Why is the rainforest lichen Methuselah's Beard (*Usnea longissima*) so rare in British Columbia's inland temperate rainforest?

Journal:	<i>Botany</i>
Manuscript ID	cjb-2021-0057.R1
Manuscript Type:	Article
Date Submitted by the Author:	26-Jun-2021
Complete List of Authors:	Strother, Ivy; University Of Northern British Columbia Ecosystem Science and Management, Ecosystem Science and Management Coxson, Darwyn; University Of Northern British Columbia Ecosystem Science and Management, Goward, Trevor; University of British Columbia, UBC Herbarium, Beaty Museum
Keyword:	temperate rainforest, old-growth forest, lichen growth, occult precipitation
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

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Title:

Why is the rainforest lichen Methuselah’s Beard (*Usnea longissima*) so rare in British Columbia’s inland temperate rainforest?

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16 Abstract

17 Coastal (CTR) and inland temperate rainforests (ITR) in western North America share a rich
18 oceanic lichen flora. The distinctive Methuselah's beard lichen (*Usnea longissima*) is an
19 exception to this pattern of shared distributions, with very few ITR locations. Does this absence
20 reflect dispersal limitations or climatic intolerance? To answer this question, we transplanted *U.*
21 *longissima* thalli from the CTR to three ITR locations, assessing growth rates against reciprocal
22 CTR transplants. Canopy microclimate measurements provided concurrent data on growth
23 conditions. Growth rate responses (length, mass and area) were evaluated after summer and full-
24 year transplants. Notwithstanding extended drought conditions during the summer period, annual
25 transplants at two of the three ITR locations supported growth rates comparable to those at the
26 CTR source *U. longissima* population, with summer dewfall and autumn rains being major ITR
27 hydration sources. Thalli transplanted to a third ITR site (summer measurements only) in a
28 location transitional to the drier interior plateau lost both mass and length. Based on these
29 findings we suggest that the absence of *U. longissima* from much of the ITR reflects the
30 combined influence of dispersal limitations and requirements for stands with long site continuity
31 and topographically induced summer wetting of thalli by dewfall.

- 32 **Keywords:** temperate rainforest, lichen biogeography, old-growth forest, lichen growth,
- 33 transplant, occult precipitation

Draft

34 Introduction

35 Coastal temperate rainforest ecosystems in western North America represent a globally
36 significant repository for oceanic lichens (Goward and Spribille 2005; Spribille et al. 2010;
37 DellaSala et al. 2011a), sharing many common species with boreal rainforests in Scandinavia
38 (DellaSala et al. 2011b; Hilmo et al. 2011). Rich epiphytic lichen communities are found in the
39 CTR, from calicioid mesolichens, to foliose and tripartite Lobarion cyanolichens, and pendulous
40 “hair” lichens (Goward et al. 1994; Goward 1999; Sillett and Antoine 2004; Goward and
41 Campbell 2005; McCune and Geiser 2009; Spribille et al. 2010).

42 Particularly notable in the CTR is the old-forest-dependant lichen species *Usnea*
43 *longissima* Ach., or Methuselah’s Beard, which can form dense curtains on ocean-front trees
44 (Brodo et al. 2001). In western North America, this species is locally abundant in maritime and
45 hypermaritime sites from southeast Alaska through British Columbia (B.C.) (Halonen et al.
46 1998, Price and Hochachka 2001), extending southward along the coast ranges into Oregon and
47 northern California (Keon 2001; Keon and Muir 2002; Carlberg and Peterson 2005). By contrast,
48 *U. longissima* is essentially absent in temperate rainforests farther inland (Goward 1999), in both
49 the Kispiox rainforest in the headwaters of the Skeena River watershed; and in the Caribou

rainforest, a geographic variant of the ITR characterized by wet-cool conditions in the headwaters of the Fraser and Columbia rivers. Within the Caribou rainforest *U. longissima* has been found at only a few widely scattered sites (Beaty Biodiversity Museum 2020; Goward and Spribille 2005; CNALH 2020; T. Spribille, pers. comm.)

Although the Caribou rainforest receives about half the mean annual precipitation of the CTR, soil moisture availability in the former is supplemented by spring snow melt and in many locations by ground water flow, especially in the wet toe slope (moisture receiving) topographic position (Radies et al. 2009). As a result, comparable ecosystems can be found in both rainforests, with western redcedar (*Thuja plicata* Donn ex D. Don) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) characteristically dominant or co-dominant in old-growth stands (Meidinger and Pojar 1991). Coastal and Caribou rainforest sites in B.C. share many vascular and non-vascular plant taxa (Gavin and Hu 2006; Björk 2010; Stevenson et al. 2011). Epiphytic macrolichens, in particular, have been proposed as shared indicators of temperate rainforest status, with more than 40% of CTR epiphytic macrolichens also found in old Caribou rainforest stands (Goward and Spribille 2005).

Factors constraining the distribution and growth of *U. longissima* have previously been studied at several locations within the southern region of the CTR (McCune et al. 1996, Keon and Muir 2002), however, we have no comparable information on constraints facing ITR populations in general and the Caribou rainforest in particular. There are at least two possible explanations for the rarity of *U. longissima* in the Caribou rainforest, one historical, the other ecological. The historical explanation pertains to the relatively recent development of the Caribou rainforest ecosystems. Palynological evidence suggests that Western Red-cedar, a tree whose presence can promote establishment of many old growth lichens (Goward & Arsenault 2018), entered the Caribou rainforest about 6,000 years ago in the extreme south and only 2,000 years ago farther north (Gavin et al. 2009). This timeline is relevant insofar as dispersal in *U. longissima* appears to depend primarily on fragmentation from existing thalli (Esseen et al. 1981, Esseen 1985, Gauslaa 1997, Rolstad et al. 2013), which is likely the least efficient form of dispersal available to lichens (Sillett and Antoine 2004).

A second explanation for the general absence of *U. longissima* from the Caribou rainforest is that environmental conditions limit its establishment and/or growth. The Caribou rainforest experiences a relatively continental climate, with a deep winter snowpack and

subfreezing weather that precludes metabolic activity in canopy lichens for extended periods during this season (Coxson and Stevenson 2007). Although *U. longissima* is found within similarly continental sites in Fennoscandia (Esseen et al. 1981, Jansson et al. 2009), the duration of the winter period and proportion of precipitation as snow is higher in the Caribou rainforest, with snow accounting for nearly 50% of annual precipitation at the Ancient Forest site vs. ca. 35% in eastern Sweden (Jansson et al. 2009, Wang et al. 2012).

