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A short contribution to Littorella uniflora (L.)Asch. germination capability

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A short contribution to *Littorella uniflora* (L.) Asch. germination capability

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ABSTRACT

The germination capability of *Littorella uniflora* was studied in relation to the conservation management. Twelve sediment samples were taken from the cultivation tanks with *L. uniflora* in the Rescue cultivation in the Institute of Botany, Academy of Science of the Czech Republic. Samples were air-dried and all *L. uniflora* seeds were collected and counted. In Experiment 1 the classical germination test was carried out. The germination capability reached 53.3% in the filterpaper and 33.6% in the sand treatment, respectively. In Eperiment 2, the capability of seeds to break through the different amount of sand was tested. While in the first treatment (1cm layer of the sand) the germination capability was comparable with the Experiment 1 (43%), in the other two treatments (3 and 5cm layer of the sand) no seedling occurred on the substrate surface. In the context of the conservation management it means, that shallow plowing management (localities with sand bottom) or partial removal of the sediment layer (localities with deep organic sediment layer) could help to stabilize or even support the *L. uniflora* population. Such management practises should be tested in some localities with gradually declining *L. uniflora* population, in addition to the summer drainage of the fishponds.

keywords: summer-drained fishpond, isoetid, seed bank, conservation management

Introduction

Littorella uniflora, a small amphibious "isoetid" waterplant is well known from the Western and Central European oligotrophic water bodies. In the second half of the 20th century a rapid decline occurred in number of sites originally inhabited by isoetids (Arts 2002). Acidification seems to be the main cause of this state (Roelofs et al., 1984; Roelofs et al.,1994; Arts, 2002). Similar decline occurred also in the area of the former Czechoslovakia (Hejný & Husák, 1978). However, the reason was an eutrophication connected with common carp (*Cyprinus carpio*) overstocking (Hejný 1967; Pokorný et al., 1990; Procházka & Husák, 1999). Historically, *L. uniflora* was reported from tens of water bodies in the Czechoslovakia (Klika 1935; Jílek 1956; Hejný 1967; Husák & Adamec, 1998). Recent population of *L. uniflora* can be found only at eight localities in the Czech Republic (Šumberová 2011), two drinking water reservoirs and six extensively managed fishponds. Most of them are located in the southern part of the Czech Republic.

L. uniflora is critically endangered in the Czech Republic (Decree 395/92), as well as protected by the governmental law in Poland and France (Act 1982; Act 2004). However very few practical measures for its rescue have been done. For example there is only one nature reserve in the whole Czech Republic with the active management of preservation of *L. uniflora* population. Studies on *Littorella uniflora* so far have provided information on its morphological and anatomical structure (Hostrup & Wiegleb, 1991; Nielsen & Sand-Jensen, 1997; Robe & Griffiths,1998) or biochemistry functioning (Smolders et al., 2002), but practical information is still missing. Our short contribution is follow up of the article about germination ecology by Arts & van der Heijden (1990) with effort to provide answers closely connected with the *L. uniflora* localities in the Czech Republic and their suitable management. According to the ongoing research (Kolář, unpublish data) it seems that at most *L. uniflora* localities even if with almost extinct population the seeds are present at different depths of the bottom sediment. Therefore, one question was from which depth of sediment the *L. uniflora* seed can break. The answer can to set up the suitable management of the *L. uniflora* localities whether the simple summer drainage can support *L. uniflora* population or any special measures like plowing are needed.

Materials and methods

Study site and seed origin

Seeds were extracted from two cultivation tanks in the Collection of Aquatic and Wetland Plants in the Institute of Botany Academy of Science of the Czech Republic in Třeboň, where the plants have been flowering regularly almost every year. The origin of the *Littorella uniflora* plants in these tanks is the Králek fishpond (South Bohemia, Czech Republic), a small, eutrophic (pH 7.9, alkalinity = 1.26mmol*l^{-1}, TN= 2.54mg*l^{-1}, TP=0.76mg*l^{-1} in the water column) fishpond used for pikeperch fry rearing. Králek fishpond is a nature reserve declareted for the *L. uniflora* conservation. Several conservation methods to support *L. uniflora* population like limiting fishstock, sediment or litoral vegetation removal were applied there.

