Botanica
ISSN 2538-8657
https://doi.org/10.35513/Botlit.2023.2.7
2023, 29(2): 96–109

Original research

Structure of *Equisetum variegatum* (Equisetaceae) populations in natural and anthropogenic habitats

Mindaugas Rasimavičius[1*], Jonas Remigijus Naujalis[2], Zigmantas Gudžinskas[3]

[1] Life Science Centre, Institute of Biosciences, Vilnius University, Saulėtekio Av. 7, 10257 Vilnius, Lithuania
[2] The Lithuanian Academy of Sciences, Gediminas Av. 3, 01103 Vilnius, Lithuania
[3] Nature Research Centre, Institute of Botany, Žaliųjų Ežerų Str. 47, 12200 Vilnius, Lithuania
*Corresponding author. E-mail: mindaugas.rasimavicius@gf.vu.lt

Abstract

Rasimavičius M., Naujalis J.R., Gudžinskas Z., 2023: Structure of *Equisetum variegatum* (Equisetaceae) populations in natural and anthropogenic habitats. – Botanica, 29(2): 96–109. https://doi.org/10.35513/Botlit.2023.2.7

Many native plant species colonise anthropogenic habitats, but relatively little information is available on the status and development of their populations under unusual conditions. The most reliable information on the status and performance of plants in particular habitats is obtained through population studies. The study aimed to determine the characteristics of *Equisetum variegatum* populations occurring in natural and anthropogenic habitats. Nine coenopopulations of *Equisetum variegatum* occurring in natural and anthropogenic habitats were studied in four administrative districts (Kelmė, Lazdijai, Trakai and Varėna) of Lithuania. The ramet density, shoot height, total number of shoots and the number of fertile and sterile shoots and buds in a ramet were assessed. The density of *Equisetum variegatum* ramets was significantly higher in natural habitats than in anthropogenic habitats. The largest mean height of shoots was found in shrubland and shallow water habitats, whereas the largest mean number of all shoots, fertile shoots and buds in a ramet was recorded in dry sands. Old shoots in natural habitats comprised a significantly smaller portion (18.1%) of all shoots than in anthropogenic habitats (42.8%). Thus, all the studied traits can be used to evaluate the status of the populations. Long-term studies of *Equisetum variegatum* populations should be performed to determine how they change in response to the succession of natural and anthropogenic habitats. As the natural habitats of *Equisetum variegatum* decline, anthropogenic habitats may be considered the refugia for their populations.

Keywords: alkaline fens, aquatic habitats, clonal plant, fertile shoot, grasslands, ramet, refugia, sands, sterile shoot.

INTRODUCTION

Environmental changes caused by intense human activity affect all its components, from individuals and populations to habitats and ecosystems (DeAngelis, Yurek, 2017; Mitchell & Whitney, 2018). Plants respond differently to anthropogenic environmental changes (Bazzaz, 1996). Plant species that are sensitive to habitat changes and have low ecological plasticity decline in population size or disappear entirely in a short time. Populations of species with high ecological plasticity adapt over time to the modified conditions and occupy heavily altered or completely new habitats, often significantly different from their natural habitats (Gratani, 2014; Martínez-Blancas et al., 2018; Vinton et al., 2022).

Plant species with high genetic, phenotypic and ecological plasticity can survive or even thrive in habitats experiencing anthropogenic pressure of varying intensity (Coleman et al., 1994; Wade, 2003; Lavergne et al., 2005; Whitham et al., 2006). Studies over the last few decades have shown that habitats exposed to continuous human impact or anthropogenic pressure are becoming particularly important for the survival of some plant species (Gaston & Fuller, 2007; Jukonienė, 2008; Sykes et al., 2020). It has been observed that artificial habitats increasingly support plants that have been considered sensitive to anthropogenic pressures and that the populations established in such habitats persist for a relatively long time (Rewicz et al., 2017, 2018; Fekete et al., 2019; Martín-Forés et al., 2022).

The most reliable information on the status and performance of plants in particular habitats is obtained through population studies (Buckley & Puy, 2022). Even single census studies of populations of a plant species under different habitat conditions provide a substantial amount of information on the status of the populations and allow to make reasonable predictions about their future development (Oostermeijer & Hartman, 2014; Gudžinskas & Rasimavičius, 2017). The assessment of changes in the demographic composition of plant populations and the state of individuals is essential both for assessing the threat posed by alien species (Levin et al., 2019; Taura & Gudžinskas, 2020; Loomis, 2022) and for determining the need for conservation and management measures for rare and endangered native species (Žalneravičius & Gudžinskas, 2016; Gudžinskas & Taura, 2021; Kazlauskas et al., 2022; Ryla et al., 2022; Taura et al., 2022).

Plants of the genus *Equisetum* L. (Equisetaceae Rich. ex DC.) are often the subject of scientific studies; however, researchers have mainly focused on the taxonomy and evolution (Spatz & Emanns, 2004; Guillon, 2004, 2007; Bennert et al., 2005; Husby, 2013), distribution (Kalinowski et al., 2016; Gudžinskas & Rasimavičius, 2017; Smith, 2023) and chemical composition (Currie & Perry, 2007; Law & Exley, 2011; Boeing et al., 2021) of species. Little research has been performed on the structure and demographic composition of populations of the genus *Equisetum*, or only individual aspects of population structure have been assessed. Only studies on the population ecology of *Equisetum arvense* L., *Equisetum fluviatile* L. and *Equisetum hyemale* L. have gained a little more attention from researchers (Rutz & Farrar, 1984; Marshall, 1986; Marsh et al., 2000).

Although *Equisetum variegatum* Schleich. ex F.Weber & D.Mohr is a widespread species (Merryweather, 2020; Smith, 2023), its populations have so far been studied only very fragmentarily (Naujalis, 1995; Czylok, 1997; Czylok & Rahmonov, 1998; Smith, 2023). This was among the reasons why the populations of *Equisetum variegatum* were chosen as the target of the study. In addition, it has been observed that *Equisetum variegatum*, formerly rare in Lithuania and mostly occurring in wetlands, is increasingly found in anthropogenic habitats, particularly in excavated sand and gravel quarries (Rasimavičius & Naujalis, 2012). The study aimed to determine the characteristics of *Equisetum variegatum* populations occurring in natural and anthropogenic habitats. The following questions were addressed: (a) what is the density of ramets in different habitats and populations? (b) what is the shoot state and ramet structure in the studied populations and habitats? (c) what is the ratio of young and old shoots in the studied populations and habitats?

MATERIALS AND METHODS

Study species

Equisetum variegatum (Equisetaceae) is a rhizomatous perennial plant with evergreen, usually unbranched, coarse shoots. The shoots are isomorphous, fertile or sterile, erect or decumbent, and rarely prostrate. *Equisetum variegatum* is widespread in the northern hemisphere, occurring in most of Europe, northern regions of Asia and North America. It commonly grows in open and sunny sites. Typical habitats of *Equisetum variegatum* are alkaline fens, river, stream and lake shores, transitional mires, wet grasslands, upland scree and dune slacks. It is more frequent and abundant in the mountains than in the lowlands (Hackney, 1981; Machon et al., 2001; Merryweather, 2020; Smith, 2023). In Lithuania, the natural habitats of *Equisetum variegatum* are in alkaline fens, wet grasslands, lakeshores, and occasionally coastal dunes and sands. Still, it is also often found in anthropogenic habitats such as sand and gravel pits, along roads and railways, and in drainage ditches (Rasimavičius & Naujalis, 2012).

