UCRL-JRNL-237189

LAWRENCE LIVERMORE NATIONAL LABORATORY

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December 11, 2007

The Lichenologist

^{14}C/C measurements support Andreev's internode method to determine lichen growth rates in *Cladina stygia* (Fr.) Ahti

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Abstract Growth rates and the ability to date an organism can greatly contribute to understanding its population biology and community dynamics. 1n 1954, Andreev proposed a method to date *Cladina*, a fruticose lichen, using total thallus length and number of internodes. No research, however, has demonstrated the reliability of this technique or compared its estimates to those derived by other means. In this study, we demonstrate the utility of $^{14}C/C$ ratios to determine lichen age and growth rate in *Cladina stygia* (Fr.) Ahti collected from northwestern Alaska, USA. The average growth rate using $^{14}C/C$ ratios was 6.5 $mm \cdot yr^{-1}$, which was not significantly different from growth rates derived by Andreev's internode method (average = 6.2 $mm \cdot yr^{-1}$); thus, suggesting the reliability of Andreev's simple field method for dating lichens. In addition, we found lichen growth rates appeared to differ with geographic location, yet did not seem related to ambient temperature and total precipitation.

Keywords Accelerator mass spectrometry – *Cladina* – Climate – Fruticose lichen – Lichen age

Introduction

Predictable, incremental growth is an important tool for understanding many organisms and their environment (e.g., Hemming 1969; Pannella 1971; McLaughlin et al. 1987). Lichen growth rates have been used to determine productivity and performance of lichen communities (e.g., Karenlampi 1971; Vasander 1981; Keon and Muir 2002), as measures of lichen response to changes in their environment (e.g., Miller 1973; Lechowicz 1981; Benedict 1990; Kytöviita and Crittenden 2002), or to estimate age of the substrate upon which they are growing (McCarthy 1999). In addition, growth curves inferred from these rates can contribute to lichen population dynamics (Yarranton 1975).

Most studies use marginal growth rates, assessing incremental change of thallus radius, perimeter or area over time. For saxicolous crusts and foliose lichens these two-dimensional methods may be adequate, but not for fruticose lichens. Change in biomass or lichen height has been implemented to account for the three-dimensional growth of these lichens (e.g., Ahti 1959; Steen 1965; Lechowicz 1981; McCune et al. 1996; Hyvarinen and Crittenden 1998; Peck et al. 2000). Another method implemented widely in Arctic systems to assess growth within the genus *Cladina* is average annual linear growth rate (Scotter 1963; Karenlampi 1970; Prince 1973; Lechowicz 1983). Although originally outlined by Salazkin (1937), Andreev (1954) popularized this technique, which relies upon *Cladina* producing a whorl of branches each year, thus enabling growth rates to be derived by dividing total lichen height by the number of internodes.

More recently, accelerator mass spectrometry (AMS) was used to precisely date lichen fragments by measuring $^{14}C/C$ ratios that compare the carbon content in a fragment to a radiocarbon standard (Clark et al. 2000; Bench et al. 2001; Clark 2001). This technique was used successfully to age the crustose lichen, *Caloplaca trachyphylla* (Tuck.) Zahlbr (Clark et al. 2000). However, similar work with the crustose lichen *Rhizocarpon geographicum* (L.) DC and fruticose lichen *Usnea longissima* Ach. showed flat ^{14}C profiles (Bench et al. 2001; Clark 2001; Bench et al. 2002). To our knowledge, this methodology has not been implemented successfully with any fruticose lichen.

Our main objectives for this study were: (1) examine the utility of $^{14}C/C$ ratios for estimating growth rates and ages of *Cladina stygia* (Fr.) Ahti. (2) Estimate average growth rate and absolute age of *Cladina stygia* in northwestern Alaska using both traditional methods (sensu Andreev) and $^{14}C/C$ ratios, and compare their results. (3) Seek trends between lichen growth rate and climatological and geographical variables.

