Aquatic Botany 119 (2014) 91–99

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Re-establishment of an extinct population of the endangered aquatic plant *Potamogeton coloratus*

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ARTICLE INFO

Article history:
Received 29 January 2014
Received in revised form 13 May 2014
Accepted 12 August 2014
Available online 21 August 2014

Keywords:
Aquatic plant
Endangered species
Germination
Population restoration
Protective management
Wetland habitats

ABSTRACT

Persistent soil seed banks play a key role in preventing extirpation of plants during periods of temporarily unsuitable conditions. They can serve as effective tools in achieving the goal of restoring wetland vegetation. *Potamogeton coloratus*, an aquatic plant of shallow, calcareous waters, has become critically endangered or vanished in large regions of its mainly European range. In the Czech Republic, it was observed in 1977 for the last time (prior to our restoration project) and has been classified as an extinct species. We successfully re-established one population of it, and also established a new one, both located in a fen complex from which it has been absent for more than 30 years. One population was restored in a desilted pool in which the species had occurred previously; the other was established in a newly created pool by means of transferring soil, including the seed bank, from a pool in which the species formerly occurred. Monitoring of these sites over subsequent years confirmed establishment and increase of the populations. Along with the target species, other rare, native aquatic plants (e.g. *Utricularia vulgaris* and *Chara hispida*) were found growing in the newly created pool. Propagules of these species were also detected in the sediment collected in the old pool prior the desilting and subjected to soil seed bank analysis. Although altogether 26 plant species were identified in the soil seed bank, no propagules of *P. coloratus* were detected, most likely due to their small number in the sediment analyzed.

1. Introduction

For many vascular plants as well as multicellular algae, persistent soil propagule banks (comprising seeds or spores, depending upon the taxa) play a key role in preventing extirpation during periods of temporarily unsuitable conditions (Baskin and Baskin, 1998; Bonis and Grillas, 2002; Fenner and Thompson, 2005). A high percentage of such species is poor competitors, and therefore require habitats having conditions (e.g. high salt concentrations or lack of nutrients) or disturbance regimes (e.g. flooding and subsequent water drawdown, grazing) that block succession by other species (Keddy, 2007, 2010). Many aquatic macrophytes fit this paradigm, occurring in habitats characterized by fast and unpredictable environmental change. Although some species cope with this challenge by means of easily dispersed propagules, species with heavy seeds and/or fruits without specific dispersal adaptations typically rely on surviving several or even dozens of years as seeds buried in the bottom sediment (Baskin and Baskin, 1998). Soil seed banks are also of importance even for easily dispersible species that are habitat specialists, as suitable sites can be too distant to reach. Wetland species that are thought to be able to survive at least several years in seed banks include *Carex bohemica*, *Crassula aquatica*, *Elatine* spp., *Juncus bufonius*, *Littorella uniflora*, *Nymphoides peltata*, *Potamogeton* spp., *Pulegium vulgare*, *Samolus valerandi* and many others (Smits et al., 1990; Thompson et al., 1997; Bekker et al., 1999; Poschlod et al., 1999; Pott and Remy, 2000; Šumberová et al., 2012a).

For many aquatic macrophytes, considerable population declines have occurred throughout much or all of their ranges as a consequence of global environmental threats including progressive eutrophication, large-scale land use changes, climate change and related to these changes overall habitat loss (e.g. Sand-Jensen et al., 2000; Kozlowski et al., 2008). One of the possible approaches in preventing the decline or even extirpation of ecologically

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http://dx.doi.org/10.1016/j.aquabot.2014.08.005
0304-3770/

specialized plant species and their communities is habitat restoration (e.g. Brouwer et al., 2002; Klimkowska et al., 2010). Soil propagule banks can serve as effective tools in achieving the goal of restoring wetland flora (although in some circumstances restoration success is more dependent on propagule dispersal from the surroundings than on the bank in the soil, see e.g. Bakker et al., 1996; Brown, 1998). It can be especially important for extremely rare species that are extinct over large areas, with soil propagule bank activation providing the last realistic possibility for population recovery. Soil propagule banks can be used both for restoration in situ (Brouwer et al., 2002; Combroux et al., 2002; Nishihiro et al., 2009), by exposing the necessary sediment layers, or they can be transported to recolonize other suitable sites (Brown and Bedford, 1997).

Lowland mires and fens are among the most threatened habitats in many parts of Europe (Council of Europe, Directorate of Culture and Cultural and Natural Heritage, 2011). Most of them have been converted to valuable arable land (Pfadenhauer and Grootjans, 1999; Klimkowska et al., 2010; Kaplan, 2012). Their remnants often suffer from a water deficit due to drainage, as well as from water uptake, eutrophication, and ruderalisation. Phosphorus-based eutrophication caused mainly by intensive agriculture (Withers and Haygarth, 2007) contributes to considerable vegetation changes of the lowland fen habitats (Lucassen et al., 2005). The fen meadow complexes in the Middle Labe River area (where also our study site is located), Czech Republic, are illustrative of this situation. Species-rich complexes of fen meadows, reeds and shallow pools were largely replaced by fields or reed stands hosting only several common wetland species. Many endangered fen species vanished from this area, including some Czech endemics (Kaplan, 2012). The pools either disappeared due to terrestrialisation or were heavily eutrophied (especially pools in fen complexes' marginal zones neighbouring arable fields). Associated with these changes, *Potamogeton coloratus*, a characteristic species that originally formed large stands in shallow calcareous waters in the Hrabanovská Černava fen, disappeared subsequent to 1977, with this representing the last occurrence (prior to this study's restoration efforts) of this species in the Czech Republic.

