reported strong increase in mycobiont respiration shortly after freezing [21]. Increased respiration may be a mechanism to repair membrane disruptions for example, which likely caused the measured rise in conductivity at the lowest temperature. Thereby, freezing has at least temporary effects on the functioning of *L. virens*.

The lacking effect of freezing on subsequent growth rates was consistent with earlier measured gas exchange rates that were reported at normal levels a few weeks after freezing in various lichens [21]. Apparently, repair mechanisms function well after freezing, even in *L. virens*. The 2.1 times higher overall growth rate in *L. pulmonaria* compared to *L. virens* corresponds well to the 2.2-ratio measured under field conditions for these two species in late summer with no frost [19]. Thereby, the lower RGR in *L. virens* is not due to species-specific differences in freezing tolerance. *Lobaria pulmonaria* has likely a higher growth potential than *L. virens*, partly because the less attached and thinner growth form in *L. pulmonaria* allows a more flexible use of

humid air and liquid water than in *L. virens* [13,28].

Exotherms are rarely measured in lichens. In the Antarctic *Umbilicaria aprina,* the exotherm was -5.4 °C [35], in coastal Californian lichens -3.0 to -5.0 °C [32], and in lichens from the Magdalena Mountains of New Mexico -2.8 to -3.7 °C [1]. No additional exotherms at lower temperatures, like those that kill plants during extreme freezing [37], were seen. Thus, when freezing starts, there was no strong barrier to subsequent spread of ice inside the thallus. The exotherms for the two *Lobaria* species fall within the range of earlier measured values for lichens, and are also within the range of supercooling [-2 to -8 °C; 37] in plants. Cooling below the exotherm leads to further dehydration of the protoplast in lichens, causing similar internal changes as those occurring during dehydration at higher temperatures [35]. The apparent disappearance of the exotherm at higher temperature in *L. virens* (Fig. 2) suggests that most water becomes frozen at higher temperatures in *L. virens* than in *L. pulmonaria*. Slightly higher freezing point indicates that *L. virens* contains less compatible solutes for lowering the freezing point than *L. pulmonaria*. Thereby, *L. virens* may be more exposed to dehydration stress than *L. pulmonaria* at a given freezing temperature. Because the freezing point depression is 1.86 °C molal^{-1}, the molality of the unfrozen water is approximately 3 molal, corresponding to a water potential of 7 MPa and a relative humidity of 93–94%. However, freezing and the associated loss in water potential does not necessarily exclude photosynthesis in lichens. In Antarctic lichens, photosynthesis [22,25] and Φ_{PSII} [16] has been measured at temperatures as low as -17 °C. However, *Lobaria* species with higher temperature optima for growth [3] and with very low growth rates in winter [27] likely have higher temperature thresholds for photosynthesis.

From the freezing tolerance literature for lichens that is mainly based on gas exchange as a viability measure, the photobiont type apparently influences lichens' freezing tolerance. Lichens with *Trentepohlia* [21,32] and *Coccomyxa* [18] are considered less tolerant than those with other green algal photobionts. However, the presence of *Trentepohlia* in Antarctic lichens at 80°S [39] suggests that it is not the *Trentepohlia* photobiont *per se* that shapes the lower freezing tolerance. Because the photobiont genera *Trentepohlia* and *Coccomyxa* mainly associate with specific taxonomic groups of lichenized fungi [10], the lower freezing resistance in lichens with such photobionts may also be shaped by their fungal partners. It is a paradox that our two lichens share the same green algal photobiont species [6] and yet their photobiont responses differ.

