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Current Distribution and Conservation Issues of Aquatic Plant Species Protected under Habitats Directive in Lithuania

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Abstract: The European Habitats Directive was adopted to halt the rapid loss of biodiversity and has become an important instrument for protecting biodiversity in the European Union. Three aquatic plant species protected under the European Habitats Directive have so far been found in Lithuania: *Aldrovanda vesiculosa*, *Caldesia parnassifolia*, and *Najas flexilis*. Our aim in this study was to evaluate the former and current distribution and the status of conservation of the target species. Screening for the above-mentioned protected aquatic plant species was performed in 73 natural lakes throughout Lithuania in 2019–2021. We confirmed extant populations of *Aldrovanda vesiculosa* in four lakes, *Caldesia parnassifolia* in two lakes, and *Najas flexilis* in four lakes in the northeastern part of the country. We studied *Aldrovanda vesiculosa* populations three times (2015, 2019, and 2022) in Lake Rūžas and once each in Lake Apvardai and Lake Dysnai (2020). The population density of *Aldrovanda vesiculosa* ranged from 193.4 ± 159.7 to 224.0 ± 211.0 individuals/m^2, the mean length of plants ranged from 12.5 ± 2.1 to 14.3 ± 2.7 cm, and the mean number of apices ranged from 2.0 ± 0.7 to 2.2 ± 0.9 per individual. The habitat of *Aldrovanda vesiculosa* in Lake Rūžas covered an area of about 3 ha. The number of generative individuals of *Caldesia parnassifolia* widely varied between years in Lake Rūžas. All populations of *Najas flexilis* were small, although the potential habitats in the studied lakes cover relatively large areas. We propose designating all lakes with populations of *Aldrovanda vesiculosa*, *Caldesia parnassifolia*, and *Najas flexilis* as special areas of conservation, as well as developing and implementing action plans for the conservation of these species and their habitats.

Keywords: *Aldrovanda vesiculosa*; *Caldesia parnassifolia*; communities; distribution; *Najas flexilis*; population size; special areas of conservation; turions; vegetative propagation

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1. Introduction

Inland freshwater bodies and wetlands are crucial ecosystems for maintaining ecological stability and preserving biodiversity [1–4]. Ecologically, economically, and socially important freshwater ecosystems are vulnerable, especially in agrarian and urbanised areas [5]. Freshwater ecosystems are changing because of both natural causes and anthropogenic pressure, particularly as a result of pollution and the resulting eutrophication of water bodies [3,6,7]. Climate change and the resulting alterations in water regimes also seriously impact the state of water bodies and wetlands [1,8]. The effects of these factors are altered habitats, reduced biodiversity, and threat of extinction in certain species [9–12].

The European Habitats Directive (Council Directive 92/43/EEC) was adopted to halt the rapid loss of biodiversity and has become an important instrument for protecting biodiversity in the European Union. The preamble to the Habitats Directive specifies that its primary objective is to ensure the restoration or maintenance of natural habitats and species of community interest at a favourable status. The Habitats Directive now aims to protect the 233 habitat types and 1389 characteristic, rare, or endangered species of flora and fauna in the European Union [13]. Twelve vascular plant species protected under the Habitats Directive are found in Lithuania, of which three (*Aldrovanda vesiculosa* L., *Caldesia parnassifolia* (L.) Parl., and *Najas flexilis* (Willd.) Rostk. & Schmidt) are aquatic plant species.

Aldrovanda vesiculosa is among the most renowned and studied aquatic plant species in the world because of its distinctive appearance, carnivorous habits, and worldwide rarity [14]. Globally, *A. vesiculosa* is considered an endangered (EN) species [15]. In North America, populations of this species are classified as non-native but not invasive [16,17]. New occurrences of the species have recently been discovered in India [18] and Mongolia [19], and several new localities have been reported in Ukraine [20]. In Europe, extant populations of *A. vesiculosa* have been recorded in 10 countries, where it occurs in natural habitats [15,21]. The species has been successfully reintroduced in the Czech Republic, and has been introduced in Germany [22], Switzerland, and the Netherlands [14]. Although the species has been recorded in 14 European countries, the continent-wide population is considered to be declining. Recently, 184 populations were confirmed as extinct, while only 49 populations were extant [15]. Relatively large and stable populations of *A. vesiculosa* persist in Poland, Lithuania, Russia, and Ukraine. More than half of the extant populations are concentrated in Ukraine, where they are currently most threatened [15,20].

Caldesia parnassifolia is widespread in the tropical and subtropical regions of Africa, Asia, Australia, and the temperate zone of Europe. Globally, the population status of the species is of least concern (LC) [23]. *C. parnassifolia* is a critically endangered (CR) species in Poland [24,25], Hungary [26], and Lithuania [27], whereas in Italy, it is declared extinct [10,28]. In Belarus, it is listed as a protected species [29].

Najas flexilis, in contrast to *Aldrovanda vesiculosa* and *Caldesia parnassifolia*, is mainly distributed in the boreal and temperate regions of Europe and North America, with isolated occurrences in Asia [19,30–32]. The status of the population of *Najas flexilis* is of least concern (LC) at the global level [31], but more than a decade ago, it was recognised as a vulnerable (VU) species [33]. *N. flexilis* is considered extinct in Germany, Switzerland, and Poland [34–36]. Most extant localities in Europe are concentrated in the British Isles [37], and a considerable number of occurrences have been recorded in Latvia [38]. The species has also been confirmed to occur in Norway, Finland, Sweden, Estonia, Lithuania, Austria, Belarus, and Russia [39–46].

The degradation and loss of suitable habitats because of natural and anthropogenic causes are among the most important drivers of species decline [7,47]. Aquatic, riparian, and wetland plants are particularly sensitive to changes in habitat conditions; therefore, their conservation and the protection of their habitats are challenging [7,47–50]. As such, precise knowledge of the distribution, habitat ecology, and population size of an endangered species is essential to ensure its favourable conservation [28].

At the initial stages of the implementation of the Habitats Directive in Lithuania, when the special areas of conservation (SACs) were first established, the focus was on the already known habitats of protected aquatic plants. Initially, experts assessed the status of the populations, and the Conservation and Action Plan for *Aldrovanda vesiculosa* [51] was prepared. Following the implementation of the nature management measures, in 2015, more extensive studies on *A. vesiculosa* were initiated. In 2019–2021, a new round of search and assessment for *A. vesiculosa*, *Caldesia parnassifolia*, and *Najas flexilis* was undertaken throughout the territory of Lithuania. The aim of this study was to assess the status of three aquatic plant species, *Aldrovanda vesiculosa*, *Caldesia parnassifolia*, and *Najas flexilis*, protected under the European Habitats Directive in Lithuania. With this study, we attempted to answer the following questions: (a) What is the current distribution of the three protected aquatic plant species in Lithuania? (b) What is the current state of the habitats of these species? (c) In which plant communities are *Aldrovanda vesiculosa* and *Caldesia parnassifolia* occurring? (d) How do the population density and morphological parameters of *Aldrovanda vesiculosa* depend on habitat conditions? (e) What are the conservation status and requirements of the target species?

2. Materials and Methods

2.1. Study Species

Aldrovanda vesiculosa L. (Droseraceae) is a perennial, rootless, free-floating carnivorous aquatic plant with a stem 6–20 cm long, and occasionally longer. The stem of a mature plant has 15–20 whorls of leaves, with 1–8 branches, or is unbranched. The leaf whorl consists of 6–9 leaves with snapping traps for small aquatic animals. In temperate regions, it can flower and produce viable seeds, but usually reproduces vegetatively. Branches detached from the parent individual in summer form a new individual, the apices of which turn into turions (wintering buds) in autumn [14,24].

This species has a wide range, extending from the tropical regions of Australia, Asia, and Africa, to the temperate regions of Europe. The northernmost occurrences of the species were recorded in northwestern Russia [15].

Caldesia parnassifolia (L.) Parl. (Alismataceae) is a perennial rhizomatous aquatic plant that grows up to 80 cm tall. Its leaves are floating or emerged, with petioles 5–100 cm long and ovate or elliptic leaf blades with a cordate base and obtuse apex. Its inflorescence is emergent and paniculate, with whorled branches. Its flowers are bisexual, with persistent sepals and ovate petals. Its fruitlets are obovoid, with 3–5 longitudinal ribs on each side. It reproduces vegetatively via turions, which form at the tips of rhizomes and sometimes in inflorescences [52–54].

This species is distributed mainly in the tropical and subtropical regions of Africa, Asia, and Australia, and occurs in the temperate regions of Europe [23]. The newly discovered locality on the border between Lithuania and Latvia [55] is the northernmost record for this species in Europe.

Najas flexilis (Willd.) Rostk. & Schmidt (Hydrocharitaceae) is an annual aquatic plant that grows completely submerged and rooted on the bottom. Its stem is 2.5–50 cm long. Its leaves are sessile, its lamina is minutely serrulate with unicellular teeth, and its apex is acute. The leaf base is slightly wider than the lamina and minutely serrulate with teeth like those of the lamina. The plants are monoecious. Female and male flowers are solitary, sessile, and enveloped in membranous involucre. Its fruits consist of one narrowly-to-broadly obovate seed. Its seed coat has 5–6 angled areolae [34,36].

This species is distributed in the boreal and temperate regions of North America and Europe, with a few isolated parts of its range known in Asia [30–32]. Many localities of *Najas flexilis* are recorded in Latvia [38]. The species also occurs in Belarus [44], whereas it is considered extinct in Poland [56].

2.2. Study Area and Sites

The study area was in the northeastern part of Lithuania, including parts of the Zarasai, Ignalina, and Visaginas administrative districts (Figure 1). The territory is in the Aukštaičiai Upland, which is a part of the Baltic Highlands. The relief of the region was shaped by two glacial flows of the last glaciation [57]. The Aukštaičiai Upland is characterised by an abundance of lakes, which occupy approximately 6% of its area [57]. Because the region has a more continental climate than other parts of Lithuania, it is characterised by a wide range of temperatures, colder winters, longer snow cover, and a shorter plant-growing season [58]. The mean annual precipitation in this area is 650 mm, approximately 65% of which falls during the warm season. Evaporation from the water surface is as high as 538 mm in May–October [58].

