# Non-destructive estimation of lichen biomass

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*Abstract*: Ground lichens constitute a vital part of reindeer winter diet, and non-destructive estimation of lichen biomass is therefore crucial for providing objective data for the management of lichen resources. The aim of this study is to compare precisions of different methods of estimating lichen biomass from ground cover and thallus heights of the four most important lichen species: *Cladonia arbuscula, C. rangiferina, C. stellaris*, and *Cetraria islandica*. The methods were validated against actual lichen biomass within 50 cm x 50 cm plots. Different methods gave remarkably similar results, except when cover estimation was based on presence/absence data with a strict criterion of 100% lichen cover. Average thallus heights within the plot explained the variation in lichen biomass as well as lichen volume (estimated from cover and average heights). Relationships between biomass and volume or height were also very similar for all four lichen species, and separation into species thus do not seem necessary for practical purposes.

Key words: ground lichens; reindeer grazing, vegetation monitoring.

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#### Introduction

An essential component in a grazing management system is information on the status and changes of key resources. In Sweden, semi-domesticated reindeer generally migrate between summer grazing grounds in the mountains and lichen-rich winter grazing grounds in the boreal forest. Lichens may constitute 35-80% of the diet during winter for reindeer (Gaare & Danell, 1999; Heggberget *et al.*, 2002), and the most important species include *Cladonia arbuscula*, *C. rangiferina*, *C. stellaris*, and *Cetraria islandica*. Reindeer will dig through the snow to reach the lichens, and snow characteristics are very important in determining accessibility of ground lichens (Skogland, 1978; Helle, 1984). Snow cratering will, on a yearly basis, create a mosaic of grazed and non-grazed lichen patches where lichens are accessible, and largely untouched lichen mats where accessibility is limited, perhaps due to adverse snow conditions. Cratering will also cause fragmentation of lichen thalli as some fragments will always be left in the snow. A typical winter grazing site will thus consist of a mix of thicker lichen patches and disturbed patches with lichen fragments of various sizes.

Reindeer husbandry in Sweden is generally limited by winter resources, except in the southernmost part of the husbandry area where summer pastures tend to be more limiting. A key resource to manage in this system is thus ground lichens on the winter grazing grounds. Non-destructive and easily achievable estimates of lichen biomass are needed to provide data on the status of winter ranges to reindeer grazing managers. However, the few relationships between lichen volume and biomass that we have found (e.g. Eriksson, 1979; Kumpula *et al.*, 2000) do not provide estimates of variance, and uncertainties in the relationships can thus not be estimated. The aim of this study is to compare various methods of estimating lichen biomass from volume or thallus height measurements.

## Methods

Four species of fruticose lichens were chosen for the study: Cladonia arbuscula, C. rangiferina, C. stellaris, and Cetraria islandica. Two similar, but less abundant, species in the area were excluded: Cladonia arbuscula ssp. mitis, and Cladonia stygia (Krog et al., 1994). The lichens were measured and collected in September 2001 from two different sites near Umeå in northern Sweden: Obbola (63°39'N, 20°17'E) and Piparböle (63°55' N, 20°6'E). The two sites were chosen to include the entire gradient from large intact lichen thalli to fragmented, grazed thalli, and both sites were pine heaths with a field layer dominated by lichens. The Obbola site is not grazed by reindeer, while the Piparböle site is grazed, at least during some winters. Grazing signs were evident in Piparböle, but the actual locations where we sampled the lichens had not been grazed for at least one winter.

For each species, 50 cm x 50 cm plots with a monoculture of the particular species were subjectively chosen to encompass as much of the gradient in lichen thallus height as possible. At each plot, a 50 cm x 50 cm frame on adjustable legs were placed a few centimeters above the vegetation (Fig. 1). The frame was divided into 36 squares. The height of the lichens was measured at each of the 25 intersections created by the 36 squares by lowering a blunt metal rod (Ø 3mm) at a perpendicular angle to the frame. The rod was lowered to the base of the lichens and not pushed into the litter and humus layer. The height of the lichens was measured to the nearest 0.5 centimeter.

