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Multiscale environmental characterization of Aldrovanda vesiculosa in the Danube Delta, Romania

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Abstract

Aldrovanda vesiculosa is a critically endangered aquatic plant in the Danube Delta, where it is vulnerable to water level fluctuations. In this context, this study established conservation measures for the species *A. vesiculosa* following investigations on the analysis of habitats and characteristics of the species in the polders of the Perișor abandoned facility in the Danube Delta.

The study was conducted in the Perișor dunes (Tulcea County), between June and August 2024. To identify habitat and phytocoenological preferences, relevés were carried out, with a sample area of $1m^2$. A ProDSS multiparametric digital water quality meter was used to analyze the physicochemical factors of the water, and molecular absorption spectrometry methods were used to analyze the physicochemical factors of the sediment. Topographic and bathymetric measurements were performed using the SonTek Acoustic Doppler Current Profiler (ADCP) RiverSurveyor M9 for the hydrological analysis.

Aldrovanda vesiculosa was reported in four EUNIS habitat types. The most favorable habitat was C1.32 free-floating vegetation, where the highest densities and individual lengths were reported. In the habitat Q53 - tall sedge beds, the lowest values for density and length of *A. vesiculosa* individuals were recorded. Cluster analysis revealed six associations, such as *Typhetum angustifoliae*, *Spirodelo-Aldrovandetum*, *Caricetum ripariae*, *Schoenoplectetum lacustris*, *Nymphaeetum albae,* and *Scirpo-Phragmitetum*. Plant length ranged from 4 to 21 cm, and the density of individuals ranged from 3 to 300 per 1 m^2. Physicochemical analysis of the water showed that *A. vesiculosa* preferred waters with moderate temperatures, from 22 °C to 27 °C, a more alkaline pH, from 8.5 to 10.5, low ammonium and nitrate concentrations and depths of up to 23 cm. Correlation analysis indicated that with increasing specific conductivity, vegetation cover, water depth and transparency, there is a decrease in the average length of *A. vesiculosa* individuals. Also, increasing vegetation cover caused a decrease in the minimum length of *A. vesiculosa* individuals. Moreover, the number of *A. vesiculosa* individuals increases with atmospheric pressure. In addition, a slight increase in individuals was recorded in waters with high nitrate concentrations. PCA analysis showed a strong correlation between the number of individuals per 1 m^2 and atmospheric pressure. Hydrological measurements in the central basin feeding the polders showed that *A. vesiculosa* growth indicated increased water levels and flow in 2024.

In conclusion, urgent interventions are required to conserve *A. vesiculosa* in the Danube Delta through transplantation. Protecting this plant requires constant monitoring and integrating measures into local environmental policies.

Highlights

Aldrovanda vesiculosa is critically endangered in the Danube Delta due to anthropogenic impact.

The highest densities and sizes of individuals were recorded in habitat C1.32, Free-floating vegetation of eutrophic waterbodies.

Plant length decreases with increasing specific conductivity, water depth, and vegetation cover.

The number of *A. vesiculosa* individuals is positively correlated with atmospheric pressure.

In the Danube Delta, *A. vesiculosa* has disappeared from some locations.

Habitat protection, hydrological regime control, and species transplantation are essential measures for conserving *A. vesiculosa*.

1. Introduction

One of the key priorities in biodiversity conservation is the protection of endangered plants (Heywood 2019; Wang et al. 2022). Thus, the most vulnerable biological resource is represented by endangered plants (Wang et al. 2022). In this context, conservation and protection measures must be established so that these plants' biological and genetic values are not lost (Salgotra and Chauhan 2023). The loss of these values will significantly impact ecosystems and people (Zhang et al. 2018b). Among the conservation measures established for these plants, the most widely used is *in situ* conservation (Godefroid et al. 2011), which involves protecting native populations, maintaining natural habitats (Volis 2016), and promoting the creation of new protected areas (Wang et al. 2022). Volis (2016, 2017) also mentions that the future of conservation is "conservation-oriented restoration", meaning *in situ* and quasi *in situ* conservation.

Freshwater ecosystems are essential for biodiversity conservation (Sinkevičienė et al. 2023) and are important from an ecological, economic, and social point of view (Mitsch and Gosselink 2000). However, these ecosystems are considered vulnerable (Sinkevičienė et al. 2023) due to natural (climatic variations) and anthropogenic (pollution, eutrophication) factors. Therefore, thoroughly understanding endangered species' distribution, habitat ecology, and population size is essential to ensure their effective conservation (Fenu et al. 2021).

Aldrovanda vesiculosa L. (Fig. 1; Adamec 2018; Płachno et al. 2020), a species of the Droseraceae Salisb. family (Jury 2009) grows in freshwater ecosystems. The name "*Aldrovanda*" comes from Ulisse Aldrovandi, the founder of the Botanical Garden of Bologna, Italy, and the species name "*vesiculosa*" comes from the Latin "*vesicula*" meaning small bladder (Adamec 2018). The genus *Aldrovanda* is a tertiary element (Płachno et al. 2020), and the species *A. vesiculosa* is a relict species (Yakubovskaya 1991; Cameron et al. 2002; Cross 2012; Adamec 2018), being considered one of the most famous aquatic plant species in the world (Sinkevičienė et al. 2023). The species is in major decline (Cross 2012; Adamec 2018; Cross

and Adamec 2020; Nishihara et al. 2023), being considered “Endangered” at the global level (Cross and Adamec 2020; EEA 2025). However, it is regarded as a non-native species in North America, but does not present an invasive character (Lamont et al. 2013; Cross et al. 2015). At the European and European Union levels, the species is classified in the “Data Deficient” category (Bilz and Lansdown 2011; EEA 2025). In Romania, the species is “Critically Endangered” (Dihoru and Negrean 2005). In the Danube Delta, at Perișor (Tulcea County), the species is “Vulnerable”. In Romania, its survival is affected by eutrophication and the lowering of the water level (Chirilă et al. 2024). In this context, *A. vesiculosa* can be considered a good indicator of water quality. The species' distribution is extensive (Płachno et al. 2020; Nishihara et al. 2023), but it is very fragmented (Cross and Adamec 2020), being recorded in Africa, Asia, Australia, and Europe (Shiga et al. 2020; Nishihara et al. 2023).

The general habitat of the species is represented by aquatic ecosystems (Adamec 2018). Wetlands represent the EUNIS habitats – code Q and Inland surface waters – code C (Chirilă et al. 2024). In Romania, the species occurs in habitats such as fish farms, marshes, shallow lakes, and water storage (Chirilă et al. 2024). The availability and quality of these habitats are decreasing (Cross and Adamec 2020). The species occupies a particular and restricted ecological niche in suitable habitats and declines rapidly following even small changes in habitat quality (Cross and Adamec 2020). The quality of the habitats in which *A. vesiculosa* grows depends on maintaining a balance between various ecological requirements (Adamec 1999a; Cross 2012). Even small changes in environmental factors, such as water chemistry or vegetation density, can dramatically affect *A. vesiculosa* populations (Adamec 2018). *A. vesiculosa* habitats require a high and constant concentration of CO_2 in the water (Adamec and Kovářová 2006; Cross et al. 2016), an optimal water level between 0.2–0.5 m (Adamec 2018), and the absence of dense macrophyte biomass (Cross et al. 2015, 2016; Adamec 2018). A layer of dead plant litter is required to optimize water chemistry and habitats rich in zooplankton (Kamiński 1987b; Adamec 2018). *A. vesiculosa* stands should be well lit, with clear, warm water, moderate nutrient levels (Kamiński 1987a, 1987b; Adamec 2018) and an optimal pH between 5.7 and 7.6 (Adamec 2018). For example, in Japan, the importance of a constant water pH between 6.4 and 7.0, as well as high water temperatures of up to cca. 30°C, has been repeatedly emphasized (Ishino 1963; Onoda 1963; Komiya 1982a; Komiya 1982b). In addition, to support the spread of *A. vesiculosa*, the areas should be close to each other, within a few kilometers (Berta 1961; Walters 1979).

Anthropogenic impacts include deterioration of water quality, wetland aridification and restoration (Walters 1979; Kamiński 1987, 2006; Adamec 1995; Cross 2012; Fleischmann et al. 2018), lack of suitable habitats (eutrophication of agricultural sites, fisheries), pollution or drying of habitats (Cross 2012; Adamec 2018; Cross et al. 2020). Losses were partly caused by the grazing of maturing floating shoots by ducks or emerging ripe shoots by small rodents (Adamec 2018). However, no emerging shoots died from severe frosts (Adamec 1999a, 1999b, 2018).

Aldrovanda vesiculosa is highly vulnerable to desiccation, even in the short term, and seasonal low water levels or drought can lead to its extinction (Cross et al. 2016). Although the factors contributing to the extinction of the species vary by region, globally, drought and eutrophication are considered the leading causes of its decline (Cross et al. 2016). For this reason, protecting the species is a major conservation concern (Adamec 2018). In Europe, eutrophication and drought have been identified as the main causes of habitat loss for this species (Chiba Prefecture 2009; Cross 2012). In Japan, only five known locations have been affected by natural disasters. In contrast, most locations have suffered from human activities such as over-collection and land conversion for agriculture, as these locations were predominantly in agricultural areas (Adamec, 2018). This species has a high sensitivity to water quality, and it has been observed that plants that initially thrived can suddenly disappear during cultivation.

The growth of *A. vesiculosa* is influenced by several abiotic factors, including irradiance, temperature, pH, CO_2 concentration, water depth, and water chemistry (Adamec 2005; Chiba Prefecture 2009; Cross 2012; Cross et al. 2016). Although the plant can survive in aquatic environments with limited food resources (Kamiński 1987; Chiba Prefecture 2009; IUCN 2012), eutrophication causes algal blooms, which can accelerate the decline or even extinction of the species (Adamec 1995, 1997).

Species extinction can be caused by different factors depending on the location, but human activities often play a major role in threatening their survival. In addition, invasive species of aquatic plants or animals can amplify the risk of extinction. To protect endangered plant species, close collaboration between the public and civil sectors is essential (Shimai and Ohmori 2023).

The main goal of this study is to explore and analyze in detail the correlations between some characteristics of the *A. vesiculosa* species and a series of environmental variables, given the complexity of the ecological interactions that influence the development and distribution of this species. The study aims to provide a deeper understanding of how environmental factors contribute to the dynamics of *A. vesiculosa* populations and to identify the essential variables that may affect the survival and prosperity of this rare species. In this sense, the study has three fundamental objectives: (1) presenting the plant associations in the analyzed areas, (2) measuring hydrological, morphological, and physico-chemical factors in the field, and (3) establishing correlations between the characteristics of *A. vesiculosa* populations and environmental variables measured in the field.

Based on data and personal observations, we believe that the population size of *A. vesiculosa* is influenced by the abundance and dynamics of zooplankton communities, especially *Daphnia* spp., which suggests that climate change, by modifying food web interactions and hydrological factors, could have an important impact on this species. The study will test this hypothesis by statistically analyzing the correlations between the average temperature recorded in the analyzed habitats and the population size of *A. vesiculosa*. Through these analyses, the study aims to contribute to developing effective conservation and management strategies for this vulnerable species.

2. Materials and methods

2.1 Study area

The study was conducted in June 2024, in the polders of the Perișor fish farm (Fig. 2; Tulcea County). The fish farm is located in the Danube Delta between the Dranov canal and the Perișor fish ferm (Almazov et al. 1963). Administratively, this farm belongs to the Murighiol locality (Tulcea County). The length of the Perișor canal is 8.23 km, and the width is 10 m.