As the restoration measure involving creation of small and shallow water body was planned for Hrabanovská Černava in autumn 2007, we decided to use this opportunity and try to re-establish a population of *P. coloratus*. Our initial hypothesis was that its viable soil seed bank might still exist in the pool where it was last observed. The present study had the following aims: (1) to test if the sediment with potential occurrence of soil seed bank can be used for re-establishment of a population of *P. coloratus* more than 30 years after the species vanished; (2) to monitor the vegetation development in order to suggest a management approach that would promote persistence of re-established *P. coloratus* and other rare aquatic and wetland species at this site; and (3) to compare the pre- and post-restoration floras with the species composition of propagules detected in the soil seed bank in order to gain insight into the abilities of populations of the seed bank species' to persist after habitat restoration.

2. Materials and methods

2.1. Target species

P. coloratus Hornem. is an aquatic species with its centre of distribution in western and south-western Europe, and rare occurrence in adjacent parts of central and south-eastern Europe and southern Scandinavia; outside of Europe it has been recorded in only a few localities in south-western Asia and northernmost Africa (Hultén and Fries, 1986; Wiegleb and Kaplan, 1998; Kaplan, 2010). It occurs in shallow, clear, base-rich, usually nutrient-poor waters, preferably in pools, runnels and drainage ditches in calcareous fens, in small rivers, and less frequently on margins of calcareous lakes and ponds (Preston, 1995; Buchwald et al., 1995; Hollingsworth et al., 1995; Gornall et al., 1998; Kaplan, 2010). It is an anemophilous, self-compatible and thus often autogamous perennial that typically sets copious seed, produced in 1.3–1.9 mm long fruits arranged in spikes (Wiegleb and Kaplan, 1998; Kaplan, 2010), but can also spread by rhizomatous growth (Hollingsworth et al., 1995) and vegetative fragments (Barrat-Segretain et al., 1999). Thanks to the extensive range of phenotypic plasticity known in many species of *Potamogeton* (Kaplan, 2002), it produces carpets of low-growing short stems in shallow waters but elongate stems up to about 1 m long in deep waters. *P. coloratus* is able to grow in both disturbed and undisturbed habitats (Trémolières, 2004). However, due to its slow growth, and thus low ability to compensate quickly (i.e. within one growing season) the biomass loss after a disturbance event, it should have its ecological optimum in habitats with low disturbance frequency and intensity (Mony et al., 2011). As a poor competitor it appears in early stages of succession of shallow wetlands (e.g. after an extensive disturbance) and disappears again in later successional stages predominated by dense marshland vegetation (Buchwald et al., 1995; Bruin, 1997). Some authors consider it to be highly ammonium sensitive (e.g. Trémolières, 2004) but according to Schneider and Melzer (2004) the increased phosphorus amount in water is a much more important factor restricting the occurrence of *P. coloratus*.

Because of the loss and degradation of suitable habitats throughout its range, *P. coloratus* has become seriously endangered in many countries (e.g. Roweck et al., 1986; Buchwald et al., 1995; Kleinsteuber and Wolff, 1996; Ludwig and Schnittler, 1996; Moser et al., 2002; Bauer, 2006). In the Czech Republic, it was recorded as early as the beginning of the 19th century but in spite of the long and intensive floristic research in this country (see Krahulec, 2012) only two other localities were discovered later (Kaplan, 2010). All three sites (Brandýs nad Labem, Čelákovice, Hrabanovská Černava) were of a relict character and no spread of *P. coloratus* to new localities has ever been observed, which is in accordance with the position of these sites on the edge of the distribution range of the species. At two of the localities (Brandýs nad Labem and Čelákovice) *P. coloratus* disappeared as a result of habitat changes long ago (in the first half of 19th and 20th century, respectively), only at the third one (Hrabanovská Černava) it persisted up to 1977 when it was recorded for the last time (Kaplan, 2010). Since then, it has not been found there despite regular targeted searching and intensive monitoring of the locality (Husáková, 2005; Husáková et al., 1988; Kaplan, 2010). Consequently, the species was classified as extinct in the Czech Republic (Holub et al., 1979; Holub, 2000; Holub and Procházka, 2000; Procházka, 2001) and only recently it was transferred among critically threatened taxa (Danihelka et al., 2012; Grulich, 2012) in consequence of the population restoration described in this paper.

2.2. Study site

Hrabanovská Černava National Nature Reserve (Fig. 1) is located near Lysá nad Labem, central Bohemia, Czech Republic. The centre of the reserve is at 50°12′58″ N, 14°49′55″ E (WGS 84). This locality has been known since the 19th century as one of the best-preserved species-rich calcareous fen complexes in Bohemia (e.g. Sitenský, 1891; Klečka, 1930; Husáková et al., 1988). Fossil records from this site indicate continuous occurrence of fen vegetation from the Late Glacial through the entire Holocene, i.e. for more than 12,000 years (Pokorný et al., 2010). Unfortunately, since 1930s the area has been used as a water source, and subsequent drastic drainage of the area in 1967–1971 caused changes in the water regime and disappearance of several fen species. Because the drainage system has become less effective over time, the level of ground water has

