



Effects of site-specific climatic conditions on the radial growth of the lichen biomonitor *Xanthoria parietina*

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Abstract

The protocols commonly applied in surveys with lichens as biomonitors of airborne trace elements require analyses of samples derived from thalli or parts of thalli grown in the last year before sampling, under the postulation that samples of the same size are of the same age. Unfortunately, the influence of ecological site-specific factors on lichen growth is still largely ignored, so that samples of the same size collected in environmentally and climatically diverse sites might actually differ in age. This work aims at quantifying the influence of climatic conditions on the radial growth rates (RaGRs) of *Xanthoria parietina*, one of the most popular lichen biomonitors. RaGR was monitored in seven populations distributed along an altitudinal transect of 30 km in the Classical Karst (NE Italy), from 20 to 500 m above sea level. For c. 17 months, lobe growth was measured seasonally with a digital calliper, and site-specific climatic variables were monitored by means of thermo-hygrometric sensors and implemented by meteorological data. Finally, the lobe growth of *X. parietina* was modelled as a function of 18 environmental variables. Results revealed that thalli of relatively dry sites had significantly lower seasonal RaGR with respect to moister ones. Considering that cumulative precipitations were equally distributed along the transect, it was concluded that RaGR of *X. parietina* is affected negatively by high air temperatures and positively by high relative humidity. The importance of RaGR variation in lichen bioaccumulation studies is critically discussed.

Keywords Altitude · Air pollution · Biomonitoring protocols · Climate · Trace elements · Water availability

Introduction

Lichens are a stable, extracellular mutualistic symbiotic association between a fungus (the so-called mycobiont), generally an ascomycete, and one or more populations of algae and/or cyanobacteria (the so-called photobionts) (Honegger 1998), plus an unknown number of further participants, ranging from

parasites to saprotrophs (Muggia et al. 2016). Lacking roots, stomata and a waxy cuticle, lichens predominantly derive water and inorganic nutrients from dry and wet atmospheric deposition (Williamson et al. 2004). This fact, along with the proven correlation between pollutant concentration in lichen thalli and atmosphere (Sloof 1995; van Dobben et al. 2001), makes lichens excellent biomonitors of airborne-persistent pollutants such as trace elements (Bargagli 1998) and polycyclic aromatic hydrocarbons (Kodnik et al. 2015; Domínguez-Morueco et al. 2017).

In foliose lichens, the intra-thalline content of most of trace elements is significantly higher in the old (central) than in the juvenile (peripheral) parts of the thallus, because the element concentration strongly depends on the exposure time (Loppi et al. 1997; Nimis et al. 2001). For this reason, in order to compare the elemental composition of lichen samples collected in a given area, these have to consist of parts as much as possible equal in age (Bargagli and Nimis 2002). Standard protocols, first adopted by national agencies (e.g. Nimis and Bargagli 1998) and now widely used in European countries (Rossbach and Lambrecht 2006; Brunialti and Frati 2007;

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Branquinho et al. 2008; Dzubaj et al. 2008), recommend the use of the outermost portions of thallus lobes, roughly corresponding to the growth of the last year. Nevertheless, only few studies have been focused on the yearly growth rate of lichens commonly used as biomonitors of trace elements (Fisher and Proctor 1978; Tretiach et al. 2013; Richardson 1967; Moxham 1981; Honegger 1996). However, none of these works has been specifically focused on the inter-site variability of seasonal or yearly radial growth rate in relation to local climatic conditions.

Foliose lichens grow radially by expanding their peripheral lobes a few millimetres per year, thanks to the activity of a pseudomeristematic rim area (Honegger 1996), characterised by dense, small mycobiont and photobiont cells and high division rates (Hill 1989). Lichens are generally slow-growing organisms (Honegger 1998). This is due to their poikilohydric lifestyle and to the relatively high percentage of time spent in a metabolically inactive state (Lange and Green 2008). In particular, when the lichen photobiont is a green alga, the thallus growth is favoured by prolonged periods with high air humidity, because green microalgae can recover from dehydration also in absence of liquid water, reaching a positive CO₂ income (Büdel and Lange 1991; Palmqvist 2000). On the contrary, increasing temperatures can rapidly decrease the thallus water content inducing lichens to a dormant state (Crabtree and Ellis 2010).

Considering that water availability and the evaporative demand are heterogeneous even at small spatial scales, the site-specific climatic conditions can strongly affect lichen growth (Gauslaa et al. 2007). This fact becomes of major importance in the context of biomonitoring surveys, since neglected differences in radial growth rates (RaGRs) among sites would correspond to differences in exposure times of the sampled materials. In this light, a correct assessment of the natural variability of annual RaGR in a given study area would significantly improve the robustness of lichen biomonitoring data.

The foliose lichen *Xanthoria parietina* (L.) Fr. Th. is a model species and is also one of the most used biomonitors of airborne trace elements, but its growth has been investigated in a few studies only (Richardson 1967; Moxham 1981; Honegger et al. 1996) and never in relation to site-specific factors. In this study, we tested the hypothesis that site-specific climatic conditions differently influence the radial growth of *X. parietina*.