Study sites

Nine coenopopulations of *Equisetum variegatum*, hereafter referred to as populations, were studied in four administrative districts (Kelmė, Lazdijai, Trakai and Varėna) of Lithuania. The studied *Equisetum variegatum* populations were named according to the nearest village or other place name (Table 1).

The Snaigynas population was studied on the northern shore of Lake Snaigynas (Lazdijai district, southern Lithuania). The population of *Equisetum variegatum* occupied a small area on a swampy shore of the lake, which was densely covered (coverage 80%) with 2–3 m tall shrubland of *Frangula alnus* Mill. and *Alnus glutinosa* (L.) Gaertn. *Equisetum variegatum* formed a discontinuous stand composed of patches of various sizes.

The Kunigiškė population was studied in the vicinity of Kunigiškė village (Kelmė district, northern Lithuania) in alkaline fen habitat. The surrounding habitat and a part of *Equisetum variegatum* stand were formerly regularly mown and sometimes grazed by livestock.

The Vilkokšnis population was studied on the southern shore of Lake Vilkokšnis (Trakai district, south-eastern Lithuania). At the study site*, Equisetum variegatum* grew in the alkaline fen on the shores of the lake and covered a relatively large area.

Three populations (Giraitė A, Giraitė B and Giraitė C) were studied in the former Giraitė sand quarry (Varėna district, southern Lithuania) in anthropogenic habitats. The first population of *Equisetum variegatum* (Giraitė A) was studied in a habitat of sparsely vegetated dry sands. The second studied population (Giraitė A) occurred in a dry sandy grassland. The third population of *Equisetum variegatum* (Giraitė C) was studied in a shallow water habitat formed at the bottom of the quarry. *Equisetum variegatum* grew in the permanently flooded area, on average 10 cm deep.

Three populations (Beržupis A, Beržupis B and Beržupis C) were studied in the former (closed in 1977) Beržupis sand and gravel quarry (Varėna district, southern Lithuania) in anthropogenic habitats. The first population of *Equisetum variegatum* (Beržupis A) was studied at the top of the south-eastern slope, in a habitat of sparsely vegetated dry sands. The second population (Beržupis B) occurred in the middle part of the slope, in a dry grassland formed on gravel. The third population (Beržupis C) was selected in damp gravel at the bottom of the quarry. The area was covered with a dense (coverage 80%) shrubland formed mainly by *Salix cinerea* L., *Salix myrsinifolia* Salisb. and *Frangula alnus* Mill.

Population studies

The field surveys of *Equisetum variegatum* populations were performed in July and August. The density and composition of the populations were investigated in sample plots of 1 m^2. For each population, five sample plots were selected and examined (a total of 45 sample plots). The sample plot was delimited by a frame, and all *Equisetum variegatum* ramets growing within the delimited area were excavated, labelled and transported to the laboratory for further analysis.

Since *Equisetum variegatum* is a clonal plant, ramet (Fig. 1) was chosen as the reference unit. The ramet is a relatively independent component of the clone that can exist independently and has all the morphological constituents characteristic of the spe-

Table 1. Name, geographical location and habitat characteristics of the studied *Equisetum variegatum* populations. Abbreviations of habitat characteristics: A – anthropogenic habitat, N – natural habitat

No.	Population name	District	Latitude (°N)	Longitude (°E)	Habitat characteristics
1.	Snaigynas	Lazdijai	54.10334	23.72756	N; shrubland
2.	Kunigiškė	Kelmė	55.78027	23.03921	N; alkaline fen
3.	Vilkokšnis	Trakai	54.50210	24.70489	N; alkaline fen
4.	Giraitė A	Varėna	54.27314	24.70174	A; sparsely vegetated dry sands
5.	Giraitė B	Varėna	54.27305	24.70233	A; dry sandy grassland
6.	Giraitė C	Varėna	54.27284	24.70488	A; shallow water body
7.	Beržupis A	Varėna	54.23504	24.59267	A; sparsely vegetated dry sands
8.	Beržupis B	Varėna	54.23505	24.59180	A; dry sandy grassland
9.	Beržupis C	Varėna	54.23510	24.59087	A; shrubland

cies (Klimešová & Klimeš, 2008; Klimešová et al., 2011; Goldberg et al., 2020).

Cleaned and separated ramets of *Equisetum variegatum* from each sample plot were analysed separately in the laboratory. Shoots and buds of each ramet were counted, and the height of the tallest shoot was measured. The height of the shoots was measured with a ruler to an accuracy of 1 mm.

Shoots of *Equisetum variegatum* were classified into sterile and fertile shoots (Fig. 1). Sterile shoots were those which did not have a strobile. Fertile shoots were those with a strobile at the apex. Shoots that had lost one or more apical metamers were not classified into sterile and fertile shoots. As the shoot of *Equisetum variegatum* ages, the apical metamers (strobiles, nodes and internodes) are the first to be lost, which is an essential diagnostic characteristic for determining the relative age of the shoot. Two age groups were distinguished based on the morphological characteristics of the shoots: young and old (Fig. 1). A shoot was considered young if it had all its morphological structures retained, with a clearly visible strobile or conical apex. A shoot was considered old if it had lost part of the metameres (nodes and internodes), but at least one metamere was above the soil level. Underground parts of *Equisetum variegatum* ramets were also examined, and the number of dormant buds was counted.

Fig. 1. The structure of *Equisetum variegatum* ramet: fertile shoot (a), sterile shoot (b), young shoot (c), old shoot (d) and bud (e)

Equisetum variegatum ramets collected from the studied populations were analysed in the laboratory of the Department of Botany and Genetics of the Institute of Biosciences, Vilnius University. A part of the collected *Equisetum variegatum* specimens representing each studied population was deposited at the Herbarium of Vilnius University (WI).

Statistical analyses

A total of 22457 *Equisetum variegatum* ramets were counted in all studied populations. Fifty ramets were measured and assessed from each study plot in each population, and their data were used for further analysis (a total of 250 ramets from each population and 2250 ramets from all nine populations). The ratio of young and old shoots in each population was calculated using the assessment data of all shoots of the 250 ramets (a total of 7464 shoots).

The Shapiro-Wilk test was used to assess the normality of the collected datasets. Some datasets (number of shoots in the ramet and shoot height) were normally distributed, while others were non-normally distributed. Therefore, the data were analysed by applying non-parametric methods. The results of the descriptive statistics on ramet density were presented as mean and standard deviation (mean ± SD), minimum and maximum values and median. In contrast, the other results were presented only as mean and standard deviation. The datasets of the populations were compared by applying the Kruskal-Wallis H test. For pairwise comparison of ramet density, shoot height and number of shoots in a ramet, the Mann-Whitney post hoc test was used. As the datasets on the number of fertile and sterile shoots and number of buds in a ramet contained many zeros, Dunn's post hoc test was used for pairwise comparison of populations. The calculations were computed, and the graphs were plotted using the Past 4.10 software (Hammer et al., 2001).

