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

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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

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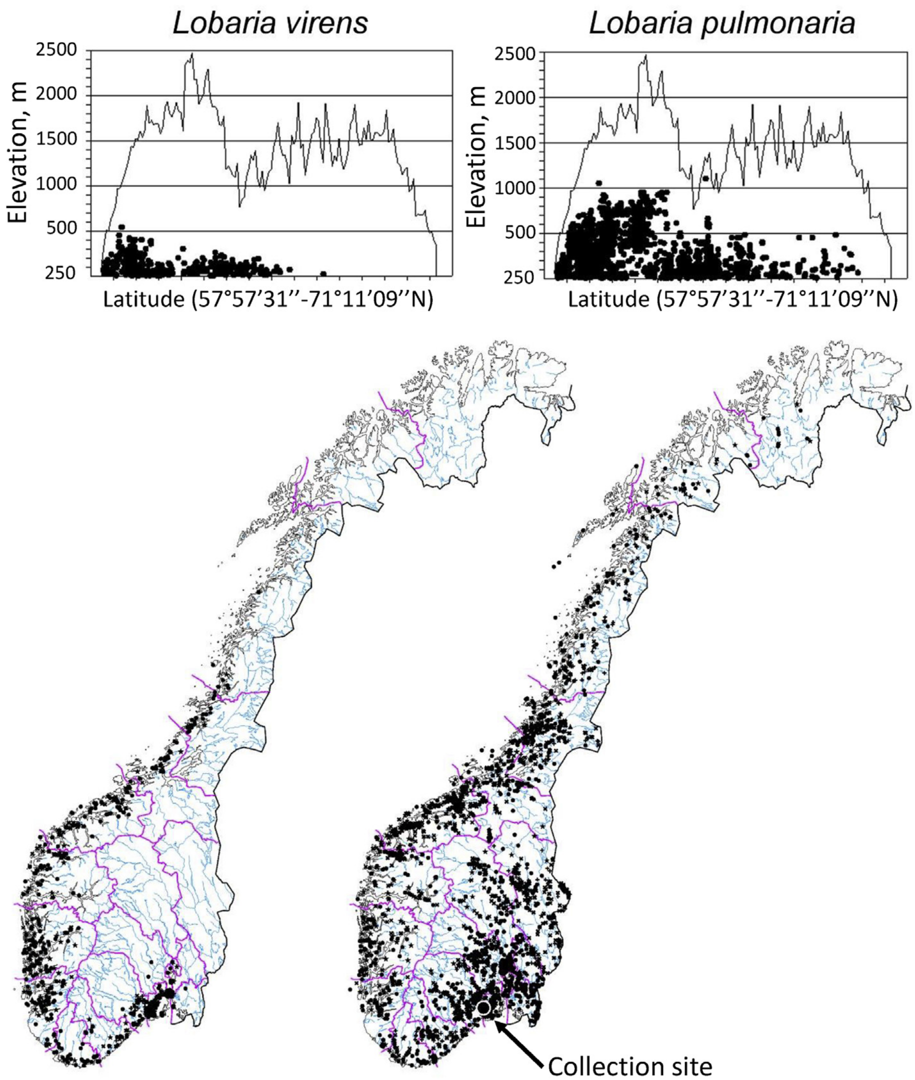

Fig. 1. Occurrences of *Lobaria virens* and *L. pulmonaria* in Norway plotted as latitude-elevation diagrams (top) and maps (bottom). In the two upper diagrams, the curve shows the highest position of the land surface at each latitude. The maps and the diagrams were based on the NLD-database at http://nhm2.uio.no/lav/web/index.html (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.