Limnological Review (2013) 13, 3: 171-179
DOI 10.2478/limre-2013-0019

Reproduction of *Hydrocharis morsus-ranae* taxa in an oxbow lake of the River Vistula

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Abstract: The aim of the research was to establish the density of specimens and shoots as well as the reproductive effort of *Hydrocharis morsus-ranae* during the whole vegetative period in a Polish oxbow lake. The following specimen features were examined: plant diameter, total length, the number of buds, flowers, young fruit, ripe fruit, turions and leaves and also dry total mass, vegetative mass, generative mass, the bud mass, the flower mass, young fruit mass and ripe fruit mass. The density of *Hydrocharis morsus-ranae* specimens per square metre ranged from 10 to 170 while the density of shoots ranged from 10 to 545. From one square metre overgrown with *Hydrocharis morsus-ranae*, a maximum of 389 turions, 50 fruit and 4000 seeds are produced. The maximum of reproductive effort is 97.8% of vegetative mass and 2.2% of generative mass in September 2010. The factors which best explain changeability of the *Hydrocharis morsus-ranae* population in time are the length and the diameter of the specimens in the population. Fruiting of *Hydrocharis morsus-ranae* in Poland is higher than in Canada, where it is an invasive taxon. *Hydrocharis morsus-ranae* is well adapted to the environment in oxbow lakes of the River Vistula and represents the S-R strategy.

Key words: density, flower, fruit, *Hydrocharis*, invasion, reproduction, seasonality, turion

Introduction

Hydrocharis morsus-ranae belongs to aquatic monocotyledon plants (Dandy 1980). Revision of the *Hydrocharis* genus was described by Cook and Luond (1982). The biology of *Hydrocharis* was described by Catling et al. (2003) and Scribailo and Posluszny (1984). The development of *Hydrocharis morsus-ranae* fruit was described by Toma (2008). Natural habitats of this species are overgrowing lake bays, oxbow lakes, ponds, clay pits filled with water, astatic waters and peat bogs (Kłosowski and Kłosowski 2001). Reports about the invasiveness of this aquatic plant indicate its significant ability to overgrow areas in a short time. In Poland, this species has a large number of localities which, according to a classification by Zarzycki et al. (2002), in the last decades, have shown the following dynamic tendency: its localities disappear and new ones appear or it is disappearing due to habitat degradation. Occurrence of *Hydrocharis morsus-ranae* is determined by the ecological indicator values. Ecological indicators: Light value L=4 – moderate light; Temperature value T=3-4 – moderately cool climatic conditions, lower mountain zone, northern division in lowlands, special microhabitats – raised bogs and moderately warm climatic conditions, most of the lowland and colline region; Trophy value Tr=4-3 – eutrophic and mesotrophic water; Water acidity value R=4-5 – neutral 6≤pH<7 and alkaline pH>7; Soil granulometric value D=5 – heavy clay and loam; Organic matter content value H=2 – mineral-humic soil (Zarzycki et al. 2002).

Hydrocharis morsus-ranae has a natural range of occurrence in Europe and northern Asia (Hegi 1981). It has been introduced in Canada and the United States, where it is regarded as an invasive plant. In Canada, *Hydrocharis morsus-ranae* was introduced in 1932 in Central Experiment Farm in Ottawa and began to spread from there (Minshall 1940; Dore 1954; Lumsden and Mclachlin 1988). The influence of the introduction of *Hydrocharis morsus-ranae* on other macrophytes (Catling et al. 1988) and the distribution of *Hydrocharis morsus-ranae* in North America have been described (Catling and Dore 1982; Catling and

Porebski 1995). Also, the distribution of this species in Canada has been presented (Catling et al. 2003).

The results of the research may be applied to assess the invasiveness of *Hydrocharis morsus-ranae* species on other habitats in Europe and in the world as well as to shape the settlement of leisure areas with this species.

The aim of the research is to establish the density of specimens and shoots of *Hydrocharis morsus-ranae* and determine the level of generative and vegetative reproduction of *Hydrocharis morsus-ranae*, its reproductive effort and also to decide which factors best explain the changeability of the *Hydrocharis morsus-ranae* population in time.