Methods

Sampling and processing

Cladina stygia is a terricolous lichen endemic to open bog environments (Ahti and Hyvönen 1985). This fruticose species occurs primarily in boreal and arctic regions, yet also extends into some temperate areas (Ahti and Hyvönen 1985; Perlmutter 2005). During the 2003 and 2004 field seasons, 12 individual thalli were collected from northwestern Alaska, USA (64°30’–65°30’N, 161°07’–165°05’W). To represent the climatic and environmental variability, thalli were collected from seven different sites on the Seward Peninsula, Alaska (Fig. 1). The seven sites were divided into two areas; east and west (see Fig. 1). Robust thalli were collected and stored in glass vials and subsequently transported to Oregon State University for sectioning.

Thalli were washed with deionized water to remove large adhering fragments and to re-hydrate for ease of processing. While moist, we measured total length of the predominant main axis and total number of internodes, or spaces between branches, along this axis. All side branches were trimmed away and unsampled. From the basal-most portion of the main axis towards the tip, fragments, ranging between 2-8 mm in length, were sampled approximately one centimeter apart. To minimize the effects of carbon turnover, the outer medulla and algal layer of each fragment was scraped away and considered a separate sample. Small segments of the inner medulla, or sterome, were the primary samples used in our analyses. These samples were stored in glass vials and transported to the Center for Accelerator Mass Spectrometry (CAMS) at Lawrence Livermore National Laboratory.

At the CAMS facility, samples were washed in 1N HCl at 80°C to remove any remaining adhering carbonate. Lichen fragments were weighed and loaded with CuO oxidizer into quartz tubes. Loaded tubes were evacuated, sealed and combusted at 900°C. Catalyzed by iron powder, graphite was condensed from CO_2 gas at a low temperature (Vogel et al. 1984). The $^{14}C/C$ isotope ratio in the graphitized sample was measured by AMS (Southon et al. 1990). Sample ages were calibrated with Levin and Kromer (2004), one-year smoothing and one-year resolution using CaliBomb (Reimer et al. 2004). For all analyses, we used the mean of the upper and lower calibrated ages.

Comparison of the isotopic ratios of lichen inner medullary fragments to ratios estimated by Levin and Kromer (2004) allows us to determine the approximate date carbon fixed by the photobiont was incorporated by the mycobiont. Although carbon turnover has been demonstrated in some lichens (Bench et al. 2002), we believe removal of the photobiont helps reduce these effects. Preliminary analyses of samples containing both photobiont and outer medulla scraped from the inner medulla, demonstrated similar or no trends of age with distance from the lichen base, which substantiates our choice of using primarily inner medullary fragments for our analyses.

Analyses

Traditional estimates of lichen growth rate were determined using Andreev's (1954) method of average annual linear growth rates (i.e., lichen height divided by the number of internodes on a single thallus). Absolute age was calculated by subtracting the number of internodes from the year of collection. Growth rates from $^{14}C/C$ ratios were derived from slope estimates of regressing the approximate distance of each sample from the base of the lichen against the mean calendar year determined from $^{14}C/C$ ratios. The absolute age from $^{14}C/C$ ratios was the mean year of measurement for the basal-most sample of each lichen. Growth rates and ages were compared with paired and two-sample *t*-tests.

To look for trends between lichen growth and climatological variables we compared the amount of material (or length of lichen) between two adjacent samples to climatic variation within the time frame elapsed between those samples. We used climate data from the nearest weather stations, located in Nome and Kotzebue, Alaska (National Climatic Data Center, National Oceanic and Atmospheric Administration). Data from both stations yielded similar patterns, so for ease of interpretability, we present only Nome climate data. Sites used in the climate analysis ranged between 66 and 210 km from the town of Nome, and fairly flat topography ensures climatic data from this station adequately represent the climate at each site. We used three climate variables; accumulated number of days below freezing, accumulated precipitation, and mean annual temperature. For every two adjacent samples on all 12 lichens (62 pairs in all), we summed or averaged the climate for the time span spanning the two adjacent samples. Accordingly, we summed the length of lichen used for each of the adjacent samples and the length of the internode between these samples. This length of lichen, representing growth over a given period of time, was then compared to the accumulated number of days below freezing, accumulated precipitation, and mean annual temperature of that same time period.