RESULTS

Ramet density

Analysis of the density of *Equisetum variegatum* ramets revealed that it was significantly higher ($p = 0.008$) in natural habitats (335.1 ± 228.6 ramets/m^2) than in anthropogenic habitats (172.4 ± 155.0 ramets/m^2). Even more remarkable differences were found when comparing the density of ramets by habitat type. The highest ramet density was found in natural alkaline fen habitats (451.3 ± 186.4 ramets/m^2), but it was not significantly different ($p = 0.075$) from anthropogenic dry grassland habitats (316.2 ± 161.4 ramets/m^2). In anthropogenic dry sands, the density of ramets (130.3 ± 106.6 ramets/m^2) was almost the same ($p = 0.850$) as in shrubland habitats (104.5 ± 59.6 ramets/m^2). The lowest ramet density was in the shallow water habitat (35.4 ± 19.2 ramets/m^2), but it was not significantly different ($p = 0.058$) from the ramet density in the anthropogenic dry sands.

The most remarkable mean density of *Equisetum variegatum* ramets was found in populations occurring in natural alkaline fen habitats (Kunigiškė and Vilkokšnis). Lower density was recorded in anthropogenic dry grassland habitats (Giraitė B and Beržupis B), but the densities of ramets were not significantly different ($p > 0.05$) from those in alkaline fens (Table 2). In shrubland habitats (Snaigynas and Beržupis C), the density of *Equisetum variegatum* ramets was significantly lower than in open alkaline fen and dry grassland habitats. The lowest density of ramets was found in populations of *Equisetum variegatum* occurring in dry sands (Beržupis A) and shallow water (Giraitė C) habitats (Table 2).

Shoot height

The height of shoots in studied *Equisetum variegatum* populations ranged from 2 cm to 44 cm, and the mean height of all shoots (n = 2250 ramets) was 12.7 ± 7.0 cm. The most considerable mean height of *Equisetum variegatum* shoots (22.0 ± 7.3 cm) was found in the Snaigynas population, which occurred in a natural shrubland habitat. The mean shoot height in the Giraitė C population (20.8 ± 7.0 cm), which occurred in anthropogenic shallow water habitat, was significantly higher than in the other populations, although significantly lower ($p = 0.026$) than the mean shoot height in the Snaigynas population (Fig. 2). The mean shoot height of *Equisetum variegatum* growing in open alkaline fen habitats (14.5 ± 5.3 cm in Vilkokšnis and 12.1 ± 4.2 cm in Kunigiškė) was almost half that of plants growing in shrubland and water habitats. Still, their shoot height was significantly higher than that of plants growing in open anthropogenic habitats (Fig. 2). The lowest mean shoot height of *Equisetum variegatum* was found in the Beržupis B (7.2 ± 3.0 cm) population, which occupied anthropogenic dry grassland habitat. It is important to note that the mean shoot height was significantly different between all populations stud-

Table 2. Mean *Equisetum variegatum* ramet density (mean ± standard deviation; ramets in one m^2), minimum, maximum and median in the studied populations. Different superscript letters indicate a significant difference ($p < 0.05$) between pairs of populations according to the Mann-Whitney post hoc test results

Population	Habitat type	Ramet density (mean ± SD)	Minimum	Maximum	Median
Snaigynas	Shrubland	102.8 ± 59.4^{ajl}	61	202	77
Kunigiškė	Alkaline fen	471.2 ± 235.4^{bko}	310	888	380
Vilkokšnis	Alkaline fen	431.4 ± 147.6^{cko}	306	681	376
Giraitė A	Dry sands	198.4 ± 109.4^{djo}	66	366	180
Giraitė B	Dry grassland	242.4 ± 143.6^{ejkno}	98	460	220
Giraitė C	Aquatic	35.4 ± 19.2^{fmn}	14	56	30
Beržupis A	Dry sands	62.2 ± 44.7^{gjlm}	15	112	51
Beržupis B	Dry grassland	390.0 ± 156.2^{ho}	230	607	310
Beržupis C	Shrubland	106.2 ± 66.8^{ijn}	20	178	132

ied, except for the Giraitė B (8.5 ± 3.4 cm) and the Beržupis A (8.7 ± 4.2 cm) populations (p = 0.550), occurring in dry sands and dry grassland habitats, respectively.

The mean shoot height of *Equisetum variegatum* in natural habitats (16.2 ± 7.1 cm) was significantly higher (p < 0.001) than in all studied anthropogenic habitats (10.9 ± 6.3 cm). Significant differences (p < 0.001) were also found in mean shoot height among all studied habitat types. Mean shoot height was ranked (in descending order) by habitats as follows: shallow water (20.8 ± 7.0 cm), scrubland (15.8 ± 8.5 cm), alkaline fen (13.3 ± 4.9 cm), anthropogenic dry sands (9.8 ± 4.2 cm) and anthropogenic dry grassland (7.8 ± 3.3 cm) habitats.

Ramet structure

Ramets of *Equisetum variegatum* had from 1 to 28 shoots in the studied populations. The mean shoot number in a ramet was 3.3 ± 2.1 (n = 2250 ramets). The largest mean number of *Equisetum variegatum* shoots in a ramet was recorded in the Giraitė A (5.1 ± 3.7 shoots) population, which occurred in dry sands (Fig. 3). The mean number of shoots in this population was significantly more prominent than in all other studied populations. The number of shoots in a ramet in populations occurring in natural habitats was comparable to the number of shoots in anthropogenic habitats. The mean number of shoots in the Snaigynas population (3.6 ± 2.1 shoots) occurring in shrubland was not significantly different (p = 0.162) from the Giraitė B population (3.1 ± 1.2 shoots), located in anthropogenic grassland habitat. The mean number of shoots in a ramet of the Vilkokšnis population (2.9 ± 1.2 shoots) occurring in an alkaline fen was not significantly different from the populations of Beržupis A (2.8 ± 0.9 shoots) and Beržupis B (3.0 ± 1.0 shoots) (p = 0.643 and p = 0.207, respectively) occurring in anthropogenic dry sands and dry grassland habitat (Fig. 3). The smallest mean number of shoots in a ramet was found in the Beržupis C population (2.5 ± 0.6 shoots), which occurred in anthropogenic shrubland habitat. The mean shoot number in a ramet of *Equisetum variegatum* growing in a shallow water habitat in the Giraitė C population (4.2 ± 2.7 shoots) was relatively high and significantly different from all other studied populations.

The mean number of all *Equisetum variegatum* shoots in a ramet in natural (3.0 ± 2.6 shoots) and anthropogenic habitats (3.5 ± 5.0 shoots) was significantly different (p < 0.001). The most pronounced