Cultivation tanks were randomly sampled with the Beeker sediment core sampler. From each tank, 6 samples 10cm deep were taken. Volume of one sample was 125ml.

Samples were air-dried and seeds were collected and counted using a magnifying glass (12x zoom). They were stored at 4°C in the dark until the start of the experiments.

Experiment 1

To estimate the germination capability, collected seeds were placed in Petri dishes in an open glasshouse with free air circulation in July-August 2014. In all dishes, seeds older than 1-year (without the perianth) were used. To create moist conditions, strips of filter paper and tap water were used. Three glass dishes only with filter paper and three with sterilized (250°C for 1hour) sand was used. Each dish contained 25 seeds. Samples were exposed to natural photoperiod and temperature shifting, mimicking the natural summer condition on the exposed fishpond bottoms. After 60 days, the germination was evaluated. A seed was considered to be germinated when the radicle emerged through the flat top of the nut.

Experiment 2

To observe from which depth the seeds can germinate, the seeds were sown in glass dishes in different depths – 20 seeds were covered by 1, 3 or 5 cm of sterilized (250°C for 1hour) sand, respectively. Each sand depth was

carried out in triplicate. Growth conditions were the same as in Experiment 1. After 60 days, the final percentage of germination was evaluated. A seed was considered to be germinated when the first leaves broke through the sand.

Results

A total of 357 seeds of *L. uniflora* was found in 12 samples taken from two cultivation tanks (total area 4.72 m^2, 2.36 m^2 each). The tanks are characterized by low substrate depth (mean depth was only 12 cm), but a huge density of *L. uniflora* plants (cca 250ex. per square meter). Each sample (125 ml) contained on average 29 seeds. This means that estimated number of all seeds in one tank equals 65 702 ex.

Experiment 1

Seeds germinated between days 5 and 50 of the experiment, but there was a significant difference between treatments ($t = 0.042316$, see Fig 1 and Table 1). At the filter paper treatment 53.3% (48-60%) of all *L. uniflora* seeds germinated, but only 33.3% (24-44%) in the sand treatment.

Experiment 2

The seeds of *L. uniflora* were able to break through only the minimum layer of sand substrate (1cm in our case, Table 1, Fig 2). Their germination capability responded to the results found out in Experiment 1, and amounted on average to 43.3% (30-50%). The treatments with 3 or 5cm of sand layer had a negative effect on seed germination. None of the seeds could break through the sand layer of this depth.

Discussion

The background of the Experiments 1 and 2 is based on the former research reported by Arts & van der Heijden (1990) about the germination ecology of *Littorella uniflora*. The authors found out the factors which significantly affected the seed´s germination, namely the moist substrate, alternating light and temperatures and dessication pre-treatment. These conditions are typical of the late spring or early summer period, when the water level could be low and the exposed bottoms could occur. In case of the managed fishponds this season can be replaced by summer drainage (Hejný 1978; Šumberová 2011).

Even if we met almost the similar conditions which Arts & van der Heijden (1990) found out as the most important ones for the succesfull gemination of *Littorella uniflora*, the germination capability in Experiment 1 did not reached as high values as they mentioned (76%). Age of the seeds could not be consider as the main limiting factor because the plants were growing not more than 10 years in the cultivation tanks and the viability of seeds longer than 30 years was reported (Wynhoff 1988).

We found out rather high number of seeds in the sediments of the cultivation tanks. However such high concentration of seeds should not be expected in the fishpond´s sediments because *L. uniflora* usually do not flower every year in its natural localities. Moreover it reproduces only generatively for decades in fishponds with intensive fish management and without regular summer-draining (Kolář unpublish data]. Estimation of the size of seed bank on natural localities is an object of our ongoing research.