Methods

Observation and material collection were carried out once a month from May to October 2010. 20 specimens were gathered each month for study. 120 *Hydrocharis morsus-ranae* specimens constituted the research material and were examined in laboratory conditions. The following specimen features were examined: plant diameter, total length, the number of buds, flowers, young fruit, ripe fruit, turions and leaves and also dry total mass, vegetative mass, generative mass, the bud mass, the flower mass, young fruit mass and ripe fruit mass. The plant density was estimated in two randomly selected places in the *Hydrocharis morsus-ranae* population with the use of a wooden frame of 1×1m. The density per square metre was calculated as a mean of two measurements of the specimen numbers. Counting the specimens was difficult due to polycormism occurring in this species as well as intertwining of specimens in places into a dense coat covering the water surface. *Hydrocharis morsus-ranae* specimens were put in plastic bags and taken to a laboratory where they were tagged. Then, their diameter and their length were measured and the buds, flowers, young and ripe fruit of the fresh material were counted. Tagged specimens were dried at a temperature of 70°C for 8 hours and the drying was repeated three times. The specimens were weighed to establish their total dry mass, their vegetative mass, generative mass, the bud mass, the flower mass, young fruit mass and ripe fruit mass. The calculations and diagrams were made with Statistica10.

Definitions of notions used in the text:

Shoot density – the number of ramets of a clonal colony which are rooted and connected with a genet.

Plant density – the number of specimens, each of which consists of a number of ramets.

Research area

The research area is located in Europe, southern Poland (Fig. 1), in the macro-region of the Oświęcim Basin, which spreads in the River Vistula basin between the Silesian-Kraków Upland in the North and the Silesian and Wieliczka Foothills in the south. It borders with Kraków Gate in the east and with Ostrava Basin in the west. The Oświęcim Basin is divided into the Pszczyna Plain, Upper Vistula Valley and Wilamowice Foothills.

Fig. 1. Locality of research area in Poland

In the Oświęcim Basin, the River Biała, the River Soła and the River Skawa flow into the River Vistula together with the River Przemsza, the River Gostynia and the River Pszczynka flowing down from the Silesian Upland.

Administratively, the research area is located on the borderline of Lesser Poland and Silesian Province. The research locality (50°03'47.92" N, 19°11'24.71" E) is located to the North-West of Oświęcim. The oxbow lake (length 220 m, width 25-40 m, average depth 2.40 m) where the sample material was taken from to be examined, is cut off from the current of the River Vistula (about 150m to the River Vistula), and has bottom sediments with substantial thickness, about 2m. The oxbow lake is eutrophic and surrounded by mixed-tree species stands. It is 150 m from the road on one side and about 800 m from buildings on the other side.

The climate of the Oświęcim Basin is conditioned by the parallel pattern of physical and geographical units which triggers the inflow of air masses from south west but also the accumulation of air from higher parts of the Carpathians connected with the local circulation conditions. Masses of polar-maritime and continental air dominate in the general circulation. Western and north-western winds prevail, which is connected with the direction of advection of the most commonly inflowing masses of polar and maritime air. The Oświęcim Basin is characterized by its unfavourable anemological conditions. It is poorly ventilated (stillness constitutes approximately 17% and together with winds of 2 m s^{-1} nearly 70% of the entire number of cases) whereas inversions and stationary air masses together with air pollution are a source of unfavourable aero-sanitary conditions. The annual precipitation in this region is 700-800mm. Mean annual temperature is 8.2°C, whereas mean annual temperature in January is –3.2°C and in July is 18.2°C. The vegetative spell lasts 210-220 days and the mean time of snow cap occurrence is 70-75 days (Bajorska and Stachnik 2007).

Results

During the six months of research, the density of *Hydrocharis morsus-ranae* ranged from 10 to 170 specimens per square metre. The density of shoots ranged from 10 to 545 per square metre (Fig. 2). The dry biomass of *Hydrocharis morsus-ranae* in the period of the highest production in September was 37.94 g m^{-2}. From one square metre overgrown with *Hydrocharis morsus-ranae*, a maximum of 389 turions, 50 fruit and 4000 seeds are produced in the Polish oxbow lake. The dynamics of the *Hydrocharis morsus-ranae* population is characterized by rapid growth of specimens and then rapid fall. The highest growth of the