Most of the studied lakes belong to the Daugava River basin, except for Lake Dūkštas, which belongs to the Nemunas River basin [59]. All the studied lakes, except Lakes Avilys and Ažvintis, are in protected areas (including SACs of the *NATURA 2000* network).

The studied lakes have at least one inflow and outflow, meandering shorelines, and sheltered shallow bays. Alksnas, Apvardai, Dysnai, and Rūžas Lakes are natural, shallow, polymictic, unstratified, and mostly surrounded by mires (Table 1, Figure 1). Because the areas around the lakes experienced intensive agriculture in the past, much of the waterlogged land in the lake basins has been drained. Deep, dimictic, stratified, mesotrophic lakes, such as Sągardas, Ažvintis, and Dūkštas Lakes are natural, and are surrounded by forests, fields, and sparsely populated settlements (Table 1, Figure 1). The bottom substrate of the lakes with *Najas flexilis* populations consist of sand and gravel with a thin layer of silt, whereas the bottom substrate of the lakes with *Aldrovanda vesiculosa* and *Caldesia parnassifolia* consist of organic sediments, silt, and clay.

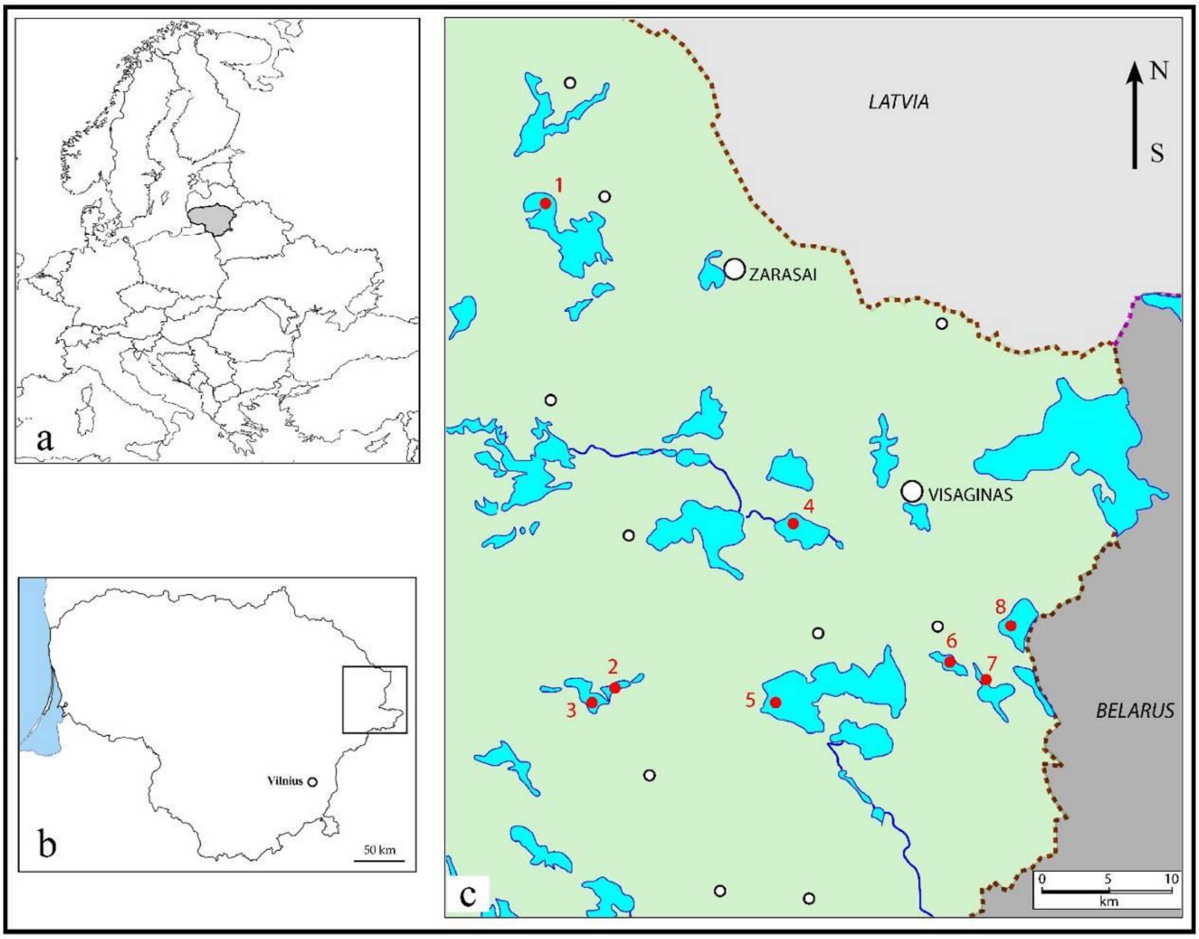

Figure 1. Position of Lithuania in Europe (**a**). A rectangle delineates the northeastern region of Lithuania (**b**). Lakes where the target species were found are marked by red dots (**c**): *Najas flexilis* in lakes Avilys (1), Ažvintis (2), Sągardas (3), and Dūkštas (4); *Aldrovanda vesiculosa* in lakes Dysnai (5) and Alksnas (6); *Aldrovanda vesiculosa* and *Caldesia parnassifolia* in lakes Rūžas (7) and Apvardai (8).

Table 1. Hydromorphological characteristics of studied lakes and physicochemical parameters of their water [60]. Physicochemical properties of water were determined in 2007 (Lake Apvardai), 2017 (Lakes Avilys and Rūžas), 2019 (Lakes Dysnai and Alksnas), 2020 (Lake Dūkštas), and 2021 (Lakes Sągardas and Ažvintis). Abbreviations: DO, dissolved oxygen; TDS, total dissolved substances; EC, electric conductivity; TN, total nitrogen; TP, total phosphorus.

No.	Lake	Area (ha)	Mean Depth (m)	Maximum Depth (m)	Secchi Depth (m)	DO (mg/L)	pH	TDS (mg/L)	EC (µS/cm)	TN (mg/L)	TP (mg/L)
1	Avilys	1224	3.0	13.5	3.7	8.8	8.6	1.7	245	0.54	0.01
2	Sągardas	114	7.6	26.5	5.2	9.9	8.4	1.0	165	0.48	0.01
3	Ažvintis	264	5.7	23.0	4.0	9.9	8.4	1.5	127	0.46	0.01
4	Dūkštas	520	5.4	10.5	4.8	9.4	8.6	1.3	207	0.53	0.01
5	Dysnai	2401	3.0	6.0	1.1	10.6	8.7	–	321	0.56	0.03
6	Alksnas	176	2.6	4.6	4.2	9.8	8.5	1.5	343	0.46	0.01
7	Rūžas	219	2.5	4.3	2.5	8.0	8.4	–	–	0.54	0.02
8	Apvardai	425	2.6	4.9	–	7.6	8.2	–	310	–	–

2.3. Historical and Current Distribution of Species

We critically reassessed the historical and current distributions of *Aldrovanda vesiculosa*, *Caldesia parnassifolia*, and *Najas flexilis* based on studies of the herbarium collections and analyses of the literature and other information sources. Because the literature in the first half of the 20th century contained inaccurate references to the locations of the studied species, we relied on the information provided by primary sources or on the labels of the herbarium specimens to assess the historical records of species. We used the plant specimens from the herbaria of the Institute of Botany of the Nature Research Centre (BILAS) and Vilnius University (WI) in this study.

We performed targeted searches for protected aquatic plant species of European importance in Lithuania from mid-July to mid-September 2019–2021. For the search for *Aldrovanda vesiculosa* populations, we selected 48 shallow lakes in the whole territory of Lithuania that were predominantly vegetated by floating-leaved plants and with banks fully or partially surrounded by mires. We selected the lakes according to orthophotographic images and previously collected information on plant species diversity and plant community composition. We also screened lakes where *A. vesiculosa* had been previously recorded. We did not perform targeted screening for *Caldesia parnassifolia*, but lakes where this species had been recorded previously were inspected.

To screen for *Najas flexilis*, we selected 25 mostly large and deep lakes with no or a weakly developed belt of floating-leaved plants and predominantly submerged vegetation consisting mainly of charophyte (or bryophyte) species in the whole territory of Lithuania. We paid particular attention to lakes where plants of the genus *Najas* had previously been found or were found during the survey. We surveyed the submerged vegetation in the lakes, searching for *N. flexilis* in transects perpendicular to the shoreline. We surveyed the shallows for *N. flexilis* using an aquascope, whereas in the deep areas, we obtained plant samples using a Bernatowicz's grab (0.4×0.4 m) or a grapnel for detailed identification and analyses. We recorded data on water and bottom sediment characteristics in each transect, and species diversity and abundance in different depth zones.

2.4. Assessment of Aldrovanda vesiculosa Populations

We started regular assessments of the population status and habitat condition of *A. vesiculosa* in Lake Rūžas in 2015, following the implementation of the Species Conservation Action Plan in 2013–2014. We conducted studies in Lake Rūžas in 2015, 2019, and 2022; we assessed Lake Apvardai and Lake Dysnai *A. vesiculosa* populations in August–September 2020. In Lake Rūžas, we performed studies at 20 sampling plots during each year of the study; in the small populations in Lakes Apvardai and Dysnai, we performed studies at 10 and 15 sampling plots, respectively. We analysed a total of 85 sampling plots.

At each survey point, we performed a phytosociological relevé using the Braun-Blanquet [61] approach. We assessed the plant community on minimum and maximum areas of 1 and 4 m^2, respectively, with uniform plant cover. After the community assessment, we placed a 0.25 m^2 floating square frame (all sides 0.5 m long) with a grid of 25 cells (10×10 cm) to delimit the sampling plot (Figure 2). We assessed the percentage cover of each plant species present in the sampling plot (with a precision of 0.1%). Then, we randomly selected ten individuals of *A. vesiculosa* from the sampling plot for length measurements and apex counts. We counted all individuals of *A. vesiculosa* in the sampling plot to determine their density. Then, we measured the water depth at the survey point. *Caldesia parnassifolia* occurred in communities with *Aldrovanda vesiculosa* in Lake Rūžas and Lake Apvardai; therefore, we did not separately assess the abundance of this species.