In contrast to vascular plants, for which abundant soil moisture supply (e.g., from seepage) can compensate for low precipitation compared to the CTR (Stevenson et al. 2011), canopy lichens like *U. longissima* depend for hydration on rainfall, snowmelt or occult precipitation in the form of dewfall or fog (Gauslaa 2014). While climate data are not available for the few ITR locations where *U. longissima* has previously been found, collection notes from herbarium records suggest that topographic influences such as cold air drainage may play a major role in creating suitable microhabitats (Spribille pers. com. 2020).

Our aim in this paper is two-fold: first to assess growth conditions for *U. longissima* in southern inland British Columbia by monitoring microclimate; and second, thereby, to evaluate the relative importance of growth limitations as an explanatory mechanism for the general absence of *U. longissima* in the Caribou rainforest. We transplanted thalli of *U. longissima* collected within its current range in the CTR to three sites within or adjacent to the Caribou rainforest, in sites where it does not presently occur. Taken together, these three sites form a climatic gradient from cool-wet to warm-dry growing season conditions. We hypothesized that

transplanted thalli of *U. longissima* would respond along this gradient, from copious growth in the wettest Caribou rainforest site to meagre growth in the driest transplant site, located adjacent to the southern boundary of the Caribou rainforest.

Materials and Methods

Study species

Usnea longissima Ach. is a pendulous epiphytic lichen, characterized by a long central main strand, from which perpendicular side branches arise. As with other *Usnea* species, the main strand of *U. longissima* thalli has an “elastic” cartilaginous central cord, which is surrounded by the medulla, often together with fragments of the outer cortex (Goward 1999). This species tends to be more oceanic in its distribution in North America than in Europe and Asia (Clerc 2011). In western North America it is found most commonly in coastal forests from Alaska to California, usually in close proximity to maritime environments as shown, e.g., by collection records from the UBC herbarium (Fig. 1) (see also Brodo et al. 2001).

Transplant sites and protocols

Thalli were collected in south coastal British Columbia at Cassiar Cannery near the mouth of the Skeena River in the Pacific Coastal Temperate Rainforest where they grow

120 abundantly on oceanfront trees and shrubs (Fig. 1). The climate at Cassiar Cannery is regarded as
121 hypermaritime, with frequent rain and fog (Meidinger and Pojar 1991). Our three transplant sites
122 were located about 500+ km inland from the BC coast, within or immediately adjacent to the
123 Caribou rainforest, as follows: 1) the Ancient Forest in the upper Fraser River Valley; 2)
124 Edgewood Blue near Wells Gray Provincial Park in the Clearwater River valley; and 3) the
125 Aleza Lake Research Forest in the upper Fraser River Valley. Aleza Lake falls just outside the
126 western Caribou rainforest boundary, in a transition zone to drier forests of the B.C. interior
127 plateau. Edgewood Blue likewise falls just outside the southern Caribou rainforest boundary, in a
128 transition zone to interior-wetbelt conditions (Coxson et al. 2019). The Ancient Forest is the
129 wettest of the three transplant sites, while Edgewood Blue is the driest (Table 1). The majority of
130 winter precipitation falls as snow in all three ITR sites (Table 1).

131 Source thalli were collected on 24 and 25 April 2018. All thalli came from branches of
132 Sitka spruce (*Picea sitchensis* (Bong.) Carriere) or western redcedar, collected within 3 m of the
133 ground. Thalli were inspected on collection to ensure uniform colour and health as well as
134 minimal side branching. Any thalli with discoloured or blackened segments were discarded.

While sorting, thalli were randomized and numbered. Thalli were held dry in a cool dark location prior to their placement on meshes in the field as described below.

Prior to transplant, individual thalli were cut into approximately 60 cm lengths (60.1 - 60.5 cm), taking care to remove the apical portions in order to avoid any confounding effects of apical versus intercalary growth (Rolstad and Rolstad 2008). The length of individual thalli was measured after pulling the central cord straight, but without being stretched. Initial measurements of mass were taken at Cassiar Cannery within 24 hours of collection after thalli had been air dried for 12 hours. Mass was recorded to within 0.001 g using an Ohaus “Explorer Pro” balance (Ohaus Corporation, Parsippany, NJ, USA). Forty sacrificial thalli, weighed at Cassiar Cannery as described above, were later reweighed under laboratory conditions at the University of Northern British Columbia, following 24-hour exposure to oven-dry conditions of 40°C. This provided a correction factor for any humidity effects on thallus mass at the time of initial measurement (at Cassiar Cannery). Mass was subsequently calculated as:

$$\text{Mass}_{\text{corrected}} = \text{Mass} * ((\text{Oven weight}_{\text{sacrificial}} / \text{Mass}_{\text{sacrificial}}) * 100)$$

as outlined in Antoine and McCune (2004). A similar procedure was carried out with the thalli sampled at the end of the experiment.

151 The surface area of our transplants was measured at the end of the transplant period based
152 on a vertical projection of the thalli following Gauslaa and Coxson (2011), modified in that
153 surface area measurements were taken on dry thalli. Photographs were taken with a Nikon D600
154 camera (24 MP) (Nikon, Melville, NY) using a Micro-Nikkor 60mm macro-lens mounted 30 cm
155 above the lichen thalli. ImageJ (<https://imagej.nih.gov/ij/>) was used to calculate the Area_{final}
156 (cm²) of thalli after Esseen et al. (2017).

157 At the transplant sites, lichen thalli were placed within the canopy on plastic mesh nets,
158 similar to methods of Phinney et al. (2021), but using a smaller mesh size, using 4x4 mm PVC
159 mesh netting attached to a 70 cm length of wooden doweling (Fig. 2). Three thalli were tied to
160 each net, for 175 nets in total. Thalli were tied to transplant nets in an arc pattern using nylon
161 thread, resulting in two loose ends hanging much as if the thallus were draped over a tree branch
162 (Fig. 2). Each net was photographed before and after transplant to assist in the identification of
163 thallus fragmentation, in cases where this occurred.