2.2. Phytocoenological analysis

Sixty-eight relevés (including 34 taxa) were collected for the phytocoenological analysis. The size of the sample areas was 1 m^2. For the classification of the vegetation, the Agglomerative Hierarchical Clustering method was applied (ß-flexible method, β = − 0.25 and Bray-Curtis dissimilarity). The data used were represented by average percentage values corresponding to the Braun-Blanquet cover-abundance scale (Cristea et al. 2004). Subsequently, the data were normalized by square root transformation. The dendrogram was made in GINKGO (Bouxin 2005), and the optimal number of clusters was determined based on the average Silhouette index (Rousseeuw 1987). The synoptic table of the analyzed communities was made by JUICE version 7.1 (Tichý 2002). The diagnostic species were determined based on the IndVal index (Dufrêne and Legendre 1997) and validated by a permutation test (de Cáceres and Legendre 2009). The nomenclature of plant species followed World Plants (2025), and the nomenclature of plant associations followed Chifu et al. (2014). The nomenclature of higher syntaxa followed Mucina et al. (2016). Conversely, the coenotaxonomic affiliation of plant associations followed Chifu et al. (2014). Habitat types were classified using the EUNIS habitat classification expert system (Chytrý et al. 2020). The nomenclature of algal species followed AlgaeBase (https://www.algaebase.org/).

2.3. Analysis of species characteristics

In each sample area, all individuals of *A. vesiculosa* were counted to determine the population density. Initially, individuals were counted per 1 m^2, was extrapolated to the entire investigated area. In addition, maximum individual length, minimum individual length, and average individual length were measured.

2.4. Hydrological analysis

The measurements on the Perișor Canal had as a starting point the intersection with the Dranov Canal and as an arrival point the Perișor fish farm. The total length measured on this canal is approximately 8.000 linear m. The topobathymetric data were collected using state-of-the-art equipment in autumn when water levels are usually low. The topographic measurements were carried out using the Global Navigation Satellite System (GNSS) SPECTRA PRECISION SP80 GPS to determine the planimetric and altimetric coordinates. With the help of this equipment, the water level elevations corresponding to the banks and the altimetric (z) and planimetric (x and y) coordinates of the bank ends were obtained. Thus, 104 points were obtained on the Perișor Canal, translated into the Stereo70 projection system with the r.M.N.S. system. The points were determined by the Static method, with horizontal accuracy: 3 mm + 0.1 ppm and vertical accuracy: 3.5 mm + 0.4 ppm (Neary and Gunawan 2011). This method involved post-processing the GPS data using GNSS records from the same time interval as the GPSs, purchased from the Tulcea Permanent Station of the national GNSS network. Post-processing was performed using the Carlson SurvCE program. Bathymetric surveys were conducted using either a 135 HP boat, a laptop for digital data collection, and the SonTek Acoustic Doppler Current Profiler (ADCP) RiverSurveyor M9 equipment (SONTEK Company, San Diego, USA) with 3 × 3 transducers, each with a different orientation. The equipment incorporates a 2-axis compass tilt sensor, a temperature sensor, an 8 GB internal storage hard drive, and a vertical acoustic beam (ultrasound) for depth measurement. The equipment used for hydrological measurements on the targeted channels is the same as that used for topo-bathymetric measurements, namely the SonTek Acoustic Doppler Current Profiler (ADCP) RiverSurveyor M9.

2.5. Physico-chemical analysis – plots

In each plot, the following physicochemical water parameters were recorded: T °C, mmHg, SPC, pH, ORP – Oxidation Reduction Potential (mV), Ammonium – NH_4, Ammonia – NH_3, and Nitrate – NO_3. These parameters were measured with a ProDSS Multiparameter Digital Water Quality Meter.

2.6. Sediment and water sample collection

Sediment and water samples were collected at three locations where *A. vesiculosa* was identified (Fig. 3). Shallow areas (maximum 30–40 cm) were chosen, close to the banks. The Eijkelkamp hand auger manual for heterogeneous soil was used for sediment sampling. The samples were placed in durable bags, labeled, and sent to the chemistry laboratory within the DDNI Tulcea for analysis. The water sampling was conducted at the exact location where a sample was collected and stored in a plastic container for analysis in the same laboratory.

For the water samples, the following parameters were determined: pH (pH units), EC - Electrical Conductivity (µs/cm), TDS – Total Dissolved Solids, NH_4^+/NH_3, $N\text{-}NO_2$, $N\text{-}NO_3$, Organic N, Total N, NH_4^+, NO_2^-, NO_3^-, $P\text{-}PO_4$, Total P, CO_3^{2-}, HCO_3^-, Cl^-, Mg^{2+}, Ca^{2+} (mg/l) while for the sediment samples, the following parameters were determined: pH (pH units), NO_2^-, NO_3^-, $N\text{-}NH_4^+$, NH_4^+, NH_3, CO_3^{2-}, HCO_3^-, Cl^-, Ca^{2+}, Mg^{2+} (mg/100g soil), Organic Carbon, Humus (%). According to applicable standards, methods used to evaluate these parameters include molecular absorption spectrometry, volumetric, and potentiometric analysis.

2.7. Statistical analyses

To determine which environmental factors influence some morphological characters of *A. vesiculosa*, the Kendall test was applied, using R Statistical Software (v4.1.4; R Core Team 2024) through the R package "ggpubr" (v0.6.0; Kassambara 2023). The dependent variables were the number of *A. vesiculosa* individuals per 1 m^2, average individual length (cm), minimum individual length (cm), and maximum individual length (cm). The independent variables were T °C, mmHg, SPC, pH, ORP, NH_4, NH_3, NO_3, water depth, water transparency, elevation, and vegetation cover. The correlation results (R- and p-values) are presented in the graphs. A statistically significant correlation between two variables is when the p-value < 0.05.

PCA analysis, made by PC-ORD software (McCune and Mefford 2006), was applied to explore the relationships between morphological characters and environmental variables.

Graph visualizations were performed in R Statistical Software (v4.1.4; R Core Team 2024), via the "ggplot2" (Wickham 2016) for graph generation, readr for data import in .csv format, and "ggrepel" (Slowikowski 2023) for displaying labels without overlapping on scatter plots.

3. Results

3.1. Habitat preference

The habitats in which *A. vesiculosa* data were recorded fall into four types according to the EUNIS classification: C1.32 Free-floating vegetation of eutrophic waterbodies, Q51 Tall-helophyte bed and N1H Atlantic and Baltic moist and wet dune slack, complemented by rare habitats, such as Q53 Tall-sedge bed (Table 1).

Table 1
Presence and ecological characteristics of *A. vesiculosa* in four EUNIS habitat types

EUNIS habitat	No. total of individuals	No. of individuals/ m^2	Water depth (cm)	Length of *A. vesiculosa* individuals (cm)	Ecological observations
C1.32 Free-floating vegetation of eutrophic waterbodies	210	210	12	11	Optimal habitat: floating vegetation, shallow water, maximum density and length, excellent light and nutrient conditions.
N1H Atlantic and Baltic moist and wet dune slack	186	37	27.6	7.43	Habitat characterized by deep, poorly oxygenated water, vegetation with floating leaves, moderate density and length, and possible presence in bright microzones.
Q51 Tall-helophyte bed	1829	30	16.8	7.78	Most common habitat; moderate density and length; eutrophic conditions; present in interspaces in reed beds; favorable but suboptimal.
Q53 Tall-sedge bed	5	5	10	6	Marginal and temporary habitat; shallow depth, limited light due to dense sedge; minimum density and length.

In habitat N1H, *A. vesiculosa* was recorded in the association *Nymphaeetum albae*. This habitat was characterized by a moderate density and length of individuals and deeper and poorly oxygenated waters compared to the other habitat types analyzed. In this context, the species' survival in this habitat is slightly tolerant of certain suboptimal conditions, such as high vegetation cover. Still, at the same time, there is a stable water level and microzones where light penetrates.

In habitat Q51, *A. vesiculosa* was recorded in associations with *Typhetum angustifoliae*, *Phragmitetum australis* and *Schoenoplectetum lacustris*. According to our data, this is the most frequent habitat where *A. vesiculosa* was recorded. The habitat was characterized by waters with moderate depths, medium density and a moderate length of individuals, which suggests favorable conditions, but is suboptimal compared to habitat C1.32.

In habitat Q53, *A. vesiculosa* occurs in the association *Caricetum ripariae*, where the lowest density and minimum mean length values were recorded. This habitat has suboptimal conditions for the species' development, represented by competition for resources, vegetation cover, and high luminosity. Moreover, the low density, reduced depth, and high cover of *C. riparia* suggest a marginal and temporary character of the habitat for *A. vesiculosa*.

In habitat C1.32, *A. vesiculosa* was identified in the *Spirodelo-Aldrovandetum* association. This is one of the most favorable habitats for developing the species *A. vesiculosa*, where the highest values for density and average length of individuals were recorded. The habitat is characterized by floating vegetation, developed in shallow waters with a high trophic level. This suggests that this habitat has the best conditions regarding the degree of brightness, the availability of nutrients, and the absence of vertical competition for light, in the absence of dense emergent vegetation.

3.2. Habitat area, percentage cover, and density of individuals

Habitats where *A. vesiculosa* occurs in a range of sizes from 4.200 m² to over 170.000 m². Overall, the habitat area occupied by *A. vesiculosa* was proportional to the total size of the habitat. In the most significant habitat, Q51 Tall-helophyte bed, the species occupied over 80% of the habitat area

(143.773 m² out of 173.966 m²). In the medium-sized habitat Q51 Tall-helophyte bed, *A. vesiculosa* occupied over 80% of the habitat area. In addition, the habitat was characterised by optimal ecological conditions, such as adequate light, optimal depth, and nutrient availability, and the number of individuals per 1 m² of *A. vesiculosa* ranged between 280 and 300. Also, in habitats with a cover of *A. vesiculosa* between 10% and 20%, the number of individuals per m² ranged between 10 and 21. In contrast, in habitats with small areas characterised by reduced light, interspecific competition, and excessive depth, the cover was below 1%, and the number of individuals per 1 m² was 3 (Fig. 4).

3.3. Cluster analysis

In the Perișor fish farm in the Danube Delta, *A. vesiculosa* occurs in six clusters (plant associations; Fig. 5): *Typhetum angustifoliae* Pignatti 1953, *Spirodelo-Aldrovandetum* Borhidi et J. Komlódi 1959, *Caricetum ripariae* Máthé et Kovács 1959, *Schoenoplectetum lacustris* Chouard 1924, *Nymphaeetum albae* Vollmar 1947, and *Phragmitetum australis* Soó 1927.

Cluster 1: *Typhetum angustifoliae*

The diagnostic species is *Typha angustifolia* (0.870, 0.001).

This cluster included 37 relevés, encompassing communities commonly found in wet habitats, such as marshes and stagnant water areas. Vegetation cover ranged from 44–60%. The habitat includes shallow waters, with depths between 12 and 21 cm. In this association, the number of *A. vesiculosa* individuals per 1 m² varied significantly, between 4 and 320. *Typha angustifolia* covered 37.5% and 62.5%, and A. *vesiculosa* covered 0.5% and 5%.

Cluster 2: *Spirodelo-Aldrovandetum*

The diagnostic species are *Lemna trisulca* (0.819, 0.031), and *Aldrovanda vesiculosa* (0.762, 0.022).

The cluster includes two relevés. Vegetation cover varied from 48–63%, and the number of *A. vesiculosa* individuals decreased from 154 to 210 individuals/ 1 m². Water depth ranged between 11 and 12 cm. *A. vesiculosa* had a cover of 37.5%.

Cluster 3: *Caricetum ripariae*

The diagnostic species is *Carex riparia* (0.977, 0.006).

In this cluster was included a relevé. The reduced number of individuals of *A. vesiculosa* was five individuals/ 1 m². *Carex riparia* had a cover of 87.5%, and *A. vesiculosa* had a cover of 0.5%. Vegetation cover was 90%. The water depth was 10 cm.

Cluster 4: *Schoenoplectetum lacustris*

The diagnostic species is *Schoenoplectus lacustris* (0.938, 0.004).

The cluster includes five relevés. Vegetation cover ranged from 46–83%. The number of *A. vesiculosa* individuals per 1 m² ranged from 3 to 176, and species cover ranged from 0.5–17.5%. *Schoenoplectus lacustris* had a cover ranging from 37.5 to 62.5%. Water depth ranged from 10 to 30 cm.

Cluster 5: *Nymphaeetum albae*

The diagnostic species are *Myriophyllum spicatum* (0.707, 0.038) and *Elodea nuttallii* (0.702, 0.047).