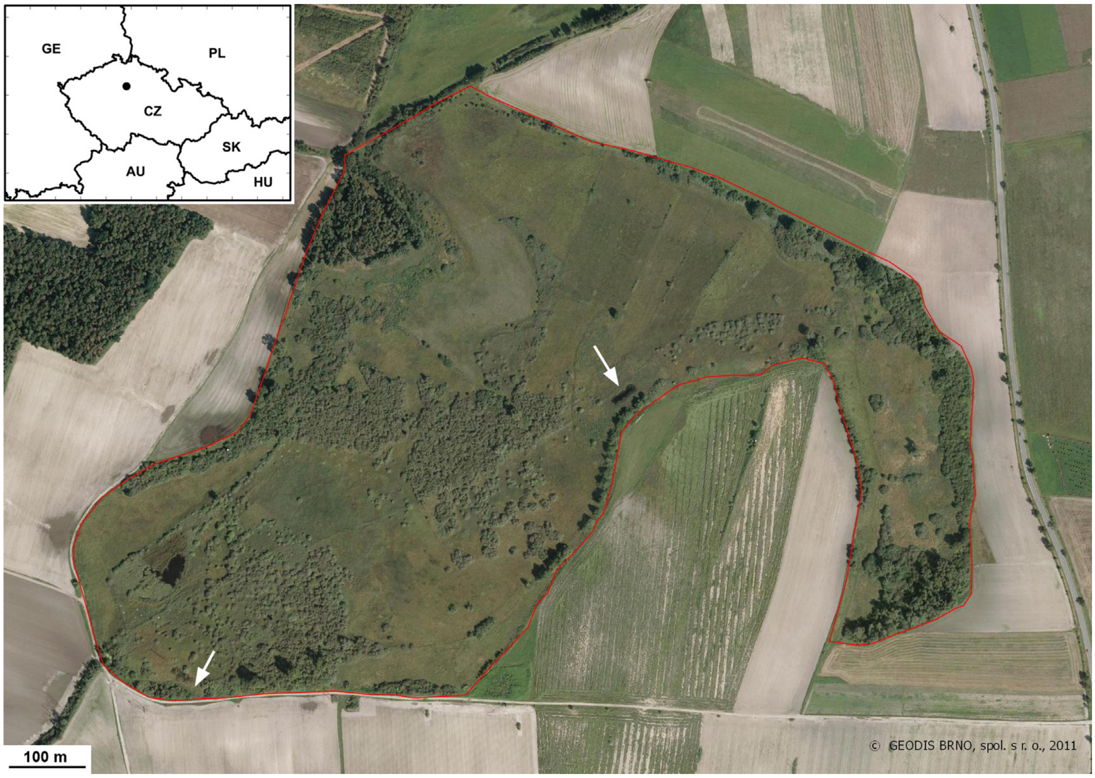

Fig. 1. Aerial photograph of the Hrabanovská Černava fen in central Bohemia, Czech Republic, surrounded by arable land. The red line indicates the borders of the Hrabanovská Černava National Nature Reserve. The arrow in the eastern part of the reserve indicates the new pool created in 2007; the arrow at the south-western edge of the reserve indicates the position of the old pool desilted in 2011. (For interpretation of the references to color in this figure legend, the reader is referred to the electronic version of the article.)

gradually increased over the last two decades. The fens are surrounded by arable field, which are sources of undesirable eutrophication in the marginal zones of the reserve. The entire fen complex is devoid of larger water bodies that would attract aquatic birds.

Historically, *P. coloratus* was recorded in this fen complex only in pools in the south-western part of the reserve. The 15×3–5 m oblong-shaped pool where *P. coloratus* was last observed (50°12′48.9″ N, 14°49′43.7″ E) had become heavily eutrophied during last decades, with most of it filled with organic sediment and overgrown with a dense stand of *Phragmites australis* until 2011, and with stands of *Urtica dioica* in its marginal zone indicating considerable eutrophication. The pool's maximum water depth was about 70 cm, but it seasonally dried in summers. During the last decade it was devoid of any submerged aquatic plants but the water surface occasionally hosted fronds of *Lemna minor*. Below the layer of *Lemna minor* the water was transparent, allowing inspection of water column and bottom characteristics. Bottom substrate conditions, i.e. deep layer of semi-liquid anoxic sapropel mud overlayed by plant detritus (mainly from *P. australis*), coupled with darkness under the dense reed stand did not allow growth of any other aquatic plant species rooting in the bottom. Regular monitoring repeatedly confirmed that conditions had become unsuitable for the survival of *P. coloratus*. Thus, it was apparent that the species had disappeared as a result of changes in the water regime and substrate chemistry, and consequent accelerated succession.

2.3. Restoration techniques

The restoration methods described in this study were applied in conformity with a management plan for the Hrabanovská Černava National Nature Reserve designed by the Nature Conservation Agency of the Czech Republic. In November 2007, to increase the habitat diversity within this fen complex, a new pool was created, by removing approximately 300 m^3 of soil from a wet fen in the eastern part (50°13′03.8″ N, 14°50′10.1″ E) of the nature reserve, an area where no pools or other bodies of open water had ever been observed. The new pool has a rectangular shape of 24×12 m, with a 70 cm average depth and, along its western edge, a maximum depth of 150 cm. The eastern edge of the pool verges into a shallow zone 12×12 m that is inundated only seasonally. The restoration works were provided by a small local company (BK služby, Ltd.) working only in the Czech Republic, in particular in central Bohemia. The use of the local company for restoration works prevented introduction of plant propagules from remote regions. The site of the new pool is 700 m northeast of the previously known site of *P. coloratus* (Fig. 1).

