‘GROWTH RINGS’ IN CRUSTOSE LICHENS: COMPARISON WITH DIRECTLY MEASURED GROWTH RATES AND IMPLICATIONS FOR LICHENOMETRY

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Abstract

Some species of crustose lichens, such as *Ochrolechia parella* (L.) Massal., exhibit concentric marginal rings, which may represent an alternative technique of measuring growth rates and potentially, a new lichenometric dating method. To examine this hypothesis, the agreement and correlation between ring widths and directly measured annual radial growth rates (RaGR, mm a^{-1}) were studied in 24 thalli of *O. parella* in north Wales, UK, using digital photography and image analysis. Variation in ring width was observed at different locations around a thallus, between thalli, and from year to year. The best agreement and correlation between ring width and lichen growth rates was between mean width of the outer two rings (measured in 2011) and mean RaGR (in 2009/10). The *O. parella* data suggest that mean width of the youngest two growth rings, averaged over a sample of thalli, is a predictor of recent growth rates and therefore could be used in lichenometry. Potential applications include: as a convenient method of comparing lichen growth rates on surfaces in different environmental settings; and as an alternative method of constructing lichen growth-rate curves, without having to revisit the same lichen thalli over many years. However, care is needed when using growth rings to estimate growth rates as: growth ring widths may not be stable; ring widths exhibit spatial and temporal variation; rings may not represent 1-year's growth in all thalli; and adjacent rings may not always represent successive year's growth.

Key Words: *Ochrolechia parella* (L.) Massal., Marginal growth ring, Radial growth rate (RaGR), Annual variation, Lichenometry

1. Introduction

Lichenometry has been used as a dating technique by earth scientists for more than 50 years. However, its usefulness and validity have been subjected to intense scrutiny. Recent studies have shown both the potential power and questionability of lichenometry as a surface-exposure dating technique. For example, diligent lichen-population studies – involving measurement and statistical analysis of several thousand thalli growing on coeval surfaces – have greatly increased our understanding of crustose lichen growth history, mortality, and longevity (Loso *et al*., 2014) and expanded the opportunities for surface dating applications. In stark contrast, others have taken a highly critical view of the technique, either by highlighting the apparent inaccuracy, imprecision, and unreliability of the ages derived (e.g. Jomelli *et al*., 2007); or, more recently, by strongly questioning the validity of the technique at a fundamental level (Osborn *et al*., 2015).

Nevertheless, there are many good reasons why the size of lichens (and some bryophytes) growing on stone surfaces can shed useful, sometimes unique, information on the exposure age (and history) of a surface. In fact, the very reasons why Knut Faegri, Roland Beschel, James Benedict, and many other early pioneers of the lichenometric technique found it so useful in the 1930s, 1950s and 1960s – and why so many still do today (e.g. McEwen & Matthews, 2013; Bull, 2014; Foulds *et al*., 2014). The fact that the monotonous slow growth of lichens can be measured (directly over time) or inferred (from surfaces of known age), allows the use of certain lichens as a form of biological chronometer – an environmental surrogate for the passage of time. In this article we explore a little studied but potentially valuable branch of lichenometry, viz. the use of marginal growth rings to estimate lichen growth rates and lichen age.

Some crustose lichens, including species of *Ochrolechia*, *Rhizocarpon*, and *Fuscidea* have concentric 'rings' at the margin of the thallus (Fig 1). Within a ring, alternating light and dark bands are often evident. The biological origin of the bands has not been established but the lighter bands appear to represent relatively rapid growth in summer while the narrow darker bands more suppressed or truncated growth in winter

(Hale, 1973; Hooker, 1980; Armstrong and Bradwell, 2010). The more growth is suppressed in winter, the more evident the dark band appears to be and the clearer the growth rings. This observationimplies that rings may be more prominent in species growing in more seasonal or more stressful climatic conditions. If each complete light/dark couplet or 'ring' represented a single year's growth, growth patterns could be traced back a number of years in some thalli. Lichen growth rings could therefore provide a potentially new *in situ* lichenometric method of determining the growth rate and, hence, approximating the age of lichen thalli (e.g. Armstrong, 1983; 2005a; 2005b, 2014; McCarthy, 2003). In short, if intra-thallus growth rings could be used to infer lichen growth rates – akin to tree rings ('dendrochronology') – they may serve as an independent measure of lichen age, provide constraint on site-specific and between-site growth rates, and possibly help to restore trust in the lichenometric technique more generally.

In a preliminary study, the widths of successive marginal rings in 25 thalli of *Ochrolechia parella* (L.) Massal. (syn. *O. pallescens* auct. brit. p.p.), growing at a maritime site in north Wales, UK, were measured (Armstrong and Bradwell, 2010). Between 3 and 7 rings were frequently present at the margin, with ring width generally varying from 1 – 2 mm, consistent with yearly variation in radial growth rate (RaGR) reported in studies by direct measurement (Phillips, 1969; Armstrong, 2005a; 2006). In addition, the same preliminary study explored the potential for using marginal growth rings to estimate the age of a crustose lichen thallus growing on a bedrock surface exposed by glacier recession between AD 1945 and 1955 inSE Iceland (Armstrong and Bradwell, 2010). . A minimum exposure age estimate of AD 1959 ±5, consistent with the known surface age, was obtained by measurement of its marginal rings and a simple 'growth rate' extrapolation.