164 Transplant nets at Cassiar Cannery and Edgewood Blue were attached to conifer branches
165 at a height of 1.5 m above the ground. Transplants at the Ancient Forest and Aleza Lake were
166 established at a height of 1.5 m above the early spring snowpack, ca. 2.5 m above the ground

after snowmelt. Conifer branches were chosen to represent microsites on the side of the tree that faced adjacent water or wetlands. Branches also had to provide sufficient space for net and thalli movement during wind events. Transplant nets were placed on Sitka spruce, western redcedar and western hemlock branches at Cassiar Cannery, on western redcedar, western hemlock, and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) at the Ancient Forest, on hybrid spruce (*Picea glauca* x *engelmannii*), Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco), western redcedar and western hemlock at Edgewood Blue, and on hybrid spruce and subalpine fir at Aleza Lake, taking care to ensure that thalli were evenly distributed between host tree species at each site.

The Cassiar Cannery and Edgewood Blue locations were both located at the base of steep mountain slopes, while the Ancient Forest site was situated at mid-slope in a broad mountain valley and Aleza Lake at the edge of the central-interior plateau. Transplanted thalli at the Ancient Forest were placed on the branches of trees at the edge of gaps facing small wetlands in the forest canopy, with relatively little direct exposure to open sky. By contrast, transplants at the Aleza Lake and Edgewood Blue sites were on trees located at the edge of small ponds and thus fully exposed to the sky. Establishment and take-down dates for all transplants are provided in Table 2. Fifty transplant nets were allocated to each site, with 25 nets collected at the end of the summer period, and the rest collected the following year in spring 2019. The one exception to

184 this was at Aleza Lake, where only 25 transplant nets were established due to logistical issues;
185 these provided data for the summer 2018 period only.

186 The response of *U. longissima* to transplant conditions was assessed by pre- and post-
187 transplant measurements of thallus length (cm) and mass (g), and post-transplant measurements
188 of thallus area (cm²). Measured values for each of these parameters were averaged for the three
189 individual thalli on each net at establishment, resulting in 25 replicates (nets) for each site during
190 the summer and annual period (except for the summer-only Aleza measurements). Only two nets
191 were lost during the transplant period.

192 Changes in the length and mass of transplant thalli were expressed as relative growth
193 rates (RGR), following Gauslaa and Coxson (2011):

194
$$\text{RGR(L)} = ((\text{Length}_{\text{final}} - \text{Length}_{\text{initial}})/\text{Length}_{\text{initial}})*100$$

195
$$\text{RGR(M)} = ((\text{Mass}_{\text{final}} - \text{Mass}_{\text{initial}})/\text{Mass}_{\text{initial}})*100$$

196 The relationship between final mass and final area for replicate thalli at each locality is expressed
197 by the slope of the linear regression (g/cm²), while the ratio of final mass (mg) per unit final
198 length (cm) for replicate thalli at each locality is expressed as $\Sigma(\text{Mass}_{\text{final}}/\text{Length}_{\text{final}})$.

Growth rate data from the transplant thalli were partitioned into two data sets. First, all thalli on each net were assessed for differences in length and mass between initial and final transplant measurements irrespective of any loss of thallus segments to fragmentation. We refer to these measurements as “ecological” growth rates, as they integrate the various factors operating on each thallus, including changes in mass derived from trade-offs between photosynthesis and respiration during the transplant period, and the loss of mass from any thallus fragmentation due to wind action, shedding of snow and/or ice, and any other disturbance. “Ecological” growth rates (presented in Supplemental materials) were denoted as $RGR(L)_e$ for length-based measurements and $RGR(M)_e$ for mass-based measurements.

Second, we excluded all thalli assumed to have significantly fragmented over the transplant period. Examination of changes in thallus length at each site (Figs. 3 and 4) revealed a marked distinction between thalli that had significantly fragmented, typically with a loss of 10 cm or more in length, and thalli that remained more or less intact. Thalli that remained intact showed a range of growth patterns, from thalli that showed a predominant increase in length, as at Cassiar Cannery (Fig. 3A), to sites where most thalli showed a decrease in length, as at Aleza Lake (Fig. 3D). The point of demarcation between thalli with marked fragmentation (dramatic

215 reductions in length) and those without (smaller reductions in length or increases in length)
216 generally exceeded one standard deviation (SD) in length change (Fig. 3) below the y-axis. This
217 threshold (one SD below y-axis) was subsequently used as a basis for exclusion of thalli
218 presumed to have undergone major fragmentation.

219 We refer to the calculated changes in length and mass of the thalli *not* subject to
220 fragmentation as yielding a potential “physiological” growth rate for each site, that is, the net
221 outcome of photosynthetic carbon gain minus respiratory loss over the transplant period. These
222 assessments were denoted as $RGR(L)_p$ for length-based measurements and $RGR(M)_p$ for mass
223 based measurements.

224 The above examination of changes in length and mass were carried out separately for
225 thalli collected in autumn 2018 (summer growth rates only) versus those collected in spring 2019
226 (annual growth rates). We subsequently calculated potential changes in length and mass during
227 the winter period by subtracting the summer values from the annual data set. This involved
228 subtracting the mean relative growth rate for the summer period at each site from the relative
229 growth rates of individual thalli in the annual transplant set. This calculation was carried out for
230 measurements of both length and mass and on the subsets of thalli used in “ecological”

assessments (all thalli) and “physiological” assessments (excluding fragmented thalli as described above).

The relationship between final mass and final area for replicate thalli at each site is expressed by linear regression (g/cm^2), while the ratio of final mass (mg) per unit final length (cm) for replicate thalli at each site is expressed as $\Sigma(\text{Mass}_{\text{final}}/\text{Length}_{\text{final}})$. Relationship comparisons were performed using the “physiological” growth rate criteria. The ratio of mass per unit length calculated from our data is the 'dry' equivalent to specific thallus mass [STM], following Esseen et al. 2017.

Climate measurements

Climate stations were installed at each site for the duration of the transplant period, each consisting of a Hobo H21-002 Microstation Datalogger (Onset Computer, Cape Cod, MA). Sensors were placed adjacent to canopy branches, at the same location as the lichen transplant nets. Temperature and relative humidity were measured using a Hobo 12-bit Temperature/Relative Humidity Smart Sensors installed in an unaspirated radiation shield. Photosynthetically active radiation (PAR) was recorded using a Hobo Photosynthetic Light (PAR) sensor. Incident precipitation was measured with a Hobo tipping bucket rain gauge (0.2 mm resolution) while the

247 duration of periods of both incident and occult precipitation (condensation of mist and fog on
248 foliage as per Weathers 1999) was measured with a Hobo Leaf Wetness Smart Sensor, installed
249 at a 45° orientation from vertical immediacy adjacent to thalli on nets. Measurements were taken
250 from all sensors at 5-minute intervals.