In April 2008, approximately 40 kg of wet mud was dug from the upper layer of the bottom of the pool in which *P. coloratus* had last been observed (henceforth referred to as the "old pool") and transferred to and spread in the newly created pool. The mud comprised a mixture of fine black organic sediment and coarse detritus from *P. australis* along with mineral sand and loam from deeper bottom layers. We presumed that, based upon the species' prior occurrence, the mud would contain dormant *P. coloratus* seeds. The new pool was monitored for the occurrence of aquatic vegetation from 2009 to 2012.

In addition to the approach of creating a new pool, we also subjected the old pool to restoration. This was accomplished by completely desilting the pool in October 2011 and leaving it to natural succession. The restored pool had open water surface throughout the year and the water depth varied in different zones from 5 to 170 cm. The development of vegetation was monitored over the subsequent year, with the expectation that some *P. coloratus* seeds that had been buried in the sediment would be exposed and thus made available for germination.

2.4. Monitoring of vegetation

The vegetation was monitored in three periods. (1) The period of monitoring of the old pool before the soil transfer and creation of the new pool (1978 – November 2007). The monitoring was focused in particular on search for *P. coloratus* and other submerged or floating plant species, which could have been hidden in the *Phragmites-Urtica* stands or under the layer of *Lemna minor*. (2) The period after the soil transfer from the old pool to the new pool (November 2007–October 2011). The monitoring was focused especially on the vegetation development in the new pool. At the same time we checked if the moderate soil disturbances caused by digging of sediment in the old pool (soil transfer, seed bank analysis) did or did not created microsites suitable for rooted aquatic plants including *P. coloratus*. (3) The period after the desilting of the old pool (October 2011–June 2013). The monitoring was focused on vegetation development in both pools.

The monitoring of the vegetation of both pools included all the vascular plant species and charophytes growing directly in the water as well as in 1 m wide margins of the pools experiencing frequent water level changes (water depth approximately between 0 and 10 cm). We always started by inspection of the vegetation from the banks and collecting of visible submerged or floating plants using an anchor. When any new species could not be found, we continued the monitoring by wading in water. In the period (2) and (3) when *P. coloratus* population was re-established, the wading was restricted on parts with transparent water only, in order to avoid damage of *P. coloratus* and other rare species potentially hidden in turbid water (the situation rather frequent in the first year after creation/restoration of pools). For *P. coloratus*, we counted number of established colonies and the ramets within each colony (colony = probably the polykormon originating from a single propagule). The quantity of the other species in vegetation was estimated according to a simple scale: d – dominant, the species formed compact stands of the size of at least 5 m^2 in total in the particular pool, f – the species occurring usually in at least several dozens of specimens but not forming compact stands in the particular pool, r – the species occurring in up to 10 individuals in the particular pool.

2.5. Soil propagule bank analysis

After the successful establishment of *P. coloratus* population as well as the populations of several other rare vascular plants and algae from the sediment transplanted into the newly created pool (see Section 3) we decided to analyze the soil propagule (mainly seed and charophyte spore) bank of the old pool in order to directly ascertain the individual species soil propagule bank density and also to infer its longevity from the percentage of seeds/spores that would germinate. In April 2011, a 10 kg sample of mud was dug from the same parts of the source pool from which the 40 kg had been collected in April 2008 for use in the new pool. This sample, having presumably the same composition as the mud collected earlier, was used for soil seed bank analysis, as follows. First, the 10 kg sample of mud was carefully homogenized and the coarse particles (e.g. large pieces of *P. australis* material) removed. A total of 3 kg of mud was then randomly taken from the sample and divided into two equal parts, with each subjected to a different soil seed bank analysis technique. For one we used the seedling emergence method and for the other applied the rinsing method (according to Gross, 1990; ter Heerdt et al., 1996). For the seedling emergence method, the sediment was cultivated between May 21, 2011 and October 26, 2012 in a greenhouse without light or temperature regulation (but generally above 0 °C). The substrate was kept flooded with shallow water (ranging in depth between 2 and 5 cm) from the beginning of the cultivation until the end of August 2011. Then, the sediment was kept exposed but moist until mid-April 2012, when it was flooded again, and maintained in the flooded condition until the end of the cultivation. The sediment was checked regularly for emerged seedlings and after identifying them they were removed from the cultivation containers. For further details on cultivation conditions, see Šumberová et al. (2012a,b). In the rinsing method, the sediment was rinsed using an automatic sieve system (AS 200 basic of Retsch, Ltd.) between May 2011 and April 2012. Sieve meshes of 4 mm, 2 mm, 1 mm, 0.25 mm and 0.1 mm were used in sequence to ensure detection of overall soil seed bank variability. The trapped sediment containing plant propagules was subjected to visual inspection under a stereoscopic microscope, sorted, and identified using literature (Cappers et al., 2006; Bojňanský and Farkašová, 2007) and the reference seed collection of the Institute of Botany, Department of Vegetation Ecology in Brno.

The results of the soil seed bank analyses are presented in Table 1. In several cases precise counting of emerged plants was impossible due to fast clonal growth (*Lemna minor*) or dense fragile stands (*Chara* spp.). In these cases symbols d (dominant – presented in hundreds propagules in a microscope-observed sample and predominating the cultivations) and f (frequent – occurring in dozens of propagules in a microscope-observed sample with up to 10% cover in cultivations) were used in the table.

