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POND RESEARCH AND MANAGEMENT

Life-history traits controlling the survival of *Tillaea aquatica*: a threatened wetland plant species in intensively managed fishpond landscapes of the Czech Republic

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Abstract *Tillaea aquatica* (Crassulaceae) is considered as annual wetland species threatened by changes in land use and progressing eutrophication in large part of its European distribution range. We summarised the historical and recent data on this species, and analysed its distribution and associated habitat changes in the Czech Republic. We used permanent plots as well as seed bank and seed dispersal studies to obtain better insight into the plant's survival strategy. During the second half of the twentieth century *T. aquatica* disappeared from most historical localities situated mainly in large fishponds. After 1999, altogether 18 new populations were found in small fry ponds and other fish-farming ponds. The largest populations of *Tillaea* were found in ponds with long-term bottom exposure where the vegetation of perennial herbs was eliminated by herbicides or grazing. Propagules easily dispersible by water, on gumboots or tyres of vehicles, and long-term soil seed bank also might contribute to persistence of the species in the habitats, diminishing the chance of extinction. As the fishpond management has changed, and so have done the original habitats of *Tillaea*, the species could survive in habitats different from those in the past. In this article, we suggest management measures aimed at promoting survival of *Tillaea* under new circumstances.

Keywords Fishpond management · Herbicide application · Seed dispersal · Species competition · Vegetation dynamics · Wetland vegetation

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Introduction

Tillaea aquatica L. (syn. *Crassula aquatica* (L.) Schönland, *Bulliardia aquatica* (L.) DC., Crassulaceae family) is an annual wetland plant species of sub-oceanic circumpolar distribution. In many European countries, the species has recently been considered as very rare, and it is listed as critically endangered or even extinct in national and regional red lists and red data books (e.g. Holub & Grulich, 1999; Kącki, 2003; Popiela, 2005; Fischer et al., 2008). The main threat factors are habitat and management changes, such as eutrophication, cessation of wetland grazing and wetland drainage (Rintanen, 1996; Stoltze & Phil, 1998). In Central Europe, where the species preferentially colonises ponds used in fish-farming, its strong decline in recent decades has been explained as a consequence of fishpond management changes (Chán, 1999; Holub & Grulich, 1999).

Similar negative trends have been documented also in populations of other wetland annuals, e.g. *Cyperus flavescens*, *Elatine hexandra*, *Gnaphalium luteo-album*, *Illecebrum verticillatum*, *Juncus tenageia* and *Radiola linoides* (Lampe, 1996; Stoltze & Phil, 1998; Holub & Grulich, 1999; Täuber, 2000; Kącki, 2003). In the past, these species had numerous populations in suitable habitat throughout Europe. Recently, many localities have been lost, especially those on the margins of ranges, thus diminishing the distributions of these species. For the majority of mudflat species, long-distance propagule dispersal via waterfowl is thought to be an efficient tool for population's natural re-establishment (Salisbury, 1970; Deil, 2005). However, for plants with specific habitat requirements, the existence of suitable habitats is usually the main factor limiting their occurrence (Rabinowitz, 1981). Therefore, having easily dispersible propagules should not be automatically considered as a factor preventing a plant's rarity (Fenner & Thompson, 2005).

Between 1999 and 2010, we recorded data on mudflat vegetation and soil seed banks in the Czech Republic, especially in various types of ponds used for fish farming. In this article, we present the results relating to *T. aquatica*, a species of high extinction risk and conservation value, especially in Central Europe. We aim to address the following topics: (1) its current and historical distribution and habitat preferences in the Czech Republic; (2) species composition of vegetation co-occurring with it in different habitats; (3) changes of its cover and overall cover categories of its co-occurring vegetation in response to herbicide application; (4) its germination in various conditions; and (5) its seed dispersal and survival in soil seed banks.

Materials and methods

Target species

Tillaea aquatica occupies periodically flooded and exposed sandy, loamy or muddy, non-calcareous substrata (Huber, 1961, pp. 124–125; Casper & Krausch, 1981; Deil, 2005). Owing to its low height and biomass and slow growth, it is a competitively poor herb. Therefore, it can survive only in habitats where competitively strong species either cannot grow because of extreme conditions (e.g. nutrient limitation; Keeley, 1998) or are eliminated by management. In Europe, *T. aquatica* has been reported especially from the short, grass-like plant communities that can be assigned to the classes *Isoëto-Nanojuncetea*, *Littorelletea*, and outside Europe it has also been found in equivalent communities (Deil, 2005; Šumberová, 2011; Šumberová et al., 2011).

Occurrence of *T. aquatica* is concentrated mainly in southern Scandinavia, but scattered localities are known also in Western and Central Europe and the European part of Russia (Huber, 1961, pp. 124–125; Hultén & Fries, 1986). Outside of Europe, the species has been documented especially frequently in Japan and in coastal regions of the USA and southern Canada. It is rarely known also from Siberia, the Russian Far East, and from inland North America (Hultén & Fries, 1986). The northernmost localities of *T. aquatica* are situated along the Arctic Circle in Central Norway and Northern Sweden. The southern European border of the species' distribution range passes through Central Europe (Huber, 1961, pp. 124–125; Casper & Krausch, 1981; Hultén & Fries, 1986).

Along with other "typical annual fishpond species" (e.g. *Coleanthus subtilis*, *Elatine triandra*, *E. hydropiper*, *E. hexandra* and *Eleocharis ovata*; Pietsch, 1973), *T. aquatica* is amongst species to which Central European origin has not been yet definitively ascribed. Originally, they could have colonised fishponds either from the remnants of natural lakes (of Tertiary or, rarely, Glacial origin) which are considered to have been incorporated into the fishponds during their construction in the Middle Ages (Pokorný & Jankovská, 2000) or they could have been transported by waterfowl from lake basins and coastal habitats in other parts of Europe (Hejný, 1969). Therefore, the classification of *T. aquatica* as native (i.e. not introduced by man) to Central Europe is justified.

In the Czech Republic, the first records of *T. aquatica* appeared in the middle of the nineteenth century, with the beginning of systematic botanical research of wetlands (Grulich, 1985). As of the mid-1980s, there were herbarium specimens of this species from 32 sites (Grulich, 1985), and additional localities were mentioned in the literature (e.g. Klika, 1935; Ambrož, 1939). Most of the sites at which the species was found were situated in South-Bohemian fishpond basins and the Bohemian-Moravian Uplands, also mostly in fish farming locations (Grulich, 1985). In both regions, *T. aquatica* was found especially on sandy margins of fishponds that were regularly exposed during summer drainage. After the 1950s, summer drainage was in large part replaced by modern intensification methods, e.g. fish feeding with cereals, fertilising fishponds with organic manure, and liming. This process has led, amongst other things, to an increase in pH, eutrophication and ruderalisation of the fishpond milieu (Hejný et al., 1982), and it is considered to be the most important cause of the decline of many wetland species, including *T. aquatica*. In the early 1990s, a single stable population of *T. aquatica* was known in South Bohemia, and other populations were briefly observed on exposed margins of two Central Bohemian water reservoirs during extremely low water levels (Grulich, 1985; Chán, 1999; Holub & Grulich, 1999).

Study area

The research was carried out throughout the Czech Republic, and included several hundred localities situated at altitudes of 160–730 m a.s.l. Sites with recent occurrences of *T. aquatica* are concentrated in south and southwestern Bohemia and Bohemian-Moravian Uplands, with the only exception being in the westernmost part of Bohemia (Fig. 1). Their altitudes ranges between 375 and 610 m a.s.l. (Table 1). The landscape in these regions consists of flat basins with large pond systems, including fishponds of an area of several hundreds hectares, or