Per cent cover was measured in each plot through several methods: 1/ The per cent hits (out of 25) by the rod (i.e. a point intercept method), 2/ The per cent of squares (out of 36) with presence of the species, 3/ The per cent of squares with at least 50% cover of the species, 4/ The per cent of squares with 100% cover of the species, and finally 5/ visual estimation of cover in the plot (on a 5% unit scale). We used the mean height of the lichens from the 25 point-frequency points as height estimations from each plot. Lichen volume was calculated as per cent cover \* plot area \* height.

At each plot, all the lichens within the 0.25 m<sup>2</sup> frame were collected by hand, and cleaned in a wet condition from litter and dead basal parts so that only live thalli remained. The lichens were dried in 80 °C for 76 hours, allowed to cool to room temperature and weighed. During weighing, the relative humidity of the room was measured as lichens can take up moisture from the air and thus become heavier. However, relative humidity was always below 36% during weighing, which is considerably lower than a suggested



Fig. 1. The sampling frame from above, placed in a mixed stand of *Cladonia arbuscula* and *C. rangiferina*.

Table 1. Regressions of biomass on volume based on different cover estimation methods. Cover estimation methods are: visual estimation of percent cover, presence/absence data based on any fragment present, at least 50 of square covered with lichens, or 100% of the square covered with lichens, and number of hits (point-frequency). Results shown are slopes (g DW cm<sup>-3</sup>) for weighted linear regressions through the origin with R<sup>2</sup>-values given in parentheses. See Methods for further details.

Cover estimation	C. arbuscula	C. rangiferina	C. stellaris	C. islandica
Visual	0.017 (0.89)	0.016 (0.96)	0.016 (0.90)	0.019 (0.88)
Presence/Absence	0.012 (0.93)	0.011 (0.96)	0.012 (0.95)	0.014 (0.95)
Pres./Abs. 50%	0.017 (0.92)	0.018 (0.91)	0.016 (0.85)	0.018 (0.84)
Pres./Abs. 100%	0.052 (0.72)	0.054 (0.52)	0.041 (0.58)	0.029 (0.74)
Point intercept	0.014 (0.88)	0.013 (0.95)	0.015 (0.91)	0.015 (0.92)

threshold of 70% over which lichen weight will deviate significantly from completely dry material (Renhorn & Esseen, 1995).

The relationships between the various measures of biomass (dry weight) and volume for each species were estimated as linear regressions through the origin. In addition we also regressed biomass on thallus height from each plot (based on the mean of 25 points). As variances seemed not to be homogenous in some cases, we used weighted regressions with the independent variable as weights (see also Bråthen & Hagberg, 2004). The weights were estimated with the Weight Estimation procedure in SPSS, ver. 12.0.1. We also calculated 95% prediction intervals with the individual case confidence interval command in SPSS. We also tried curvi-linear and reciprocal polynomial regressions (results not shown), but these methods gave very small increases in fit in all cases and we thus only report results from the weighted linear regressions for the individual species. For all species together a power function regression of biomass on volume gave better fit than linear regression.

#### Results

Most linear regressions between lichen volume and biomass showed good fits with high  $R^2$  values (0.84 -0.96). Slopes (g DW cm<sup>-3</sup>) were also in general similar, both for different methods and for the four different species (Table 1). Another general pattern is that different methods for estimating cover gave remarkably similar results, except where cover is measured as frequency of 100% cover in squares, which seemed to strongly underestimate lichen cover.

The linear regressions of mean thallus height (based on 25 points in each plot) on biomass gave equally good fits as regressions based on volume (Table 2, Fig. 2). Slopes (g DW cm<sup>-3</sup>) were remarkably similar, although the tight fit of the regressions made the slope for *C. islandica* significantly different from the slopes for *C. arbuscula* and *C. rangiferina*, and the slope of *C. rangiferina* significantly different from that of *C. stellaris*.

Table 2. Regressions of biomass (g DW  $/0.25m^2$ ) on mean thallus height (cm). See Table 1 for details.

