



# Fitness and growth of the ephemeral mudflat species *Cyperus fuscus* in river and anthropogenic habitats in response to fluctuating water-levels



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## ABSTRACT

*Cyperus fuscus* is a representative of threatened ephemeral wetland plant communities in summer-dry shoreline habitats. We compared variation and plasticity in traits related to fitness and growth of plants germinating from the soil seed bank and established plants from river and secondary anthropogenic habitats. Plants from sites at rivers, fishponds and fish storage ponds were cultivated and selfed to get homogenous seed material for a germination and an environmental manipulation experiment involving three different water regimes. Differences in traits and their plasticities were evaluated by means of linear mixed models. *Cyperus fuscus* followed a low-oxygen escape strategy when flooded. Seeds of plants derived from the soil seed bank germinated faster than seeds of plants derived from established plants suggesting that short-term selection of genotypes is mediated by the particular conditions on the site during germination. The experiment revealed significant differences between river and secondary habitats as well as between the soil seed bank and established plants. For example, plants from river habitats produced the highest number of culms with inflorescences. The difference was most evident under partial submergence. Plants from fish storage ponds rapidly reached the reproductive phase, but produced less culms with inflorescences. This seemingly allows them to cope with numerous and irregular disturbances and intensive substrate moisture changes. Our results suggest that populations have adapted to conditions at secondary habitats provided by fish farming during the last centuries.

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## 1. Introduction

Habitat loss and degradation are the primary environmental causes of biodiversity decline at local, regional and global scales (van Vuuren et al., 2006). For both species and ecosystems, many of the changes assumed for the 21st century can be best described as shifts in potential distribution, with favorable conditions vanishing

in some places, which may cause local extinctions, and appearing in new places, which may result in colonizations (Pereira et al., 2010). Possible secondary “rescue” (substitute) habitats of endangered species are therefore crucial for the conservation of biodiversity. However, when species from an endangered habitat find refuge in a different habitat type, conditions in the new environment might select for genotypes, which differ from those suitable for the original habitat. Consequently, species persist, but with a modified genetic structure.

Reciprocal transplant and common garden experiments have repeatedly shown that plant populations are adapted to local environmental conditions (e.g., Clausen et al., 1941; Leimu and Fischer, 2008; Rice and Knapp, 2008; Ågren and Schemske, 2012). Evolution can lead to specialization, generalization or adaptive phenotypic plasticity (Anderson et al., 2014). In strongly divergent environ-

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ments, particularly when conditions change slowly relative to the lifespan of an organism, specialization through local adaptation of ecotypes to a narrow range of conditions is favorable. Generalization and adaptive phenotypic plasticity may evolve, when the individuals must face multiple environmental conditions during their or their parents' lifetime (Anderson et al., 2014).

Flooding stress occurring in wetland ecosystems varies in seasonal timing, duration, water depth, and frequency. For many plant species, flooding has a devastating effect on performance, but wetland species are adapted to survive temporary submergence. Evolution of suites of traits is evident in wild wetland species and in rice, adapted to particular flooding regimes (Colmer and Voeselek, 2009; Voeselek et al., 2014). The main reason why flooding hampers plant performance is a negative effect on photosynthesis due to slower gas exchange and reduced light availability. Wetland species are thus usually characterized by an adaptive plastic response to flooding involving rapid shoot elongation to restore air contact and the formation of large portions of aerenchyma to facilitate internal gas diffusion (low-oxygen escape strategy; Kende et al., 1998; Vretare et al., 2001; Bailey-Serres and Voeselek, 2008). This response seems to be favorable in environments with shallow and prolonged floods (Voeselek et al., 2004). Alternatively, wetland plants may adopt a quiescence strategy by dampening energetically expensive processes such as growth, allowing endurance of deep floods or floods short in duration (e.g., Voeselek et al., 2004; Akman et al., 2012; van Veen et al., 2013).

Annual mudflat species survive the flooded conditions by maintaining an enormous soil seed bank as one of their key life history traits (Bernhardt, 1993; von Lampe, 1996; Deil, 2005; Baskin and Baskin, 2014). Soil seed banks may function as a “genetic memory” by storing genetic variability and hence local adaptation to habitat conditions in viable seeds buried in the soil (Leck et al., 1989; Mandák et al., 2012). The mudflats are usually exposed in summer, enabling the short-lived terrestrial species to complete their life cycle (Hejný, 1960). However, the exposed substrate along rivers may be suddenly flooded by summer flood pulses during the growing season as well and should therefore favor a low-oxygen escape strategy as a plastic response to flooding (Voeselek et al., 2004). Regulation of the majority of the world's rivers, however, has led to a dramatic loss of floodplain habitats, so that a large portion of the aquatic and temporary wetland biodiversity is nowadays concentrated in small water bodies distributed in the landscape (e.g., Davies et al., 2008; Hein et al., 2016; Richert et al., 2016). Fishpond and fish storage pond systems with a historical Central European distribution hotspot in the Czech Republic provide a rich mosaic of different wetland habitats with relatively natural features suitable as substitute habitats for threatened mudflat species (Květ et al., 2002; Šumberová et al., 2012; Wezel et al., 2014; Richert et al., 2016). The water level along the amphibious shorelines of fishponds depends on both weather conditions and management, but the hydrologic regime of fish storage ponds is decoupled from weather conditions and only depends on management. The modified hydrologic regime in the anthropogenic habitats might interfere with the ability of the plants to respond to temporary flooding.

*Cyperus fuscus* L. is a typical representative of ephemeral mudflat communities. Using *C. fuscus* as a model, we aimed at testing the hypothesis that divergent selective pressures in three habitat types, amphibious shorelines of rivers, fishponds, and fish storage ponds, each with specific hydrologic regimes, lead to local adaptation and phenotypic differentiation. Specifically, we asked the following questions: (1) What is the plastic response of *C. fuscus* to temporary flooding? (2) Are there any differences detectable between plants derived from the soil seed bank and plants derived from the above-ground population? (3) Are populations adapted to the specific conditions in the three habitat types? Do plants from the

three habitat types persistently vary in their response to flooding, i.e. in their plasticity in growth parameters? To answer our questions and assess phenotypic plasticity, we applied three different water treatments in an environmental manipulation experiment by using plants grown from seeds collected from soil seed bank and above-ground populations.