Figure 2. Sampling plot was delimited by a graded wooden frame to study *Aldrovanda vesiculosa* populations (photo by Z. Sinkevičienė).

The nomenclature of phytocenoses followed Šumberová [62]. The names of vascular plants were determined according to Euro+Med PlantBase [63].

2.5. Statistical Analyses

The results of the Shapiro–Wilk test showed that parts of the dataset (plant length, number of apices, number of individuals per sampling plot, cover of species or their groups, and depth) did not meet the criteria of normal distribution. Therefore, we compared the datasets using nonparametric statistical analysis methods. We used the Kruskal–Wallis H-test to detect differences between plant length, number of apices, number of individuals per sampling plot, and cover of *Aldrovanda vesiculosa* from different study sites and years, and we applied the Mann–Whitney U-test for post hoc pairwise comparison. When presenting descriptive statistics, we report the mean and the standard deviation (mean $\pm$ SD), and the minimum, maximum, and median values. Relationships between the number of individuals and water depth, and between the coverage of individuals and water depth, were estimated using Spearman's rank-order correlation. Our results and data on the length of *A. vesiculosa* individuals obtained from references were compared using a *t*-test. Because we counted individuals of *A. vesiculosa* in 0.25 m^2 sampling plots during the study, we multiplied the number of individuals in each plot by four, and we calculated the mean density of individuals per 1 m^2 from the result. We performed principal component analysis (PCA) using the number of individuals of *A. vesiculosa*, the cover of submerged, floating, and emergent plants, and the water depth in the sample plot. We used a correlation matrix between groups in the analysis. We considered each study in different years in the same lake or in different lakes as a separate group. We performed a cluster analysis of the communities with *A. vesiculosa* and *Caldesia parnassifolia*, and we created a dendrogram using the paired group (UPGMA) algorithm and Euclidean distance. We performed all calculations using PAST 4.10 software [64].

3. Results

3.1. Historical and Current Distribution of Species

Our comprehensive analysis of the literature and herbarium data showed that *Aldrovanda vesiculosa* was first recorded in Lithuania in 1955 in Lake Dysnai (WI) [65]. An earlier reference [66] to the occurrence of *A. vesiculosa* in the vicinity of Vilnius in the 19th century is a misinterpretation, and this error has been repeated by several recent authors [15,67]. Diels [66] cited Gorski [68] as a source of information, but he was referring to the locality of *A. vesiculosa* in the Pinsk region (present-day southern Belarus), not in the vicinity of Vilnius. The discovery of this species in 1821 in the Pinsk region was reported by Wolfgang [69], who was probably the primary source of information.

The search for *A. vesiculosa* in Lake Dysnai, which lasted almost half a century, was unsuccessful. A relatively large population of the species was discovered only in 2001 in Lake Rūžas, a few kilometres northeast of Lake Dysnai [70]. During subsequent investigations of lakes in the northeastern part of Lithuania, in 2005, *A. vesiculosa* was rediscovered in Lake Dysnai [51], but in a different part of the lake to which it had been found in 1955 [65]. Furthermore, new localities of the species were found in Lake Alksnas [51] and Lake Apvardai [71] in 2005 and 2006, respectively. The record of *A. vesiculosa* in the Ignalina district in Lake Daržinėlė [71] was not confirmed by herbarium specimens and has not been confirmed in any subsequent study.

Our extensive searches for *A. vesiculosa* in 48 selected potential lakes between 2019 and 2021 did not reveal any new localities of this species. Considering the similarity of the vegetation and ecological conditions to the already known localities, we found ten lakes to be suitable for *A. vesiculosa*, but we did not record the species. Despite diligent searching, we did not find the species in any of the suitable bays of Lake Dysnai, except for the northwestern bay. The surveys confirmed that *A. vesiculosa* is currently growing in previously recorded localities in Lakes Rūžas, Apvardai, and Alksnas.

Caldesia parnassifolia was first found in present-day Lithuania in the early 19th century [72]. We confirmed the record of the species in the vicinity of the city of Vilnius through herbarium specimens (WI). In the first half of the 19th century, another locality of this species was registered in western Lithuania, in Kretinga, in a pond near a monastery (BILAS), but whether the plant grew naturally or was planted remains unclear [73,74]. In the middle of the 20th century, two localities of *C. parnassifolia* were recorded in southern Lithuania, in Lakes Daugai [75] and Ilgis [65]. The species was subsequently not detected at these sites and was considered extinct in Lithuania [27,76,77]. More than half a century later, *C. parnassifolia* was rediscovered at a new site in northeastern Lithuania, in Lake Rūžas [78]. Another locality of this species was found in the nearby Lake Apvardai during a survey of an *Aldrovanda vesiculosa* habitat. Both populations of *Caldesia parnassifolia* that are currently known in Lithuania are more than 100 km to the north of the previously recorded localities. In 2021, *C. parnassifolia* was found in Lake Kampiniškiai in the territorial waters of Latvia and Lithuania [55]. The lake is divided into two parts by the state border between Latvia and Lithuania; in Latvia, it is named Lake Lielais Kumpanišķi (Medumi municipality).

Najas flexilis was first found in Lithuania in the second half of the 20th century. Fragments of plant parts of this species were intermixed in the herbarium specimen of *Chara aspera* Willd. (BILAS) collected in 1966 in Lake Germantas (western Lithuania) [40,76]. During subsequent studies, *Najas flexilis* was not rediscovered in this lake. A new locality of the species was discovered in 1998 in Lake Sągardas (eastern Lithuania) [40,76]. During an extended search for the species in 2019–2021, we identified three new localities in Lakes Avilys, Ažvintis, and Dūkštas, and we confirmed that *N. flexilis* still occurs in Lake Sągardas.

3.2. Assessment of *Aldrovanda vesiculosa* and *Caldesia parnassifolia* Populations

3.2.1. Habitats and Communities

Aldrovanda vesiculosa in Lake Rūžas (Figure 3) occurred in an approximately 730 m long northern inlet, with a width ranging from 15 to 80 m, and a total area of about 33,000 m^2 (Table 2). The inlet was connected by a stream with Lake Žilmas to the north. *A. vesiculosa* was distributed throughout the length of the inlet, but mainly concentrated along its shores and amongst floating-leaved plants (ca. 11,000 m^2). The water depth in the inlet varied from (0.1) 0.5 m at the coast of the mire to 2 m at its deepest points. The water was clear, slightly brownish, and transparent to the bottom.

Table 2. Area of *Aldrovanda vesiculosa* habitats, area of occurrence, and calculated number of individuals (mean ± SD) in the studied lakes.

Lake	Year	Area of Habitat (m^2)	Area of Occupancy (m^2)	Number of Individuals
Rūžas	2015	33,000	11,000	2,127,400 ± 1,756,700
Rūžas	2019	33,000	11,000	2,464,000 ± 2,321,000
Rūžas	2022	33,000	11,000	2,248,400 ± 1,005,400
Apvardai	2020	6400	2400	219,840 ± 81,840
Dysnai	2020	10,000	3300	306,240 ± 145,200
Alksnas	2021	<10	1	5

In Lake Dysnai, the species occurred in the southwestern bay at the mouth of the Svetyčia stream. The plants were scattered in a shallow part of the bay, up to 1 m deep, and the habitat covered approximately 10,000 m^2 (Table 2). This shallow area was protected from direct wave action by a wide belt of *Nuphar lutea* (L.) Sm. stands. Loose stands of *Typha angustifolia* L., floating-leaved plants, and swampy shores were the main refuge for *Aldrovanda vesiculosa*. The water was turbid and greenish or yellowish.

In Lake Apvardai, *A. vesiculosa* was fragmentarily distributed along the southwestern and western shoreline and occupied a total area of approximately 6400 m^2 (Table 2). We found it in open areas between floating swampy islets and sparse stands of helophytes (*Typha angustifolia* and *Schoenoplectus lacustris* (L.) Palla). These areas were also separated from the main part of the lake by a belt of *Nuphar lutea* stands. The water was slightly greenish or brownish and moderately turbid.

We confirmed only a few individuals of *Aldrovanda vesiculosa* occurring in Lake Alksnas at the mouth of the stream that connected it to Lake Liūneliai in a small area (Table 2). The dominant vegetation in Lake Alksnas was charophytes, and it was therefore unsuitable for *A. vesiculosa*; it can only grow in shallow water in a mire. In all the other lakes, the localities of *A. vesiculosa* were surrounded by transitional mires or quaking bogs and at the mouths of inflowing or outflowing streams. These parts of mainly eutrophic lakes showed signs of dystrophy. The bottoms of the lakes were composed of thick blackish organic sediments.

We recorded 37 plant species in the 85 phytosociological relevés with *A. vesiculosa* from Lakes Rūžas, Dysnai, and Apvardai (Appendix A; Supplementary Material, Table S1). The communities were dominated by vascular plants, and only one species, *Nitellopsis obtusa* (Desv.) J. Groves belonged to the charophytes. We did not identify the species of filamentous algae, so they were not included in the number of species, but they are an important indicator of the ecological conditions of habitats. We combined *Utricularia australis* R. Br. and *Utricularia vulgaris* s.str., which are rarely found with the flowers necessary for accurate identification, into a single taxon: *Utricularia vulgaris* s.l. We recorded the highest number of species, 27, in Lakes Apvardai and Rūžas in 2020 and 2022, respectively. We recorded floating-leaved and free-floating plants (*Nuphar lutea*, *Potamogeton natans* L., *Nymphaea candida* J. Presl & C. Presl., *Stratiotes aloides* L., *Hydrocharis morsus-ranae* L., and *Utricularia vulgaris* s.l.), as well as submerged plants (*Potamogeton* × *bambergesnis* Fisch. and *Myriophyllum* cf. *verticillatum* L.), in all the studied communities (Figure 3). We assumed that *Myriophyllum* cf. *verticillatum* included not only the species itself but also its hybrids.