Material and methods

The species

X. parietina is a very common dorsiventral foliose lichen with coccoid green algae of the genus *Trebouxia* as photobionts

(Ahmadjian 2001). The thallus is greenish-grey to vivid yellow-orange, more or less orbicular in outline and appressed to the substratum, with apothecia (the sexual reproductive structures of the fungus) in the central parts, and well-developed lobes at the margins. *X. parietina* is rather xerophilous and usually colonises nitrogen-rich environments with high solar irradiation (Fрати et al. 2007). It has been used as model organism in several studies concerning desiccation tolerance (e.g. Honegger 2003; Brandt et al. 2015), regenerative capacity (Honegger 1996; Honegger et al. 1996), photo-oxidative tolerance (Piccotto et al. 2011; Bertuzzi et al. 2013, 2017), sexual reproduction (Scherrer et al. 2005; Itten and Honegger 2010) and lichen re-synthesis (Bubrick and Galun 1986). Due to its worldwide distribution, *X. parietina* has frequently been used as a biomonitor of persistent airborne pollutants (Cuny et al. 2004; Branquinho et al. 2008; Demiray et al. 2012; Augusto et al. 2015) and eutrophication phenomena (Olsen et al. 2010; Munzi et al. 2017).

Study sites

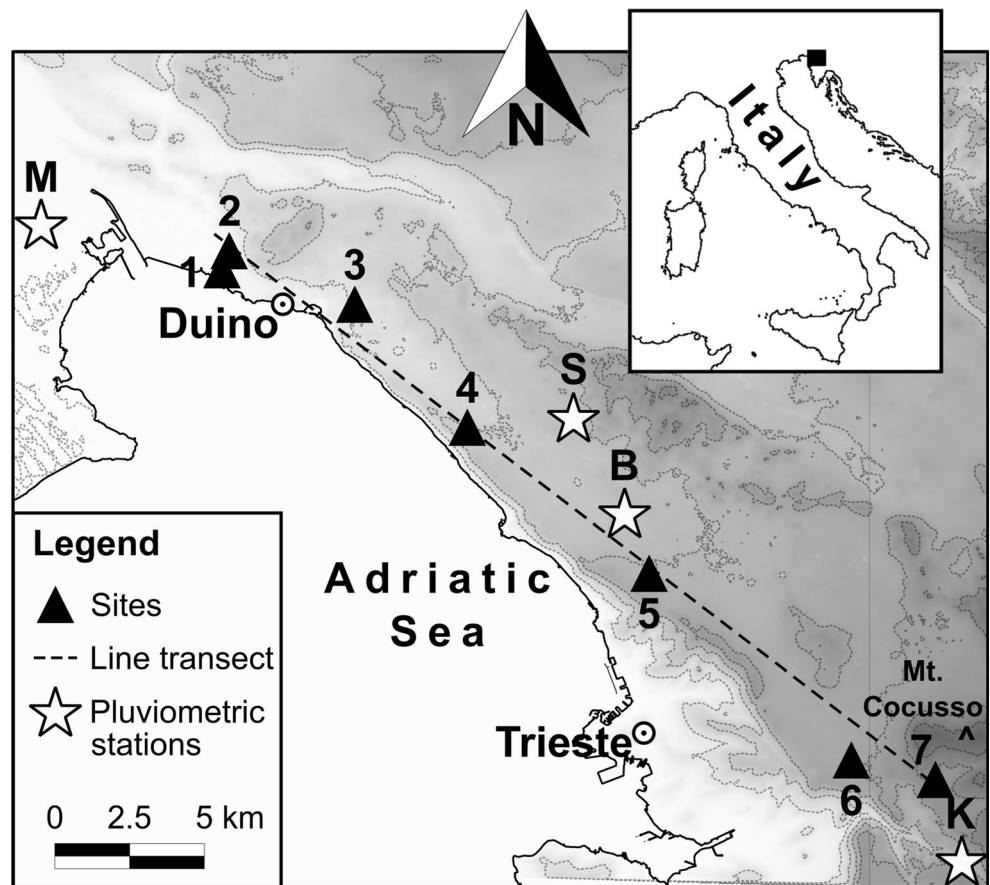
This study was carried out in the Classical Karst, a NW-SE-directed anticline near Trieste (NE Italy; Fig. 1). Here, seven sites, similar in terms of land use and epiphytic lichen flora, were selected along a 30-km-long altitudinal transect extended from the coast near Duino (16 m a.s.l.; 13°35' 23.21" E, 45° 46' 52.10" N) to the base of Mt. Cocusso (500 m a.s.l.; 13° 53' 59.37" E, 45° 37' 50.27" N). All the sites were selected in proximity of *Quercus-Ostrya* forest edges localised in semi-natural areas far from known pollution sources (Regione FVG 2007). According to the classification of Köppen-Geiger, the climate of the area is fully humid, warm temperate, with hot summers on the coast and warm ones in the interior, being transitional between sub-Mediterranean and pre-Alpine (Kottek et al. 2006) climates. Average rainfall and annual temperature are 1190 mm and 13 °C, respectively, with marked seasonal changes (Osmer FVG 2016; weather station of Sgonico, 268 m a.s.l.; 13° 45' 0.00" E, 45° 44' 24.00" N). Cold, dry, intense ENE winds ("Bora") are frequent, especially between October and April, and can reach a speed of 44–50 m s⁻¹.