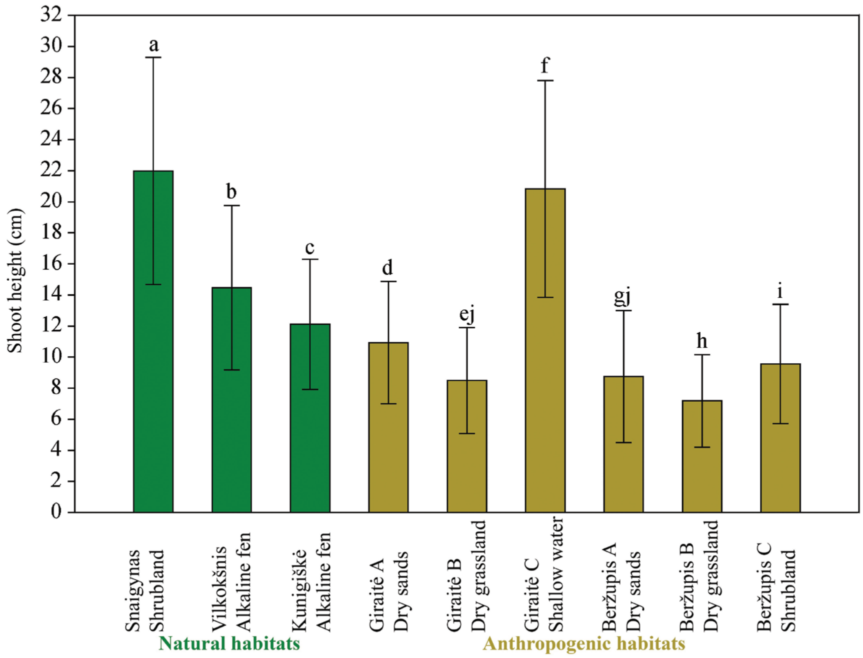

Fig. 2. Mean height of *Equisetum variegatum* shoots in the studied populations. Different letters above the whiskers indicate a significant difference (p < 0.05) between pairs of populations according to the Mann-Whitney post hoc test results

mean number of shoots in a ramet was in the shallow water habitat (4.2 ± 2.7 shoots). A similar but significantly lower (p = 0.003) mean number of shoots in a ramet was found in anthropogenic dry sands (4.0 ± 2.9 shoots). The smallest number of shoots in a ramet was found in alkaline fen (2.7 ± 1.2 shoots) and shrubland (3.0 ± 1.7 shoots) habitats, and the mean number of shoots in a ramet was not significantly different between these habitat types (p = 0.092). Thus, the mean number of shoots in a ramet is significantly larger in anthropogenic habitats than in natural habitats.

In the studied populations of *Equisetum variegatum*, ramets had from 0 to 10 fertile shoots. All analysed ramets (n = 2250) had a mean of 0.4 ± 0.8 fertile shoots. The largest mean number of fertile shoots

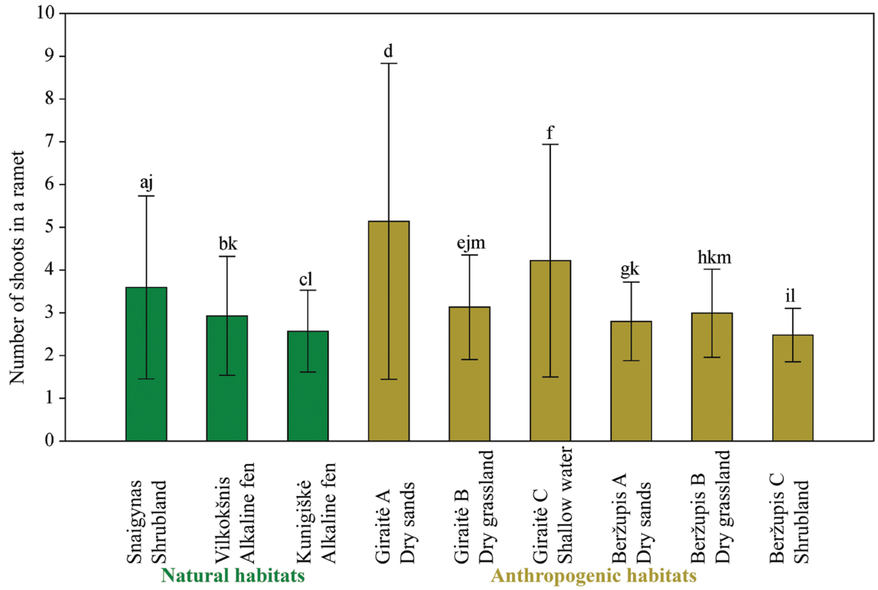

Fig. 3. Mean number of shoots in a ramet of *Equisetum variegatum* in the studied populations. Different letters above the whiskers indicate a significant difference ($p < 0.05$) between pairs of populations according to the Mann-Whitney post hoc test results

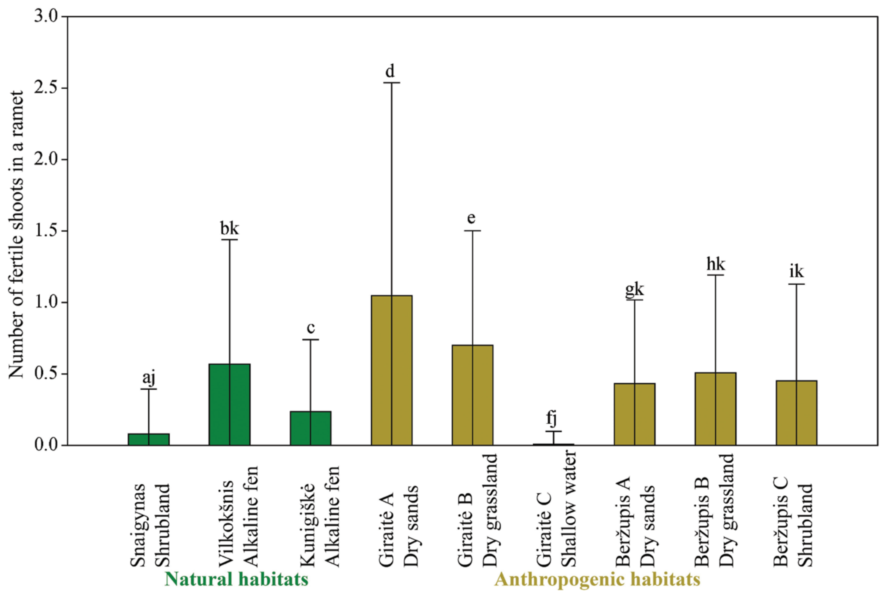

Fig. 4. Mean number of fertile shoots in a ramet of *Equisetum variegatum* in the studied populations. Different letters above the whiskers indicate a significant difference ($p < 0.05$) between population pairs, according to Dunn's post hoc test results

of *Equisetum variegatum* was found in the Giraitė A population (1.0 ± 1.5 shoots), which occurred in dry sands (Fig. 4). The number of fertile shoots in the shallow water habitat in the Giraitė C population (0.0 ± 0.1 shoots) was exceedingly small and significantly smaller than the number of fertile shoots in all other studied populations. The number of fertile shoots was significantly larger ($p < 0.001$) in the Vilkokšnis population (0.6 ± 0.9 shoots) than in the Kunigiškė population (0.2 ± 0.5 shoots), even though both populations occurred in alkaline fen habitats (Fig. 4). Notably, the number of fertile shoots in the Vilkokšnis population was similar ($p > 0.05$) to the number of fertile shoots in the Beržupis A (0.4 ± 0.6 shoots), Beržupis B (0.5 ± 0.7 shoots) and Beržupis C (0.5 ± 0.7 shoots) populations, all of which occurred in anthropogenic habitats.

The mean number of fertile shoots in a ramet of *Equisetum variegatum* in all natural habitats (0.3 ± 0.6 shoots) was significantly smaller ($p < 0.001$) than in all anthropogenic habitats (0.5 ± 0.9 shoots). The largest mean number of fertile shoots in a ramet was found in the anthropogenic dry sands (0.7 ± 1.2 shoots) and anthropogenic dry grassland (0.6 ± 0.8 shoots) habitats, and there was no significant difference ($p = 0.901$) between the mean number of fertile shoots. The smallest mean number of fertile shoots in a ramet was in the shallow water habitat (0.0 ± 0.1 shoots).

