THE DEMOGRAPHIC SUCCESS OF *MARSILEA QUADRIFOLIA* L. IN A MAN-MADE WATER BODY FROM DANUBE DELTA BIOSPHERE RESERVATION

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ABSTRACT

Marsilea quadrifolia L. (water clover) is a unique species from Romanian flora that belongs to the Marsileaceae family and is one of the most vulnerable plants in Europe due to the loss of its habitats. In this paper, the demographic evolution of a new *M. quadrifolia* population in the Danube Delta Biosphere Reserve and its natural and anthropic threats are reported. The aquatic fern, *M. quadrifolia,* has colonized a suitable man-made water body. After a decade of monitoring, the demographic trend is positive but the support capacity of habitat for the water clover remains limited in the long term. The population is vulnerable due to succession of vegetation and its vicinity with a human settlement. The establishment of a micro-reserve represents a suitable method to protect and maintain this threatened *M. quadrifolia* population.

ZUSAMMENFASSUNG: Der Ansiedlungs- und Ausbreitungserfolg von *Marsilea quadrifolia* L. in einem anthropogenen Lebensraum des Biosphärenreservats Donaudelta.

Marsilea quadrifolia L. ist die einzige Art der Familie Marsileaceae, die in der Flora Rumäniens vorkommt und aufgrund der Zerstörung ihrer natürlichen, charakteristischen Lebensräume zu den europaweit am stärksten gefährdeten Pflanzenarten gehört. In der vorliegenden Arbeit wird über den demografischen Erfolg einer Population von *M. quadrifolia* berichet, im Biosphärenreservat Donaudelta identifiziert wurde. Obwohl die demografische Entwicklung positiv ist, der langfristige Bestand des Lebensraumes begrenzt ist. Die Dauerhaftigkeit dieser Population wird sowohl von natürlichen Faktoren und anthropogenen Faktoren gefährdet. Die Einrichtung eines Mikroreservates ist eine mögliche praktische Lösung, die für den Schutz dieser Population von *M. quadrifolia* vorgeschlagen wurde.

REZUMAT: Succesul demografic al speciei *Marsilea quadrifolia* L. într-un habitat antropic din Rezervaţia Biosferei Delta Dunării.

Marsilea quadrifolia L. (trifoi cu patru foi) este singura specie de ferigă din familia Marsileacea care este prezentă în flora României și una dintre speciile de plante vulnerabile. În această lucrare este raportat succesul demografic al unei populații de *M. quadrifolia* care a colonizat un habitat acvatic antropic din Rezervația Biosferei Delta Dunării. După un deceniu de monitorizare, trendul demografic este pozitiv, dar capacitatea de suport a habitatului pe termen lung este limitată. Persistența populației este amenințată deopotrivă de factorii naturali şi de cei antropici. Crearea unei micro-rezervaţii poate fi soluția practică pentru protecția şi conservarea acestei populaţii vulnerabile de *M. quadrifolia*.

INTRODUCTION

Marsilea quadrifolia L. (known by the vernacular names “four-leaf clovers”, “water clover”, or “water shamrock”) is an aquatic and amphibious pteridophyte which occurs especially in standing shallow waters. This heterosporous and floating-leaved fern is considered the lectotype of the generic name (Grolle, 1988; Johnson, 1988; Iamonico, 2012). The oldest genus of Marsileaceae family (Lupia et al., 2000), has about 52 extant species worldwide, and is prevalent in intertropical regions (Lin and Johnson, 2013).

In Europe, only four species of *Marsilea* genus occur: *M. quadrifolia* L., *M. strigosa* Willd., *M. batardae* Launert, and *M. aegyptica* Willd., the latter is only found in the Lower Volga region (Crabbe and Akeroyd, 1993). Due to the habitat destruction as a consequence of increased human activities, all the other *Marsilea* species have become rare, vulnerable, endangered or extinct in the wild. Therefore, in the European Union they all are protected and are being nominated as species of community interest whose conservation requires the designation of special areas of conservation.

According to the map of European distribution of *M. quadrifolia* species (Jalas and Suominen, 1972), this specific fern is prevalent between 45º and 50º north latitude. Consequently, the native range corresponds to the lowlands and flood plains of the main rivers, and hygrophilous grasslands of Europe. In the past, this plant has been quite common in the area of its distribution from Western Europe; the historical floras called it a “pig pasture weed” (Poschlod et al., 2005).

