Species composition of freshwater lichens in temperate mountain streams: the effect of site, habitat and local spatial isolation

Druhové složení lišejníků v temperátních horských potocích: vliv lokality, stanoviště a prostorové izolace

Beata Krzewicka[1], Natalia Matura[1], Edyta Adamska[2] & Piotr Osyczka[3]*

[1]*W. Szafer Institute of Botany Polish Academy of Sciences, Lubicz 46, 31-512 Kraków, Poland;* [2]*Nicolaus Copernicus University, Department of Geobotany and Landscape Planning, Faculty of Biology and Veterinary Sciences, Lwowska 1, 87-100 Toruń, Poland;* [3]*Institute of Botany, Faculty of Biology, Jagiellonian University, Gronostajowa 3, 30-387 Kraków, Poland, e-mail: piotr.osyczka@uj.edu.pl*
**corresponding author*

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Lichens associated with aquatic and semi-aquatic habitats are a specific ecological group of symbiotic organisms. Distribution patterns, especially those of freshwater lichens and factors determining their occurrence, are poorly recognized. The species richness and composition of lichens were studied in the splash and submerged zones of Carpathian mountain streams. Habitat parameters, including pH, water conductivity, dissolved oxygen content, silting and light intensity at sampling sites, were used in the analysis. The streams differed greatly in terms of the species composition; only three lichens (*Thelidium minutulum*, *Verrucaria hydrophila* and *V. praetermissa*) of the entire pool of 29 recorded species were found in all streams. This fact does not directly relate to the habitat parameters measured either at the level of individual streams or considering all the streams studied. Instead, the differences in the species composition of lichens increased with the geographical distance between streams, even locally. This means that the occurrence of lichens in mountain streams is strongly site-dependent and the variability in the habitat is of less importance for species presence. Presumably lack of effective natural vectors and weak dispersal ability are strong limiting factors for freshwater lichens. Nevertheless, increased ion concentration in water can considerably promote the development of the thalli of some species of lichens, as in the case of *Verrucaria praetermissa*, while it can be a limiting factor for others, as in the case of *V. hydrophila*.

Keywords: aquatic habitat, distribution, ecology, flysch watercourses, lichenized fungi, species diversity

Introduction

Understanding the combined effect of environmental factors on organisms is still regarded as one of the greatest deficiencies in the ecological conservation of water environments (Sala et al. 2000, Darling & Côté 2008). Aquatic lichens, especially freshwater ones, are one of the least studied groups (Tierno de Figueroa et al. 2013). The occurrence of species is usually reported, but rarely data on their habitat preferences and distribution are available (Rosentreter 1984, Gilbert 1996, Thüs 2002, Harada & Wang 2006,

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Nascimbene & Nimis 2006, Krzewicka & Hachułka 2008, Krzewicka 2009). Lichens constitute a symbiotic association of two units, a lichen-forming fungus (mycobiont) and an alga and/or a cyanobacterium (photobiont), forming a combined organism (Ahmadjian 1993). The vast majority of lichens colonize terrestrial ecosystems and are incapable of surviving under strongly hydrated conditions (Thüs et al. 2014). However, there are species that occur in aquatic or semi-aquatic freshwater and marine habitats. Lichen species directly related to water constitute a relatively small group worldwide (Thüs & Schultz 2009). Due to high demands on the water clarity and sensitivity to pollution, many of the aquatic lichen species are considered to be endangered or rare in some regions of Europe (Cieśliński et al. 2006, Nascimbene et al. 2007, Liška et al. 2008, Krzewicka et al. 2017, Matura 2020). They constitute about 6.5% of the global number of lichen species but only few (approximately 1.5%) occur in freshwater (Thüs et al. 2014). Certain species of lichens in this ecological group seem to be non-specific and widespread (see e.g. McCarthy 1991, Harada 1996, Krzewicka 2012, Orange 2014); on the other hand, species richness and composition often differs in adjacent natural-flowing watercourses and the factors affecting the local distribution of such lichens are poorly known (Pentecost 1977, Gilbert & Giavarini 1997, Krzewicka et al. 2017).

Lichen biota associated with mountain rivers and streams is characterized by zonal distribution of species, which is generally determined by the distance from the main current (Santesson 1939, Ried 1960, Gilbert 1996, Krzewicka & Galas 2006, Coste 2010, Krzewicka et al. 2017). Depending on the seasonal dynamics of the water flow, splashing intensity and duration of flooding resulting in the submergence of thalli, various patterns of zonation have been distinguished. Based on the duration of the annual floods in submontane and montane streams, Ried (1960) distinguished four main zones of occurrence of lichens relating to water requirements and tolerance of a species, i.e. aquatic lichens, amphibious lichens, tolerant of submergence lichens, and terrestrial lichens clearly sensitive to submergence. Gilbert (1996) recognized various zones on the basis of the duration of the total submergence of lichen thalli. On the other hand, Coste (2010) designated zones based on the duration of the periods for which they remained dry or in close contact with water. The dynamics of water flow in mountain streams in central Europe is usually high and the complete drying of the bedrock in the bed of a stream is very rare. A simple division based on thallus intimacy with water or exposition to air is often used (Nascimbene et al. 2007, Krzewicka et al. 2017). In this approach, the first zone usually comprises the permanently submerged bottom of a stream and the second the splashed or occasionally flooded and wet rocks and stones on the lower bank (Krzewicka et al. 2017).

In addition, to the dynamics of stream currents, it is widely believed that the diversity of freshwater lichens may be largely dependent on various other habitat factors, including the type and stability of the substrate (Thüs 2002, Krzewicka & Galas 2006), light availability (Pentecost 1977, Nascimbene & Nimis 2006), degree of silting (Gilbert 1996, Gilbert & Giavarini 1997) and physical and chemical parameters of the water (Nascimbene & Nimis 2006, Nascimbene et al. 2009, Krzewicka et al. 2017). However, the actual effect of basic water and external habitat parameters still remains poorly studied. Pentecost (1977) was the first to draw attention to the fact that the occurrence of species and development of thalli are strongly determined by the mineral composition of water. Strong attachment to a given type of substrate, either alkaline (calcareous rocks) or acidic (granite crystalline rocks), is often an attribute of epilithic lichens (Thüs 2002). However, the presence of typically acidophilous lichens on alkaline substrates in acidic water has also been reported for freshwater lichens (Gilbert & Giavarini 1997).

During field research on freshwater lichens in mountain streams, we observed that despite apparently similar habitat conditions and close proximity of streams, the species composition of lichen assemblages differed considerably. The main purpose of this study was to examine the freshwater lichen assemblages associated with mountain streams in terms of their richness, species composition and habitat requirements. We tested the following hypotheses: (i) the differences in the composition of assemblages of lichens between streams are due to differences in the habitat conditions they provide; and (ii) the pattern in the distribution of the species in streams is largely determined by changing habitat conditions downstream. In addition, we intended to identify lichens with a narrow ecological amplitudes to define specific conditions they prefer and to verify differences in the species richness and composition between the submerged and splash zones. The final purpose of this study was to verify whether the abundance of the thalli of selected common lichens in a stream can serve as an indicator of water quality.