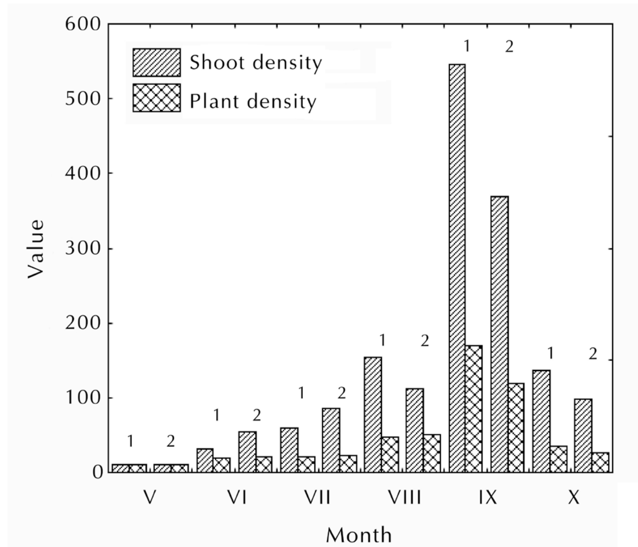

Fig. 2. Density of *Hydrocharis morsus-ranae* in an oxbow lake of the River Visula. Numerals 1 and 2 denote sampling sites

specimens occurred in September, and higher changeability was found in shoot density than in plant density (Fig. 2).

ANOVA analysis and Tukey's test show significant differences in density between September, May, June, July and August (Table 1). There was a rapid growth in the density of *Hydrocharis morsus-ranae* specimens in September. ANOVA analysis was carried out to establish significant differences in the diameter and length of the specimens. The analysis showed significant differences (Table 2, Fig. 3). Tukey's test was carried out to establish in which months the differences were significant.

ANOVA analysis was made for the number of buds, flowers, young fruit, ripe fruit, turions and leaves; the analysis of vegetative, generative and general mass showed significant differences in all traits except young fruit (Table 3). Tukey's test showed significant differences between May and the other months: June and May, July, August; July and May, June and October; August and May, June and October; September and May, October and May, July and August (Table 4).

Table 1. Analysis of variance of *Hydrocharis morsus-ranae* abundance. *Results marked in bold* correspond to *p*-values less than 0.05. SS – sum of squares, df – degree of freedom number, MS – mean square, F-test –Anova analysis, p-probability – Anova analysis

Variable	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Shoot abundance	263643.4	5	52728.68	17711.50	6	2951.917	17.86252	0.001523
Plant abundance	24999.0	5	4999.80	1353.00	6	225.500	22.17206	0.000837

Table 2. Tukey's HSD (Honestly Significant Difference) post-hoc test for shoot density of *Hydrocharis morsus-ranae*. *Results marked in bold* correspond to *p*-values less than 0.05

Month	{1} M=10.000	{2} M=43.500	{3} M=72.500	{4} M=133.50	{5} M=457.00	{6} M=117.00
5 {1}		0.985486	0.845005	0.327607	**0.001502**	0.447795
6 {2}	0.985486		0.992311	0.597422	**0.002213**	0.751621
7 {3}	0.845005	0.992311		0.856455	**0.003208**	0.953262
8 {4}	0.327607	0.597422	0.856455		**0.007695**	0.999456
9 {5}	0.001502	0.002213	0.003208	0.007695		0.005991
10 {6}	0.447795	0.751621	0.953262	0.999456	**0.005991**	

Table 3. Analysis of variance for features of *Hydrocharis morsus-ranae* population. *Results marked in bold* correspond to *p*-values less than 0.05. SS – sum of squares, df – degree of freedom number, MS – mean square, F-test –Anova analysis, p-probability – Anova analysis