However, in a study of lichen growth rates on the South Orkney Islands in the maritime Antarctic, Hooker (1980) emphasized caution in the use of lichen rings to estimate growth rates. Hence, in *Buellia russa* (Hue.) Darb., rings were present only in the non-lichenised hypothallus but each concentric ring did represent one year's growth. By contrast, in *Buellia coniops* (Wahlenb.) Th. Fr. and *Caloplaca cirrochrooides* (Vainio) Zahbr., 'pseudoannual rings' were present in which each new ring that developed appeared to represent two or more growing seasons. The rings in *Caloplaca* were also not as distinct as those of other species and no new marginal rings appeared to form during two subsequent growing seasons (Hooker, 1980). Hence, further research is clearly needed before marginal zonation or rings can be used as a reliable measure of lichen growth rate and longevity.

For intra-thallus rings to be of optimal use in lichenometry, they would need to be distinct; stable from year to year; easily measurable; consistently represent one year's growth; and show close agreement and correlation with directly measured RaGR. Hence, to examine the feasibility of using marginal zonation rings as an estimate of lichen growth, the degree of agreement and correlation between growth estimated from rings and by direct measurement of RaGR was studied in a sample of 24 thalli of *O. parella* growing in a maritime environment in north Wales, UK. The other principle objectives of the study were to determine: (1) whether the rings were detectable and easily measurable, (2) whether rings were stable from year to year, (3) whether successive growth rings represented a consecutive series of 1-year growth increments, and (4) the degree of agreement and correlation between rings and directly measured RaGR.

2. Materials and Methods

2.1 Site

The study site was a series of south-facing maritime rock surfaces located in the Dyfi estuary at 'Picnic island,' Aberdyfi, north Wales, UK (Grid Ref. SN 6196) in an area of Ordovician slate rock described previously (Armstrong, 1974). These surfaces possess a rich lichen flora characteristic of maritime siliceous rock in the west of the UK (James *et al.*, 1977), have a high proportion of crustose species (Armstrong, 1974), and include a large population of *O. parella* with marginal rings (Armstrong, 1974; Armstrong and Bradwell, 2010). *O. parella* is a relatively common lichen and is member of several different communities in north Wales, including those on north- and south-facing rock surfaces, and rocks with steep or shallow surface slopes. It is a potentially useful species for lichenometric dating studies.

2.2 Measurement of rings

The concentric marginal rings of 24 randomly-selected thalli of *O. parella*, with largest diameters 18 – 118 mm were studied. Each thallus was photographed in its entirety using a Canon IXUS 70 digital camera (7.1 Megapixels, Focal length 5.8 – 17.4 mm, Closest focusing distance 30 mm), which incorporates a x12 zoom lens, providing a particularly clear image of the rings (Fig 2a). A scale measure marked in mm was placed adjacent to each thallus. Width of each distinct growth ring was measured using ‘Image J' software developed by the National Institute of Health, Bethesda, USA (Syed *et al*., 2000; Girish and Vijayalakshmi, 2004) and available as a free download. The width of each easily identifiable marginal ring was measured at five randomly selected points around each thallus, using the outer edge of each dark band as a baseline, and then averaged. Rings were measured in three successive years, viz. at the beginning of January 2009, 2010, and 2011. Rings were numbered from the edge towards the centre of the thalli and also identified according to year of measurement, i.e., ring 10.3 would identify the third ring from the margin measured in 2010.

2.3 Measurement of growth

To measure RaGR of each thallus, the advance of the thallus margin was measured in relation to fixed points marked on the rock located at 1 mm intervals from the thallus edge (Hale, 1970; Armstrong 1973; 1975; 2013). Between eight and ten randomly chosen locations were measured around each thallus. Growth increments were measured with ‘Image J' software using the method described previously (Armstrong, 2013, 2014). Hence, each lichen image was magnified to clearly reveal the fixed markers and the scale measure. The image was then calibrated using the scale measure and the distance from the margin to the fixed marker measured. Subsequent measurements of these distances were made from photographs taken on 1, January 2009, 2010, and 2011 enabling estimates of RaGR (mm a^{-1}), averaged over all thallus locations, to be made for each thallus in 2009 and 2010.

2.4 Data analysis

Comparisons of mean RaGR and ring width were performed using 't' tests. Correlations between the width of concentric rings and directly measured RaGR were studied using Pearson's correlation coefficient ('r') and regression methods (Armstrong and Hilton, 2011). For this analysis, thalli exhibiting either zero growth during the period under study or in which rings were indistinct or lost were excluded. Correlation is not the same as 'agreement', i.e., two quantities may be highly correlated but not agree in the quantity that they estimate. Hence, the extent of the 'agreement' between the two measures of growth in individual thalli was assessed using the Bland and Altman graphical method (Bland and Altman, 1986; 1996). This method measures by how much the results obtained using two methods differ and how far apart the two estimates of growth should be before there is significant 'disagreement'. The essential feature of a Bland/Altman plot is that the two estimates of growth, from marginal rings and direct measurement, are subtracted for each thallus and these differences are plotted against the mean of the two measurements. The 'mean difference' averaged over thalli, is known as the degree of 'bias' and is the central 'bias line' on a Bland/Altman plot. Either side of the bias line are plotted the 95% confidence intervals (CI) in which 95% of the differences in growth as estimated by the two methods for the sample of thalli would be expected to fall.

3. Results

Fig 2a shows three concentric rings of *O. parella* measured in 2009 which are relatively distinct over at least part of the thallus. Fig 2b shows rings in more detail revealing the characteristic narrow dark and wider light bands. In addition, further more subtle, banding is evident especially within the first ring. Fig 2c and 2d show the same rings observed in 2009 and 2011, suggesting that the first two rings had largely disappeared over this period as a result of marginal erosion.