Ramets of *Equisetum variegatum* had between 0 and 13 sterile shoots in the studied populations. The mean number of sterile shoots in all the ramets analysed (n = 2250) was 1.3 ± 1.5. The most remarkable mean number of sterile shoots was found in the Giraitė C population (2.6 ± 1.8 shoots), which occupied anthropogenic shallow water habitat (Fig. 5). In the Vilkokšnis population (0.7 ± 0.9 shoots), which occurred in alkaline fen habitat, the mean number of sterile shoots of *Equisetum variegatum* ramets was not significantly different ($p > 0.05$) from the populations Beržupis A (0.8 ± 0.9 shoots) and Beržupis B (0.7 ± 0.8 shoots), occupying anthropogenic dry sands and dry grassland habitat, respectively. The mean number of sterile shoots (0.9 ± 0.8 shoots) in the ramets of the Kunigiškė population, which occurred in a natural alkaline fen habitat, was almost the same ($p = 0.822$) as that of the Beržupis C (0.9 ± 0.8 shoots) population, which occupied an anthropogenic shrubland habitat (Fig. 5). Relatively large number of sterile shoots was observed in *Equisetum variegatum* ramets of the Snaigynas population (1.7 ± 1.3 shoots), which occurred in a natural shrubland habitat. The mean number of sterile shoots in a ramet in this population significantly differed ($p < 0.01$) from all other studied populations.

The mean number of sterile shoots of *Equisetum variegatum* in a ramet in natural habitats (1.1 ± 1.1 shoots) was significantly lower ($p = 0.001$) than

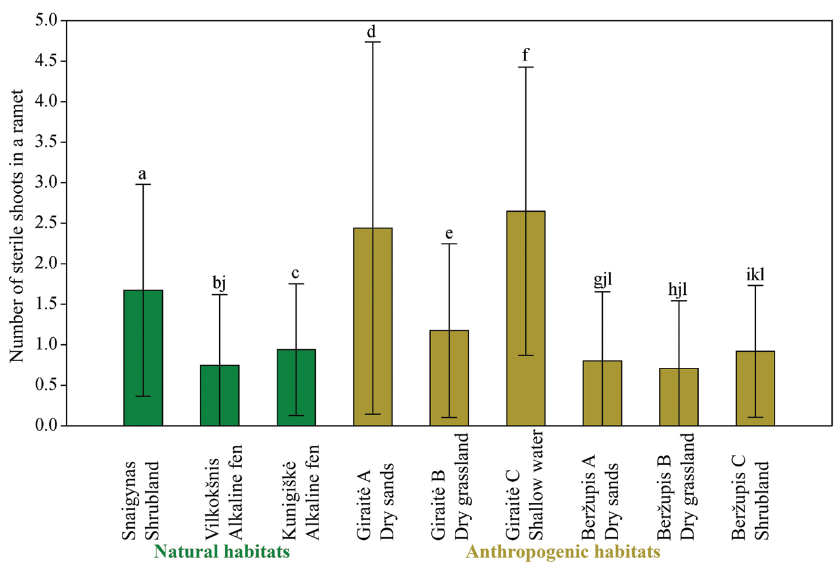

Fig. 5. Mean number of sterile shoots in a ramet of *Equisetum variegatum* in the studied populations. Different letters above the whiskers indicate a significant difference ($p < 0.05$) between population pairs, according to Dunn's post hoc test results

in all anthropogenic habitats (1.4 ± 1.6 shoots). The most prominent mean number of sterile shoots in a ramet was found in the shallow water habitat (2.6 ± 1.8 shoots), and the number of sterile shoots was significantly larger ($p < 0.001$) than in all other habitats. In the alkaline fen (0.8 ± 0.8 shoots) and anthropogenic dry grassland (0.9 ± 1.0 shoots) habitats, the number of sterile shoots in a ramet was not significantly different ($p = 0.250$). The mean number of sterile shoots in a ramet was similar ($p = 0.726$) between shrubland habitats (1.3 ± 1.2 shoots) and dry sands (1.6 ± 1.9 shoots).

The number of buds in a ramet ranged between 0 and 4 in the studied populations of *Equisetum variegatum*. The mean number of buds was 0.2 ± 0.4 in all the ramets analysed (n = 2250). The largest mean number of *Equisetum variegatum* buds in a ramet was recorded in the Beržupis B population (0.3 ± 0.7 buds), which occurred in anthropogenic dry grassland habitat (Fig. 6). This population was significantly different ($p < 0.05$) by the mean number of buds in a ramet from all other studied populations. The smallest number of buds in a ramet was found in the Kunigiškė population (0.0 ± 0.2 buds), which occurred in alkaline fen habitat (Fig. 6). Ramets of this population had significantly fewer buds ($p < 0.01$) than ramets of all other studied populations, except for the Vilkokšnis population which occupied alkaline fen habitat.

The mean number of buds in a ramet of *Equisetum variegatum* growing in anthropogenic habitats (0.2 ± 0.5 buds) was significantly larger ($p < 0.001$) than in natural habitats (0.1 ± 0.4 buds). Analysis of the number of buds in a ramet from different habitats revealed that the mean number was the same in shrubland, anthropogenic dry grassland and shallow water (all with 0.2 ± 0.5 buds) habitats. Only ramets of *Equisetum variegatum* occurring in alkaline fens (0.1 ± 0.3 buds) had significantly ($p < 0.001$) fewer buds than in all the other habitats.

Ratio of young and old shoots

Examination of the shoots comprising ramets of *Equisetum variegatum* according to their age groups revealed significant differences between populations (Fig. 7). In natural alkaline fen habitats, young shoots were predominant. In contrast, the fraction of old shoots was relatively small. Young shoots comprised 88.6% of the total number of shoots analysed in the Vilkokšnis population and 83.6% in the Kunigiškė population, occurring in alkaline fen habitats. In the Snaigynas population, which occurred in a shrubland habitat, young shoots comprised 75.2% of the total number of shoots. In contrast, in the Giraitė A population, which occurred in anthropogenic dry sands, young shoots comprised 72.4% (Fig. 7). In the popu-

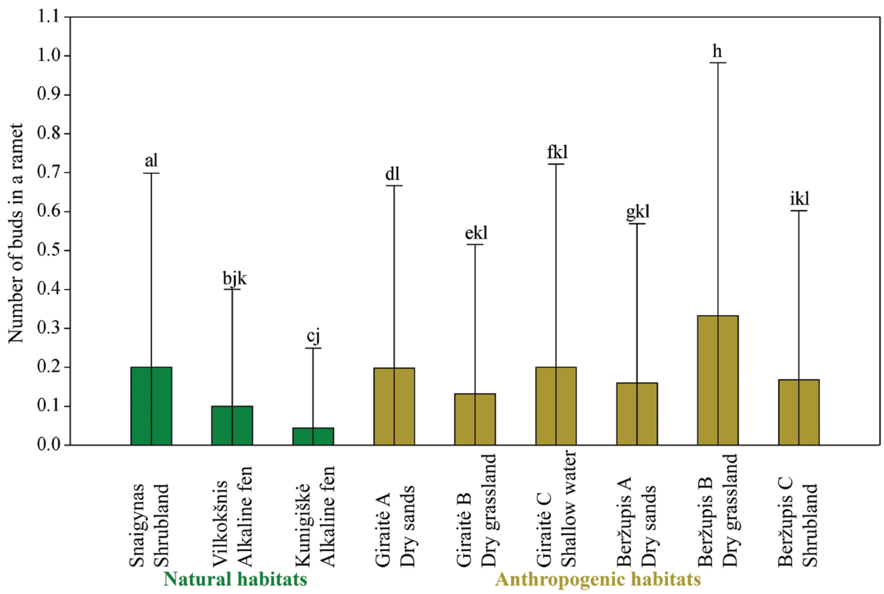

Fig. 6. Mean number of buds in a ramet of *Equisetum variegatum* in the studied populations. Different letters above the whiskers indicate a significant difference ($p < 0.05$) between population pairs, according to Dunn's post hoc test results