The ecological implications of the contrasting long- and short-term effects are not clear. Based on our data, we can set up two mutually exclusive interpretations or hypotheses: 1: Both species are highly freezing tolerant because growth rate, representing a reliable measure of lichen fitness, was not influenced by freezing. According to this interpretation, the strictly coastal distribution of *L. virens* must be explained by other factors than low freezing tolerance. 2: The significant short-term adverse effects in *L. virens* after one freezing event may accumulate during a winter with many repeated freezing-thawing cycles and eventually lead to long-term adverse effects. Because the diurnal temperature amplitudes are larger in inland than in coastal areas, freezing-thawing cycles likely cause more damage in inland sites. So far, there is evidence that the coastal, more southern lichen with *Trentepohlia* photobionts (*Roccella* and *Dendrographa* spp.) were more susceptible to freezing than lichens with other green algal photobionts [21,32]. For the coastal *L. virens*, the higher exotherm and the stronger short-term reduced viability are consistent with the oceanic distribution pattern of this species. Yet, because the long-term lichen functioning was not affected in any of the two species, we cannot conclude that the freezing tolerance plays a role in shaping the distribution of *L. virens*. Nevertheless, our results invite to future studies not only testing the effects of repeated freezing-drying cycles, but also to e.g. assessments of intraspecific photo- and mycobiont-responses for thalli from different climatic zones. Freezing tolerance is likely a more important factor for
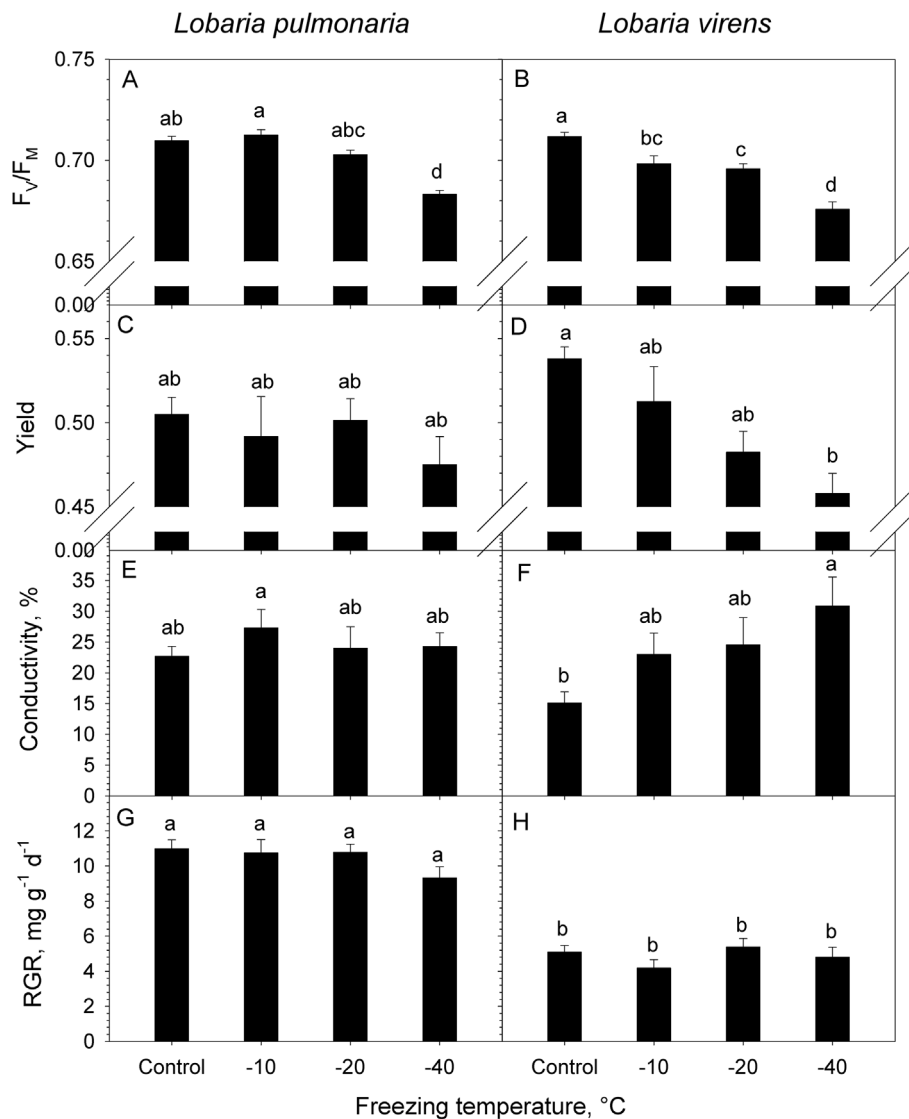
Fig. 3. Short- (A–F) and long-term (G–H) responses to freezing of hydrated *Lobaria pulmonaria* and *L. virens* thalli to −10, −20 and −40 °C. A–B: The maximal photosystem II efficiency (F_V/F_M; $n = 25$). C–D: The effective PSII yield (Φ_{PSII}; $n = 10$). E–F: A conductivity index ($n = 10$). G–H: Relative growth rate (RGR; $n = 15$). Within a parameter, means sharing the same superscript letter do not differ significantly ($P < 0.05$) from each other (Tukey multiple comparison test).

lichen distributions in general than previously thought.