3. Results

3.1. Pool vegetation and management

No aquatic plants were observed in the new pool during the first season, 2008, but they could have been overlooked because of slightly turbid water. During the summer of 2009, only *Chara* sp. was detected. In September 2009, during an unusually prolonged seasonal dry period, portions of the bottom of the pool were exposed. Eighteen short ramets of *P. coloratus*, possibly growing from a few connected rhizome systems, were detected in shallow water or on the wet mud of the exposed bottom of the middle of the pool. The water level rose later in the autumn, and the plants successfully survived the winter. Moreover, many new shoots of *P. coloratus* were observed in the pool in June 2010. These included 86 well-developed ramets growing in a rather compact colony (growing from a single or a few rhizome systems) in the middle of the pool and 6 shoots at its western edge. Many of these plants reached the water surface, flowered freely, and set fruit. They increased in size and abundance in 2011 and formed 5 compact colonies by August 2011. The largest colony, in the middle of the pool, covered about 7 m^2. Altogether 151 fruiting ramets reaching or close to the water surface were counted, with many, short, sterile ramets occurring either in the colonies or as isolated individuals along the pool's margin. These sterile ramets could not be counted because they were mostly concentrated near the bottom and within the dense stands of adult plants. The highest density of shoots was in water 82–102 cm deep, in the middle of the pool. There, *P. coloratus* cover ranged between 80 and 90%. The plants along the pool's margin were in about 20 cm of water. Shoots of *P. coloratus* overwintering under the ice were noted in February 2012, and large stands were observed later in the growing season. The only other submerged vascular plants found in the pool were *Utricularia australis* and *U. vulgaris*, which occurred sporadically in the pool. Two algae species, *Chara hispida* and *C. globularis*, formed dense masses locally near the bottom. Individual plants of *P. australis* and, to a lesser extent, *Juncus articulatus* and several other helophytes penetrated from the banks to the shallows of the pool (Table 1). The expansion of *P. australis* into the pool occurred despite the fact that the surrounding fen meadows are mown regularly, and might have been enabled by the lack of mowing of the pool's banks during a period immediately after its creation (due to bank instability).

Table 1
Plant species found in vegetation and soil seed bank of the old and new study pools in the Hrabanovská Černava fen in 2011(–2012). The data for the old pool include species before and after the restoration and species found in the soil seed bank by both used methods of analysis. For the new pool only the species occurring in vegetation are given. Remarks and explanations: d – dominant; f – frequent, but occurring with low cover; r – rare, only few individuals (for details see the Methods); * – related species difficult to distinguish as seeds or sterile plants and/or species difficult to quantify due to rapid clonal growth. The number of seeds/spores is given for the total amount of cultivated or rinsed sediment (i.e. 2 × 1.5 kg). The method which contributed to detection of a particular species in soil seed bank is given as a superscript of the number of propagules: s – seedling emergence method, r – rinsing method, b – both methods. The viability of seeds found in the rinsing method was estimated only visually (undamaged seeds considered as viable), no tetrazolium or germination tests were carried out.

Species name	Vegetation			Propagule bank
	Old pool (before rest.)	Old pool (after rest.)	New pool	
Potamogeton coloratus	–	r	d	–
Phragmites australis	d	–	f	13^{b}
Urtica dioica	d	–	–	712^{b}
Lemna minor	d	–	–	f^{*b}
Chara hispida	–	–	d	d^{*b}
Chara fragilis	–	–	f	f^{*b}
Utricularia australis	–	–	f	–
Utricularia vulgaris	–	–	f	3^{s}
Lysimachia vulgaris	–	–	f	–
Mentha aquatica	–	–	f	–
Lycopus europaeus	–	–	f	3^{r}
Juncus articulatus	–	–	f	11^{*b}
Juncus alpino-articulatus	–	–	f	
Carex buxbaumii	–	–	f	–
Carex lepidocarpa	–	–	r	–
Chenopodium rubrum	–	–	–	95^{b}
Schoenoplectus tabernaemontani	–	–	–	55^{b}
Rumex maritimus	–	–	–	42^{b}
Carex pseudocyperus	–	–	–	29^{b}
Typha latifolia	–	–	–	26^{b}
Chenopodium album agg.	–	–	–	15^{r}
Batrachium sp.	–	–	–	11^{b}
Carex elata	–	–	–	5^{s}
Cladium mariscus	–	–	–	4^{r}
Iris pseudacorus	–	–	–	4^{b}
Sambucus nigra	–	–	–	4^{r}
Alisma plantago-aquatica	–	–	–	3^{r}
Eleocharis ovata agg.	–	–	–	2^{s}
Carex panicea	–	–	–	1^{r}
Lythrum salicaria	–	–	–	1^{s}
Myosoton aquaticum	–	–	–	1^{r}
Ranunculus flammula	–	–	–	1^{r}
Ranunculus repens	–	–	–	1^{r}
Not identified	–	–	–	34^{b}
Total (only for propagule bank)	–	–	–	1065

Because *P. australis* tends to overgrow small shallow pools and produce monodominant stands, the shoots projecting above the ice were cut in February 2012 to suppress its expansion. The suppression of *P. australis* has been the only management measure we have used in the newly created pool since it was "seeded" with the mud from the old pool.