In the Czech Republic, the summer fishpond drainage was a regular part of the standard common carp (*Cyprinus carpio*) production. The summer drainage, as a source of nutrient recovery, especially in nutrient poor fishponds, was fully replaced by the fertilization during the last 40 years. Additionally, the sediment accretion rate up to 4 cm per year is typical of the Czech fishponds (Petříček 1999).

Therefore the aim of this short contribution is rather practical. It is known, that *L. uniflora* has a persistent seed bank and the seeds keep their germination viability for more than 30 years (Wynhoff 1988). *L. uniflora* seeds were found in the sediment after many years by Bekker et al. (1999) who studied sediments in 80 years old dune slacks in the Netherlands, but they did not study germinative ability of the seeds. The question remains, if the seeds present in the fishpond bottom are able, in case of optimal growing condition, to grow up and set a new population? Therefore we germinated seeds covered with different thickness of the sediment. The results show that the seeds were able to geminate only when exposed directly on the bottom surface or covered by very shallow layer of the sediment (1 cm). The seed germination was totally blocked by only the layer of 3 cm of

sand. Therefore it seems that only the summer drainage and the presence of exposed bottoms could not support the germination of *L. uniflora* seeds. According to the sediment age and accretion rate, seeds can be buried at the depth from five to fifteen centimeters (Thompson et al. 1997; Bekker et al. 1999). In case of the fishpond reservoirs it is necessary to take into consideration the depth up to fifty centimeters (Šumberová III, 2013, pers. comm.). Therefore it is important to choose a technologically appropriate method, such as shallow plowing to turn over the sediment. This method could be useful in the reservoirs with the sand bottom without a thick layer of organic sediment. In fishponds with such deep organic sediments, the better way to enhance *L. uniflora* population is the partial sediment removal. This measure was successfully used in the Králek fishpond in 2007 (Hesoun et al., 2008). After removal of 20 cm of the mud sediment, *L. uniflora* seedlings appeared very soon (Kolář, unpublish data).

In the Czech Republic, the main problem is to make sure that the fishpond holders will perform summer drainage or partial sediment removal. The law for nature protection contains the possibility to compensate losses of the fish production, however this measure has been used only occassionally. The successful example is the Králek fishpond, the nature reserve declareted in 2000 for the protection of the *L. uniflora* population as well as of the other unique waterplant species. The step forward hopefully will be the rescue programme for *L.uniflora* in the Czech Republic which is prepared nowadays.

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Conflict of interest

Experiments were done at the critically endangered species and their parts, therefore the permition from the Czech Ministry of Environment was needed. Numbers of the authorizations are SR/0008/TR/2014_3 and KUJI 33224/2014.

Conclusions

We found out rather high concentration of seeds (250 seeds per $1m^2$) in the sediment of the rescue cultivation tanks of *Littorella uniflora* in the Institute of Botany, Czech Republic where the plants were flowering almost every year during the last decade. The germination capability of these seeds was 53.3% (on the surface of wet filter paper) and 33.3% (on the surface of wet sand), respectively. The seeds of *Littorella uniflora* were able to germinate when covered with 1 cm of sand however no seed was able to germinate when covered with 3 or 5 cm of sand.

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	substrate	mean±stand.deviation
Experiment 1	filter paper	53.3±5%
	sand	33.3±8.2%
Experiment 2	sand 1 cm	43.3±9.4%
	sand 3 cm	0±0
	sand 5 cm	0±0

Table 1 Germination capability of *Littorella uniflora* seeds in Experiment 1 and 2