251 Climate data were analyzed to determine the interaction of three different binary
252 conditions under field growth, asking: 1) Were thalli wet or dry? (using leaf wetness and RH
253 sensor values as a proxy for thallus hydration); 2) Were thalli in the light or in the dark?
254 (PAR=0); and 3) Were thalli frozen (less than or equal to 0°C) or not frozen? (above 0°C). Thalli
255 were considered hydrated when ambient RH values exceeded 97% and/or when leaf wetness
256 sensor values were greater than 50%. Subsequent field observations conducted at Edgewood
257 Blue in summer 2020 confirmed that this 50% threshold corresponded with the point at which *U.*
258 *longissima* thalli became dry after previous dewfall wetting (see Supplemental data).

259 Using these binary subsets, total transplant time was divided into three categories of
260 growth: 1) periods when thalli were presumed to be physiologically inactive by virtue of being
261 frozen or dry; 2) periods when thalli would be metabolically active and photosynthesizing, i.e.
262 not frozen, but hydrated, and exposed to daylight; and 3) periods when thalli would be

metabolically active, i.e. not frozen, but hydrated, and in the dark). Finally, the frequency distribution of these three growth conditions was assessed against ambient temperature at each site.

Statistical Analyses

Statistical analysis was performed in R version 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism version 8.0.0 (GraphPad Software, San Diego, USA) and SYSTAT Ver 13. (SYSTAT, Santa Clara, CA). In R $Mass_{initial}$, $Length_{initial}$, and the ratio of $\frac{Final\ Mass}{Final\ Length}$ were tested for treatment effect using ANOVA. SYSTAT was used to test treatment effects of RGR(M) and RGR(L) (in both “ecological” and “physiological” thalli subsets) with Kruskal-Wallis or ANOVA, depending on the normality of the dataset. Pairwise Wilcoxon or Tukey’s tests were performed respectively. The difference between regression slopes, where used, were evaluated in GraphPad Prism. All p values were considered significant at a confidence level of 95%.

Results

Growth Rates

The summer growth rates of individual thalli varied considerably among the four sites (Fig. 3). At Cassiar Cannery, most thalli grew 5 to 10 cm, while one thallus grew nearly 22 cm (Fig. 3A). Only two of the thalli at Cassiar were shorter at the end of the summer, both due to fragmentation (with one of the fragmented thalli falling just below our 1 SD cut-off, hence included in the “physiological” data set). The majority of thalli at the three transplant sites grew in length, with Aleza lake being the exception (Figs. 3B – 3D). Loss of length due to fragmentation affected more replicates at the driest site (Edgewood Blue) as compared to the wetter sites (Fig. 3D). Of the thalli left in the field for the full annual period, Cassiar Cannery had the greatest proportion of individual thalli showing positive sustained growth rates, with over one-third growing more than 10 cm and one thallus growing 29.5 cm (Fig. 4A). A smaller proportion of thalli showed positive length changes at the Edgewood Blue and Ancient Forest sites.

These patterns of change in the length of individual thalli are reflected in plots of median percent relative growth rates (Figs. 5). When fragmented thalli were excluded from the data set analysis (hence not subjected to a “physiological” assessment of growth potential), differences in relative growth rates between sites were pronounced. Increases in median length were greatest at Cassiar Cannery and Edgewood Blue. Only small changes in median RGR(L)p were seen at the Ancient Forest site. All three sites showed positive median annual RGR(L)p (Fig. 5C), with values from the Cassiar Cannery site slightly higher than those at Edgewood Blue. Changes in mass (RGR(M)p) in summer transplants was greatest at Cassiar Cannery and Edgewood Blue, with Ancient Forest and Aleza Lake transplants showing virtually no change in mass (Fig. 5D). Only Edgewood Blue and Ancient Forest sites had significantly different median RGR(M)p

values (Fig. 5F). An overview of these changes in mass in transplanted thalli (summer period) can be seen from comparison of the relative size of pie chart plots (Fig. 1). Similar trends were observed in calculations where all thalli (included fragmented thalli) were included (“ecological” growth rates; see Supplemental materials).

The ratio of mass per unit length for summer thalli was highest at Aleza Lake (8.20 mg/cm) followed by Edgewood Blue (7.59 mg/cm), Ancient Forest (6.80 mg/cm) and Cassiar Cannery (6.25 mg/cm) (Fig. 6). Values for Aleza Lake were significantly higher than those for Cassiar Cannery and Ancient Forest, with values for Edgewood Blue thalli significantly higher than those from Cassiar Cannery. At the end of the annual growth period, Ancient Forest thalli (7.91 mg/cm) had the highest mass per unit length ratio, followed by Edgewood Blue thalli (7.80 mg/cm), with Cassiar Cannery thalli having the lowest mass per unit length ratio (6.92 mg/cm). No site was statistically different from the others in annual growth rate comparisons of mass per unit length. The relationship between mass and area for annual thalli measurements resulted in r^2 values that ranged from 0.389 at Edgewood Blue to 0.794 at Cassiar Cannery (Fig. 7). Summer-only results showed similar patterns.

Climate

At Cassiar Cannery air temperatures during the summer period (April 28th – September 28th) were generally between 5 and 15°C, with thalli hydrated in the light (as indicated by wetting sensors – Fig. 8A) 22.5% of the time, and hydrated in the dark 28.2% of the time (Fig. 7B). Thalli at Cassiar Cannery were active 50.6% of the time during the summer period (Fig. 8C). The Ancient Forest had similar growth conditions with thalli active 48.4% of the time (Fig. 8C), with a roughly equal split between active periods in light and dark. Thalli at Aleza Lake

were active a much lower proportion of time during the summer period (23.5%), while Edgewood Blue was similar to Ancient Forest, with thalli active 47.5% of the time. Edgewood Blue was the warmest site, with thalli subject to temperatures above 20°C 17.7% of the time, compared to 16.8 % at Aleza Lake, 8.7% at Ancient Forest, and 1.6 % at Cassiar Cannery.

In the winter period *U. longissima* transplants faced long periods of inactivity due to their being frozen, especially at the Ancient Forest and Edgewood Blue sites (81.4 and 82.5% of the time respectively) (Fig. 9C). The proportion of time thalli experienced hydrated light conditions was low at all sites during the winter period, though greater at Cassiar Cannery (12.6%) than at Ancient Forest or Edgewood Blue (6.1 and 7.6% respectively) (Fig. 9A). The longest periods of thallus hydration at the Ancient Forest and Edgewood Blue sites occurred during the autumn and spring periods, when extended periods of precipitation occurred. In contrast, wetting sensors indicated long periods of hydration during winter rains at Cassiar Cannery.