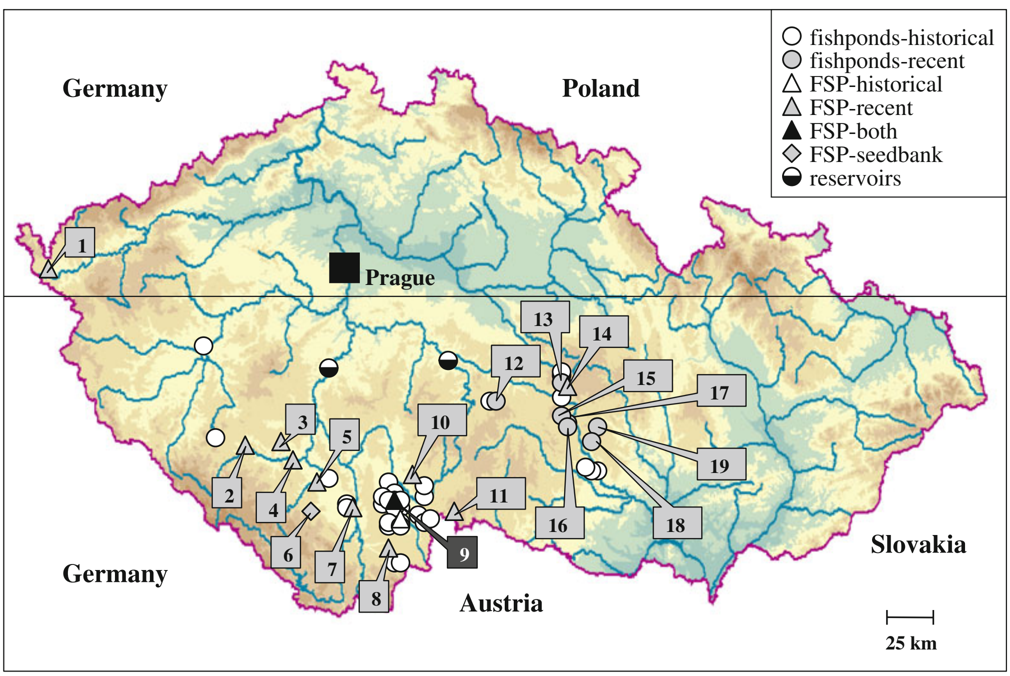

Fig. 1 Historical and recent distributions of *T. aquatica* in the Czech Republic. The recent localities are numbered and further described in Table 1 and in the text. *FSP* fish storage ponds; *FSP-both* historically known, still existing localities; *FSP-seedbank* fish storage ponds with recent occurrence only in soil seed bank; *reservoirs* water reservoirs, only historical records available

Table 1 Recent occurrence of *T. aquatica*: summary of localities, their management and size of *T. aquatica* population

No.	Habitat	Locality	Coordinates	Altitude	Management	Population size	Data recording	Source	Remarks
1	FSP	Krapice fish storage pond complex	50°6′44″N, 12°18′36″E	445	LSD, G-S	>1,000	2002, 2008	O	
2	FSP	Horažďovice fish storage pond complex	49°19′33″N, 13°40′39″E	440	LSD, H	>100	2008	O	Historical data only without exact location
3	FSP	Rojice fish storage pond complex	49°20′50″N, 13°56′55″E	450	LSD, M, G-B	>100	2001, 2006, 2008	O	
4	FSP	Čejetice fish storage pond complex	49°14′59″N, 14°1′23″E	380	LSD, M	>10	2001	O	During visits in 2002–2008 not confirmed
5	FSP	Vodňany, fish storage pond complex of the town Vodňany	49°9′6″N, 14°10′31″E	390	LSD, M, G-B	>100	2001, 2003, 2008	O	
6	FSP	Hrbov fish storage pond complex near Netolice	49°1′20″N, 14°9′14″E	460	LSD, M	>100 ?	2010	O	Detected only in soil seed bank
7	FSP	Hluboká nad Vltavou fish storage pond complex	49°2′45″N, 14°25′58″E	375	LSD, H, M	>10,000	2001–2010	O	Population size considerably increasing
8	FSP	Lhotka fish storage pond complex	48°51′39″N, 14°41′39″E	460	LSD, G-S	>1,000	2008	O	
9	FSP	Šaloun fish storage pond complex near Lomnice nad Lužnicí	49°4′6″N, 14°42′44″E	425	SSD-LSD, M	>100 ?	1999	Filípková (2001)	Also historical records available (Grulich, 1985); during visits in 2002 and 2008 not confirmed; strong elimination of summer drying in last decade
10	FSP	Kardašova Řečice fish storage pond complex	49°11′4″N, 14°51′21″E	440	SSD-LSD, H	>100	2001, 2003, 2008	O	
11	FSP	Albeř, old fish storage pond complex (in the village)	49°1′49″N, 15°8′8″E	610	SSD-LSD, H	>10	2003	O	During the visit in 2010 not confirmed, probably due to exchange of bottom substrate

Table 1 continued

No.	Habitat	Locality	Coordinates	Altitude	Management	Population size	Data recording	Source	Remarks
12	FP	Kamenický fishpond near Herálec	49°31′37″N, 15°26′7″E	570	SSD-LSD, F	>1,000	2007, 2010	Čech in Hadinec & Lustyk (2006); O	Duration of exposure is dependent on water availability
13	FP	Kamenný fishpond near Polnička	49°36′56″N, 15°54′7″E	600	SSD-LSD, F	>1,000	2000	O	Duration of exposure is dependent on water availability
14	FSP	Žďár nad Sázavou new fish storage pond complex (under Pilská water reservoir)	49°35′8″N, 15°55′47″E	570	H (surrounding of ponds)	>10	2008	O	Puddle on road margin amongst the ponds under direct herbicide impact
15	FP	Staropavlovský fishpond near Pavlov	49°27′29″N, 15°54′55″E	545	SSD-LSD, F	>10,000	2002	Němcová (2004); O	
16	FP	Nový fishpond near Zadní Zhořec	49°25′43″N, 15°55′57″E	540	SSD-LSD, ??	>100 ?	2002	Němcová (2004)	
17	FP	Křenčák fishpond near Netín	49°24′43″N, 15°56′15″E	535	SSD-LSD, ??	>100 ?	2003	Němcová (2004)	
18	FP	Obecník fishpond near Dolní Hlíny settlement	49°24′57″N, 16°8′31″E	510	SSD-LSD, ??	>100 ?	2002	Němcová (2004)	Retreat after the bottom cleaning in the last decade
19	FP	Holánek fishpond near Březejc	49°20′41″N, 16°6′35″E	455	SSD-LSD, ??	>100 ?	2002	Němcová (2004)	

The locality numbering corresponds with that on the map (Fig. 1)

FP fishpond, *FSP* fish storage pond, *LSD* long summer drainage, *SSD* short summer drainage, *G-S* grazing of sheep, *G-B* grazing of domestic birds, *H* herbicide spraying, *M* mowing, *F* fertilisation with organic manure, *O* own data

?? = further practices are supposed, but data are missing; >10 = tens of individuals, etc.; ? = exact data are missing, the population size was estimated on the basis of phytosociological relevés or soil seed bank density; for full citations of other sources see the reference list hilly country with chains of smaller fishponds in stream valleys. The geology of the basins and stream valleys is dominated by unconsolidated non-calcareous limnic sediments. Surrounding hills are mainly formed by granitoids and other crystalline bedrocks (Demek & Mackovčin, 2006). Regions with *T. aquatica* have mean annual temperatures between 6 and 8°C, and mean (April–September) growing season temperatures from about 12 to 15°C. The mean annual precipitation ranges between approximately 550 and 800 mm, and of this, ca. 350–500 mm fall during the April–September period (Tolasz, 2007). *T. aquatica* has never been recorded in the warm, dry lowland parts of the Czech Republic (Holub & Grulich, 1999; see also Fig. 1).

Fish ponds and fish storage ponds: characteristics, use and management differences

A fishpond is a water body primarily used for fish breeding. Fishponds in the Czech Republic are primarily used for breeding common carp (*Cyprinus carpio* L.) as a commercial product. Some other fish are added to ponds to improve the ecosystem function and productivity (e.g. pike—*Esox lucius* L., and tench—*Tinca tinca* L.). Once fish are harvested from the fishponds, they are stored before sale in special storage ponds, usually oblong or square with sides 5–30 m and depth 1–2 m, which connect via ditches to the fishponds and/or watercourses. Fish are sorted into storage ponds by species and size and held from the end of September until Christmas, and occasionally up to spring.

One of the oldest management practices in fishponds and fish storage ponds is intentional summer drainage. In fishponds it helps to improve fertility and to eliminate submerged plants and fish parasites. In the past, production increases for several years after the fishpond summer drainage even compensated for the absence of fish production in the year the fishpond was empty (Šusta, 1995). In the fish storage ponds, summer drainage functions especially to eliminate fish parasites and diseases.

After the 1950s, modern intensification practices increased strongly (see "Target Species" section) and summer drainage, especially in the large fishponds with marketable fish, was no longer profitable. Therefore, it has been largely eliminated and nowadays it is most often used only in fishponds with carp fry (i.e. fry ponds). There, the shallowly flooded mudflat vegetation provides an optimal milieu for development of small phytoplankton, which is the most effective food source for the youngest fish.