Material and methods

Study area

Mountain streams in the Beskid Sądecki Mts (Western Carpathians), which consist of sedimentary rocks, known as the Carpathian flysch, were studied. Sandstones, shales and marl deposited in this area were folded in the Tertiary forming a dam (Birkenmajer & Oszczypko 1989). The streams and main rivers in the Beskid Mts all flow northwards to the Baltic Sea. The river system is characterized by a high density and its development is favoured by high rainfall, considerable slope in the terrain and a low permeability of the flysch substrate. The density of the river system ranges from 1.5 to 4.0 km per km^2 and the fall in the streams can reach 130‰. A high variability in the level of water is typical of Carpathian rivers and streams. Flooding in the study area occurs mainly in two seasons: in spring during the melting of the snow cover and in summer, usually in late June and early July, caused by heavy rain. The water level fluctuates up to 3 m in small streams and even up to 4 m in large streams in the Beskid Mts (Ziemońska 1973, Radecki-Pawlik 2006).

Field studies and data sampling

Ten mountain streams were selected for study (the abbreviations given here are used subsequently in this paper): Potok Baraniecki (BA), Potok Bliszcze (BL), Potok Czaczowiec (CZ), Potok Kozłecki (KO), Potok Młodowski (ML), Potok Przysietnica (PR), Potok Szczawniczek (SZ), Potok Uhryński (UH), Potok Wierchomlanka (WI) and Potok Wojkowski (WO) (Electronic Appendix 1). They flow through the lower mountain zone covered by seminatural beech and mixed coniferous forests and are not directly affected by human settlement and agriculture.

The research was carried out in summer 2017 when the weather was relatively uniform and stable. Data was collected after a minimum of a three-day rainless period. The sampling sites were nearly equally spaced out and included the upper, middle and lower parts of each stream. Altogether, data from 43 sampling sites was collected. Each site included approximately a 20 meter section of a stream. Lichen thalli were examined in two hydrological zones: the submerged zone (A; permanently submerged stream bottom, aquatic habitat) and splash zone (B; permanently splashed and frequently flooded rocks on lower bank of stream). The number of individuals of each species was estimated at each sampling site. The entire surface of each site was thoroughly examined, including all substrate types (boulders, rocks, small stones, rocky substrates, solid surfaces).

Identification of the species

Specimens collected were identified using standard lichenological techniques based on the appropriate keys and taxonomic treatments (i.e. Smith et al. 2009, Thüs & Schultz 2009, Krzewicka 2012, Orange 2013, Wirth et al. 2013). Specimens are housed at the herbarium of the W. Szafer Institute of Botany, Polish Academy of Sciences in Kraków (KRAM) and are available on request from the curator.

Habitat parameters measured

The following parameters, routinely used as baseline indicators of water quality, were measured and included in subsequent analyses: pH, electrical conductivity of water [µS/cm] (HI 9811-5 meter, Hanna Instruments), dissolved oxygen content [%] (CO-105 oxygen meter, Elemtron) and silting [% of the rock surface]. In addition, light intensity (photosynthetic active radiation; $\mu mol\ m^{-2}\ s^{-1}$) was measured at each sampling site using Kipp & Zonen PAR Quantum Sensor. Five measurements at different points at each site were taken on three consecutive days and the mean value was considered as one observation. Measurements were made in the morning on a clear day (previous three days rainless). The percentage of the surface covered with silt is the mean value of the measurements taken in a straight line across each stream in an area of 1 m^2 at sites on the left and right hand bank and in the middle of the stream.

Evaluation of the abundance lichen thalli

The abundance of thalli at the sampling sites was estimated for three hygrophilous species, i.e. *Thelidium minutulum*, *Verrucaria hydrophila* and *V. praetermissa*. These lichens form relatively well visible and recognizable thalli and are commonly found in Carpathian streams (Krzewicka et al. 2017, Matura 2020, see also Table 1). The percentage of the host substrate covered by the thallus of a species in an area of 25 cm^2 was assessed in the splash and submerged zones using a 5 × 5 cm frame. The frame was positioned on the surface of a stone so that most of the thallus was contained within it.

Table 1. – List of lichen species associated with mountain streams in the Beskid Sądecki Mts (Western Carpathians) and their general characteristics. Functional groups: crust. sex. – crustose growth form and mainly sexual reproductive strategy; per – perithecia; ap – apothecia; green algae – other than *Trentepohlia,* mainly *Trebouxia*. Habitat: Sub – submerged zone (A), Spl – splash zone (B); I, II, III – habitat classes (Hab-I, Hab-II, Hab-III), for more details see Fig. 2; ○ absent, ● present. Zone preference: based on data obtained from this study. Red list category: CR – critically endangered, EN – endangered, VU – vulnerable, NT – near threatened, LC – least concern, DD – data deficient ▶

Species	Species abbreviations	Functional group / Photobiont	Occurrence in habitat classes		Zone preference	Frequency in relation to		Category in Red list of lichens in Poland
			Sub (zone A)	Spl (zone B)		Streams	Sampling sites	
Bacidina inundata (Fr.) Vězda	Bac inu	crust. sex. ap/ green algae	I○ II○ III○	I● II● III●	spl (B)	50%	19%	
Hydropunctaria rheitrophila (Zschacke) C. Keller, Gueidan et Thüs	Hyd rhe	crust. sex. per/ green algae	I● II● III●	I● II○ III○	mainly sub (A)	30%	9%	VU
Ionaspis lacustris (With.) Lutzoni	Ion lac	crust. sex.ap/ green algae	I○ II○ III○	I○ II● III○	spl (B)	10%	2%	DD
Porina guentheri (Flot.) Zahlbr.	Por gue	crust. sex. per/ *Trentepohlia*	I○ II○ III○	I○ II● III○	spl (B)	10%	2%	CR
Porpidia crustulata (Ach.) Hertel et Knoph	Por cru	crust. sex. ap/ green algae	I○ II○ III○	I○ II● III○	spl (B)	10%	2%	
Thelidium aquaticum Servít	The aqu	crust. sex. per/ green algae	I○ II○ III○	I● II○ III○	spl (B)	20%	5%	DD
Thelidium klementii Servít	The kle	crust. sex. per/ green algae	I● II○ III●	I○ II○ III○	sub (B)	20%	5%	
Thelidium minutulum Körb.	The min	crust. sex. per/ green algae	I● II● III●	I● II● III●	sub (A)/spl (B)	100%	56%	NT
Thelidium rehmii Zschacke	The reh	crust. sex. per/ green algae	I○ II○ III○	I● II● III○	spl (B)	20%	5%	
Thelidium zahlbrucknerii Servít	The zah	crust. sex.per / green algae	I○ II● III○	I● II● III●	mainly spl (B) possible sub (A)	30%	21%	
Thelidium zwackhii (Hepp) A. Massal.	The zwa	crust. sex.per/ green algae	I● II● III○	I● II● III●	sub (A)/spl (B)	50%	21%	
Trapelia coarctata (Sm.) M. Choisy	Tra coa	crust. sex. ap/ green algae	I○ II○ III○	I● II● III○	spl (B)	30%	7%	
Verrucaria aethiobola Wahlenb.	Ver aet	crust. sex. per/ green algae	I○ II○ III○	I● II● III○	spl (B)	20%	5%	EN
Verrucaria aquatilis Mudd	Ver aqu	crust. sex. / green algae	I● II● III●	I● II● III●	sub (A)/spl (B)	70%	42%	VU
Verrucaria cernaensis Zschacke	Ver cer	crust. sex. per/ green algae	I○ II○ III○	I● II○ III●	spl (B)	20%	5%	