Detailed examination of patterns of wetting and drying showed major differences between sites seasonally. Although thalli at Cassiar Cannery were frequently wet by mist and fog under fair weather conditions during the summer period (e.g. see data for July 26th to 31st in Supplemental materials), the majority of thallus hydration, in both summer and winter was from wetting by rainfall (see Fig. 1 inset pie charts). Summertime wetting of thalli at Edgewood Blue, by contrast, was strongly influenced by dewfall wetting, accounting for the majority of thallus hydration episodes (Fig. 1). A detailed plot showing representative dewfall wetting episodes under summer conditions (July 10th to 15th, 2018) is shown in Supplemental materials. Wetting of thalli by dewfall condensation also occurred during the summer period at Aleza Lake, however the duration of wetting events was typically much shorter than that at Edgewood Blue

(Fig. 1). Summertime wetting of thalli at the Ancient Forest site was predominantly from rainfall (Fig. 1).

Discussion

Growth Rate Assessments

A familiar sight in exposed ocean-front rainforests in coastal western North America is the branches of trees and shrubs festooned with Methuselah's Beard lichen *Usnea longissima*, often to the point of obscuring the underlying foliage. Sustaining such high levels of standing biomass in a canopy environment where thalli are often stripped from branches by storms, translocated onto lower branches or the forest floor, suggests appreciable annual growth rates. This was certainly apparent in transplanted thalli from our source population at Cassiar Cannery, where mean annual growth rates (changes in both length and mass) exceeded 10%, and where individual thalli increased by up to 50%. During the summer period, especially in fair weather, much of this growth was sustained by fog interception, while in fall and winter the major hydration source was rain.

The question our study was designed to answer is: what would happen when thalli from the coast were transplanted to sites within BC's Caribou rainforest. Would similar rates of growth be sustained (suggesting that their general absence from this region was due to dispersal limitations), or would growth be appreciably reduced in these more continental locations? The short answer is that *U. longissima* can grow just as well in the Caribou rainforest as along the Pacific coast, with the proviso that topographic position in the inland sites is more important than regional climate. Thus, thalli transplanted to the driest of the four inland sites, Edgewood Blue,

sustained the highest growth rates, comparable to or even higher than those in the source Cassiar Cannery population. Notably, the primary summer moisture source for thalli transplanted to Edgewood Blue was morning dewfall. At Ancient Forest, by contrast, the most important summer moisture source was rainfall, which sustained slightly lower mean growth rates. Although summer dewfall events were common at Aleza Lake, they were less intense than at Edgewood Blue, resulting in much shorter diurnal hydration episodes and, associated with this, very low growth rates (summer data only), with many thalli losing mass and actually shrinking during the five-month transplant period.

Our measured growth rates fell within the range of previous studies on *U. longissima*. Growth rates in “hair” lichen transplants (e.g., *Alectoria*, *Bryoria*, *Evernia divaricata*, *Nodobryoria*, *Ramalina thrausta*, *Usnea*) which have commonly been assessed based on two criteria: on the basis of changes in length, and on the basis of changes in mass (McCune et al. 1996, Keon and Muir 2002, Stevenson and Coxson 2003, Rolstad and Rolstad 2008, Phinney et al. 2021). Rolstad and Rolstad (2008) reported annual increases in thallus length from 24.5 to 61.5% in sites ranging from Oregon to Alaska, while Keon and Muir (2002), working in Oregon, reported an even wider range from 21 to 123%. Rates were lower in central Sweden, where Jansson et al. (2009) recorded annual increases in thallus length of 11%, and annual increases in thallus mass from 12 to 31%, depending on the light exposure (from adjacent edges) faced by thalli. Gauslaa et al. (2007) found that changes in mass during the June to October growing season were highly variable in Norway and Sweden, ranging from 1.1 to 38.2%, with the highest growth rates occurring in hypermaritime regions.

Climatic limitations on growth.

The summer of 2018 represented a period of severe climate stress for our transplanted thalli. The city of Prince George ca. 110 km west of the Ancient Forest site, recorded its fifth driest summer on record, with August being the driest since 1943 (Pawliw 2018). The Aleza Lake and Edgewood Blue sites similarly experienced extended drought conditions, with few precipitation events and wildfires widespread in the region. Growth rate assessments of our transplanted thalli may thus provide a valuable test of the resilience of *U. longissima* in inland southern BC - an important consideration in light of climate-change predictions, which suggest that extreme drought events may become more frequent over time (Hebda 1997).

Our climate measurements suggest that successful summer growth (length and mass) of *U. longissima* at Cassiar Cannery is facilitated by morning fog versus dewfall at Edgewood Blue. Intriguingly, Matthes-Sears and Nash (1986) have shown that morning fog is similarly a major driver of summer growth in *Ramalina menziesii* Taylor - another epiphytic lichen of the Pacific coast (Brodo et al. 2001) – though they also note that the cool, low-light conditions associated with fog at the same time limit the growth potential of that species. A similar observation applies also to *U. longissima* at Cassiar Cannery (see Supplemental data). Turning to Edgewood Blue, thalli hydrated by dewfall experience a shorter period of diurnal hydration yet at the same time experience higher temperatures and improved light exposure - conditions optimal for growth. Although dewfall has long been known to constitute an important moisture source for ground-dwelling lichens in deserts and grasslands (Kappen et al. 1979), its importance for epiphytic lichens has only recently begun to be explored in detail (Gauslaa 2014).

At Edgewood Blue, located in a deep glacially scoured mountain valley (over 1900 m elevation difference from mountain-top to valley-bottom), nighttime cooling under clear sky conditions (again with dominant high-pressure conditions in summer 2018) provided a different

417 moisture source for *U. longissima* thalli, with heavy dewfall observed on many summer
418 mornings (see also Bidussi et al. 2013). This hydration pulse was more transient in nature than
419 morning fog at Cassiar Cannery, with wetting sensors and hydrated thalli drying rapidly as the
420 sun rose, resulting in potential hydration periods of 2-3 hours after sunrise each day; see below.
421 Condensation of mist and fog on foliage is referred to as “occult” precipitation (Weathers 1999).
422 Although occult precipitation in the form of dewfall has long been known as an important
423 moisture source for ground-dwelling lichens in deserts and grasslands (Kappen et al. 1979), its
424 importance for epiphytic lichens specifically has only recently begun to be explored in detail
425 (Gauslaa 2014).