Results and Discussion

Growth rate and absolute age

Using traditional methods (sensu Andreev 1954), the average growth rate of *Cladina stygia* in northwestern Alaska was 6.2 mm·yr^{-1}. Our estimates of growth are slightly faster than reported elsewhere for other species within *Cladina* using the same method (e.g., Andreev 1954; Scotter 1963; Pegau 1968; Prince 1973; Boudreau and Payette 2004). Vasander's (1981) measurements of *C. arbuscula* (Wallr.) Hale & Culb and *C. rangiferina* (L.) Nyl in southern Finland, however, were fairly similar to our growth rates using this traditional internode method.

^{14}C/C ratios measured in *Cladina stygia* revealed linear trends in lichen growth rate (Fig. 2). The average growth rate of our lichens, using data from ^{14}C/C ratios, was 6.5 mm·yr^{-1}. We found the two methods, internode-based growth rates and carbon ratios, did not significantly differ from one another (t = -0.75, p = 0.47). Moreover, the variability in rates was not systematically biased between the two methods (Fig. 3). Although we do not know the "true" growth rate of these lichens, both methods appear to have roughly equal bias and error. Therefore, this concordance supports further use of Andreev's internode method as a reliable field practice to estimate growth rates of *Cladina*.

The oldest lichen we found, using either method, was 30 years old. We found lichen age did not significantly differ between the two methods (t = -0.59, p = 0.60); although, trends in the data suggest ^{14}C/C ratios may underestimate age of younger lichens, while the internode method may underestimate age of older lichens (Fig. 4). Due to the indeterminate growth of lichens, these individuals may have been much older, yet the older portions at the base had decayed or broke off and were unsampled.

Our ^{14}C/C ratios for *Cladina stygia* contrast with those reported for the fruticose lichen *Usnea longissima*, which showed flat ^{14}C profiles (Clark 2001). Clark (2001) proposed that complex carbon transfer and subsequent turnover in fruticose and foliose lichens manifest as uniform carbon profiles, inhibiting their usefulness in dating studies. These disparate results raise the question: is carbon simply not turning over as quickly in *Cladina*, or does the climate of the surrounding environment (i.e., our sites were colder and shorter-seasoned than those of Clark (2001)) reduce the degree of turnover? Future studies incorporating different species, within *Cladina* and from other fruticose genera, may yield insights into this disparity of success with $^{14}C/C$ ratios.

Climate and geography

Using growth rates derived from both $^{14}C/C$ ratios and internode counts, we found lichens collected in the eastern portion of the Seward Peninsula had significantly faster growth rates, than those from the western portions ($t = 4.76$, $p < 0.01$; Fig. 2). Average growth rates based on $^{14}C/C$ ratios and internode counts for the eastern sites were 7.8 and 7.0 $mm \cdot yr^{-1}$, and 5.2 and 5.5 $mm \cdot yr^{-1}$ for the western sites, respectively. The eastern sites are located at the junction of the Seward Peninsula and mainland Alaska; thus, these sites tend to be more continental while those to the west are more oceanic. Elsewhere, lichen growth rates have been linked to continentality of climate (e.g., Andreev 1954); with faster lichen growth rates in more oceanic climates, where temperature and precipitation are more favorable. Conversely, our results depict increased growth rates with increased continentality. The eastern and western regions sampled appeared fairly similar in climate (Fig. 5). However, due to lack of detailed local climate data for each collection site, we are unable to determine whether climate accounts for the observed differences in lichen growth rates.