## 2. Material and methods

### 2.1. Study species

The brown galingale *Cyperus fuscus* (Cyperaceae) is a summer-annual, self-compatible graminoid native to the Mediterranean Region and temperate Eurasia (East, 1940; McKenzie et al., 1998; Bryson and Carter, 2010). The small achenes have no particular dispersal features and are supposedly dispersed by wind, water, and in mud carried away by animals (e.g., waterfowl) or humans (e.g., on footwear or vehicles; von Lampe, 1996; Šumberová et al., 2012). *Cyperus fuscus* grows on muddy, sandy or gravelly substrates in habitats subjected to changing water levels and characterized by low competition of perennial plants. As a small ephemerohyte, it is a typical component of amphibious short annual vegetation, and a pioneer of land interface zones of rivers, lakes, pools and ponds, where it grows during periodic drying of these waters (class Isoëto-Nanojuncetea Br.-Bl. et Tx. 1943). The species can also be found in secondary habitats like gravel pits, wet fields as well as traditionally used fishponds and fish storage ponds, which are mainly used for breeding of common carp (*Cyprinus carpio* L.) in the Czech Republic and some other regions of Europe. In the Old World, the species itself is not threatened, but its habitat is a priority habitat of the European Habitats Directive (code: 3130) and includes many rare species (Deil, 2005; Altenfelder et al., 2014). As other mudflat species, *C. fuscus* establishes a persistent soil seed bank, out of which seeds germinate under favorable conditions (Baskin and Baskin, 2014).

### 2.2. Characterization of the studied habitats

From July to October 2012, we visited 33 localities of the three different habitat types, rivers (11 localities), fishponds (10), and fish storage ponds (12), in Austria, the Czech Republic, Poland and Slovakia (Table 1, Fig. 1). Every sampled locality is unique, but still a typical variant of one of the three habitat types – amphibious shorelines of rivers, fishponds, and fish storage ponds – that face different hydrologic regimes. A microsatellite analysis shows that there is no clear neutral genetic differentiation among the three habitat types (Böckelmann et al., unpublished data). Suitable river habitats have become rare due to regulation of the water flow in the last centuries (e.g., Hein et al., 2016). The level of the streaming water is changing fast and dynamically and is therefore unpredictable, but the mudbanks are usually exposed in later summer (Hejný, 1960). River habitats in the South Bohemian Region, where the density of anthropogenic habitats is highest, are not suitable for *C. fuscus* because of the acidic granite substrate and the small size of the rivers. The two secondary habitats differ from rivers and from each other. Historical, semi-intensively managed fishponds, as relatively natural habitats for this species, face regular and managed changes of water level by man and climate (Květ et al., 2002). The sapropelic mud is usually thick, nutrient rich and exposed for a few months in summer. After fish harvest in autumn, the fish is stored alive in the fish storage ponds before it is sold. Flooding and management is based on ad hoc decisions of the fish farmers and so is less predictable. Most of the year, however, these concrete tanks with stony ground are without water. Management of fish storage ponds is aimed at maintenance of low-nutrient conditions and

**Table 1**  
Locations of the 33 populations of *Cyperus fuscus* sampled in Central Europe.

Locality, collectors, voucher number (WHB <sup>a</sup> ) and collection date	Coordinates	Sea level	Germination and flooding experiment
<b>Rivers</b>			
Czech Republic, Central Bohemian Region, Zárby: shoreline of river Labe (JB, KŠ, KT, PK, SP, ZH <sup>b</sup> ; WHB 62957; 21.08.2012)	N 50° 13.424' E 14° 37.717'	168 m	Included
Czech Republic, South Moravian Region, Lanžhot: shoreline of river Dyje (JB, KŠ, KT; WHB 62982; 10.09.2012)	N 48° 42.710' E 16° 54.169'	152 m	Not included
Czech Republic, South Moravian Region, Lanžhot: river arm of river Dyje (JB, KŠ, KT <sup>b</sup> ; WHB 62981; 10.09.2012)	N 48° 40.354' E 16° 55.442'	150 m	Included
Czech Republic, South Moravian Region, Moravská Nová Ves: oxbow of river Morava (JB, KŠ, KT <sup>b</sup> ; WHB 62980; 11.09.2012)	N 48° 47.079' E 17° 4.793'	162 m	Not included
Czech Republic, South Moravian Region, Velké Němčice: alluvial sediments of river Svratka (JB, KŠ, KT <sup>b</sup> ; WHB 62979; 11.09.2012)	N 48° 59.056' E 16° 39.894'	183 m	Included
Czech Republic, Ústí nad Labem Region, Nebočady: artificial pool of river Labe (JB, KŠ, PK, SP <sup>b</sup> ; WHB 62978; 13.09.2012)	N 50° 43.767' E 14° 11.222'	130 m	Not included
Austria, Lower Austria, Zwentendorf: shoreline of river Traisen <sup>a,c</sup> (JB <sup>b</sup> ; WHB 62956; 18.09.2012)	N 48° 22.245' E 15° 50.283'	183 m	Included
Austria, Lower Austria, Markthof: sidearm of river March (JB, KŠ, PK <sup>b</sup> ; WHB 62974; 05.10.2012)	N 48° 11.485' E 16° 58.302'	126 m	Not included
Poland, Lower Silesia, Borków: artificial pool of river Odra (JB, KŠ, PK <sup>b</sup> ; WHB 62973; 02.10.2012)	N 51° 40.477' E 16° 12.239'	76 m	Included
Poland, Lower Silesia, Cigalice: artificial pool of river Odra (JB, KŠ, PK <sup>b</sup> ; WHB 62955; 03.10.2012)	N 52° 1.883' E 15° 36.659'	55 m	Included (only germination experiment)
Slovakia, Bratislava Region, Vysoká pri Morave: river arm of river Morava (JB, KŠ, PK <sup>b</sup> ; WHB 62972; 05.10.2012)	N 48° 18.739' E 16° 54.224'	155 m	Not included
<b>Fishponds</b>			
Czech Republic, Liberec Region, Zahradky: fishpond Novozámecký, used for marketable fish, dried after many years due to dam reconstruction (JB, KŠ, KT <sup>b</sup> ; WHB 62967; 10.07.2012)	N 50° 37.687' E 14° 32.595'	252 m	Included
Czech Republic, Central Bohemian Region, Petrovice: fishpond Horní Petrovický, used for fish fry, regularly dried in summer (JB, KŠ, KT <sup>b</sup> ; WHB 62965; 11.07.2012)	N 49° 43.099' E 14° 39.030'	400 m	Included
Czech Republic, Central Bohemian Region, Libohošť: fishpond Libohošťský, used for fish fry, regularly dried in summer (JB, KŠ, KT <sup>b</sup> ; WHB 62964; 11.07.2012)	N 49° 42.057' E 14° 35.398'	374 m	Not included
Czech Republic, South Bohemian Region, Chrástovice: fishpond Chválovec, used for fish fry, regularly dried in summer (JB, KŠ, KT <sup>b</sup> ; WHB 62962; 13.07.2012)	N 49° 19.262' E 13° 53.836'	466 m	Not included
Czech Republic, South Bohemian Region, Rojice: fishpond Velkorojický, used for marketable fish, low water level after about 5 years (JB, KŠ, KT <sup>b</sup> ; WHB 62961; 13.07.2012)	N 49° 20.998' E 13° 56.540'	457 m	Included
Czech Republic, Plzeň Region, Smrkovec: fishpond Velký Smrkovec, used for marketable fish, regularly with low water level due to small inflow (JB, KŠ <sup>b</sup> ; WHB 62997; 24.07.2012)	N 49° 20.211' E 13° 35.915'	473 m	Not included
Czech Republic, South Bohemian Region, Skaličany: fishpond Pýcha, used for fish fry, regularly dried in summer (JB, KŠ <sup>b</sup> ; WHB 62996; 24.07.2012)	N 49° 26.078' E 13° 54.699'	489 m	Not included
Czech Republic, Central Bohemian Region, Sedlečko: fishpond Velký Sedlečský, used for marketable fish, in biennial intervals with low water level (JB, KB <sup>b</sup> ; WHB 62994; 27.07.2012)	N 49° 41.614' E 14° 32.091'	447 m	Not included
Czech Republic, Central Bohemian Region, Solopysky: fishpond Dolní Solopyský, management unknown, very often low water level (JB, KB <sup>b</sup> ; WHB 62992; 02.08.2012)	N 49° 39.217' E 14° 23.095'	379 m	Included
Czech Republic, South Moravian Region, Křepice: unnamed small village fishpond, drained after many years due to dam reconstruction (KB <sup>b</sup> ; WHB 62983; 05.09.2012)	N 48° 59.193' E 16° 5.641'	334 m	Included
<b>Fish storage ponds</b>			
Czech Republic, Pardubice Region, Lázně Bohdaneč: fish storage ponds with long summer drainage, mowing and herbicide spraying (started 2012) (JB, KŠ, KT <sup>b</sup> ; WHB 62970; 09.07./20.08.2012)	N 50° 4.994' E 15° 39.887'	220 m	Not included
Czech Republic, Liberec Region, Doksy: fish storage ponds with long summer drainage and grazing (JB, KŠ, KT <sup>b</sup> ; WHB 62969; 10.07.2012)	N 50° 33.830' E 14° 39.532'	266 m	Included
Czech Republic, Central Bohemian Region, Mšec: fish storage ponds with long summer drainage and herbicide spraying (JB, KŠ, KT <sup>b</sup> ; WHB 62963; 12.07.2012)	N 50° 11.815' E 13° 54.651'	410 m	Not included
Czech Republic, South Bohemian Region, Hluboká nad Vltavou: fish storage pond with short summer drainage and herbicide spraying (JB, KŠ, KT <sup>b</sup> ; WHB 62960; 14.07.2012)	N 49° 2.624' E 14° 25.952'	376 m	Included
Czech Republic, Central Bohemian Region, Nedrahovice: fish storage ponds with short to long summer drainage and herbicide spraying (JB, KB <sup>b</sup> ; WHB 62993; 01.08.2012)	N 49° 36.856' E 14° 27.600'	360 m	Not included
Czech Republic, Central Bohemian Region, Semovice: fish storage pond with short summer drainage and occasional herbicide spraying	N 49° 45.067'	357 m	Included