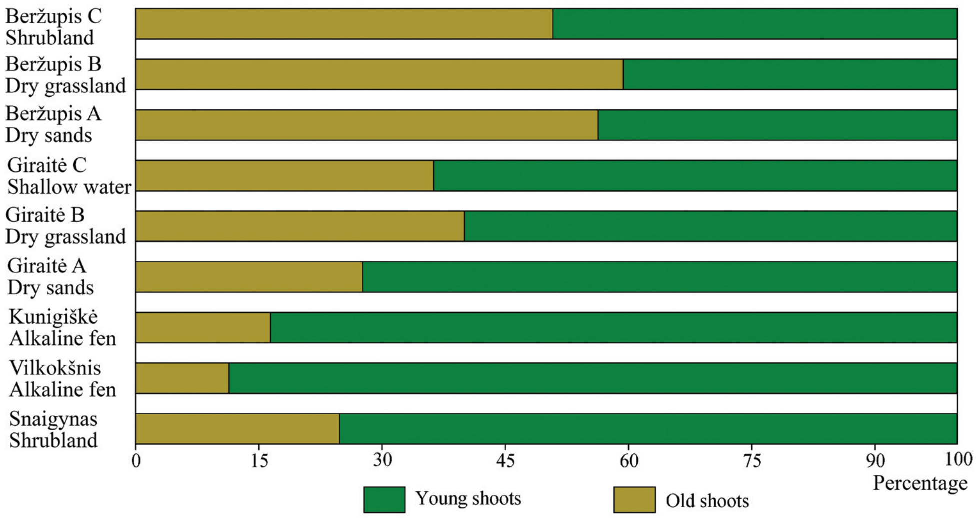

Fig. 7. The ratio of young to old shoots (%) in the studied populations of *Equisetum variegatum*

lations of Beržupis A, Beržupis B and Beržupis C, occupying anthropogenic habitats, more than a half of the total number of shoots in the analysed *Equisetum variegatum* ramets were old (56.3%, 59.4% and 50.8% of shoots, respectively).

Analysis of the ratio of young and old shoots of *Equisetum variegatum* in natural and anthropogenic habitats revealed considerable differences. Old shoots in natural habitats comprised 18.1% of all shoots, while in anthropogenic habitats, they comprised 42.8%. Analysis of the proportion of old and young shoots by habitat type showed slightly different patterns. The smallest percentage of old shoots (13.9%) was found in alkaline fen habitats, while the largest percentage was found in anthropogenic dry grassland habitats (49.5%). Old shoots comprised a similar proportion in shrublands, dry sands and shallow water habitats (38.9%, 38.2% and 36.3%, respectively). Thus, young shoots dominated in the alkaline fen habitats, whereas in the anthropogenic grassland habitats, the proportions of young and old shoots were approximately equal.

DISCUSSION

Equisetum variegatum is a species characteristic of alkaline and spring fens, transitional mires and other permanently wet habitats (Machon et al., 2001; Kaplan et al., 2020; Smith, 2023). Since human activities have significantly reduced natural habitats, *Equisetum variegatum* is increasingly inhabiting anthropogenic environments, mainly sand and gravel pits, ditches and railway embankments (Rasimavičius & Naujalis, 2012; Świerkosz & Reczyńska, 2017; Kaplan et al., 2020; Smith, 2023). A study performed more than a decade ago showed that only 30% of the known populations in Lithuania occupy natural habitats. In contrast, the rest of the populations occur in heavily modified or artificial habitats (Rasimavičius & Naujalis, 2012). Therefore, the present study was initiated to determine whether there are differences between the composition of *Equisetum variegatum* populations and the ramet structure in natural and anthropogenic habitats.

The results of the *Equisetum variegatum* population analysis showed that the highest density of ramets was found in open alkaline fen habitats. Nevertheless, there were no significant differences in the density of ramets between the individual populations studied in the alkaline fen and anthropogenic dry grassland habitats. Low ramet density was found in natural and anthropogenic habitats with dense shrubs (coverage was about 80%). Thus, it can be concluded that the density of *Equisetum variegatum* ramets is strongly influenced by the amount of sunlight reaching the herb layer. *Equisetum variegatum* is a light-loving plant (Merryweather, 2020; Smith, 2023); therefore, the most favourable conditions for its growth occur in open habitats, whereas in the case of a lack of light in scrubland habitats, the population of these clonal plants decline. Still, they persist for quite a long time. Shrub encroachment in alkaline fen and wet dune slack habitats is considered a significant threat to *Equisetum variegatum* populations in many European countries (Machon et al., 2001; Kaplan et al., 2020; Smith, 2023).

The density of *Equisetum variegatum* ramets was lowest in anthropogenic dry sands and shallow water habitats. Both habitats are unusual for the species, but long rhizomatous *Equisetum variegatum*, when established, can form stands and persist for a long time. The significantly lower density of ramets in anthropogenic dry sands than in anthropogenic dry grassland habitats may have also been influenced by differences in the age of clones. Unfortunately, the age of establishment of any studied population was not known. It can only be assumed that the populations in natural habitats have been established for a long time. In contrast, in anthropogenic habitats, they were established only after the end of sand and gravel quarrying about four decades ago. The density of *Equisetum variegatum* in the anthropogenic grassland of the Beržupis quarry was studied in the late 1980s, and a mean density of 17 ramets/m^2 was found (Naujalis, 1995). The study, conducted three decades later, revealed a significantly higher mean density of ramets, suggesting that their density increases as the population ages. Results obtained on the ramet density of *Equisetum variegatum* cannot be compared with ramet density in other regions, as no results of similar studies have been published.

The analysis of the shoot height of *Equisetum variegatum* revealed that the tallest shoots were found in extreme habitat conditions, in shrubland and shallow water habitats. Very tall shoots are an indicator of unfavourable growing conditions, mainly insufficient light. This assumption is supported by the low number of fertile shoots in the ramets under light stress. Even fewer fertile shoots were present in the shallow water habitat. The results of this study support the assertion that the stability of *Equisetum variegatum* populations is being threatened by shrub encroachment (Smith, 2023). Conversely, exceptionally low shoots also indicate that habitat conditions are unfavourable for the plant. The lowest shoots were found in anthropogenic dry sands with constant water deficits. It is essential to highlight that the plants had significantly more fertile shoots in permanently dry habitats. This suggests that *Equisetum variegatum*, like other plants, allocates more energy to reproduction under conditions of permanent environmental stress (Cramer et al., 2011; Prasch & Sonnewald, 2015).