The appearance of *P. coloratus* in the old pool was faster, with the first signs of the restored presence of this species appearing in May 2012, in the first growing season after desilting. At that point, three small detached leaves and a fragment of a young stem were found floating on the water surface or drifting along the banks. Then, in August, six detached, floating shoots were observed. As no rooted shoots were observed in the shallow, marginal areas of the pool with sufficiently transparent water to see the bottom, we assume that the plants appeared from seeds that germinated in deeper zones of the pool. The exact number of seedlings and rooted young shoots could not be counted because of slightly turbid water. At least 127 ramets of *P. coloratus* occurred in the pool in May 2013. Most of them grew from a few connected rhizome systems that formed four more or less compact colonies with cover up to 80%. Sixteen shoots approached the water surface and produced young inflorescences.

Besides *P. coloratus*, no other aquatic or wetland plants have been visible at the early successional stage of the desilted old pool, although some green filamentous algae have been seen there. However, it is possible that other aquatic plants could be present, but hidden in the deeper turbid water. Expansion of reed has not yet been observed in this pool and no active management has been undertaken since it was desilted.

3.2. Soil propagule bank and its relationship to vegetation

The species found in the soil propagule bank of the old pool are presented in Table 1 along with lists of the species occurring in the vegetation of each pool.

Comparisons of the vegetation of the new pool with both the soil propagule bank and vegetation of the old pool before it was desilted showed only few similarities (see Table 1). Two rare species, *C. hispida* and *U. vulgaris* were identified in cultivation of the propagule bank and also found in the new pool. Seeds of *P. coloratus* were not found in analyzed sediment sample from the old pool, even though this species re-established its population in the old pool and also colonized the new pool. In contrast, *U. dioica*, which was a dominant of the vegetation and soil seed bank in the old pool, was not confirmed in the vegetation of the new pool. Several other species, either ruderals (*Chenopodium album* agg., *Sambucus nigra*) or wetland perennials (e.g. *Carex pseudocyperus*, *Cladium mariscus*, *Schoenoplectus tabernaemontani*), were found in the soil seed bank of the old pool, but occurred only in the surrounding vegetation (arable fields, fen meadow) without being recorded in the vegetation of either pool.

4. Discussion

4.1. Effectiveness of soil seed bank techniques for restoration and possible role of long-distance dispersal

Both approaches using the soil seed bank – in situ and translocation – were effective in yielding populations of *P. coloratus* in an area where the species had not been observed for more than thirty years. In particular, *P. coloratus* now flourishes in the new pool, with hundreds of spikes with fruits produced each season. The species did, however, reappear more quickly in the old pool than the new one, likely due to the old pool having more propagules present in its seed bank than the number of seeds that would have been introduced to the new pool via the mud transfer. We can only speculate about this, however, as neither the seedling emergence method nor the rinsing method enabled us to actually find propagules of this species in the mud sample from the source pool.

We carefully analyzed other possible sources of *P. coloratus* propagules for both pools. Besides the presence, although not confirmed, of viable seeds in soil seed bank of old pool, three other theoretical possibilities should be discussed. (1) "A small, hidden population of *P. coloratus*, not producing seeds in sub-optimal conditions survived in the old pool. The desilting of the old pool thus only supported success of this population. The re-established populations have their origin in rhizome fragments more likely than in viable seeds, which are not present in the seed bank anymore." Although this explanation could seem realistic for some reasons (e.g. relatively fast establishment of the populations), we consider it to be highly improbable. As already explained (see Sections 2.2 and 2.4), the habitat conditions in the old pool before the restoration were totally unsuitable not only for *P. coloratus* but also for any other hydrophytes except for *Lemna minor*. Strong eutrophication, toxic substrate, accumulation of dead biomass, frequent drying-up in summers and shade caused by *P. australis*–*U. dioica* stands continued for at least 18 years before the restoration. Our experience from cultivation indicate that rhizomes of *P. coloratus* not connected with photosynthetically active shoots are not capable to survive in the sediment for years and their dying is even accelerated by anoxic conditions, such as occurred in the old pool prior to restoration.

(2) "The population of *P. coloratus* originates from deliberate plantation because the Hrabanovská Černava Nature Reserve is not fenced and anyone can enter and re-introduce the extinct species." Although the deliberate plantations of endangered aquatic plants started to be popular in the last two decades, this is very unlikely in the case of *P. coloratus* in the Czech Republic. Prior to our restoration project, the living plants of the target species were only available in the collection of aquatic plants at the Institute of Botany, Třeboň. The collection is not freely accessible and does not provide plant material for public use. Additionally, only a limited number of people were informed about the restoration project, in order to avoid the undesirable visits of the pools shortly after the restoration, and possible disturbances of establishing vegetation.

(3) "The propagules of *P. coloratus* could be dispersed from other existing populations or viable soil seed banks by means of long-distance transport (e.g. ornithochory)." In the case of both pools in the Hrabanovská Černava Nature Reserve there was no possibility of the species' dispersal from the surroundings. As discussed above (see Section 2.1), two other historically known populations of *P. coloratus* in the Czech Republic became extinct many decades before the population in Hrabanovská Černava disappeared. Unlike the Hrabanovská Černava Nature Reserve, the two other localities were not subjected to any restoration measures or other soil manipulations, and therefore it is highly improbable they would release the seeds from soil seed bank, if there still exist any. However, if it had happened, it would have documented the survival of viable *P. coloratus* seeds not after 30 years but after 70 or even 170 years, respectively (see Kaplan, 2010).