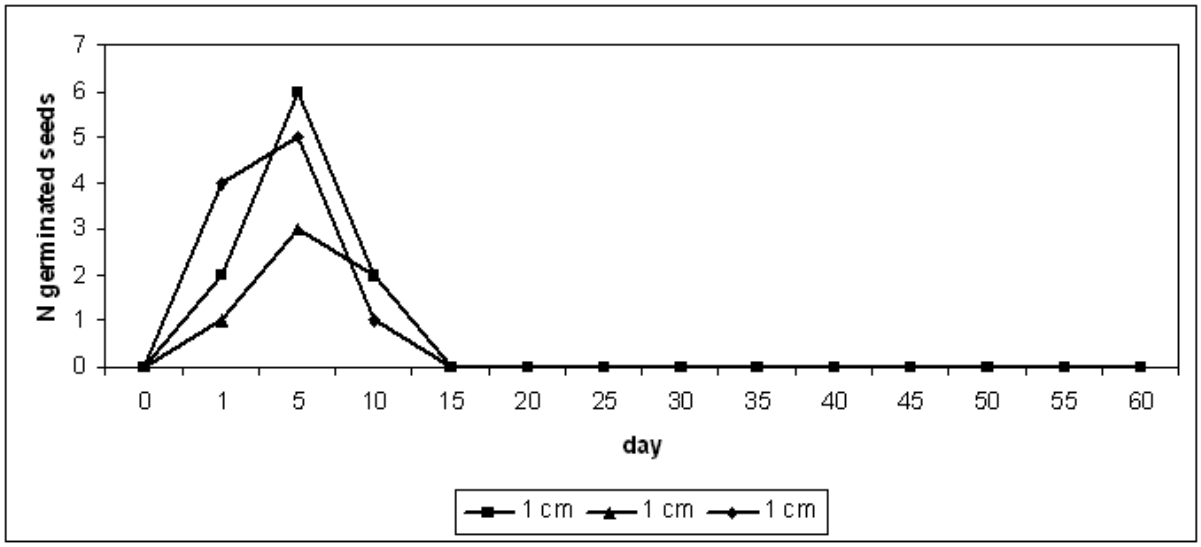

Fig 1 Germination of *L. uniflora* seeds at filterpaper (1F-3F) and sand treatment (1S-3S)