Radial growth rate measurements

In each site (c. 20–50 m²), four to six thalli of *X. parietina* (average diameter = 4.5 ± 1.2 cm) growing on one to three sub-vertical trunks of manna ash (*Fraxinus ornus*) trees at 70–180 cm above the ground were selected, described in a field sheet and individually photographed with a digital device (iPad mini; Apple, California, USA).

Measurements of linear lobe lengthening were carried out according to Tretiach et al. (2013). A stainless nail was fixed in the centre of each thallus and eight to ten marginal lobes

Fig. 1 Geographic location of the sites selected for the growth measurements of the epiphytic lichen *X. parietina* along a 30-km-long transect in the Classical Karst (NE Italy). Variation in grey scale reflects the increasing altitude, from sea level (white) to 500 m (dark grey) as calculated by digital elevation modelling (see section “Site-specific environmental variables”). The sites are numbered according to their increasing altitude. Thermo-hygrometric sensors were installed in sites 2, 4, 5 and 7. The pluviometric stations are indicated by a star (B = Borgo Grotta Gigante; K = Kozina; M = Monfalcone; S = Sgonico). The distance of each site from the sea was 330, 990, 1200, 970, 2250, 5840 and 8200 m for sites 1–7, respectively



growing up or downwards were selected. Lateral lobes were excluded a priori in order to avoid interference due to the radial growth of the lichen-hosting trunks. The radial length of each lobe was measured from the nail to the lobe margin with a digital calliper equipped with a Vernier scale (Maurer, Italy; sensitivity ± 0.01 mm). The lobe radial measurements were carried out between February 14, 2015 and June 29, 2016, every 100 ± 10 days from t_0 to t_5 , so as to define five seasonal time intervals (STI₁₋₅) and two long-term periods, herein named as LT₁ and LT₂ with a duration of 400 and 504 days, respectively. Since the thallus size is influenced by the water content, measurements were carried out in sunny days, when air relative humidity was between 60 and 70%. If snails and arthropods grazed some of the selected lobes between two consecutive seasonal intervals, these were discharged and new lobes were selected as explained before. The measurements of seasonal radial growth rate (SRaGR) were calculated for each thallus over the j th STI as follows:

$$SRaGR_j = \frac{\sum_i^{N_i} [(L_i^{t_{n+1}} - L_i^{t_n}) / (t_{n+1} - t_n)]}{N_i} \times 1000 \quad (1)$$

where $L_i^{t_{n+1}}$ and $L_i^{t_n}$ are the radial measurements of the i th lobe, respectively, collected at time t_{n+1} and t_n and N_i is the number

of lobes measured in a single thallus. SRaGR were expressed in micrometres per day. Overall, according to a factorial design based on 8–10 lobe marginal measurements collected on 4–6 thalli in 7 sites over 5 seasonal intervals, more than 1500 measurements were collected in the field.

The long-term radial growth (LTRaG₁₋₂) was calculated taking into account only those lobes which had not been grazed. LTRaG₁ and LTRaG₂ were expressed in millimetres and refer to the average lengthening of the lobes observed between t_0 and t_4 (LT₁, 400 days) and between t_0 and t_5 (LT₂, 504 days), respectively.

Site-specific environmental variables

In order to check whether the site-specific climatic conditions differed along the altitudinal transect during the study period, the air temperature (T), relative humidity (RH) and dew point (DP) values were continuously monitored every 30 min by thermo-hygrometric sensors (EL-USB-1, Lascar Electronics, UK) installed at sites 2, 4, 5 and 7, at intervals of 100 m in altitude. Sensors were fixed on N-exposed inner branches of the lichen-hosting trees, so as to shield them from direct solar irradiation. Furtherly, the hourly values of saturation deficit (SD) and air water potential (Ψ_{wv}) were calculated as:

$$SD = e_{(s)} \times (1 - RH/100) \quad (2)$$

$$\Psi_{wv} = (R \times T/V_w) \times \ln(RH/100) \quad (3)$$

where $e_{(s)}$ is the saturation vapour pressure (kPa), R is the gas constant ($J K^{-1} mol^{-1}$) and V_w is the partial molar volume of water. Overall, each site was described as a function of 15 climatic variables that correspond to the mean minimum, average and maximum values of T , RH , DP , SD and Ψ_{wv} calculated for each seasonal interval. Along with these climatic variables, the distribution of the seasonal cumulative precipitations (cP) along the altitudinal transect was also evaluated as a function of the data collected by four pluviometric stations present in the study area (Fig. 1; ARPA FVG–OSMER and GRN 2018). Finally, along with climatic variables, the site-specific altitude and distance from the sea were calculated in QGIS environment (QGIS Brighton version 2.6) by applying digital elevation modelling and *v.distance* algorithm, respectively. Overall, in this study, the 7 selected sites were characterised in terms of 18 environmental descriptors, i.e. the above mentioned 15 climatic variables, plus cP, altitude and distance from the sea.

Data analysis

A first factorial ANOVA was implemented to assess the site-specific differences among sites 2, 4, 5 and 7 in terms of T and local air water availability (RH , DP , SD and Ψ_{wv}) within and between seasonal intervals. This analysis was improved by exploring the climatic differences over the day course by subdividing the 24-h time period in six intervals of 4 h each, starting from midnight. The lobe radial growth data were first analysed by Tukey's test in order to remove site-specific outliers. Then, the values of seasonal and long-term radial growth were analysed by a further factorial ANOVAs using "site" and "time intervals" (i.e. STI_{1-5} and LT_{1-2}) as categorical factors. The "site" \times "time interval" interaction term and the LSD post hoc test were used to evaluate the significant inter-site differences in terms of both SRaGR and LTRaG within the respective time intervals. Afterwards, the relationships between the seasonal radial growth rate and the 18 environmental variables measured over the five seasonal time intervals were evaluated by Pearson's correlation coefficient, limited to those sites equipped with thermo-hygrometric sensors.