At the end of the twentieth century, the studied species became a rare taxa in its natural European range (Bruni et al., 2013; Estrelles et al., 2001; Godreau et al., 1999; Lozano et al., 1996). The declining trend related to the significant loss of natural habitats, especially due to river courses affected by human induced changes such as: channelization and embankment, drainage of floodplains and others wetlands, high fertilization of the agricultural lands, and changes of agricultural practice (Schneider-Binder, 2014; Godreau et al., 1999). Furthermore, the lack of any long-distance dispersal mechanism limits its dissemination in other possible and suitable habitats, despite the fact that it has the ability to colonize new habitats (Burk et al., 1976), and its pioneer capabilities that were observed in disturbed habitats are a result of the capacity for vegetative reproduction (Dallai et al., 2010). On the other hand, because it is a light loving species, along to the ecological succession it is expected to be eliminated by the higher and faster growing plant species.

New sites with *M. quadrifolia* in natural habitats from its known European natural range (Conrad, 2005; Hulina, 1998; Pistoja et al., 2006), and new populations, spontaneously colonized in former sites where it was mentioned, were extinct previously (Pistoja et al., 2006), or in some man-made wetlands that have replaced abandoned farmlands located outside of its natural range (Bremer, 2003; Bremer, 2007; Drok and Weeda, 1999), have been reported in the last years.

The habitats of this pteridophyte are wetlands with heavy clay to sandy substrates. It grows mainly in shallow permanent lakes and at the edges of ponds, as well as in small rivers with slower water flow and lower force. Man-made water bodies (ditches, rice fields) are colonized (Schneider-Binder, 2014). As a hydrophyte, *Marsilea quadrifolia* prefers mesotrophic and eutrophic waters (Lin and Johnson, 2013). Thus *M. quadrifolia* has the ability to remove nitrite from contaminated water (Rawat et al., 2009), that it is beneficial for nutrient mitigation from the fresh water lake and for wetland restoration (Khan and Manzoor, 2010). It occurs also in conditions of light to medium salinity (Schneider-Binder, 2014). Due to the fact that it is sensitive to aquatic environmental pollutant exposures, this fern has been chosen for ecotoxicogenomics research (Miranda et al., 2014; Snape et al., 2004).

According to the Braun-Blanquet approach, this fern occurs in communities that belong to Isoëto – Littorelletea (BR. – Bl. Et. Vlieger 1937), class of vegetation (Mucina, 1997), and recently, in a new conquered habitat, it was found in Eleocharito acicularis – Limoselletum (Malcuit, 1929), association (Bremer, 2013). A review of plant communities where *M. quadrifolia* was found has been made by Schneider-Binder (2014).

Due to populations' decline throughout Western Europe, the protection and conservation of *Marsilea quadrifolia* in European Union states (UE) has started since 1979, when the Bern Convention on the Conservation of European Wildlife and Natural Habitats has been ratified. In the European Habitats Directive (Council Directive 92/43/EEC, 1992), it is listed as a strictly protected species (Council of Europe, 2010), and its habitat which requires protection and conservation is encoded 3130 – "oligotrophic to mesotrophic standing waters with vegetation of Littorelletea uniflorae and/or Isoëto-Nanojuncetea". For this taxa there have been 94 Natura 2000 sites designed in all UE member states.

Also, this taxa is legally protected in non UE countries such as Albania (Kashta, 2007; Xhulaj and Mullaj, 2002), Bosnia and Herzegovina (Schneider-Binder, 2014), Montenegro (Radovic et al., 2008), and Ukraina (Witkowski et al., 2003).

In Romania, *Marsilea quadrifolia* has been a protected species since 1993, after the Government ratified Berne Convention (Law 13/March 11/1993). It is listed as an endangered species on the national red list of plant species (Oltean et al., 1994), but it is not mentioned in the most recent published Red Book of Romanian flora (Dihoru and Negrean, 2009).

In the man-made habitats (rice fields) from South Asia, water clover is a noxious and undesirable weed (Chang, 1970; Luo and Ikeda, 2007; Satapathy and Singh, 1985), difficult to control because it is tolerant to most of the grass killing herbicides used (Kathiresan, 2006), and the anthropogenic habitats (especially drain ditches) from Europe are temporary shelters rather than mainstays, hence the plant can spread further (Nowak and Nowak, 2006). Additionally, the anthropogenic habitats could be a suitable refuge for the plant because they are analogous habitats, broadly similar to natural ones.

