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Original Research

Study on the Rare Semiaquatic Plant *Elatine hydropiper* (Elatinaceae) in Lithuania: Population Density, Seed Bank and Conservation Challenges

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Abstract

Background: Wet, intermittently flooded freshwater coastal and shallow water habitats are notable for their high biodiversity. Many of the usually semiaquatic annuals or small perennials common in such habitats are in decline due to the high sensitivity of such habitats to changes caused by anthropogenic and natural factors. The Euro-Siberian semiaquatic *Elatine hydropiper* rarely occurs in Lithuania and is protected there and in other Baltic and Central European countries. In 2020, we found a large population of *Elatine hydropiper* in southern Lithuania (Varėna district) in Lake Pabezninkai and its exposed shores. The water level of the lake has receded by about 1.5–1.6 m since 2018, exposing wide, wet, and sandy or silty shores. The aim of this study was to determine the status of the *Elatine hydropiper* population and to assess its potential for long-term survival. The objectives of the study were to (a) determine the size and quantitative characteristics of the population, (b) assess the seed bank in the shallows and exposed shores of the lake, (c) assess the species diversity in the communities with *Elatine hydropiper*, (d) evaluate the conservation value of the studied species and the lake habitat. **Methods**: The studies were carried out between 2020 and 2021. The number and coverage of plant patches as well as seed bank studies were carried out at three sites with different shoreline substrate. At each site, the number of patches and coverage was assessed in 50 sample plots of 100 cm^2. The seed bank was examined in the top 5 cm of the substrate. A total of 90 substrate samples were analyzed. **Results**: We found that *Elatine hydropiper* grows on ca. 0.38 ha in the lake and on ca. 0.95 ha on the shores. On the exposed shores, the mean number of *Elatine hydropiper* patches per 1 m^2 was 2155 $\pm$ 1241.5 and their mean cover was 23.17% of the surface. Seed bank analyses revealed that the seeds and seed shells were significantly more abundant in the bottom sediments than in the shore sand or silt. The mean number of seeds per liter of soil was 85.8 $\pm$ 210.2, and the mean number of seed shells was 217.0 $\pm$ 265.8. **Conclusions**: The assessment of the status of the species in Lithuania according to the IUCN (International Union for Conservation of Nature) criteria has led us to conclude that it should now be classified as vulnerable [A3; B1ab(iii); C2a(ii); D2]. The key to protecting *Elatine hydropiper* and other co-occurring rare species with similar ecological and biological characteristics, as well as the plant communities of shallows and exposed wet banks, is to protect their habitats. The survival of the species and their habitats could be assured by recurring significant water level fluctuations in the lake, resulting in periodic emergence of patches of wet sand or silt free from perennial vegetation.

Keywords: annual plants; amphibious species; communities; habitats; IUCN criteria; mesotrophic lakes; vulnerable species

1. Introduction

Freshwater coastal habitats of rivers and lakes, which form the transition zone between aquatic and terrestrial habitats, are particularly important for riverine and lacustrine biodiversity [1–3]. Unfortunately, these ecosystems have been heavily impacted by human activities and are among the most threatened habitats in Europe and worldwide [4]. In addition to direct habitat destruction related to drainage, straightening and regulation of water courses, bank stabilization, etc. [5], water pollution is among the biggest threats. Many researchers stress that increasing eutrophication of water bodies and their shores causes significant habitat changes and leads to a decline in the diversity and abundance of specialized species [6,7]. Eutrophicated waterside habitats are also highly vulnerable to invasions by alien plants [8–10] and to the expansion of native perennial tall plants [11,12]. Mesotrophic habitats of periodically exposed shores with pioneer and ephemeral vegetation are considered vulnerable at the European level and are included in the European Red List of Habitats [13].

Changes in precipitation and drought regimes due to climate change are also contributing to the rapid loss of coastal habitats [14,15]. When water levels in reservoirs drop and do not recover for several years, the open banks are quickly occupied by perennial plant communities, which create unfavorable conditions for the growth of low-competitive annual plants [4,16]. Although many of the small plants characteristic of shorelines and shallows form an abundant and long-lasting seed bank, where seeds remain viable for 50 years or more [4], many of them are

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becoming increasingly rare in natural habitats and are only found in anthropogenic habitats [17]. Such changes in distribution pose many problems for the selection and application of conservation approaches [18].

Many plant species growing on the banks or shallows of periodically receding water bodies are threatened and have been included in national or regional Red Lists [19–23]. Among such species is *Elatine hydropiper* L. (Elatinaceae), the Euro-Siberian species with the largest concentration of sites of occurrence in Central and Northern Europe. The density of sites towards the east decreases significantly [21]. It is frequent in southern regions of Norway, Sweden, Finland, and around the Gulf of Finland, however only single occurrences are known in the Baltic Countries [21,24]. Despite being considered as a species of least concern (LC) at European level [25], it is endangered in many regions of Central Europe and the Baltic States [19–23,26]. One of the most important reasons for rarity and decline is the lack of suitable habitats and changes to existing habitats caused by various reasons [9]. In Lithuania, *Elatine hydropiper* is a very rare species, the occurrence of which has been confirmed in 1988 and until now only this small population is known [24,27].

Species of the genus *Elatine* are minute semiaquatic therophytes adapted to grow in a constantly changing environment [21,28]. Species of the genus are characterized by high seed production and an extremely long-term seed bank. It has been reported that the seeds of these plants remain viable in soil for at least 50 years [4,29]. Seeds require light to germinate and therefore only seeds that are fully exposed to the soil surface will germinate [30]. Seeds in water are thought to germinate almost simultaneously because they receive sufficient light once the water level drops to a certain threshold [31].

The seed shells of *Elatine* species are well preserved in deep layers in the form of subfossils or fossils and are relatively easy to identify due to their distinctive shape and surface pattern [28,29,32]. This makes the remains of seeds of both extant and extinct *Elatine* species significant for paleobotanical and archeobotanical studies [32,33]. Seed remains help to reconstruct environmental conditions during certain historical or geological periods with a high degree of accuracy [32–34].