Species	Species abbreviations	Functional group / Photobiont	Occurrence in habitat classes		Zone preference	Frequency in relation to		Category in Red list of lichens in Poland
			Sub (zone A)	Spl (zone B)		Streams	Sampling sites	
Verrucaria devensis Orange	Ver dev	crust. sex. per/ green algae	I○ II○ III○	I● II○ III●	spl (B)	20%	5%	
Verrucaria dolosa Hepp	Ver dol	crust. sex. per/ green algae	I● II○ III○	I● II● III●	mainly spl (B) possible sub (A)	50%	16%	
Verrucaria elaeina Borrer	Ver ela	crust. sex. per/ green algae	I○ II○ III●	I● II● III●	mainly spl (B) possible sub (A)	60%	30%	
Verrucaria elaeomelaena (A. Massal.) Arnold	Ver ena	crust. sex. per/ green algae	I○ II○ III○	I○ II● III●	spl (B)	20%	5%	
Verrucaria funckii (Spreng.) Zahlbr.	Ver fun	crust. sex. / green algae	I○ II○ III○	I○ II● III●	spl (B)	20%	5%	
Verrucaria humida Orange	Ver hum	crust. sex. per/ green algae	I● II○ III●	I● II○ III●	sub (A)/spl (B)	40%	14%	
Verrucaria hydrophila Orange	Ver hyd	crust. sex. per/ green algae	I● II● III●	I● II● III●	sub (A)/spl (B)	90%	60%	VU
errucaria margacea (Wahlenb.) Wahlenb.	Ver mar	crust. sex. per/ green algae	I○ II○ III○	I● II● III●	spl (B)	60%	16%	
Verrucaria muralis Ach.	Ver mur	crust. sex. per/ green algae	I○ II○ III○	I● II● III●	spl (B)	60%	23%	
Verrucaria murina Leight.	Ver ina	crust. sex. per/ green algae	I○ II○ III○	I● II● III●	spl (B)	50%	19%	DD
Verrucaria praetermissa (Trevis.) Anzi	Ver pra	crust. sex.per / green algae	I● II● III●	I● II● III●	sub (A)/spl (B)	100%	74%	NT
Verrucaria sublobulata Eitner ex Servít	Ver sub	crust. sex. per/ green algae	I● II○ III○	I● II● III●	mainly spl (B) possible sub (A)	80%	26%	DD
Verrucaria submauroides Zschacke	Ver des	crust. sex. per/ green algae	I○ II○ III○	I● II● III●	spl (B)	60%	21%	
Verrucaria submersella Servít	Ver lla	crust. sex. per/ green algae	I○ II○ III○	I○ II● III○	spl (B)	20%	5%	

Data processing and statistical analysis

Cluster analysis using a hierarchical clustering routine based on an unweighted pair-group average (UPGMA) algorithm was used to group the sampling sites according to their pH, water conductivity, dissolved oxygen content and silting. Non-metric multidimensional scaling (NMDS) was used (Taguchi & Oono 2005) for the same purposes. As an external factor not directly related to aquatic attributes, light intensity at the sampling sites was not included in these analyses. Sampling sites were assigned to one of three general habitat classes: Hab-I, Hab-II and Hab-III (Fig. 1) based on the comparison of the results.

The Kruskal-Wallis test followed by Dunn's post hoc test were used to test the differences in parameters across the streams. After Levene's test, used to assess the equality of variances, a one-way analysis of variance (ANOVA), together with Tukey's test (the unequal n HSD modification) for posterior comparisons, were performed in order to reveal significant differences in parameters across habitat classes. Prior to this, the normality of the distribution of the variables was verified using the Kolmogorov-Smirnov test. The variables were Box-Cox-transformed if necessary.

The seriation of species presence/absence at all sampling sites was done using a constrained algorithm (Brower & Kile 1988). The Mantel test (Mantel & Valand 1970) was used for the correlation between geographical distance and lichen species composition dissimilarity matrices. Coordinates of the sampling sites that were located in the middle part of the streams and the presence/absence of species in individual streams were considered.

The correlation matrix was computed in order to determine the relationship between all habitat parameters. In addition, relationships between values of all parameters measured at sampling sites and the distance of these sites from the source of each stream were evaluated by means of Pearson correlation coefficient (R). Since some significant differences in water conductivity and dissolved oxygen content were revealed between the streams, the coefficients were calculated separately for streams with comparable values of these parameters in accordance with the results of Dunn's test.

Non-metric multidimensional scaling (NMDS) was used to determine the similarities between examined sampling sites in terms of lichen composition. This was done both for species occurrence in habitat classes and streams. The presence or absence of a species was a priority and the scaling was based on the Jaccard coefficient. Detrended correspondence analysis (DCA) was used to determine the association of individual lichens with different habitat types, i.e. habitat classes (Hab-I, Hab-II and Hab-III) and hydrological zones (A and B). This analysis was done using the mean frequency of species calculated for particular habitat types. Canonical correspondence analysis (CCA; Legendre & Legendre 1998) was used to relate the abundance of individual lichens to habitat parameters measured at the sampling sites. A Monte Carlo permutation test based on 9999 random permutations was done in order to assess the statistical significance of the relationships between species and habitat factors as well as canonical axes (ter Braak & Šmilauer 2002).

Relationships between thallus abundance and habitat parameters at the sites where lichens were growing were estimated using Pearson correlation coefficient. The analyses were done for both hydrological zones (A and B). Statistical calculations were carried out using CANOCO 5, Past 3.21 (Hammer et al. 2001) and STATISTICA 12.

