

***Lobaria amplissima* thalli with external cephalodia need more rain than thalli without**

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Abstract: Hydration traits determine much of a lichen's distribution pattern along a climatic gradient but the mechanisms involved are still incompletely known. A higher abundance of large external cephalodia in wet oceanic than in drier climates has previously been reported in *Lobaria amplissima*. This study aims to quantify how much more rain *L. amplissima* thalli with external cephalodia would need to fill their internal water holding capacity (WHC_{internal}) than thalli without. The mean WHC_{internal} was 1.8 times higher in thalli with external cephalodia than in those without. The WHC_{internal} when converted to mm rain needed to saturate an average specimen was 1.37 mm (min–max: 0.55–3.8 mm) for a cephalodiate thallus, whereas an average thallus without external cephalodia needed just 0.76 mm (min–max: 0.36–1.3 mm). Known dewfall rates and rates of water uptake from humid air are far below what is needed to saturate even the cephalodiate thallus with the lowest WHC_{internal}, implying a stronger dependency on rain for thalli with external cephalodia. Thus, the observed trends in this study are consistent with earlier reports of decreasing frequency of external cephalodia from wet to drier climates.

Key words: cephalolichens, cyanobacteria, hydration traits, specific thallus mass, water storage

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Introduction

Morphological traits in lichens are important in the understanding of functional adaptations, for example regarding lichen water relations. Also, traits with other well-documented functions, such as N-fixing cephalodia (Millbank & Kershaw 1969, 1970), may influence lichen hydration patterns, although this is poorly known. Cephalolichens are characterized by colonies of cyanobacteria (cephalodia) inhabiting an otherwise green algal-dominated thallus. Although cephalolichens share some traits with chloro- and cyanolichens, the cyano- and chlorolichen morphs of a cephalolichen

are functionally fundamentally different. For example, whereas the chloromorph can be photosynthetically activated by humid air alone, the cyanomorph needs liquid water to initiate photosynthesis (Schlensog *et al.* 2000; Green *et al.* 2002). Furthermore, cyanolichens have substantially greater ratios of internal water holding capacity (WHC_{internal}) to specific thallus mass (STM) than chlorolichens, with cephalolichens at intermediate levels (Gauslaa & Coxson 2011).

The species for our study belongs to the *Lobariaceae*, a family with many cephalo- and cyanolichens currently subjected to a number of taxonomic revisions. Moncada *et al.* (2013) recently proposed to resurrect the old genus *Ricasolia* for some members of the genus *Lobaria* including *Lobaria amplissima* (Scop.) Forssell (= *Ricasolia amplissima* (Scop.) De Not.). This is primarily a western European species with a preference for temperate rainforest climates (Ellis 2016), but was recently reported (in the form of a new subspecies) from north-west America (Cornejo *et al.* 2017). Its primary photobiont

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belongs to the green-algal genus *Dictyochochloropsis* (Dal Grande *et al.* 2014), presently referred to as *Symbiochloris reticulata* (Škaloud *et al.* 2016), while its secondary cyanobacterial photobiont (*Nostoc*) occurs as invisible smaller internal cephalodia, sometimes with additional fruticose and spectacular external cephalodia (Jordan 1970). Compared to the cephalolichens *L. pulmonaria* and *L. virens*, *L. amplissima* is thicker with higher water storage and slower desiccation (Gauslaa *et al.* 2017; Longinotti *et al.* 2017). A thicker thallus also implies a need for more water to activate its photosystem II (PSII) (Phinney *et al.* 2018). Furthermore, because lichen tissues with a cyanobacterium hold more water than those with green alga (Gauslaa & Coxson 2011), the presence of many cephalodia should create a need for more water to fill the lichen's water storage. It is possible that two genetically slightly different varieties of *L. amplissima* may exist (Blom & Lindblom 2010). The variety mainly occurring in oceanic climates usually has many external cephalodia, whereas the variety in drier parts of Scandinavia often lacks such cephalodia (Blom & Lindblom 2010). However, there is no evidence as yet to suggest that genetic differences between the two varieties determine the frequency of external cephalodia in these two types, since a correlation does not prove causality. If an abundance of large external cephalodia has a major impact on the water storage pools, it probably affects lichen fitness along a moisture gradient, implying that it is not easy to know whether the presence of external cephalodia results from acclimation to wet climates or from genetic differences.