Four relevés were included in this cluster. *A. vesiculosa* had several individuals per 1 m² ranging from 3 to 15 and a cover of 0.5%. Water depth ranged from 10 to 80 cm. *Nymphaea alba* had covers from 37.5–62.5%, and *Elodea nuttallii* had covers from 0.5–17.5%. Vegetation cover ranged from 39–67%.

Cluster 6: *Phragmitetum australis*

The diagnostic species is *Phragmites australis* (0.785, 0.001).

This cluster included 19 relevés. The species forming the association, namely *Phragmites australis*, had a cover ranging from 37.5–62.5%. Vegetation cover ranged from 38–83%. *A. vesiculosa* had covers ranging from 0.5–5%, and the number of individuals per 1 m² ranged from 3 to 280. Water depth ranged from 8 to 25 cm.

3.4. Morphological characters

The average length of *A. vesiculosa* individuals varied from 4 cm to 12 cm. In polders where the average length was 12 cm, the habitat is characterized by favorable conditions for the development of the species. In contrast, the reduced average lengths show limiting factors, such as water eutrophication and limited trophic resources. The differences between the minimum (4 cm) and maximum (21 cm) lengths indicate a high population heterogeneity in the analyzed polders. In addition, this shows the presence of several development stages. In polders where the length of the individuals was between 7 and 8 cm, the environmental conditions are uniform. Also, the average length tends to increase in habitats where the

number of individuals is high. Moreover, in polders where a small number of individuals were recorded, considerable average lengths of *A. vesiculosa* individuals were recorded (Fig. 7).

3.5. Physico-chemical parameters of water – plots with *A. vesiculosa*

In the polders where *A. vesiculosa* grows, the physicochemical parameters of the water vary considerably (Fig. 8). Thus, the water temperature fluctuated between 22.4°C and 28°C. The atmospheric pressure was relatively constant, 763–764 mmHg, which shows similar altitudes between the polders. The specific conductivity (SPC) had values between 354.3 and 1557 µS/cm, indicating variations in the mineral load of the waters. This is an important factor for the oligotrophic environments preferred by *A. vesiculosa*. The pH of the water varied between 7.82 and 11.67, which indicates the presence of habitats where conditions are alkaline, as well as the existence of neutral or slightly acidic conditions. The oxidation-reduction potential (ORP) had values between − 126.5 and 155.5 mV and showed a wide range of chemical processes. These processes vary from low to highly oxygenated environments and may affect the development of the species' traps. Concentrations of nitrogen compounds (NH_4, NH_3, NO_3) showed differences between polders. Thus, extremely high values of NH_4 and NH_3 could have come from local sources of pollution, which affect the oligotrophic habitat required for *A. vesiculosa*.

3.6. Relationship between some morphological characters and environmental variables

The correlation between average individual length and independent variables (specific conductivity, vegetation cover, water depth, and water transparency) is negative. Thus, as average individual length increases, independent variables decrease. These results show that *A. vesiculosa* prefers waters with lower conductivity for optimal development. Regarding vegetation cover, *A. vesiculosa* prefers habitats with less dense vegetation. Thus, in habitats where vegetation cover is high, the increase in individual size is limited, possibly due to competition for resources. Regarding water depth and transparency, *A. vesiculosa* prefers shallower waters and moderate transparency (Fig. 9).

The correlation between the minimum individual length of *A. vesiculosa* and the independent variables (vegetation cover and specific conductivity) is negative. Thus, with the increase in minimum individual length, there is a decrease in vegetation cover and specific conductivity (Fig. 10). These results indicate that *A. vesiculosa* prefers more open habitats with low vegetation cover. Regarding specific conductivity, better-developed *A. vesiculosa* individuals are found in waters with low mineralization.

The correlation between the number of individuals of *A. vesiculosa* per m^2 and atmospheric pressure is optimistic and slightly pessimistic with the nitrate concentration (Fig. 11). Thus, the number of individuals increases with the atmospheric pressure (mmHg). These results show that atmospheric pressure influences the dynamics of water, which affects the availability of oxygen. Regarding the concentration of nitrates (NO_3), it was observed that a slight increase in the number of individuals was recorded in waters with high nitrate concentrations. In this context, *A. vesiculosa* can tolerate moderate nutrient concentrations.

3.7. Principal Component Analysis (PCA)

Ordination PCA (Fig. 12) indicated a strong correlation between the no. of individuals per 1 m^2 and atmospheric pressure. These results confirm the statistical correlation obtained previously, where R = 0.46 and p = 0.00058, showing that the no. of individuals is influenced by atmospheric pressure. This may be due to changes in water level and dissolved oxygen.

3.8. Hydrological measurements

The water level increased in 2024, possibly due to precipitation, snowmelt, and changes in the water's hydrological regime. The water flow rate increased in 2024, showing a higher water input into the channel. This may be due to external sources, such as seasonal tributaries and nearby lakes. In contrast, the average water velocity decreased in 2024 (Fig. 13).

3.9. Morphological analysis

3.9.1. Substrate description (bank sediment)

The drills at the three locations identified the same type of sediment, with slight variations in color or composition - mica-rich sand with a significant organic component, dark grey to black, containing relatively few shells, wood remnants and reed fragments.

3.9.2. Analysis of the physico-chemical parameters of water and soil

Laboratory results did not indicate significant variations in chemical composition, but some differences were observed. The chemical composition of the sediments (Table 2) does not differ significantly, with similar values recorded for NO_2^-, NO_3^-, N-NH_4, NH_4^-, NH_3, HCO_3^-, Cl^-, and Ca^{2+}. Differences are recorded at Mg^{2+} (2.4, respectively 3.3 higher in the PER01s sample) and organic carbon (OC) (2.1, respectively 2.5 times lower in the Per03s sample).

Table 2
Chemical composition of the sediments at sampling points in Perişor

Sediments samples	pH	NO_2	NO_3	N-NH_4	NH_4	NH_3	CO_3-	HCO_3-	Chlorides	Cl	Mg	Organic carbon	Humus
		(mg/ 100g soil)										%	
Per01s	8.33	0.0008	0.373	0.007	0.009	0.008	0.000	3.050	35.450	3.407	4.013	2.923	5.040
Per02s	8.46	0.0009	0.326	0.007	0.009	0.008	0.000	4.575	23.040	3.607	1.702	3.480	6.000
Per03s	8.56	0.0006	0.362	0.006	0.008	0.007	0.000	2.898	33.680	3.607	1.216	1.392	2.400

The water samples (Table 3) indicate brackish water with more evident values in the PER03w sample (due to the proximity to the Black Sea). This sample generally contains higher percentages of N-NO_3 (2.3 and 2.6 times respectively), Cl^- (2.4 and 3.6 times respectively) or Mg^{2+} (3.8 and 3.3 times respectively).

Table 3
Physico-chemical parameters of the water at sampling points in Perişor

Water samples	pH	T	EC	TDS	NH_4^+	N-NO_2	N-NO_3	Organic N	N total	Inorganic N	P-PO_4 mg/l	Total P	H CO_3^-	Chlorides	Cl
		(ºC)	(µs/ cm)	(mg/ L)											
Per01w	7.77	21	1285	643	0.126	0.026	0.034	4.552	4.612	0.186	0.016	0.054	335.5	230.4	84.97
Per02w	7.75	21	999	499	0.133	0.008	0.030	3.068	3.106	0.171	0.015	0.046	335.5	156.0	59.31
Per03w	7.78	21	2130	1065	0.217	0.035	0.079	5.132	5.246	0.331	0.022	0.057	445.3	553.1	73.74

4. Discussions

4.1. Habitat and phytocoenology preferences

From a physicochemical point of view, *A. vesiculosa* survives in deeper waters than in other habitats analysed, which may reduce competition with different aquatic plants with more stringent light or oxygen requirements. Hydrological stability plays an important role in the NIH habitat for this species, as sudden fluctuations can affect buoyancy and light-capturing capacity. The species appears tolerant to low dissolved oxygen concentrations, probably due to its efficient metabolism and ability to obtain nutrients by capturing and digesting small aquatic organisms. Although light may be partially obstructed by the surrounding tall vegetation (helophytes and other large aquatic species), *A. vesiculosa* persists due to the presence of brighter microzones, places where light manages to penetrate to the water surface. *Aldrovanda vesiculosa* has moderate ecological plasticity; it is not a strictly heliophilous species nor completely shade-tolerant. It does not compete with vigorous species (e.g., dominant helophytes), but persists in transition zones or niches where competitive pressure is lower. *A. vesiculosa* forms hibernacula (turions) in late summer, which sink and overwinter on the bottom of the water. In spring, it develops again if conditions become favorable.

Habitat is dominated by tall, emergent helophyte plants (e.g., reeds, rushes) and dense vegetation. Water depth is moderate and sufficient for plant buoyancy. In terms of stability, the habitat is relatively stable, but *A. vesiculosa* can be affected seasonally by the development of plant mass. Tall vegetation shades the water, but *A. vesiculosa* develops better in bright microzones at the edge of reed beds. The density of the species is medium, and the plant is not dominant but is constantly present. The length of the individuals is moderate, compared to the data analysed in other habitats, which indicates good development. In the short term, within the habitat, the species thrives up to several hundred individuals per square meter in low water level conditions and high brightness. Dissolved oxygen is generally of a medium level but not optimal. The level of nutrients can vary, affecting the species in the long term. *A. vesiculosa* develops in this habitat even if nitrogen and phosphorus concentrations are low, but this can be a limiting factor; for this reason, habitat Q51 does not offer development conditions for *A. vesiculosa*.

Habitat Q53 has a shallow depth that may limit the development of the species *A. vesiculosa*. The water level is fluctuating and unstable, which may be detrimental to the development of the species. The brightness at the water level is low due to the shadow created by the dense vegetation. Another factor influencing the population size indicates a poor environment for colonisation and regeneration. Individuals have limited development, probably due to physiological stress caused by inadequate oxygenation and rapid changes in the aquatic microclimate. Because of these changes, food and light are not enough. *A. vesiculosa* cannot compete effectively with *Carex riparia* for light and space; it only occurs in successional stages or in water holes where light can penetrate. Habitat Q53 offers the poorest conditions among those analysed so far. Individuals' low density and poor development reflect high stress on the species, and competition with *C. riparia* and lack of light play a critical role. In this context, *A. vesiculosa* here

occasionally depends on disturbing factors (e.g., openings in vegetation, higher water levels in certain seasons). Thus, it cannot support sustainable populations and only serve as a temporary refuge or dispersal area.

During the growing season, the water level is low but stable, and the water temperature fluctuates up to 3℃. The lack of emergent vegetation and the exclusive presence of floating vegetation allow for efficient light penetration. *A. vesiculosa* develops here in dense colonies, reflecting optimal conditions, has vigorous development, the possibility of branching, and rapid regeneration. The microinvertebrate fauna is well represented in eutrophic habitats, supporting the trophic base of the plant. The analysed chemical parameters show moderate variations. Habitat C1.32 offers the best ecological conditions for *A. vesiculosa*: abundant light, available nutrients, no vertical competition, and stable hydrological conditions. The *Spirodelo-Aldrovandetum* association functions as a true optimal ecological refuge, in which the species manifests its maximum development and reproduction potential.

4.2 Environmental conditions analysis

The water temperature recorded in the polders where *A. vesiculosa* develops ranged from 22.4°C and 28°C, with an average temperature of 24.2°C. These results fall within the limits Cross (2012) recorded for biomass growth in populations in Europe and North America, in which the optimal range is between 22°C and 36°C. Thus, our data support the idea that the populations of *A. vesiculosa* analyzed have adequate conditions for the development of the species. Moreover, temperature represented a limiting factor in flowering. According to the literature (Adamec 1999c, 2018; Cross et al. 2016), flowering is triggered by 3–4 weeks, when the water temperature exceeds 26°C. In this case, in our study, only six (17%) of the polders analyzed had water temperatures higher than 26°C, which shows that thermal conditions for flowering are rarely encountered. A study conducted in the Czech Republic (Cross et al. 2016) observed that flowering was preceded by several days when the temperature was between 28 and 33°C. The current conditions recorded are closer to those in areas with the marginal distribution of the species *A. vesiculosa*. An example is represented by the populations of *A. vesiculosa* in the Netherlands or north-west Germany, where the maximum recorded water temperature rarely exceeds 23°C (Cross 2012). In this case, major adaptive factors are the ecological plasticity of the species and the dominance of vegetative reproduction.