In order to assess whether the selected 18 environmental variables affect the SRaGR of *X. parietina*, a principal component regression analysis (PCR; see Jolliffe 2002) was performed on lichenometric and climatic data collected at sites 2, 4, 5 and 7. The advantage of this statistical approach lies in overcoming the multicollinearity problems among predictor variables, when, as in the case of climatic variables, these are strongly mutually correlated. A principal component analysis (PCA) was carried out on the 16 climatic variables and the resulting first two principal components of the PCA (i.e. PC1

and PC2, respectively) along with the site-specific altitude and distance from the sea were then used as predictors in a generalised linear mixed model (herein named as growth rate model (GRM)). Furthermore, in order to take into account the effect of the biological variability on site-specific SRaGR, the thallus was used as random effect. The significance of the GRM model was tested by comparing its residuals with those of a null model by one-way ANOVA. Similarly, the statistical significance of the random effect (i.e. thallus) was tested by comparing the GRM residuals with those of a second generalised linear model in which the selected fixed effects were the same of the GRM but the random effect related to the thallus was not considered. Finally, the goodness of fit of GRM was assessed by testing the significance of the coefficient of determination (R^2) between the estimated and the observed SRaGR values collected in the four sites.

Results

Climatic conditions

Cumulative precipitations were equally distributed along the transect (Fig. 2a) during the same seasonal interval. Those observed in summer (385 ± 29 mm) and autumn 2015 (387 ± 52 mm) and spring 2016 (359 ± 25 mm) were quite similar, whereas during winter 2015, the precipitation slightly increased (452 ± 34 mm). Spring 2015, with only 158 ± 13 mm, was significantly less rainy than the other seasons, and its seasonal average RH value was relatively more similar to that of summer 2015 than to that of spring 2016 (Fig. 2c).

The factorial ANOVA performed on climatic data revealed a pronounced, relatively stable thermometric gradient along the transect (Fig. 2, left panel, and Supplementary Table 1). Over the course of the day, the sites closest to the coast (sites 1 and 2) were significantly warmer ($+5$ °C) than those at higher altitudes, in good accordance with the adiabatic lapse rate (1 °C per 100 m altitude). This means that, during summer daytime (8–12 h), the evaporative demand along the transect markedly varied between the transect extremes: from the 3.29 kPa of the coastal sites (sites 1 and 2) to the 2.04 kPa of the opposite transect extreme, i.e. site 7 (Fig. 2b). On the contrary, during winter, the frequent occurrence of cold Bora wind gusts characterised site 7 as the driest one, since, throughout the entire course of the day, its average dew point value was relatively lower with respect to those recorded at lower altitude (Fig. 2d).

The average seasonal values of both RH and Ψ_{wv} were quite similar along the transect, but site 5 had the highest air humidity in autumn and winter 2015. In particular, during the latter seasonal intervals, site 5 was significantly moister than sites 2 and 4 (Fig. 2c and Supplementary Table 1).