During investigations in southern Lithuania in 2020, we found a large and abundant population of *Elatine hydropiper* on the exposed shores of Lake Pabezninkai (Varėna district), where *Scirpus radicans* and *Eleocharis ovata*, previously unreported in the country, and several other protected plant species were found [35,36]. The discovery of the *Elatine hydropiper* population in Lake Pabezninkai and its shores prompted us to perform detailed studies with the aim of determining the status of the population and assessing its potential for long-term survival. The objectives of the study were to (a) determine the size and quantitative characteristics of the population, (b) assess the seed bank in the shallows and exposed shores of the lake, (c) assess the species diversity in the communities with *Elatine hydropiper*, (d) evaluate the conservation value of the studied species and the lake habitat.

2. Materials and Methods

2.1 Study Species

Elatine hydropiper (Elatinaceae) is annual plant, 0.5–2 cm tall, with branched shoots that are creeping on substrate and rooting at nodes. Leaves opposite, oblong, oblong-elliptic, or subspatulate, 2–5 mm long and ca. 0.5 mm wide. The tetramerous flowers are solitary in leaf axils, one or two flowers per node, subsessile or very shortly pedicellate. Sepals 4, oblong, 0.6–0.7 mm long and ca. 0.3 mm wide, with rounded apex. Petals 4, obovate or broadly elliptic, 0.8–1 mm long and ca. 0.4 mm wide, slightly longer and wider than sepals, with rounded apex. Stamens 8, shorter than petals. Styles 4. Capsule compressed globose, ca. 2 mm in diameter. Seeds horseshoe-shaped, 0.5–0.7 mm long, with fine and dense hexagonal reticulation [21,27].

2.2 Study Area

Studies on *Elatine hydropiper* were carried out in southern Lithuania, ca. 15 km north of Varėna, in Lake Pabezninkai and on its exposed shores (Varėna district). The lake is situated on the southern edge of the Dzūkai Upland (a part of the Baltic Uplands). The relief of the area consists of moraine hills which form a complex with limnoglacial formations [37]. The lake is surrounded by forests in the north, sparsely populated areas with extensively used arable lands and grasslands in the west and south, and intensively grazed pastures in the east (Fig. 1). The partially abandoned livestock farms on the south-eastern shore of the lake may have been a significant source of pollution in the past.

The standard mean annual temperature in the study area is 6.8 °C. The coldest month in the area is January with a standard mean temperature of –3.7 °C, whereas July is the warmest month with a mean temperature of 17.9 °C. The standard mean annual sum of precipitation is 701 mm with the largest amount of precipitation occurring during the summer months (242 mm) and the lowest amount falls in winter (145 mm) [38].

Lake Pabezninkai is of glacial origin and covers an area of 61.4 ha. The lake is slightly elongated, 1 km long and 0.7 km wide and shallow, with an average depth of 1.9 m (the maximum recorded depth is 3 m) and polymictic. The shore is gently sloping, sandy, in some places consisting of silty sand or silt and admixture of pebbles, with some scattered boulders. There are periodic fluctuations in water level. At the time of the lowest water level (by 1.5–1.6 m), the area of the lake decreases to 38.2 ha, exposing sandy or silty plains 30–80 m wide [35]. The water in the lake is brownish, with mean transparency of 0.87 ± 0.15 m (Secchi disk), with low concentration of dissolved minerals (con-

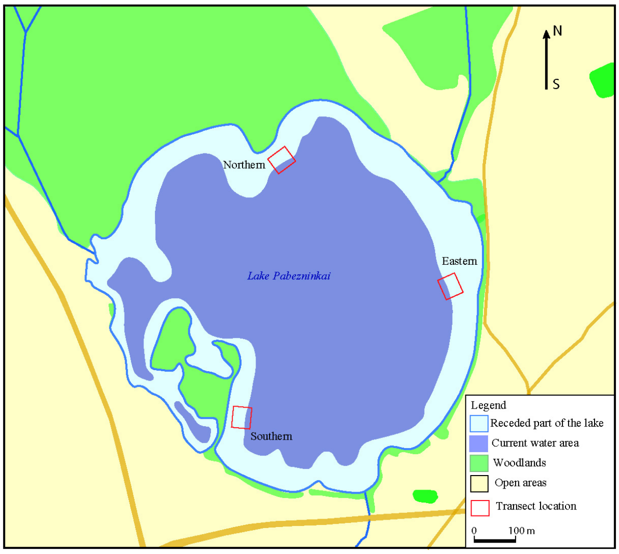

Fig. 1. Lake Pabezninkai with its surroundings and sampling sites where *Elatine hydropiper* abundance and seed bank were assessed.

ductivity 43 $\pm$ 4 μs cm^{-1}) at pH 6.13 $\pm$ 0.4 [39]. The mean concentration of dissolved oxygen from May to November has been recorded as 9.51 $\pm$ 2.21 mg L^{-1}, the concentration of dissolved inorganic nitrogen as 0.57 $\pm$ 0.70 mg L^{-1}, the total concentration of nitrogen as 1.64 $\pm$ 0.71 mg L^{-1}, and the total concentration of phosphorus as 0.037 $\pm$ 0.010 mg L^{-1} [39].

2.3 Distribution and Abundance

Field surveys on the exposed shores and shallows of Lake Pabezninkai were carried out in August–September 2020 and April–July 2021. Data from previous studies and literature sources were also used to assess the distribution of *Elatine hydropiper* in Lithuania. Voucher specimens of *Elatine hydropiper* collected during this research were deposited at the Herbarium of the Institute of Botany of the Nature Research Centre, Vilnius (BILAS). A distribution map of *Elatine hydropiper* in Lithuania was compiled by applying a system of grid cells, which were arranged according to geographical coordinates with sides of 6′ of latitude and 10′ of longitude.

The abundance of *Elatine hydropiper* was assessed in September 2020 on three 30 m long transects selected on the northern, eastern, and southern shores of the lake (Fig. 1). The survey sites were selected depending on substrate type. The transect was selected on wet sand on the eastern shore, on silty sand with pebbles on the northern shore and on silt on the southern shore. The transects were placed 2 m from the edge of water at the study time. Each transect consisted of 50 sampling plots, spaced in a single line at 0.5 m intervals. The sampling plots were delimited by a wooden frame with sides of 0.1 $\times$ 0.1 m (area 0.01 m^2). In order to determine the number of individuals of *Elatine hydropiper*, it is necessary to excavate plants from the substrate. Since this species is rare in Lithuania, we decided to use non-destructive methods by counting plant patches rather than individuals. Each patch usually consisted of one to a several individuals, but larger patches sometimes contained more than ten individuals. Therefore, each sampling plot was photographed from above (Canon EOS 550D, EF-S 18–55 mm lens, Tokyo, Japan) with the camera mounted on a tripod, maintaining a uniform distance (0.3 m) from the lens to the ground surface.