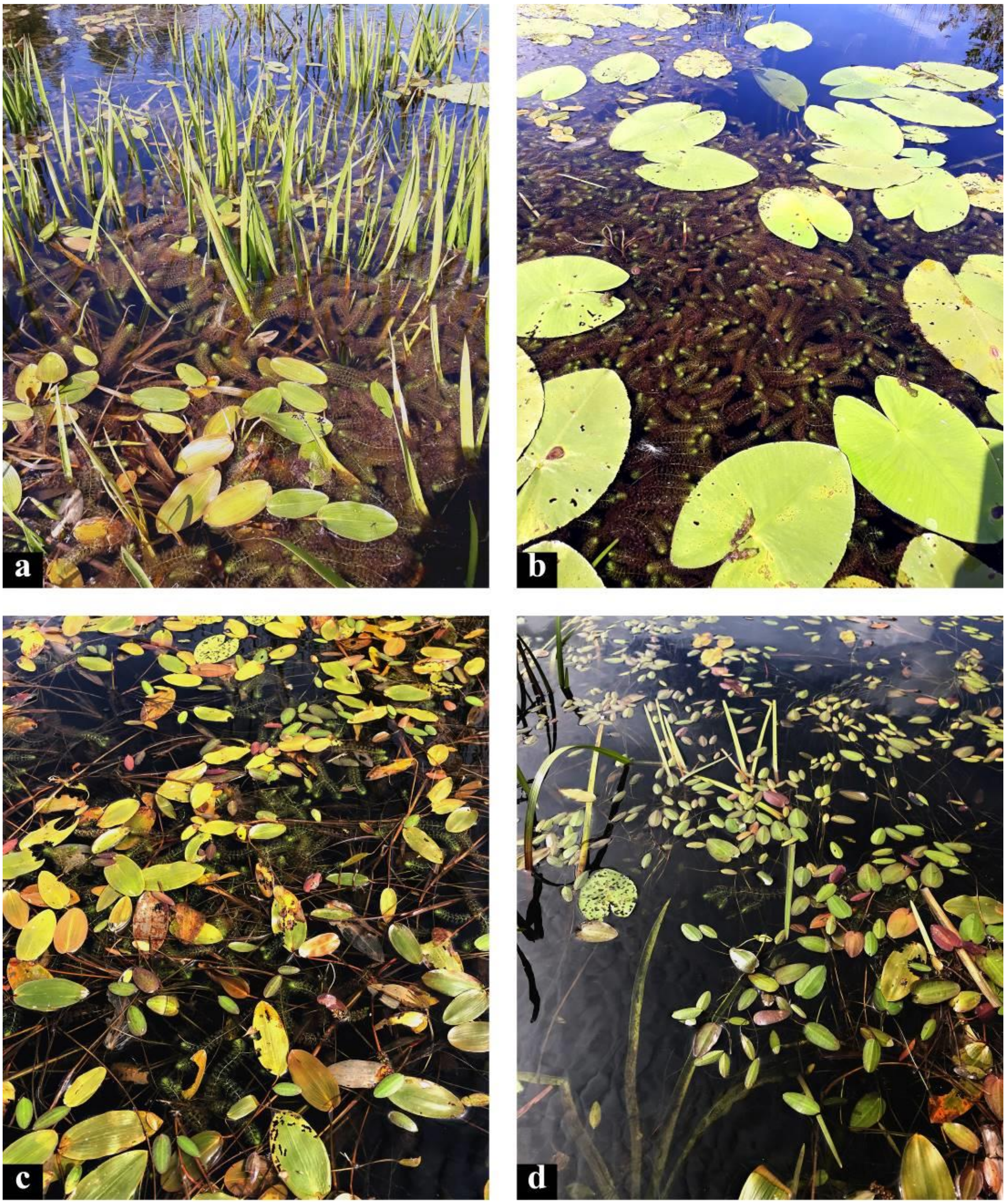

Figure 3. Plant communities of floating-leaved and floating plants with dominant *Aldrovanda vesiculosa* (**a**,**b**) and communities with *Aldrovanda vesiculosa* and *Caldesia parnassifolia* (**c**,**d**) in Lake Rūžas. Photos by Z. Sinkevičienė (**a**,**b**) and L. Petrulaitis (**c**,**d**).

We found *Aldrovanda vesiculosa* in all 85 phytosociological relevés, which was usually abundant. Most of the phytocenoses with dominant *A. vesiculosa* were grouped in one cluster (Figure 4, cluster C). We could hardly attribute these relevés to the association *Spirodelo-Aldrovandetum vesiculosae* Borhidi et Járai-Komlódi 1959, as the presence of the characteristic species of the *Lemnetea* class (*Utricularion vulgaris* alliance) was negligible compared with that of the species of the *Potametea* class (*Nymphaeion alliance*). Some of the relevés from this cluster, especially those recorded in the shallows of Lakes Apvardai and Dysnai, were similar to the relevés from cluster J (Figure 4) recorded in the same lakes, where none of the species present were dominant.

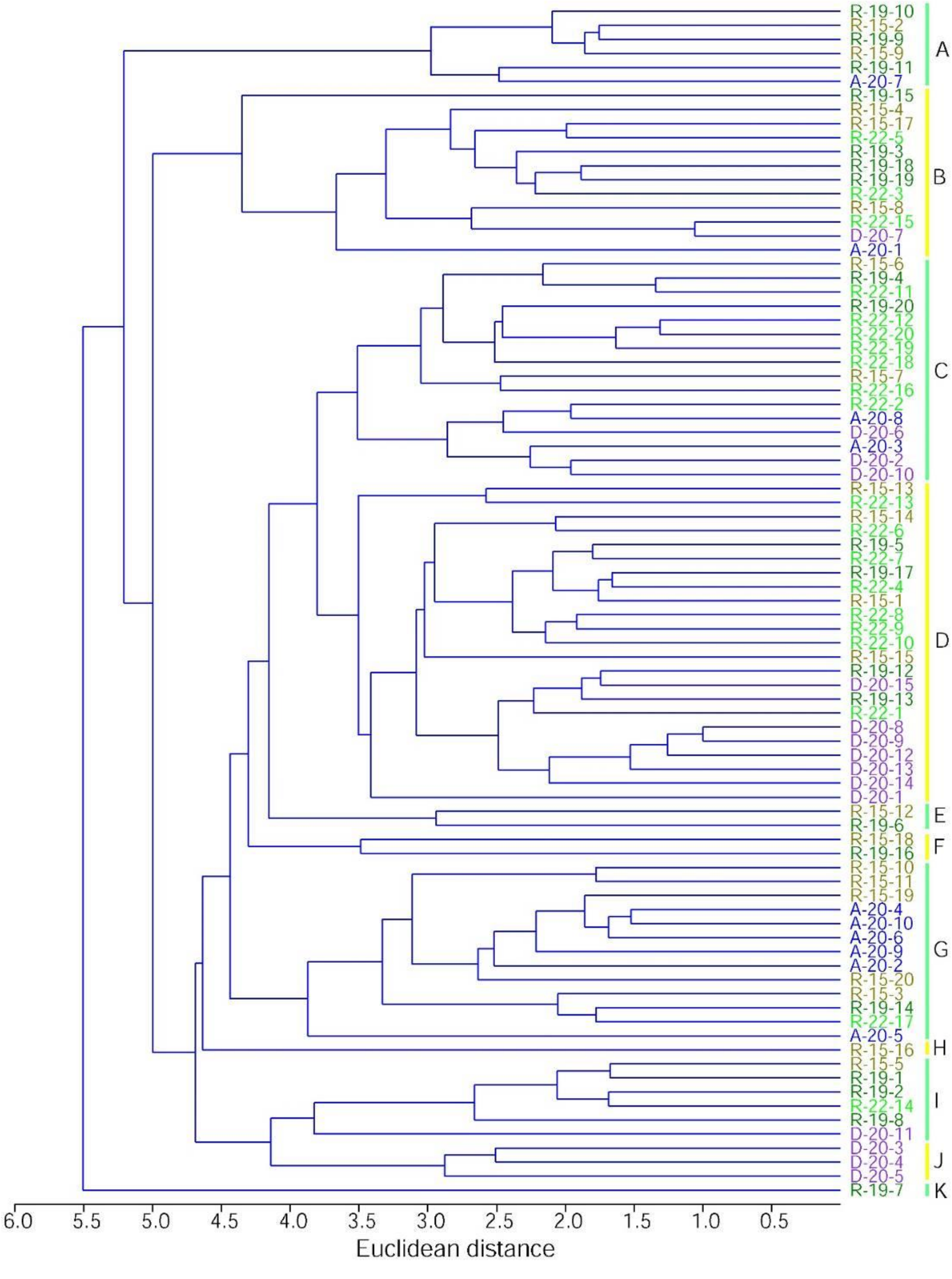

Figure 4. Dendrogram of communities with *Aldrovanda vesiculosa*, created using paired-group (UPGMA) algorithm and Euclidean distance. Different colours indicate different study years and lakes: Lake Rūžas (olive, green, and light green in 2015 (R-15), 2019 (R-19), and 2022 (R-22), respectively), Lake Apvardai (blue in 2020 (A-20)), and Lake Dysnai (dark purple in 2020 (D-20)). In relevé numbers, last two digits indicate sequence number of relevé (Supplementary Material, Table S1). Capital letters on the right refer to clusters explained in the text.

We commonly found *A. vesiculosa* in stands dominated by *Nuphar lutea* (association *Nymphaeo albae-Nupharetum luteae* Nowińsky 1927), especially in Lakes Rūžas and Dysnai (Figure 4, cluster D). Less frequently, this species occurred in phytocenoses of the associations *Potametum natantis* Hild 1959 (Figure 4, cluster G) and *Stratiotetum aloidis* Miljan 1933 (Figure 4, cluster B,), mainly recorded in Lakes Rūžas and Apvardai. We also found *Aldrovanda vesiculosa* in the association *Myriophylletum verticillati* Gaudet ex Šumberová in Chytrý 2011 (Figure 4, cluster I) in Lakes Rūžas and Dysnai. Only occasionally was the study species recorded in stands of *Sparganium emersum* L. (cluster E), *Ceratophyllum demersum* L. (cluster H), or *Sparganium natans* L. (cluster K) in Lake Rūžas (Figure 4).

Caldesia parnassifolia was a constant and sometimes abundant (Figure 3) co-occurring species with *Aldrovanda vesiculosa* in Lakes Rūžas and Apvardai. Phytosociological relevés with dominant *Caldesia parnassifolia* were clustered together (Figure 4, cluster A). We inventoried communities dominated by flowering individuals of *C. parnassifolia* in Lake Rūžas in 2015 and 2019, but we recorded no such stands in 2022. Flowering individuals were mainly located along the swampy shores up to a depth of 0.5 m in the lake and were most abundant in 2019. Nonflowering individuals of *C. parnassifolia* were widespread throughout the inlet and were a quite constant component of the communities surveyed in all years of the study.