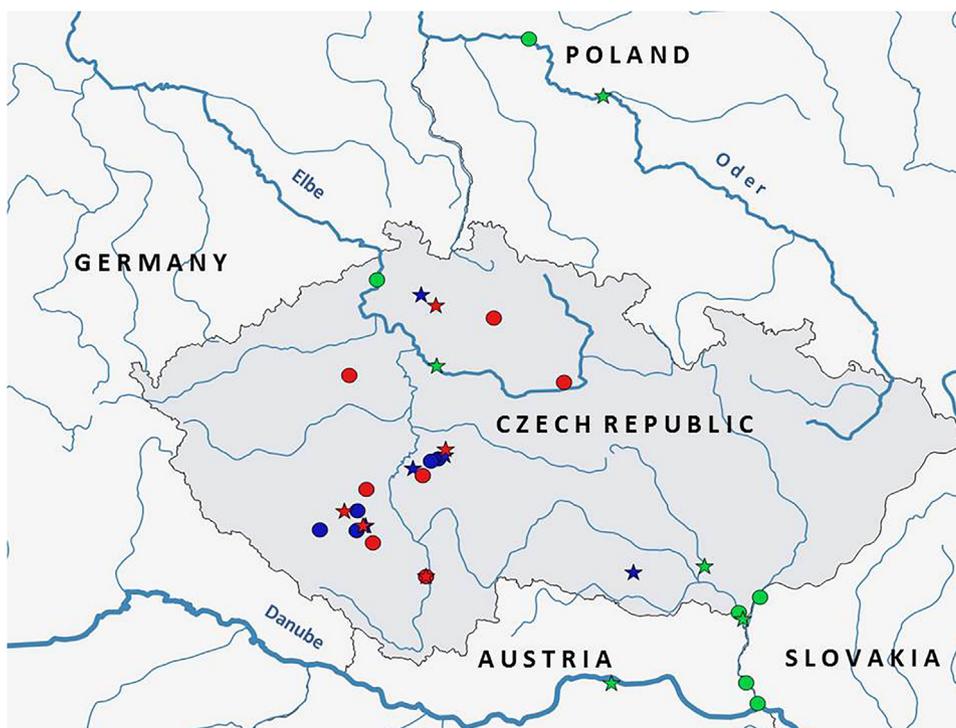
Table 1 (Continued)

Locality, collectors, voucher number (WHB <sup>a</sup> ) and collection date	Coordinates	Sea level	Germination and flooding experiment
(JB, KB <sup>b</sup> ; WHB 62959; 06.08.2012) Czech Republic, South Bohemian Region, Hluboká nad Vltavou: fish storage pond with long summer drainage and herbicide spraying	E 14° 39.655' N 49° 2.686'	372 m	Not included
(JB, KB, KŠ <sup>b</sup> ; WHB 62991; 07.08.2012) Czech Republic, South Bohemian Region, Čejetice: fish storage pond with long summer drainage and mowing	E 14° 25.991' N 49° 14.973'	387 m	Not included
(JB, KŠ <sup>b</sup> ; WHB 62958; 08.08.2012) Czech Republic, South Bohemian Region, Rojčice: fish storage pond with long summer drainage, mowing and low-intensity poultry grazing	E 14° 1.331' N 49° 20.842'	450 m	Included
(JB, KŠ <sup>b</sup> ; WHB 62989; 08.08.2012) Czech Republic, Central Bohemian Region, Dobrá Voda: fish storage pond with long summer drainage and sheep grazing	E 13° 56.903' N 49° 33.247'	449 m	Not included
(JB <sup>b</sup> ; WHB 62988; 09.08.2012) Czech Republic, South Bohemian Region, Tchořovice: fish storage pond with long summer drainage and mowing	E 13° 59.790' N 49° 26.115'	447 m	Included
(JB <sup>b</sup> ; WHB 62987; 09.08.2012) Czech Republic, Central Bohemian Region, Střehom: fish storage ponds with short summer drainage, mowing and herbicide spraying	E 13° 48.442' N 50° 28.341'	252 m	Not included
(JB, KŠ, KT, SP <sup>b</sup> ; WHB 62985; 23.08.2012)	E 15° 7.952'		

<sup>c</sup>Traits of plants collected in the field not measured.