Conflicts of interest

None.

References

[1] E.N. Ashworth, T.L. Kieft, Measurement of ice nucleation in lichens using thermal analysis, Cryobiology 29 (1992) 400–406.
[2] J. Asplund, P. Larsson, S. Vatne, Y. Gauslaa, Gastropod grazing shapes the vertical distribution of epiphytic lichens in forest canopies, J. Ecol. 98 (2010) 218–225.
[3] M. Bidussi, Y. Gauslaa, K.A. Solhaug, Prolonging the hydration and active metabolism from light periods into nights substantially enhances lichen growth, Planta 237 (2013) 1359–1366.
[4] M. Bidussi, K.A. Solhaug, Y. Gauslaa, Increased snow accumulation reduces survival and growth in dominant mat-forming arctic-alpine lichens, Lichenologist (Oxf.) 48 (2016) 237–247.
[5] E. Dahl, The Phytogeography of Northern Europe, Cambridge University Press, Cambridge, 1998.
[6] F. Dal Grande, A. Beck, C. Cornejo, G. Singh, S. Cheenacharoen, M.P. Nelsen, C. Scheidegger, Molecular phylogeny and symbiotic selectivity of the green algal genus *Dictyochloropsis* s. l. (Trebouxiophyceae): a polyphyletic and widespread group forming photobiont-mediated guilds in the lichen family Lobariaceae, New Phytol. 202 (2014) 455–470.
[7] C.J. Ellis, Oceanic and temperate rainforest climates and their epiphyte indicators in Britain, Ecol. Indicat. 70 (2016) 125–133.
[8] G.C. Evans, The Quantitative Analysis of Plant Growth, Blackwell Scientific Publications, Oxford, 1972.
[9] J.F. Farrar, Physiological buffering, in: M. Galun (Ed.), CRC Handbook of Lichenology, vol. II, CRC Press Inc, Boca Raton, Florida, 1988, pp. 101–105.
[10] T. Friedl, B. Büdel, Photobionts, in: T.H. NashIII (Ed.), Lichen Biology, Cambridge University Press, Cambridge, 2008, pp. 9–26.
[11] Y. Gauslaa, D.S. Coxson, K.A. Solhaug, The paradox of higher light tolerance during desiccation in rare old forest cyanolichens than in more widespread co-occurring chloro- and cephalolichens, New Phytol. 195 (2012) 812–822.
[12] Y. Gauslaa, M.A. Alam, K.A. Solhaug, How to optimize lichen relative growth rates in growth cabinets, Lichenologist (Oxf.) 48 (2016) 305–310.
[13] Y. Gauslaa, K.A. Solhaug, S. Longinotti, Functional traits prolonging photo-synthetically active periods in epiphytic cephalolichens during desiccation, Environ. Exp. Bot. 141 (2017) 83–91.
[14] T.G.A. Green, B. Schroeter, L. Kappen, R.D. Seppelt, K. Maseyk, An assessment of the relationship between chlorophyll fluorescence and CO2 gas exchange from field measurements on a moss and a lichen, Planta 206 (1998) 611–618.
[15] J. Hájek, P. Váczi, M. Barták, L. Jahnová, Interspecific differences in cryoresistance of lichen symbiotic algae of genus *Trebouxia* assessed by cell viability and chlor-ophyll fluorescence, Cryobiology 64 (2012) 215–222.
[16] J. Hájek, M. Barták, M. Hazdrová, M. Forbelská, Sensitivity of photosynthetic pro-cesses to freezing temperature in extremophilic lichens evaluated by linear cooling and chlorophyll fluorescence, Cryobiology 73 (2016) 329–334.
[17] P.M. Jørgensen, The oceanic element in the Scandinavian lichen flora revisited, Symb. Bot. Ups. 31 (1996) 297–317.
[18] P. Kallio, S. Heinonen, Influence of short-term low temperature on net

photosynthesis in some subarctic lichens, Rep. Kevo Subarct. Res. Stn. 8 (1971) 63–72.