Variable	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Plant diameter	1618	5	324	727	114	6.38	50.7411	0.000000
Plant length	13965	5	2793	3011	114	26.41	105.7551	0.000000
Number of buds	1	5	0	7	114	0.006	4.5972	0.000739
Number of flowers	16	5	3	9	114	0.08	41.2789	0.000000
Number of young fruit	2	5	0	19	114	0.16	2.0321	0.079319
Number of mature fruit	2	5	0	12	114	0.11	3.6739	0.004047
Number of turions	84	5	17	94	114	0.83	20.3258	0.000000
Number of leaves	756	5	151	934	114	8.19	18.4582	0.000000
Plant dry total mass of one individual	851589	5	170318	425747	114	3734.62	45.6051	0.000000
Generative dry plant mass	609	5	122	2700	114	23.68	5.1421	0.000273
Vegetative dry plant mass	812842	5	162568	426892	114	3744.67	43.4133	0.000000
Bud dry mass	1	5	0	5	114	0.04	4.8511	0.000464
Flower dry mass	25	5	5	23	114	0.20	24.0612	0.000000
Young fruit dry mass	63	5	13	777	114	6.81	1.8410	0.110367
Mature fruit dry mass	274	5	55	1730	114	15.17	3.6122	0.004534
Turion dry mass	3164	5	633	5494	113	48.62	13.0123	0.000000
Number of shoots	102	5	20	153	114	1.34	15.1566	0.000000
Full dry generative mass	1021	5	204	3703	114	32.48	6.2859	0.000035
Full dry vegetative mass	4964551	5	992910	2912861	114	25551.41	38.8593	0.000000
Full fruit dry mass	1094	5	219	4886	114	42,86	5,1032	0,000293
Full turion dry mass	12818	5	2564	17548	114	153,93	16,6535	0,000000
Full dry mass of one complex of individuals	5087769	5	1017554	2911021	114	25535,27	39,8490	0,000000

Table 4. Tukey's HSD test for plant diameter of *Hydrocharis morsus-ranae*. *Results marked in bold* correspond to *p*-values less than 0.05

Month	{1} M=10.000	{2} M=43.500	{3} M=72.500	{4} M=133.50	{5} M=457.00	{6} M=117.00
5 {1}		0.000119	0.000119	0.000119	0.000119	0.000119
6 {2}	0.000119		**0.000200**	**0.000133**	0.072635	0.974906
7 {3}	0.000119	0.000200		0.999055	0.346923	**0.001614**
8 {4}	**0.000119**	**0.000133**	0.999055		0.172476	**0.000483**
9 {5}	**0.000119**	0.072635	0.346923	0.172476		0.346923
10 {6}	**0.000119**	0.974906	**0.001614**	**0.000483**	0.346923	

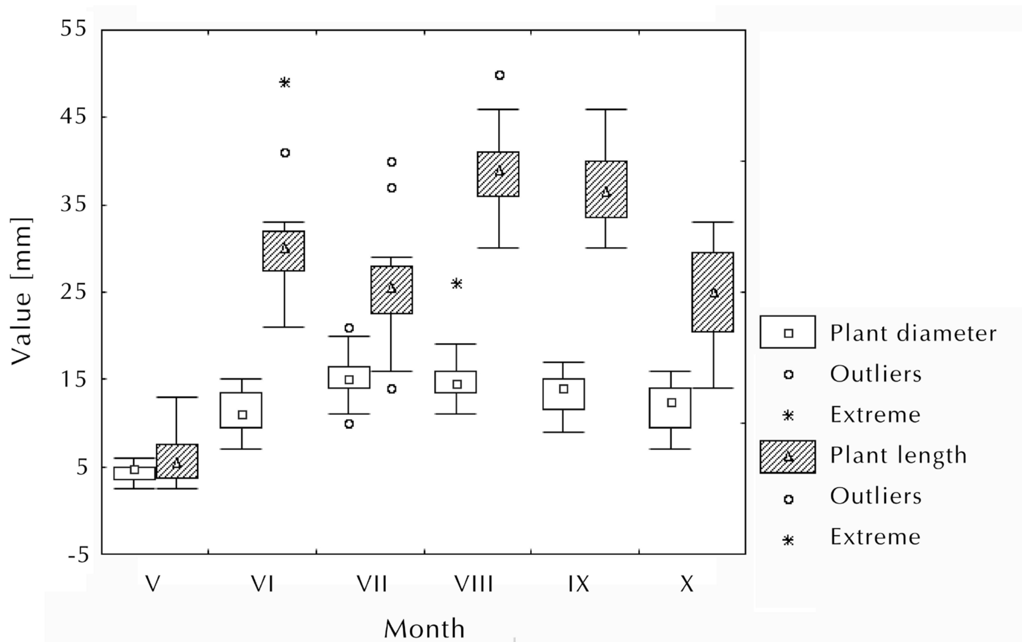

Fig. 3. Descriptive statistics for plant diameter and plant length of *Hydrocharis morsus-ranae*

The reproductive effort in each month is as follows: 100% of vegetative mass and 0.0% of generative mass in May; 100% of vegetative mass and 0.0% of generative mass in June; 100% of vegetative mass and 0.0% of generative mass in July; 98.7% of vegetative mass and 1.3% of generative mass in August; 97.8% of vegetative mass and 2.2% of generative mass in September; 98.5% of vegetative mass and 1.5% of generative mass in October. Average dry mass of fruit and turions was compared in time. The fruit and turions gain maximum mass in September (Fig. 4).