<sup>a</sup> WHB = Herbarium of the University of Natural Resources and Life Sciences, Vienna.

<sup>b</sup> Collectors: JB = Jörg Böckelmann, KB = Kateřina Bubíková, KŠ = Kateřina Šumberová, KT = Karin Tremetsberger, PK = Pavel Kúr, SP = Soňa Pířová, ZH = Zdenka Hroudová, ZK = Zygmont Kački.



**Fig. 1.** Map of study sites of *Cyperus fuscus* populations in Central Europe. Circles represent sites investigated in the field only. Stars represent sites further analyzed in the germination and environmental (water level) manipulation experiment. Green symbols represent near-natural sites along rivers (n = 11), blue symbols fishponds (n = 10), and red symbols fish storage ponds (n = 12). Details of the corresponding localities are shown in Table 1.

elimination of muddy sediments with high water capacity. High ground temperatures during summer may create drought stress. To maintain the basins, the ground vegetation is removed by grazing, mowing or, more recently, herbicides (Šumberová et al., 2006, 2012).

### 2.3. Field studies and sampling for experiments

At each locality, we established three 1 m<sup>2</sup> plots within the *C. fuscus* population. On each of these 1 m<sup>2</sup> plots, preferably twelve

ripe plants were collected. The roots of these plants were rinsed out in the field and the shoot height was measured. Thereafter, the plants (1162 in total) were dried at ambient temperature. Their shoot and root biomass as well as the number of culms with inflorescences were determined. After that, plants were stored at 6 °C in the dark for six months.

Further, five soil samples, each with two fractions, 0–5 cm deep soil (shallow seed bank) and 5–15 cm deep soil (deep seed bank), were taken in every 1 m<sup>2</sup> plot, four samples in the corners and the fifth in the middle. The first 5 mm of topsoil were removed

and the shallow seed bank was sampled with a metal square (10 cm × 10 cm). The deep seed bank was sampled with a shovel. After removal of plant fragments and stones, the soil samples were cooled for the rest of the field trip and then stored at 6 °C in the dark for approximately six months.

#### 2.4. Preparation of seed material for experiments

In April 2013, the five soil samples of the same plot and fraction were pooled and carefully homogenized. Six hundred grams of the resulting mixed soil sample were spread out as a 5 mm thin layer on a 3 cm layer of sterile sand in the glasshouse of the University of Natural Resources and Life Sciences, Vienna, Austria, in a single 54 cm × 31 cm plastic tray (seedling emergence method; Thompson et al., 1997; Bernhardt et al., 2008). The temperature was set to 28 °C to stimulate the germination of seeds of *C. fuscus* (von Lampe, 1996; Pietsch, 1999) and the trays were watered daily until August 2013. The number of seedlings emerging from the soil samples was counted. When possible, 15 individuals of *C. fuscus* from every pooled soil sample of all 33 localities were pricked and individually grown in plastic pots with a diameter of 9 cm filled with standardized soil mixture (two parts fertilized peat soil and one part sand). Plastic cups with a volume of 500 ml and self-made vents closed with tea filters were used to prevent outcrossing to obtain homogenous seed material for the experiments. The cup fitted perfectly on the pots, so that the transfer of pollen between plants was very unlikely. At the same time, seeds from the plants collected in the field were germinated under the same conditions, pricked and subjected to the same procedure as the plants that emerged from the soil samples. The ripe seeds were harvested from the selfed mother plants in October/November 2013 and stored at 6 °C in the dark until May 2014.

#### 2.5. Germination and environmental manipulation experiments

In May 2014, we chose 16 localities (five resp. six of each habitat type; Table 1) with at least five mother plants with ripe seeds from every fraction (established plants, shallow seed bank, and deep seed bank). A germination experiment was performed on possibly 50 seeds of each of 238 mother plants in plastic Petri dishes with a diameter of 9 cm on filter paper watered with Milli-Q® (Merck Millipore, Darmstadt, Germany) water. The experiment was performed in a Versatile Environmental Test Chamber MLR-352-PE (Panasonic, Osaka, Japan) with a day/night rhythm of 14/10 h and a high difference of 35 °C day and 10 °C night temperature to trigger germination (von Lampe, 1996). Germination was monitored for four weeks, daily in the first week and every two days later on.

The first 18 germinated seeds of each mother plant were planted in three 9 cm plastic pots with the same substrate as above and grown in the glasshouse under similar conditions. In June, six daughter plants of each of 225 (out of the 238) mother plants from 15 localities (five of each habitat type; Table 1) were planted into individual 9 cm pots, again with similar substrate. To further reduce possible maternal effects, seedlings of the same size were selected. This set of 1350 plants corresponding to 225 families (15 families, each consisting of six daughter plants of one mother plant, of each of the 15 chosen localities) was used for an environmental manipulation experiment (Fig. 2). For each locality, 15 plants (one daughter plant from each family of the respective locality) were placed in a plastic tray (six such trays per locality). Two of the six trays per locality were subjected to one of three different watering treatments (no flooding, moderate flooding, and severe flooding).

The watering treatments were chosen to simulate field conditions in the different habitats. In the treatment without flooding, the plastic trays had draining holes 1 cm above the ground. The

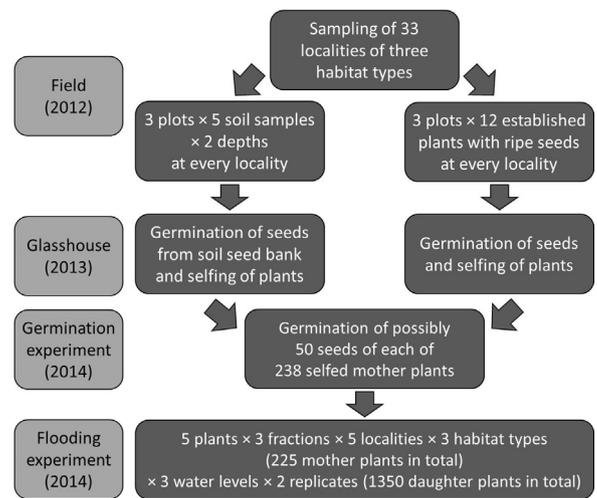


Fig. 2. Flowchart of the experimental steps.