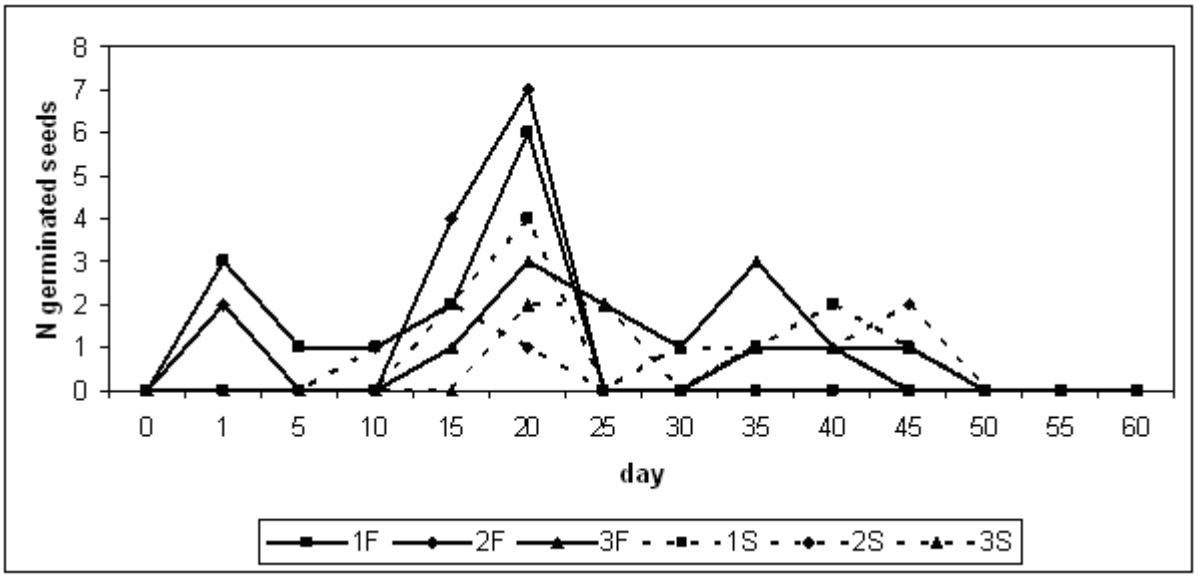

Fig 2 Germination of *L.uniflora* seeds though 1 cm of sand

Figure
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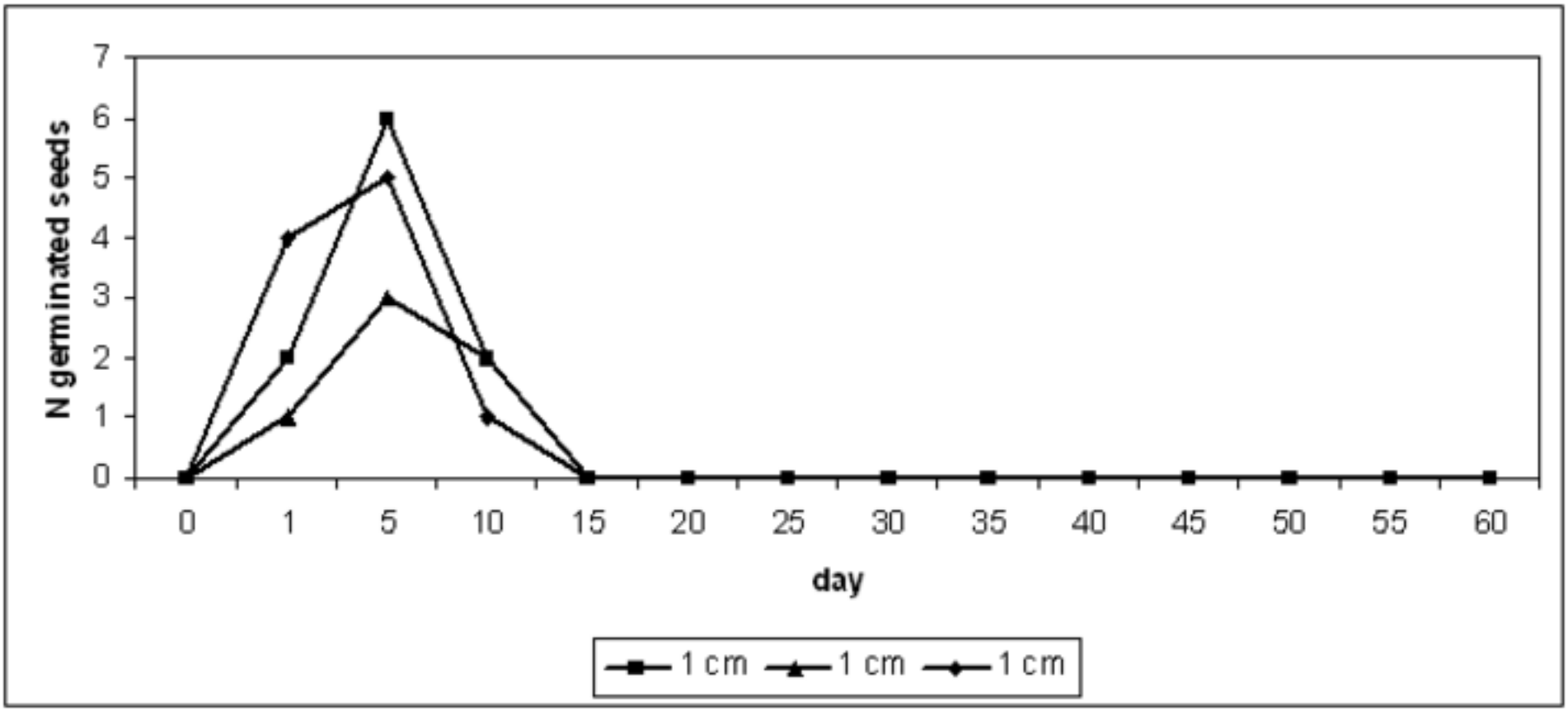

Figure
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