The number of patches of *Elatine hydropiper* and the area covered by the patches (with precision of 0.01 cm^2) in the sampling plot were calculated using Digimizer software 5.7.2 (MedCalc Software Ltd, Ostend, Belgium). In the uploaded image with the field frame used for scaling, all distinct outlines of *Elatine hydropiper* patches were delineated (Fig. 2). Other plant species and other objects within the sampling plot were ignored.

2.4 Habitat and Communities

Surveys of plant communities and habitats of *Elatine hydropiper* were performed in September 2020 and July 2021 on all exposed shores and in shallows of Lake Pabezninkai. Phytosociological relevés of the plant communities with *Elatine hydropiper* were conducted applying the Braun-Blanquet [40] approach. Communities were described in the water up to the distribution limit of plants, in the zone of water level fluctuations and on land. A total of 31 relevés of plant communities were used for the analysis. The area of the relevés was chosen by considering the natural boundaries of the ecological conditions and community and ranged from 2 m^2 to 10 m^2. The nomenclature of vascular plant taxa follows the Euro+Med PlantBase (https://europlusmed.org/).

2.5 Seed Bank Study

The sampling areas for the study of *Elatine hydropiper* seed bank in substrate were selected at the same sites of the lake where the abundance of individuals was assessed in 2020 (Fig. 1). Samples of the substrate were collected in April 2021. Three transects parallel to the shoreline were established at each site. The first transect was located 12 m from the shoreline, the second 2 m from the shoreline and the third in the water, 1 m from the current shoreline, at an average depth of 10 cm (Fig. 3). At each transect, 5 cm of upper substrate layer was sampled every 5 m using 50 mm diameter tubular sampler. The area of each sample was 19.63 cm^2 and its volume was 98.17 cm^3. The collected substrate samples were immediately placed in labelled bags. The samples were stored in a refrigerator at +4 °C until the analysis. Each transect was sampled with 10 substrate samples and a total of 30 samples were taken at the site. A total of 90 samples were used for the seed bank analysis.

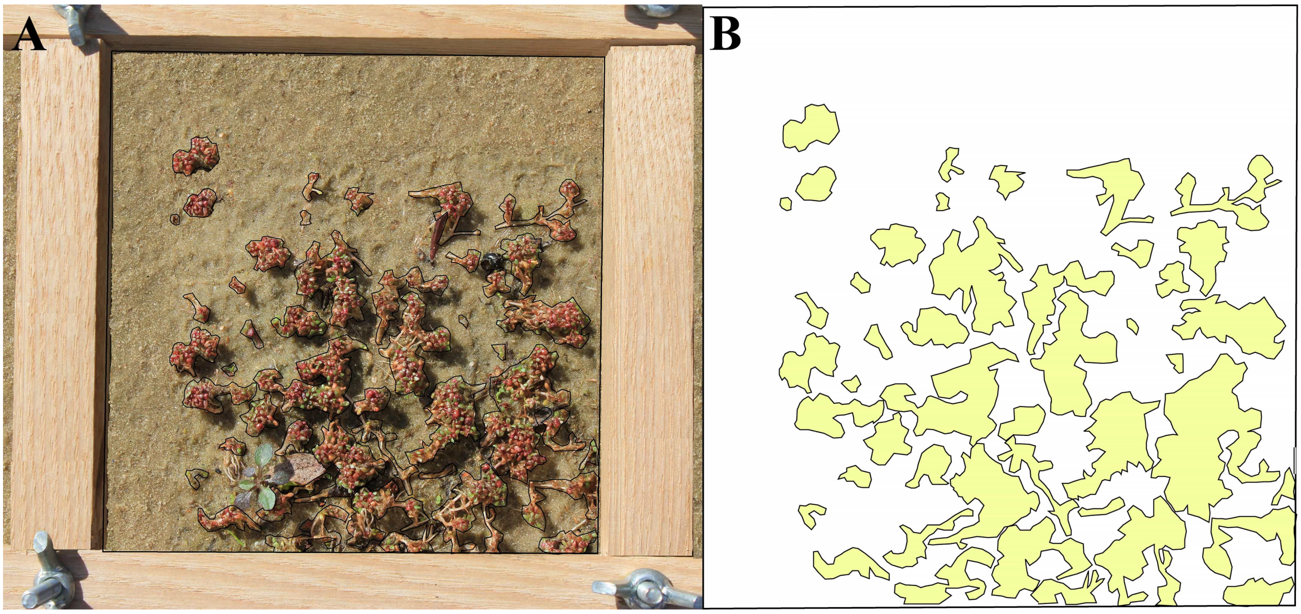

Fig. 2. Assessment of *Elatine hydropiper* coverage in the sampling plot. (A) Patches of *Elatine hydropiper* with outlines. (B) Arrangement of *Elatine hydropipe*r patches in the sampling plot.

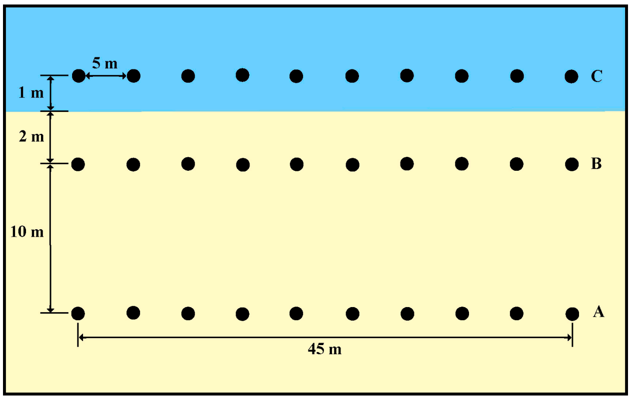

Fig. 3. Scheme of the arrangement of the *Elatine hydropiper* seed bank sampling plots. The blue background represents water, the yellow background shore.