The pH of the water ranged from 7.8 to 11.6, with an average of 10.05, which means an alkaline environment. These data show a major deviation from the optimal range reported in the literature, ranging from 5.7–7.6 (Adamec 1997; Cross et al. 2016). Even though the species survives in a wide pH range, the optimal development of the species is associated with a slightly acidic to slightly neutral pH with adequate concentrations of dissolved CO_2 (Kamiński 1987a; Adamec and Kovářová 2006). The high pH value may mean a decrease in CO_2. This may be correlated with intense photosynthesis or water eutrophication. Thus, the alkaline conditions recorded at Perisor are not optimal for the development of the species and may contribute to a decrease in long-term ecological success.

The nutrient concentrations recorded at Perișor are higher than the data reported in the literature (Adamec 1999a; Cross et al. 2016), where the optimal values for NH_4-N were 10–30µg/L, and for NO_3-N were 0–20µg/L. Our results show an average for NH_3 of ~ 169µg/L, for NO_3 of ~ 144µg/L, and the maximum values being 3619.46µg/L (NH_3) and 647.61µg/L (NO_3). This shows a high degree of eutrophication. In this context, the reported conditions can support the growth of *A. vesiculosa* in the short term, but simultaneously favor the appearance of algae, and the stability of populations is endangered (Kamiński 1987a; Adamec and Kovářová 2006). The high NH_4:NO_3 ratio shown in our data is also characteristic of dystrophic habitats preferred by *A. vesiculosa* and corresponds to the plant's preference for low nitrogen concentrations (Adamec 2000). At the same time, high nutrient concentrations can also mean ecological stress, where competition and turbidity can affect photosynthesis and prey capture capacity.

Water depth varied between 7 and 30 cm, with an average of 16 cm, which means that *A. vesiculosa* is below the optimal range of 20–50 cm reported in the literature (Adamec 1999a; Cross 2012). Thus, waters with depths < 10 cm were frequently associated with a high rate of eutrophication, rapid development of helophytic plant species and filamentous algae, etc. (Adamec and Lev 1999; Cross et al. 2016).

According to the literature (Cross 2012), frequent fluctuations and a decrease in water level below 10 cm represent some of the greatest threats to *A. vesiculosa*. As such, water levels below 15 cm indicate a high vulnerability of the habitats where *A. vesiculosa* develops at Perisor, under conditions of severe drought. Water transparency, which presented values approximately equal to the water depth, showed clear and illuminated waters favorable for photosynthesis.

4.3. Conservation implications

Aldrovanda vesiculosa was recorded in six vegetation associations, where the density and cover of this species differed. Thus, the highest number of individuals was recorded in communities with medium, free-floating vegetation, such as *Spirodelo-Aldrovandetum*. Within this association, competition with other species with high biomass is low. The lowest number of individuals was recorded in communities with tall vegetation, such as *Phragmitetum australis* and *Caricetum ripariae*. Within these associations, reduced water oxygenation and substrate shading may limit the development of the analyzed species.

Sediment analysis shows organic sand with slight variations in color or organic components. The proximity to the Black Sea makes the water brackish, highlighting the role of salt water for the habitat.

The ecology of *A. vesiculosa* reflects a high degree of specialization and a certain ecological tolerance under suboptimal conditions. The C1.32 habitat has the best conditions for *A. vesiculosa*, which is important for conservation, while the Q51 habitat can function as a complementary support for this species. Habitats N1H and Q53 are marginal, and the species' occurrence in these conditions suggests a capacity to adapt to local microvariations, but it cannot support stable populations without interventions.

In the Danube Delta, *A. vesiculosa* grows in a private fish farm, an artificial habitat. In this case, the species is subject to a high risk of local extinction in the future, especially if other constructions or hydrotechnical modifications take place in place of that fish farm. Because *A. vesiculosa* is strictly protected internationally, it is necessary to take conservation measures. One of the most important conservation measures is the ecological monitoring of the current habitat. It is also essential to transplant shoots in rigorously selected locations with environmental characteristics (constant hydrological regime, low trophic level, hydrophytic vegetation) similar to *A. vesiculosa*. Also, to involve local decision-makers in conserving this species, it is necessary to carry out information and ecological education campaigns. As such, for the long-term conservation of the *A. vesiculosa* species, an integrated approach is necessary, combining proactive relocation measures regarding habitat protection and community awareness.

Conclusions

The presence of *Aldrovanda vesiculosa* in the Danube Delta isn't evenly spread out — not even close. Some vegetation types support it fairly well, while others hardly seem to let it survive. The best spots, based on current observations, are those with floating, mid-height vegetation. *Spirodelo-Aldrovandetum* is a good example. It looks like these areas give the plant just enough light and space. Probably more importantly, there's not a lot of competition from aggressive, fast-growing species.

In contrast, where vegetation gets tall and dense — like in *Phragmitetum australis* or *Caricetum*

ripariae — *A. vesiculosa* becomes rare. It's easy to see why. These types of plants create a lot of shade, and oxygen levels in the water drop as a result. It's not the kind of place where a small, light-sensitive carnivorous plant is going to thrive. The sediment in most of the sites is organic sand. It doesn't vary much, though here and there you'll see some difference in color or organic content. Nothing too dramatic. What might matter more is the water. Being relatively close to the Black Sea, some of these waters are slightly brackish. It's subtle, but the salt influence is there. Whether that helps or hinders the species isn't entirely clear, but it's likely part of the equation. Now, while this species is definitely specialized, it's shown some resilience in unexpected places. There are small populations in less ideal habitats — like N1H and Q53. Conditions there aren't great, and it's doubtful those populations can sustain themselves long-term. But the fact that the plant's there at all hints at some ability to adapt, at least to micro-level changes. A bigger concern is a population growing in an artificial setting — a fish farm such as Perișor polder. On paper, it's a habitat. In reality, it's extremely vulnerable. Any changes to the water system or future construction work, and that group could disappear entirely. And considering *Aldrovanda vesiculosa* is under strict international protection, that's not a small risk. So what's the path forward? First, ongoing monitoring. We need to keep close track of the locations where it's still surviving — or thriving. That's basic but critical. In parallel, it may be necessary to transplant shoots into better-suited areas. That has to be done carefully — selecting spots with low nutrient levels, steady hydrology, and the kind of aquatic plant communities *A.vesiculosa* prefers. It's not just about the plant — the whole ecosystem around it has to fit. Also, and this part often gets overlooked, local involvement is important. Without engaging landowners, fish farm managers, or local authorities, any effort might stall out. People on the ground can either make or break a conservation strategy. In short, there's no single solution. But a mix of steady monitoring, smart relocation, and clear communication with local stakeholders gives this species its best shot. We've already lost *A.vesiculosa* in much of Europe. It would be a serious failure to let it disappear here too.

Declarations

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contribution

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Data availability

The data that support the findings of this study are included within this paper and its supplementary information. Any other data are available from the corresponding author upon request.

References

1. Adamec L, Lev J (1999) The introduction of the aquatic carnivorous plant *Aldrovanda vesiculosa* L. to new potential sites in the Czech Republic: A five-year investigation. Folia Geobot. 34:299–305. https://doi.org/10.1007/BF02912816
2. Adamec L (2018) Biological flora of Central Europe: *Aldrovanda vesiculosa* L. Perspect Plant Ecol Evol Syst. 35:8–21. doi: 10.1016/j.ppees.2018.10.001.
3. Adamec L (2005) Ten years after the introduction of *Aldrovanda vesiculosa* to the Czech Republic. Acta botanica gallica 152(2):239–245. https://doi.org/10.1080/12538078.2005.10515475
4. Adamec L, Kovářová M (2006) Field growth characteristics of two aquatic carnivorous plants, *Aldrovanda vesiculosa* and *Utricularia australis*. Folia Geobotanica 41:395–406. https://doi.org/10.1007/BF02806556
5. Adamec L (1995) Ecological requirements and the European distribution of the aquatic carnivorous plant *Aldrovanda vesiculosa* L. Folia Geobot Phytotax. 30:53–61. https://doi.org/10.1007/BF02813219
6. Adamec L (1997) How to grow *Aldrovanda vesiculosa* outdoors. Carniv Plant Newslett. 26:85–88.
7. Adamec L (1999a) Seasonal growth dynamics and overwintering of the aquatic carnivorous plant *Aldrovanda vesiculosa* at experimental field sites. Folia Geobot. 34:287–297.
8. Adamec L (1999b) Turion overwintering of aquatic carnivorous plants. Carniv Plant Newslett. 28:19–24.
9. Adamec L (1999c) Further notes on flowering and seed set of *Aldrovanda vesiculosa*. Flytrap News (Sydney) 12(4):9–12
10. Adamec L (2000) Rootless aquatic plant *Aldrovanda vesiculosa*: physiological polarity, mineral nutrition, and importance of carnivory. Biol Plant. 43:113–119. https://doi.org/10.1023/A:1026567300241
11. Almazov AA, Bondar C, Diaconu C, Ghederim VETURIA, Mihailov AN, Mita P, … Vaghin NF (1963) Zona de varsare a Dunarii. Morfografie hidrologica, Bucharest, pp. 396.
12. Berta J (1961) Beitrag zur Ökologie and Verbreitung von *Aldrovanda vesiculosa* L. Biológia (Bratislava), 16:561–573.
13. Bilz M, Lansdown RV (2011) *Aldrovanda vesiculosa*. The IUCN Red List of Threatened Species 2011: e.T162834A5625679. https://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T162834A5625679.en
14. Bouxin G (2005) Ginkgo, a multivariate analysis package. Journal of vegetation Science 16(3):355–359.
15. Cameron KM, Wurdack KJ, Jobson RW (2002) Molecular evidence for the common origin of snap-traps among carnivorous plants. Am J Bot. 89:1503–1509. https://doi.org/10.3732/ajb.89.9.1503
16. Chiba Prefecture (2009) Red Data Book Chiba: Vascular Plants. Chiba, Japan: Nature Conservation Division.
17. Chifu T, Irimia I (2014) Diversitatea fitosociologică a vegetației României II: Vegetația erbacee antropizată. Tom 2. Iași: Institutul European.
18. Chirilă SD, Doroftei M, Vassilev K, Covaliov S (2024) The phytocoenology, distribution, and habitat preferences of the species *Aldrovanda vesiculosa* (Droseraceae) in Romania. Botanica Serbica 48(1):47–60. https://doi.org/10.2298/BOTSERB2401047C
19. Chytrý M, Tichý L, Hennekens SM, Knollová I, Janssen JA, Rodwell JS, …Schaminée JH (2020) EUNIS Habitat Classification: Expert system, characteristic species combinations and distribution maps of European habitats. Applied Vegetation Science 23(4):648–675. https://doi.org/10.1111/avsc.12519
20. Cristea V, Gafta D, Pedrotti F (2004) Fitosociologie. Presa Universitară Clujeană Cluj-Napoca