In the fish storage pond systems, management did not change so markedly because the ponds usually remain without water when not used. In any given fish storage pond system, different ponds are generally empty at different times, with these differences in bottom exposure strongly reflected in the structure and species composition of bottom vegetation (Šumberová et al., 2005). Before being stocked, a storage pond has to be clean, i.e. without tall-forb stands which would take up oxygen, and cause, amongst other things, problems in harvesting the fish. Various practices exist to suppress vegetation succession during the whole growing season. Probably the oldest one, still widely used, is mowing the vegetation stands and raking the biomass out of the ponds. Owing to the amount of labour involved in regular mowing, many fish farms use other ways to eliminate vegetation, such as grazing of domestic animals (sheep or others), herbicide application or only very short exposure of the bottom during the growing season. Some fish farms even use mechanical removal of the vegetation together with the muddy bottom sediment every year.

Field data collection

Field data collection proceeded in the 1999–2010 growing seasons. The following types of data were collected:

(1) Vascular plant species composition. These were assessed separately for each locality (and in fish storage pond systems for each individual pond), mainly in terms of absence-presence data, but for threatened species, population sizes were estimated. In small populations (of up to 20) the number of individuals was precisely counted, whereas in larger populations a part of population was counted and then the overall population size estimated according to the area occupied by the species. In species with procumbent growth (including *T. aquatica*), the counting of individuals—even in smaller populations—was difficult because of their intertanglement, and precise counting would have necessitated uprooting

them. In such cases, therefore, estimation on the scale <10, >10, >100, etc. individuals was used.

(2) Standard phytosociological relevés (Braun-Blanquet, 1964). These were done on plots of size varying from 1 to 25 m^2 according to vegetation type, i.e. 1–4 m^2 in *Lemnetea*, *Potametea*, *Bidentetea*, *Isoëto-Nanojuncetea* and *Littorelletea* communities, and 9–25 m^2 in *Phragmito-Magnocaricetea* and species poor types of *Molinio-Arrhenatheretea* pond bottoms. A modified Braun-Blanquet scale (van der Maarel, 1979) was used to record relative species abundance.

(3) Phytosociological relevés on permanent plots. These 1 × 1 m plots were established in autumn 2001/spring 2002 in fish storage ponds in Hluboká nad Vltavou (loc. 7 in Fig. 1 and Table 1) and monitored regularly during summer drainage in the years 2002–2009. In 2002 and 2003 this monitoring was done every 2 weeks, whereas in 2004 and 2005 it was done once per month. Typically, summer drainage would begin in March and end in October, and for 2002–2005, vegetation recording would typically be done during these months, although if particular ponds were drained for shorter periods, their vegetation was recorded just during those months the ponds were actually drained. From 2006 to 2009, vegetation was recorded twice a year (June and August). A modified Braun-Blanquet scale (van der Maarel, 1979) was used to record relative species abundance.

(4) Habitat type, use, and management data. These were gathered by personal observations and/or interviewing fish farmers. Data recorded included information about use of fertilisers, liming, fish feeding, herbicides (type, frequency of application), mowing, grazing, and periodicity of flooding and exposure. As these data from some localities were incomplete, we used them only in interpretation of the results and not directly in the statistics.

Seed bank and seed dispersal analyses: germination experiments

Seed bank and seed dispersal analyses were carried out in 2008–2010. In total, 87 sediment samples from 24 localities were collected. The samples were collected either before the growing season in March and April (63 samples) or after it in September–November (24 samples). The following types of substrate were analysed (number of samples and localities in individual categories see in Table 3): bottom sediment from fishponds, fish storage ponds, puddles on the roads amongst the fish storage ponds, connecting ditches and drainage channels; material (typically particles up to 1 × 1 cm including seeds as well as fragments of plants) floating on the water surface of supply ditches and fish storage ponds, and sediments (mainly muddy or clayey with admixture of sand) attached to vehicles, gumboots and other equipment (e.g. boats, fishing nets, etc.) used in fishpond management. Almost all samples were collected in regions with historical or recent occurrence of *T. aquatica* (South-Western and Southern Bohemia, Bohemian-Moravian Uplands). About 80% of all samples were collected from ponds and equipment on the property of a single fish farm, Rybářství Hluboká CZ Ltd (see loc. 7 in Fig. 1 and Tables 1, 3). This approach was chosen to obtain better insight into the processes of propagule exchange amongst fishponds, fish storage ponds and the surrounding landscape. Therefore, more than one type of sample was usually collected from a given locality, resulting in a "sediment series". For example, during the fish harvest at a particular fishpond we would sample not only from the pond bottom, but also vehicles, gumboots, and equipment used in that pond. For each item from which sampling was done (pond bottom, wheels and frame of the lorry used for fish transportation, etc.), sediment was collected randomly from different parts of it. These materials obtained from different parts of a given item would then be carefully homogenised to yield a mixed sample. Coarse particles (stones, wood pieces, roots, etc.) were removed. If possible (e.g. fish storage pond bottoms), 450–475 ml of homogenised sediment was collected in total per item. In some cases, the amount of sediment had to be reduced because of the scarcity of appropriate sediment on the items (e.g. gumboots).

The seedling emergence method was used for the analysis of sediments (e.g. Gross, 1990; Thompson et al., 1997). The samples were stored in the refrigerator at 4–5°C until they were prepared for cultivation. The storage time ranged from several days (spring samples) to about 6 months (autumn samples not cultivated until the next spring). To achieve the highest possible germination rate, each sample was divided into sub-samples of 40–100 ml (with the number of sub-samples depending on the source of the material, as some yielded more than others). Each sub-sample was diluted with tap water and discharged in a thin layer (2–6 mm) into plastic cultivation containers (12 × 19 × 11 cm) filled with about 4–6.5 cm of cultivation substrate. The substrate was a mixture of fine sand, silty loam and peat (2:2:1), and had been sterilised for 3 h at 100°C. Usually, the majority of sub-samples from each sample were kept moist (terrestrial treatment), and the rest were shallowly flooded (submerged treatment; water level ranging from 2 to 5 cm). For very small samples, only the terrestrial treatment was used at the beginning of cultivation. For the samples from 2008 and 2009, the moisture conditions were changed in the second year of their attempted germination, and for the samples from 2010 this change was done in the year of collection, to allow germination of species with different moisture requirements. The containers were covered with fine, unwoven fabric to protect against the influx of local propagules. Cultivation was performed in a greenhouse without temperature and daylight regulation, thus simulating natural germination conditions. The temperatures ranged between 5 and 35°C. All seedlings that emerged were identified, counted and then removed from the container. Difficult to identify taxa (e.g. Poaceae) were transplanted to and cultivated in separate pots until their identities could be determined. Some seedlings died before they could be completely identified, and in these cases only approximate identification is given.

The samples containing *T. aquatica* seeds were summarised in the table (Table 4). For more detail on the samples see the Appendix 1—Supplementary Material. Following codes were used: F—floating material from fish storage ponds, C—drainage channels (bottom sediments), V—vehicles, G—gumboots, FSP—fish storage ponds (bottom sediments).

To obtain better comparison of germination rates of species occurring in larger quantities in our sediment samples and representing different ecobiological groups (in terms of habitat and life-history strategy), we used direct sowing on the cultivation substrate described above. The experiment was carried out on 12 plant species, including *T. aquatica*, all of which are known to grow on exposed pond bottoms. For each species, 300 seeds were used, with 100 allotted to each of the three cultivation treatments: (1) 10-week storage in tap water in refrigerator, spring sowing in greenhouse; (2) winter sowing in greenhouse, exposure to frost; (3) spring sowing of dry seeds stored in room temperature. These seeds had been obtained either in the field or from multiple samples of cultivated plants.

Data processing and analysis

We stored our phytosociological relevés in the Czech National Phytosociological Database (CNPD), using Turboveg database software (Hennekens & Schaminée, 2001; Chytrý & Rafajová, 2003). We then used published and unpublished relevés, stored in CNPD, and done by Kateřina Šumberová and other authors, both for comparison between historic and recent vegetation occurring with *T. aquatica* and for completion of the target species' distribution map. These relevés originated from 15 recent and 14 historical localities (29 localities in total), and the sources included Klika (1935), Ambrož (1939), Jílek (1956, 1960), Gazda (1958), Pešout (1996), Filípková (2001), Němcová (2004), Hejný (unpublished, coll. 1945), Chytrý (unpublished, coll. 2001). The distribution map (Fig. 1) also included floristic data summarised by Grulich (1985), and Holub & Grulich (1999), as well as our own floristic data. In organising the data for vegetation comparison and mapping purposes, we assembled two datasets of phytosociological relevés and a dataset of 60 localities for mapping (including 19 recent localities—see Table 1, and 41 historical localities).