The return of *Marsilea quadrifolia* in sites where formerly it has been declared extinct could be interpreted, by the conservation in those sites of sporocarps, as highly resistant to dryness which can "germinate" even after long periods of time of dormancy if the proper environments are re-established (Nagalingum et al., 2006).

Although there is no overwhelming evidence, it is believed that ducks and other waterfowl unintentionally disseminate this species between different bodies of water (Johnson, 1986). The major and frequent flooding of meadows substantially increase the chances of dissemination of sporocarps and rhizome fragments from a site to other suitable habitats.

The objective of this paper is to present an assessment of the ecological success of *Marsilea quadrifolia* population which was found in a drainage channel (Fig. 2) from the Danube Delta in 2004. The possible circumstances of its occurrence in this place are discussed; an introduction is deliberately excluded. The main threats of this new *M. quadrifolia* population from the Danube Delta are assessed due to a very close vicinity of its conquered habitat with a human settlement.

MATERIAL AND METHODS

In order to establish the occurrence and distribution of *Marsilea quadrifolia* in the Danube Delta, the national flora literature was surveyed (Ciocârlan, 1990, 2000; Ţopa, 1952; Dihoru and Negrean, 1976; Sârbu et al., 2013). The distribution of *M. quadrifolia* in the Danube Delta was mapped on 10 x 10 km squares in the UTM grid system, according to bio-cartographic code from Romania (Lehrer, 1977).

The demographical success of the population (λ) was estimated using the evolution of cover area over the 2004-2014 period, based on following formula:

$$\log \lambda \sim [(\log (1 + popsize_{actual})) - (\log (1 + popsize_{initial}))]/year \quad (1),$$

here $popsize_{actual}$ is the population size in 2014, $popsize_{initial}$ is the initial size when it was discovered (in 2004), and year represents the number of years since it was discovered. The population size was estimated as surface cover (m^2).

The nomenclature of plants taxa associated with *Marsilea quadrifolia* is in accordance to Ciocârlan (2000), Sârbu et al. (2013), and Tutin et al. (1993).

Possible ways of colonization of the site from Sfântu Gheorghe were analysed based on information published in relation with the species. The main threats were assessed due to the proximity of a human settlement.

RESULTS

The chorology of *Marsilea quadrifolia* L. in the Danube Delta Biosphere Reserve

For Romanian flora, *Marsilea quadrifolia* is the sole member of the Marsileaceae family (Ciocârlan, 2000; Ţopa, 1952; Sârbu et al., 2013). According to Ţopa (1952), until the middle of 20th century *Marsilea quadrifolia* had not been reported in the Danube Delta although, theoretically at least, in this large wetland there are suitable habitats. Subsequently, it was reported at Sulina (Ciocârlan, 1990; Dihoru and Negrean, 1976; Sârbu et al., 2006), Gârla Magearu, and Canal Rusca (Fig. 1), the last site being estimated with "the largest population of *Marsilea quadrifolia* in the Danube Delta" (Sârbu 2007, Sârbu et al., 2006). Schneider-Binder (2014) mentioned *Marsilea quadrifolia* at Sfântul Gheorghe in 2011. Another two sites are reported in the Ukrainean part of the Danube Delta (Klokow and Dyachenko, 1988).

Study area, habitat description, and population estimation

During floristic investigations at Sărăturile beach ridge in the Danube Delta, a very small population of *Marsilea quadrifolia* was discovered in July of 2004 in the eastern part of Sfântul Gheorghe Village, very close to the mouth of the Sfântul Gheorghe distributary. The geographic coordinates of this population are: 44º53'26" N, 29º36'18" E, UTM grid QK 07.

Sfântul Gheorghe Village is located in the south of Sărăturile beach ridges plain, known as "Grindul Sărăturile", at the mouth of the Sfântul Gheorghe arm of the Danube River. The climate of this area is temperate continental. In the first decade of XXI century the mean annual temperature was 11.3ºC, with 0.3ºC more than average of the 1896-1960 period and the mean annual rainfall amount decreased from 363 mm to 274 mm. According to the Martonne index of the aridity value, the climate is close to semi-arid type (Strat, 2010).