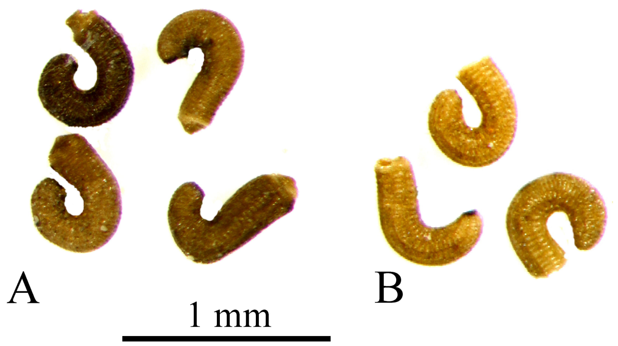

Fig. 4. Intact seeds (A) and seeed shells (B) of *Elatine hydropiper* extracted from the seed bank.

The collected samples were washed in the laboratory under a stream of running water using a sieve with a mesh diameter of 0.25 mm to remove the fine fraction of the substrate. The washed substrate with the remaining seeds was poured into a Petri dish and analyzed under a Konus Crystal-Pro Stereo Microscope (Verona, Italy). The seeds collected from the sample were divided into two groups: intact seeds and empty seed shells (Fig. 4). Seeds that were opaque or dark when viewed under the light microscope and had operculum were considered as intact seeds (hereafter, seeds), while those that were translucent or semi-translucent and without operculum were classified as seed shells. Seed shell fragments were excluded from the seed shell count.

2.6 Assessment of Threats

Assessment of the threat for *Elatine hydropiper* populations in Lithuania were performed following the IUCN Guidelines and Criteria [41,42] and based on data of earlier investigations and the results of this study.

2.7 Statistical Analysis

The results of descriptive statistics are presented as mean and standard deviation (mean ± SD). The normality of the data (number of patches, patch area, number of seeds and number of seed shells) was assessed using the Shapiro-Wilk test. The results for the number of *Elatine hydropiper* patches and the area covered in the test plots (100 cm^2) were converted for a square metre by multiplying the original data by 100. The number of patches and occupied area were normally distributed in the transect plots, but due to the different sample sizes, nonparametric methods

(Kruskal-Wallis H-test and Dunn's z post-hoc) were used for comparison. The results of the seed bank tests were non-normally distributed and therefore the above nonparametric methods of statistical analysis were applied. Since the density of seeds and seed shells was assessed on samples of 98.17 cm^3, the results obtained were converted for a liter of substrate by multiplying the original data by 10.19. Relationships between the number of seeds and the number of seed shells were tested by employing Spearman's rank-order correlation (r_s). All calculations were performed using the PAST 4.08 software (Natural History Museum, University of Oslo, Norway) [43].

3. Results

3.1 Distribution and Abundance

Elatine hydropiper was first confirmed to occur in Lithuania in 1988 on the muddy shore of Lake Merguva (environs of Bitėnai village, Rambynas Regional Park, Pagėgiai district, western Lithuania; Fig. 5). A small group of individuals was found, scattered over an area of less than 0.5 m^2. Despite continuous efforts to find *Elatine hydropiper* in this area and to assess the status of the population, searches were unsuccessful until 2018. In that year, a few individuals of this species were found at the previous location, on the muddy shore of Lake Merguva, rather densely overgrown by nitrophilous plants.

In 2020, in southern Lithuania (Fig. 5), we found a population of *Elatine hydropiper* on the exposed shores and shallows of Lake Pabezninkai (Varėna district, environs of Sarapiniškės village). During September and October of 2020, we also screened the shores of Lakes Lavysas, Glėbas, and Glūkas (southern Lithuania), which are characterized by significant fluctuations of the water level and wide exposed shores similar to Lake Pabezninkai; however, *Elatine hydropiper* was not found.

Elatine hydropiper was found in 2020 on the shores of almost the entire perimeter of Lake Pabezninkai, with a length of approximately 3830 m. The plants form stands of varying widths, usually 2–5 m, sometimes up to 10 m, on the shores and belts of approximately 1 m wide in the water at a depth of approximately 0.1–0.3 m, occasionally to 0.5 m. The total estimated area covered by *Elatine hydropiper* stands on the shores of the lake was therefore approximately 9500 m^2 and in the water approximately 3800 m^2. The total estimated area covered by this species was approximately 1.34 ha.

The lowest number of *Elatine hydropiper* patches in all sampling plots ($n = 50$) was recorded in the Eastern transect ($n = 634$), while the highest number of patches was recorded in the Southern transect ($n = 1311$; Table 1). Statistically significant differences were found in the number of patches between all transects (Kruskal-Wallis H = 32.71; $p < 0.001$). Pairwise comparisons showed that the number of patches was significantly lower in the Eastern transect than in the Northern transect (Dunn's $z = 3.55$; $p < 0.001$) and significantly lower than in the Southern transect ($z = 5.66$; $p < 0.001$). There were smaller but still significant differences between the Northern and Southern transects in the number of *Elatine hydropiper* patches ($z = 2.11$; $p = 0.035$). In the Eastern and Northern transects, the lowest number of patches in the study plots was 0, whereas in the Southern transect 11 patches. The highest number of patches (61) per individual plot was recorded in the Northern transect.

The Eastern transect had the smallest mean patch area and the Southern transect had the largest mean patch area (Table 1). The area of *Elatine hydropiper* patches varied from 0.01 cm^2 to 16.85 cm^2 in all transects surveyed. There were significant differences between transects in *Elatine hydropiper* patch area (H = 439.0; $p < 0.001$), and there were significant differences between pairs of transects in terms of mean *Elatine hydropiper* patch area. The largest patches were found in the Southern transect, which was located on the silty lake shore.

The area occupied by *Elatine hydropiper* patches was significantly different between the three transects (H = 68.14; $p < 0.001$). Pairwise comparisons between transects showed significant differences in patch area. The highest cover was found at the Southern site, which was surveyed on a silty shore, and the lowest cover was found at the Eastern site, which was studied on a wet sandy shore (Table 1). Thus, the mean coverage of *Elatine hydropiper* was 7.58% on the eastern shore of the lake in the wet sand, 20.44% on the northern shore in the sand with pebbles, and 36.87% of the surface on the southern shore in the wet silt.