Interestingly, lichens from the eastern portion of the Seward Peninsula were younger on average than those from the west (comparing intercepts, $t = -4.77$, $p < 0.01$; Fig. 2). These geographic differences in age and growth rate may be better explained by biotic interactions rather than climatic factors. Average total lichen cover and lichen species richness of the eastern sites (52% cover and 32 species) tended to be greater than those to the west (14% cover and 23 species). Faster growing lichens in a highly competitive environment, such as sites to the east, would likely more easily succeed.

While lichen growth is likely dependent upon climate, we found no trend in lichen growth with changes in accumulated number of days below freezing, accumulated precipitation or mean annual temperatures ($r \leq 0.25$; Fig. 5). Our null conclusions could be attributed to scaling differences (climate data is coarse-grained, while our lichen measurements are very fine-grained), which may contribute to our inability to see such a relationship. It is plausible that lichens' plasticity allows a similar amount of growth in various climatic conditions, until a threshold is reached—a threshold we did not measure in our data.

Conclusions

$^{14}C/C$ ratios can successfully estimate growth rate and age of the fruticose lichen, *Cladina stygia*. We also found growth rates derived from $^{14}C/C$ ratios closely coincided to those from Andreev's internode method. This correspondence justifies future use of the internode method as a quick yet reliable field method. Finally, growth rates were significantly faster on the eastern Seward Peninsula than in specimens collected farther west. Growth rates, however, did not appear correlated to our climate variables.

Acknowledgements The authors thank Scott Miller, Sarah Nunn and Amanda Hardman for their support in the field, and Tom Guilderson and the CAMS staff for their help in the lab. We are also grateful to Peter Neitlich and the National Park Service who provided support and additional funding for this study. This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

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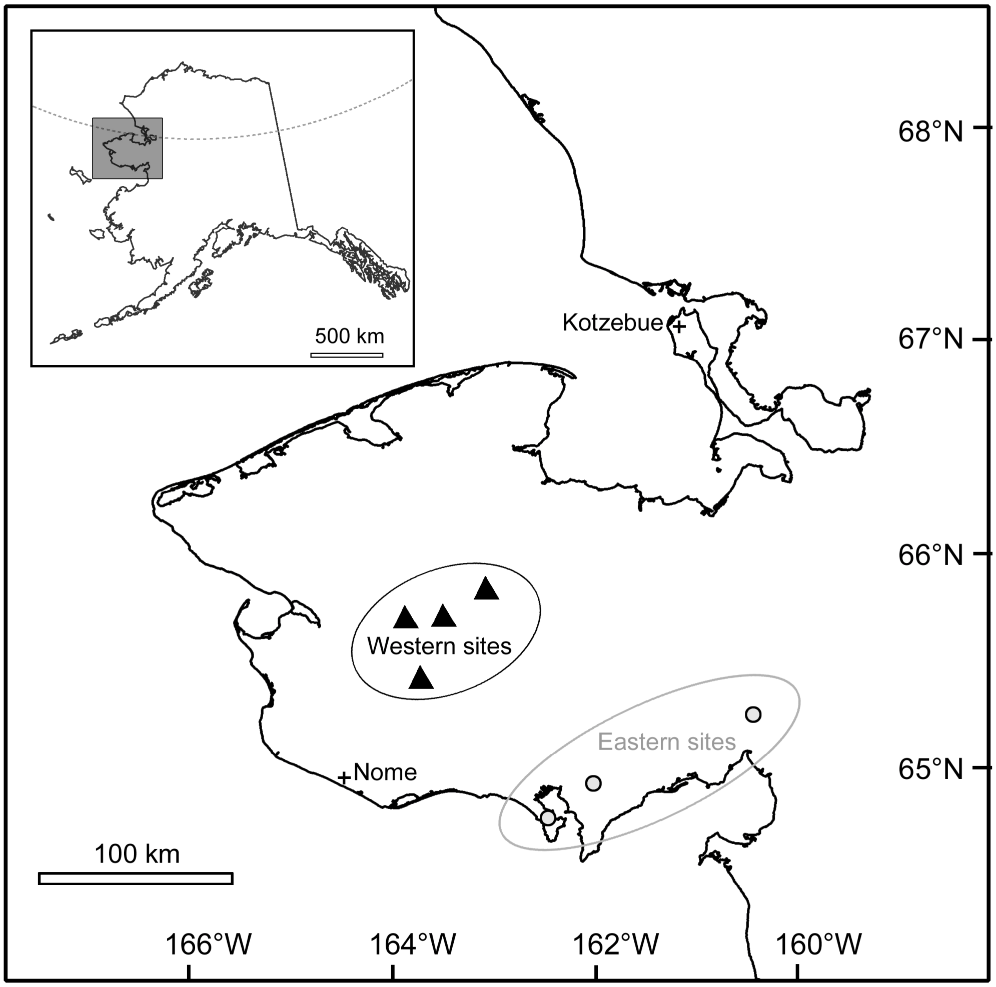