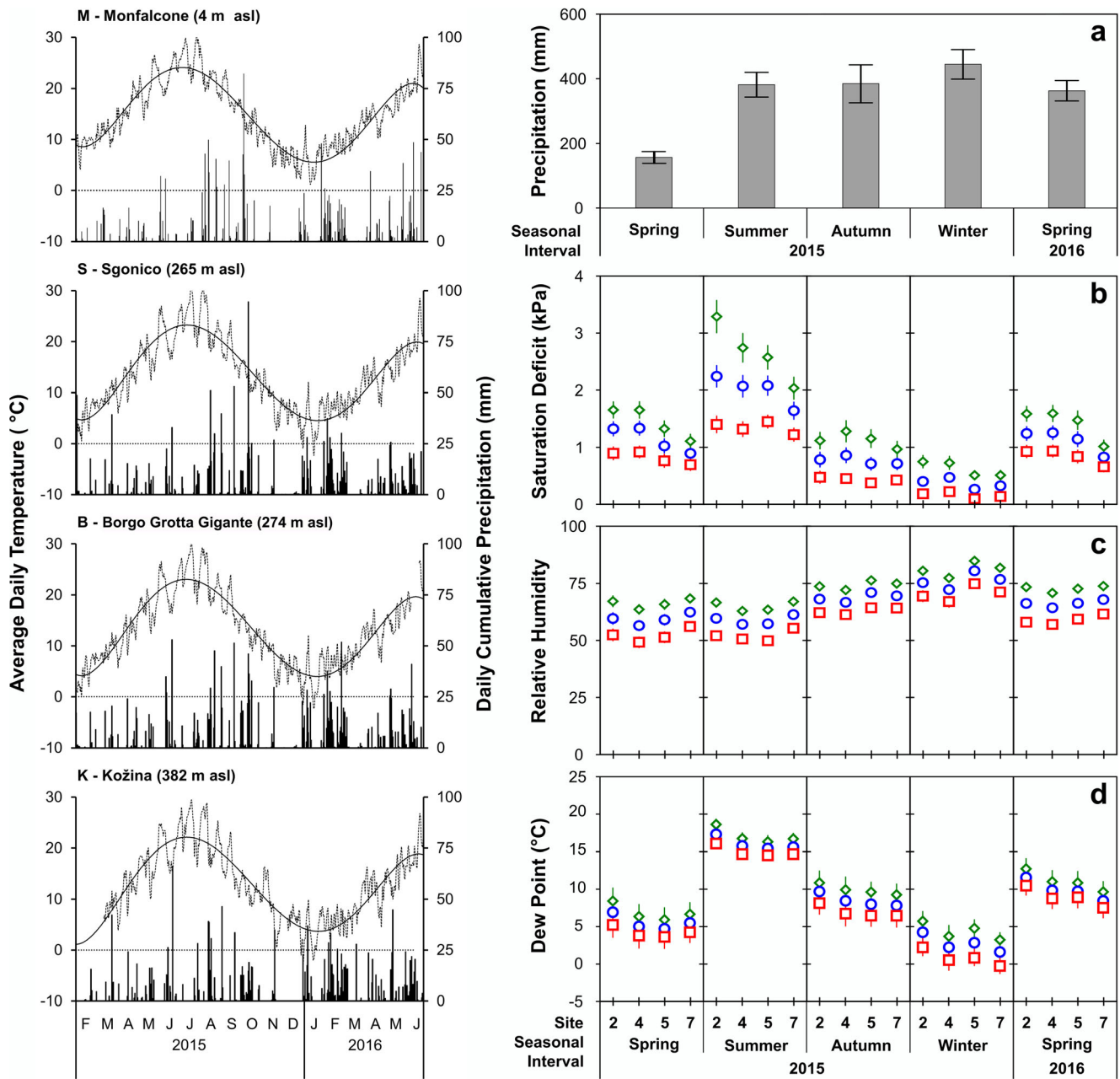


Fig. 2 Left panel: average daily temperature (dotted lines) and daily precipitations (black bars) measured in the four pluviometric stations of Fig. 1 in the study period. Right panel: values of average cumulative precipitation (a); saturation deficit (b), relative humidity (c) and dew

point (d), directly measured with thermohygrometric sensors at sites 2, 4, 5 and 7 in the study period. Diamonds, circles and squares indicate the mean maximum, average and minimum values

Seasonal and long-term radial growth

The summary of factorial ANOVA (Table 1) revealed that the radial growth of *X. parietina* was not constant during the study period, being the “time interval” factor highly significant. The SRaGR was lower in the dry, warm seasons (i.e. spring and summer 2015) and higher in the rainy, moist, cold ones (i.e. autumn and winter 2015 and spring 2016) (Fig. 3). In agreement with these results, SRaGR measured at sites 2, 4, 5 and 7 were negatively correlated with the seasonal average values of

T , DP and SD and positively correlated with those of cP, RH and Ψ_{wv} (Supplementary Table 2). The highly significant correlations between SRaGR and climatic data, coupled with the fact that the outcomes of factorial ANOVA showed significant interaction effect “site \times time interval” (Table 1), suggest that the inter-site SRaGR variability was related to the significant climatic differences observed among sites. Inter-site variability was lower in the dry, warm spring and summer 2015 (Fig. 3 and Supplementary Table 3). In summer 2015, although the cumulative precipitation doubled along the entire

Table 1 Summary of factorial ANOVA analyses performed on seasonal radial growth rate (SRaGR) and long-term radial growth (LTRaG) values

	SRaGR			LTRaG		
	df	F	P	df	F	P
Time interval (TI)	4	36.96	0.000	1	18.23	0.000
Site (S)	6	19.23	0.000	6	11.64	0.000
TI × S	24	3.82	0.000	6	0.40	0.873
Error	117			46		
Total	151			59		
r_{adj}^2		0.700			0.580	

The results of the LSD's post hoc test are given in Supplementary Table 3 for SRaGR data, whereas those of LTRaG data are reported in Fig. 4

df degree of freedom, F F statistic, P p value

transect with respect to spring 2015, SRaGR slightly decreased in five sites, due to the increased seasonal average values of T and SD. In autumn and winter 2015, inter-site variability increased, although cumulative precipitations were still equally distributed along the transect. In fact, according to the site-specific water availability (see previous section), thalli at the transect extremes and at site 4 had significant lower SRaGR values than at site 5 (Supplementary Table 3). During spring 2016, SRaGR sharply decreased in all selected sites in parallel to the average temperature increase. Compared to spring 2015, the inter-site variability of SRaGR increased during 2016, in line with the seasonal SD gradient between the two transect extremes ($\Delta SD = 0.903$ kPa) and the high values of both cumulative precipitations and average seasonal RH.