3.2 Seed Bank

We found that the substrate of the exposed shores and the bottom sediments of Lake Pabezninkai contain an abundant *Elatine hydropiper* seed bank. Seeds were found even in soil samples taken in an area where no *Elatine hydropiper* individuals were present in 2020 (Eastern site, transect A). A pairwise comparison of seed bank density in the substrate of the transects furthest from the lake shore (A transects) showed that the substrate at the Northern and Southern sites did not differ in seed density ($z = 0.16$; $p = 0.874$), but seed density in both was significantly higher than in the A transect of the Eastern site (Table 2). The same pattern was found when examining the seed bank density in the B transects, which were located on the exposed shore of the lake, 2 m from the shoreline. There were no differences in seed bank density in the soil of the B transects at the Northern and Southern sites ($z = 1.09$; $p = 0.273$), but they were both significantly different from the B transect at the Eastern site. Sediment samples collected from the water in the C transects showed no significant differences in seed bank density between the Northern and Eastern sites ($z = 0.51$; $p = 0.609$), but they had significantly lower seed densities than the C transect at the Southern site (Table 2).

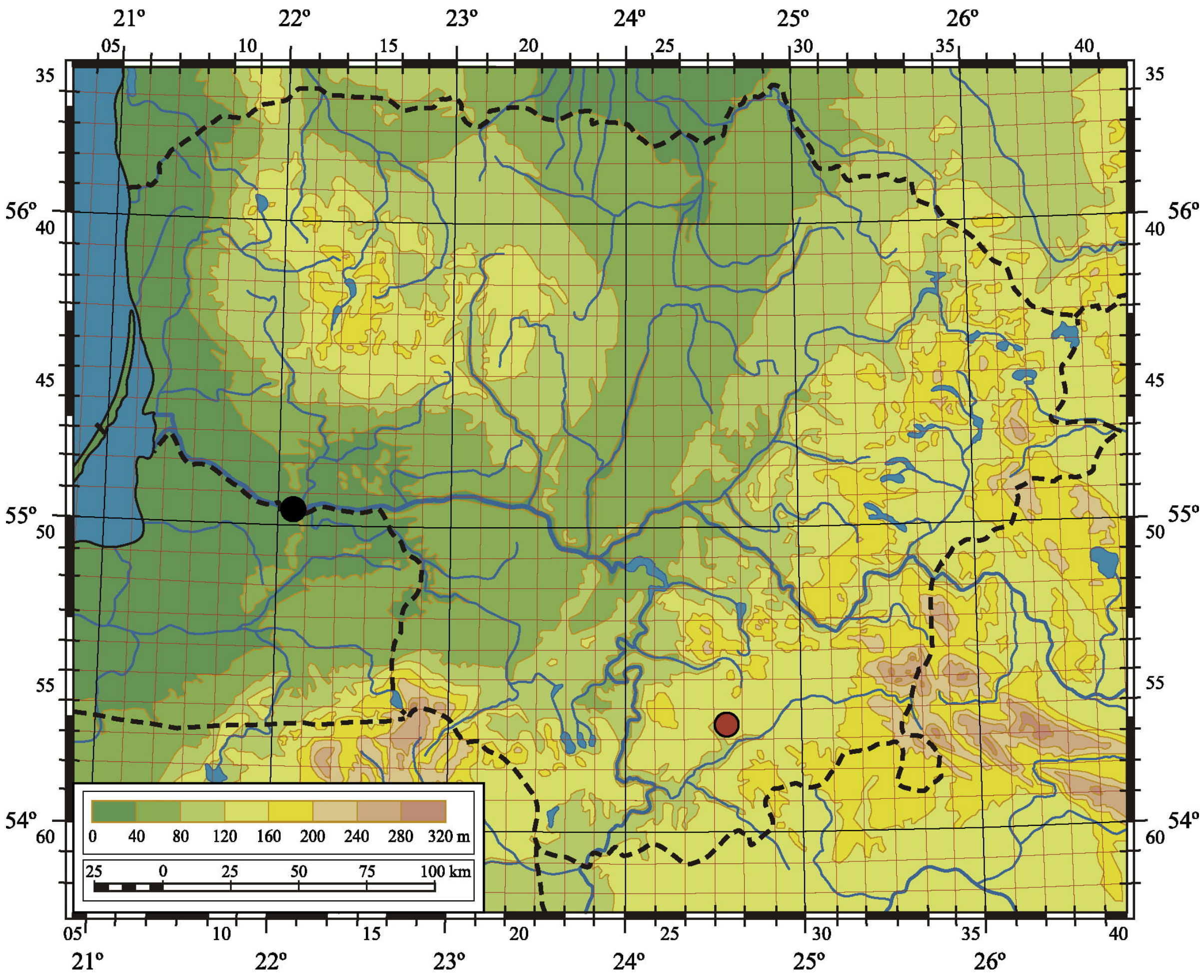

Fig. 5. Sites of occurrence of *Elatine hydropiper* in Lithuania. Red dot marks the site of the analyzed population.

The analysis of the number of seeds from the pooled sampling site data revealed that the highest mean density was found in the Southern site and the lowest in the Eastern site, but there was no significant difference in seed density between the Southern and Northern sites (z = 0.76; p = 0.446). The seed density in the Eastern site was significantly lower than in the other two sites (Table 2).

The analysis of *Elatine hydropiper* seed shell density in substrate revealed the same trends as those found for seed density. There were no significant differences in seed shell density between the A transects of the Northern and Southern sites (z = 0.52; p = 0.601), but both transects had significantly higher seed shell density than the transect at the Eastern site. There were no significant differences in mean seed shell density between the substrate of the B transects of the Northern and Southern sites (z = 0.44; p = 0.663), but both sites had significantly more seed shells than the B transect at the Eastern site (Table 3). The same patterns of seed shell density were found in the lake bottom sediments. There were no significant differences in seed shell density between the C transects at the Northern and Southern sites (z = 1.52; p = 0.127), but both had significantly more seed shells than the C transect at the Eastern site.