The number and mean widths of the concentric rings in each of the three years in all thalli are summarized in Table 1. Thalli exhibited between one and six rings in 2009 (mean = 3.7, SD = 1.37), between one and seven rings in 2010 (mean = 3.7, SD = 1.59), and between two and seven rings in 2011 (mean 3.8, SD = 1.21). Comparisons between successive years suggested that some rings clearly visible in 2009 had become indistinct or had disappeared by 2011 in 6/24 and 3/24 thalli respectively. In some thalli, marginal erosion resulted in the loss of rings followed by the development of a new ring at the newly exposed thallus edge. Mean width of all rings was in the range 0.48 – 0.77 mm (Standard deviation = 0.41 – 0.68), mean width in 2011 being less than in 2009/2010. The frequency distribution of ring number did not exhibit significant skew or kurtosis in any year but the distribution of ring widths was markedly asymmetric with a significant degree of skew in each of the three years studied. A complete new ring with light and dark bands was observed to develop in one or both years in eight thalli, while in the remainder, a partial ring or a complete ring plus part of a new ring were formed.

Spatial and temporal variations in marginal ring width around an individual thallus are shown in Fig 3. Several sources of variation in width are evident: (1) within a single ring at different locations around the thallus, as indicated by the large standard deviations, (2) between successive rings within a thallus, e.g., between rings 9.2 and 9.3 and (c) between the same ring measured in successive years, e.g., between rings 9.2 and 10.3, suggesting rings may continue to increase in width after the year of formation.

A summary of thalli RaGR measurements is shown in Table 2. Mean RaGR was 0.44 mm a^{-1} (range 0 -1.08, Standard deviation = 0.32) in 2009 and 0.34 mm a^{-1} (range 0 – 1.16, SD = 0.31) in 2010. There was no significant differences in RaGR measured in the two years (Paired ‘t’ = 0.81, $P > 0.05$).

The correlation between the number and width of rings and thallus size is shown in Table 3. There were no significant correlations between the number of rings present and thallus diameter in any of the three years studied ($r = 0.15 – 0.34$, $P > 0.05$). However, there was a significant positive correlation between ring width and thallus diameter for the average of all rings ($r = 0.50$, $P < 0.01$) and for the average of the first two rings ($r = 0.57$, $P < 0.01$) suggesting increasing growth rates with size. Directly measured RaGR also increased with thallus diameter but exhibited a weaker relationship with size than the growth rings ($r = 0.43$, $P < 0.05$).

Comparison and correlation between growth means derived from marginal rings and direct measurement are shown in Table 4. There was no significant difference in RaGR and ring widths when comparing RaGR in 2009 and width of the first ring in 2010 (10.1) ($t = -0.01$, $P > 0.05$), RaGR in 2010 and width of ring 11.1 ($t = 0.24$, $P > 0.05$), between RaGR in 2009 and mean width of rings 11.1 and 11.2 ($t = 0.15$, $P > 0.05$), and between mean RaGR in 2009/10 and mean width of rings 11.1 and 11.2 ($t = -0.53$, $P > 0.05$) suggesting good agreement between these estimates. However, there was a significant difference between mean RaGR in 2009/10 and mean width of all rings present ($t = 3.01$, $P < 0.01$). There was no significant correlation between mean RaGR in 2009/10, and the average width of all rings present ($r = 0.39$, $P > 0.05$) and there were no significant correlations between RaGR in either 2009 ($r = 0.39$, $P > 0.05$) and 2010 ($r = 0.24$, $P > 0.05$) and width of the most recent ring. However, the best combination of agreement and correlation was between mean RaGR in 2009/10 and the mean width of rings 11.1 and 11.2 ($r = 0.60$, $P < 0.05$). Fig 4 shows the linear correlation between mean RaGR in 2009/10 and the mean width of rings 11.1 and 11.2 revealing, despite the significant correlation, a considerable degree of scatter about the line.

A Bland and Altman plot of the same data shown in Fig 5 indicates the degree of agreement/disagreement between the measures of ring width and growth. The bias line is located at 0.02 indicating that averaged over all 15 thalli included in this analysis, width of the most recent rings and actual growth measurements are estimating essentially the same quantity. However, the degree of error for individual thalli is large, the 95% confidence intervals being ±0.48 mm. Eight out of 15 thalli were located fairly close to the bias line (within 0.2 mm) suggesting good agreement between the two methods. In addition, there were a further seven thalli in which agreement was weaker; in three of these thalli ring widths overestimated growth compared with RaGR and in four thalli ring widths underestimated growth. Hence, averaged over thalli, the two methods show close agreement, but agreement is poor for an individual thallus.

4. Discussion

Lichens are a potentially valuable dating tool for geoscientists and archaeologists. However, the validity of lichenometry as a geochronological technique has been the subject of intense criticism on a number of grounds (e.g. Jochimsen, 1973; McCarthy, 1999; Osborn *et al*., 2015). Although the most recent critical review focuses on existing techniques (use of the largest, or several largest, lichens; calibration curves; and non-reproducibility of lichenometric ages) the arguments presented by Osborn *et al*. (2015) may serve to undermine trust in lichenometric dating more generally. The following discussion explores a potential new branch of the lichenometric technique using marginal growth rings as an independent measure of lichen growth rate and potentially lichen age; and also explores thepossible usefulness of lichen growth rings in lichenometric dating.