21. Cross A, Adamec L, Turner SR, Dixon KW, Merritt DJ (2016) Seed reproductive biology of the rare aquatic carnivorous plant *Aldrovanda vesiculosa* (Droseraceae). Botanical Journal of the Linnean Society 180(4):515–529.
22. Cross A (2012) *Aldrovanda*: The Waterwheel Plant. Redfern Natural History Productions Ltd.; Poole, UK.
23. Cross A, Adamec L (2020) *Aldrovanda vesiculosa* (errata version published in 2025). *The IUCN Red List of Threatened Species* 2020: e.T162346A275442310. Accessed 28 May 2025.
24. Cross AT, Adamec L, Turner SR, Dixon KW, Merritt DJ (2016) Seed reproductive biology of the rare aquatic carnivorous plant *Aldrovanda vesiculosa* (Droseraceae). Bot J Linn Soc. 180:515–529. https://doi.org/10.1111/boj.12391
25. Cross AT, Skates LM, Adamec L, Hammond CM, Sheridan PM, Dixon KW (2015) Population ecology of the endangered aquatic carnivorous macrophyte *Aldrovanda vesiculosa* at a naturalised site in North America. Freshwater Biol. 60:1772–1783. https://doi.org/10.1111/fwb.12609
26. De Cáceres M, Legendre P (2009) Associations between species and groups of sites: indices and statistical inference. Ecology 90:3566–3574.
27. Dihoru G, Negrean G (2009) Cartea roşie a plantelor vasculare din România (Red book of vascular plants from Romania) [in Romanian]. Academia Română p. 290.
28. Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345–366. https://doi.org/10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2
29. EEA (2025) *Aldrovanda vesiculosa* L. https://eunis.eea.europa.eu/species/168992. Accessed 05 April 2025
30. Fenu G, Siniscalco C, Bacchetta G, Cogoni D, Pinna MS, Sarigu M, Abeli T, Barni E, Bartolucci F, Bouvet D … Ercole S (2021) Conservation status of the Italian flora under the 92/43/EEC 'Habitats' Directive. Plant Biosyst. 155:1168–1173.
31. Fleischmann A, Cross AT, Gibson R, Gonella PM, Dixon KW (2018) Systematics and evolution of Droseraceae. In: Ellison AM, Adamec L (Eds.), Carnivorous Plants: Physiology, Ecology, and Evolution. Oxford University Press, Oxford, U.K, pp. 45–57.
32. Godefroid S, Rivière S, Waldren S, Boretos N, Eastwood R, Vanderborght T (2011) To what extent are threatened European plant species conserved in seed banks?. Biological Conservation 144(5):1494–1498.
33. Guiry MD, Guiry GM (2024) AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org
34. Heywood VH (2019) Conserving plants within and beyond protected areas–still problematic and future uncertain. Plant Diversity 41(2):36–49. https://doi.org/10.1016/j.pld.2018.10.001
35. Ishino S (1963) Water Temperature and pH at Hozoji-numa Pond. In: Hanyu City *Aldrovanda* Conservation Group (Ed.), *Aldrovandain* Hanyu City. Hanyu, pp. 10–12. (in Japanese).
36. IUCN (2012) IUCN Red List Categories and Criteria, Ver. 3.1, Second ed. Gland and Cambridge.
37. Jury S (2009) Droseraceae. In: Euro + Med Plantbase - the information resource for Euro-Mediterranean plant diversity.
38. Kamiński R (1987a) Studies on the ecology of *Aldrovanda vesiculosa* L. I. Ecological differentiation of *A. vesiculosa* population under the influence of chemical factors in the habitat. Ekol Pol 35:559–590.
39. Kamiński R (1987b) Studies on the ecology of *Aldrovanda vesiculosa* L. II. Organic substances, physical and biotic factors and the growth and development of *A. vesiculosa*. Ekol. Pol. 35:591–609.
40. Kamiński R (2006) Restitution of Waterwheel plant (*Aldrovanda vesiculosa* L.) in Poland and recognition of factors deciding on its survival in temperate climate. In: Polish. Prace Ogr. Bot. Uniw. Wrocł. 8:105.
41. Kassambara A (2023) ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.6.0 https://rpkgs.datanovia.com/ggpubr/
42. Komiya S (1982a) The history of the discovery of *Aldrovanda* and the extinction. Hanyu City Board of Education (Ed.), *Aldrovanda* and Its Growing Environment, Hanyu pp. 9–14.
43. Komiya S (1982b) Release experiments of *Aldrovanda* and other aquatic plants at the habitat. Hanyu City Board of Education (Ed.), *Aldrovanda* and Its Growing Environment, Hanyu pp. 21–54.
44. Lamont EE, Sivertsen R, Doyle C, Adamec L (2013) Extant populations of *Aldrovanda vesiculosa* (Droseraceae) in the New World. J Torrey Bot Soc. 140:517–522.
45. McCune B, Mefford MJ (2006) PC-ORD. Multivariate analysis of ecological data. Version 5.32 MjM Software, Gleneden Beach, Oregon, U.S.A.
46. Mitsch WJ, Gosselink JG (2000) The value of wetlands: Importance of scale and landscape setting. Ecol Econ. 35:25–33.
47. Mucina L, Bültmann H, Dierssen K, Theurillat J-P, Raus T, Arni AČ, Šumberová KŠ, Willner W, Dengler J, Gavilán García R, Chytrý M, Hájek M, Di Pietro R, Iakushenko D, Pallas J, Daniëls FJA, Bergmeier E, Santos Guerra A, Ermakov N, Valachovič M, Schaminée JHJ, Lysenko T, Didukh YP, Pignatti S, Rodwell JS, Capelo J, Weber HE, Solomeshch A, Dimopoulos P, Aguiar C, Hennekens SM, Tichý L (2016) Vegetation of Europe: hierarchical floristic classification system of vascular plant, bryophyte, lichen and algal communities. Applied Vegetation Science 19:3–264. https://doi.org/10.1111/avsc.12257
48. Neary VS, Gunawan B (2011) Field measurements at river and tidal current sites for hydrokinetic energy development: best practices manual (no. ORNL/TM-2011/419). Oak Ridge National Lab.(ORNL), Oak Ridge, TN (United States).
49. Nishihara S, Shiga T, Nishihiro J (2023) The discovery of a new locality for *Aldrovanda vesiculosa* (Droseraceae), a critically endangered free-floating plant in Japan. Journal of Asia-Pacific Biodiversity 16(2):227–233.

50. Onoda K (1963) Overview of the *Aldrovanda* Habitat. Hanyu City Aldrovanda Conservation Group (Ed.), *Aldrovanda* in Hanyu City. Hanyu pp. 6–7.
51. Płachno BJ, Strzemski M, Dresler S, Adamec L, Wojas-Krawczyk K, Sowa I … Mirand VF (2020) A chemometry of *Aldrovanda vesiculosa* l. (Waterwheel, Droseraceae) populations. Molecules 26(1):72.
52. R Core Team (2025) RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. http://www.rstudio.com/.
53. Rousseeuw PJ (1987) Silhouettes: a Graphical Aid to the Interpretation and Validation of Cluster Analysis. Journal of Computational and Applied Mathematics 20:53–65.
54. Salgotra RK, Chauhan BS (2023) Genetic diversity, conservation, and utilization of plant genetic resources. Genes 14(1):174.
55. Shiga T, Baasanmunkh S, Oyuntsetseg B, Khaliunaa K, Kato S, Choi HJ (2020) Aquatic macrophyte flora of Numrug strictly protected area and some parts of eastern Mongolia. Bulletin of Water Plant Society Japan 109:31–45.
56. Shimai H, Ohmori T (2023) Threatened aquatic plant *Aldrovanda vesiculosa* L.: a review of its discovery and extinction in Japan. Aquatic Botany 103682.
57. Sinkevičienė Z, Kamaitytė-Bukelskienė L, Petrulaitis L, Gudžinskas Z (2023) Current distribution and conservation issues of aquatic plant species protected under habitats directive in Lithuania. Diversity 15(2):185.
58. Slowikowski K (2023) ggrepel: Automatically position non-overlapping text labels with 'ggplot2'. R package version 0.9.3.
59. Tichý L (2002) JUICE, software for vegetation classification. Journal of Vegetation Science 13:451–453.
60. Volis S (2016) Conservation meets restoration – rescuing threat-ened plant species by restoring their environments andrestoring environments using threatened plant species. Isr. J. Plant Sci 63:262–275.
61. Volis S (2017) Complementarities of two existing intermediate conservation approaches. Plant Diversity 39(6):379–382.
62. Walters SM (1979) Conservation of the European Flora: *Aldrovanda vesiculosa* L., a documented case-history of threatened species. Almqvist.
63. Wang Y, Fukuda H, Zhang P, Wang T, Yang G, Gao W, Lu Y (2022) Urban wetlands as a potential habitat for an endangered aquatic plant, *Isoetes sinensis*. Global Ecology and Conservation 34: e02012.
64. Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.
65. WorldPlants (2025) Plant List. https://www.worldplants.de/world-plants-complete-list/complete-plant-list. Accessed 23 March 2025.
66. Yakubovskaya TV (1991) The genus *Aldrovanda* (Droseraceae) in the Pleistocene of the Belorussian SSR. Bot Zhurnal. 76:109–118.
67. Zhang Z, Guo Y, He JS, Tang Z (2018b) Conservation status of wild plant species with extremely small populations in China. Biodiversity Science 26(6):572.

Figures

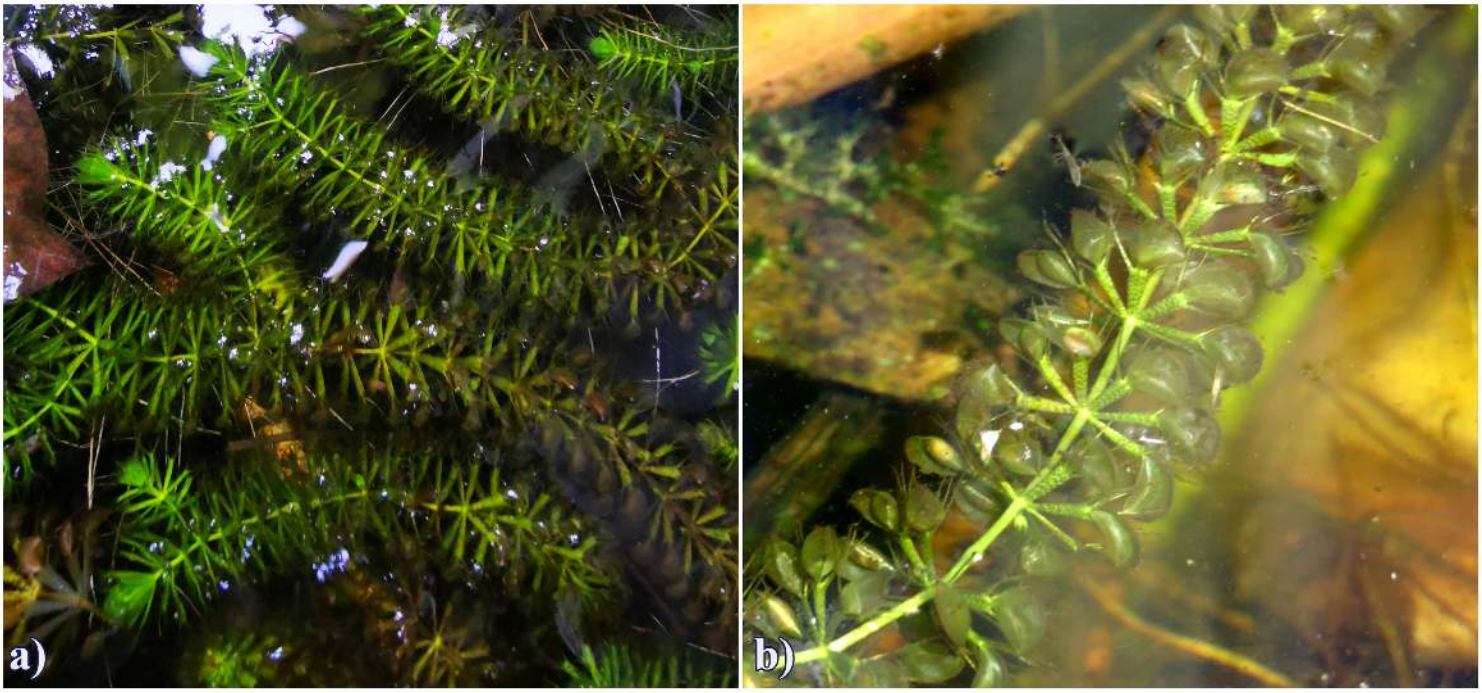


Figure 1

Aldrovanda vesiculosa (A, B) (Photographs: Mihai Doroftei – A, Simona Chirilă – B)