Comparison of the pooled data showed that there was no significant difference in seed shell density between the Northern and Southern sites (z = 0.14; p = 0.892), but they had a significantly higher seed shell density than at the Eastern site. A pairwise comparison of the pooled seed shell densities of the A, B, and C transects revealed that there were no significant differences between the terrestrial (A and B) transects (z = 0.41; p = 0.683), but both had significantly lower seed shell densities than the pooled aquatic (C) transects. Correlation analysis of pooled data showed that there is a strong reliable relationship between the number of seeds and the number of seed shells in the substrate samples (r_s = 0.83, $p < 0.001$).

In absolute numbers, seed shells were more abundant than seeds at all study sites, but there was no significant difference between seed and seed shell densities in the Eastern transect (z = 1.09, p = 0.274). Transects A, B, and C were all found to contain significantly more seed shells than seeds.

3.3 Habitat and Communities

Communities with *Elatine hydropiper* were described in water (0.1–0.5 m depth), in intermittently flooded coastal depressions, in the contact zone of water and shore (less

Table 1. Characteristics of *Elatine hydropiper* patches in three studied transects, mean number of patches re-calculated for square meter and coverage of plants per square meter (mean ± SD).

Transect	Number of patches (n)	Mean area of patch (cm^2)	Mean number of patches per m^2	Mean coverage (cm^2) of *Elatine hydropiper* per m^2
Northern	1069	0.96 ± 1.42^a	2140 ± 1416.9^a	2044.0 ± 1632.1^a
Eastern	634	0.58 ± 1.10^b	1270 ± 1258.5^b	758.1 ± 869.3^b
Southern	1311	1.40 ± 1.22^c	2624 ± 839.5^c	3687.8 ± 1525.4^c
Pooled	3014	1.07 ± 1.31	2155 ± 1241.5	2317.8 ± 1793.4

Different letters (a,b,c) denote statistically significant differences between the means among the transects applying the Dunn's post hoc test.

Table 2. Mean number of *Elatine hydropiper* seeds per liter of the substrate in the studied transects and at study sites.

Site	Transects			Pooled
	A	B	C	
Northern	95.8 ± 145.0^a	49.1 ± 34.0^a	49.9 ± 68.2^a	64.9 ± 93.9^A
Eastern	1.0 ± 3.2^b	30.5 ± 89.5^b	64.1 ± 80.7^a	31.9 ± 72.1^B
Southern	145.7 ± 334.3^a	45.7 ± 76.4^a	290.4 ± 459.0^b	160.6 ± 335.1^A
Pooled	80.8 ± 212.0^A	41.8 ± 68.7^A	134.8 ± 285.3^B	85.8 ± 210.2

Different lower-case and capital letters (a,b,A,B) denote statistically significant differences between the means among the transects and pooled data, respectively, applying the Dunn's post hoc test.

than 0.1 m depth), and in permanently wet exposed shores. A total of 40 cooccurring plant species were recorded with *Elatine hydropiper* in the 31 relevés examined. The analysis of the species composition of the communities revealed two large groups of relevés. One group includes terrestrial communities formed in wet sand or silt, while the second group unites communities in permanently submerged or intermittently flooded areas (**Supplementary Tables 1–3**).

The most constant (occurring in more than 50% of relevés) and abundant species were *Alisma plantago-aquatica*, *Alopecurus geniculatus*, *Eleocharis palustris*, and *Limosella aquatica*, while *Rorippa palustris*, *Rumex maritimus*, *Callitriche palustris*, *Eleocharis acicularis* were slightly less constant. Most of these species are characteristic of the class *Isoëto-Nanojuncetea* Br.-Bl. et Tüxen ex Br.-Bl. *et al*. 1952 and the alliance *Eleocharition acicularis* Pietsch ex Dierßen 1975 from the class *Littorelletea uniflorae* Br.-Bl. et Tüxen ex Westhoff *et al*. 1946.

Almost monospecific stands of *Elatine hydropiper* with only occasionally occurring juvenile individuals of *Typha* sp. were recorded in permanently submerged areas. In the zone of water level fluctuations, the diversity of co-occurring species was much higher. *Elatine hydropiper* was least abundant in communities belonging to the association *Limosello aquaticae-Eleocharitetum acicularis* Wendelberger-Zelinka 1952 (class *Littorelletea uniflorae*). Some of the communities with a predominance of *Alopecurus geniculatus* are classified in the initial stage of the association *Ranunculo repentis-Alopecuretum geniculati* Tüxen 1937, which belongs to the pioneer grassland communities of the alliance *Agropyro-Rumicion crispi* Nordhagen 1940 em Tüxen 1950. *Elatine hydropiper* was abundant both on land and in the water level fluctuation zone. The lowest abundance was recorded in the communities of the association *Scirpetum radicantis* Nowiński 1930 (class *Phragmito-Magnocaricetea* Klika in Klika et Novák 1941) formed furthest from the water line.

3.4 Assessment under IUCN Criteria

The available information on *Elatine hydropiper* in Lithuania shows that two populations are currently known in the country: on the exposed shores of Lake Merguva (Pagėgiai district) and on the exposed shores and shallows of Lake Pabezninkai (Varėna district). The population on the shores of Lake Merguva is very small, consisting of only a few individuals, while the majority of individuals (almost 100%) are concentrated in a single site on the shores and in the shallows of Lake Pabezninkai. With further successional changes along the shores of Lake Merguva, the current extent of occurrence (EOO) of less than 5000 km^2 is likely to be reduced by half in the future. For the same reasons, the current area of occupancy (AOO) may also decrease. The status of the population on the shores and in shallows of Lake Pabezninkai will depend on the further development of the lake. According to current data, the species should be considered vulnerable (VU) [A3; B1ab(iii); C2a(ii); D2], but may be classified as endangered (EN) in the near future.

Table 3. Mean number of *Elatine hydropiper* seed shells per liter of substrate in the studied transects and at the study sites.