The first dataset, with 88 standard phytosociological relevés, included historical and recent relevés from all known localities of *T. aquatica* in the Czech Republic. This dataset was used to show the variability of vegetation types in which *T. aquatica* has been documented. It included 19 recent relevés from fish storage ponds, 17 recent, and 51 historical (1930s–1950s) relevés from fishponds, and a single relevé from a water reservoir (1990, assigned as historical).

The second dataset was from our repeated sampling of the 42 permanent plots in 19 fish storage ponds of the Hluboká nad Vltavou fish storage pond system (managed by a single fish farm, Rybářství Hluboká CZ Ltd.). These data allow us to discern the dynamics of *T. aquatica* and its responses to different management practices. This dataset contained 1521 relevés collected in 2001–2009, of which only 153 relevés included the target species.

All the relevés were first processed using JUICE 7 software (Tichý, 2002). The first dataset was then classified using modified TWINSPAN software (Hill, 1979; Roleček et al., 2009), which split the dataset into the number of groups pre-defined by the user (and if the resulting categorisation is not meaningful or interpretable, the number of groups can be redefined and the analysis run again). Sørensen's index was used as a measure of dissimilarity amongst the clusters. The clusters yielded by the TWINSPAN classification were displayed in a DCA ordination diagram of plots (see Fig. 2).

Detrended correspondence analysis (DCA) in the CANOCO program (ter Braak & Šmilauer, 2002) was used to assess the main pattern of species composition and to visualise ecological requirements of *T. aquatica*. To interpret the DCA results, unweighted average Ellenberg indicator values for light, temperature, continentality, soil reaction, moisture and nutrients (Ellenberg et al., 1992) were calculated for each plot. Species cover values were transformed to percentages and their square roots calculated. Ellenberg indicator values, together with sampling year, were passively projected as vectors onto the ordination diagram.

To discern the population dynamics (reflected in changes in cover) of *T. aquatica* under strong herbicide control, we analysed only the second dataset from the permanent plots. The dataset contained 237 taxa of plants. Changes in dominance of *T. aquatica*, expressed as percentage cover on individual plots, were related to the above mentioned plot characteristics using a set of individual regression models with Poisson distributions. In these models, cover of *T. aquatica* was the dependent variable, and the vegetation characteristics were used as individual explanatory variables. Calculations were performed using STATISTICA 9 software (www.statsoft.com).

The taxonomy and nomenclature of all vascular plant species and bryophytes in this article follow Kubát et al. (2002) and Kučera & Váňa (2003). The algae were not identified, with the exception of *Botrydium granulatum* Grev. and *Nostoc commune* Vaux ex Born. et Flach.

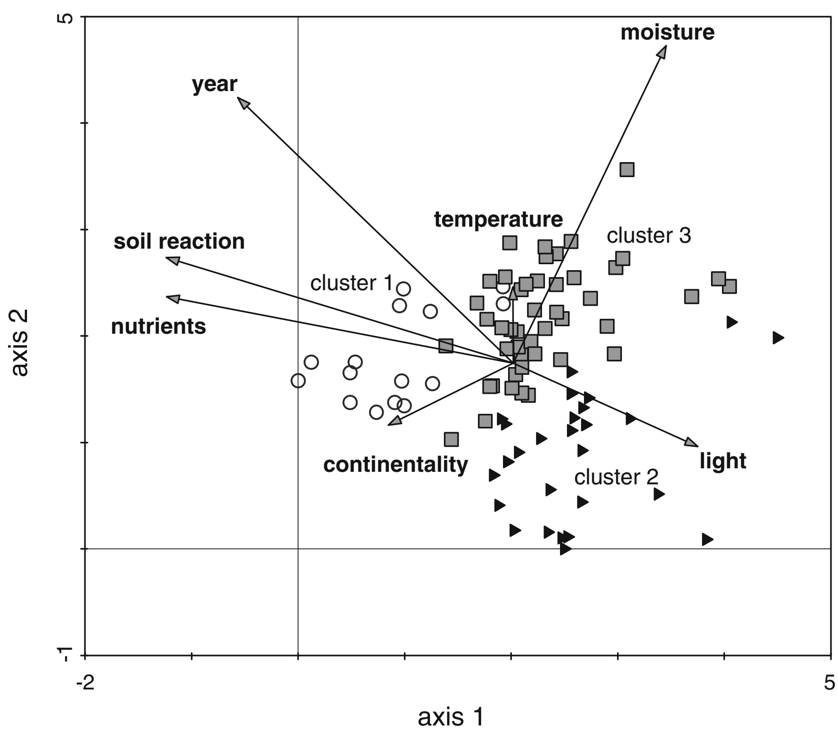

Fig. 2 DCA ordination, plots with passively projected sampling year and mean Ellenberg indicator values for each plot. *Eigenvalues* first axis 0.445, second axis 0.396. *Cluster 1* recent relevés from fish storage pond bottoms, *Cluster 2* historical relevés from sandy fishpond margins, *Cluster 3* recent and historical relevés from wet muddy bottoms (mainly of fishponds)

Results

Recent and historical distribution and population size of *T. aquatica*

The distribution map (Fig. 1) shows both the historical and the recently recorded occurrence of *T. aquatica* in the Czech Republic. Although the historical and recent distributions overlap, the frequency of the species has changed. Up until 1990 inclusive, 41 localities had been documented, whereas afterwards 19 localities were recorded (including loc. 6 in Fig. 1 and Table 1, where the species was detected only in the soil seed bank). The greatest proportional change between number of historical and recent localities was in the fishpond basin near the town Třeboň (see the extensive cluster of historical fishpond localities in Fig. 1). In the past, more than 1/2 of all known localities of the species in the national territory were concentrated there. A single locality now remains there.

A considerable shift was detected also in habitat types colonised by *T. aquatica*. Historical occurrence was documented mainly from fishponds, including large water bodies of areas of 100 or more hectares. However, recent populations were mostly found in fish storage ponds. The only region with currently known populations of the species in fishponds is the Bohemian-Moravian Uplands (locs. 11–19 in Fig. 1 and Table 1). In addition, all the recent sites harbouring *T. aquatica* are small-sized in comparison to the majority of those documented in the past. Thus, none of the recent exceeds 10 ha (for fish storage pond systems sum of areas of all individual ponds are considered).

The population size of *T. aquatica* at recent localities ranges from two individuals to more than 10,000. At 13 of the 19 localities, the populations were small or scattered, estimated mostly to be several hundred individuals. Some of these populations have not been confirmed in the last 5 years despite the intensive field investigations. Populations at six of the documented localities numbered more than thousand individuals. They were found in fish storage ponds that were exposed relatively long and under herbicide control or sheep grazing, and in some cases, in fishponds (for which management does not include these practices) (Table 1).

Vegetation associated with *T. aquatica*

As a result of TWINSPAN analysis the dataset of 88 relevés was split into three groups that could be well interpreted and which are shown in the DCA ordination diagram (Fig. 2). Cluster 1 includes 15 recent relevés, all collected in fish storage ponds. According to Ellenberg indicator values, this vegetation type shows a relationship with relatively nutrient rich substrata with higher soil reaction. Species such as *Gnaphalium uliginosum*, *Plantago uliginosa* and cyanobacteria *Nostoc commune* were abundant in this vegetation type (Fig. 3). The second group of relevés (cluster 2) contains 27 historical records. Cluster 2 appears on the opposite side of the nutrient and pH gradient from cluster 1 (Fig. 2). This vegetation is associated with relatively dry sites. Relevés in this cluster are characterised by species of nutrient poor, acidic substrata, e.g. *Gypsophila muralis* and *Juncus tenageia* (Fig. 3). The third relevé group (cluster 3; 46 relevés) includes 41 recent and historical relevés from fishponds, four relevés from fish storage ponds and a single relevé from a water reservoir. This cluster occupies the central and right upper parts of the DCA ordination diagram (Fig. 2) and shows a relationship with relatively high moisture and temperature. Species preferring wet muddy substrata, e.g. *Coleanthus subtilis*, *Elatine hydropiper*, *E. triandra* and *Limosella aquatica*, are typical of this vegetation type (Fig. 3).

Monitoring of vegetation on permanent plots in fish storage ponds in Hluboká nad Vltavou

The cover of *T. aquatica* significantly increased during the 8-year monitoring of permanent plots in fish storage ponds in Hluboká nad Vltavou between 2001 and 2009 (Table 2). The increased cover of *T. aquatica* was positively associated with increasing species richness, especially of bryophytes, and with higher cover in the herb layer and moss layer.