Marginal zonation or concentric 'growth' rings have been reported in many crustose lichen species from several genera (e.g. Hale, 1973; Hooker, 1980; Benedict, 1990) but as yet have not been used in lichenometry to estimate the age of lichen thalli or derive the exposure age of rock surfaces. If marginal 'growth' rings were found to be demonstrably annual – and therefore be deemed a reliable measure of radial growth from year to year – certain lichen species could provide a potentially valuable, previously unexploited, lichenometric dating tool. We have explored the fundamental premise of this potentially new lichenometric method by examining the relationship between marginal 'growth' ring widths and directly measured growth rates over a period of years.

Data are presented on the widths of marginal rings within a sample of the common crustose lichen thalli *(O. parella)* growing at a maritime site in north Wales which are then compared with directly measured growth rates. In this population, a high proportion of thalli exhibited at least two distinct growth rings while a smaller number of thalli exhibited four or more rings. These data agree with those of Armstrong and Bradwell (2010) suggesting that averaging marginal rings is a possible alternative method of studying the growth of crustose lichens.

The number of rings present in thalli appears to be independent of thallus size probably because rings are only clearly evident at the margin of the thalli. However, there was a (statistically?) significant increase in ring width with thallus size which suggests increased growth rates in larger thalli. Various growth models have been proposed for the shape of the growth curve of crustose lichens. Hence, Proctor (1977) studied the growth curve of the placodioid species *Buellia* (*Diploicia) canescens* (Dicks.) DNot. It was assumed that RaGR was proportional to an area of thallus in an annulus of constant width within the growing margin and that the shape of the growth curve was essentially asymptotic. By contrast, a number of studies (Armstrong, 1983; Haworth, et al., 1986; Bradwell and Armstrong, 2007) have suggested that in *Rhizocarpon geographicum* (L.) DC., the growth curve is not asymptotic, but approximates to a second-order (parabolic) curve: RaGR increasing in smaller thalli to a maximum and then declining in larger thalli. However, Trenbirth and Matthews (2010) have proposed several models for the growth curve of *R. geographicum* including models in which growth increases with size, as in *O. parella,* remains relatively constant or is parabolic with a declining phase. The present preliminary data provide no evidence for a declining phase of growth in *O. parella*, instead growth seems to be slow in individuals 20-40 mm in diameter and then increases rapidly in individuals greater than about 40 mm in diameter. The relationship between ring widths and thallus diameter suggests that rings measured over a sample of thalli of different size could be used to rapidly construct an age-size curve for certaincrustose lichens and therefore constitute an alternative method of direct lichenometry – without the need to re-measure thalli over periods of many years (Trenbirth and Matthews, 2010, Armstrong 2014)

Directly measured annual radial growth of lichen thalli over 2 years at the study site was found, in most cases, to equate to marginal ring widths over the same time period – showing that marginal rings in *Ochrolechia parella* are generally a good proxy for growth rate. In this crustose species, the best combination of agreement and correlation was achieved between the widths of the outer two rings measured in 2011 and mean RaGR over the previous two years. Agreement and correlation was poor, however, when all visible rings were included probably because either growth in earlier years was distinctly different from that measured in 2009 and 2010 or possibly because of subsequent changes in width of older rings. Poor agreement and correlation at the level of an individual thallus, could be attributable to errors in identifying and measuring rings, changes in ring morphology after they were formed, uncertainties in the measurement of RaGR, or intrinsic variation among thalli regardingthe extent to which a single ring actually represents a single year's growth (Hooker, 1980). This problem, together with the observation that some thalli of *O. parella* exhibited zero growth or even marginal erosion over the period of the study, suggests a relatively largenumber of lichens , probably at least 20-30 thalli, should be used in studies using growth rings to estimate growth rates.

In addition to these findings, this study has highlighted several problems that should be taken into account when attempting to use marginal rings to estimate growth rates in any lichen population. First, there can be problems in identifying and measuring the rings.For instance, rings were clearly evident and measureable at the thallus margin but were increasingly obscured and difficult to measure behind the margin as a result of variable amounts of growth in thickness causing thalli to 'wrinkle', and then become further obscured by the formation of reproductive structures (apothecia) in the centre (Fig 2a). In addition, the dark band, which may represent winter growth and which was used as a baseline to measure each ring, was not always distinct enough to be clearly identified in all thalli. Additional sub-mm banding was often evident within a ring, which may represent intra-annual (seasonal) variations in growth (Rydzak, 1961; Hale, 1970; Armstrong, 1993; Lawrey and Hale, 1971; Fisher and Proctor, 1975; Moxham, 1981; Benedict, 1990), making 'annual' ring identification difficult in some thalli. It is also easier to identify and measure rings of larger than smaller thalli, the rings being narrower and more crowded together in smaller thalli. Identification of tree rings can also be complex in dendrochronology (Fritts, 1976) with optical magnification often used in such studies (Jomelli *et al.*, 2012). In the present study, the lichen rings were easy to identify on digital photographic images, and can be magnified on screen to the required extent, making them easy to measure using computer software (Armstrong and Bradwell, 2010; Armstrong, 2013; 2014).

Second, the width of a ring varied at different locations around thalli, which was also observed in the study by Armstrong and Bradwell (2010), and is consistent with peripheral growth variations observed in many studies (Armstrong and Smith, 1992; Armstrong and Bradwell, 2001; 2011). Consequently, in measuring ring width, a mean of several measurements, between 5 and 10, should be taken at random locations around each thallus.