Site	Transects			Pooled
	A	B	C	
Northern	290.4 ± 315.7^a	216.9 ± 136.2^a	133.6 ± 290.5^a	290.7 ± 309.6^A
Eastern	6.1 ± 9.9^b	91.8 ± 268.8^b	119.2 ± 76.5^a	72.4 ± 168.0^B
Southern	224.3 ± 275.9^a	228.2 ± 262.1^a	411.6 ± 438.0^b	288.0 ± 245.7^A
Pooled	173.6 ± 264.3^A	179.0 ± 231.2^A	298.5 ± 288.6^B	217.0 ± 265.8

Different lower-case and capital letters (a,b,A,B) denote statistically significant differences between the means among the transects and pooled data, respectively, applying the Dunn's post hoc test.

4. Discussion

4.1 Distribution and Abundance

In Lithuania, *Elatine hydropiper* has been recorded for a long time in only one locality, in the western part of the country [24,27]. Although the therophyte communities occupying wet and periodically flooded habitats, which are characteristic for *Elatine hydropiper*, have been studied in Lithuania in considerable detail, this species has not been recorded [44]. *Elatine hydropiper* has also not been detected during large-scale studies of lake vegetation, monitoring of water bodies and inventories of habitats of European importance throughout Lithuania [45–48]. Thus, *Elatine hydropiper* is a truly rare species in Lithuania and the rarity cannot be explained by insufficient investigations. This suggests that the species is restricted to specific habitats, shallows and wet shores of mesotrophic or oligotrophic lakes characterized by periodic fluctuations in water level. Very few habitats are known in Lithuania that could be favorable for the growth of *Elatine hydropiper*. Our targeted surveys have clearly shown that this species does not grow on the wet shores and shallows of other lakes that appear as suitable by all relevant habitat characteristics (e.g., lakes Lavysas, Glėbas, Glūkas). An assessment of the distribution of the species in the Baltic States and adjacent countries, despite the abundance of lakes, found the species to be rare or only occurring in restricted areas [21–24]. In Estonia, the largest and most abundant population of the species is concentrated in Lake Peipsi and its sandy shores, while in other localities the species is scarce [23]. *Elatine hydropiper* can be expected to be found in other locations in Lithuania in the future, but a significant increase in the number of localities, assessing the rarity of suitable habitats, cannot be anticipated.

We found that the area and number of patches of *Elatine hydropiper* were significantly lower on exposed sandy lake shores than on sand with pebbles and silty sand. Furthermore, the coverage of plants growing in silty sand was significantly higher than those growing in sand with pebbles and sand. We believe that the nature of the substrate determines both the accumulation and germination of *Elatine hydropiper* seeds and the establishment of seedlings. Seeds from mobile sand may be more easily washed into water during the cool season and some seedlings may be killed by drought. Furthermore, plants growing in sand can be easily uprooted by lake waves. We think this may explain the abundance of uprooted individuals found in the water near the sandy shoreline at the eastern site in 2020. In contrast, no or few uprooted individuals were found in the water at the northern site, where the shore is composed of sand with pebbles, and at the southern site, where the shore is composed of silt. We assume that sand with pebbles and silt has also a higher seed retention rate than pure sand and the resulting seedlings are better protected from uprooting by waves. Despite the variation in the number of patches and the variation in coverage on different substrates, the average cover of *Elatine hydropiper* on the exposed shores of Lake Pabezninkai is 23.17%. As this species was present on 0.95 ha of exposed shores, according to our estimates, a total of more than 20.4 million *Elatine hydropiper* patches and at least twice as many individuals could have been present in 2020.

4.2 Seed Bank

Seed bank analyses have shown that the substrate of the exposed shores of Lake Pabezninkai and the sediments of the lake bed contain a large number of *Elatine hydropiper* seeds and seed shells. Comparison of the study sites showed that the eastern site, which had clean sand without silt, had significantly fewer seeds and seed shells than the other study sites. There was also a significantly lower mean number of *Elatine hydropiper* patches and their coverage was comparable to those on sand with pebbles (Northern site) and silt (Southern site) shorelines. This suggests that the nature of the substrate has an impact on the abundance of individuals and hence on the accumulation of seeds in the soil. Some of the seeds that mature on land are washed into the lake by rising water during the cold season, during heavy rains, or by snowmelt water [49,50]. This is confirmed by the fact that the number of seeds and seed shells found in the bottom sediments is significantly higher than in the exposed shore substrate. It is important to add that the *Elatine hydropiper* growing in water was mostly sterile or of low fertility, while plants on the shore were highly fertile. Thus, the seed production of individuals growing in the water should be significantly lower than on land. This assumption is supported by the results of other studies. Seed production of amphibious plants that can tolerate water level fluctuations has been found to be much higher on wet shores than when growing underwater [50]. The high number of seeds in the bottom sediments can only be explained by the movement of seeds from the coast to the water.

We found that the number of seed shells of *Elatine hydropiper* was significantly higher than the number of seeds. These patterns indicate that the seed shells, or a large proportion of them, do not decompose and accumulate in the soil. The strongly significant correlation between the number of seeds and the number of seed shells in the soil indicates that there is a certain balance between the annual seed production and the accumulation in the seed bank and the depletion of the seed bank.

Poschlod and Rosbakh [4] have studied the bottom sediments of fish ponds and found that they contain a large seed bank of annual plants typical of wet shores and shallows of water bodies. However, the abundant seed bank and the potential for population recovery do not guarantee that this opportunity will be realized. Abandoned fish ponds usually are being reclaimed and converted to forests or grasslands. As a result, the seeds in the soil seed bank may never have the opportunity to establish a viable population, despite the long viability of the seeds. Furthermore, it has been estimated that at least 860 lakes in Lithuania were degraded during the 20th century, most of which were less than 0.5 ha in area [51,52]. Some of the drained and overgrown lakes have turned into wetlands, but 24% of the lake beds have become forested areas, 24% converted into grasslands, and 18% converted into arable lands [52]. There is very little chance that, even within a few decades, favorable conditions will ever be created for populations to recover from the seed bank of wet shore plants that used to be present in the substrate of the lake or pond sediments [4,53].