[19] I.K. Kann, Ultravaiolet Radiation Affects Growth and Photosynthesis in the Old Forest Lichens *Lobaria pulmonaria* and *L. virens* (M.Sc. thesis), Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Ås, 2016.

[20] L. Kappen, O.L. Lange, The cold resistance of phycobionts from macrolichens of various habitats, Lichenologist (Oxf.) 4 (1970) 289–293.

[21] L. Kappen, O.L. Lange, Die Kälteresistenz einiger Makrolichenen, Flora 161 (1972) 1–29.

[22] L. Kappen, B. Schroeter, C. Scheidegger, M. Sommerkorn, G. Hestmark, Cold resistance and metabolic activity of lichens below 0 °C, Adv. Space Res. 18 (1996) 119–128.

[23] I. Kranner, R.P. Beckett, A. Hochman, T.H. Nash III, Desiccation-tolerance in lichens: a review, Bryologist 111 (2008) 576–593.

[24] O.L. Lange, Hitze- und Trockenresistenz der Flechten in Beziehung zu ihrer Verbreitung, Flora 140 (1953) 39–97.

[25] O.L. Lange, Pionierpflanzen in Kältewüsten, Umschau 72 (1972) 650–654.

[26] W. Larcher, H. Bauer, Ecological significance of resistance to low temperature, in: O.L. Lange, P.S. Nobel, C.B. Osmond, H. Ziegler (Eds.), Physiological Plant Ecology I. Responses to the Physical Environment, Springer, Berlin, 1981, pp. 403–437.

[27] P. Larsson, Y. Gauslaa, Rapid juvenile development in old forest lichens, Botany 89 (2011) 65–72.

[28] S. Longinotti, K.A. Solhaug, Y. Gauslaa, Hydration traits in cephalolichen members of the epiphytic old forest genus *Lobaria* (s. lat.), Lichenologist (Oxf.) 49 (2017) 493–506.

[29] R.E. Longton, Biology of Polar Bryophytes and Lichens, Cambridge University Press, Cambridge, 1988.

[30] A. Moen, National Atlas of Norway: Vegetation, Norwegian Mapping Authority, Hønefoss, 1999.

[31] S. Munzi, T. Pisani, S. Loppi, The integrity of lichen cell membrane as a suitable parameter for monitoring biological effects of acute nitrogen pollution, Ecotoxicol. Environ. Saf. 72 (2009) 2009–2012.

[32] T.H. Nash III, L. Kappen, R. Lösch, D.W. Larson, U. Matthes-Sears, Cold resistance of lichens with *Trentepohlia*- or *Trebouxia*-photobionts from the North American west coast, Flora 179 (1987) 241–251.

[33] A. Sadowsky, S. Ott, Photosynthetic symbionts in Antarctic terrestrial ecosystems: the physiological response of lichen photobionts to drought and cold, Symbiosis 58 (2012) 81–90.

[34] P.F. Scholander, W. Flagg, R.J. Hock, L. Irving, Studies on the physiology of frozen plants and animals in the Arctic, J. Cell. Physiol. Supplement 42 (1953) 1–56.

[35] B. Schroeter, C. Scheidegger, Water relations in lichens at subzero temperatures: structural changes and carbon dioxide exchange in the lichen *Umbilicaria aprina* from continental Antarctica, New Phytol. 131 (1995) 273–285.

[36] K.A. Solhaug, Low-light recovery effects on assessment of photoinhibition with chlorophyll fluorescence in lichens, Lichenologist (Oxf.) 50 (2018) 139–145.

[37] C.J. Weiser, Cold resistance an injury in woody plants, Science 169 (1970) 1269 &.

[38] O.A. Yemets, Y. Gauslaa, K.A. Solhaug, Monitoring with lichens – conductivity methods assess salt and heavy metal damage more efficiently than chlorophyll fluorescence, Ecol. Indicat. 55 (2015) 59–64.

[39] D.O. Øvstedal, C.E.G.R. Schaefer, A new lichen species from the Heritage Range, Ellsworth Mountains, Antarctica, Hoehnea 40 (2013) 361–364.