426 Another potential hydration source for *U. longissima* and other chlorolichens is
427 rehydration directly from high atmospheric humidity. Here we call attention to a recent study by
428 Eriksson et al. (2018), in which it is suggested that long, pendulous “hair” lichens like *U.*
429 *longissima* may be specially adapted to water uptake from humid air. Consistent with this
430 hypothesis, Phinney et al. (2018) demonstrated rapid (< 10 minutes) reactivation of
431 photosynthesis in *U. dasopoga* (Ach.) Nyl. and other “old-forest” chlorolichens exposed to high
432 relative humidity. While we included hydration of *U. longissima* thalli from high ambient
433 humidity as an assumption in our treatment of microclimate data, in practice it can be difficult to
434 separate the contribution of different wetting sources.

435 The seasonal occurrence of precipitation in the coastal and inland temperate rainforests of
436 BC differs considerably (Stevenson et al 2011). In the coastal temperate rainforest prolonged
437 periods of winter rain are common; in contrast, winter precipitation in the inland temperate
438 rainforest mainly falls as snow, resulting in deep winter snowpacks. The importance of winter
439 rains to growth of lichens in coastal temperate rainforests was highlighted by Muir et al. (1997),

who noted that a majority of growth in the canopy epiphyte *Lobaria pulmonaria* (L.) Hoffm. occurred during the wetter months (November to June) in western Oregon. In contrast, thalli of *U. longissima* in the Caribou rainforest sites were inactive (dry or frozen) over 80% of the time during the winter months (October to March). The shoulder seasons (October and March), however, did provide periods when growth could occur in the inland sites during the winter period, especially at the Ancient Forest site.

Lichen morphological responses.

In addition to differences in relative growth rates (mass or length) between coastal and inland transplant sites, we also observed major differences in the patterns of growth. Thus, the inland transplants at the end of the experiment had a higher ratio of mass per unit length than their counterparts at Cassiar Cannery. These differences were especially notable during the summer transplant period at Edgewood Blue and Aleza Lake, the driest of our Caribou rainforest sites (by rainfall). We would speculate that repeated short duration hydration episodes may have resulted in thalli at Edgewood Blue allocating more growth to lateral branches and/or the medullary tissue of their main strand than did their counterparts at Cassiar Cannery, which allocated more growth to increasing the length of their central strands.

Rolstad and Rolstad (2008) note that while most epiphytic lichens grow primarily at their apices (with pseudomeristomatic tissue confined to lobe margins and branch tips), *U. longissima* is capable of diffuse or intercalary growth, with a potential doubling time of less than a year under favorable conditions. Sanders and Rios (2012) found that the emergence of lateral branches in *U. longissima* commonly occurs in thallus portions where the cortex has been disrupted, that is, coincident with regions where intercalary growth would be greatest. The

functional significance of these morphological differences between wetter and drier sites is not fully understood. Nybakken and Gauslaa (2007) describe short side-branches (fibrils) in *U. longissima* as the functional equivalent of photosynthetic organs; thus thalli allocating growth to increases in mass within existing thallus strands (as at Edgewood Blue and Aleza Lake) may facilitate more efficient light capture and growth. In contrast, Gauslaa et al. (2009) hypothesize that where thalli preferentially allocate carbon to length growth, thalli will better be able to harvest light within complex three-dimensional forest canopies.

Although significant changes occurred between thalli at different transplant sites with respect to mass per unit length relationships, we did not find significant differences in mass per unit area relationships. Presumably this was owing to shared constraints on the allocation of carbon to surface area, that is, whether to lateral branches along the main strand (hypothesized for Edgewood Blue and Aleza Lake) or to length growth of the thallus as a whole (at Cassiar Cannery). It is worth noting that Gauslaa et al. (2009) found that carbon allocation in small *U. longissima* thalli resulted in preferentially greater gains in area, compared to larger thalli, which showed preferential gains in mass. However, this phenomenon of size dependent growth allocation (area versus mass) is unlikely to play a major role in our data set, given the standardization of our initial transplant segment lengths.

A common challenge facing researchers assessing growth rates is the tendency of “hair” lichens to fragment under field conditions, an attribute that facilitates short-range dispersal, but can lead to high rates of variability in lichen transplant experiments. Gauslaa (1997) noted that the size class distribution of *U. longissima* thalli sampled with the canopy did not differ significantly from that of thalli in litterfall traps, suggesting frequent fragmentation at their point of attachment. Gauslaa attributed the ready fragmentation of *U. longissima* thalli to “a

combination of weak axes and lack of holdfasts” resulting in “a population of repeatedly displaced thalli”, where “fragmentation normally takes place in weak, apparently dead and discoloured segments along the main axis”. This observation is important to the interpretation of our own data set, where transplant thalli were screened to exclude any strands with pre-existing dark or discoloured segments. The most likely mechanism for fragmentation of thalli in our transplants would thus be wind action at the point of attachments to transplant nets – a prediction consistent with observed changes in length of a small subset of our thalli in each of our data sets.

One of the more interesting facets of the study data set is the growth rate response of the Aleza Lake thalli, where most thalli shrank during the transplant period. This phenomenon, of lichen thalli catabolizing tissue under conditions where respiration exceeds photosynthesis, was previously observed in fruticose thalli of *Flavocetraria cucullata* (Bellardi) Kärnefelt & Thell by Lechowicz (1981), using concurrent measurements of gas exchange and changes in mass. The Lechowicz study did not assess the impacts of the observed mass loss on thallus morphology. Similar experiments have not been conducted in “hair” lichens; however, there is no *prima facie* reason to believe that thalli could not lose mass under conditions of prolonged stress, as at Aleza Lake, where the duration of summer hydration events was much lower than at our other sites.

Supporting evidence for mass loss in *Usnea* comes from the study of Baker et al. (1979), who suggested that the chondroid central cord in *Usnea* plays a major role as storage tissue for carbohydrate energy reserves. Shrinkage, under conditions where thalli had a chronic unfavorable carbon balance, could thus be envisaged as a mechanism driving observed changes in length. The shrinkage process in this case would be facilitated by the lack of cortex over the central strand in *U. longissima*. Gauslaa et al. (2009) hypothesized that reallocation of mass from the central cord supported short-term changes in area-based growth in transplanted thalli of *U.*

509 *longissima*. Side-by-side comparisons with sympatric corticate *Usnea* species at our study sites,
510 such as *U. cavernosa* Tuck., might provide an interesting test of this hypothesis.