Fig. 1 Map of Seward Peninsula and seven sites from which lichens were collected. The dashed line in the Alaska inset in the upper left corner represents the Arctic Circle. Black triangles represent the four western sites (6 lichens were collected from these sites) and grey circles represent the three eastern sites (6 lichens). Plus signs indicate nearby towns, including Nome and Kotzebue.

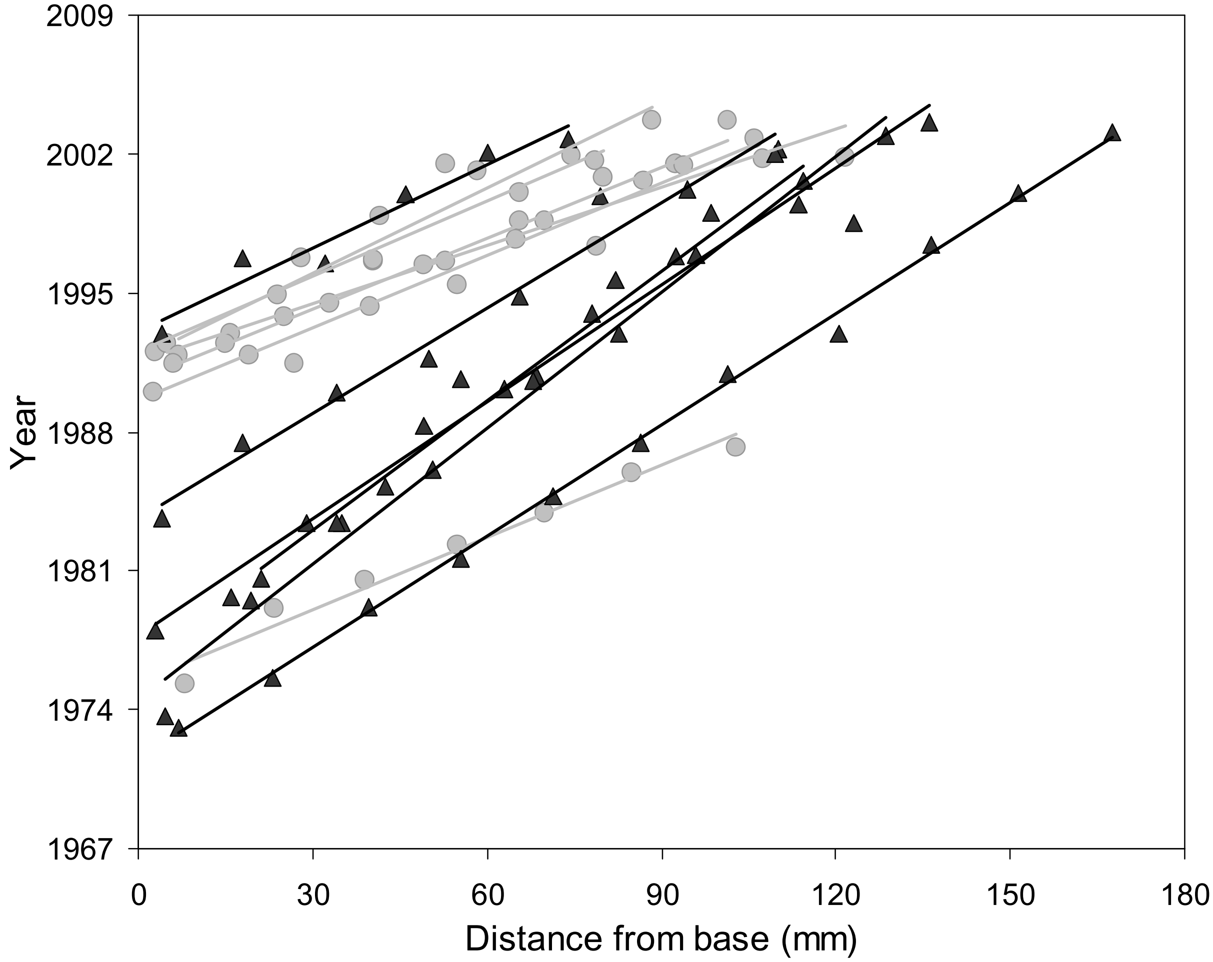