Here we test the hypothesis that thalli with external cephalodia have a higher WHC than those without. A higher WHC of cephalodiate thalli would be consistent with a higher competitive advantage of the variety with external cephalodia in a rainy climate, whereas a lower WHC in the thalli without external cephalodia would allow more frequent and more rapid PSII activation with just humid air and dewfall (Gauslaa 2014; Phinney *et al.* 2018). The latter play an important role as hydration sources for

lichens in more continental climates characterized by strong nocturnal cooling.

Material and Methods

We collected a number of separate *L. amplissima* thalli with varying amounts of external cephalodia from a large population of the western variety in the wet oceanic Kvinnherad, Hordaland, western Norway (59° 58' 41.41202''N, 6° 3' 39.49946''E, 350 m a.s.l.), in October 2013, from each of eight old pollarded trunks of *Fraxinus excelsior*. Pieces with and without external cephalodia were taken from each of the thalli collected. Debris, tree bark and bryophytes were removed. All pieces (120 with and 120 without external cephalodia), hereafter called thalli, had actively growing lobes with intact lobe ends and a minimum of cut edges. It was possible to select lobes of the two categories that were relatively similar with respect to the proportion of apical and central parts because the presence and number of external cephalodia varied across most thalli. In the laboratory, thalli were saturated in deionized water until their mass did not increase further. Their hydrated area (A_{wet}) was determined from photographs using ImageJ 1.48 (Rasband 2014). We measured wet mass (WM_{blotting}) immediately after blotting all external water on the surface with dry filter paper. Afterwards, we dried the thalli at room temperature and weighed them. At this weighing session, five additional control thalli of each species were also weighed air-dried then oven-dried (24 h at 70 °C) before measuring their oven-dry mass (DM). We used the mean reduction factor in mass from the air-dry to the oven-dry state for each category to convert air-dry mass to DM for all specimens and estimated specific thallus mass (STM) as $STM = DM/A_{\text{wet}}$. The internal water holding capacity was calculated as $WHC_{\text{internal}} = (WM_{\text{blotting}} - DM)/A_{\text{wet}}$.

We computed means, standard errors and medians. For each category of thalli, linear regressions with 95% confidence intervals were calculated to evaluate species-specific differences in the STM-dependency of WHC_{internal} . Log-transformation was applied to improve data distributions for the regressions but a non-parametric test (Kruskal-Wallis one-way analysis of variance on ranks with all pairwise multiple comparison procedures, Dunn's Method) was used to assess differences between the two categories. These analyses were run in SigmaPlot (Systat Software, San Jose, CA).

Results

Sampled *L. amplissima* thalli with and without external cephalodia had broadly similar (Fig. 1A) yet slightly different surface areas (Table 1). The lack of significant regression between thallus area and STM in any of the two categories (Fig. 1A) shows that STM was not size-dependent for the limited range