Seed bank and seed dispersal vector analysis, germination in various conditions

Tillaea aquatica seeds were detected in 8 (9%) of the total of 87 analysed sediment samples (Tables 3 and 4; for more detail on the samples see Appendix 1—Supplementary Material). The seeds were found in one pond bottom sample and seven samples from

Fig. 3 DCA ordination of vascular plants. Only plant species with the highest weights in the analysis are shown. *Eigenvalues* first axis 0.445, second axis 0.396

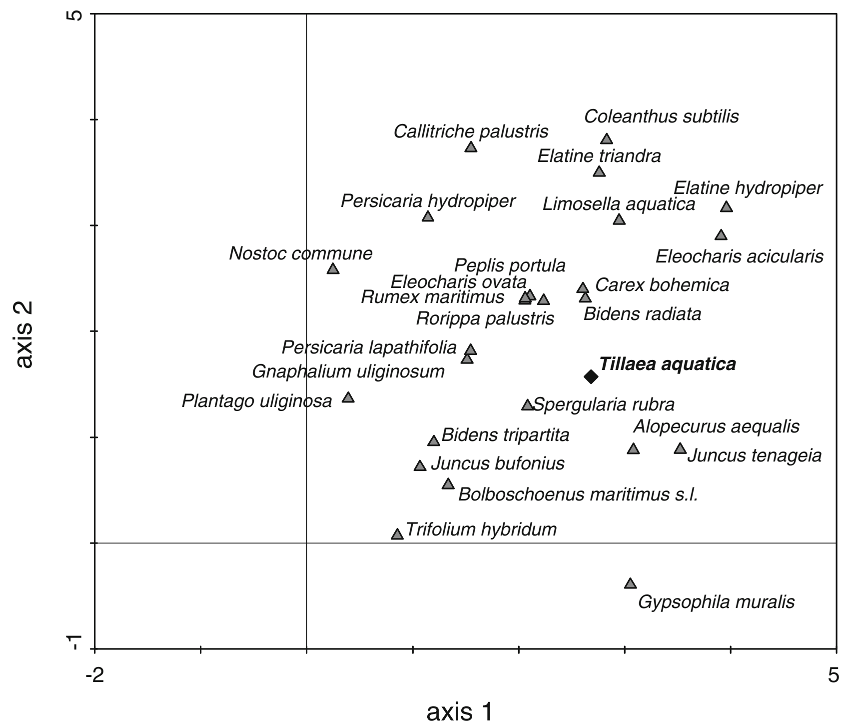

Table 2 Relationship between cover of *T. aquatica* and individual vegetation characteristics analysed by linear regressions with *T. aquatica* percentage cover as the dependent variable and the vegetation characteristics as explanatory variables

	Cover of *T. aquatica*
Number of all species	0.09*
Number of herb species	0.06*
Number of moss species	0.11**
Month	n.s.
Year (2001–2009)	0.18***
Herb cover	0.11***
Mosses cover	0.12***
Algae cover	n.s.
Mean Ellenberg indicator values calculated for herb species occurred on a plot	
Light	n.s.
Temperature	n.s.
Continentality	n.s.
Moisture	−0.07*
Soil reaction	n.s.
Nutrients	−0.05**

Slope and significance level are given. *n.s.* non significant, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

seed dispersal vectors. The highest seed density of *T. aquatica* was detected in floating material samples F1 and F2 from Hluboká fish storage ponds (Table 4; these floating material consisted mainly of biomass of *T. aquatica*). These samples, produced by division of an original, larger sample into two equal parts that were each stored in different conditions, showed significant differences in numbers of emerged seedlings. The number of seedlings in F2, which was allowed to dry in a refrigerator, was more than twice as high as in F1, which was stored in cold but without the possibility of drying (Table 4). Similarly, in the direct sowing experiment, *T. aquatica* germinated best in the treatment comprising cold and relatively dry storage (Fig. 4). There, it had a 100% germination rate, which was not attained in any other species in any treatment.

Despite the existence of large sources of propagules in Hluboká, other samples from this fish storage pond system and the equipment used in managing the ponds were much poorer in producing germinating *T. aquatica* (see samples F3, C1, C2, V1 and G1 in Table 4). Especially in bottom sediment from drainage channels (C1 and C2), there were more wetland annual species, e.g. *Cyperus fuscus* and *Callitriche palustris*,

Table 3 Summary of sediment samples from various items, showing representation of samples from Hluboká nad Vltavou in the entire sediment collection

Sampled item	Number of all				With *Tillaea* only			
	Samples		Localities		Samples		Localities	
	All	Hluboká f. f.	All	Hluboká f. f.	All	Hluboká f. f.	All	Hluboká f. f.
Fish storage ponds (bottoms)	15	2	8	1	1	0	1	0
Fishponds (bottoms)	22	22	10	10	0	0	0	0
Puddles on the roads	3	3	1	1	0	0	0	0
Canals and ditches (bottom sediments)	4	4	1	1	2	2	1	1
Floating material (canals, fish storage ponds)	6	6	1	1	3	3	1	1
Vehicles	18	17	9	8	1	1	1	1
Gumboots	8	8	6	6	1	1	1	1
Fish farming equipment	11	9	8	6	0	0	0	0
Total	87	71	24	15	8	7	2	1

In the second part of the table, only the samples with *T. aquatica* seeds detected are summarised. Usually more than one sample was taken from a given locality, thus the total numbers of localities do not correspond to sums in particular locality columns

Hluboká f. f. Hluboká nad Vltavou fish farm

with greater numbers of germinating seeds occurring for these species than for *T. aquatica* (Table 4). In 63 of the total of 71 samples from Hluboká, including 22 samples from 10 fishponds, we did not detect *T. aquatica* at all (Table 3). There was only one sample outside of Hluboká having *T. aquatica* (FSP1 in Table 1). This sample was taken from below stands of *Carex acuta* at loc. 6 (Table 1). *T. aquatica* and some other wetland annuals (e.g. *Lindernia procumbens* and *Pulicaria vulgaris*) were found there only in the soil seed bank, not in the vegetation. In 14 other samples from seven fish storage pond complexes, including recent and historic localities of *T. aquatica*, we did not detect any seed of this species (Table 3).

In all the samples with *T. aquatica,* non-wetland species, especially common nitrophilous weeds and neophytes, were also found. Sometimes they even formed an important part of the overall species spectrum, e.g. in sample V1 (Table 4).

Discussion

Changes in frequency and habitat preference, and their implications for threat status assessment of *T. aquatica*

Our results have shown considerable changes in occurrence of *T. aquatica* in the Czech Republic during the twentieth century. The most remarkable is the retreat of *T. aquatica* from South Bohemian fishponds where the species in the past formed large stands (Ambrož, 1939). Together with *T. aquatica*, typical vegetation of sandy fishpond margins with the species such as *Gypsophila muralis* and *Illecebrum verticillatum* also disappeared. However, the annual vegetation of shallow mud, on which *T. aquatica* can also grow, still occurs in the region quite frequently. Despite this, recently the species has not been confirmed in either the vegetation or bottom sediment seed bank of any South Bohemian fishpond, even though the region was investigated intensively. The most important reason for this retreat has probably been the widespread elimination of summer-long drying of fishponds, with drying now, if done, typically lasting just from March/April till May/June. Two months or even less is sufficient time for reproduction of short living annuals; e.g. *Coleanthus subtilis*, *Limosella aquatica* or *Elatine* spp. (Šumberová et al., 2005, 2006), but not for *T. aquatica*.

Large *T. aquatica* populations still survive in several South- and one Western-Bohemian fish storage pond complexes. It is likely that the species occurred there in the past as well, but historical data for the majority of these localities are missing because of the low research intensity on this habitat type in the past (Šumberová et al., 2005, 2006).