Fig. 2 Scatterplot of distance of lichen segment from base, in millimeters, and age of that segment derived from $^{14}C/C$ ratios. Lines represent least square regressions of twelve separate lichens. Black lines and triangles are lichens from the western Seward Peninsula and grey lines and circles are from the eastern Seward Peninsula.

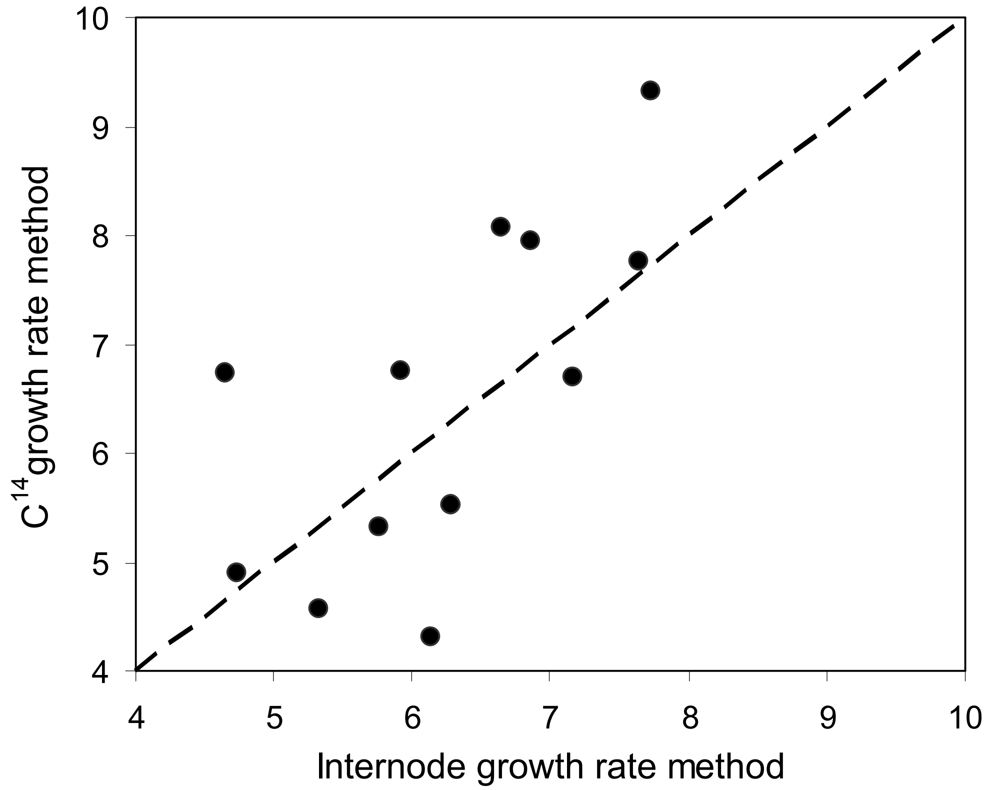


Fig. 3 Scatterplot of growth rates (mm·yr^{-1}) of 12 individual lichens using two different methods; internode growth rates (sensu Andreev 1954) and ^{14}C/C ratios (slopes from Fig. 2).