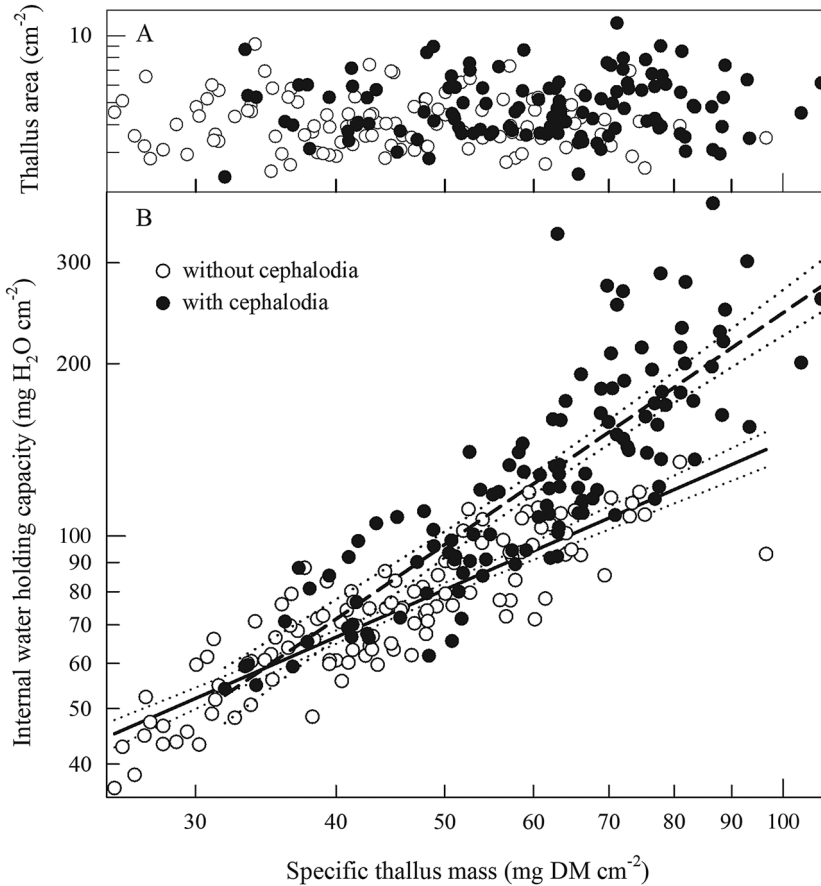


FIG. 1. A, thallus size of 240 prepared pieces of *Lobaria amplissima* thalli with (filled symbols) and without (open symbols) external cephalodia across specific thallus mass; B, dependency of internal water holding capacity ($WHC_{internal}$) on specific thallus mass for the *L. amplissima* thalli shown in Fig. 1A. The regression equations were $\log_{10} WHC_{internal} = 0.454 + 0.855 \times \log_{10} STM$ ($r_{adj}^2 = 0.768$; $P < 0.001$; solid line) for thalli without cephalodia and $\log_{10} WHC_{internal} = -0.297 + 1.343 \times \log_{10} STM$ ($r_{adj}^2 = 0.697$; $P < 0.001$; hatched line) for cephalodiate thalli. Dotted lines indicate 95% confidence intervals.

of sizes used in this study, facilitating comparisons between the two categories of thalli. The STM and $WHC_{internal}$ were higher for the

majority of thalli with external cephalodia than for those without (Table 1, Fig. 1B). $WHC_{internal}$ was particularly great in thalli

TABLE 1. Thallus size, specific thallus mass (STM) and internal water holding capacity ($WHC_{internal}$) in *Lobaria amplissima* with and without external cephalodia. Median (25–75%) and mean values are provided (n = 120). Differences between the medians were analysed by the Kruskal-Wallis One-Way Analysis of Variance on Ranks.

	Without external cephalodia		With external cephalodia		ANOVA on ranks P - value
	Median (25–75%)	Mean ± SE	Median (25–75%)	Mean ± SE	
Thallus area, cm ²	4.00 (3.33–4.83)	4.22 ± 0.11	4.58 (3.74–5.89)	5.00 ± 0.15	<0.001
STM, mg cm ⁻²	44.8 (36.4–57.2)	46.9 ± 1.3	63.0 (50.8–72.9)	62.4 ± 1.5	<0.001
$WHC_{internal}$, mg H ₂ O cm ⁻²	74.1 (60.9–91.6)	76.2 ± 2.0	120.4 (91.5–169.2)	136.7 ± 5.8	<0.001

with higher STM. The mean STM was 1.3 times higher in thalli with external cephalodia than in those without; the mean WHC_{internal} was 1.8 times higher in cephalodiate thalli (Table 1). The $WHC_{\text{internal}}/STM$ -ratio was 1.64 ± 0.014 (mean \pm 1SE, $n = 120$) for the non-cephalodiate thalli versus 2.13 ± 0.014 for those with cephalodia. The slope for the respective regression lines in linear plots (not shown) was as high as 2.99 ± 0.24 for thalli with external cephalodia and just 1.31 ± 0.08 for thalli without. For individual thalli, there was more than a ten-fold total variation in WHC_{internal} from 35 (without external cephalodia) to $381 \text{ mg H}_2\text{O cm}^{-2}$ (with external cephalodia). Converted to mm of rain, this means that the non-cephalodiate thallus with the lowest STM could fill up its entire internal water storage with just 0.35 mm of rain or even dewfall. By contrast, at least 3.8 mm of rain would be needed to saturate the cephalodiate thallus with the highest WHC_{internal} , provided that all the rain hits the thallus.

Discussion

This study reports a functional significance of external cephalodia as hydration traits. Clear differences in water storage were found between thalli with and without external cephalodia, which might explain the respective western and eastern distributions of the two *L. amplissima* varieties described by Blom & Lindblom (2010). The WHC_{internal} even in *L. amplissima* without external cephalodia (Fig. 1), is high compared to measured chloro- (Esseen *et al.* 2015) and cephalolichens (Longinotti *et al.* 2017). It even lies above the 2:1 line (the slope of the regression line between STM and WHC_{internal}) (Fig. 2) that characterizes cyanolichen members of the *Lobariaceae* (Gauslaa & Coxson 2011), implying that every mg DM of an average *L. amplissima* with external cephalodia can store 2.2 mg water (Table 1). *Lobaria amplissima* holds small amounts of external water (Longinotti *et al.* 2017), therefore the WHC_{internal} accounts for most of its total water storage capacity. Our data do not allow an estimation of how much the