Table 4 Seed bank and seed dispersal vectors analysis: results of cultivation

	number of seedlings in samples															
	F1		F2		F3		C1		C2		V1		G1		FSP1	
species	sample	1l	sample	1l	sample	1l	sample	1l	sample	1l	sample	1l	sample	1l	sample	1l
Tillaea aquatica	**3580**	**7537**	**8127**	**17109**	**3**	**6**	**58**	**129**	**2**	**4**	**2**	**4**	**1**	**20**	**21**	**47**
Bidens frondosa (incl. cf.)	2	4	3	6	57	120	0	0	0	0	0	0	0	0	0	0
Limosella aquatica	0	0	0	0	0	0	73	162	29	65	0	0	0	0	20	44
Lindernia dubia (incl. cf.)	0	0	25	53	0	0	464	1031	361	801	0	0	0	0	0	0
Cyperus fuscus	0	0	0	0	1	2	346	768	73	163	0	0	2	40	510	1133
Callitriche palustris	0	0	0	0	0	0	283	629	88	195	1	2	1	20	127	282
Juncus bufonius (incl. cf.)	0	0	0	0	0	0	122	270	47	104	27	57	2	40	111	247
Juncus tenuis (incl. cf.)	0	0	0	0	0	0	1	2	0	0	67	141	0	0	1	2
Plantago major/uliginosa	0	0	0	0	0	0	5	11	0	0	27	56	8	160	131	291
Peplis portula	0	0	0	0	0	0	11	24	14	31	0	0	0	0	298	662
Lindernia procumbens	0	0	0	0	0	0	7	16	3	7	0	0	0	0	129	287
Gnaphalium uliginosum	0	0	0	0	0	0	13	29	6	13	0	0	0	0	112	249
Juncus compressus (incl. cf.)	0	0	0	0	0	0	0	0	0	0	5	11	0	0	111	247
Juncus sp.	0	0	0	0	1	2	9	20	4	9	39	82	0	0	72	160
Lemna minor/gibba	20	42	0	0	33	69	24	54	9	20	0	0	0	0	0	0
Juncus articulatus (incl. cf.)	0	0	0	0	1	2	44	97	8	18	1	2	0	0	0	0
Alisma plantago-aquatica (incl. cf.)	0	0	0	0	33	70	5	11	1	2	0	0	0	0	0	0
Spirodela polyrhiza	0	0	0	0	2	4	26	58	3	7	0	0	0	0	0	0
Poa annua	0	0	0	0	0	0	1	2	0	0	24	51	3	60	0	0
Lythrum salicaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	60
Lindernia sp.	0	0	0	0	0	0	3	7	40	89	0	0	1	20	0	0
seedlings in sample	3613	7606	8167	17194	177	373	1662	3693	765	1700	284	598	23	460	1840	4089
identified taxa in sample	9	–	10	–	23	–	49	–	37	–	34	–	10	–	36	–

Only the species with more than 50 seeds per litre of sample and the samples with *T. aquatica* detected are displayed. The total number of seedlings includes unidentified seedlings. Only in those samples for which no individuals were identified to the species level was genus level identification (or, if genus level identification was impossible, family level) included in the total number of identified taxa. The numbers of emerged seedlings of individual species and their recalculation per litre of sediment are given for each sample. Values higher than 1,000 seeds per litre, 500 seeds per litre, and 100 per litre are highlighted in colour. Values for *T. aquatica* are printed in bold. Species for which some of the individuals were only tentatively identified are indicated with "incl. cf." in columns. Codes: F – floating material, C – drainage channels (bottom sediments), V – vehicles, G – gumboots, FSP – fish storage ponds (bottom sediments)

Other species detected in the samples: *Agrostis tenuis* V1—1/2; *Alnus glutinosa* F2—2/4, F3—6/13; *Alopecurus aequalis* C1—4/9, V1—1/2; *Alopecurus geniculatus* C1—2/4, C2—2/4; *Betula pendula* C1—1/2, V1—5/11; *Bidens tripartita* F3—1/2, FSP1—7/16; *Calamagrostis epigejos* V1—1/2; *Capsella bursa-pastoris* F3—7/15, V1—1/2, G1—1/20; *Carex acuta* FSP1—10/22; *Carex bohemica* (incl. cf.) F1—1/2, F3—1/2, C1—3/7, C2—3/7, V1—2/4; *Carex* cf. *elongata* V1—1/2; *Carex nigra* FSP1—2/4; *Cerastium* cf. *holosteoides* C1—1/2; *Chenopodium album* agg. F3—2/4; *Chenopodium polyspermum* C2—3/7, FSP1—1/2; *Cyperus flavescens* C1—2/4; *Deschampsia cespitosa* V1—4/8; *Digitaria sanguinalis* F2—1/2; *Echinochloa crus-galli* C1—5/11, C2—2/4, V1—1/2, FSP1—2/4; *Elatine hydropiper* C1—7/16, C2—3/7; *Elatine triandra* (incl. cf.) C1—12/26, C2—4/9, FSP1—3/7; *Eleocharis acicularis* (incl. cf.) F3—2/4, C1—11/24, C2—1/2, FSP1—11/24; *Eleocharis ovata* F3—1/2, C1—18/40, C2—1/2; *Eleocharis palustris* agg. FSP1—1/2; *Epilobium ciliatum* C1—1/2, C2—7/16, FSP1—1/2; *Epilobium hirsutum* V1—1/2; *Epilobium tetragonum* agg. V1—1/2; *Equisetum arvense* F3—1/2; *Eragrostis minor* V1—1/2; *Erigeron annuus* agg. C1—1/2; *Galinsoga quadriradiata* F3—2/4; *Holcus lanatus* C2—3/7; *Juncus effusus* C1—3/7, C2—4/9, V1—19/40; *Juncus filiformis* FSP1—18/40; *Leersia oryzoides* C1—5/11, C2—3/7, FSP1—17/38; *Lycopus europaeus* F1—1/2, F3—5/11, V1—1/2; *Matricaria discoidea* V1—3/6; *Medicago lupulina* F3—1/2; *Mentha arvensis* FSP1—2/4; *Molinia arundinacea* V1—1/2; *Myosotis caespitosa* F1—1/2, F2—4/8, FSP1—1/2; *Myosoton aquaticum* C1—3/7; *Najas minor* C1—3/7, C2—2/4; *Persicaria hydropiper* C2—1/2, V1—2/4, FSP1—12/27; *Persicaria lapathifolia* FSP1—9/20; *Persicaria minor* F2—1/2, C1—2/4, V1—1/2, FSP1—8/18; *Phragmites australis* F1—1/2; *Plantago lanceolata* V1—1/2; *Poa palustris* C1—1/2, C2—1/2, V1—2/4; *Poa pratensis* C1—1/2; *Poa trivialis* (incl. cf.) C1—8/17, C2—7/16; *Polygonum aviculare* agg. C2—1/2, V1—16/34, G1—2/40, FSP1—2/4; *Potamogeton pusillus* s. str. C1—2/4, C2—3/7; *Pulicaria vulgaris* FSP1—2/4; *Ranunculus sceleratus* (incl. cf.) F1—3/6, F2—1/2, F3—2/4, C1—2/4, C2—2/4; *Rorippa palustris* (incl. cf.) F1—2/4, F2—1/2, F3—6/13, C1—20/44, C2—9/20, FSP1—12/27; *Rumex crispus* F3—1/2, FSP1—1/2; *Rumex maritimus* F2—1/2, C2—1/2; *Sagina procumbens* FSP1—11/24; *Scirpus sylvaticus* C1—1/2; *Solidago gigantea* C1—1/2; *Spergularia echinosperma/rubra* C1—5/11, C2—2/4, FSP1—11/24; *Stellaria alsine* V1—2/4; *Tanacetum vulgare* C2—1/2; *Trifolium hybridum* C1—1/2, FSP1—1/2; *Trifolium repens* V1—1/2; *Tripleurospermum inodorum* F3—1/2; *Urtica dioica* C1—3/7, C2—4/9, V1—2/4, FSP1—1/2; *Veronica anagallis-aquatica* C1—1/2; *Zannichellia palustris* C1—4/9; *Bidens* sp. F3—1/2, FSP1—1/2; *Callitriche* sp. (incl. *C.* cf. *stagnalis*) V1—3/6; *Chenopodium* sp. C1—2/4; *Epilobium* sp. C1—5/11; *Myosotis* sp. V1—1/2; *Persicaria* sp. FSP1—2/4; *Rumex* sp. C1—1/2; *Typha* sp. V1—2/4; *Verbascum* sp. G1—1/20; Poaceae indet. F3—1/2, C1—4/8, C2—1/2, V1—6/13, FSP1—1/2; not identified F1—2/4, F2—1/2, F3—5/11, C1—21/46, C2—6/13, V1—7/15, G1—1/20, FSP1—21/47