3.2.2. Morphology of *Aldrovanda vesiculosa*

We recorded the longest individual (23 cm) of *A. vesiculosa* in Lake Rūžas in 2015. The mean length of *Aldrovanda vesiculosa* individuals only slightly varied between study years (Table 3). We found the highest mean length of individuals in Lake Rūžas in 2015 (14.3 ± 2.7 cm), and their mean length was significantly longer ($p < 0.01$) than that in other years in Lake Rūžas and in Lakes Apvardai and Dysnai. We found no significant differences ($p > 0.05$) between the lengths of *A. vesiculosa* individuals in Lake Rūžas in 2019 and 2022 and the lengths of individuals in Lakes Apvardai and Dysnai in 2020 (Table 3).

Table 3. Descriptive statistics of *Aldrovanda vesiculosa* individuals in different years and lakes (mean ± SD, minimum and maximum values, median). Different lower-case letters in superscript indicate significant differences ($p < 0.05$) across rows according to Mann–Whitney pairwise comparison.

Lake	Rūžas	Rūžas	Rūžas	Apvardai	Dysnai
Study year	2015	2019	2022	2020	2020
Number of studied individuals	197	196	200	100	150
Plant length (cm)	14.3 ± 2.7^{a}	12.5 ± 2.5^{b}	12.5 ± 2.1^{b}	13.2 ± 3.2^{b}	12.6 ± 2.6^{b}
Minimum–maximum	8–23	7–20	6–19	7–21	8–22
Median	14.5	12.0	12.5	13.0	12.0
Number of apices	2.2 ± 0.9^{a}	2.0 ± 0.9^{b}	2.0 ± 0.7^{b}	3.4 ± 1.2^{c}	3.6 ± 1.2^{c}
Minimum–maximum	1–4	1–5	1–4	1–8	1–7
Median	2	2	2	3	3

The number of shoots, and thus, the number of apices of *A. vesiculosa* indicates their reproductive capacity because each apex forms a turion from which a new plant may grow the following year. During the study, we found that individuals had a maximum of eight apices. *A. vesiculosa* had significantly fewer apices (Table 3) in Lake Rūžas than in Lakes Apvardai and Dysnai ($p < 0.001$), and we found no difference between the populations of the latter two lakes in the number of apices ($p = 0.939$). In Lake Rūžas, the number of apices of *A. vesiculosa* individuals was significantly higher (Table 3) in 2015 than in 2019 ($p = 0.023$) and 2022 ($p = 0.044$), whereas the number of apices did not significantly differ between 2019 and 2022 ($p = 0.570$).

3.2.3. Density and Cover of *Aldrovanda vesiculosa* Individuals

The density of *A. vesiculosa* individuals varied more between the lakes studied than between years in Lake Rūžas (Table 4). In this lake, the density ranged from 193.4 ± 159.7

to 224.0 ± 211.0 individuals/m^2, but the density did not significantly differ between years ($p > 0.05$). The density of *A. vesiculosa* in Lakes Apvardai and Dysnai was significantly ($p < 0.01$) lower than that in Lake Rūžas in all years of the study (Table 4). We found the same patterns when assessing the coverage of *A. vesiculosa* individuals in the study plots (Table 4). In Lake Rūžas, we found no significant differences between the coverage of individuals in the study plots in any survey year ($p > 0.05$). In the individual study plots, *A. vesiculosa* covered between 3.2% and 75.5% of the surface area (Table 4). We found a weak significant correlation between the number of individuals in the study plot and water depth ($r_s = 0.32$; $p = 0.003$) and between the coverage of individuals and water depth ($r_s = 0.35$; $p = 0.001$).

Table 4. Characteristics of sampling plots and descriptive statistics of *Aldrovanda vesiculosa* density and cover in different years and lakes (mean ± SD, minimum and maximum values, median). Different lower-case letters in superscript indicate significant differences across rows according to Mann–Whitney pairwise comparison.

Lake	Rūžas	Rūžas	Rūžas	Apvardai	Dysnai
Study year	2015	2019	2022	2020	2020
Number of sampling plots	20	20	20	10	15
Mean depth (m)	1.0 ± 0.2 ^{a}	0.9 ± 0.2 ^{a}	1.0 ± 0.2 ^{a}	0.6 ± 0.2 ^{b}	0.4 ± 0.1 ^{c}
Density of individuals per m^2	193.4 ± 159.7 ^{a}	224.0 ± 211.0 ^{ab}	204.4± 91.4 ^{a}	91.6 ± 34.1 ^{b}	92.8 ± 44.0 ^{b}
Minimum–maximum	32–564	24–744	79–400	40–140	44–212
Median	146	158	165	90	84
Mean cover (%)	28.8 ± 19.5 ^{a}	25.4 ± 18.7 ^{ab}	29.7± 13.2 ^{a}	14.3 ± 5.9 ^{b}	14.0 ± 6.5 ^{b}
Minimum–maximum	7.5–75.5	3.2–65.0	11.5–58.0	5.1–26.9	7.3–28.9
Median	24.0	18.1	24.0	12.9	11.7

The results of the PCA performed using data from 85 sampling plots showed that the first component (PC1) explained 70.1% (eigenvalue: 3.506) and the second component (PC2) explained 15.2% (eigenvalue: 0.761) of the variance (Figure 5). The loadings for each factor in PC1 and PC2 are presented in Table 5.

Table 5. Loadings of the analysed factors in first two principal components.

Component	PC1	PC2
Number of *Aldrovanda vesiculosa* individuals per sampling plot	0.459	0.437
Cover of submerged plants (%)	−0.319	0.716
Cover of floating plants (%)	0.436	−0.372
Cover of emergent plants (%)	−0.506	0.009
Water depth in the sampling plot (m)	0.491	0.397

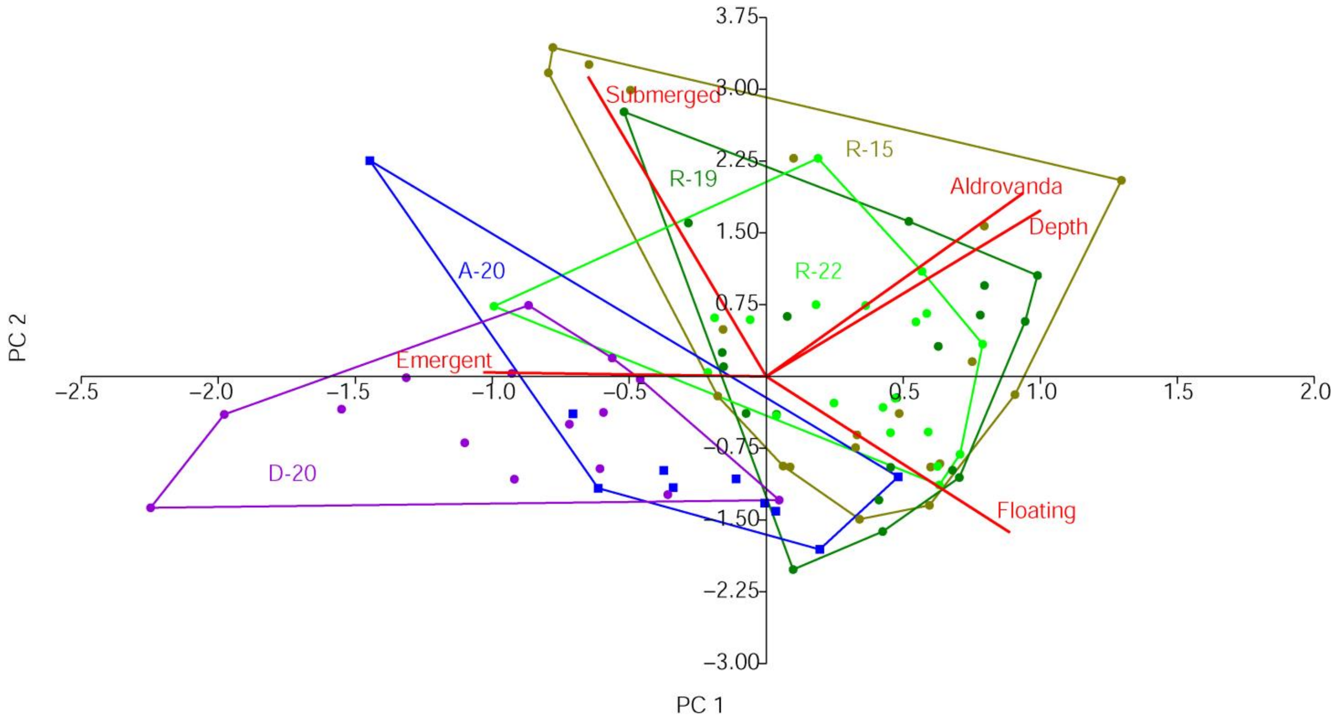

Figure 5. Principal component analysis of studied *Aldrovanda vesiculosa* populations based on density of individuals; cover of submerged, floating-leaved (excluding *Aldrovanda vesiculosa*), and emergent plants; and water depth in sampling plots. Different-coloured symbols and convex hulls indicate different study years and lakes: Lake Rūžas (olive, green, and light green in 2015 (R-15), 2019 (R-19), and 2022 (R-22), respectively), Lake Apvardai (blue in 2020 (A-20)), and Lake Dysnai (dark purple in 2020 (D-20)).

3.3. Assessment of Najas Flexilis Habitats

At all the studied sites, the abundance of *N. flexilis* was very low. At two sites in Lake Ažvintis, we found only solitary individuals of *N. flexilis* growing at a depth of 1.5 m on a sparsely vegetated bottom, in the transition zone between helophytes and submerged plants. We recorded a slightly more abundant population of this species at two sites in Lake Avilys. We found sparse *N. flexilis* at a depth of 1.2 m among sparse swards of *Fontinalis antipyretica* Hedw., *Hydrilla verticillata* (L. f.) Royle, *Potamogeton rutilus* Wolfg., *Potamogeton perfoliatus* L., and *Schoenoplectus lacustris*. *Najas flexilis* was somewhat more frequent in Lake Dūkštas, but, as in other areas, it was not abundant. We usually observed it at depths of 1–3 m in stands of charophytes (*Chara filiformis* A. Braun in Hertzsch, *Chara globularis* Thuill., *Chara virgata* Kütz., and *Chara tomentosa* L.) and bryophytes (*Fontinalis antipyretica*, *Drepanocladus aduncus* (Hedw.) Warnst., and *Rhynchostegium riparioides* Hedw.), often together with *Najas minor* All.