4.3 Habitat and Communities

We found that the population of *Elatine hydropiper* in Lake Pabezninkai and its exposed shores covered a large area. A smaller proportion of the population was submerged during the growth period and *Elatine hydropiper* formed submerged monospecific or species-poor communities. On the exposed shores of the lake, *Elatine hydropiper* rarely formed monospecific stands and grew in association with other plant species, mainly forming communities of the classes *Isoëto-Nanojuncetea* and *Littorelletea uniflorae*. Comprehensive studies of ephemeral vegetation of intermittently flooded wetlands in Poland [54], the Czech Republic, and Slovakia [55] have shown that *Elatine hydropiper* is most closely associated with the communities of the association *Polygono-Eleocharitetum ovatae* Eggler 1933. However, these communities do not occur in natural habitats in the above-mentioned Central European countries. Such communities are mostly found in fishponds, and *Elatine hydropiper* is sometimes referred to as a specific species of fishpond [56]. It should be added that *Eleocharis ovata*, a characteristic species of the *Polygono-Eleocharitetum ovatae* association, was also found in Lake Pabezninkai for the first time in Lithuania [35,36]. As the water level in Lake Pabezninkai has receded by about 1.5–1.6 m over the last four years, *Elatine hydropiper* was found in communities of different developmental stages. The lowest abundance of this species was found in the communities of *Scirpetum radicantis* Noviński 1930. These communities were recorded for the first time in Lithuania on the shores of this lake [35]. The occurrence of *Scirpetum radicantis* communities once again confirms the uniqueness and nature conservation value of this natural lake.

Considering the physical and chemical characteristics of the water and the vegetation, Lake Pabezninkai belongs to the habitat type of European importance (3130 Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae* and/or of the *Isoëto-Nanojuncetea*). The conservation status of this habitat type, with a few exceptions, has been assessed as bad or poor in most countries of the European Union [25]. Furthermore, mesotrophic habitats of periodically exposed shores with pioneer and ephemeral vegetation are classified as vulnerable in the European Red List of Habitats [13]. Lakes with low mineral content are highly vulnerable because biogenic substances from the environment cannot be quickly neutralized and cause irreversible processes [57,58]. There are no clear signs of pollution of Lake Pabezninkai at the present time, but until 1990 the lake was affected by pollution from nearby livestock farms. Despite the former pollution, the lake bottom sediments are not rich in nutrients, as the tall helophytes (*Phragmites australis*, *Typha latifolia*) grow fragmentary on the shores and the nitrophilous plants (*Bidens*, *Persicaria*) are scarce on the wet exposed shores [35,36].

4.4 Assessment under IUCN Criteria

Until 2020, only one very small population of *Elatine hydropiper* was known in Lithuania. Therefore, it was assessed as a data deficient (DD) species according to the IUCN criteria and was not included in the most recent list of protected species [59]. After the discovery of the new population and the assessment of the species applying the IUCN criteria, we found that it should be classified as a vulnerable (VU) species. Although the status of the species in Europe has been assessed as least concern (LC), it was included in Red Lists in most neighboring countries. In Poland, Denmark, and the Netherlands, *Elatine hydropiper* is considered vulnerable (VU) [26,60,61], in Estonia and Latvia endangered (EN) [19,23], in Switzerland critically endangered (CR) [22] and it is considered extinct in the Kaliningrad region (Russia) [20]. In Sweden and Finland, where many relatively shallow oligotrophic lakes remain, *Elatine hydropiper* is classified as a species of least concern [62].

The need to protect the Lake Pabezninkai as a vulnerable natural habitat of European importance is indisputable. The abundant population of *Elatine hydropiper*, a species rare in Lithuania, as well as other very rare (*Eleocharis ovata* and *Scirpus radicans*) and quite rare (*Juncus bulbosus* and *Ranunculus reptans*) plant species add to the conservation value of the lake. Unfortunately, there is still a lack of data on fluctuations in water level of the lake and their frequency. It is not known whether the current drop in water levels is only a short-term phenomenon or whether it is due to ongoing processes of groundwater level decline and will continue for decades. If the water level of the lake recovers, the area of suitable shore habitats will be reduced, but the conditions for the plant to grow in shallow water and, during periods of low water level, on exposed shores will improve. However, if the decline in water levels persists for a prolonged period, adverse habitat changes are possible, as the currently open banks may be overgrown by perennial plants and shrubs, and the conditions will not be suitable for the growth of *Elatine hydropiper*. Furthermore, if the decline in water levels continues, the lake could be in danger of disappearing, as happened to Lake Vazgirdonys (3.4 ha), just 0.5 km south of Lake Pabezninkai.

The greatest challenge for the protection of *Elatine hydropiper* and other annual or short-lived species typical of the transition zone between aquatic and terrestrial habitats is the low choice of active conservation measures and the lack of economically feasible good practice [2,3,7].

5. Conclusions

The population of *Elatine hydropiper* found in southern Lithuania, in Lake Pabezninkai and its shores, is one of the largest populations of this species in the Baltic States, both by area and abundance. The high abundance of *Elatine hydropiper* on exposed shores and in the water indicates that conditions are favorable for the species to exist in this area. Studies have confirmed that the lake bottom sediments and the substrate of the exposed shorelines contain an abundant seed bank of *Elatine hydropiper*, which can ensure the long-term survival of the population in the course of annual and successional habitat changes. The large amount of seed shells in the substrate suggests that the abundance of *Elatine hydropiper* individuals in the habitat is not a random event, but that there is a balance between accumulation and depletion of the seed bank.

The studied population of *Elatine hydropiper* occupies a natural habitat of lakes with low mineral content, which is classified as a habitat of European importance (3130 Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae* and/or of the *Isoëto-Nanojuncetea*). The population of *Elatine hydropiper* in Lake Pabezninkai is unique not only because of its size and abundance, but also because of its relatively intact habitat. In most Central European countries this plant is now commonly confined to anthropogenic habitats, mostly fish ponds.

We found that *Elatine hydropiper* is a vulnerable species in Lithuania and that its conservation is inseparable from habitat conservation. We do not yet know whether the significant drop in the lake's water level five years ago is a temporary phenomenon or resulting from climate change and a general decrease in precipitation, which has changed hydrological conditions over a large area. Further research should help to answer this question. If the water level in the lake continues to decline, there will be major challenges to protect not only *Elatine hydropiper* and other rare plant species, but also the entire habitat.

Author Contributions

ZG designed the research study. LT, LKB, ZS and ZG performed the research, analyzed the data and wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

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