Species	Slope	$R^2$
C. arbuscula	26.35	0.92
C. rangiferina	23.07	0.96
C. stellaris	29.29	0.94
Cetr. islandica	31.98	0.96
All species	28.39	0.92



Fig. 2. Linear regressions through the origin of lichen biomass (g DW/0,25m<sup>2</sup>) on mean lichen thallus height (cm). Each point represents one monospecific sampling plot where mean thallus height was estimated from 25 sampling points. Slopes and  $R^2$ -values are given in Table 2.



Fig. 3. Linear regressions through the origin of lichen biomass (g DW/0,25m<sup>2</sup>) on mean lichen thallus height (cm). Each point represents one monospecific sampling plot where mean thallus height was estimated from 25 sampling points. The thick line is the weighted linear regression (slope = 28.39,  $R^2 = 0.94$ ) based on all plots and species, and the thin lines are 95% prediction confidence intervals (± 42.4% in the upper end; i.e. the error when predicting a single biomass value from an estimation of mean thallus height).



Fig. 4. A comparison between our study and data from Kumpula *et al.* (2000). For our data, we used all four species and calculated volume based on visual estimation of cover and the mean thallus height for all 25 sampling points per plot. The thick solid line is the linear regression through the origin with our data (slope = 15.54 g DW dm<sup>-3</sup>,  $R^2$  = 0.81), and the dashed line is the same with Kumpula *et al.*'s data (slope = 7.99 g DW dm<sup>-3</sup>,  $R^2$  = 0,78). Our data suggests a diminishing relationship between biomass and volume. Regression on volume raised to 0.7508 gave the best fit (slope = 28.93 g DW dm<sup>-3\*0.7508</sup>,  $R^2$  = 0.90), showed as a thin solid line.

#### Discussion

As the different methods of estimating lichen volume gave very similar results, this may give the impression that they are interchangeable. However, this is not the case; different methods of estimating cover have different advantages and disadvantages. Studies show that visual estimation of cover is usually the fastest method (Johansson & Moen 2003), but it is also the method with the largest subjectivity (Sykes et al., 1983; Tonteri, 1990). Thus, to achieve repeatable results, the personnel doing the analyses have to be both trained and calibrated (Dethier et al., 1993). Further, increased precision in the cover estimate through visual estimation cannot be increased by increased effort in contrast with the other methods where a higher number of points or plots will cause the estimation to be closer to the true value. Many studies recommends that point intercept methods should be used to estimate biomass (e.g. Floyd & Anderson, 1987; Stampfli, 1991; Bråthen & Hagberg, 2004), since they are faster than presence/absence methods and less subjective than visual estimates. However, these methods are more suited for common species since a very large number of points may be used to record rare species (Jonasson, 1988; Økland, 1990). The choice of method should thus reflect the specific question and the resources available. It should further be mentioned that various photographic techniques have been successfully used for estimating cover in single-layer vegetation (e.g. Dietz & Steinlein, 1996; Luscier et al., 2006), including in lichen-rich vegetation (Gaare & Tømmervik, 2000). However, if a separation of species is needed, the colour separation of different Cladonia (Cladina) species is not large enough to show up on photographs so these species have to be grouped (Vanha-Majamaa et al., 2000).

A crucial aspect of using mean thallus height (as a proxy for volume) as a predictor for lichen biomass is that the zero heights, i.e. where there are no lichens, are included in calculating the mean. If so, then this method incorporates a cover estimate without the need for acually measuring cover. This would thus be a fast and efficient method for estimating lichen biomass. It is imperative that the sampling scheme for obtaining data for this method is such that a large number of points is measured over the entire sampling area, and that the points are placed so that any subjective choice over the exact location where thallus height is measured is minimized. This is to ensure both that the entire sampling area is covered and that points with both presence and absence of lichens are measured. The higher the aggregation of lichen patches in the sampling area, the more number of points is needed to characterize the site. However, the power of the method should be checked in the field as we have no data on the spatial pattern of lichen patches in our sites. A simple sampling scheme may be to lay transects over the site and measure lichen height at pre-determined intervals. The mean thallus height (with confidence intervals) can then be converted to lichen biomass per  $0.25 \text{ m}^2$  by using our equations. This has then to be multiplied by the area of the site divided by  $0.25 \text{ m}^2$ .