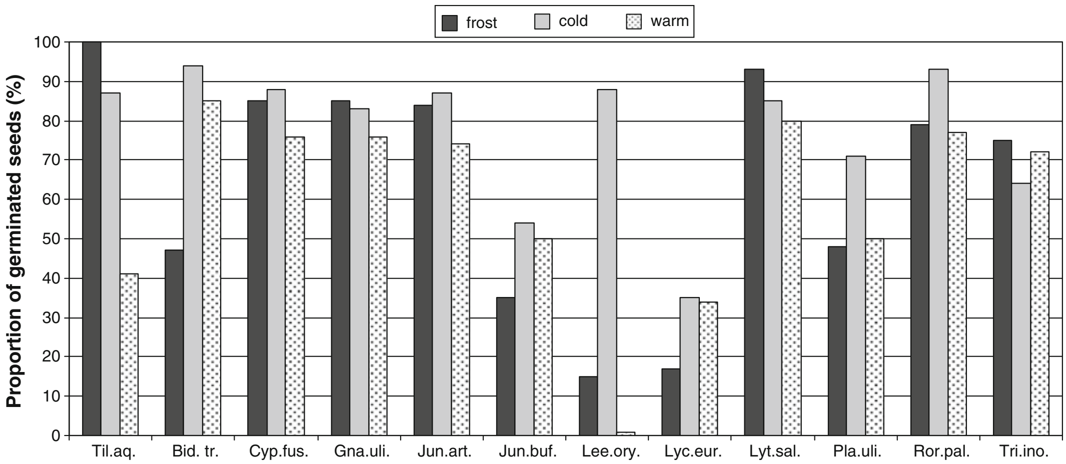

Fig. 4 Greenhouse germinability of *T. aquatica* and 11 other species found in similar habitats. Three various pre-treatments were used (see details in "Materials and methods"). Til.aq. = *Tillaea aquatica*, Bid.tr. = *Bidens tripartita*, Cyp.fus. = *Cyperus fuscus*, Gna.uli. = *Gnaphalium uliginosum*, Jun.art. = *Juncus articulatus*, Jun.buf. = *Juncus bufonius*, Lee.ory. = *Leersia oryzoides*, Lyc.eur. = *Lycopus europaeus*, Lyt.sal. = *Lythrum salicaria*, Pla.uli. = *Plantago uliginosa*, Ror.pal. = *Rorippa palustris*, Tri.ino. = *Tripleurospermum inodorum*

In the Bohemian-Moravian Uplands the situation is different. There, *T. aquatica* mainly occupies fishpond bottoms. The fishponds in this region are smaller and situated in higher altitudes, which does not allow for such intensive management. Many of them are still regularly dried for several months in summer. Owing to the colder climate, succession of taller and competitively stronger herbs on exposed pond bottoms is relatively slow and thus does not interfere with the growth of *T. aquatica*. In contrast, the fish storage ponds in this area are usually flooded during the whole growing season or dried for only a few weeks. In addition, it is common practice of local fish farms every few years to clean out fine muddy sediment from pond bottoms, leaving behind just the sandy substrate or to replace this substrate as well. These practices would explain the small sizes of the populations of *T. aquatica* recorded in fish storage pond systems in the Bohemian-Moravian Uplands, as they were probably temporary. At loc. 14, the population was not even found in the pond itself, but in a puddle on the edge of a road amongst the ponds.

Nearly all the recently known localities of *T. aquatica* were discovered in the last 10 years. As shown already in other articles (e.g. Šumberová, 2003, 2005; Šumberová et al., 2006), the scarcity of records of rare wetland annuals in the second half of the twentieth century was probably, to some extent, a consequence of insufficient field investigations of specific wetland habitats. Similar experiences with *T. aquatica* were also noted by Norwegian researchers (Often et al., 2005), who recently mapped coastal habitats and compared the results with an older and less systematic investigation. However, even in Scandinavia, where the species has a centre of its European distribution, it has experienced an actual decline because of eutrophication and land use changes, e.g. elimination of grazing (Rintanen, 1996; Stoltze & Phil, 1998). According to the IUCN classification criteria (IUCN, 2001), *T. aquatica* has recently been listed as nearly threatened (NT) in Finland and Sweden (Rassi et al., 2001; Heino & Toivonen, 2008; Gärdenfors, 2010), vulnerable (VU) in Norway (Kålås et al., 2010), and endangered (EN) in Denmark (Stoltze & Phil, 1998).

It is obvious that ecologically specialised plant species may become threatened even if they have large distribution ranges (Rabinowitz, 1981). Habitat destruction, eutrophication and global climate change (with, e.g. changes in flooding and exposure dynamics in natural habitats, or enhanced succession due to higher temperatures), may all contribute to further decline of *T. aquatica* and other low-growing and competitively poor rare wetland annuals. Given these long-term threats, taking steps to promote survival of these species, even if on man-made habitats associated with particular management regimes, e.g. fishponds and fish storage ponds, is of high importance (Květ et al., 2002; Šumberová, 2003; Deil, 2005).

For the assessment of wetland annual conservation status, monitoring of populations and distribution, it should be taken into account that for many of them, it might be difficult to discern changes in their frequency. This is due to the following two issues:

(1) Occurrence of these species is often small-scale, therefore requiring fine coordinate grids for their mapping. However, historical data are usually not so precisely localised that they would allow detailed analyses of temporary changes in species occurrence. For instance, large water bodies of several hundred hectares might need to be divided between two or more mapping fields, but if in historical sources the species is localised only according to the pond's name, then only one mapping field of the historical occurrence would likely be considered. Actual declines of the species could then be overlooked or underestimated because of the smaller number of mapping fields treated as occupied in the past.
(2) Many wetland annuals (besides *T. aquatica* also e.g. *Cyperus michelianus*, *Elatine* spp., *Isolepis setacea* and *Lindernia procumbens*) could easily be overlooked during usual floristic investigations, especially in the case of very small and scattered populations. In particular, these populations might be hidden to some extent in soil seed banks for a long time (Poschlod et al., 1999; Bernhardt et al., 2008). Doing relevés, with their required attention to detail, and analysing sediments possibly harbouring propagules of these species might give us further important information about their population dynamics.

Tillaea aquatica and herbicide use

Despite the endangerment of *T. aquatica* in many European countries, some intensive management practices that eliminate competition from tall herbs, especially perennials, can, in certain circumstances, actually favour its survival. Although both the use of herbicides and intensive grazing can eliminate these competitors, we focus here on herbicide application, as our data have revealed potentially important interactions between it and *T. aquatica* populations.

Our long-term monitoring of permanent plots confirmed the resistance of *T. aquatica* to herbicides having as their active substance glyphosate. These are known under the trade names Roundup, Roundup Bio, Clinic and others, and commonly used in fishpond management to eliminate weeds. In the Hluboká nad Vltavou fish storage ponds, this herbicide started to be used regularly in about 2000. Over the course of several years during our study, the vegetation largely changed, with the strong competitors that still occupied the plots in 2002 (see Šumberová et al., 2005), decreasing. The number and sizes of populations of *T. aquatica* at this locality multiplied, with the species contributing substantially to overall herb layer cover and even becoming dominant in several ponds. We interpret the increasing cover of *T. aquatica* (Table 2) as a result of the Roundup application.

At the same time as the increase in *T. aquatica*, the cover and species number of mosses in the permanent plots also increased. Like *T. aquatica*, mosses require longer bottom exposure and competition elimination for optimal growth. The association of *T. aquatica* with mosses is thus probably largely explained by their common preferences for these growing conditions. The low-growing bryophyte stands, unlike stands of tall vascular plants, do not represent important competition for *T. aquatica*.

As far as we know, our data are the first which have documented an increase in population density of a rare species as a consequence of herbicide application. However, it is not the only example of herbicide resistance in the genus *Tillaea*. This property has also been reported in *Tilllaea recurva*, an Australian species more commonly known under the name *Crassula helmsii* and considered as invasive in some parts of Europe (Dawson, 1996). It might be due to similar, specific physiological processes that occur in these related taxa.

However, it is important to keep in mind that the population effects on *T. aquatica* likely largely depend upon the identity of the herbicide used. This, too, is shown by data from our permanent plots. There, in 2009, the herbicide Reglone, containing diquat dibromide as its active substance, was used instead of Roundup for vegetation elimination. Although we observed sensitivity of *T. aquatica* to this herbicide and an apparently associated decline of this species in several ponds, the effect was not sufficiently strong to be reflected in the data analysis. Further monitoring is necessary to evaluate the long-term influence of such an event. It is possible that more important than the partial die-off of *T. aquatica* populations is the selective support of other species, especially some tall grasses which survived better after Reglone application than they would after use of Roundup.