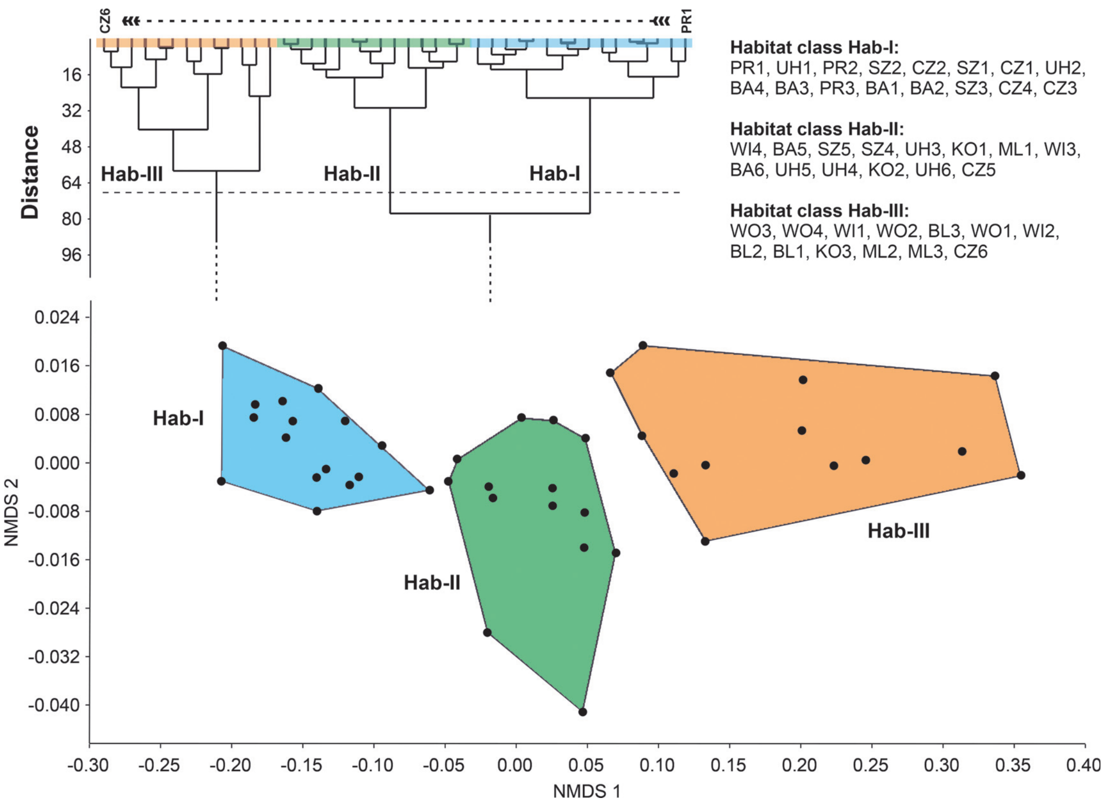


Fig. 1. – Cluster analysis dendrogram (UPGMA) and non-metric multidimensional scaling scatterplot (NMDS) of the sampling sites representing three different habitat classes (Hab-I, Hab-II, Hab-III) separated by pH, conductivity, dissolved oxygen content and silting. The affiliation of sampling sites to a habitat class is provided alongside the dendrogram; sampling sites are listed in order of appearance on the dendrogram. See Material and methods for stream abbreviations and Electronic Appendix 1 for stream locations.

Results

Variability of the habitat

Although there were significant differences in the conductivity and dissolved oxygen content of the water in the streams the parameters measured were similar (Electronic Appendix 1). A dendrogram and NMDS diagram revealed three distinct groups, accordingly the sampling sites were assigned to the hydrological habitat classes: Hab-I, Hab-II and Hab-III (Fig. 1). Water pH did not differentiate significantly between them, however, pH in Hab-III was slightly higher than in Hab-I and Hab-II. Water conductivity varied significantly between the classes; the lowest was recorded in Hab-I and the highest in Hab-III. Dissolved oxygen content was similar in Hab-I and Hab-II but different in Hab-III, whereas degree of silting was similar in Hab-II and Hab-III but different in Hab-I (Fig. 2). Light-exposure at the sampling sites varied and, although more shady localities occurred more frequently in Hab-I (Fig. 2), insolation did not affect the classification. The same class could be found to occur at different streams and between two and three

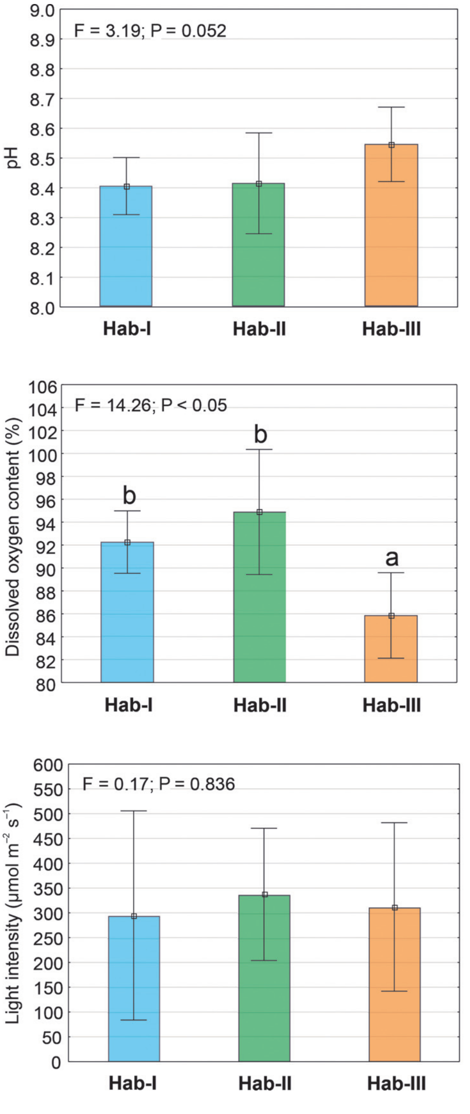


Fig. 2. – Box-and-whisker plots of the parameters of the habitat classes (Hab-I, n = 16; Hab-II, n = 14; Hab-III, n = 13); points indicate mean values, whiskers indicate standard deviations. Results of ANOVA (F- and P-values) and the Kruskal-Wallis test (H- and P-values) are provided. Letters denote the results of Tukey's test and Dunn's test with different letters indicating significant differences for $P < 0.05$.

habitat classes occurred along the same stream. Habitat conditions along some streams were, however, homogenous and all the sampling sites along these streams belonged to only one habitat class. For instance, only Hab-I was recorded in the PR stream whereas in the BL and WO streams it was only Hab-III (Fig. 1).

There was a significant positive correlation between pH, water conductivity and light intensity at the sampling sites and their distance from the source (Electronic Appendix 1). This means that values of chemical parameters gradually increased downstream and shading decreased. The pH value and conductivity of water were generally correlated positively with each other ($R = 0.39$; $P < 0.05$) while dissolved oxygen content correlated negatively with conductivity ($R = -0.50$; $P < 0.05$). Local siltation was not found to correlate with other factors.