If only overall lichen biomass is needed (and if observers who are not trained botanists are used), it might be advantageous to estimate total lichen biomass of an area without separating the species. We thus also calculated the regression between mean thallus height and biomass for all four species combined (Table 2, Fig. 3). This relationship was also strong ( $R^2 = 0.92$ ) and may suffice for most practical purposes, especially considering the uncertainties both in the regression and in the estimation of mean thallus height from a large sampling area. Please note that this is only relevant for estimations of lichen standing crop. If growth and productivity estimates are needed, it is necessary to separate the species as different species will have different growth potentials, depending on for instance chlorophyll content and distribution (Palmqvist & Sundberg, 2000).

Various estimates of lichen volume vs. biomass have been published earlier, and some have been in practical use. For instance, our results corresponds well with relationships used in Norway (E. Gaare, pers. comm.; Norway: slope = 0.0216 g cm<sup>-3</sup>, our study: slope = 0.02839 g cm<sup>-3</sup>). However, other relationships are more diverging. Eriksson (1979) gives a slope that is about 30 times higher than ours (slope = 0.62 g cm<sup>-3</sup>). Another example comes from Kumpula et al. (2000). A comparison with their data and ours show significantly different regressions (Fig. 4; Kumpula et *al.*: slope = 7.99,  $R^2$ =0.78; Our study: slope = 15.54,  $R^2$ =0.81). These differences are of course not satisfactory, but it is difficult to find a clear explanation. Possible candidates are differences in the cleaning of lichen thalli and the definition of live and dead parts. In our case, we cleaned the lichens in a wet condition, removed all debris such as pine needles, and removed all decomposed and partly decomposed parts of the thalli. No attempt was made to estimate if intact parts were live or dead. The information in Kumpula et al. (2000) is incomplete, but they point out that "only the living part of lichens was considered when classifying and measuring the percentage cover and height of lichens". If they applied a more strict definition than we did, it would give a lower biomass-volume regression slope, as shown in Fig. 4. Eriksson (1979), on the other hand, calculated his slope from volume estimates based on vegetation descriptions of "the actual forest types in question" (Eriksson, 1979, p. 9; our translation) and biomass samples from those vegetation types. As our study shows, volume estimates from even

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the same plot give different results. Thus, the method of Eriksson cannot be considered exact enough. However, given the differences between different studies, we advocate some caution in choosing an equation for estimating lichen biomass, or recommend verification of the equation versus methods used for recording lichen height or volume.

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#### Icke-destruktiv skattning av lavbiomassa

*Abstract in Swedish / Sammanfattning*: Marklavar utgör en kritisk del av renars vinterföda, och det blir därmed väsentligt att kunna skatta lavbiomassa på ett icke-destruktivt sätt för att kunna förvalta lavresursen. Syftet med denna studie är att jämföra precisionen hos olika metoder för att skatta lavbiomassa baserat på volym- och bålhöjdsmätningar för de fyra viktigaste arterna: *Cladonia arbuscula, C. rangiferina, C. stellaris,* and *Cetraria islandica*. Metoderna validerades mot lavbiomassa mätt i 50 cm x 50 cm provytor. Olika metoder gav anmärkningsvärt lika resultat, förutom när täckning skattades med förekomstdata med ett strikt kriterium av 100% lavförekomst. Medelhöjd på lavbålarna i provytan förklarade lika mycket av variationen i lavbiomassa som lavvolym (baserat på täckning och medelhöjd). Förhållandet mellan biomassa och volym eller bålhöjd var också mycket lika mellan arterna vilket medför att det, för praktiska ändamål, inte är nödvändigt att separera arterna vid skattningar av total biomassa.