plants were watered daily without being flooded. On very hot summer days, it could happen that the soil dried up superficially, as it may occur in fish storage ponds. The two flooding treatments were chosen to simulate conditions at fishpond and river habitats. The severe flooding treatment with two consecutive phases of submergence may occasionally occur at river habitats, where the water level can vary greatly and rapidly. To simulate flooding, three plastic trays with vents at the bottom were placed on a free hanging metal rack in a 400 l plastic tank (twenty such tanks in total). The height of the rack was adjustable with an accuracy of 1 cm with a galvanized steel chain on the four corners. The tanks were filled with water and the water level was kept stable by a draining hole on a fixed height. The water in the tanks was oxygenized with an air compressor for 8 h per day. Further, we replenished the tanks with fresh water for twenty minutes every two weeks. Replicates of the same locality were always placed in different tanks. In the moderate flooding treatment, plants were flooded for 6 cm (measured from the top of the pot) for four weeks after slowly submerging the trays (1 cm per day). In the severe flooding treatment, the same procedure was adopted, but the plants were lowered to a depth of 12 cm. After four weeks of submergence, the plants were brought to the surface at the same speed (1 cm per day). After two weeks at the surface, the plants of the severe flooding treatment were again flooded, now with to a depth of 6 cm for four weeks, whereas the plants of the moderate flooding treatment stayed at the surface. Every two weeks, a liquid organic-mineral fertilizer (Blumendünger mit Guano, Compo, Münster, Germany) containing a high portion of guano, 4% total N (nitrate and ammonium), 5% P<sub>2</sub>O<sub>5</sub>, 6% K<sub>2</sub>O, 0.01% B, 0.002% Cu, 0.02% Fe, 0.01% Mn, 0.001% Mo, and 0.002% Zn was applied. In the control treatment, 30 ml of fertilizer was given to each tray. The 400 l tanks got the quadruple amount.

In the three months of the experiment, there were three monitoring dates, the first before any treatment was carried out (12–18 June 2014), the second, when the plants were brought to the surface after four weeks (21–31 July 2014), and the third at the end of the experiment (26 August–10 September 2014). At every monitoring date, we measured various plant traits to assess fitness and growth under different watering treatments, namely the height of the erected plant (i.e. the height of the highest culm), the number of culms, the number of leaves, the number of culms with inflorescences, the length of the longest leaf, and the width of the two widest leaves at the basis. A vitality index based on the overall impression (color, health status) ranging from 1 (nearly dead) to 9 (vigorous) was determined for each plant at every monitoring

date. At the last monitoring date, the shoots were harvested and dried at 70 °C, and the shoot biomass was determined.

## 2.6. Statistical analysis

Differences in traits and their plasticity were evaluated by means of linear mixed models and generalized linear mixed models. For analysis of field data, habitat was treated as fixed factor and site within habitat as random factor. For analysis of germination data, habitat, fraction and their interaction were treated as fixed factors, and site within habitat as random factor. Both data sets were not normally distributed, even after the usual transformation, and the variances were heterogeneous. Therefore, generalized linear mixed models were calculated using the procedure GLIMMIX of SAS version 9.4 (SAS Institute, Cary, North Carolina, USA). A gamma distribution and a log link function were assumed and a Wald-Z test for random factors was performed. Differences between least square means were tested for significance by Tukey-Kramer post-hoc tests.

Residuals of the data of the flooding experiment were normally distributed, so that this data set was analyzed by linear mixed models (procedure MIXED of SAS 9.4) using treatment as third fixed factor in addition to habitat and fraction. The two-way interactions habitat  $\times$  treatment, fraction  $\times$  treatment and habitat  $\times$  fraction were also included. Site within habitat and its interactions with treatment and fraction were treated as random factors. Since the date when the individual plant was measured and so the number of days since germination had a significant influence, it was used as a covariate. The use of means of the two biological replicates in different tanks did not change the results of the statistical analysis, so that the original data set of 1350 plants was used for analysis. For every plant trait measured in the experiment, the individual statistical model with the smallest Akaike Information Criterion (AIC) was chosen. To examine the significance of the random factors, a likelihood ratio test was performed (Galwey, 2006).