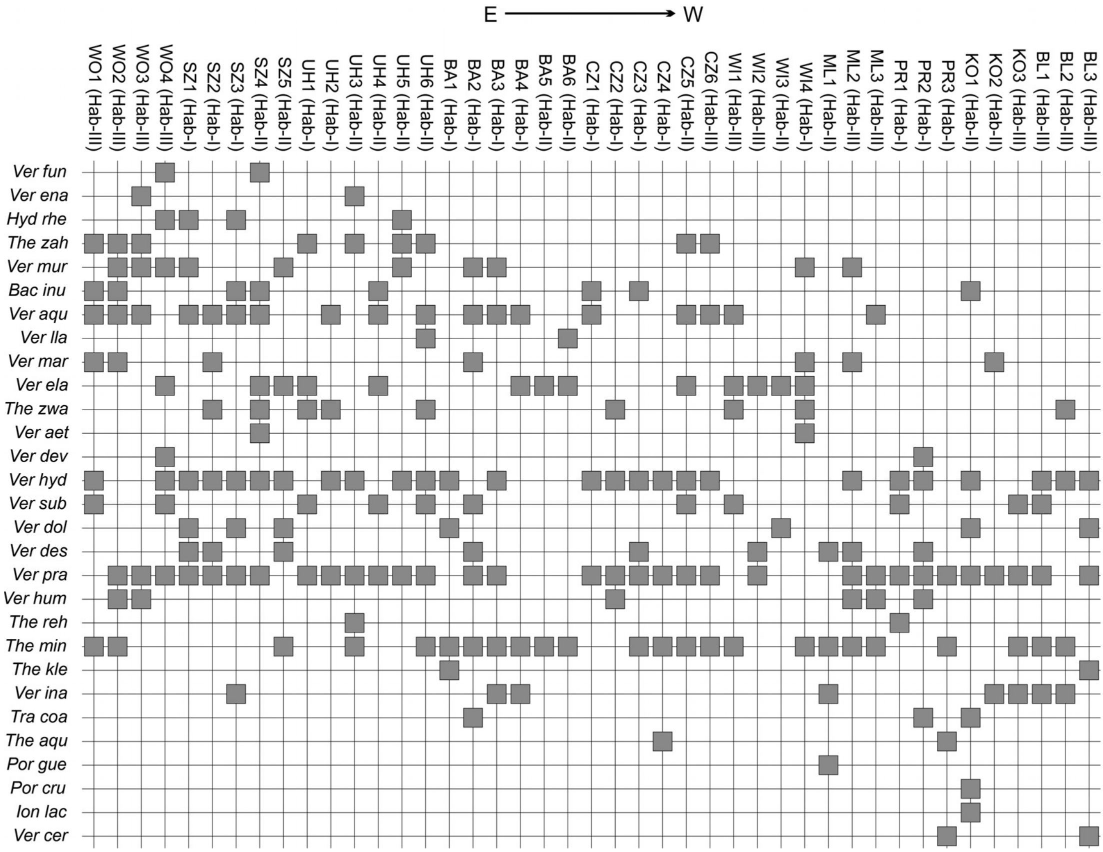


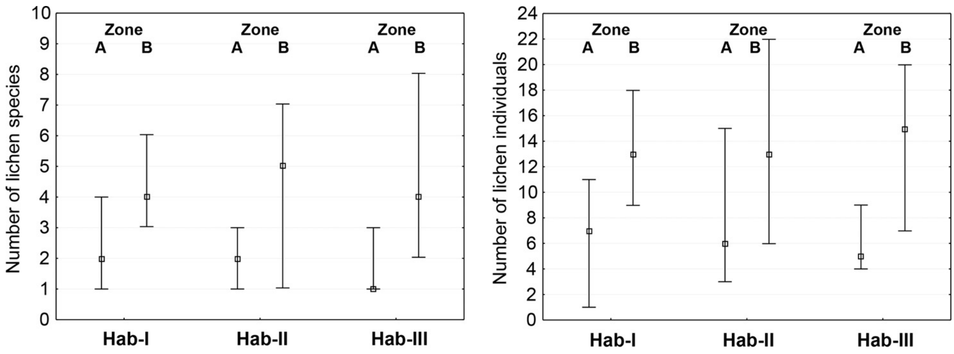
Fig. 3. – The seriation diagram of the species absence-presence at the sampling sites of particular streams; habitat classes are given in parentheses. The streams are arranged from east to west. See Table 1 for species abbreviations, Material and methods for stream abbreviations.

Richness of lichens in terms of species

A total of 29 hygrophilous lichens were recorded (see also Fig. 3 and Electronic Appendix 1); the number of species ranges from nine to 15 in individual streams. The richness of the lichen biota did not differ greatly between habitat classes. However, the number of species and individuals and the Shannon index were generally twice as large in the splash zone (B) than in the submerged zone (A). This disparity between zones was the smallest in Hab-I and greatest in Hab-III (Fig. 4). Twelve species were found to be able to exist permanently under water and one lichen, *Thelidium klementii*, was recorded only in this habitat.

Patterns of lichen species composition

NMDS diagrams illustrate general patterns of similarity between the sampling sites for the composition of lichens across habitat classes and streams. A relatively continuous



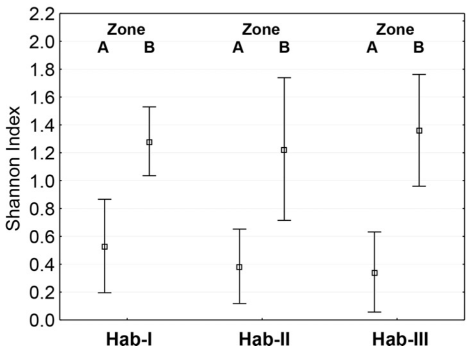


Fig. 4. – Richness of the lichen biota in habitat classes (Hab-I, Hab-II, Hab-III) expressed as the number of lichen species, the number of lichen individuals and the Shannon diversity index. Points indicate the median, whiskers indicate minimum and maximum; data are presented separately for the submerged (A) and splash (B) zones.

distribution of sites is depicted in Electronic Appendix 1 and groups relating to specific habitat classes are not differentiated. Points on the diagrams are unorganized and the classes overlap. The greatest dispersion is recorded for Hab-II sites while most of the sites within Hab-I and Hab-III classes are contained in Hab-II.

Differences in the lichen biota are more apparent for individual streams as shown in Electronic Appendix 1. Non-adjacent streams such as WO and BL or SZ and KO are also clearly separated in the diagram while points representing sampling sites that are close to one another such as WO, SZ, UH or PR, KO, BL are also close together on the diagram. The species composition at the sampling sites along WI differed from that for other streams and these sites are completely separate.

According to the Mantel test, the correlation coefficient between all the entries in the geographical distance matrix and lichen species composition dissimilarity matrix was positive and significant; $R = 0.52$, $P < 0.05$. Dissimilarity in the species composition of lichens in streams increased as the geographical distance between them increased (Fig. 5); the species composition of adjacent streams were the most similar.