The nearest localities of recent *P. coloratus* occurrence are in Germany, about 300 km from our study site (Roweck et al., 1986; Kohler et al., 1987). Although long-distance dispersal of seeds via aquatic birds probably plays an important role in wetland ecosystems (e.g. Figuerola et al., 2002; Santamaría et al., 2002), coincidence of several factors is necessary if the seed dispersal, seed germination and new population establishment should be successful (e.g. seeds must be dispersed to habitat suitable for their germination and further development; Clausen et al., 2002). Therefore, ornithochory is much more likely to contribute to the dispersal of aquatic plant species which are frequent in the landscape, form relatively large biomass with numerous seeds and possess of broad ecological range than to the dispersal of rare species with very specific habitat demands. Analyses of aquatic bird gut usually detected the propagules of those plant species which are common over large areas, often representing dominants of the vegetation (Figuerola et al., 2002; Figuerola and Green, 2002b; Green et al., 2002; Brochet et al., 2009, 2010). Among the members of the genus *Potamogeton* there were represented only *P. pusillus* and *P. pectinatus*, which are the most common species of the genus in Europe (Figuerola and Green, 2002b; Brochet et al., 2009, 2010). In contrast, *P. coloratus* is a rare species, a habitat specialist, and its localities are rather scattered and particularly rare towards the edges of its distribution range. Although ornithochory in this species cannot be excluded, it is probably particularly important on the local scale in the regions with higher density of its existing localities. However, Hrabanovská Černava is a wetland complex with high level of isolation not only from the other European *P. coloratus* localities but also from the other wetland complexes in the Czech Republic. It has only a few, very small water bodies, and thus does not offer suitable conditions for wild ducks and geese, which are considered the major vectors of long-distance plant dispersal (Figuerola and Green, 2002a). In addition, small, newly created or re-constructed wetlands do not attract aquatic birds because of lack of food sources and the vegetation providing safe refuges and/or nesting places. Particularly important is the fact that the first plants of *P. coloratus* already appeared during the second year in the new pool and even during the first year (first spring) in the old pool, only 7 months after it was desilted in late autumn. Moreover, no newly established population of *P. coloratus* has ever been recorded in the Czech Republic even in suitable wetlands frequently visited by migrating birds.

It should be discussed that not only aquatic birds but also various cars and other vehicles can serve as dispersal vector over larger distances (Bakker et al., 1996; Ansong and Pickering, 2014). However, as we found during our own research of seed dispersal in pond systems (Šumberová et al., 2012a,b; Šumberová and Ducháček, unpublished data), vehicles do not serve as aquatic plant dispersers, with the exception of amphibious plant species like *Callitriche* spp. During this dispersal, the seeds occurring in wet mud attached to the wheels and wheelframe of the vehicle are probably subjected to conditions very unsuitable for aquatic plant species (perhaps strong moisture fluctuation), which might lead to the loss of their viability. In other way we are not able to explain the fact that the vehicles serve as effective dispersers of the plant species with various seed size and shape, and from various ecological groups except for aquatic plants. Although further experiments are necessary to clear this fact, we can already now exclude the possibility of *P. coloratus* introduction via vehicles into Hrabanovská Černava. In addition to the reasons mentioned above, the only vehicles moving directly in the nature reserves were the excavators used for the construction of the new pool and the desilting of the old pool. They have never been used for similar works in other countries than in the Czech Republic and thus they could not come into contact with *P. coloratus* seeds prior to the restoration works on Hrabanovská Černava.

On the basis of our experience and literature data we concluded that the coincidence of arrival of *P. coloratus* seeds from one of the distant localities directly to the small pools on Hrabanovská Černava and their successful germination and recruitment within the given short time slot is highly unlikely. Also the probability of the establishment of *P. coloratus* population from the other discussed sources (1 and 2) is much less probable than its recovery from soil seed bank present on the restored locality. We did not detect *P. coloratus* propagules in our sediment sample likely because the density of seeds was too low or the sediment sample too small (although it was 3× larger than the samples standardly used in our previous studies and large enough to detect broad spectrum of species in wetland soil seed banks, see e.g. Šumberová et al., 2012a,b). The latter would have been particularly problematic if the particular spot or vertical layer from which we took the sample in 2011 lacked seeds, in contrast to the exact location from which the mud was collected for translocation. Given this possibility, we might have been especially fortunate to have obtained seed-rich mud for the translocation, and, in applying this approach in future restoration projects, it might be helpful to use soil taken from various locations within the source site. However, even when using such sampling design, it cannot be ensured that the seeds of the target species would be collected, in particular in low-density soil seed banks. To increase the volume analyzed might be problematic too as the soil seed bank analysis is quite time-consuming and – in the case of the seedling emergence method – also space-demanding procedure. Our restoration project was successful not only in terms of producing the two resulting populations of the target species *P. coloratus* but also in its positive effects on other rare species. This included helping to establish a large new population of *C. hispida*, recently considered endangered in the Czech Republic (Caisová and Gąbka, 2009). It also included the establishment of the critically endangered *U. vulgaris* (Danihelka et al., 2012; Grulich, 2012) in the new pool.

4.2. Soil seed bank longevity

A major determinant of the success of any project using a soil seed bank for population restoration will be the relationship between the longevity of the target species' seed bank and the length of time since the species last reproduced at the site in question. Thus, for those species for which soil seed banks offer the last hope of population restoration, knowledge of seed bank longevity is crucial in assessing how much time is available for restoration efforts. However, data on the longevity of aquatic plant seed banks are scarce. The most comprehensive European seed bank data compilation (Thompson et al., 1997), which focuses on north-western European species (the majority of which occur also elsewhere in Europe), contains information on relatively few wetland or aquatic plants. For example, for *Potamogeton*, only four species – *P. pectinatus*, *P. perfoliatus*, *P. pusillus* and *P. vaginatus* – are included. Of these, this source lists confirmation of permanent soil seed banks for only *P. pectinatus* and *P. pusillus*, without any particular data about seed longevity. Kaplan and Štěpánek (2003) have presented results from longevity research on *Potamogeton berchtoldii*. In the case of our target species, *P. coloratus*, we could not find precise published seed bank longevity data.