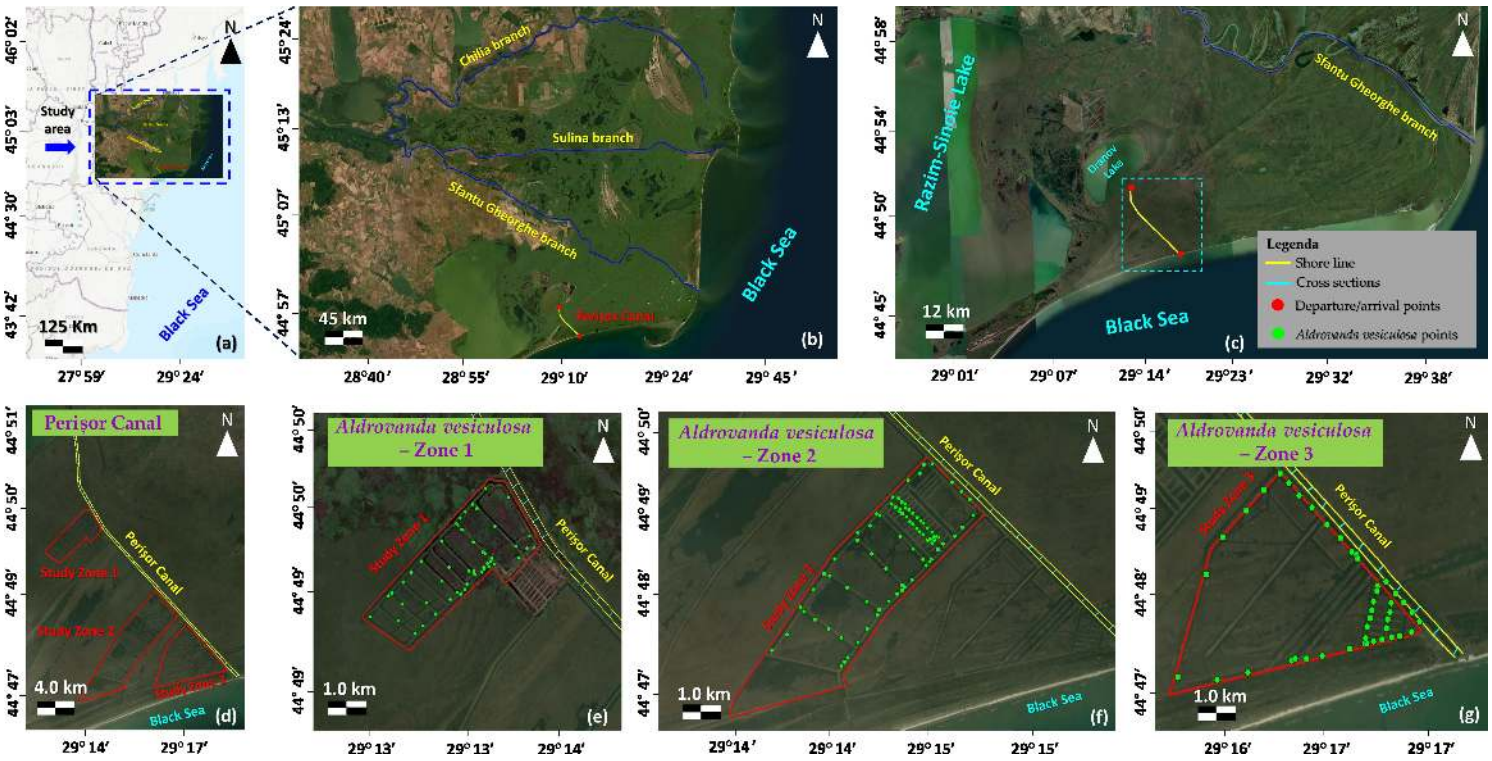


Figure 2

Field data collection – hydrological measurements on the Perișor Canal in the Danube Delta: (a) view of the study area superimposed on the topographic map; (b) view of the study area superimposed on the Danube Delta orthophotomap; (c) view of the Perișor Canal superimposed on the Danube Delta orthophotomap; (d) view of study area 1 and study area 2; (e) view of topo-bathymetric data collection on the Perișor Canal near study area 1; (f) view of topo-bathymetric data collection on the Perișor Canal near study area 2 (Map: Alexandru Bănescu)



Figure 3

Sediment and water sample collection (Map:Oliver Livanov)

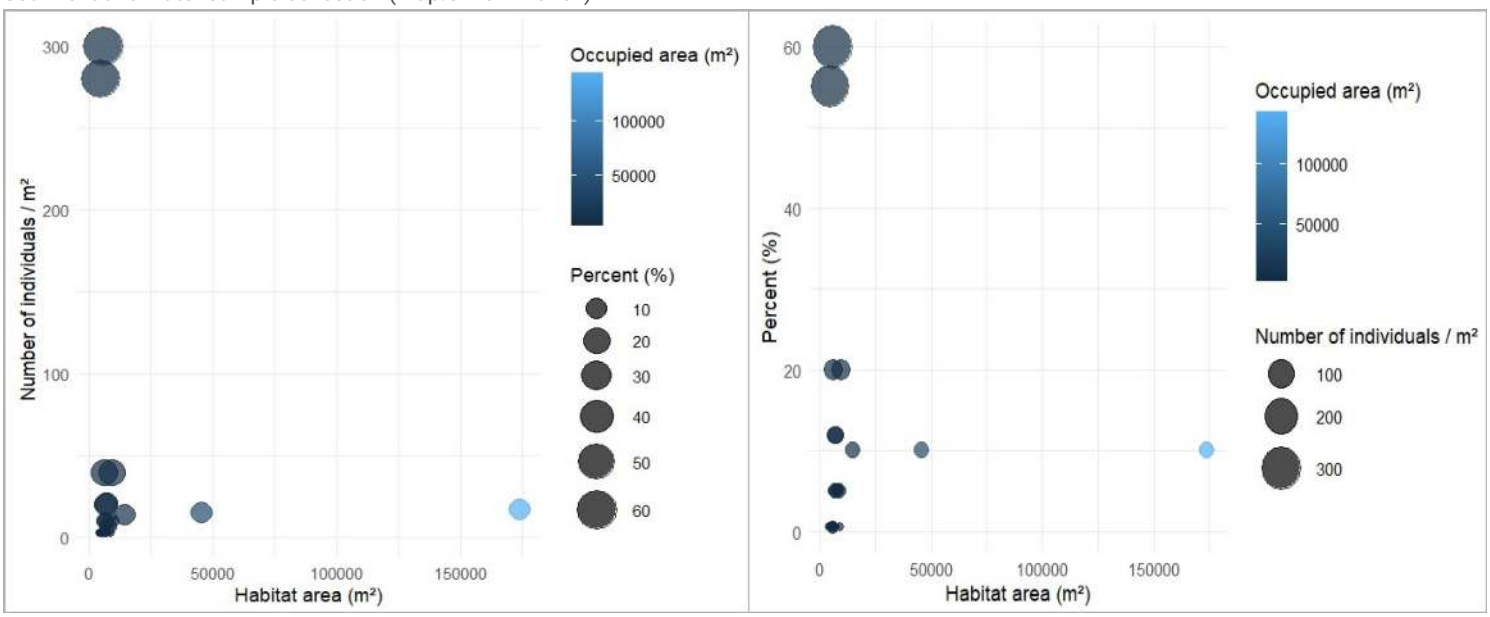


Figure 4

The relationship between habitat area and population structure: density of individuals and occupancy percentage