The population of *Najas flexilis* in Lake Sągardas has been known for more than two decades. Our current studies showed that the species grows in this lake at a depth of 1.5–3.5 m, together with *Nitella flexilis* (L.) C. Agardh, *Chara strigosa* A. Braun, and *Elodea canadensis* Michx., although it is also not abundant.

Most of the assessed populations of *Najas flexilis* occurred in lakes with bottom sediments consisting mainly of gravel or sand and a thin layer of silt. The bottom vegetation in these lakes was relatively sparse, formed mainly by mosses and macroalgae.

3.4. Conservation Status and Prospects

3.4.1. Conservation of *Aldrovanda vesiculosa*

Two special areas of conservation (SACs) of the *NATURA 2000* network were established in Lithuania within the conservation objectives of protecting *A. vesiculosa* populations (the *Lake Rūžas* SAC and the *Environs of Ažušilė–Didžiagiris* SAC). However, this species has not been confirmed in Lake Daržinėlė, situated in the *Environs of Ažušilė–Didžiagiris* SAC. Despite the proposals, no SACs have yet been established for the protection of the species in Lakes Apvardai and Dysnai. Lake Alksnas is in the *Pušnis Bog* SAC; however, objectives for the conservation of *A. vesiculosa* populations have not been defined in this SAC.

A conservation plan for *A. vesiculosa* has been developed to restore and maintain the favourable conservation status of its habitats in Lithuania [51]. Based on this document, a management plan for the habitat of the species in Lake Rūžas was developed in 2012 and implemented in 2013–2014. Based on the results of an expert assessment of the habitat, the management plan for *A. vesiculosa* included the following measures: (a) the removal of *Nuphar lutea* rhizomes forming floating islands on the surface of the water; (b) the removal of floating islets formed on rhizome confluences or detached from the shore from the lake (Figure 6); (c) the removal of tree and shrub thickets on the shores and on large islets, which provide shade in the lake; and (d) the removal of obstructions on the bed of the stream linking Lakes Rūžas and Žilmas to re-establish the natural circulation of water.

Figure 6. Aggregations of uprooted *Nuphar lutea* rhizomes (**a**), and uprooted individuals of *Caldesia parnassifolia* (**b**). Photos by Z. Sinkevičienė.

In late autumn, after the growing season had ended and *Aldrovanda vesiculosa* turions had sunk to the bottom, floating aggregations of *Nuphar lutea* rhizomes were removed from the lake and the stream bed using a special floating technique. Small floating islets and aggregations of floating islets and dams were also removed from the stream bed. Trees growing on the banks of the lake and larger islets were felled and all their biomass removed from the habitats. This improved the light regime of the habitat, as the trees growing on the shores of the small bays, where most of the *Aldrovanda vesiculosa* individuals were concentrated, provided strong shade.

Following the implementation of the measures outlined in the action plan for the conservation of *A. vesiculosa* in Lake Rūžas, we assessed the species population in 2015, 2019, and 2020 (Table 2). The results of the studies showed that the number of individuals in the population had remained relatively stable. The state of this population could be assessed as favourable; however, further regular observation and assessment of the state is necessary to analyse the effect of filamentous algae and assemblages of uprooted floating *Nuphar lutea* rhizomes.

The populations of *Aldrovanda vesiculosa* in Lakes Apvardai and Dysnai were much smaller and contained fewer individuals than those in Lake Rūžas (Table 2). We constantly observed the presence of filamentous algae in both lakes. They had been severely affected in the past by agricultural activities, such as the draining of the surrounding wetlands and the presence of livestock farms in the catchment area of the lakes. The negative effects of agricultural activities are continuing, as drainage water from cultivated fields is still flowing into the western part of Lake Apvardai. The state of both populations could be assessed as unfavourable.

The true state of the entire population in Lake Alksnas remains unknown due to its inaccessibility. By 2021, *A. vesiculosa* was almost extinct in the accessible part of the site at the mouth of the stream flowing from Lake Liūneliai.

3.4.2. Conservation of *Caldesia parnassifolia*

In Lithuania, *C. parnassifolia* occurs in one of the previously designated SACs, the *Lake Rūžas SAC*, but the species has not yet been included in the conservation targets. We propose amending the conservation targets of the *Lake Rūžas* SAC to include *C. parnassifolia*. We also propose designating Lake Apvardai as an SAC for the conservation of the habitats and populations of *C. parnassifolia* and *Aldrovanda vesiculosa*.

The habitat of *Caldesia parnassifolia* in Lake Rūžas partially coincided with the habitat of *Aldrovanda vesiculosa*, but it covered a larger area outside the narrow inlet. We recorded the largest population conditions in 2015 and 2019. In those years, both vegetative and generative individuals were abundant in the lake. We observed the highest number of flowering plants and individuals forming turions in the inflorescence on 1 September 2019. A few plants had fully developed but their seeds were immature. During the study in 2022, only one generative individual was present in the whole population of *Caldesia parnassifolia*, but it had not produced mature seeds or formed turions in the inflorescence. Many vegetative individuals had been uprooted and floated on the surface of the water (Figure 6).

We observed the negative impacts of the increasing eutrophication of water and of the abundance of filamentous algae in Lake Apvardai, where *C. parnassifolia* together with *Aldrovanda vesiculosa* occurred in only two locations.

3.4.3. Conservation of *Najas flexilis*

The *Lake Sągardas* SAC is the only area that has been purposefully designated for the conservation of *N. flexilis*. The population of this species in Lake Dūkštas belongs to a previously designated *Gražutė Regional Park* SAC, and additional objectives for the conservation of *N. flexilis* have been established. The historical locality of this species in Lake Germantas (Telšiai district, western Lithuania) is in the *Lake Germantas* SAC, but *N. flexilis* has not been detected in recent decades and is therefore not listed in the objectives for conservation. No SACs have been designated for the conservation of populations of *N. flexilis* in Lake Avilys or Ažvintis.

After screening for *N. flexilis* in the selected lakes and assessing the status of the previously recorded populations, we found that the population of the species in Lithuania is scarce. In most cases, we found a few solitary individuals at the observed sited. We observed the most favourable habitat and the largest currently known population in Lake Dūkštas, where we found *N. flexilis* at several sites. The area of habitat suitable for *N. flexilis* in the lakes was several times larger than the current area of its occurrence. More lakes in Lithuania may be suitable for *N. flexilis*, but the species has not yet been surveyed in these lakes.

4. Discussion

4.1. Historical and Current Distribution of Species

The aquatic plant species protected under the European Habitats Directive, *Aldrovanda vesiculosa*, *Caldesia parnassifolia*, and *Najas flexilis*, currently occur only in the northeastern part of Lithuania, in a relatively small area. These species are also unevenly distributed throughout the world, and this distribution pattern is probably mainly determined by their specific requirements for habitat conditions and sensitivity to environmental changes [14,79]. The northeastern part of Lithuania, as well as the adjacent regions in Latvia and Belarus, which are part of the Baltic Uplands, has a high concentration of lakes of different sizes and trophic levels [80,81]. Therefore, we assumed that the abundance of diverse lakes was the main factor determining the high concentration of rare aquatic plant species both in this region of Lithuania and in adjacent areas in Latvia [38,55] and Belarus [44,82].

In their historical localities, *Caldesia parnassifolia* [78] and *Najas flexilis* [40] in other parts of Lithuania are probably extinct because of changes in these habitats caused by natural and anthropogenic factors. The populations of *Caldesia parnassifolia* in the southern part of Lithuania were small [65,75] and may have disappeared because of natural succession. Small populations may have become extinct because of several consecutive unfavourable growing seasons, or the activities of aquatic plant-feeding birds, fish, or other animals [83–85], which may have consumed most or all of the formed turions. The current known populations of *C. parnassifolia* from northeastern Lithuania and southern Latvia are located more than 100 km north of the historic sites [78]. However, whether the species was previously unnoticed in the current sites or whether it has naturally spread to these sites remains unclear. We think that small populations of the species may have existed for a long time in these locations but were overlooked. With climate change, the warmer and longer growing season [86] may have led to an increase in its abundance and a spread to larger parts of the lakes. Additionally, *C. parnassifolia* was only discovered in Lake Rūžas after management measures had been implemented [78]. This suggests that the improved habitat conditions have led to a significant increase in the plant population and increased the ease of detecting the species.

The new records of *Najas flexilis* from three localities in this region suggest that the species may be more widespread in northeastern Lithuania, as quite abundant populations exist in the adjacent territories of Latvia and Belarus [38,44]. The species may also occur in other parts of the country, but detailed studies on potential habitats are required. The current surveys identified several water bodies with a combination of ecological and geomorphological characteristics suitable for *N. flexilis*, but we found no individuals in these lakes. Among the reasons why the species has not been detected may be the annual variation in population size due to the life history of *N. flexilis*, as well as the small size of the individuals and the difficulty in detecting them, especially at greater depths. In Lithuania, most surveys have been conducted from a boat using a grapnel, preventing sufficiently accurate assessments of species diversity and abundance [79].

4.2. Assessment of Aldrovanda vesiculosa and Caldesia parnassifolia Populations

4.2.1. Habitats and Communities

Throughout its wide range, *Aldrovanda vesiculosa* grows in a variety of habitats, ranging from shallow and stagnant to slow-flowing natural and artificial water bodies [14]. In Europe, *A. vesiculosa* is commonly found in nutrient-poor oligo-mesotrophic and dystrophic (humic) wetland systems, such as small marshes, peat bog pools, and dystrophic or peaty lakes, as well as lagoons and river deltas [14]. Sometimes, it occurs in highly eutrophic habitats such as fishponds and rice paddies [15]. The main habitats of *A. vesiculosa* at the northern edge of its range (e.g., Poland) are eutrophic–dystrophic lakes [24]. This type of habitat is also typical for *A. vesiculosa* in Lithuania. Notably, all the lakes in Lithuania where this species has been found are a part of wetland systems with various-sized mires. The mires around the lakes are crucial for the maintenance of habitats and for the protection of plants from the direct negative effects of agricultural pollution in the lake basins. Streams flowing into lakes or from lakes, which ensure water turnover, are also vital for the maintenance of favourable conditions in the habitats of *A. vesiculosa*.