A small patch of *Marsilea quadrifolia* was found in 2004 at the edge of a heavily clogged and muddy drain channel, exposed to full sunlight, and with an extremely favourable light thermal regime. This channel is part of the network of channels and ditches built around Sfântul Gheorghe Village to drain excess moisture and temporary stagnant waters to the Sfântul Gheorghe distributary due to the shallow water table that is near the topographic surface. The channel waters flow very slowly and have periods of stagnation. The water level in the channel is the highest in spring and the lowest at summer's end, in close connection with the climate area, Danube level oscillations, and water table oscillation. Also, the drainage

channel has an asymmetrical cross-section, with a steep and high bank on one side and a low, flat bank, on the other. Thus, water level changes are more visible on the right bank where the area of shallow waters becomes larger during floods. Because of that, on the right side of the channel, *Marsilea quadrifolia* is adapted to amphibious conditions and there are two forms: *Marsilea quadrifolia* L. f. *natans* Kaulf. and *Marsilea quadrifolia* L. f. *terrestris* Hayek.

The channel, which has not been dredged in the last 25 years, is heavily silted and supports an aquatic flora composed of native emergent, floating leaf plants, and submerged macrophytes species (such as *Ranunculus aquatilis* L., *Cerathophyllum demersum* L., *Myriophyllum spicatum* L., *Potamogeton pectinatus* L., *Potamogeton perfoliatus* L.) in the deeper part.

The total number of extant individuals of *M. quadrifolia* population was difficult to estimate due to the clonal nature of species, but the total area initially covered by fern when it was discovered in 2004 was less than 0.5 m^2.

The initial population was marginally accompanied by *Nymphoides peltata* (S. G. Gmel.), Kuntze, *Ranunculus aquatilis* L., *Butomus umbellatus* L., *Mentha aquatica* L., *Rumex hydropalathum* Huds., *Glyceria fluitans* L., *Ceratophyllum submersum* L.

In the subsequent years, *Marsilea quadrifolia* has migrated downstream the channel and initial nucleus according to shallow water gradient. Therefore, the area occupied by it was estimated to be around 200 m² in September 2014, both *natans* and *terrestris* forms being present. Thus, the calculated value of demographical success (log λ) is 0.45.

The fern was found in a few monotypic stands whereas in the rest of the surface individuals were spread in scattered clumps. It has not been determined if the adjacent individuals are clonally or sexually established, but if in the first field works, *Marsilea quadrifolia* was found in aquatic conditions with floating leaves; since 2009 it has also been found in terrestrial form on the right bank of the channel. In aquatic environments it was accompanied by the following enrooted aquatic plants: *Hydrocharis morsus-ranae* L., *Lemna minor*, *Lemna trisulca* and two other ferns – *Salvinia natans* and *Azolla filiculoides*, the last one being an invasive species in the Danube Delta which threaten the communities of *Marsilea quadrifolia* (Anastasiu and Negrean, 2006). In the littoral zone of the channel, the following rooted plants were identified: *Butomus umbellatus* L., *Sparganium erectum* L., *Cicuta virosa* L., *Alisma plantago-aquatica* L., *Mentha aquatica* L., *Schoenoplectus littoralis* (Schrader) Palla, *Typha angustifolia* L., and *Phragmites australis* (Cav.) Trin. ex Steud., *Juncus maritimus* Lam.

DISCUSSION

It is difficult to determine the circumstances and the time when *Marsilea quadrifolia* reached the Sfântul Gheorghe site of the Danube Delta. On the other hand, because the initial *Marsilea quadrifolia* population was very small and then it increased, this fact could explain a recent colonization of the new habitat. Because the value of log λ exceeds zero, this fact could be assessed as a success of population establishing, which means that the conquered habitat – the heavily silted drain channel – ecologically is very similar to the natural ones. This success of colonization could be taken into consideration if the Romanian authorities will decide the appropriateness of reintroduction programs of this rare fern species in the wild just as in other European countries (Estrelles et al., 2001; Nöel et al., 2011). But because the calculated value of log λ is less than one (only 0.45), the habitat has no long-term support capacity for plant population growth and survival. If any human intervention is excluded, ecological succession (transformation of habitat in a reed marsh) is the main threat.