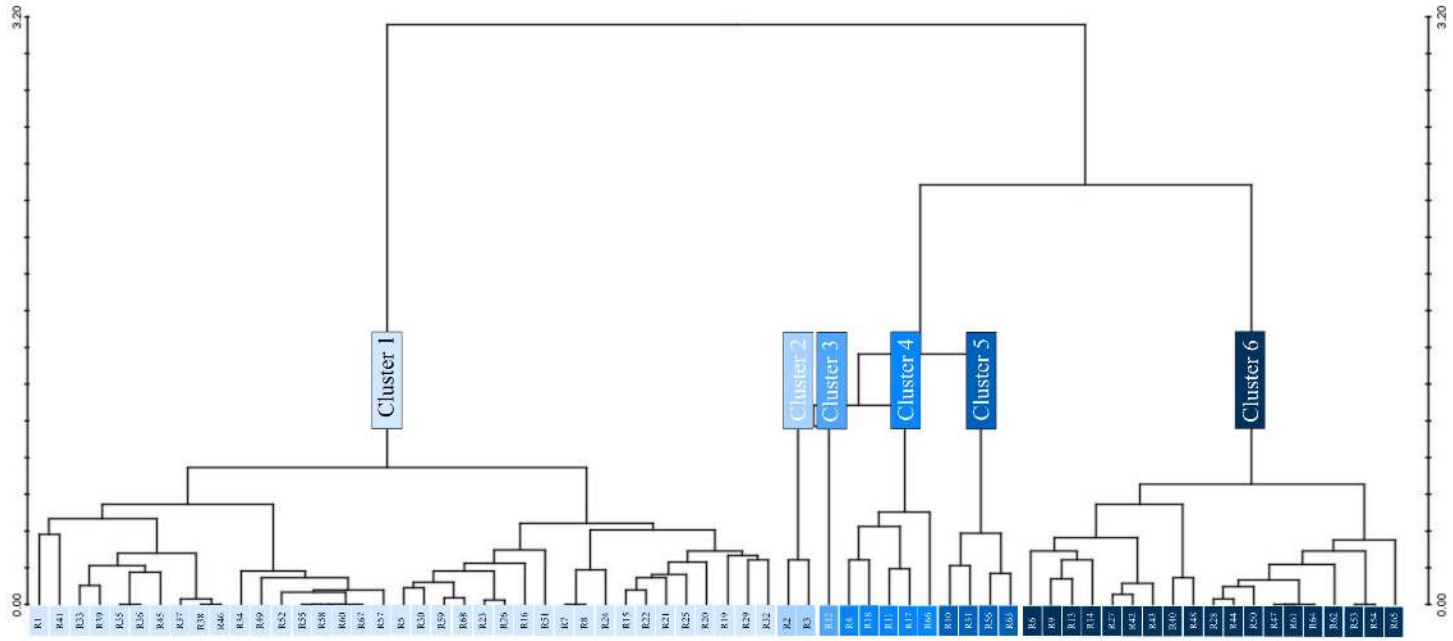


Figure 5

Dendrogram of associations with *Aldrovanda vesiculosa*

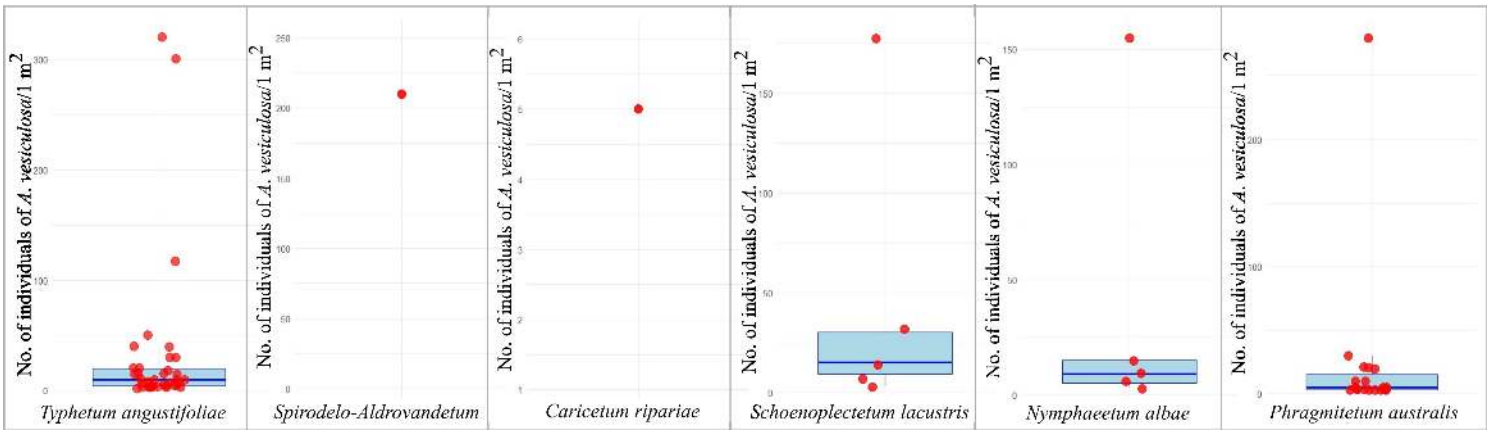


Figure 6

Number of *A. vesiculosa* individuals depending on the association

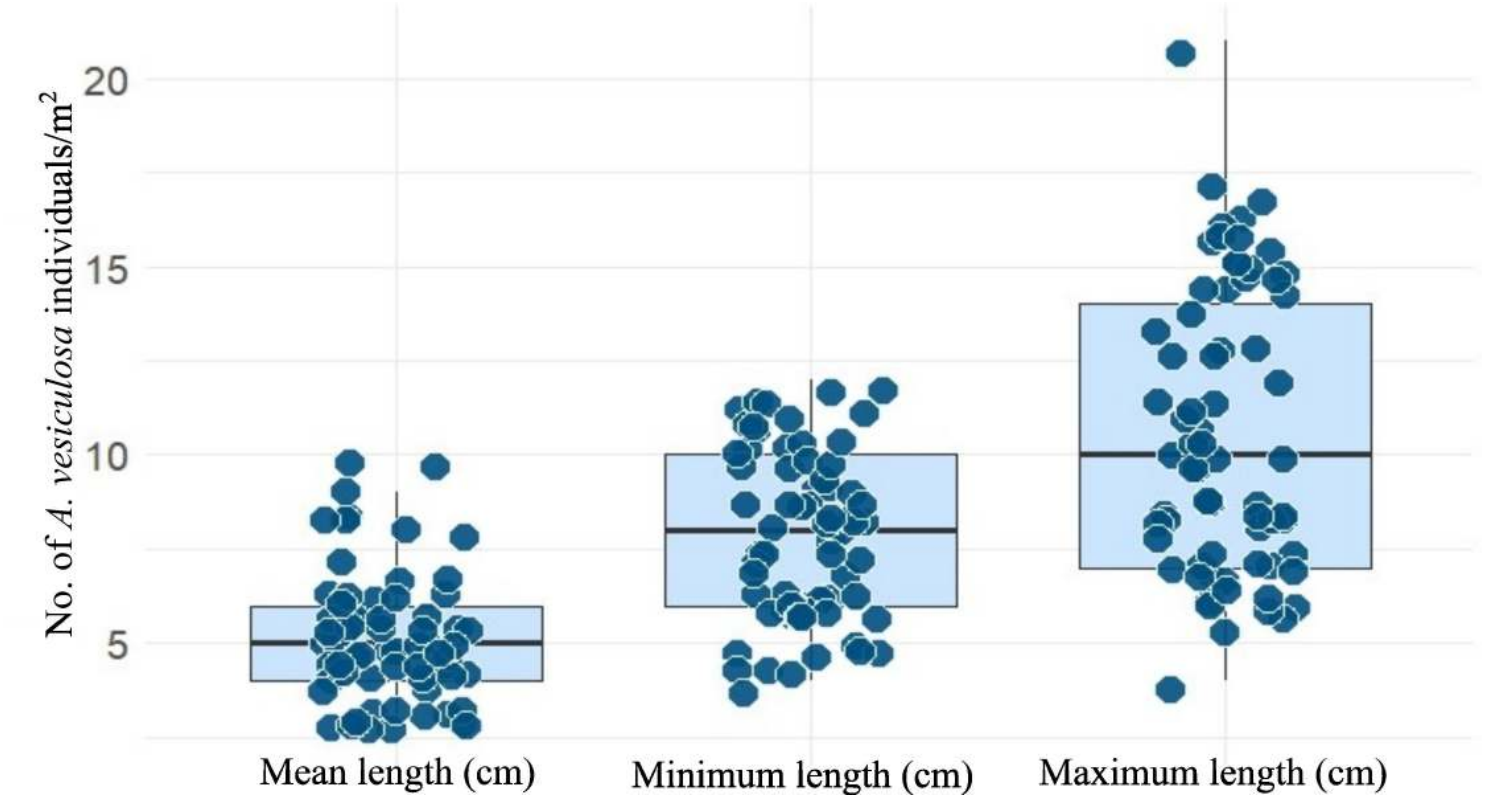


Figure 7

Variation in mean, minimum, and maximum length of *A. vesiculosa* individuals about density (individuals/ 1 m²)

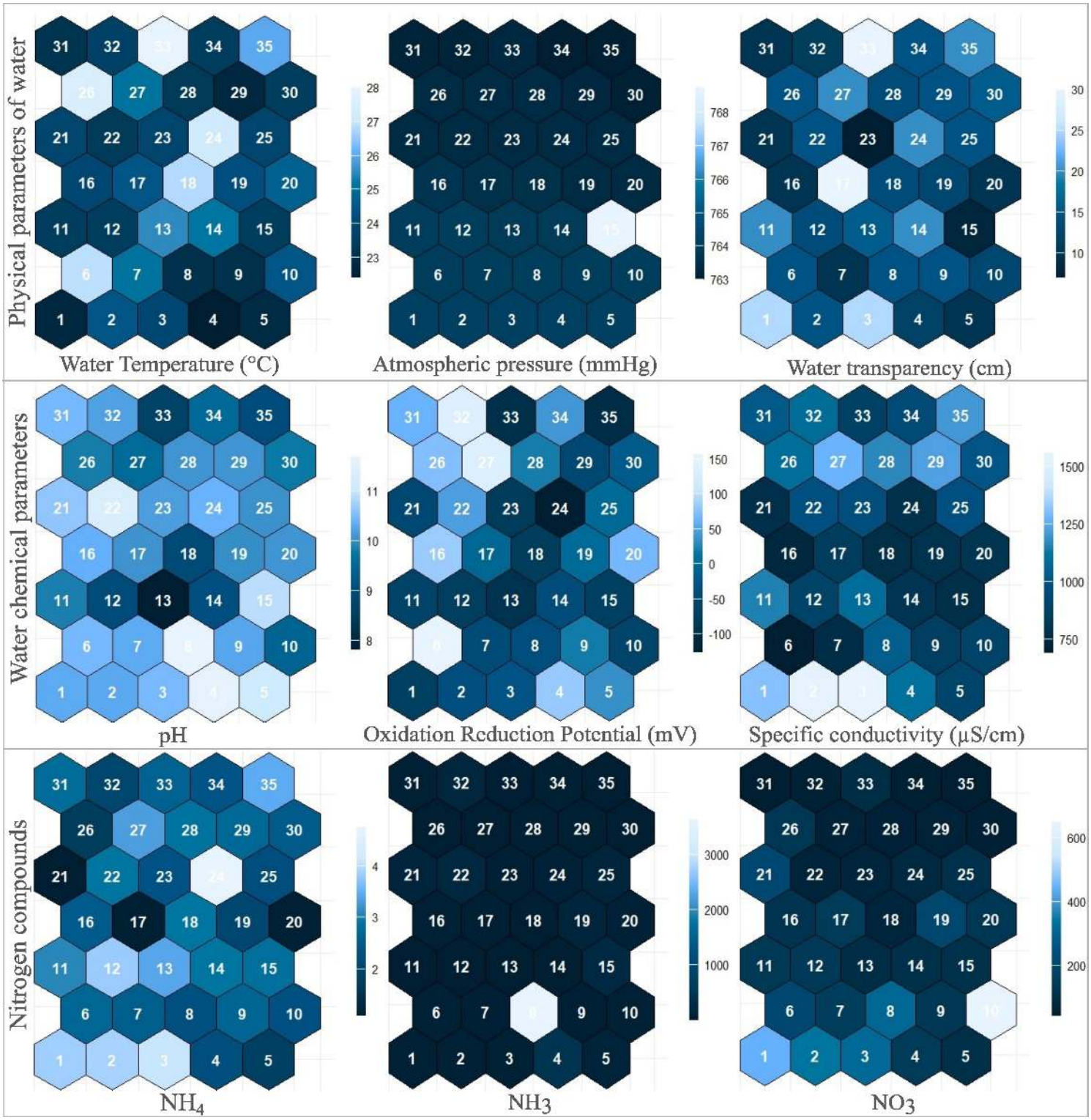


Figure 8

Self-Organizing Maps – abiotic and biotic factors (1-35: plots)