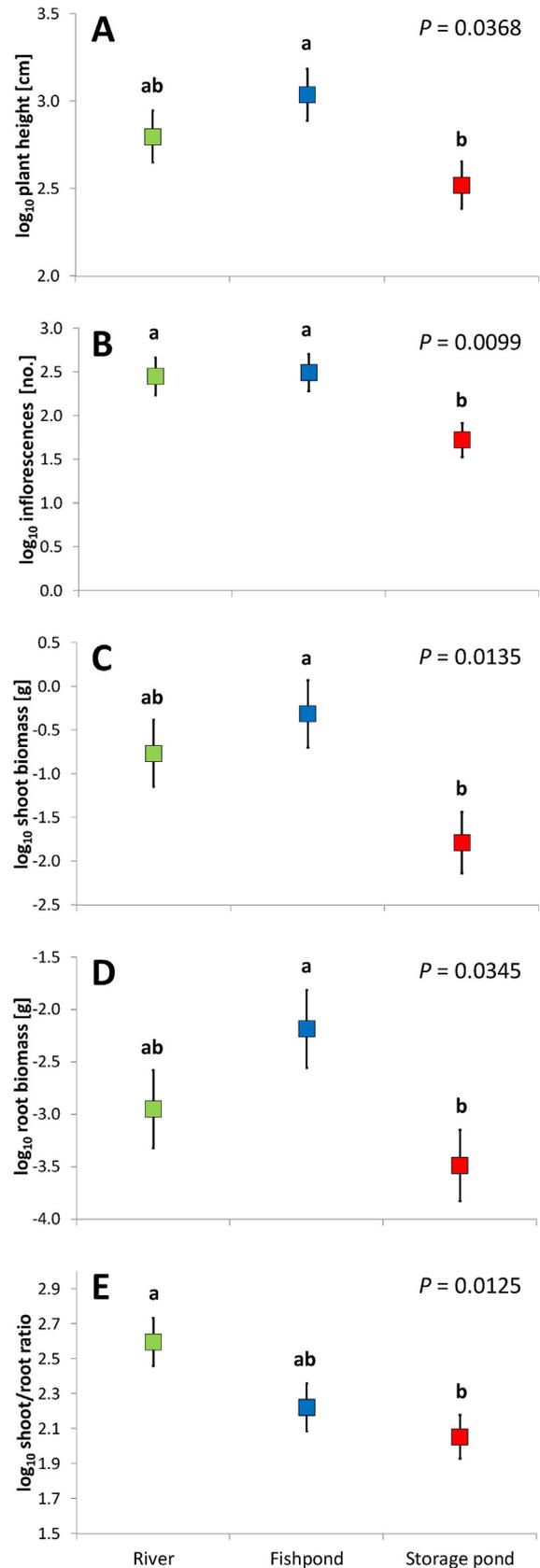
## 3. Results

### 3.1. Traits of plants sampled in the field

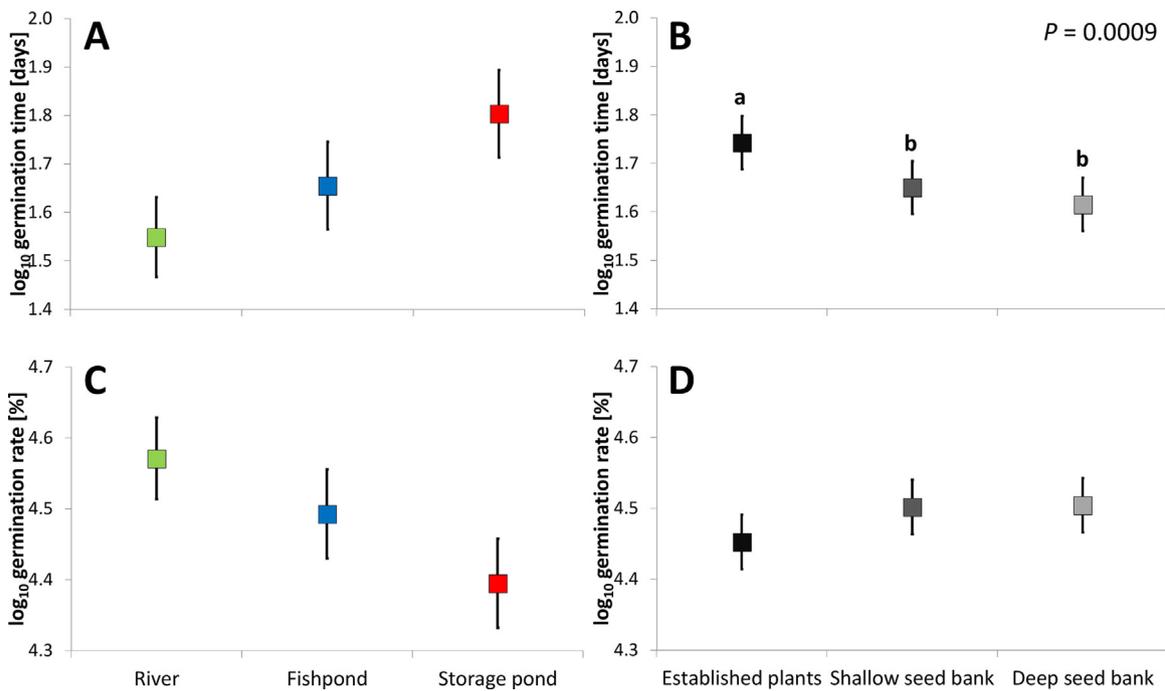
The generalized linear mixed models revealed a significant effect of habitat on all traits of plants sampled in the field (Appendix A). The sampling site also had a highly significant influence in all cases. The height of plants from fishponds was significantly greater than that of plants from fish storage ponds ( $\chi^2_{2,29.01} = 6.60$ ,  $P = 0.0368$ ; Fig. 3). Plants from rivers had an intermediate height. The number of culms with inflorescences ( $\chi^2_{2,28.98} = 9.23$ ,  $P = 0.0099$ ) was significantly greater in plants from fishponds and rivers than in plants from fish storage ponds. Shoot biomass ( $\chi^2_{2,28.99} = 8.60$ ,  $P = 0.0135$ ) and root biomass ( $\chi^2_{2,28.98} = 6.74$ ,  $P = 0.0345$ ) both showed the same pattern as plant height, with plants from fishponds having a significantly greater shoot and root biomass than plants from fish storage ponds, and plants from rivers having an intermediate biomass. The shoot/root ratio ( $\chi^2_{2,29.02} = 8.76$ ,  $P = 0.0125$ ) was significantly greater in plants from rivers than in plants from fish storage ponds, with plants harvested in fishponds having an intermediate ratio.

### 3.2. Soil seed bank and germination

We grew  $6.6 \pm 8.7$  seedlings (mean  $\pm$  standard deviation) from 10 ml of shallow soil compared to  $4.2 \pm 6.9$  seedlings in the same volume of deeper soil. The generalized linear mixed models did not reveal a significant effect of habitat on germination time or rate (Appendix B). Fraction, on the other hand, had a significant effect on germination time ( $\chi^2_{2,216.1} = 13.93$ ,  $P = 0.0009$ ) and a marginally



**Fig. 3.** Significant differences among plants of the three habitat types harvested in the field. Least square means and standard errors of the generalized linear mixed models are shown: (A) plant height, (B) number of culms with inflorescences, (C) shoot biomass, (D) root biomass, and (E) shoot/root ratio. Letters denote significant differences in least square means (Tukey-Kramer adjustment for multiple comparisons:  $P < 0.05$ ).



**Fig. 4.** Germination time and rate of seeds in relation to their habitat (river, fishpond and fish storage pond; A,C) and seed origin (established plants, shallow seed bank, deep seed bank; B,D). Least square means and standard errors of the generalized linear mixed models are shown. Letters denote significant differences in least square means of the generalized linear mixed models (Tukey-Kramer adjustment for multiple comparisons:  $P < 0.05$ ).

significant effect on germination rate ( $\chi^2_{2,216.1} = 5.09$ ,  $P = 0.0784$ ). The sampling site also had a significant effect. Seeds of plants derived from the deep and shallow soil seed bank germinated significantly faster than seeds of plants derived from established plants (Fig. 4). Similarly, seeds of plants derived from the soil seed bank germinated at a higher rate than seeds of plants derived from established plants.

### 3.3. Environmental manipulation (flooding) experiment

Just three of the 900 flooded plants (out of the 1350 total plants) died in the experiment and all other plants completed their life cycle (Table 2). There were no significant differences between habitats at the first monitoring, i.e. before the plants were submerged (Appendix C). The covariate “days since germination” was highly significant in most models. The random factor site as well as its interaction with treatment and fraction were also significant in most models.

#### 3.3.1. Effect of water level

At both the second and third monitoring, treatment had a highly significant effect on all examined variables. Plant height was highest under severe flooding and lowest without flooding, at both the second and third monitoring (Fig. 5A,B). At both monitoring dates, moderately flooded plants had the longest leaves, and non-flooded plants the shortest (Fig. 5C,D). The latter plants had the widest leaves and were most vital, whereas severely flooded plants had the narrowest leaves and were least vital (Fig. 5E–H). At the second monitoring, non-flooded and moderately flooded plants had the highest number of culms (Fig. 6A), but at the third monitoring, the moderately flooded plants had the highest number of culms compared to the other two treatments (Fig. 6B). Non-flooded plants had most culms with inflorescences and most leaves at the second monitoring (Fig. 6C,E). At the third monitoring, however, the moderately flooded plants caught up with the non-flooded plants in this

respect (Fig. 6D,F). Regarding shoot biomass, moderately flooded plants performed best and non-flooded plants worst (Fig. 6G).

#### 3.3.2. Effect of fraction

The fractions did not show any significant differences in the flooding experiment (Appendix D). Merely the interaction of fraction with habitat showed some effects, but no significant differences were detectable after Tukey-Kramer adjustment for multiple comparisons between least square means.

#### 3.3.3. Effect of habitat

At the first monitoring, no significant effects of habitat were found. Interestingly, however, the share of already flowering plants was greatest for plants from fish storage ponds (least square mean  $\pm$  standard error =  $63.5 \pm 11.8\%$ ), followed by plants from fishponds ( $47.5 \pm 11.8\%$ ) and, lastly, rivers ( $31.3 \pm 11.8\%$ ; Appendix C). With a  $P$ -value of 0.1575, these differences were, however, not significant.