PCA analysis was carried out for the following features of specimens in the population: plant diameter, length, the number of buds, flowers, young fruit, ripe fruit, turions and leaves. The aim of the analysis was to establish which factor best explains the change-

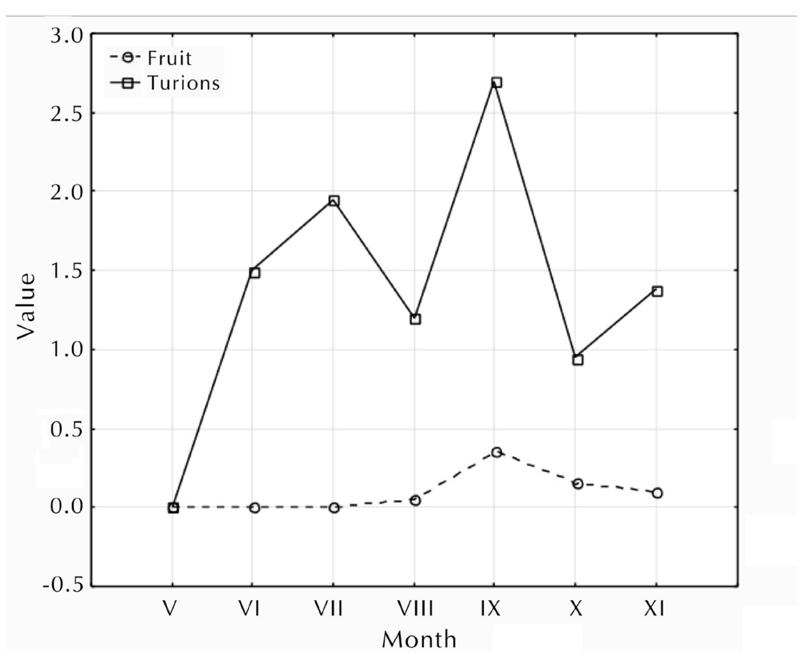

Fig. 4. Mean dry mass of fruit and turions of *Hydrocharis morsus-ranae* during the vegetative period

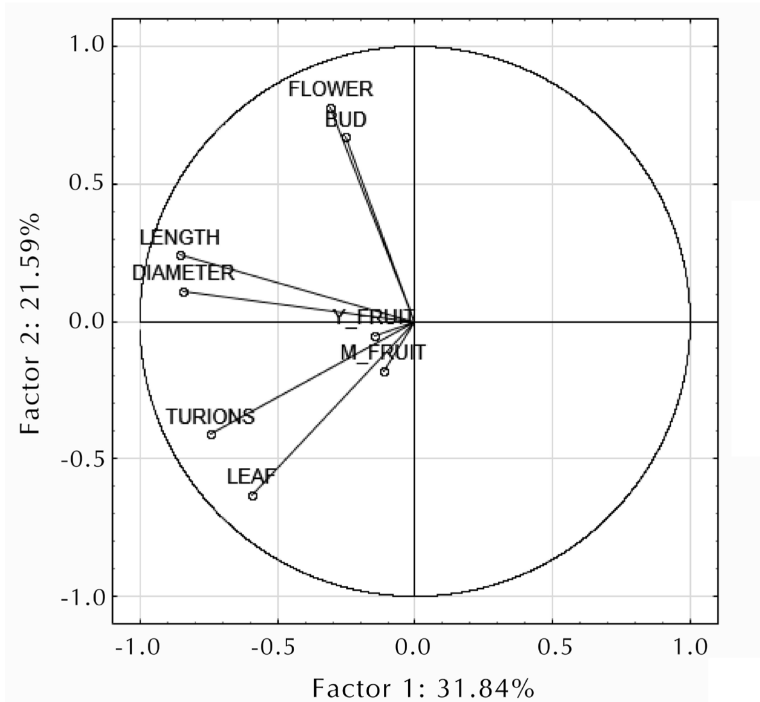

Fig. 5. PCA analysis for features of the *Hydrocharis morsus-ranae* population

ability of the *Hydrocharis morsus-ranae* population in time. The factors which best explain the changeability of the population in time are the length and the diameter of the specimens in the population (Fig. 5). The number of leaves is positively correlated with the number of turions, whereas the plant diameter is correlated with the plant length and the number of flowers is correlated with the number of buds.