The habitat of the largest population of *A. vesiculosa* in Lake Rūžas is remarkable for its species richness, low eutrophication, and relatively deep water. The relatively deep water protects the plant from the negative effects of fluctuations in water levels, provides favourable wintering conditions for turions during the cold season, and ensures the longevity of the population [53]. The relatively deep parts of a lake overgrow much slower than the shallows. Adamec [14] stated that *A. vesiculosa* grows faster in shallow areas, but shallow areas are at increased risk of drying out, faster eutrophication, the faster development of filamentous algae mats, and faster growth of helophytes. We observed such natural vegetation succession in the shallowest part of Lake Alksnas, where *A. vesiculosa* had almost vanished within a decade.

Water depth also determines the dominant vegetation. At three studied sites in Lithuania, *A. vesiculosa* usually occurred in communities of floating-leaved (alliances *Nymphaeion* and *Potamion*) or free-floating (association *Stratiotetum aloidis*) plants. This species occupies similar communities in Poland [24]. The communities of tall sedges (alliance *Magnocaricion*) were indicated as being particularly important habitats for the species in Poland [24], but such communities are not common in Lithuania. In Lake Rūžas, communities of tall helophytes (*Typha angustifolia* and *Phragmites australis*) were restricted to marshy shores (such as *Thelypterido palustris-Phragmitetum australis*), and *Aldrovanda vesiculosa* was absent in those areas. Adamec [14] assumed that *A. vesiculosa* habitats at all European sites may be stable, only occurring among or near tall helophytes such as *Phragmites australis* and *Typha angustifolia*, and tall species of *Carex*. The habitat in Lake Rūžas was protected by the surrounding marsh, whereas in Lakes Apvardai and Dysnai, tall helophytes (*Typha angustifolia*, *Schoenoplectus lacustris*) were the most notable shelter formation, providing a wind- and wave-protected microenvironment. In addition, these microenvironments were protected from the direct impact of waves by a wide belt of *Nuphar lutea* stands. Typical communities in which *Aldrovanda vesiculosa* occurs belong to the association *Spirodelo-Aldrovandetum vesiculosae*, with the presence of characteristic species of the class *Lemnetea* and the alliance *Utricullarion vulgaris* (*Spirodela polyrhiza*, *Riccia fluitans*, *Salvinia natans*, *Utricularia vulgaris*, and *Utricularia australis*). However, in such communities, the species is likely to occur only in the southern regions of its range, e.g., in Ukraine [87], but has not been recorded in the northern parts of its range, such as in Poland [24], the Czech Republic [62], or Lithuania.

We investigated communities of *Caldesia parnassifolia* in parallel with those of *Aldrovanda vesiculosa*, as the two species co-existed in the two lakes. We found no records to confirm that the two species occur in the same communities in other regions of Europe. Lake Rūžas supports the largest population of *Caldesia parnassifolia* compared with other historical and current localities. In this lake, we found only vegetating plants at depths greater than 0.5 m, which we mainly found in stands of floating-leaved plants (*Nuphar lutea* and *Potamogeton natans*). We observed similar species composition in the phytocenoses of Lake Apvardai and in the historical locality of Lake Daugai [75], as well as in the recently recorded locality of Lake Kampiniškiai on both sides of the Latvian–Lithuanian border [55]. We observed stands with dominant and flowering *Caldesia parnassifolia* in shallow (up to 0.5 m deep) areas of Lake Rūžas. Under similar conditions among helophytes (*Carex* spp., *Phragmites australis*, *Menyanthes trifoliata*, and *Comarum palustre*), it occurred at the historical site in Lake Ilgis [65]. Among helophytes (*Phragmites australis* and *Schoenoplectus lacustris*), the species has also been observed in Lakes Apvardai and Kampiniškiai [55]. In general, *Caldesia parnassifolia* is mainly distributed in communities of the class *Potametea* (alliance *Nymphaeion*) at the northern edge of the range; in shallow parts of the lakes, it may occur in helophyte communities (class *Phragmito-Magno-Caricetea*). During surveys in fishponds in France [52], *C. parnassifolia* was recorded in communities of five vegetation classes, but mainly in communities of the classes *Littorelletea* and *Potametea*, and less frequently in communities of the *Nymphaeion* alliance.

The flowering of *C. parnassifolia* is limited by water depths greater than 0.5 m [88]. This phenomenon has been confirmed by studies in northern populations in Lithuania [78] and Latvia [55], as well as in much more southerly populations in France [52]. Throughout the temperate zone of Europe, reproduction via turions is the main mode of reproduction, even though plants may sometimes produce mature seeds [52]. We observed stands of flowering and turion-forming *C. parnassifolia* plants in Lake Rūžas in 2015 and particularly in 2019, but in 2022, we noted few flowering individuals. This finding may be associated with the relatively low water levels in the lake in the first two years and the much higher water levels in the latter years. The ability of plants to grow at water depths greater than 0.5 m and to form turions has not been assessed and requires further investigation.

4.2.2. Morphology of *Aldrovanda vesiculosa*

The mean lengths of *A. vesiculosa* individuals were similar in the lakes studied, but only in Lake Rūžas in 2015 were the plants significantly longer than in other years in the same and different lakes. This suggests that more favourable conditions for plant growth have been created following the implementation of habitat management measures in 2014. The mean length of individuals in Lake Rūžas in 2015 was virtually the same ($t = 1.6$; $p = 0.107$) as that measured in the naturalised population of *A. vesiculosa* in Florida [17]. The mean length of individuals in the populations studied in Lithuania was significantly larger ($t = 28.9$; $p < 0.001$) than in the native population in Poland [89] and significantly larger than in the naturalised populations in Germany ($t = 19.5$; $p < 0.001$), but almost the same ($t = 1.3$; $p = 0.190$) as in the naturalised populations in the Czech Republic [90]. This suggests that the habitats in Lithuania are favourable for the growth of *A. vesiculosa* and that individuals develop similarly to those in much warmer climates. The significantly lower mean length of the individuals in the populations studied in Poland could also have been caused by meteorological conditions, as those populations were studied in the 1980s, before any signs of climate warming appeared.

The results of the assessment of the apices of *A. vesiculosa* individuals showed that in Lakes Apvardai and Dysnai, they were significantly more branched, and thus, larger in number. We assumed that habitat conditions have the strongest effect on the branching of individuals. In the shallow and wave-protected areas of Lakes Apvardai and Dysnai, individuals were more branched than in the deeper habitats of Lake Rūžas, where wave action can be quite strong. Waves can fragment individuals of *A. vesiculosa*, resulting in smaller numbers of branches. In addition, the water in shallow areas more strongly and quickly warms up than in the deeper parts of the lake; higher temperatures may encourage branching [89]. Individuals of *A. vesiculosa* are reported to have two–four branches; individuals with eight branches are rare [14]. We assessed the total number of apices, which directly reflects the number of turions formed by an individual. The number of apices in the populations we studied did not differ from the highest values found in naturalised populations in Florida [17]. According to Kamiński [89], individuals of *A. vesiculosa* in populations studied in Poland in the 1980s were less branched than those in all the populations studied in Lithuania. However, because of differences in study methods, we cannot reliably compare Kamiński's [89] data with ours.

Summarising the results, we conclude that individuals of *A. vesiculosa* in Lithuania are well-developed, branch abundantly, and produce many turions, which are essential for the recovery of the population after the cold season and for its long-term stability.

4.2.3. Density and Cover of *Aldrovanda vesiculosa* Individuals

The density of individuals per unit area and the overall size of a population are important indicators of its status. The results of the study revealed that the density of *A. vesiculosa* individuals in Lake Rūžas changed little over the entire study period, and that these changes are probably the result of natural population fluctuations. We found significantly lower densities of individuals in Lakes Apvardai and Dysnai. Whether the lower densities were caused by less favourable habitat conditions or for other reasons that could not be identified after a single survey remains unclear. We found a weak but significant relationship between water depth and density, as well as cover of individuals. We also found that individual *A. vesiculosa* densities were positively affected by the cover of floating-leaved plants, but negatively affected by the cover of submerged and emergent plants (Figure 5, Table 5). We think that floating-leaved plants growing abundantly at greater depths create the micro-conditions required for *A. vesiculosa* individuals to establish. However, in shallow habitats without or with only a few floating-leaved plants, *A. vesiculosa* individuals have nothing on which to anchor and are easily disturbed by waves, so their density is lower. In addition, in shallow habitats, more turions are likely to be lost during the cold season and are easily reached and consumed by plant-eating water birds.

The largest population of *A. vesiculosa* in Lithuania, and possibly in Europe, is in Lake Rūžas. The results of this study indicated that the habitat, which covers approximately 1.1 ha, contained between one and three million individuals in 2022 (Table 2). In Lakes Dysnai and Apvardai, where the suitable habitat and the density of individuals are much smaller than in Lake Rūžas, the total population in 2020 was between 160,000 and 450,000 and between 140,000 and 301,000 individuals, respectively. Thus, the total population of *A. vesiculosa* in Lithuania comprised between 1.30 and 3.75 million individuals. The results of this study are markedly different from the population size estimated by experts in 2012, when the population was assessed to be around 110,000 individuals [51]. The population size estimates differ because we accurately estimated the area covered by the population and the density of individuals, whereas the expert assessment was based on preliminary and small-sample data.