The results of this study suggest that natural alkaline fen habitats are the most favourable for *Equisetum variegatum*. In anthropogenic habitats, the status of populations varies considerably and depends on several factors such as moisture availability, light levels and the age of the population. Indicators of a favourable status of *Equisetum variegatum* populations may include a high density of ramets and a higher proportion of young shoots than old shoots. A significantly higher or lower than the mean shoot height, a high total number of shoots in a ramet and a high number of fertile shoots may indicate environmental stress to which *Equisetum variegatum* is exposed. Long-term studies of *Equisetum variegatum* populations should be performed to determine how they change in response to the succession of natural and anthropogenic habitats. As the natural habitats of *Equisetum variegatum* decline, anthropogenic habitats may be considered the refugia for their populations.

REFERENCES

Bazzaz F.A., 1996: Plants in Changing Environments. Linking Physiological, Population, and Community Ecology. Cambridge.

Bennert W., Lubienski M., Körner S., Steinberg M., 2005: Triploidy in *Equisetum* subgenus *Hippochaete* (Equisetaceae, Pteridophyta). – Annals of Botany, 95: 807–815. https://doi.org/10.1093/aob/mci084

Boeing T., Moreno K.G.T., Gasparotto A., Silva L.M., Souza P., 2021. Phytochemistry and pharmacology of the genus *Equisetum* (Equisetaceae): a narrative review of the species with therapeutic potential for kidney diseases. – Evidence-Based Complementary and Alternative Medicine, 2021: 1–17. https://doi.org/10.1155/2021/6658434

Buckley Y.M., Puy J., 2022: The macroecology of plant populations from local to global scales. – New Phytologist, 233(3): 1038–1050. https://doi.org/10.1111/nph.17749

Coleman J.S., McConnaughay K.D.M., Ackerly D.D., 1994: Interpreting phenotypic variation in plants. – Trends in Ecology and Evolution, 9(5): 187–191. https://doi.org/10.1016/0169-5347(94)90087-6

Cramer G.R., Urano K., Deirot S., Pezzoti M., Shinozaki K., 2011: Effects of abiotic stress on plants: a systems biology perspective. – BMC Plant Biology, 11: 163. https://doi.org/10.1186/1471-2229-11-163

Currie H.A., Perry C.C., 2007: Silica in plants: biological, biochemical and chemical studies. – Annals of Botany, 100: 1383–1389. https://doi.org/10.1093/aob/mcm247

Czylok A., 1997: Pionierskie zbiorowiska ze skrzypem pestrym *Equisetum variegatum* Schleich. w wyrobiskach po eksploatacji piasku. – In: Wika S. (ed.), Roślinność obszarów piaszczystych: 61–67. Katowice.

Czylok A., Rahmonov O., 1998: The initial stages of succession with variegated horsetail *Equisetum variegatum* Schleich. on wet sands of surface excavations. – In: Szabó J., Wach J. (eds), Anthropogenic aspects of geographical environment transformations: 81–87. Debrecen–Sosnowiec.

DeAngelis D.L., Yurek S., 2017: Spatially explicit modelling in ecology: a review. – Ecosystems, 20(2): 284–300. https://doi.org/10.1007/s10021-016-0066-z

Fekete R., Löki V., Urgyán R., Süveges K., Lovas-Kiss Á., Vincze O., Molnár A.V., 2019: Roadside verges and cemeteries: Comparative analysis of anthropogenic orchid habitats in the Eastern Mediterranean. – Ecology and Evolution, 9(11): 6655–6664. https://doi.org/10.1002/ece3.5245

Gaston K.J., Fuller R.A., 2007: Biodiversity and extinction: losing the common and the widespread. – Progress in Physical Geography, 31: 213–225.

Goldberg D.E., Batzer E., Elgersma K., Martina J., Klimešová J., 2020: Allocation to clonal growth: Critical questions and protocols to answer them. – Perspectives in Plant Ecology, Evolution and Systematics, 43: 125511. https://doi.org/10.1016/j.ppees.2020.125511

Gratani L., 2014: Plant phenotypic plasticity in response to environmental factors. – Advances in Botany, 2014: 208747. https://doi.org/10.1155/2014/208747

Gudžinskas Z., Rasimavičius M., 2017: Distribution, state and conservation of *Equisetum telmateia* in Lithuania. – Botanica Lithuanica, 23(1): 17–32. https://doi.org/10.1515/botlit-2017-0002

Gudžinskas Z., Taura L., 2021: *Scirpus radicans* (Cyperaceae), a newly-discovered native species in Lithuania: population, habitats and threats. – Biodiversity Data Journal, 9: e65674. https://doi.org/10.3897/BDJ.9.e65674

Guillon J.M., 2004: Phylogeny of horsetails (*Equisetum*) based on the chloroplast *rps*4 gene and adjacent noncoding sequences. – Systematic Botany, 29(2): 251–259. https://doi.org/10.1600/036364404774195467

Guillon J.M., 2007: Molecular phylogeny of horsetails (*Equisetum*) including chloroplast *atp*B sequences. – Journal of Plant Research, 120: 569–574. https://doi.org/10.1007/s10265-007-0088-x

Hackney P., 1981: *Equisetum variegatum* Web. & Mohr: A note on its occurrence in Co Down and a comparison of some of its sites in N. Ireland – The Irish Naturalists' Journal, 20(5): 180–184.

Hammer Ø., Harper D.A.T., Ryan P.D., 2001: PAST: Paleontological statistics software package for education and data analysis. – Palaeontologia Electronica, 4(1): 9.

Husby C., 2013: Biology and functional ecology of *Equisetum* with emphasis on the giant horsetails. – The Botanical Review, 79(2): 147–177.

Jukonienė I., 2008: The impact of anthropogenic habitats on rare bryophyte species in Lithuania. – Folia Cryptogamica Estonica, 44: 55–62.

Kalinowski P., Sliwinska E., Kruk J., 2016: *Equisetum ×moorei* Newman (Equisetaceae) – a 'new' nothotaxon in the Polish flora. – Biodiversity Research and Conservation, 41(1): 11–18. https://doi.org/10.1515/biorc-2016-0003

Kaplan Z., Danihelka J., Ekrt L., Štech M., Řepka R., Chrtek J. Jr., Grulich V., Rotreklová O., Dřevojan P., Šumberová K., Wild J., 2020: Distributions of vascular plants in the Czech Republic. Part 9. – Preslia, 92: 255–340. https://doi.org/10.23855/preslia.2020.255

Kazlauskas M., Taura L., Gudžinskas Z., 2022: Current state of critically endangered *Neotinea ustulata* (Orchidaceae) in Lithuania and report on a new record of the species. – Botanica, 28(2): 91–101. https://doi.org/10.35513/Botlit.2022.2.2

Klimešová J., Klimeš L., 2008: Clonal growth diversity and bud banks in the Chech flora: an evaluation using the CLO-PLA3 database. – Preslia, 80: 225–275.

Klimešová J., Doležal J., Dvorský M., De Bello F., Klimeš L., 2011: Clonal growth forms in Eastern Ladakh, Western Himalayas: classification and habitat preferences. – Folia Geobotanica, 46(2–3): 191–217.

Lavergne S., Thuiller W., Molina J., Debussche M., 2005: Environmental and human factors influencing rare plant local occurrence, extinction and persistence: a 115-year study in the Mediterranean region. – Journal of Biogeography, 32: 799–811.