Third, rings varied in measured width in successive years, some rings expanding while others appearing to slightly contract. These variations could result from further growth or contraction behind the margin attributable to wrinkling (Hale, 1970). Hence, growth does not appear to cease at the end of a growth year which therefore contrasts with dendrochronology in which tree rings exhibit 'annual termination' (Fritts, 1976). Thiscould be one explanation for the relatively poor correlation between RaGR and ring width in lichens.

Fourth, there is considerable variation in width of 'analogous' rings in different thalli. These results also suggest that local differences in microclimate over the rock surface, e.g., associated with aspect (Armstrong, 1975; 2002; 2005a), slope, or microtographical variations, could influence ring width and should be investigated further (Armstrong, 2014).

Fifth, the margins of some thalli became eroded over the study period resulting in loss of rings. Marginal erosion has been observed in many crustose lichens including *Rhizocarpon geographicum* (L.) DC, in north Wales (Armstrong and Smith, 1987) and in the north Cascades, Washington state (Armstrong, 2005a) and may be caused by environmental stress and/or competition. Marginal erosion in lichens is usually followed by regrowth (Armstrong and Smith, 1987). Hence, in some thalli of *O. parella*, the margin was eroded back to an earlier ring and then a new ring was formed as growth resumed at the new location. These observations suggest that a series of successive rings may not always necessarily represent consecutive growth increments and caution is therefore required in identifying 'analogous' rings in different thalli.

A further problem in using marginal rings as a measure of growth is determining what each marginal ring actually represents. Early studies suggested that the lighter bands represented rapid summer growth and the narrow dark bands growth in winter (Hale, 1973) and therefore, that each 'ring' represented a single year's growth. In eight thalli, one complete growth ring did appear to be formed in a single year. In the remaining (16) thalli, however, either an incomplete ring or a complete ring and part of a second ring were formed in a single year. The factors responsible for these variations are currently unknown and require investigation. Hence, these data agree with the study by Hooker (1980), who identified a more complex relationship between marginal rings and growth. Hence, we would recommend that in any proposed lichenometric study of lichen growth or growth rings, each new species will need to be calibrated against *in situ* directly measured growth rates using a sample of at least 20 – 30 thalli.

Implications for lichenometry

Not all crustose lichens have growth rings but marginal rings have now been recorded in sufficient species from several genera to make them potentially useful in lichenometry (e.g. including *Rhizocarpon*, *Ochrolechia*, *Pertusaria*, *Fuscidea*, *Buellia*, and *Caloplaca* (Hale, 1973; Hooker, 1980; Armstrong and Bradwell, 2010)) Where lichen growth rings are present and suitably calibrated, they may offer a number of potential applications for lichenometric dating work. Primarily, growth rings provide an alternative *in situ* method of estimating lichen growth rates and hence lichen age, which could prove particularly valuable in regions where it is not possible or not practical to calibrate lichen age-size curves or generate lichen demographic growth-rate data.

Where present, growth rings could be used to supplement indirect lichenometric dating studies by providing a measure of radial growth rate on independently dated surfaces and surfaces of unknown age. Crucially this opens the possibility of examining growth rate variation between sites and quantifying this variability (or uncertainty) when deriving lichenometric ages. Much debate still surrounds the micro- and macro-environmental effect of climate on lichen growth rates and the implications for lichenometric dating studies (e.g. Beschel, 1961; Jochimsen, 1973;

Innes, 1985; Bradwell & Armstrong, 2007; Osborn et al., 2015). For instance, glacial moraines situated in a precipitation-dominated environment cannot be accurately dated using a lichenometric curve calibrated in a precipitation-starved setting. Careful work by Matthews (2005) showed a growth rate differential of ~20% existed in lichen growth rates along a west-east gradient in southern Norway. Matthews (2005) recommends the use of regionally controlled dating curves when conducting lichenometric assessments across areas with differing climates or high levels of environmental heterogeneity. This recommendation is backed up by direct measurements of lichen growth rates, spanning more than a decade, along an extreme climatic gradient in Antarctica (Sancho et al., 2007). Unfortunately, calibrating regional dating (age-size) curves is often impractical due to the absence or scarcity of control surfaces at high-latitude high-altitude sites. This can lead to adaptation or adjustment of existing lichenometric dating curves (e.g. Erikstad & Sollid, 1986; Winkler, 2004; Principato, 2008), sometimes without justification. We suggest that annual growth rings could allow lichen growth to be assessed quantifiably and conveniently across climatic provinces and between study sites without the need to set up time-consuming lichen growth station experiments.

Owing to the slow growth of crustose lichens, directly measured growth rate data take several years or even decades to collect depending on the climatic setting (e.g. Benedict, 1990; McCarthy, 2002; Trenbirth & Matthews, 2010; Armstrong, 2014) Growth rings, where present, may offer a relatively rapid, cost effective and non-destructive, way to estimates lichen growth rates across a wide range of thallus sizes and across a wide range of environmental settings. With more research into their formation and evolution, growth rings may offer an alternative method of constructing lichen growth-rate curves and assembling demographic growth rate data, especially in remote or extreme environments, and thereby add to the growing literature on this topic. In due course, it is hoped the multi-faceted approaches to the study of lichen growth may help to deepen our understanding and reduce the uncertainties currently surrounding the biological and ecological basis of lichenometry.