Discussion

The data obtained on the biology of *Hydrocharis morsus-ranae* confirmed the membership of this species of the group of floating leaves and non-rooting aquatic plants (Szennikow 1952). *Hydrocharis morsus-ranae* is included in the pleustophyte group and hydrocharid subgroup – the plants float free at least during part of the vegetative period. They are equipped with leaves owing to which they are able to float on water (Hartog and Segal 1964; Segal 1970) and which also classifies them in the pleustophyte group and lemnid subgroup (Szmeja 2006). The research on Polish *Hydrocharis morsus-ranae* showed that the plants float free during a part of the vegetative period and are rooted in the muddy bottom during the other part of the vegetative period. The rooting of *Hydrocharis morsus-ranae* is connected with the accessibility of the roots to the muddy bottom and could be connected with the formation of a land form which is described by Arber (Arber 1972). To conclude, subsuming *Hydrocharis morsus-ranae* to the hydrocharid group seems more justified due to the semi-rooting observed in the specimens.

The two-level organization of the *Hydrocharis morsus-ranae* population was confirmed (Falińska 2002). The first level comprises the number of plants whereas the second level is the number of shoots (rooted structural units). Aquatic macrophytes are divided into two groups: 1. "living in motion" (pleustophytes) and 2. "remaining still" (rhizophytes) (Szmeja 2006). The population of *Hydrocharis morsus-ranae* shows characteristics of pleustophytes such as quick growth, short lifetime, and domination of vegetative progeny. However, the wintering form of endospores observed in *Hydrocharis morsus-ranae* is subsumed under the "remaining still" strategy.

The "living in motion" strategy is represented by the following species: *Lemna minor*, *Spirodela polyrhiza*, *Ceratophyllum demersum*. These species float on the water just beneath its surface. They can be rootless or can have roots submerged in water (Szmeja 2006). Examples of plants "remaining still" among the rhizoids are, for example, nympheids – plants with large and long leaves which float on water and act as floats which hold the shoot in an upright position as in *Trapa natans*, which quickly cover the surface of the water reservoir with rosettes; or as with *Stratiotes aloides* with two forms – an emergent form and a submerged form (Toma 2006) – and a varied production of turions and fruit in specimens of Poland and Finland (Toma 2012).

In the rhizophyte group there are magnopotamides – plants with long stiff or supple shoots usually fully submerged in water, e.g. *Hottonia palustris* (Szmeja 2006) or *Potamogeton crispus*, whose leaves are located on the water-air border (Toma 2002). The reproductive effort of *Potamogeton crispus* of fruit formation is 10% and turions 5.8% in Poland, whereas the reproductive effort of fruit formation is 2.04% and turions 7.1% in Finland (Toma 2005). The evergreen species from the isoetid group present a different strategy as they invest 30-50% of the dry mass of the specimen in the roots, e.g. *Lobelia dortmanna* (Szmeja 2006). The reproductive effort of *Hydrocharis morsus-ranae* of the generative organs is 2.2% and is comparable with the reproductive effort of creating generative parts in *Potamogeton crispus* in Finland but a lot less than in *Potamogeton crispus* in Poland. It has been ascertained on the basis of *Stratiotes aloides* that climatic and habitat factors have a great influence on the strategy of the species (Toma 2012).

Let's have a closer look at the characteristics of the plant selection labelled "r" or "K" (Pianka 1981). Such features of the *Hydrocharis morsus-ranae* population as quick growth of specimens, production of many small seeds and short lifetime support the thesis that *Hydrocharis morsus-ranae* represents "r" type organisms; on the other hand, developed vegetative parts, late reproduction stage and an expectancy curve type I support the thesis that *Hydrocharis morsus-ranae* belongs to "K" type organisms.