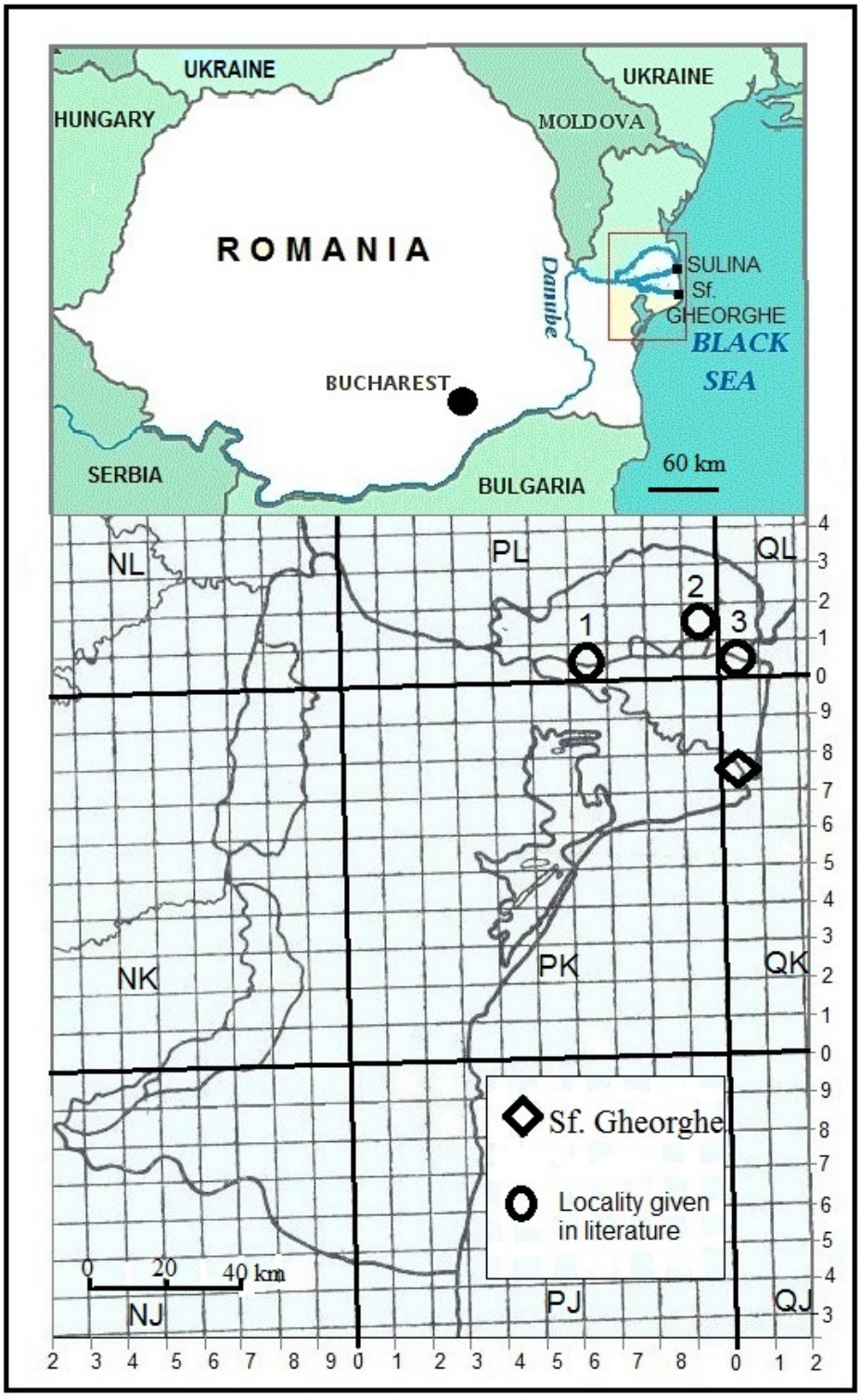

Figure 1: Distribution of *Marsilea quadrifolia* in the Danube Delta, Romania; 1 – Channel Rusca; 2 – Gârla Magearu; 3 – Sulina.

Figure 2: The drainage channel with a dense stand of *Marsilea quadrifolia* in the foreground.

With respect to the origin of this *Marsilea quadrifolia* population, it is difficult to determine the source and vector of propagules. Many Marsileaceous ferns, *M. quadrifolia*, can grow from sporocarps and rhizome fragments. The methods of dispersal of *Marsilea quadrifolia* are not clearly documented, although other species are dispersed by waterfowls (Serviss and Peck, 2008). The reproductive biology of Marsileaceous ferns was described (Johnson, 1986; Schneider and Pryer, 2002).