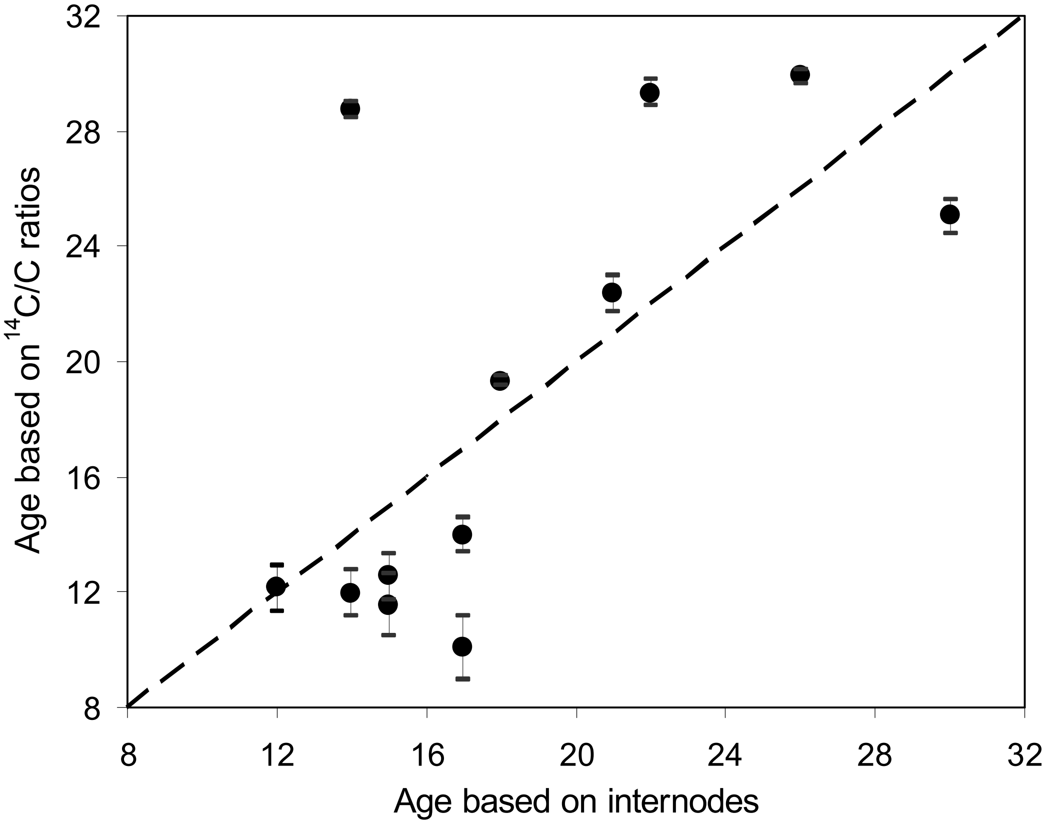
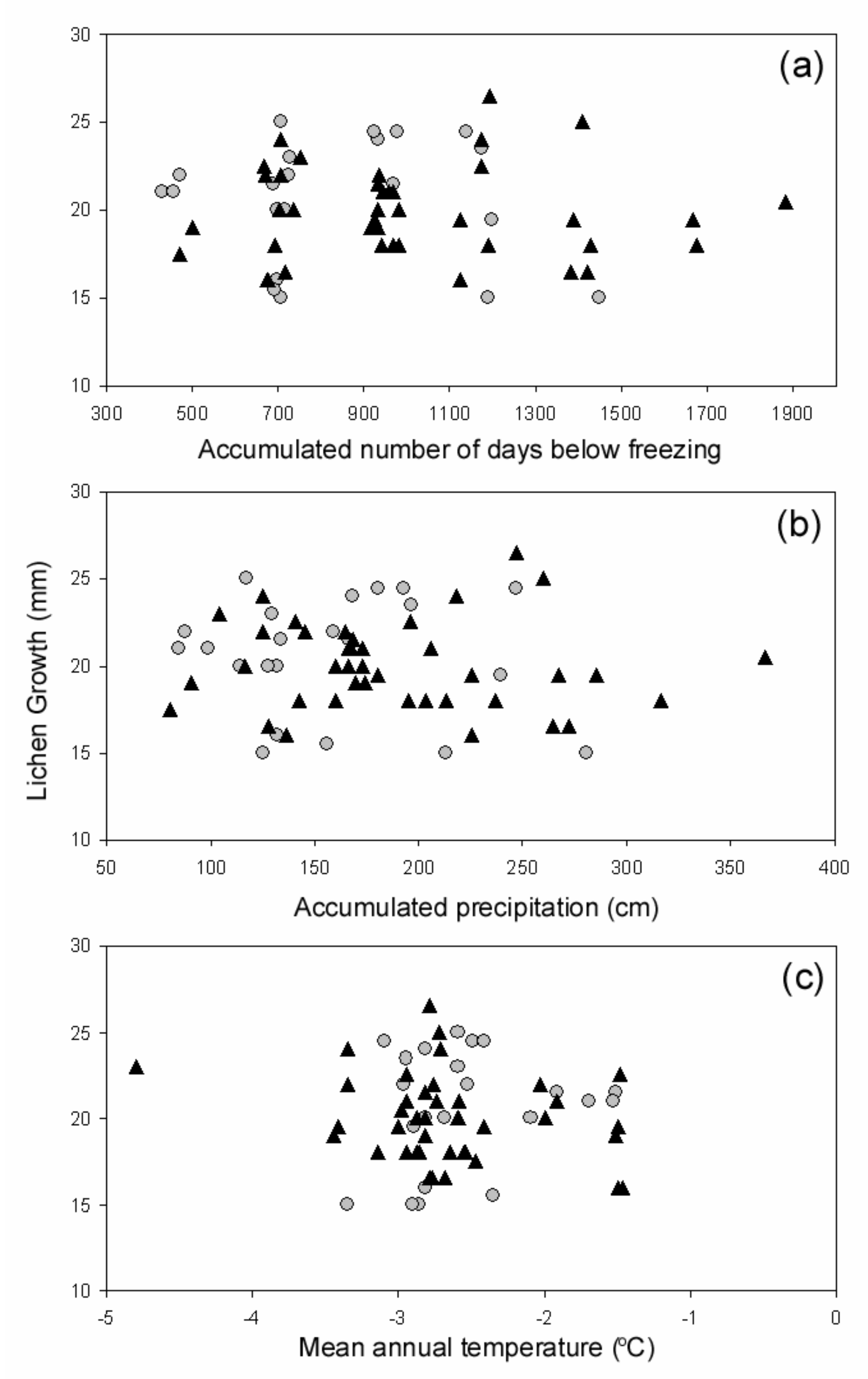


Fig. 4 Scatterplot of lichen age of 12 individual lichens using two different methods; traditional internode method (sensu Andreev 1954) and ^{14}C/C ratios. Brackets represent the confidence interval surrounding the mean calibrated age based on ^{14}C/C ratios from CaliBomb (Reimer et al. 2004). Younger lichens tended to be underestimated by ^{14}C/C ratios, while older lichens tended to be overestimated by internode counts.



(a)
Accumulated number of days below freezing
(b)
Lichen Growth (mm)
Accumulated precipitation (cm)
(c)
Mean annual temperature (ºC)

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Fig 5 (previous page) Scatterplots of lichen growth (length of two adjacent samples plus the intervening segment) and (a) accumulated number of days below freezing, (b) accumulated precipitation (cm), and (c) mean annual temperature (°C). Black triangles indicate adjacent sample pairs from lichens collected in the western sites and grey circles from the eastern sites.