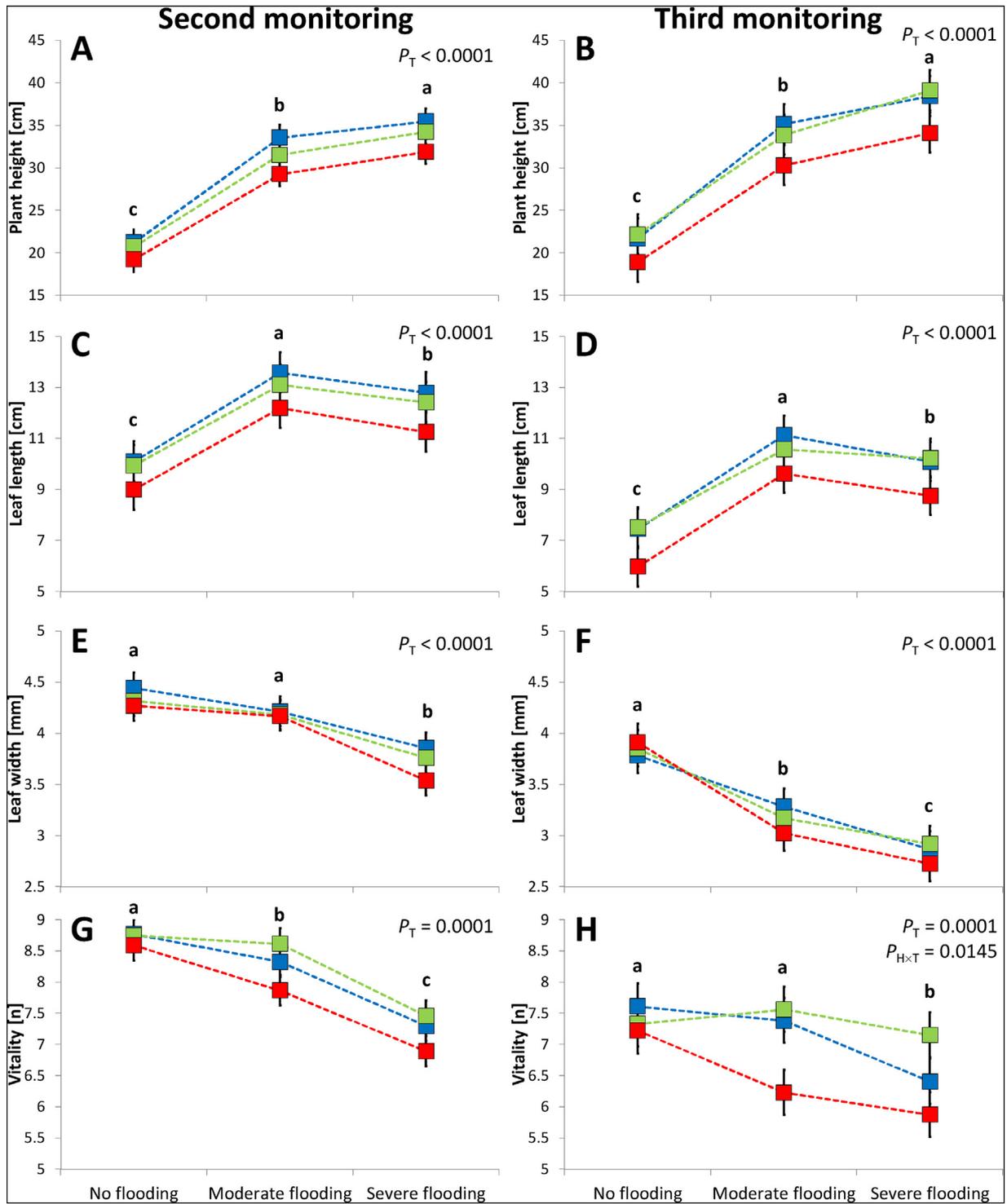
At the second monitoring, a significant effect of habitat on the number of culms was found. Plants from rivers had a significantly higher number of culms than plants from fish storage ponds (Fig. 6A). Both groups did not significantly differ from fishpond plants, which had an intermediate number of culms. At the harvest of the plants (third monitoring), a significant effect of habitat was found on the number of culms (as at the second monitoring) as well as on the number of culms with c (Fig. 6B,D). The pattern found for both traits was the same as at the second monitoring.

Significant interactions of habitat and treatment were found for the number of leaves and vitality at the third monitoring. Both traits showed a similar pattern (Figs. 5 H, 6 F). Remarkably, plants from fishponds performed best in the treatment without any flooding, but plants from rivers performed best in both the moderate and severe flooding treatments. Several other traits related to fitness, namely the number of culms, the number of culms with inflorescences, and shoot biomass, showed the same pattern, but the differences were not statistically significant (Fig. 6B,D,G).

**Table 2**  
Results of the linear mixed models used to investigate the effects of habitat, fraction and treatment on plant traits in the environmental manipulation experiment. For random, fixed factors and their interactions  $\chi^2$ -values and associated *P*-values (in brackets) are reported. Days since germination was used as a covariate.