Survival of *T. aquatica* in soil seed bank and possibilities of seed dispersal

Dense soil seed banks can prevent complete disappearance of species during temporary unsuitable conditions. In particular, for many small wetland annuals, long-term survival in soil seed banks has been confirmed and is considered a typical trait of this ecological group (Thompson et al., 1997; Poschlod et al., 1999). Moreover, in the case of *T. aquatica*, finding it occurring on the bottoms of large water reservoirs exposed rarely during extremely dry years or dam reconstruction (Jarolímek & Zaliberová, 1991; Pešout, 1996) suggests that this species can survive in the soil seed bank for many years. However, precise data about longevity of its seeds are lacking.

We found viable seeds of *T. aquatica*, and also two other threatened species, *Lindernia procumbens* and *Pulicaria vulgaris*, in the soil seed bank of a fish storage pond in a complex in which the pond bottoms were overgrown by dense stands of *Carex acuta*, with these seeds representing the first recorded occurrences of these species at this locality. At least for *T. aquatica* and *Lindernia procumbens*, in the one pond that we sampled, the inferred seed bank densities were quite high, likely representing stable populations rather than seeds recently introduced from elsewhere.

However, in interpreting the results of our germination studies, it should be taken into account that the samples we used were not very large due to space limitation in the greenhouse. Therefore, population sizes we determined could be either overestimates or underestimates. Moreover, it cannot be excluded that, in some of the South Bohemian fish storage pond complexes that we examined, small populations of *T. aquatica* still exist, even though the species was not detected in either vegetation or the soil seed bank. Nevertheless, large, persistent, recent populations would surely have been detected even in small-sized samples.

We did not analyse bottom sediment from any fish storage pond with a recently known, rich *T. aquatica* population, and therefore do not have exact knowledge about soil seed bank densities of the species at such localities. However, we estimate it to be several thousand seeds per litre of bottom sediment (i.e. several dozens of thousands on 20 l which corresponds to 2-cm layer of sediment on 1 m^2 of bottom), as these amounts were detected in the floating material taken from one of the fish storage ponds in Hluboká (loc. 7). Although some of this floating material, with attached seeds, is always removed from the pond during the cleaning of the outlet and some is carried out by water after the cleaning briefly permits freer water flow, it is likely that most of the *T. aquatica* seeds stay in the pond. According to our observations, the fruits of *T. aquatica* mature continually during the summer and autumn, and thus their seeds would be released and lodged in the soil at the bottom before pond flooding. Moreover, the relatively low number of seeds found in drainage ditches also indicates few of them are transported from fish storage ponds via water. Milberg & Stridh (1994) upon analysing the soil seed bank of *T. aquatica* in natural wetlands in central Sweden, estimated 51,800 seeds/m^2 of topsoil. Seed bank density of *T. aquatica* in Hluboká fish storage ponds may be similar or even higher, as 1 m^2 of topsoil in the above-cited study corresponds to 60 l (the sediment was collected up to 6-cm depth). Therefore, the number of seeds that might be there is 863 per litre.

Although some *T. aquatica* seeds are transported via water or on gumboots from fish storage ponds into their surroundings (including drainage canals from which they can reach a river, or puddles from which they can be picked up by vehicle wheels), the number of such transported seeds is probably not very high in species such as *T. aquatica* that lack specific dispersal adaptations (e.g. buoyant seeds, adhesive testa). However, in annual species characterised by short reproductive cycles and high seed production, even a small number of seeds is sufficient for successful colonisation of new localities.

We hypothesise that in wetland annuals, which can be dispersed by many vectors, including water, waterfowl (Hejný, 1969; Salisbury, 1970; Lampe, 1996), fish (Šumberová et al., unpublished data) and—in fish farming facilities—also with farming equipment and vehicles, colonisation success is limited mainly by the existence of suitable habitat. The environmental limitations on habitat suitability may impinge on any part of the life cycle, e.g. during the seed entrapment and germination phase (Fenner & Thompson, 2005) or the reproductive phase. *E. hexandra*, like *T. aquatica*, a rare mudflat species with disjunctive distribution range, has a large mismatch between its abundance and seed dispersal possibilities (Salisbury, 1967). This species is ecologically close to *T. aquatica*, frequently being found in the same communities (Ambrož, 1939; Pietsch, 1973).

In the case of the slow-growing *T. aquatica*, suitable conditions for its full establishment are provided only by sites where the substrate is exposed or very shallowly flooded for several months at a time and tall forb vegetation is absent. Moreover, the timing of flooding is critical. *T. aquatica* germinates in the spring, and as shown in our germination experiment, cold stratification significantly improves its germination rate. Thus, on pond bottoms exposed during the winter, the germination rate will probably be very high in the following spring. However, if the pond is flooded in March or April, these seedlings will die and the soil seed bank can be rapidly exhausted. Although keeping the pond dry for the entire spring and at least part of summer could enable *T. aquatica* to mature and contribute to the seed bank, maintaining its population, such a site also provides habitat for competitively superior tall forbs. This competition can result in reduced viability, reproductive ability and exclusion of *T. aquatica*. The impact from such superior competitors is exacerbated by eutrophication because of fish pond fertilisation, because, unlike those competitors, *T. aquatica* does not increase its biomass in response to nutrient enrichment.

In considering the effects of interspecific competition on *T. aquatica* in particular and rare wetland annuals in general, we should recognise that potential competitors are not limited to those individuals already present at a site in its soil seed bank or vegetation, but also those seeds of competing species that are transported simultaneously with those of the rare species of interest. These simultaneous arrivals (see a number of ruderals in Table 4) could spatially or temporally limit the development of rare species populations.

Connectivity of European distribution ranges of *T. aquatica* and other wetland annuals

The disjunctive distribution range of wetland annuals such as *Carex bohemica*, *Coleanthus subtilis*, *Elatine hydropiper*, *T. aquatica*, etc., are usually explained as a consequence of long-distance dispersal of these species via waterfowl. However, we do not yet have unequivocal evidence of this mode of long-distance dispersal occurring in mudflat species (Deil, 2005). Thus, although the occurrence of mud containing seeds of wetland annuals on feet and bills of waterfowl has been reported (e.g. Salisbury, 1970), it is not clear how far this was transported. Kącki (2005) compared the distribution range of *T. aquatica* and routes of waterfowl migrations and found that they overlap. He considered, therefore, the European populations of *T. aquatica* outside of Scandinavia as ephemeral and regularly re-established by the continuous introduction of seeds by waterfowl. Although in the Czech Republic an influence of migrating birds cannot be excluded, historic populations of *T. aquatica* reported for some large fishponds were numerous and stable for many years (Ambrož, 1939; Grulich, 1985). Most likely, they were also sources of propagules for fish storage ponds and small fishponds where the species survived until recent times. Resolving the question of the role of long-distance dispersal in the origin of local populations of *T. aquatica* and other rare wetland annuals will require genetic analysis of existing European populations of these plants.

Conclusions: management recommendations for further survival of *T. aquatica*

The threatened wetland plant species *T. aquatica* has a chance to survive in the current landscape, even in the limiting conditions on the margin of its range. In Central Europe, survival of this and many other mudflat species (e.g. *Carex bohemica*, *Coleanthus subtilis*, *Elatine* spp. and *Eleocharis ovata*) is strongly dependent on the maintenance of fish farming at least in its present form. Thus, management intensification, or large-scale changes in the use of the ponds, such as entirely omitting summer drainage (as an unprofitable practice), could eliminate *T. aquatica*. Moreover, in order for summer drainage to be effective in maintaining *T. aquatica* populations, in those fishponds potentially harbouring this species the water level should be lowered for at least 3–4 consecutive months every few years, as this is necessary for successful reproduction of the species. When the water level is lowered, it should expose a strip of the pond bottom, at least 2 m wide, along the edge of the fishpond, with summer drainage of the entire pond not necessary.

In addition to these recommendations regarding fish farm management, we make the following general observations and recommendations concerning the maintenance of favourable conditions for *T. aquatica*, based on its traits:

(1) The habitat must have low cover of competitively strong species during the period that *T. aquatica* germination occurs (i.e. in March–April in Central Europe, probably later in Northern Europe), and be waterlogged or very shallowly flooded.
(2) In habitats with longer exposure phases and higher nutrient amounts, elimination of competitively strong herbs is necessary. It can be performed by herbicide application, grazing, or physically removing the competing vegetation.
(3) At some localities, population recovery can proceed from the soil seed bank. Also, floating material containing high amounts of *T. aquatica* parts with associated mature seeds would provide a readily available source of propagules to establish new, nearby populations.

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