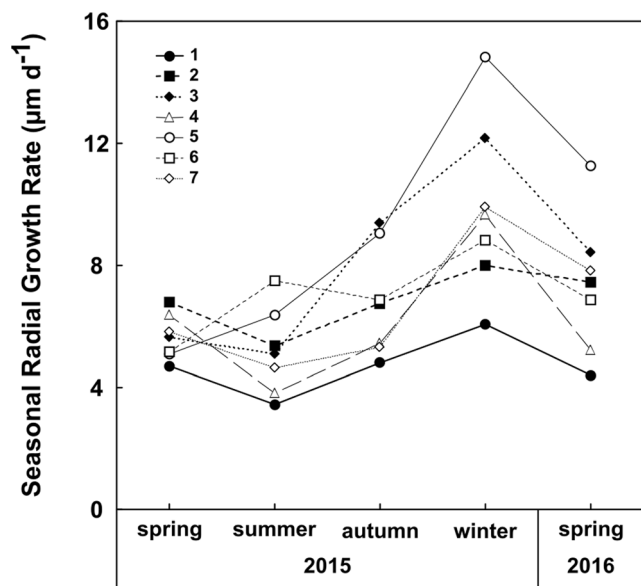


Fig. 3 Average values of site-specific seasonal radial growth rates of the epiphytic lichen *X. parietina* in five successive seasons in the seven sites of Fig. 1. The statistical differences among site-specific average values are given in Supplementary Table 3

The factorial ANOVA performed on the long-term radial growth values (LTRaG₁ and LTRaG₂) revealed a high statistical significance of the factor “site” (Table 1). In particular, the site-specific LTRaG values ranged between 1.27 and 3.19 mm during the first long-term interval (LT₁) and between 1.79 and 4.44 mm during the second one (LT₂). During both LT₁ and LT₂, the radial growths of thalli at sites 1 and 7 (the transect extremes) were significantly lower than at site 5 (Fig. 4). The site-specific differences in long-term radial growth were clearly due to the fact that site 5 was characterised by high air humidity in summer, autumn and winter. Nevertheless, the “site × time interval” interaction term was not significant (Table 1), suggesting that within the same site LTRaG₁₋₂ values were relatively homogeneous. On an annual basis, the radial growth of *X. parietina* was similar at inland sites (2.6 ± 0.3 mm year⁻¹; Fig. 5), whereas it was significantly lower (1.65 ± 0.17 mm year⁻¹) at site 1, in close proximity to the coast.

Effects of environmental variables

The results of the PCA performed on the climatic variables measured at sites 2, 4, 5 and 7 are reported in Supplementary Fig. 1. The outcomes of the principal component regression analysis revealed that the GRM satisfactorily fitted the SRaGR values measured in situ (Table 2). In fact, GRM was highly significant as described by the results of the one-way ANOVA-based comparison between the residuals of GRM and those of the null model (Table 2; $F = 53.60$; $P < 0.001$). Besides, predicted and observed SRaGR values were significantly correlated among them ($R = 0.768$), indicating that selected fixed and random effects explained 60% of SRaGR data variance ($r_{adj}^2 = 0.596$). Similarly, the residuals of GRM were significantly different from those of the second generalised linear model ($F = 4.82$; $P = 0.028$), highlighting that the selected random effect (i.e. thallus) was statistically significant. This supports the fact that the site-specific seasonal radial growth rate of *X. parietina* is partially affected by the inherent biological variability of lichen populations.

Discussion

Most of the recent studies concerning lichen growth in relation to climatic variables have been carried out on the macrolichen *Lobaria pulmonaria* (Eaton and Ellis 2012; Larsson et al. 2012; Bidussi et al. 2013a), in areas with oceanic (Gaioliveira et al. 2004) and boreal (Gauslaa et al. 2006; Bidussi et al. 2013b) climates. On the other hand, there are only few studies focusing on the radial or relative growth rate of *X. parietina* (Richardson 1967; Moxham 1981; Honegger 1996), and none of them has been carried out in Mediterranean or sub-Mediterranean environments. Our

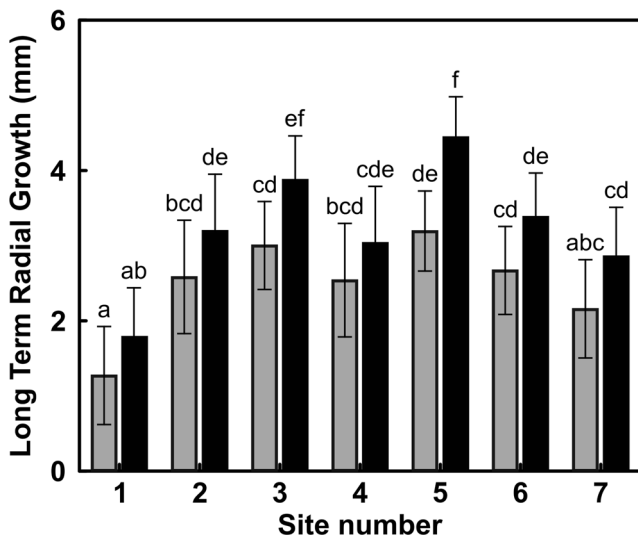


Fig. 4 Average values of site-specific long-term radial growth values of the epiphytic lichen *X. parietina* in the long-term periods LT₁ (grey bars, 400 days) and LT₂ (black bars, 504 days), in the seven sites of Fig. 1. Sites were ordered according to their increasing altitude (see Fig. 1). Whiskers indicate standard deviations. Different letters indicate significant statistical differences as evaluated by factorial ANOVA and following LSD's post hoc test

results clearly demonstrate that the seasonal radial growth of *X. parietina* increased and decreased accordingly to the seasonal rainfall (Figs. 2a and 3), which was relatively lower in spring 2015 and higher in autumn and winter 2015 and in spring 2016. This is in agreement with the fact that the growth of lichens with green algae as main photobiont is affected primarily by rainfall events (Palmqvist et al. 2008; Gauslaa 2014). Similar seasonal radial growth patterns were observed in other epiphytic foliose lichens, such as *Flavoparmelia caperata* (Fisher and Proctor 1978) and *Parmelia sulcata* (Tretiach et al. 2013). Our results as well as those reported in Fisher and Proctor (1978) revealed that the rainfall events were positively correlated with the seasonal growth rate. The