Habitat preferences of lichens

The DCA determined the general direction and range in the variability of lichen vegetation in the habitat classes and hydrological zones (Fig. 6). The eigenvalues of axes 1 and 2 were 0.26 and 0.05, respectively. Only four lichens (*Hydropunctaria rheitrophila*,

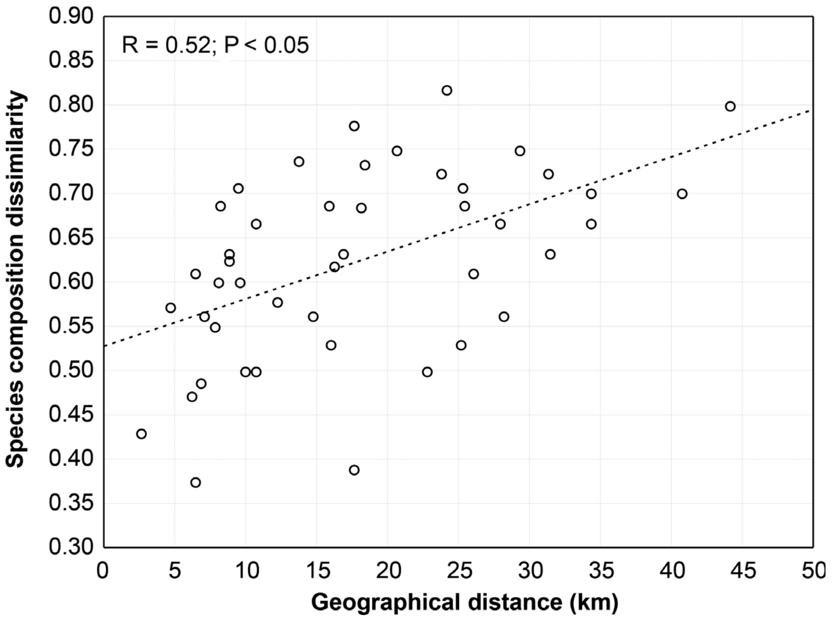


Fig. 5. – The result of the Mantel test; the correlation between species composition dissimilarity and geographical distance matrices for the streams.

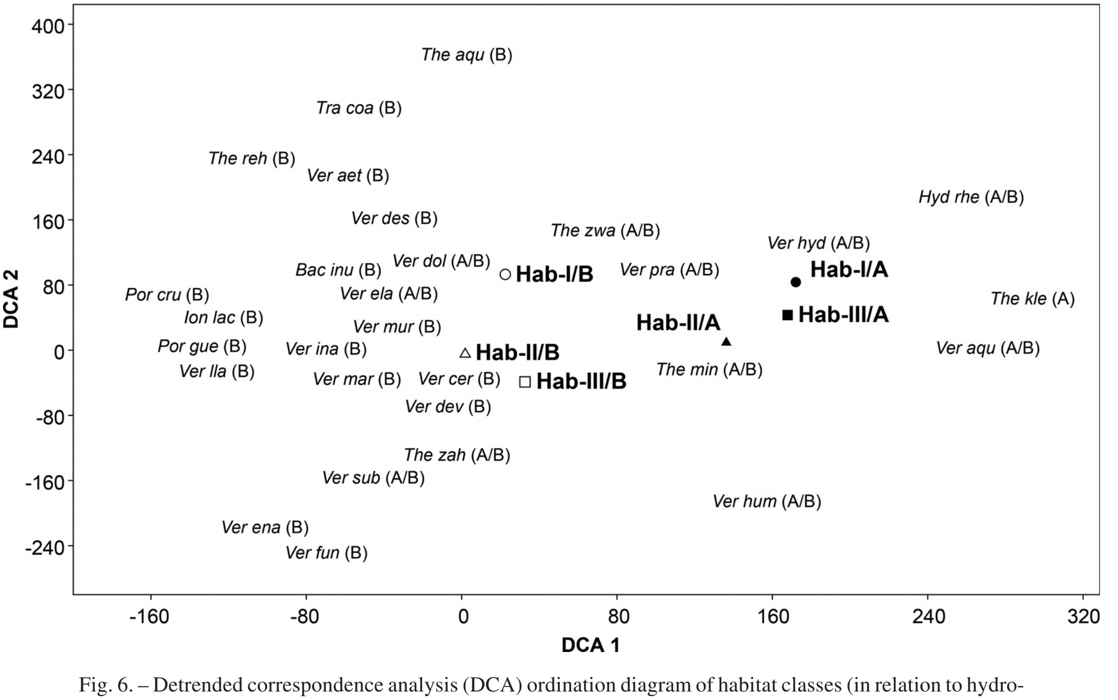


Fig. 6. – Detrended correspondence analysis (DCA) ordination diagram of habitat classes (in relation to hydrological zones; Hab-I/A, Hab-II/A, Hab-III/A and Hab-I/B, Hab-II/B, Hab-III/B) and associated species of lichens. The zone (A and/or B) in which a species of lichen was recorded is given in parentheses. See Table 1 for species abbreviations.

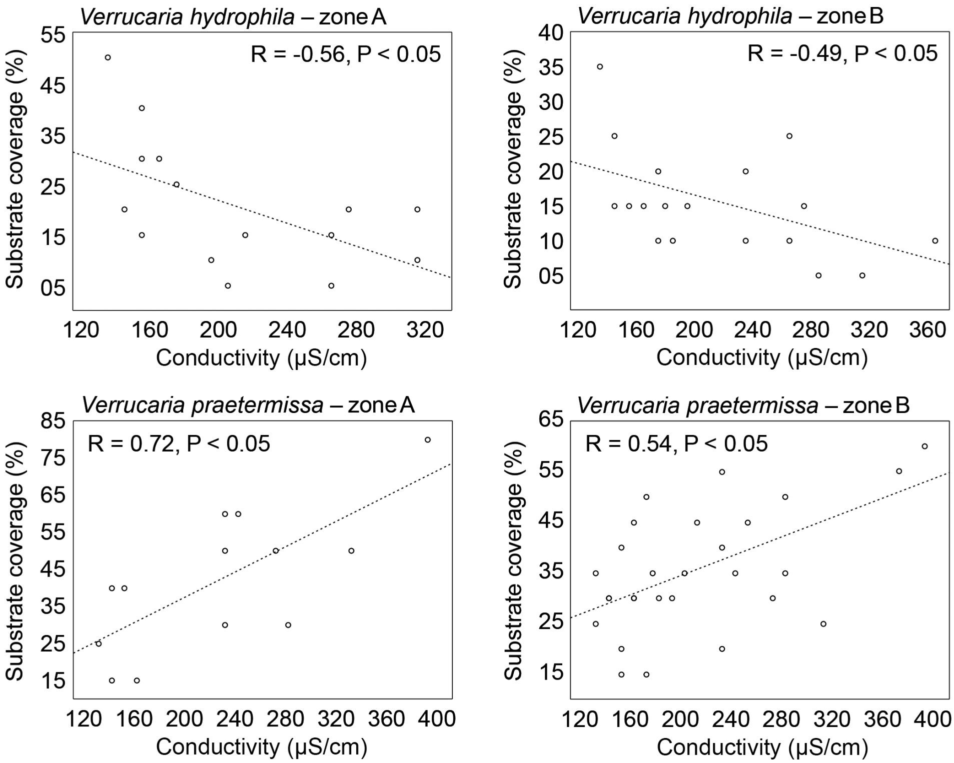


Fig. 7. – Scatterplots showing the relationship between the abundance of lichen thalli at the sampling site (average percentage coverage of 25 cm^2 stone surface by thallus) and conductivity of water measured at this site; Pearson coefficients (R) and P values are provided.

Thelidium klementii, *Verrucaria aquatilis*, *V. hydrophila*) were strongly associated with the submerged zone (Hab-I/A, Hab-II/A, Hab-III/A). Most of the other species are scattered on the left side of the graph and occurred mostly (or only) in the splash zone (Hab-I/B, Hab-II/B, Hab-III/B). It is difficult to determine accurately the affinity of some lichens to a certain habitat class; however, the species in the upper part of the graph are more associated with Hab-I whereas those on the lower part with Hab-II and Hab-III.