511 Long-term persistence by *U. longissima* or other hair lichens requires not only a positive
512 net annual carbon balance (an excess of photosynthesis over respiration) but also annual
513 productivity that at least offsets or exceeds loss to fragmentation, typically inferred by
514 measurements of litterfall deposition. Esseen (1985) estimated that 7 to 10% of the standing crop
515 of *U. longissima* was lost each year in old *Picea abies* (L.) H. Karst. stands in Sweden. Given
516 that thalli at the Edgewood Blue site demonstrated “ecological” growth rates equivalent to those
517 from Cassiar Cannery, we would suggest that the lack of *U. longissima* thalli at Edgewood Blue
518 is likely due to the combined effects of dispersal limitations and the limited number of sites with
519 long-term habitat continuity, where thalli could survive disturbance events such as forest fires;
520 see, for example, Edwards (1954).

521 Analysis of lake-bed pollen cores (Gavin et al. 2009) indicates that the northern portion
522 of the Caribou rainforest emerged much more recently than its southern portion, as noted above.

523 We suggest that the absence of *U. longissima* in the northern portion of the Caribou rainforest
524 may partly reflect insufficient time for its dispersal from adjacent source populations, whether on
525 the BC coast, where this lichen is locally common, or from northern Idaho, where it is now
526 highly restricted (CNALH 2020). The few Caribou rainforest sites known to support *U.*

527 *longissima* are in the south, where ecosystems have been available for colonization much longer.

528

529 **Lichen dispersal and biogeography.**

Most authors regard dispersal in *U. longissima* as a critical limitation in colonizing new habitat. Dispersal in *U. longissima* can arise from three main sources: ascospore release, soredial dispersal, and fragmentation of thalli (Gauslaa 1997, Rolstad et al. 2013). Ascospore release from apothecia is perhaps the most effective of these three mechanisms for long-distance dispersal (Pentecost 1981, Seymour et al. 2005, Ronnås et al. 2017). In *U. longissima*, however, dispersal from ascospores is highly unlikely, at least in western North America, where most populations consist only of sterile thalli in which apothecia are either lacking or non-reproductive (Keon and Muir 2002, Rolstad et al. 2013). Similarly, few thalli of *U. longissima* in western North America develop isidia or soredia (Keon and Muir 2002), making this an unlikely dispersal method. The most common form of dispersal in *U. longissima* is the transport of large thallus fragments from one (adjacent) tree to another within the canopy (Gauslaa 1997, Keon 2001). This provides a mechanism for repeated colonization within forest stands, and can lead to significant local accumulation of biomass (Gauslaa et al. 2008). Stands with high biomass loading will have greater resilience to unfavorable conditions, allowing some thalli to persist until conditions improve. Newly established propagules, however, would need extended periods of favorable conditions to achieve these levels of stand-level biomass accumulation; see also Goward and Campbell (2005).

Conclusions

In conclusion, our data suggests that *U. longissima* thalli should be able to persist and grow at two of our three transplant sites in inland southern British Columbia. Although Edgewood Blue was the driest of the three transplant locations, cold air drainage in a deep mountain valley at this site resulted in frequent summer dewfall events, providing a major

hydration source for transplanted *U. longissima* thalli. Interestingly, the dependence of transplanted ITR thalli at Edgewood Blue on occult precipitation (dewfall) mirrored that seen in the source CTR population at Cassiar Cannery, where occult precipitation (in this case fog) was likewise a contributing source of summer hydration. The Ancient Forest site did not experience the same frequency of summer dewfall events; however, as the wettest of the ITR locations, growth of the transplanted thalli at this site was sustained by precipitation events. Mean annual precipitation at the Aleza Lake ITR transplant location was intermediate to that of the other two ITR sites; however, thalli at this site (summer transplant data only) experienced much shorter hydration intervals during the study period. The recent discovery of two new (previously unknown) naturally occurring populations of *U. longissima* near the southern limit of the Caribou rainforest (Spribille pers. comm. 2018) is interesting in this regard, as both occur in deep valleys with cold air drainage, similar to Edgewood Blue.

Although these findings support our hypothesis that dispersal limitations have likely been a major past constraint on the establishment of *U. longissima* populations in the ITR, they also point to the importance of local microsite conditions in sustaining populations, once established. Given the rarity of *U. longissima* within BCs inland temperate rainforest, any sites where successful dispersal and establishment have occurred should be regarded as high conservation priorities for protection.

Acknowledgements

We thank the Aleza Lake Research Forest, the University of Northern British Columbia, and NSERC for funding support. Discussion and comments on manuscript drafts by Art Fredeen are

576 gratefully appreciated as are contributions of supplemental climate data for the Ancient Forest
577 site by Stephen Dery. Logistic support by Justine Crawford (Cassiar Cannery) assisted project
578 completion. We thank Keaton Freel, Emma Arvidsson, Fraser Melville, Sam McKoy, Dara
579 Campbell, Nina Grigat and North Ross for support as field assistants. We thank two anonymous
580 reviewers for valuable comments made during review.

Draft

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List of Tables

Table 1 Estimated climate normals (1961-1990) for transplant sites. Data from Climate B.C.
<http://www.climatewna.com/ClimateWNA.aspx> (Accessed June 8, 2020). Biogeoclimatic (BEC) zones from Meidinger and Pojar (1991).

Table 2 *Usnea longissima* transplant sites and establishment dates by season.

List of Figures

Figure 1. The location of *Usnea longissima* transplant study sites within British Columbia, showing the distribution of the Caribou rainforest (wet and very wet subzones of the Interior Cedar-Hemlock biogeoclimatic [BEC] zone), the Kispiox rainforest (the combined “Very Wet Cold,” “Wet Cold,” and “Moist Cold” Interior Cedar Hemlock BEC zones in the upper Skeena and Nass River watersheds.), and the Pacific coastal temperate rainforest (Coastal Western Hemlock BEC zone) ecosystems (following Coxson et al. 2019) and collection locations for specimens of *U. longissima* at the Beaty Biodiversity museum herbarium (UBC). The hydration source for *U. longissima* thalli at these sites during the summer period, differentiating between the proportion of wetting from rainfall or wetting from dewfall and/or fog interception (occult precipitation), is shown in the inset pie charts. The size of the inset pie charts is proportional to their changes in mass during the summer period (see Fig. 5). BEC zone boundaries were obtained from DataBC (<https://catalogue.data.gov.bc.ca/dataset/f358a53b-ffde-4830-a325-a5a03ff672c3>). Provincial base-map boundaries were obtained from Statistics Canada (<https://www12.statcan.gc.ca/census-recensement/2011/geo/bound-limit/bound-limit-2016-eng.cfm>). State base-map boundaries were obtained from the US Census Bureau

(<https://www.census.gov/geographies/mapping-files/time-series/geo/carto-boundary-file.html>).