Fig. 5 Lobe exposure time as a function of the annual cumulative growth observed in six sites of Fig. 1. Data of site 1 are not reported. The horizontal grey area represents the exposure time (9.5 ± 2 months) of marginal lobes of the epiphytic lichen *X. parietina* according to the threshold (1.5–2 mm; vertical dashed lines) recommended in the sample preparation for the biomonitoring of trace elements and other airborne persistent pollutants (Nimis and Bargagli 1998). The black horizontal continuous line corresponds to an exposure time of 1 year

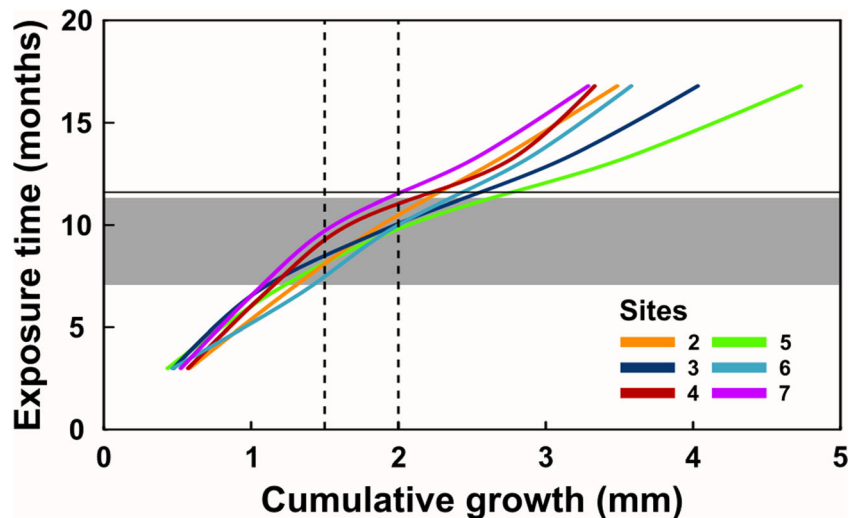


Table 2 Summary of growth rate model (GRM; see section “Data analysis”) performed on SRaGR values collected at sites 2, 4, 5 and 7 assuming altitude, distance from the sea and the first two principal factors of PCA (PC 1 and PC 2, respectively) as fixed effects and “Thallus” as random effect

	β	SE	t	P	
Intercept		6.80	0.61	11.12	0.000
Fixed effects					
<i>Altitude</i>	6.78×10^{-3}	3.2×10^{-3}	2.10	0.039	
<i>Distance from the sea</i>	-3.78×10^{-4}	2.0×10^{-4}	-1.89	0.063	
<i>PC 1</i>	-5.40×10^{-1}	7.0×10^{-2}	-7.73	0.000	
<i>PC 2</i>	-3.9×10^{-1}	1.5×10^{-1}	-2.75	0.008	
Random effect			F	P	
“Thallus”			4.82	0.028	
Overall statistics			F	P	
Model significance			53.60	<0.001	
Goodness of fit			129.75	<0.001	
$R = 0.768$					
$R^2 = 0.591$					
$r_{adj}^2 = 0.596$					
<i>Residual standard error</i>		$2.20 \mu\text{m day}^{-1}$			

The statistical significance of random effect as well as that of the GRM was tested by one-way ANOVA (see section “Data analysis”). The goodness of fit of GRM is reported as multiple regression coefficient (R), coefficient of determination (R^2) and adjusted R^2 (r_{adj}^2) calculated between predicted and observed SRaGR values. Main GLM parameters are indicated in italic-bold, whereas different fixed effects are reported in italic. β beta coefficients; SE standard error of β ; t t value of the β ; P P value; $F(10,73)$: F F statistic; W Shapiro-Wilk test for residual normality

noticeable seasonal difference of thalline radial growth observed between spring and winter 2015 (Fig. 3 and Table 1) was well explained by the different precipitation values recorded in those two periods. On the other hand, the homogeneous distribution of seasonal precipitations along the transect did not explain the observed differences in site-specific

SRaGRs within the same season (e.g. site 1 vs site 5), suggesting that further factors affected the lobe lengthening of *X. parietina* at site scale. Since *X. parietina* is a nitrophytic species, it might be argued that its growth rate may be conditioned by nitrogen availability. However, in order to minimise such potential confounding factor, we purposely selected a study area not impacted by agricultural activities and with a low, homogeneous NO_x pollution load (Regione FVG, 2007), and therefore this factor can be excluded.

Our data revealed that SRaGR was negatively correlated with *T*, DP and SD ($r < -0.40$; $p < 0.001$). Hence, the pronounced SRaGR differences between site 1 and site 5 (Fig. 3) could be explained by the site-specific air temperature, since this positively affects the environmental evaporative demand by increasing both air saturation vapour pressure and dew point. This means that thalli occurring in coastal sites were subjected to a relative higher air saturation deficit, which could rapidly reduce their water content (Kershaw 1985), and thus the time span in which lichens are photosynthetically active (Lange et al. 1986; Jonsson Čabrajić et al. 2010). In addition, hydrated thalli exposed to high temperature increase the so-called resaturation respiration, with a further reduction in net carbon gain (Green and Lange 1995; Sundberg et al. 1999).