According to Johnson (1985, 1986), the dispersal of *Marsilea* ferns differs from most ferns because they use biotic rather than abiotic agents, but the wind dispersal is excluded because of the size and weight of sporocarps and because these reproductive structures are submerged. Whereas the entire process of reproduction exclusively occurs in the water and spores are released from sporocarps only in the water. If they previously went through a process of scarification, we presume that there are two plausible possibilities: hydrochory and zoochory. It is not therefore excluded that, during the floods, the disseminules (sporocarps or rhizomes fragments) derived from other *Marsilea quadrifolia* populations of the Danube drainage basin to reach a drainage channel, thus leading to the establishment of a new population.

Also, it is not unreasonable to suppose that the migratory water birds could be responsible for the presence of the water clover in the new site, especially if studies show that the water birds are not only potentially important as agents of long distance dispersal and of colonization of new habitats (Guppy, 1906; Carlquist, 1967), but also as agents of dispersal at a local scale (Figuerola and Green, 2002; Green et al., 2002; Green et al., 2008).

However, endozoochory by waterfowl seems to be a much more important mode of dispersal for aquatic plants, including *Marsilea* ferns, than exozoochory (De Vlaming and Proctor, 1968; Brochet et al., 2010), although there are opinions according to which, this means of transportations of propagules is slightly less efficient when very great distances are involved (Carlquist, 1967).

Considering the Danube Delta is one of the largest wetlands in Europe (with high biodiversity of brooding migratory and sedentary waterfowl, and non-brooding waterfowl in winter) propagules could have been brought from anywhere to the Sfântul Gheorghe site, closer or farther away from this new site. Hypothetically, the nearest potential source is the *Marsilea* population from Sulina, located 30 km north from Sfântul Gheorghe. This possibility is in accordance with the maximum dispersal distance calculated for *M. quadrifolia* for a fixed time period (30 years), if there is climatic suitability and the landscape connectivity increases (Alagador et al., 2011). Thus, the short distance movements of water birds over the Danube Delta could be responsible for dispersal of *Marsilea quadrifolia* propagules.

The assumption concerning the genetic distance between these two *Marsilea quadrifolia* populations, and if there was an eventual gene flow, can be demonstrated only using genetic studies, in the same way as it was done for the *Marsilea strigosa* (Vitalis et al., 2002). However, after all circumstantial suppositions, the following question arises: if the water birds are agents of dispersal of the *M. quadrifolia* and if the Danube Delta is a suitable habitat for it, why are there just several populations in this large and well preserved natural wetland?

For the newly occurred *Marsilea quadrifolia* species population, there are no managed activities in order to protect and preserve it. Besides, within the Danube Delta Biosphere Reserve, its habitat is not located in a strictly protected area; on the contrary it is a zone with economic activities, right next to a human settlement. Consequently, this interesting new established population is highly threatened by the local human activities. The main threat is its specific habitat destruction by mechanical dredging of the channel in order to keep its actual function. Traditional practices of grazing cattle and poultry are other threats. Although cattle did not appear to feed on *Marsilea quadrifolia*, the trampling could cause permanent damage to individual plants, especially for terrestrial form that has only clonal reproduction. But on the other hand, cattle trampling can increase the chances of dissemination of sporocarps and rhizome fragments attached to the hooves from a site to other suitable habitats from this area.

To protect the habitat and to stabilize the currently extant population, the immediate actions are required: develop a management plan for the Sfântul Gheorghe site, and monitor/control all types of socio-economic activities, such as development projects that could affect the site of population. Currently, there is no known development projects planned that would affect the *Marsilea quadrifolia* population, but this area may be considered for development in the future, especially for touristic activities. Hence, in perfect accordance with national legislation and local circumstances, the authorities need to find the adequate solutions to protect this fern species of European interest.

A plant micro-reserve might represent an excellent measure for the in situ conservation, especially the idea of creating small protected areas for singular plant species, which initially started in Spain, is successfully spreading in other European countries (Laguna, 2001; Laguna et al., 2013).

CONCLUSIONS

This paper provides basic information about a new site with *M. quadrifolia* in the Danube Delta Biosphere Reserve, Romania. This threatened fern has colonized the end part of a heavily silted drainage channel. The surface colonized by *M. quadrifolia* had increased in size which means a success of the population occurred and a positive trend for it, but the habitat is not suitable in a long-term view.

The threats of this population include relatively small population size, ecological succession, and human activities which may lead to habitat destruction. Mechanical dredging works of the drainage channel is a major risk.

A possible successful measure to preserve this vulnerable *M. quadrifolia* population could be setting up of a plant micro-reserve.

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