Usnea longissima locations (latitude and longitude, NAD83 projection) come from the Fungi and Lichens Collection database (Beatty Biodiversity Museum 2020).

Figure 2. Assembled lichen net attached to Sitka spruce (*Picea sitchensis*) branch at Cassiar Cannery (source population) with three attached *Usnea longissima* thalli. Additional thalli can be seen growing on branches in the background.

Figure 3. The change in length of individual *Usnea longissima* transplant thalli by site (A. Cassiar Cannery; B. Ancient Forest; C. Edgewood Blue; and D. Aleza Lake) at the end of the summer 2018 transplant period. The grey line denotes one standard deviation in length below the axis by site.

Figure 4. The change in length of individual *Usnea longissima* transplant thalli by site (A. Cassiar Cannery; B. Ancient Forest; and C. Edgewood Blue) at the end of the annual (2018-2019) transplant period. The grey line denotes one standard deviation in length below the axis by site.

Figure 5. Relative growth rate (%) of *Usnea longissima* transplant thalli by length ($RGR(L)_p$) for A) summer, B) winter, and C) annual periods; and by mass ($RGR(M)_p$) for D) summer, E) winter, and F) annual periods. Dataset excludes fragmented thalli (see methods) from each site (denoted by “p” subscript as “physiological” growth rate assessments). The midpoint of each box plot indicates the median value, the lower and upper box limits the 25th and 75th percentiles

respectively, the lines indicate 10th and 90th percentiles, and closed symbols, where present, outliers. Transplant sites: Cassiar Cannery (CC), Ancient Forest (AF), Edgewood Blue (EB), and Aleza Lake (AL). Pairs of letters denote statistically significant differences between comparisons.

Figure 6. Ratio of mass per unit length (mg/cm) for *Usnea longissima* transplant thalli after A) summer and B) annual transplant periods at Cassiar Cannery (CC), Ancient Forest (AF), Edgewood Blue (EB), and Aleza Lake (AL). Pairs of letters beside bars denote statistically significant differences between comparisons.

Figure 7. The relationship between mass (mg) and area (cm²) for intact annual *Usnea longissima* transplant thalli at Cassiar Cannery (CC), Ancient Forest (AF), Edgewood Blue (EB), and Aleza Lake (AL). Regression slopes were not significantly different by site in pairwise comparisons.

Figure 8. Frequency distribution of potential thallus state: A) hydrated in light (excluding any periods when frozen); B) hydrated in dark (excluding any periods when frozen); C) desiccated (all temperatures) or frozen (any hydration status) by temperature class intervals during the summer period at Cassiar Cannery, Ancient Forest, Edgewood Blue, and Aleza Lake. Numbers in brackets indicate the proportion of the total time period experienced by thalli under each activity state.

Figure 9. Frequency distribution of potential thallus state: A) hydrated in light (excluding any periods when frozen); B) hydrated in dark (excluding any periods when frozen); C) desiccated

836 (all temperatures) or frozen (any hydration status) by temperature class intervals during the
837 winter period at Cassiar Cannery, Ancient Forest, and Edgewood Blue. Numbers in brackets
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190x254mm (96 x 96 DPI)

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1422x1066mm (72 x 72 DPI)

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174x197mm (300 x 300 DPI)

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164x184mm (300 x 300 DPI)

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252x180mm (300 x 300 DPI)

Figure 6. Ratio of mass per unit length (mg/cm) for *Usnea longissima* transplant thalli after A) summer and B) annual transplant periods at Cassiar Cannery (CC), Ancient Forest (AF), Edgewood Blue (EB), and Aleza Lake (AL). Pairs of letters beside bars denote statistically significant differences between comparisons.

104x157mm (300 x 300 DPI)

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171x116mm (300 x 300 DPI)

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170x216mm (300 x 300 DPI)

Figure 9. Frequency distribution of potential thallus state: A) hydrated in light (excluding any periods when frozen); B) hydrated in dark (excluding any periods when frozen); C) desiccated (all temperatures) or frozen (any hydration status) by temperature class intervals during the winter period at Cassiar Cannery, Ancient Forest, and Edgewood Blue. Numbers in brackets indicate the proportion of the total time period experienced by thalli under each activity state.

193x207mm (300 x 300 DPI)

Table 1 Location and estimated climate normals (1961-1990) for transplant sites. Data from Climate B.C.

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Locality	Latitude - Longitude	BEC zone	Mean Annual Temperature (°C)	Summer ¹ Mean Temperature (°C)	Winter ² Mean Temperature (°C)	Mean Annual Precipitation (mm)	Summer Mean Precipitation (mm)	Winter Mean Precipitation (mm)	Winter Mean Precipitation as snow (mm)
Cassiar	54.177° N,	Coastal	7.1	11.3	3.0	2644	958	1686	221
Cannery	-130.175° W	Western Hemlock (CWH)							
Ancient	53.771° N,	wet-cool	3.0	10.1	-4.0	1046	443	603	431
Forest	-121.226° W	Interior Cedar-							

		Hemlock							
Edgewood	51.869° N,	dry	4.7	11.9	-2.6	636	324	312	200
Blue	-120.024°	warm							
	W	interior							
		cedar							
		hemlock							
Aleza Lake	54.076° N,	wet-cool	3.3	10.8	-4.3	895	385	510	363
	-122.102°	Sub-							
	W	Boreal							
		Spruce							

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1. April to September inclusive to correspond generally with transplant dates.
October to March inclusive to correspond generally with transplant dates.

Table 2 *Usnea longissima* transplant localities and establishment dates by season.

Locality	Season	Start Date	End Date	Number of Days
Cassiar Cannery	Summer	April 28, 2018	September 28, 2018	153
	Annual	April 28, 2018	April 7, 2019	344
Ancient Forest	Summer	May 1, 2018	October 6, 2018	158
	Annual	May 1, 2018	April 10, 2019	344
Edgewood Blue	Summer	May 12, 2018	October 13, 2018	154
	Annual	May 12, 2018	April 14, 2019	337
Aleza Lake	Summer	May 7, 2018	October 4, 2018	150