5. Conclusions

Marginal, concentric, growth rings occur in numerous crustose lichen species. Our data show that the average widths of the outer two rings obtained from a reasonable sample of thalli provide a good estimate of the recent radial growth rate (in *O. parella).* However, our data also indicate that caution must be exercised; for example marginal rings cannot always simply be assumed to accurately represent the annual growth rate of any individual thallus. Neither can successive rings always be assumed to necessarily reflect consecutive yearly growth increments. In addition, the assumption that one complete ring is formed each year may not be true for all thalli,. These caveats aside, comparisons of directly measured lichen growth from year to year with marginal ring widths over the same period, do suggest that most marginal growth rings, in *O. parella* at least, form annually and are a good proxy for radial growth rate at the time of ring formation. This relationship is encouraging for those wishing to ascertain the age of surfaces using crustose lichens, as marginal zonation is present in many different genera and could potentially provide a previously unexploited dating tool. We suggest that marginal growth rings could be of use in lichenometry as an alternative *in situ* method of estimating the recent growth rate, and potentially the age, of thalli growing on surfaces, and also as a rapid means of comparing lichen growth rate variations between sites.

6. References

Armstrong RA. 1973. Seasonal growth and growth rate colony size relationships in six species of saxicolous lichens. *New Phytologist* 72: 1023-1030.DOI:10.1111/j.1469-8137.1973.tb02078.x

Armstrong RA. 1974. The descriptive ecology of saxicolous lichens in an area of South Merionethshire, Wales. *Journal of Ecology* **62**: 33-45.

Armstrong R.A. 1975. The influence of aspect on the pattern of seasonal growth in the lichen *Parmelia glabratula ssp. fuliginosa*. *New Phytologist* **75**, 245-251. DOI:10.1111/j.1469-8137.1975.tb01393.x

Armstrong RA. 1983. Growth curve of the lichen *Rhizocarpon geographicum*. *New Phytologist 94*: 619-622.

Armstrong RA 1993. Seasonal growth of foliose lichens in successive years in South Gwynedd, Wales. *Environmental and Experimental Botany* **33**: 225-232.

Armstrong RA. 2002. The effect of rock surface aspect on growth, size structure and competition in the lichen *Rhizocarpon geographicum*. *Environmental and Experimental Botany* **48**: 187-194.

Armstrong RA. 2005a. Radial growth of *Rhizocarpon* section *Rhizocarpon* lichen thalli over six years at Snoqualmie Pass in the Cascade Range, Washington State. *Arctic, Antarctic and Alpine Research* **37**: 411-415. DOI:10.1657/1523-0430(2005)037[0411:RGORSR]2.0.CO;2

Armstrong RA. 2005b. Growth curves of four crustose lichens. *Symbiosis* **38**: 45-57.

Armstrong RA. 2006. Seasonal growth of the crustose lichen *Rhizocarpon geographicum* (L.) DC. in south Gwynedd, Wales. *Symbiosis* **41**: 97-102.

Armstrong RA. 2013. Development of areolae and growth of the peripheral prothallus in the crustose lichen *Rhizocarpon geographicum*: an image analysis study. *Symbiosis* **60**: 7-15. DOI:10.1007/s13199-013-0234-2

Armstrong RA. 2014. Within-site variation in lichen growth rates and its implications for direct licheonometry. *Geografiska Annaler, Series A, Physical Geography* **96**: 217-226. DOI:10.1111/geoa.12043

Armstrong RA, Smith SN. 1987. Development and growth of the lichen *Rhizocarpon geographicum*. *Symbiosis* **3**: 287-300.

Armstrong RA, Smith SN. 1992. Lobe growth variation and the maintenance of symmetry in foliose lichen thalli. *Symbiosis* **12**: 145-158.

Armstrong RA, Bradwell T. 2001. Variation in hypothallus width and the growth of the lichen *Rhizocarpon geographicum* (L.) DC. *Symbiosis* **30**: 317-328.

Armstrong RA, Bradwell T. 2010. The use of lichen growth rings in lichenometry: Some preliminary findings. *Geografiska Annaler, Series A, Physical Geography* **92A**: 141-147.

Armstrong RA, Bradwell T. 2011. Growth of foliose lichens: a review. *Symbiosis* **53**: 1-16. DOI:10.1007/s13199-011-0108-4

Armstrong RA, Hilton AC. 2011. *Statistical Analysis in Microbiology: Statnotes*. Wiley-Blackwell, Hoboken, New Jersey.

Benedict JB. 1990. Experiments on lichen growth. I. Seasonal patterns and environmental controls. *Arctic and Alpine Research* **22**: 244-254.

Beschel RE, 1961. Dating rock surfaces by lichen growth and its application to glaciology and physiography (lichenometry). In: Raasch, G.O. (Ed.), Geology of the Arctic: Proceedings of the First International Symposium on Arctic Geology. University of Toronto Press, Toronto, pp. 1044–1062.

Bland JM, Altman DG. 1986. Statistical method for assessing agreement between two methods of clinical measurement. *Lancet* **I**: 307-310.

Bland JM, Altman DG. 1996. Measurement error and correlation coefficients. *BMJ* **313**: 41-42.

Bradwell T, Armstrong RA. 2007. Growth rates of *Rhizocarpon geographicum* lichens: a review with new data from Iceland. *Journal of Quaternary Science* **22**: 311-320. DOI:10.1002/jqs.1058

Bull WB. 2014. Using earthquakes to assess lichen growth rates. *Geografiska Annaler, Series A, Physical Geography* **96A**: 117-133.

Erikstad L, Sollid JL. 1986. Neoglaciation in South Norway using lichenometric methods. Norsk Geografisk Tidsskrift / Norwegian Journal of Geography, 40: 85–105.