The present study, in indicating that some – albeit unknown – proportion of the seeds of *P. coloratus* can survive upwards of 30 years in the soil seed bank contributes to our knowledge of the longevity of this species' seed bank. The results of other restoration efforts (especially when coupled with data from seed bank analyses) can similarly yield insights into longevity of other aquatic and wetland species' seed bank longevity.

A neglected study from the Netherlands suggests that *P. coloratus* seeds might maintain germinability even for about 100 years (Bruin, 1997). Similarly as in our case, the species appeared in the Netherlands after a long time being considered as extinct. Besides its historical localities, new localities were found in regions where peat started to be excavated and by this activity in a landscape were created small pools with clean water. They were quickly colonized by *P. coloratus*, as assumed by the author, from the seeds stored for decades in the soil seed bank. Bruin (1997) argues that the populations appeared on several sites at the same time, some of them amounting already in the first year up to 22 plant individuals. Similarly as in our locality on Hrabanovská Černava, also the localities in the Netherlands were subjected to succession of perennial herbs and water level fluctuations (including periodic desiccation). However, as the Dutch localities occurred in the dune slacks without agricultural activity, they were not impacted by strong eutrophication. Results of the restoration measure on Hrabanovská Černava indicate that *P. coloratus* seeds are able to survive for decades even under highly eutrophic conditions of wetland surrounded by arable land.

4.3. Ongoing evaluation and maintenance of restoration success

Ensuring the persistence of populations requires both long-term monitoring as well as implementing management responses to ongoing threats. Whereas monitoring vegetation cover can serve as one measure of ongoing success, important information on long-term success can come from monitoring soil seed banks over time (e.g. Combroux et al., 2002; Neff et al., 2009; Diggory and Parker, 2011). In general, production of dense soil seed banks by the target species in restored wetlands is a basic requirement for the species' further survival in case of temporarily unsuitable conditions (Baskin and Baskin, 1998; Fenner and Thompson, 2005). In our case in particular, a seed bank study of the new pool will be useful after more years have passed since its creation, when more seeds will have been produced by more abundant adult plants.

In the pools of the Hrabanovská Černava fen complex, the major threat to the developing *P. coloratus* populations might be from the poor water quality – especially in terms of low transparency – and the overall eutrophication due to the neighbouring agriculture landscape. Moreover, high nutrient amount enhances succession of competitive aquatic and wetland plants, in Hrabanovská Černava mainly of reed (*P. australis*). Similar processes have been documented also on other European localities of *P. coloratus* (e.g. Buchwald et al., 1995; Sburlino et al., 2008). In this regard, the charophytes (in our study present in the new pool can be beneficial), as large charophyte stands can improve significantly water quality (Królikowska, 1997; Nõges et al., 2003). Regular management interventions have to be performed as prevention of *P. australis* succession and forming dense stands in water bodies (Ritterbusch, 2007), otherwise the shading, abundant organic detritus, and resulting rapid terrestrialisation would lead again to extinction of aquatic vegetation.

The most effective way to restrict *P. australis* in nature reserves is by regularly mowing it during the growing season (Güsewell, 2003; Engloner, 2009) – once before its intensive growth in late May/early June, and then again in late July/early August (Čítek et al., 1998). Cutting the stands below the water surface yields more effective control, as it causes die-back of the stands (Čítek et al., 1998; Engloner, 2009). Unlike growing season mowing, mowing reeds in winter (as conducted in Hrabanovská Černava in February 2012) is reported to result in removal of only dead plant parts, allowing further reed development (Ritterbusch, 2007; Englоner, 2009). Winter reed cutting is conducted at Hrabanovská Černava for practical reasons (better accessibility). In a period of unusually severe frosts in 2006 it did lead to complete eradication of the reed colony at a pool outside of the present study, but this outcome was due to this rare, extreme weather. Management plan for Hrabanovská Černava also includes summer mowing of the surrounding fen meadows, which is crucial, as this can help limit the ability of reed beds located there to expand into the pools (Pfadenhauer and Grootjans, 1999; Güsewell, 2003). Grazing of the stands, recommended by some authors (Pfadenhauer and Grootjans, 1999; Engloner, 2009) would not be probably applicable due to small area of Hrabanovská Černava and danger of further pool's eutrophication.

Acknowledgements

We are grateful to Luboš Beran for his help during fieldwork and for his active approach to restoration of wetlands. Jonathan Rosenthal kindly improved English of an earlier version of the manuscript. Comments of two anonymous reviewers helped to improve the paper. The research and elaboration of this paper was supported by grants no. 206/09/0291, 206/09/0329 and 14-36079G (Centre of Excellence PLADIAS) from the Czech Science Foundation, by the long-term research development project no. RVO 67985939 from the Academy of Sciences of the Czech Republic, and by the long-term research plan of the National Museum Prague no. DKRVO 2013/08 00023272 from the Ministry of Culture of the Czech Republic. Management of the Hrabanovská Černava National Nature Reserve is funded by the Ministry of the Environment of the Czech Republic.

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