The relationship between the abundance of lichen species and habitat parameters was verified by CCA (Electronic Appendix 1). The Monte Carlo permutation test revealed that both the first axis and all canonical axes together were statistically significant (P = 0.042 and P = 0.040, respectively). The eigenvalue of axes 1 and 2 were 0.18 and 0.14, respectively.

Responses of lichens to habitat factors

Potential effects of the habitat parameters on the abundance of thalli of three common lichens (*Verrucaria hydrophila*, *V. praetermissa*, *Thelidium minutulum*) at particular sampling sites were examined. Significant relationships ($P < 0.05$) were found only for water conductivity (Fig. 7). Pearson correlation coefficients revealed that *V. hydrophila* and *V. praetermissa* responded markedly to water conductivity although in different ways. Responses were detected in both zones and have the same direction. Thalli of *V. hydrophila* decreased in size as conductivity increased; R = –0.56 and R = –0.49 for zones A and B*,* respectively. Thalli of *V. praetermissa* increased in size with increase in conductivity; R = 0.72 and R = 0.54 for zones A and B, respectively, with the positive correlation being particularly evident in the submerged zone (A). The size of *T. minutulum* thalli was not dependent on the conductivity of water in either zone (R = –0.30, P = 0.37; for zone A and R = –0.25, P = 0.29; for zone B).

Discussion

Habitat conditions in streams at a local-scale

Structural diversity of the habitat, often referred to as habitat heterogeneity or complexity, reflects the range of available resources used by species with different niche requirements and, therefore, determines species diversity in a certain environment (Gorman & Karr 1978, Lepori et al. 2005, Laub et al. 2012). Various environmental factors differently affect different groups of organisms. In freshwater habitats, lichens are influenced by several factors related to the duration of submergence, light availability, lithology and stability of substratum, and water chemistry. The substrate is particularly important for lichens and their occurrence in streams depends on the type of bedrock and size of the rocks (Keller 2005, Krzewicka & Galas 2006, Nascimbene & Nimis 2006, Nascimbene et al. 2009). Some aquatic lichens, although inherently saxicolous, are able to inhabit submerged roots in places with little rocky substrate (Motiejűnaitë 2003, Hachułka 2011). The increase in geomorphological diversity of the structure of stream beds by the presence of large boulders, rocky thresholds, gravel, rocky banks or logs, backwaters (abandoned channels and chutes) will increase the diversity of the space that could be colonized by lichens (Thüs & Schultz 2009). The morphology of the streams studied was similar as it was shaped mainly by Carpathian flysch (Birkenmajer & Oszczypko 1989). A wide spectrum of more or less stable rocky forms, such as boulders, rocks, small stones, thresholds and concrete culverts, occur along their entire length providing potentially suitable substrates for various species of lichens.

We identified three distinct habitat classes based on fundamental and routinely measured parameters that are easy to determine in the field, which may affect the occurrence of freshwater lichens (Fig. 1). In Hab-I there is a low concentration of ions in the water and little silt on the bedrock. On the other hand, Hab-III is characterized by approximately twice the concentration of ions, lower dissolved oxygen content, a relatively higher pH value and usually more pronounced silting (Fig. 2). The first class (Hab-I) is associated with the upper part of the streams of BA, CZ, PR, SZ and UH while all the sampling sites along BL, KO, ML, WI and WO, from their source to the lower stretches were classified in Hab-III or Hab-II/Hab-III classes (Fig. 1). WO and BL have an electrolytic conductivity inherently higher than other flysch streams in very good ecological state and of uniform chemical composition (e.g. Policht-Latawiec et al. 2014). Nevertheless, we assume that the habitat parameters measured are representative of natural state undisturbed streams in the area studied. A gradual increase in pH and water conductivity downstream and the high dissolved oxygen content recorded at the sampling sites, usually oscillating around 90% (Electronic Appendix 1), clearly indicate that the changes in parameters result from natural processes, e.g. soluble nature of water, geology, rain and evaporation (Wysocka-Czubaszek & Wojno 2014). Light intensity differs between sampling sites and insolation is not directly related to the habitat class (Fig. 2). However, some of the sampling sites close to the source of the streams were often more shaded than those in the lower parts of the streams (Electronic Appendix 1). This most probably results from the local topography, with the channel recessed more into the slope and a denser tree canopy.

Habitat preferences and distribution of freshwater lichens

The lack or scarcity of data on the occurrence of aquatic species in many parts of the world, especially Africa and South America, is presumably due to insufficient recognition of the freshwater lichen biota than to the actual absence or rarity of certain lichens. Therefore, it is difficult to compare various geographical regions in terms of species diversity at either continental or regional scales. Nevertheless, the range in species richness recorded by us in the streams in the Beskid Sądecki Mts (from nine to 15) is similar to that reported from other mountain regions in Europe, such as the Alps (Nascimbene et al. 2007) or Eastern Carpathians (Krzewicka et al. 2017); thus, the biodiversity of freshwater lichens can be considered high in this area. Although three habitat classes were distinguished, they are all in the first class of water purity (Kancelaria Sejmu RP 2011). High diversities of lichens are recorded in watercourses characterized by pure water whereas the diversity is low in those subject to eutrophication (Nascimbene et al. 2013, Krzewicka et al. 2017). Nascimbene et al. (2007) reported that the sensitivity of freshwater lichens to water parameters, such as pH, conductivity, temperature and dissolved oxygen content differ. However, a natural ionic enrichment of water and slight change in other parameters downstream (Electronic Appendix 1) does not have a strong effect on the species richness and composition of lichens (Figs 4 and 5).

There is a vertical zonation in the distribution of freshwater lichens (Ried 1960, Gilbert 1996, Krzewicka et al. 2017). Most species have a low tolerance of complete and continuous immersion in water and often or only occur in the splash zone. Lichen thalli derive their mechanical stability from conglutinate pseudoparenchyma which is hydrophilic and passively absorbs water and dissolved nutrients. Plectenchyma consists of gas filled zones built up by aerial hyphae with hydrophobic surfaces (Honegger 2006). Some of these lichens are able to survive under water and were also recorded in the submerged zone (Table 1, Fig. 6). Independent of the habitat class, the number of species and their abundance were approximately twice as high in the splash zone than the submerged zone (Fig. 4). The lichen *Thelidium klementii* is unique in this respect and was found only underwater in the two non-neighbouring BA and BL streams. Outside Poland, *T. klementii* is a very rare species known only from the type locality in Germany (Thüs & Nascimbene 2008). However, this lichen may be overlooked, which makes it difficult to ascertain the limits of its distribution.