Evans DJA, Archer S, Wilson DJH, 1999. A comparison of the lichenometric and Schmidt hammer dating techniques based on data from the proglacial areas of some Icelandic glaciers. *Quaternary Science Reviews* **18**: 13–41.

Fisher PJ, Proctor MCF. 1978. Observations on a season's growth of *Parmelia caperata* and *P. sulcata* in South Devon. *Lichenologist* **10**: 81-89.doi:10.1017/S0024282978000092

Fritts H. 1976. *Tree Rings and Climate*. Cambridge University Press, Cambridge & London, 567 pp.

Foulds SA, Griffiths HM, Macklin MG, Brewer PA. 2014. Geomorphological records of extreme floods and their relationship to decadal-scale climate change. *Geomorphology* **216**: 193-207.doi:10.1016/j.geomorph.2014.04.003

Girish V, Vijayalakshmi A., 2004. Affordable image analysis using NIH Image/Image J. *Indian Journal of Cancer* **41**: 47

Hale ME. 1970. Single-lobe growth-rate patterns in the lichen *Parmelia caperata*. *Bryologist* **73**: 72-81

Hale ME. 1973. Growth. In: *The Lichens*. Ahmadjian V and Hale ME (eds). Academic Press, New York, pp 473-492.

Haworth LA, Calkin PE, Ellis JM. 1986. Direct measurement of lichen growth in the central Brooks Range, Alaska USA, and its application to lichenometric dating. *Arctic and Alpine Research* **18**: 289-296. DOI:10.2307/1550886

Hooker TN. 1980. Lobe growth and marginal zonation in crustose lichens. *Lichenologist* **12**: 313-323.

Innes, JL. 1985. Lichenometry. *Progress in Physical Geography*, **9**: 187-254.

James PW, Hawksworth DL, Rose F. 1977. *Lichen communities in the British Isles: A preliminary conspectus*. In: MRD Seaward, ed. Lichen Ecology, pp. 295-419, Academic Press, New York.

Jochimsen M, 1973. Does the size of lichen thalli really constitute a valid measure for dating glacial deposits? *Arctic and Alpine Research,* **5**: 417–424.

Jomelli V, Grancher D, Naveau P, Cooley D, Brunstein D. 2007. Assessment study of lichenometric methods for dating surfaces. *Geomorphology* **86**: 131–143.doi:10.1016/j.geomorph.2006.08.010

Jomelli V, Pavlova I, Guin O, Soliz-Gamboa C, Contreras A, Toivonen JM, Zetterberg P. 2012. Analysis of the dendroclimatic potential of Pollepis Pepei, P. subsericans and P. rugulosa in the tropical Andes (Peru-Bolivia). Tree-Ring Research **68**: 91-103.

Lawrey JD, Hale ME. 1977. Studies on lichen growth rates at Plummers Island, Maryland. *Proceedings of the Biological Soceity of Washington* **90**: 698-725.

Loso MG, Doak DF, Anderson RS. 2014. Lichenometric dating of Little Ice Age glacier moraines using explicit demographic models of lichen colonization, growth and survival. *Geografiska Annaler, Series A, Physical Geography* **96A**: 21-41.doi:10.1111/geoa.12022

Matthews JA. 2005. 'Little Ice Age' glacier variations in Jotunheimen, southern Norway: a study in regionally controlled lichenometric dating of recessional moraines with implications for climate and lichen growth rates. The Holocene, 15: 1-19.

McCarthy, D.P., 1999. A biological basis for lichenometry? *Journal of Biogeography* **26**, 379–386.

McCarthy DP. 2003. Estimating lichenometric ages by direct and indirect measurement of radial growth: a case study of *Rhizocarpon* agg. at the Illecillewaet Glacier, British Columbia. *Arctic, Antarctic and Alpine Research* **35**: 203-213. DOI:10.1657/1523-0430(2003)035[0203:ELABDA]2.0.CO;2

McEwen LJ, Matthews JA. 2013. Sensitivity, persistence and resolution of the geomorphological record of valley-floor floods in an alpine glacier fed catchment, Leirdalen, Jotunheimen, southern Norway. *The Holocene* **23**: 974-989.doi:10.1177/0959683612475144

Moxham TH. 1981. Growth rates of *Xanthoria parietina* and their relationship to substrate texture. *Cryptogemie Bryologique Lichenologique* **2**: 171-180.

Osborn G, McCarthy D, LaBrie A, Burke R. 2015. Lichenometric dating: Science or pseudoscience? *Quaternary Research* **83**: 1-12. DOI:10.1016/j.yqres.2014.09.006

Phillips HC. 1969. Annual growth rates of three species of foliose lichens determined photographically. *Bulletin of the Torrey Botanical Club* **96**: 202-206.

Principato, SM. 2008. Geomorphic evidence for Holocene glacial advances and sea level fluctutaions on eastern Vestfirdir, northwest Iceland. *Boreas*, **37**: 132-145.

Proctor MCF. 1977. The growth curve of the crustose lichen *Buellia canescens* (Dicks) De Not. *New Phytologist* **79**: 659-663. DOI:10.1111/j.1469-8137.tb02250.x

Rydzak J. 1961. Investigations on the growth rate of lichens. *Annales Universitatis Mariae Curie-Sklodowska (Lublin, Poland)* sec C **16**: 1-15.