4.3. Habitats of Najas flexilis

We found *N. flexilis* growing in relatively large, stratified, mesotrophic lakes, with bottom sediments mainly consisting of mud with an admixture of fine gravel and stones, at depths ranging from 0.5 m to 3.5 m. *N. flexilis* usually occurred in sparse swards of charophytes and bryophytes. This species has been found under similar conditions in Estonia, where it occurs mostly in mesotrophic lakes in association with *Potamogeton rutilus* [46]. In adjacent Latvia, this species occurs in lakes of varying sizes and depths, even in shallow lakes [38]. In Switzerland, *Najas flexilis* has been recorded mainly in the transition zone between sparse *Potamogeton* stands and shallow vegetation [42]; in Norway, it has been found in mesotrophic water bodies, at depths of 2.9 m to 3.8 m [39]. Further detailed studies are required to assess the distribution of *N. flexilis* and the statuses of its habitats and populations in Lithuania.

4.4. Conservation Status and Proposals

4.4.1. Conservation of *Aldrovanda vesiculosa*

Approximately 90% of the historically known localities of *A. vesiculosa* worldwide were estimated to have become extinct within a century and a half [15]. The main causes of the extinction of many *A. vesiculosa* populations include direct anthropogenic impacts (habitat destruction, drainage, intensive agriculture, eutrophication, municipal pollution, etc.), as well as natural habitat succession, mainly associated with habitat degradation and climate change [14,48,50,91].

In Lithuania, only two special conservation areas have been established for *A. vesiculosa*. A management plan for the habitat of this species in Lake Rūžas was prepared and implemented in 2013–2014. The results of the subsequent studies showed that the positive effects of the management measures have been maintained to date. However, during surveys in September 2022, in some parts of the Lake Rūžas inlet, some *Nuphar lutea* rhizomes had started to be uprooted by methane gas released from soft-bottom sediments or by aquatic animals (*Castor fiber* and *Lutra lutra*) again. The removal of these islands was one of the management measures implemented to reduce the overgrowth of the inlet and ensure increased water movement in the outflowing streamlet.

A concern exists that mats of filamentous algae are re-establishing in the northern part of the inlet, which are particularly abundant in stands of *Myriophyllum verticillatum*. Mats of filamentous algae are an indicator of eutrophication and create unfavourable conditions for the growth of *Aldrovanda vesiculosa* [14]. We found the species to be only sporadic or absent at sites where these algae were abundant. The growth of *Cladophora* and other filamentous algae may be influenced by the slowing of the flow of the watercourse due to the presence of re-established obstacles in the stream bed. In Lakes Apvardai and Dysnai, we also observed the negative effects of filamentous algae on *A. vesiculosa*. We therefore consider that habitat management measures should be implemented every 5–10 years, depending on the results of the periodic assessment of the habitat.

The conservation of *A. vesiculosa* in Lake Alksnas is challenging because the true status of the population remains unknown due to its inaccessibility. A survey of *A. vesiculosa* in the accessible part of the site at the mouth of the stream flowing out of Lake Liūneliai revealed that it was almost extinct. The drastic decline in the abundance of *A. vesiculosa* was caused by the overgrowth of the shallow stream bed and its transformation into a swamp. The survival of the species in this area is limited, as all the surrounding habitats are protected under the Habitats Directive, and drastic measures of management (e.g., restoration of the stream), which are incompatible with the requirements of the other habitats, cannot be applied.

To conserve *A. vesiculosa* populations, periodic assessments must be conducted of the status of their populations. Regular monitoring of the status and abundance of the species is required, as are systematic surveys of the hydrochemical parameters of the water in the habitat of the species. The hydrochemical parameters established from water samples obtained from the deepest part of the lake according to the requirements of the Water Directive do not reflect the conditions in enclosed shallow inlets. When adverse changes in the habitat and population are detected, a comprehensive management plan must be developed, and the measures outlined in the plan must be rigorously implemented.

4.4.2. Conservation of *Caldesia parnassifolia*

C. parnassifolia was recently assessed as a species of least concern globally [23], as it occurs in many countries and has a wide range. Nevertheless, the species is declining and is protected in many European countries under the Habitats Directive [24]. The need for detailed surveys to identify population trends and threats was also highlighted [23]. In Lithuania, the species has been recently rediscovered and is now considered as critically endangered (CE) on the Red List [71]. To date, no special areas of conservation have been established for the conservation of *C. parnassifolia* in the country.

We found *C. parnassifolia* in Lake Rūžas in a special conservation area designated for *Aldrovanda vesiculosa*. The population of *Caldesia parnassifolia* was in favourable condition in 2015 and 2019; however, in 2022, almost all plants were in a vegetative state and much smaller than in previous years, and many uprooted individuals were floating on the surface (Figure 6). We think that *C. parnassifolia*, as well as other aquatic plants, have been uprooted by *Lutra lutra*, which searches the lake bottom for bivalves for food. Because the individuals of *C. parnassifolia* had formed few turions, the population density will likely continue to widely fluctuate in the future. Furthermore, how fluctuations in water level affect the condition of the plants is unclear.

We recorded a small population of *C. parnassifolia* in Lake Apvardai, which has also been proposed as a special conservation area for *Aldrovanda vesiculosa*. Detailed surveys of the species have not been carried out in this lake. In addition to the designation of special conservation areas in existing localities of *Caldesia parnassifolia*, detailed studies on its biology are needed, and the search for new localities in the southern part of Lithuania needs to be extended.

4.4.3. Conservation of *Najas flexilis*

Despite the results of the latest studies, knowledge is still lacking about the environmental preferences of *N. flexilis*, as they vary from region to region and generally depend on a combination of factors. Regular monitoring is required to obtain additional data for future predictions of the distribution of the species and to implement appropriate conservation measures. Approaches to *N. flexilis* monitoring vary between countries, but, in most cases, surveys should be performed annually for a fixed period, with a break of several years thereafter [79]. Because the plant is small and the accuracy of boat-based surveys is low, monitoring should be performed by diving or scuba diving, but this requires special training and additional resources [79]. The probability of finding rare species in aquatic environments is low, which additionally hinders the searches [92]. All these factors complicate the conservation of annual aquatic plant species. Monitoring of the hydrochemical parameters of the lakes in which *N. flexilis* grows and strict protection from anthropogenic pollution are essential prerequisites for its conservation.

5. Conclusions

Three aquatic plant species protected under the European Habitat Directive, *Aldrovanda vesiculosa*, *Caldesia parnassifolia*, and *Najas flexilis*, currently occur in Lithuania. All their localities are concentrated in the northeastern part of the country.

The results of targeted surveys in various lakes between 2019 and 2021 confirmed the presence of *Aldrovanda vesiculosa* in four lakes (Lakes Rūžas, Apvardai, Dysnai, and Alksnas) where it was previously recorded; we detected no new populations. We recently found *Caldesia parnassifolia* in two lakes (Lakes Apvardai and Kampiniškiai) in addition to the previously recorded occurrence in Lake Rūžas. We discovered three new localities of *Najas flexilis* (Lakes Avilys, Ažvintis, and Dūkštas), in addition to the previously recorded locality in Lake Sągardas.

The habitats of *Aldrovanda vesiculosa* and *Caldesia parnassifolia* include shallow eutrophic and dystrophic lakes that are completely or partially surrounded by mires. We mainly found both species in communities of floating-leaved (ass. *Nymphaeo albae-Nupharetum luteae* and ass. *Potametum natantis*) and free-floating (ass. *Stratiotetum aloidis*) plants, which were formed at depths of 1 m or more. The identified habitats of *Najas flexilis* were relatively deep, dimictic, stratified, mesotrophic lakes with limited anthropogenic pressure.

The habitat of *Aldrovanda vesiculosa* in Lake Rūžas covered an area of approximately 3 ha, and we estimated the total population to be one–three million individuals. Over the eight-year study period, the population fluctuated little in terms of the density of individuals. The population of *Caldesia parnassifolia* in Lake Rūžas was abundant; however, the number of generative individuals significantly varied between years. All populations of *Najas flexilis* were small, although the potential habitats in the study lakes covered relatively large areas.

The population of *Aldrovanda vesiculosa* has a favourable conservation status in Lake Rūžas. This lake was already designated as an SAC, and a habitat management plan has been implemented. The populations of *Najas flexilis* are now considered to have an unfavourable conservation status, and all lakes in which the species occurs need to be designated as SACs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15020185/s1, Table S1: Species diversity of plant communities with *Aldrovanda vesiculosa* and *Caldesia parnassifolia* in Lithuania.

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Appendix A

Table A1. Diversity and frequency of species recorded in communities with *Aldrovanda vesiculosa* and *Caldesia parnassifolia* in studied lakes and in different years of the study.

Species		**Rūžas**		**Apvardai**	**Dysnai**	**Frequency (%)**
Study year	2015	2019	2022	2020	2020	
Number of relevés	20	20	20	10	15	
Aldrovanda vesiculosa	100	100	100	100	100	100
Caldesia parnassifolia	40	40	30	40		80
Hydrocharis morsus-ranae	5	10	10	30	53	100
Myriophyllum cf. *verticillatum*	70	50	50	20	93	100
Nymphaea candida	40	10	25	50	20	100
Nuphar lutea	90	80	100	50	53	100
Potamogeton natans	85	85	80	100	87	100
Potamogeton × *bambergensis*	45	60	60	60	47	100
Stratiotes aloides	65	75	55	70	73	100
Utricularia vulgaris s.l.	30	25	5	20	7	100
Ceratophyllum demersum	10	5		10	53	80
Elodea canadensis	5	10	25	20		80
Hottonia palustris	80	70	90	50		80
Myriophyllum cf. *spicatum*	10	55	20	10		80
Sagittaria sagittifolia	20	20	25	20		80
Sparganium emersum	55	45	25	10		80
Nitellopsis obtusa	10	5	10			60
Phragmites australis	5	5		20		60
Potamogeton lucens	5		5		7	60
Ranunculus circinatus	30	5	25			60
Sparganium natans	20	40	20			60
Typha angustifolia		5	10		47	60
Carex lasiocarpa			5	20		40
Carex rostrata			5	10		40
Comarum palustre			5	10		40
Lemna minor				10	20	40
Lemna trisulca			5		20	40
Spirodela polyrhiza			10	10	13	40
Thelypteris palustris			5	30		40
Utricularia minor				10	13	40
Carex pseudocyperus					7	20
Cicuta virosa					13	20
Potamogeton crispus		5				20
Potamogeton perfoliatus					7	20
Potamogeton praelongus		5				20
Schoenoplectus lacustris				10		20
Typha latifolia				10		20
Filamentous algae				40	2	20

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