Only three species, *Verrucaria hydrophila*, *V. praetermissa* and *Thelidium minutulum*, occurred in all streams and habitat classes (Fig. 3). These lichens are frequently reported in the Eastern and Western Carpathians (Krzewicka & Galas 2006, Krzewicka 2009, 2012, Krzewicka et al. 2017) and can be considered as widespread and consistent inhabitants of clean mountain streams in this region. Other species only appear in some streams and habitat classes. It is difficult to specify any particular preferences of these species for habitat factors (Electronic Appendix 1). They tend to grow in parts of streams where the water has a low ionic content. Strong exposure to light, especially where the water is calmer, can promote the development of autonomous algae, including filamentous algae and due to their size they can occupy a large area of stabile substrate. On the other hand, silting makes it difficult for lichens to overgrow the substrate and reduces light penetration. Interestingly, some lichens exhibit atypical preferences. For example, the relatively frequent *Thelidium zahlbrucknerii* seems to prefer sites with increased water conductivity (Electronic Appendix 1) and as a rule it was found in Hab-II and Hab-III classes (Fig. 3).

High distinctiveness of streams in terms of species composition of lichens

Lichen assemblages noted at the sampling sites along the streams were not differentiated by changes in habitat parameters and the level of change was too low to affect the lichen biota along some of the streams. However, the composition of lichens in individual streams was often unique regardless of water quality. This was earlier reported for acid watercourses in England in which there were a more or less constant number of species of lichen per stream but the species composition in individual watercourses differed (Gilbert & Giavarini 1997). Some differences in the species composition can be easily explained by the type of substrate and the preference of a particular lichen for either calcareous or siliceous rocks (Gilbert 1996, Gilbert & Giavarini 1997). However, distinct communities also appear in streams with similar geomorphology (Birkenmajer & Oszczypko 1989, Radecki-Pawlik 2006). This also applies to streams with first class water purity (Nascimbene et al. 2007, Krzewicka et al. 2017). For example, Hab-III occurs along the entire length of WO and BL streams and the water conductivity and silting recorded in them is higher than in most clean flysch watercourses. We expected these streams to be inhabited by similar sets of lichens and to be different from other streams; however, the greatest differences of species composition were recorded for these streams (Figs 3 and 5). They are located at the greatest distance from each other and we observed that the species composition was similar in adjacent streams (Fig. 3). Variations in the biota of freshwater lichens appears to depend on the distance between streams and their isolation (Fig. 5).

We assume that the weak dispersal ability of the freshwater lichens recorded in this study is a more limiting factor than the habitat parameters. For example, *Hydropunctaria rheitrophila* was present in all habitat classes and hydrological zones (Table 1), does not have a clear preference for any factor (Electronic Appendix 1) and was recorded only in three neighbouring streams (Fig. 3). All the lichens recorded are capable of sexual reproduction, mainly creating perithecia, while apothecia occur sporadically as they are less adapted to water (Table 1, Thüs et al. 2014). However, unlike other typical terrestrial lichens, the spread and migration of spores and vegetative propagules of freshwater lichens between streambeds are very difficult. Effective dispersal via natural vectors, such as wind and animals, is very limited. The water flow constitutes the main and permanent means of dispersal but acts only one way within a stream. This may be the reason for the conservative species composition in mountain streams. This is supported by the fact that all the sampling sites along WI stream are at the greatest distance from the others on the NMDS ordination diagram (Electronic Appendix 1). Although all habitat classes occur along the WI stream, only a small number of lichens have colonized the bed of this stream (Fig. 3).

Effect of water parameters on the abundance of lichen thalli

Various environmental factors differently affect the growth rate of lichen thalli. For example, Thüs (2002) notes that silting can severely limit lichen growth, both because it reduces light intensity and covers the thalli. Most freshwater lichens are very sensitive to the deposition of silt-like sediments on their surface and only a few species tolerate moderate silting, such as *Bacidina inundata* and *Verrucaria praetermissa* (Thüs 2002, Nascimbene et al. 2013). We attempted to estimate the effect of the factors studied by us on the thallus abundance/size of three common freshwater lichens. We found a clear and, interestingly, opposite responses in two species in relation to ionic water enrichment (Fig. 7). The size of *V. praetermissa* thalli was correlated with water conductivity and the cover of this lichen on the substrate increased with increase in water conductivity. Good survival of this species under harsh conditions is reported by Thüs (2002). The reverse trend (decreased size of thalli) was recorded for *V. hydrophila*. It should be pointed out that these relationships are for the size of the thallus, not the number of species individuals at a sampling site.

Conclusion

Based on data collected from 10 flysch mountain streams in the Beskid Sądecki Mts (Western Carpathians), we draw the following conclusions: (i) The presence of species of freshwater lichens in mountain streams is more strongly site-dependent than habitat factor-dependent. (ii) Altogether 29 species were recorded, only three of which (*Thelidium minutulum*, *Verrucaria hydrophila* and *V. praetermissa*) can be considered as a permanent lichen component of mountain streams in the area studied. The other species have weak dispersal abilities and their occurrence is limited to certain streams or even one stream. (iii) The streams are highly specific in terms of species composition of lichens. Differences in lichen composition increase with increase in the geographical distance between streams. (iv) Differences in the species composition in a stream do not result directly from a gradual change in the habitat parameters. (v) Only four species (*Hydropunctaria rheitrophila*, *Thelidium klementii*, *Verrucaria aquatilis* and *V. hydrophila*) tend to be totally submerged and most freshwater lichens prefer the splash zone. (vi) Increased ion content in the water can considerably promote the development of lichen thalli, as in the case of *Verrucaria praetermissa*, but can limit its development of others, as is the case for *V. hydrophila*. (vii) Most of the lichens clearly preferred to inhabit the splash zone, thus the species richness there is considerable higher than at submerged localities. On the other hand, none of the species occupied a narrow ecological niche in terms of water quality; however, the results suggest that the presence of *Thelidium aquaticum* may indicate high purity of water in a stream.

See www.preslia.cz for Electronic Appendix 1

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Souhrn

O rozšíření sladkovodních lišejníků a faktorech, které je podmiňují, se ví poměrně málo. V článku přinášíme výsledky studia druhové bohatosti a složení lišejníkových společenstev v karpatských horských potocích. Parametry stanoviště (pH, konduktivita, obsah rozpuštěného kyslíku, naplavené částice a světelná intenzita) byly měřeny na studovaných lokalitách a použity jako vysvětlující proměnné v analýzách. Druhové složení jednotlivých potoků se velmi lišilo – pouze tři druhy (*Thelidium minutulum*, *Verrucaria hydrophila* and *V. praetermissa*) z celkového počtu 29 zjištěných se vyskytovaly všude. Tato heterogenita však nebyla podmíněna - variabilitou studovaných stanovištních parametrů; rozdíly v druhovém složení vzrůstaly s tím, jak byly jednotlivé potoky od sebe vzdáleny, a tento efekt se projevoval i v lokálním měřítku. Naše výsledky tedy ukazují, že výskyt lišejníků v horských potocích silně závisí na konkrétní lokalitě a méně na stanovištních parametrech dotyčného toku. Absence přirozených vektorů a slabá schopnost rozšiřování jsou faktory silně limitující šíření sladkovodních lišejníků. Zvýšená koncentrace iontů ve vodě může nicméně významně podpořit rozvoj stélek některých druhů, například *Verrucaria praetermissa*, a naopak působit jako omezující faktor u jiných, jako třeba *V. hydrophila*.

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