Syed A, Armstrong RA, Smith CUM. 2000. Quantification of axonal loss in Alzheimer's disease: an image analysis study. *Alzheimer's Reports* **3**: 19-24

Winkler, S. 2004. Lichenometric dating of the 'Little Ice Age' maximum in Mt Cook National Park, Southern Alps, New Zealand. The Holocene, 14: 911-920.

Table 1. The number (N), mean width (mm), range (mm), standard deviation (SD), and degree of skew and kurtosis of marginal growth rings in a sample of thalli of the crustose lichen *Ochrolechia parella* (L.) Massal. in three successive years (** $P < 0.01$).

Year	Variable	N	Mean	Range	SD	Skew	Kurtosis
2009	Number	24	3.7	1 - 6	1.37	0.11	-0.55
						(0.47)	(0.92)
	Width	232	0.77	0.08 - 2.93	0.68	1.03**	0.03
						(0.16)	(0.32)
2010	Number	20	3.7	1 - 7	1.59	0.03	-0.28
						(0.51)	(0.99)
	Width	187	0.80	0.11 – 3.39	0.72	1.25**	0.58
						(0.18)	(0.35)
2011	Number	13	3.8	2 - 7	1.21	0.01	-0.65
						(0.62)	(1.19)
	Width	128	0.48	0.08 – 2.06	0.41	1.86**	3.16
						(0.21)	(0.42)

Table 2. Direct measurement of radial growth rate (RaGR, mm a^{-1}) of the crustose lichen *Ochrolechia parella* (L.) Massal. in two successive years (2009 and 2010) at a maritime site in north Wales, UK (N = Number of thalli measured, SD = Standard deviation).

Year	N	Mean	Range	SD
2009	24	0.44	0 – 1.08	0.32
2010	22	0.34	0 – 1.16	0.31

Comparisons between RaGR in 2009 and 2010: Paired ‘t’ = 0.81 (P > 0.05)

Table 3. Correlations (Pearson's 'r') between number and width of growth rings, directly measured RaGR and thallus size.

Correlation	'r'	'P'
Number of rings/Thallus diameter 2009	0.15	P > 0.05
Number of rings/Thallus diameter 2010	-0.04	P > 0.05
Number of rings/Thallus diameter 2011	0.34	P > 0.05
Mean width of all rings/Thallus diameter	0.50	P < 0.01
Mean of first two rings/Thallus diameter	0.57	P < 0.001
Mean RaGR in 2009 and 2010/Thallus diameter	0.43	P < 0.05

Table 4. Comparison between means ('t' tests) and correlation (Pearson's 'r') between directly measured annual radial growth rates (RaGR, mm a^{-1}) of *Ochrolechia parella* and peripheral growth rings (* P < 0.05, ** P < 0.01, ns = not significant)

Comparison/Correlation	't'	'r'
RaGR in 2009 with width of ring 10.1	-0.01 ns	0.39 ns
RaGR in 2010 with width of ring 11.1	0.24 ns	0.24 ns
Mean RaGR in 2009 and 2010 with mean of all visible growth rings	3.01**	0.39 ns
RaGR in 2009 and mean of rings 11.1 and 11.2	0.15 ns	0.33 ns
Mean RaGR in 2009 and 2010 with mean of rings 11.1 and 11.2	-0.53 ns	*0.60

Fig 1. Examples of clear marginal zonation or 'growth rings' in crustose lichens. (A) a large *Ochrolechia parella* (L.) Massal. thallus on a gravestone, Inchnadamph. NW Scotland showing several rings, (B) a thallus of *Fuscidea cyathoides* (Ach.) V.Wirth, Vḝzda, growing on a quartzite boulder, shore of Loch Eriboll, NW Scotland with young apothecia, (C) a thallus, possibly a species of *Rhizocarpon or Fuscidea* growing near shore of Breidalon, SE Iceland, (D) a thallus of *Fuscidea cyathoides* (Ach.) V.Wirth, Vḝzda, with pycnidia growing on Basalt boulder, near Svinafellsjökull, SE Iceland.

Fig 2. Marginal rings in thalli of the crustose lichen *Ochrolechia parella* (L.) Massal. growing at a maritime site in north Wales: (A) overall view of a thallus showing three distinct rings over a part of the thallus; apothecia are also visible towards the centre of the thallus, (B) the rings in more detail revealing the characteristic dark and wider light bands with some additional banding evident within the first ring, (C) rings of a thallus in 2009, and (D) rings of the same thallus in 2010 after marginal erosion.

Fig. 3. Variation in ring width of a single thallus of *Ochrolechia parella* (L.) Massal. Rings were numbered from the edge towards the centre of the thalli and also identified according to year of measurement, e.g., ring 10.3 indicates the third ring from the margin measured in 2010. Bars indicate standard deviation (SD).

Fig 4. The relationship between mean width of the rings 11.1 and 11.2 and mean radial growth rate (RaGR) (mm a^{-1}) in 2009/10 in the crustose lichen *Ochrolechia parella* (L.) Massal. growing at a maritime site in north Wales (Pearson's 'r' = 0.56, $P < 0.05$, Linear regression: Y = 0.165 + 0.6103X with 95% confidence intervals).

Fig 5. A Bland and Altman plot showing the degree of agreement/disagreement between mean radial growth rate (RaGR) (mm a^{-1}) in the crustose lichen *Ochrolechia parella* (L.) Massal.measured over two years (2009/10) measured directly and the width of marginal rings 11.1 and 11.2 (Bias line (BL) = 0.02; SD = 0.25; Cl = 95% confidence intervals