The modularity of the specimens in the *Hydrocharis morsus-ranae* population and a varied life cycle make it difficult to classify the plant as having a definite life strategy. However, using the triangular model of plant life strategy (Grime 1977) which includes primary strategies (C- competition, R- disturbance, S-stress) and mixed strategies (C-R, S-R, C-S, and C-S-R), we can establish the strategy for the *Hydrocharis morsus-ranae* population in the oxbow lakes of the River Vistula. The pattern obtained of shoots and specimen numbers in time but also their biomass point to the S-R strategy of *Hydrocharis morus-ranae*, which is indicative of plants adapted to low disturbance and extreme environments.

There are few studies concerning the generative reproduction of *Hydrocharis morsus-ranae* (Scribailo and Posluszny 1983). The diagnostic features of the fruit and seeds of *Hydrocharis morsus-ranae*, the development of fruit and ecological aspects of drying *Hydrocharis morsus-ranae* fruit and seeds have been described (Toma 2008). Drying the fruit and seeds of *Hydrocharis morsus-ranae* has a great influence on the outer epidermis of fruit and the outer surface of the seed coat, which is important for their diagnostics. The occurrence of tubureles on the surface of fresh *Hydrocharis morsus-ranae* seeds was confirmed (Scribailo 1984; Scribailo and Posluszny 1984; Scribailo and Posluszny 1985; Toma 2008). It was also confirmed that generative *Hydrocharis morsus-ranae* shoots with fruit bend down while ripening (Scribailo and Posluszny 1984).

In some studies, it was shown that dense floating mats of *Hydrocharis morsus-ranae* reduced growth of native submerged aquatic plants in Canada (Catling et al. 1988). *Hydrocharis morsus-ranae* is one of five invasive alien plants that have been reported to have a major impact on the natural ecosystem in Canada (Catling et al. 2003). *Hydrocharis morsus-ranae* was one of six primary invasive plants selected for initiation of a database project that would provide information for management (Haber 1995).

On the other hand, other sources on the influence of various species, i.a. *Hydrocharis morsus-ranae*, on the biodiversity of local flora near Ontario talk about its neutral influence. It does not appear that exotic species invade and competitively exclude native species to a greater degree than other native species do (Houlahan and Findlay 2004).

In Poland, *Hydrocharis morsus-ranae* occurs as a native species and does not pose a threat connected with invasion. In studies to date on important issues regarding the spreading of this invasive species in Canada, the scale of the threat, the species distribution and potential ways of prevention were described. In propagation of invasive species, it is important to examine the scale of vegetative and generative reproduction in time. In the ecological research on *Hydrocharis morsus-ranae* carried out in Poland, not only its ability to create polycormons, but also its ability to produce turions and fruit were studied. There have been a few reports about *Hydrocharis morsus-ranae* sprouting in natural conditions in Romania (Serbanescu-Jitariu 1972). The first report about sprouting of this species' turions was given by Terras (1900). The details on *Hydrocharis morsus-ranae* propagation in Canada were given by Catling et al. (2003) and the propagation in Eurasia was described by Cook and Luond (1982). *Hydrocharis morsus-ranae* is a taxon clearly adopted to a wide range of climatic conditions (Catling et al. 2003).

The authors give a different number of seeds in *Hydrocharis morsus-ranae fruit*. On average, there are 26-42 seeds in one fruit, 74 maximum (Scribailo and Posluszny 1985; Preston and March 1996); 15-20 seeds in one fruit in low density mats of *Hydrocharis morsus-ranae* and 55-60 in one fruit in higher density mats (Burnham 1998).

The production of *Hydrocharis morsus-ranae* seeds in Rondeau Park in Ontario is 250 per square metre (Scribailo and Posluszny 1984). The production of *Hydrocharis morsus-ranae* seeds in Poland is 4000 per square metre. Burnham (1998) obtained similar data of 3000 seeds per square metre in Lake Opinicon.

To this day, there has been a lack of investigations of reproduction in *Hydrocharis morsus-ranae* in Poland. The research may be useful for comparison of the reproduction of this species with other countries where the plant is invasive. Seed germination of *Hydrocharis morsus ranae* in natural conditions gives valuable information about the rate of colonization of this species in water recreational areas.

Conclusion

Reproduction of *Hydrocharis morsus-ranae* on the River Vistula oxbow lakes are characterized by high growth, high density and high production of seeds and turions. Fruiting of *Hydrocharis morsus-ranae* in Poland is higher than in Canada, where it is an invasive taxon. *Hydrocharis morsus-ranae* is well adapted to the environment in oxbow lakes of the River Vistula and represents the S-R strategy.

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