Law C., Exley C., 2011: New insight into silica deposition in horsetail (*Equisetum arvense*). – BMC Plant Biology, 11: 112. https://doi.org/10.1186/1471-2229-11-112

Levin S.C., Crandall R.M., Knight T.M., 2019: Population projection models for 14 alien plant species in the presence and absence of aboveground competition. – Ecology, 100(6): e02681. https://doi.org/10.1002/ecy.2681

Loomis A., 2022: Balancing the good and the bad: Assessing the positive and negative effects of alien species on native plant demography. Dissertation, Duke University. Duke.

Machon N., Guillon J.M., Dobigny G., Cadre S.L., Moret J., 2001: Genetic variation in the horsetail *Equisetum variegatum* Schleich., an endangered species in the Parisian region. – Biodiversity and Conservation, 10: 1543–1554. https://doi.org/10.1023/A:1011816610775

Marsh A.S., Arnone J.A., Bormanes B.T., Gordon G.C., 2000: The role of *Equisetum* in nutrient cycling in an Alaskan Shrub wetland. – Journal of Ecology, 88: 999–1011.

Marshall G., 1986: Growth and development of field horsetail *Equisetum arvense*. – Weed Science, 34(2): 271–275.

Martínez-Blancas A., Paz H., Salazar G.A., Martorell C., 2018: Related plant species respond similarly to chronic anthropogenic disturbance: Implications for conservation decision-making. – Journal of Applied Ecology, 55(4): 1860–1870. https://doi.org/10.1111/1365-2664.13151

Martín-Forés I., Bywaters S.L., Sparrow B., Guerin G.R., 2022: Simultaneous effect of habitat remnancy, exotic species, and anthropogenic disturbance on orchid diversity in South Australia. – Conservation Science and Practice, 4(4): e12652. https://doi.org/10.1111/csp2.12652

Merryweather J., 2020: Britain's ferns: a field guide to the clubmosses, quillworts, horsetails and ferns of Great Britain and Ireland. Woodstock.

Mitchell N., Whitney K.D., 2018: Can plants evolve to meet a changing climate? The potential of field experimental evolution studies. – American Journal of Botany, 105(10): 1613–1616. https://doi.org/10.1002/ajb2.1170

Naujalis J., 1995: Sporiniai induočiai kaip augalų bendrijų komponentai. Pteridophytes as components of plant communities. Vilnius.

Oostermeijer J.G.B., Hartman Y., 2014: Inferring population and metapopulation dynamics of *Liparis loeselii* from single-census and inventory data. – Acta Oecologica, 60: 30–39. https://doi.org/10.1016/j.actao.2014.08.002

Prasch C., Sonnewald U., 2015: Signalling events in plants: Stress factors in combination change the picture. – Environmental and Experimental Botany, 114: 4–14. https://doi.org/10.1016/j.envexpbot.2014.06.020

Rasimavičius M., Naujalis J.R., 2012: *Equisetum variegatum* Schleich. ex Weber et Mohr in Lithuania: habitat diversity, distribution patterns and environmental status based on herbarium collection. – Ekologija, 58(4): 413–425. https://doi.org/10.6001/ekologija.v58i4.2610

Rewicz A., Bomanowska A., Shevera M.V., Kurowski J.K., Krasoń K., Zielińska K.M., 2017: Cities and disturbed areas as man-made shelters for orchid communities. – Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 45(1): 126–139. https://doi.org/10.15835/nbha45110519

Rewicz A., Rewers M., Jędrzejczyk I., Rewicz T., Kołodziejek J., Jakubska-Busse A., 2018: Morphology and genome size of *Epipactis helleborine* (L.) Crantz (Orchidaceae) growing in anthropogenic and natural habitats. – PeerJ, 6: e5992. https://doi.org/10.7717/peerj.5992

Rutz L.M., Farrar D.R., 1984: The habitat characteristics and abundance of *Equisetum ferrissi* and its parent species, *Equisetum hyemale* and *Equisetum laevigatum*, in Iowa. – American Fern Journal, 74(3): 65–76.

Ryla M., Kinduris R., Nurczyński B., Žilinskienė A., Pranaitis A., 2022: *Epipactis albensis* (Orchidaceae) species new to the flora of Lithuania. Data from the northeastern limit of the species distribution area. – Botanica, 28(1): 46–59. https://doi.org/10.35513/Botlit.2022.1.6

Smith H.P., 2023: Distribution and ecology of *Equisetum variegatum* (variegated horsetail) (Equisetaceae) on the Sefton Coast sand-dunes, north Merseyside, UK. – British & Irish Botany, 5(2): 114–130. https://doi.org/10.33928/bib.2023.05.114

Spatz H.-Ch., Emanns A., 2004: The mechanical role of the endodermis in *Equisetum* plant stems. – American Journal of Botany, 91(11): 1936–1938. https://doi.org/10.3732/ajb.91.11.1936

Świerkosz K., Reczyńska K., 2017: Nowe stanowisko skrzypu pstrego *Equisetum variegatum* Schleich. ex Weber & Mohr w Masywie Śnieżnika (Sudety Wschodnie). – Przyroda Sudetów, 20: 13–16.

Sykes L., Santini L., Etard A., Newbold T., 2020: Effects of rarity form on species' responses to land use. – Conservation Biology, 34(3): 688–696. https://doi.org/10.1111/cobi.13419

Taura L., Gudžinskas Z., 2020: Life stages and demography of invasive shrub *Cytisus scoparius* (Fabaceae) in Lithuania. – Botanica, 26(1): 1–14. https://doi.org/10.2478/botlit-2020-0001

Taura L., Kamaitytė-Bukelskienė L., Sinkevičienė Z., Gudžinskas Z., 2022: Study on the rare semiaquatic plant *Elatine hydropiper* (Elatinaceae) in Lithuania: population density, seed bank and conservation challenges. – Frontiers in Biosciences (Landmark), 27(5): 162. https://doi.org/10.31083/j.fbl2705162

Vinton A.C., Gascoigne S.J.L., Sepil I., Salguero-Gómez R., 2022: Plasticity's role in adaptive evolution depends on environmental change components. – Trends in Ecology and Evolution, 37(12): 1067–1078. https://doi.org/10.1016/j.tree.2022.08.008

Wade M.J., 2003: Community genetics and species interactions. – Ecology, 84: 583–585. https://doi.org/10.1890/0012-9658-(2003)084[0583:CGASI]2.0.CO;2

Whitham T., Bailey J., Schweitzer J., Shuster S.M., Bangert R.K., LeRoy C.J., Lonsdorf E.V., Allan G.J., DiFazio S.P., Potts B.M., Fischer D.G., Gehring C.A., Lindroth R.L., Marks J.C., Hart S.C., Wimp G.M., Wooley S.C., 2006: A framework for community and ecosystem genetics: from genes to ecosystems. – Nature Reviews Genetics, 7: 510–523. https://doi.org/10.1038/nrg1877

Žalneravičius E., Gudžinskas Z., 2016: Assessment of the data on distribution, habitats and population size of *Liparis loeselii* (Orchidaceae) in Lithuania. – Botanica Lithuanica, 22(1): 3–15. https://doi.org/10.1515/botlit-2016-0001

MR https://orcid.org/0000-0001-5770-1009
JRN https://orcid.org/0000-0003-1008-0721
ZG https://orcid.org/0000-0001-6230-5924