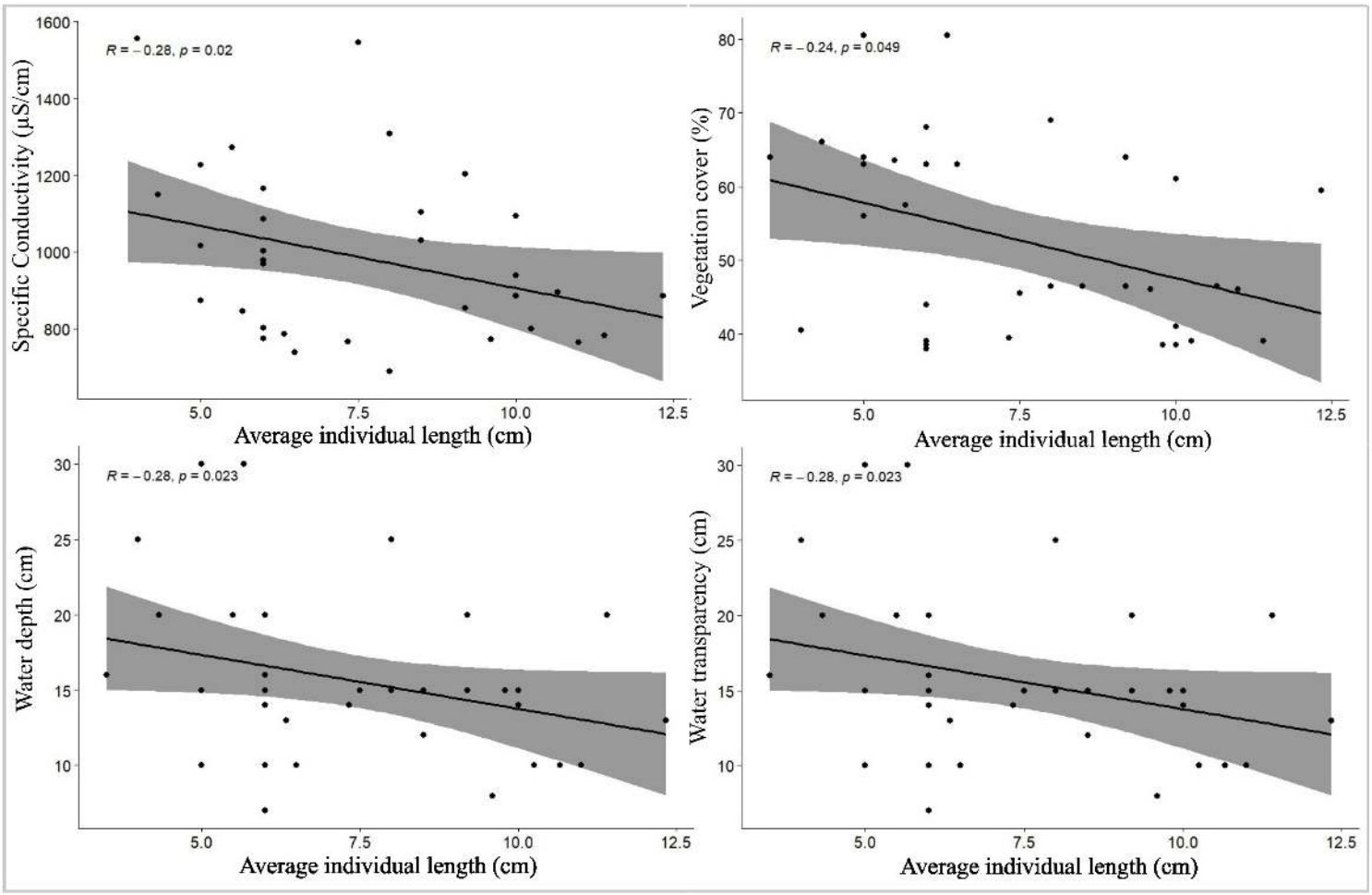


Figure 9

Relationship between average individual length and some environmental variables

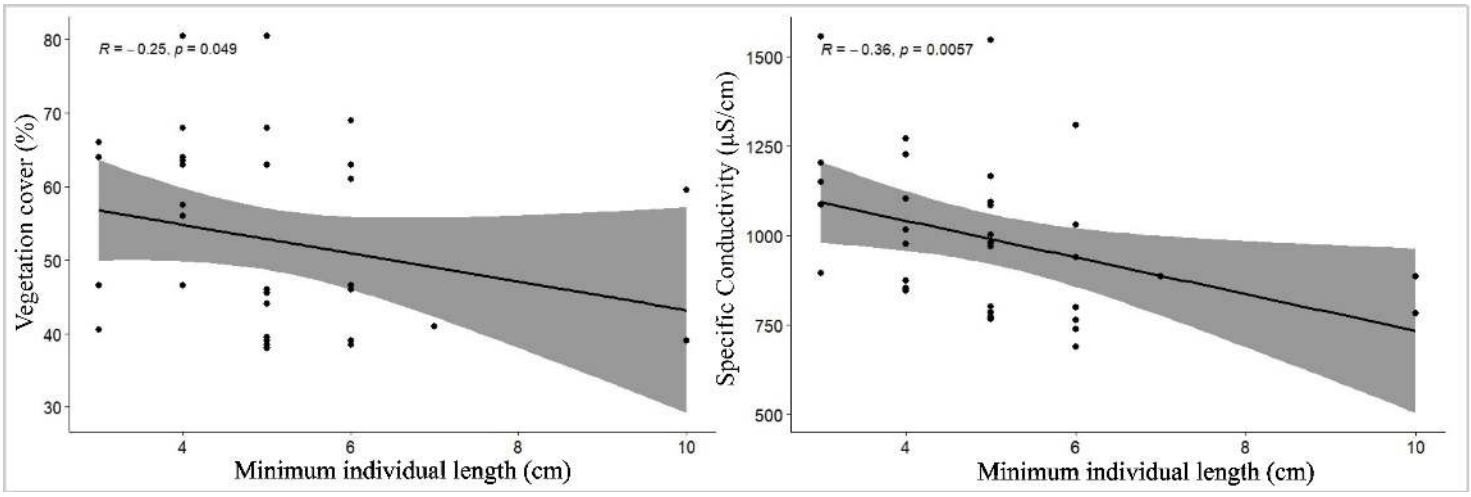


Figure 10

Relationship between minimum individual length and some environmental variables

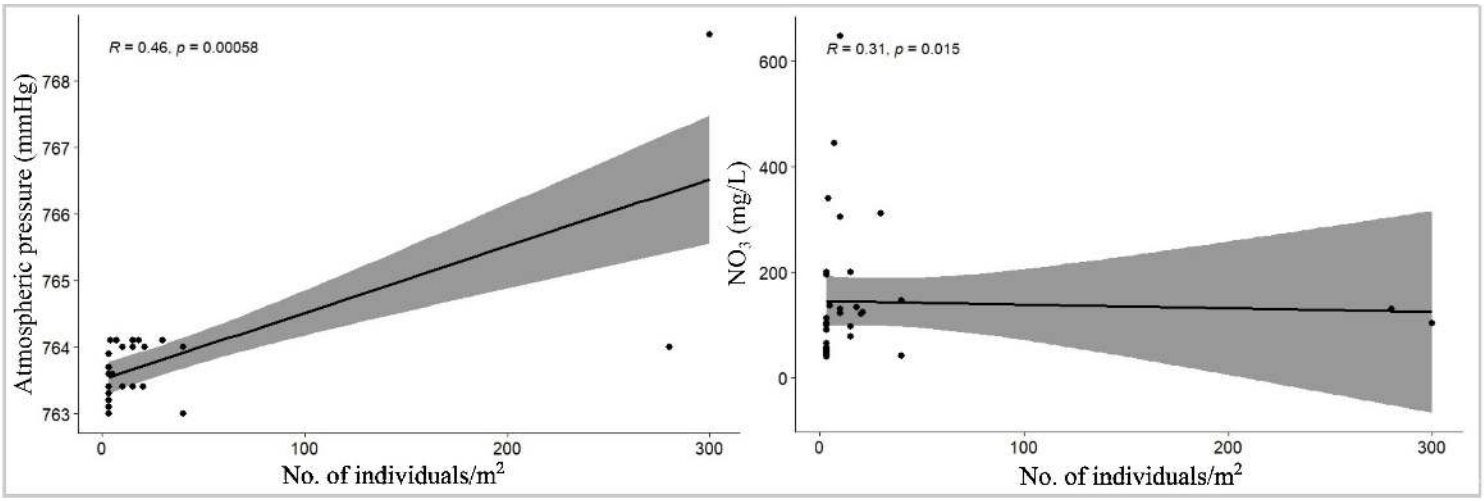


Figure 11

Relationship between the number of individuals per 1 m^2 and some environmental variables

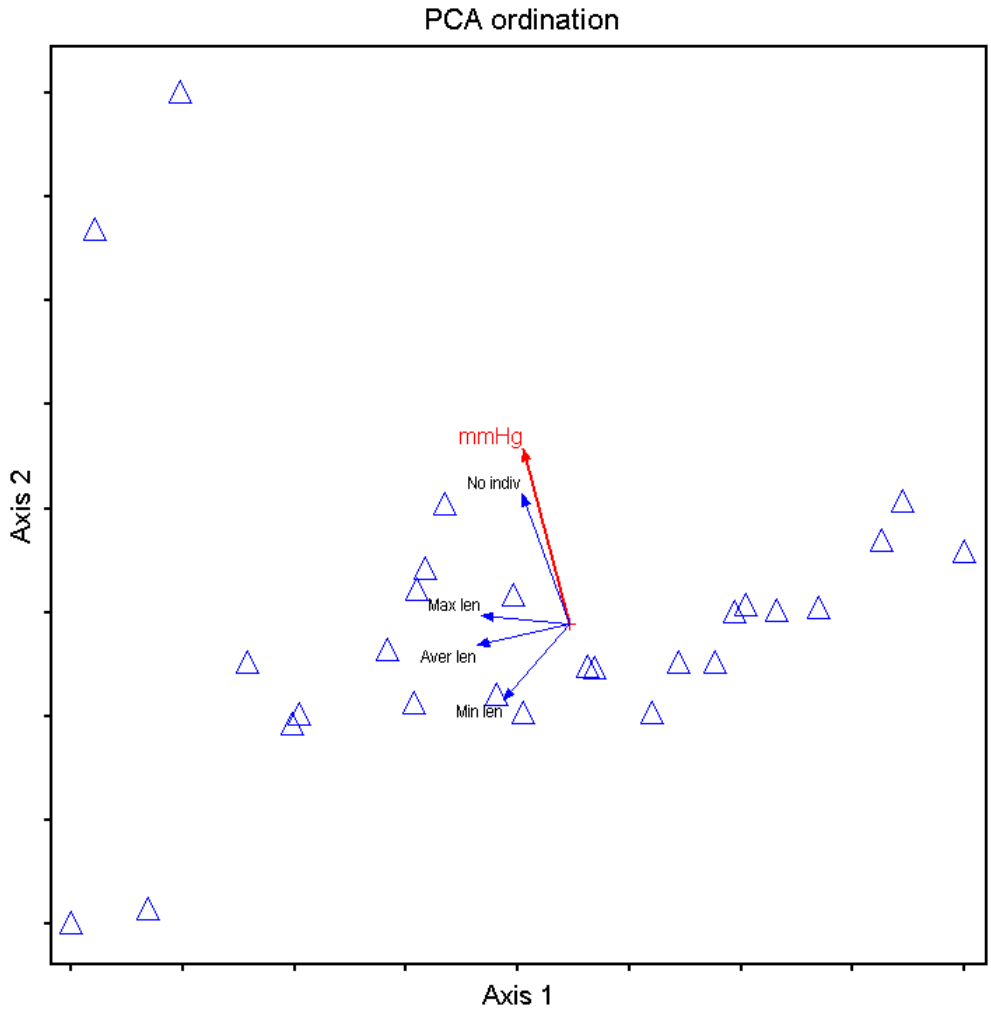


Figure 12

Principal Component Analysis. Triangles – vegetation plots

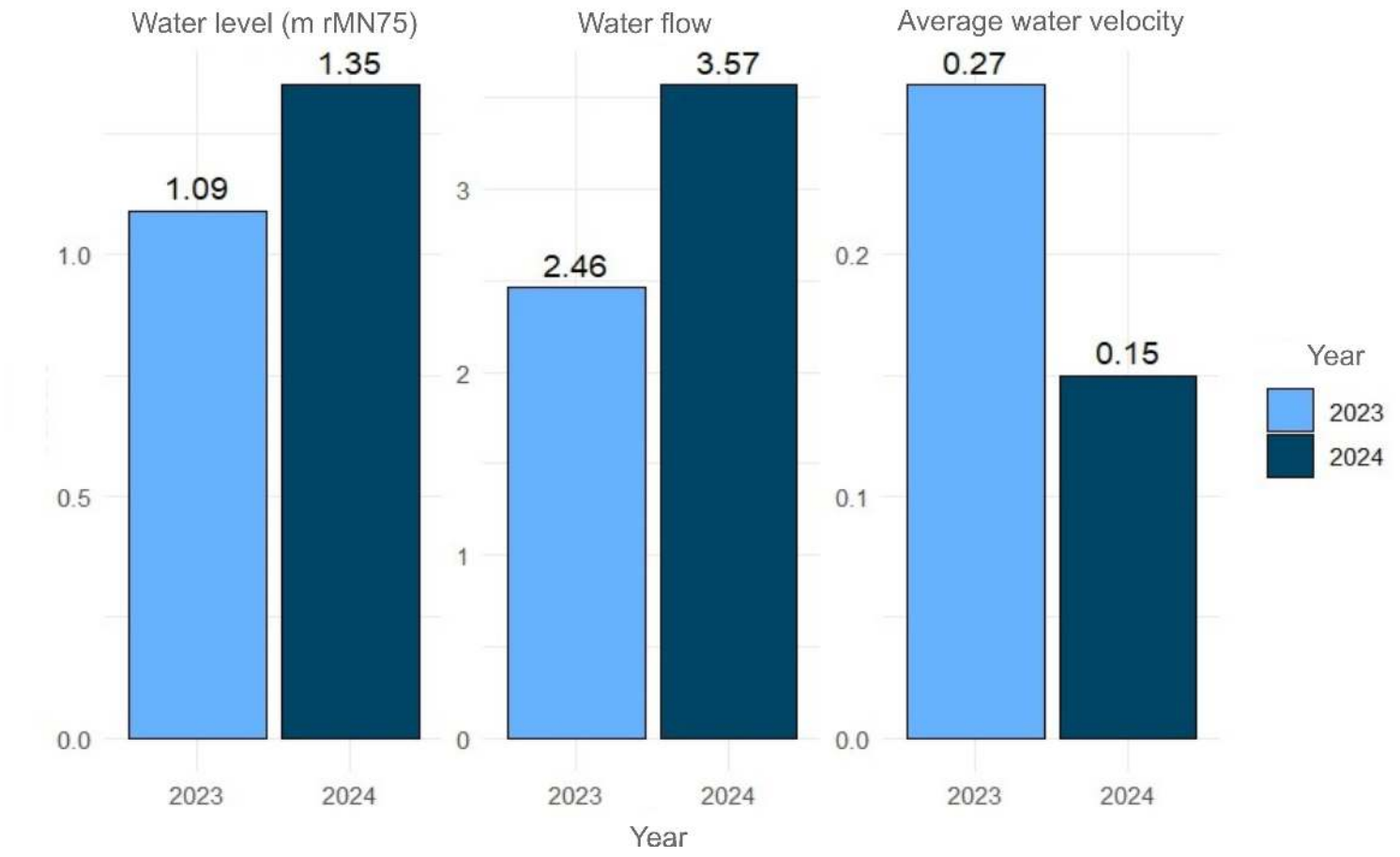


Figure 13

Hydrological measurements carried out on the Perișor canal



Figure 51

Sediment and water sample collection (Map:Oliver Livanov)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.xlsx