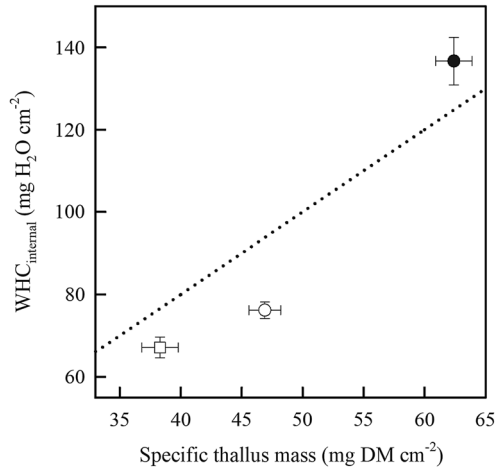


FIG. 2. Specific thallus mass and internal water holding capacity (WHC_{internal}) in *Lobaria amplissima* from a wet oceanic site (>2000 mm rain; this study) with (filled circle) and without (open circle) external cephalodia, compared with another set of thalli ($n = 109$) with few or no external cephalodia sampled from drier sub-oceanic sites with an annual rainfall of 1000–1300 mm (open square; Longinotti *et al.* 2017). Means and standard errors are provided. The dotted line shows the 2:1 line (the slope of the regression line between STM and WHC_{internal}) that characterizes cyanolichen members of the *Lobariaceae* (Gauslaa & Coxson 2011).

cephalodia *per se* contribute to the WHC_{internal} . Such an estimation would require artificial removal of the cephalodia and recording of their water holding capacity. This could not be done because the thalli were used in a subsequent reintroduction experiment during which the external cephalodia in particular happened to be grazed by gastropods (Gauslaa *et al.* 2018). The high STM and WHC_{internal} in cephalodiate thalli could be explained in the following, not necessarily mutually exclusive, ways: 1) the STM, and WHC_{internal} in particular, is higher in the external cephalodia themselves because cyanolichens store more water than chlorolichens (Lange *et al.* 1988; Gauslaa & Coxson 2011); 2) green algae-dominated lobes are thicker beneath cephalodia; 3) internal cephalodia might be more abundant in thalli with external cephalodia.

Regardless of the explanation, the much higher WHC_{internal} in thalli with external

cephalodia than in those without has ecological implications. For comparison, Longinotti *et al.* (2017) studying hydration traits in the eastern morph of *L. amplissima* with few or no external cephalodia (Blom & Lindblom 2010) reported an average STM of 38 mg DM cm⁻², which is lower than in our non-cephalodiate thalli belonging to the western morph in high rainfall areas (Fig. 2). *Lobaria amplissima* with an average STM of 40 mg DM cm⁻² needed as much as 8.8 h to reach 95% of full PSII activation in air close to 100% relative humidity (Hovind 2018) but, during this vapour uptake period, these thalli reached a relative water content of just 18.9% of their full internal hydration storage capacity. Such a low water content will rapidly be depleted after sunrise. Under optimal but realistic humidity conditions for nocturnal vapour uptake in nature, the cephalodiate specimens with much higher STM and WHC_{internal} (Fig. 2) would have filled up much less of their relative water storage capacity. Therefore, they would presumably reach not only a lower level of PSII activation (Hovind 2018), but probably also lower levels of net photosynthesis (Lange *et al.* 1986). With respect to the wetting capability of dewfall, maximal dewfall rates seem to be <0.04 mm h⁻¹ (Xiao *et al.* 2013) and a good, average nocturnal dewfall is just 0.2 mm (as reviewed by Gauslaa 2014), which would not be enough for *L. amplissima*. Therefore, *L. amplissima* with external cephalodia should have an advantage in a rainy climate with relatively frequent showers of >2 mm rainfall, consistent with their preference for rainforest climates (Ellis 2016). Only under such conditions can they fully benefit from their large WHC_{internal}, allowing long active periods after rain has ended.

In conclusion, cephalodia in cephalodiate lichens do not only have an important role in nitrogen fixation (Millbank & Kershaw 1969, 1970), but might also substantially influence functional hydration traits. An abundance of external cephalodia influences the duration of active hydration periods in rainy climates. Therefore, the trends observed in this study should imply a decreasing frequency of cephalodia from wet to drier

climates, consistent with earlier reported data (Blom & Lindblom 2010).

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