With GRM, we tested the effects of the climatic variables—these summarised up by the first two PCs—altitude and distance from the sea on the seasonal growth of the selected *X. parietina* populations. In particular, the GRM outputs indicated a significant positive effect of altitude on the seasonal growth of *X. parietina* (Table 2), suggesting that thalli occurring at low altitude had lower SRaGR, probably in relation to the high evaporative demand observed in the coastal sites 1 and 2. This is also supported by the significant relationship between the coefficients of climatic predictors (i.e. PC1 and PC2; Table 2) and the SRaGR values predicted by GRM. In particular, PC1 was positively correlated with *T*, DP and SD and negatively correlated with RH and Ψ_{wv} , whereas PC2 was negatively correlated with seasonal cumulative precipitations (Table 2). These relationships confirmed once again that SRaGR of *X. parietina* is higher in areas with low evaporative demand, especially when humid conditions prevail as it was observed at site 5.

However, a further environmental factor limiting the growth of *X. parietina* and all other lichens is certainly the frequent, dry ENE winds, which have a noteworthy drying effect on the vegetation of the Classical Karst (Poldini 1989), and certainly increase the rate of thallus water loss from lichens. Furthermore, dry winds also limit the pre-dawn dew formation, which is the pivotal factor for the re-activation of lichen photosynthesis in the absence of rain (Lange 2003). Due to the geo-morphology of the area, the sites are not uniformly exposed to the Bora wind. For instance, this is certainly more intense at sites 6 and 7 than at sites 3, 4 and 5, as testified

by the data of anemometric stations distributed along the transect (available upon request).

In summary, our data highlighted that in the study area the yearly radial growth of *X. parietina* mostly reflects water availability, whereas differences among sites during the same season are mostly due to the different evaporative demand, which depends on topological differences in air temperatures and wind frequency and/or intensity.

Long-term radial growth and implication for biomonitoring surveys

In six sites (i.e. sites 2–7), the thalline radial growth of both long-term periods (i.e. LTRaG₁ and LTRaG₂) were similar, although the prolonged humid conditions of winter 2015 and spring 2016 promoted a higher radial growth at sites 3 and 8 than at sites 2, 4, 6 and 7. With the exclusion of site 1, anomalous for the unusually high evaporative demand, the average annual growth of *X. parietina* along the transect can be determined in $2.6 \pm 0.3 \text{ mm year}^{-1}$. This estimation is consistent with the value reported by Moxham (1981) (2.7 mm year^{-1}) for thalli occurring on concrete pavers in Bath, UK and with those of Richardson (1967), who observed an increase of the thalline radius between 3.5 and 6.7 mm over a period of 18 months in Oxford, UK. On the contrary, our annual estimation significantly differs from that of Honegger et al. (1996) ($6\text{--}7 \text{ mm year}^{-1}$) for thalli on sandstone in sub-montane environments (Switzerland).

To date, from a methodological point of view, most of the surveys based on autochthonous lichens as bioaccumulators of airborne pollutants are unfortunately still performed by sampling whole thalli of approximately the same size without discerning between old and juvenile thalline parts. The element content values derived from these samples cannot be referred to a specific period of time. However, for *X. parietina*, the standard protocol of Nimis and Bargagli (1998), as modified by Bargagli and Nimis (2002), recommends to measure element concentrations in the juvenile outermost portions of thalli, i.e. the last 2 mm from the lobe tips. This lobe length would roughly correspond, in the intention of the authors, to the last year of growth. By strictly applying this protocol in our study area, the lichen material collected at site 1 would be 14 ± 2 months old, whereas the material collected at the other sites would be only 9.5 ± 2 months old (Fig. 5), due to the different yearly growth rate. In this study, the estimated average daily radial growth rate of *X. parietina* thalli along the transect was $7 \mu\text{m day}^{-1}$. Hence, in order to select lichen material grown exactly in the last year, we should select the last 2.6 mm of the thallus margin (see above). If the error of this sampling is $\pm 0.1 \text{ mm}$, fully compatible with a careful manipulation under a stereomicroscope, the error of the estimated exposure time (age) would be limited to ± 15 days.

In light of all these results, it seems appropriate to suggest that surveys based on autochthonous lichens as bioaccumulators of trace elements should preferentially be limited to species whose lobe lengthening is easily measurable. Moreover, particular attention should be paid when passive biomonitoring surveys are performed in areas with spatially heterogeneous conditions of evaporative demand, because it is mandatory to reduce potential differences in the growth rates of thalli. In this way, the comparison of samples with comparable exposure periods would be ensured. This is of primary importance when the aim of the study is to monitor the activity of a point source pollution in a given period of time or when the aim is to test the predictability of pollutant dispersion models, because these simulations are referred to specific periods of time, usually 1 or 2 years. Finally, it is worth noting that much more work is needed to estimate the growth of those lichens which are used as biomonitors of airborne pollutants, because quantitative data are missing for most of them and several growth-related issues must still be clarified.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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