Source of variation	Plantheight	Leaflength	Leafwidth	Vitality	No. of culms	Prop. offlow. plants	No. of culmswith infloresc.	No. ofleaves	Shootbiomass
First monitoring ( <i>n</i> = 1350)									
Habitat	1.64	1.42	0.10	0.44	0.53	3.70	0.55	0.23	n. a.
(df=2)	(0.4401)	(0.4909)	(0.9494)	(0.8025)	(0.7659)	(0.1575)	(0.7584)	(0.8912)	
Fraction	0.40	0.82	1.28	0.64	0.19	0.15	1.39	0.29	n. a.
(df=2)	(0.8171)	(0.6649)	(0.5262)	(0.7258)	(0.9075)	(0.9258)	(0.4987)	(0.8656)	
Habitat × fraction	<b>17.54</b>	<b>14.23</b>	<b>13.55</b>	7.40	3.26	2.58	5.72	1.23	n. a.
(df=4)	<b>(0.0015)</b>	<b>(0.0066)</b>	<b>(0.0089)</b>	(0.1161)	(0.5159)	(0.6311)	(0.2212)	(0.8730)	
Site (within habitat)	<b>17.0</b>	<b>22.5</b>	<b>34.3</b>	<b>13.2</b>	<b>6.3</b>	<b>11.8</b>	<b>29.7</b>	<b>12.0</b>	n. a.
(df=1)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(0.0003)</b>	<b>(0.0121)</b>	<b>(0.0006)</b>	<b>(&lt;.0001)</b>	<b>(0.0005)</b>	
Site × fraction	<b>54.3</b>	<b>53.1</b>	<b>6.3</b>	<b>17.4</b>	<b>42.4</b>	<b>104.1</b>	<b>72.5</b>	<b>39.2</b>	n. a.
(df=2)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(0.0121)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	
Days since germ.	<b>364.81</b>	<b>187.59</b>	<b>180.83</b>	<b>648.38</b>	<b>591.38</b>	<b>34.70</b>	<b>75.02</b>	<b>722.60</b>	n. a.
(df=1)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	
Second monitoring ( <i>n</i> = 1349)									
Habitat	2.77	1.71	1.09	2.63	<b>13.93</b>	n. a.	2.14	4.55	n. a.
(df=2)	(0.2498)	(0.4256)	(0.5798)	(0.2686)	<b>(0.0009)</b>		(0.3428)	(0.1030)	
Fraction	0.69	0.69	2.20	0.75	1.01	n. a.	3.18	0.98	n. a.
(df=2)	(0.7071)	(0.7094)	(0.3335)	(0.6875)	(0.6039)		(0.2035)	(0.6130)	
Treatment	<b>769.03</b>	<b>149.73</b>	<b>51.64</b>	<b>154.96</b>	<b>16.86</b>	n. a.	<b>57.95</b>	<b>78.24</b>	n. a.
(df=2)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(0.0002)</b>		<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	
Habitat × treatment	6.42	0.78	2.25	5.26	1.32	n. a.	1.51	4.80	n. a.
(df=4)	(0.1699)	(0.9416)	(0.6907)	(0.2616)	(0.8584)		(0.8256)	(0.3086)	
Fraction × treatment	0.58	4.12	2.25	0.41	4.73	n. a.	3.30	1.94	n. a.
(df=4)	(0.9651)	(0.3905)	(0.6893)	(0.9816)	(0.3157)		(0.5096)	(0.7474)	
Habitat × fraction	2.82	9.16	<b>13.65</b>	6.30	1.23	n. a.	6.04	5.44	n. a.
(df=4)	(0.5878)	(0.0573)	<b>(0.0085)</b>	(0.1782)	(0.8738)		(0.1960)	(0.2455)	
Site (within habitat)	<b>28.6</b>	<b>28.6</b>	<b>6.6</b>	<b>15.9</b>	.	n. a.	2.6	<b>10.4</b>	n. a.
(df=1)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(0.0102)</b>	<b>(0.0001)</b>	.		(0.1069)	<b>(0.0013)</b>	
Site × treatment	<b>12.3</b>	<b>29.3</b>	<b>44.3</b>	<b>40.3</b>	<b>84.5</b>	n. a.	<b>117.3</b>	<b>78.0</b>	n. a.
(df=2)	<b>(0.0005)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>		<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	
Site × fraction	<b>42.7</b>	<b>50.1</b>	<b>19.7</b>	<b>22.0</b>	<b>51.0</b>	n. a.	<b>33.8</b>	<b>43.7</b>	n. a.
(df=2)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>		<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	
Days since germ.	<b>11.37</b>	<b>32.92</b>	<b>16.96</b>	<b>4.84</b>	<b>4.78</b>	n. a.	<b>17.11</b>	<b>7.86</b>	n. a.
(df=1)	<b>(0.0007)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(0.0279)</b>	<b>(0.0287)</b>		<b>(&lt;0.0001)</b>	<b>(0.0051)</b>	
Third monitoring ( <i>n</i> = 1347)									
Habitat	2.12	2.53	0.24	4.71	<b>6.97</b>	n. a.	<b>8.15</b>	5.34	3.32
(df=2)	(0.3464)	(0.2819)	(0.8886)	(0.0947)	<b>(0.0307)</b>		<b>(0.0170)</b>	(0.0692)	(0.1901)
Fraction	1.03	1.05	1.72	0.80	1.77	n. a.	1.64	1.45	0.79
(df=2)	(0.5984)	(0.5902)	(0.4233)	(0.6688)	(0.4119)		(0.4403)	(0.4834)	(0.6724)
Treatment	<b>319.64</b>	<b>108.23</b>	<b>104.26</b>	<b>18.37</b>	<b>12.19</b>	n. a.	<b>13.00</b>	<b>23.81</b>	<b>33.59</b>
(df=2)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(0.0001)</b>	<b>(0.0023)</b>		<b>(0.0015)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>
Habitat × treatment	2.95	1.72	5.06	<b>12.42</b>	5.75	n. a.	5.04	<b>10.34</b>	4.84
(df=4)	(0.5661)	(0.7877)	(0.2816)	<b>(0.0145)</b>	(0.2190)		(0.2830)	<b>(0.0350)</b>	(0.3040)
Fraction × treatment	0.32	2.42	1.53	0.68	3.48	n. a.	4.07	2.15	1.77
(df=4)	(0.9886)	(0.6583)	(0.8211)	(0.9535)	(0.4811)		(0.3967)	(0.7073)	(0.7781)
Habitat × fraction	2.23	6.31	<b>10.22</b>	2.21	1.20	n. a.	1.02	1.76	2.67
(df=4)	(0.6932)	(0.1773)	<b>(0.0369)</b>	(0.6964)	(0.8773)		(0.9064)	(0.7795)	(0.6153)
Site (within habitat)	<b>27.2</b>	<b>27.6</b>	<b>22.6</b>	<b>7.8</b>	.	n. a.	.	3.0	<b>7.3</b>
(df=1)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(0.0052)</b>	.		.	(0.0833)	<b>(0.0069)</b>
Site × treatment	<b>40.8</b>	<b>29.7</b>	<b>47.9</b>	<b>62.3</b>	<b>39.6</b>	n. a.	<b>37.7</b>	<b>25.6</b>	<b>29.8</b>
(df=2)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>		<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>
Site × fraction	<b>73.6</b>	<b>22.2</b>	<b>20.8</b>	<b>60.7</b>	<b>64.7</b>	n. a.	<b>56.1</b>	<b>64.5</b>	<b>150.6</b>
(df=2)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>		<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>
Days since germ.	<b>26.39</b>	<b>135.58</b>	<b>0.17</b>	<b>35.25</b>	<b>14.57</b>	n. a.	<b>10.51</b>	<b>22.96</b>	<b>12.44</b>
(df=1)	<b>(&lt;0.0001)</b>	<b>(&lt;0.0001)</b>	<b>(0.6801)</b>	<b>(&lt;0.0001)</b>	<b>(0.0001)</b>		<b>(0.0012)</b>	<b>(&lt;0.0001)</b>	<b>(0.0004)</b>

df = ° of freedom; n. a. = not available.



**Fig. 5.** Interaction of habitat with water treatment in the flooding experiment at the second and third monitoring (harvest; green, rivers; blue, fishponds; red, fish storage ponds; see Fig. 6 for legend). Least square means and standard errors of the linear mixed models are shown for traits reflecting response to flooding – plant height (A,B), leaf length (C,D), and leaf width (E,F) – and vitality (G, H). Letters denote significant differences in least square means (Tukey-Kramer adjustment for multiple comparisons:  $P < 0.05$ ;  $P_T$ ,  $P$ -value of treatment;  $P_{H \times T}$ ,  $P$ -value of the interaction of habitat and treatment).

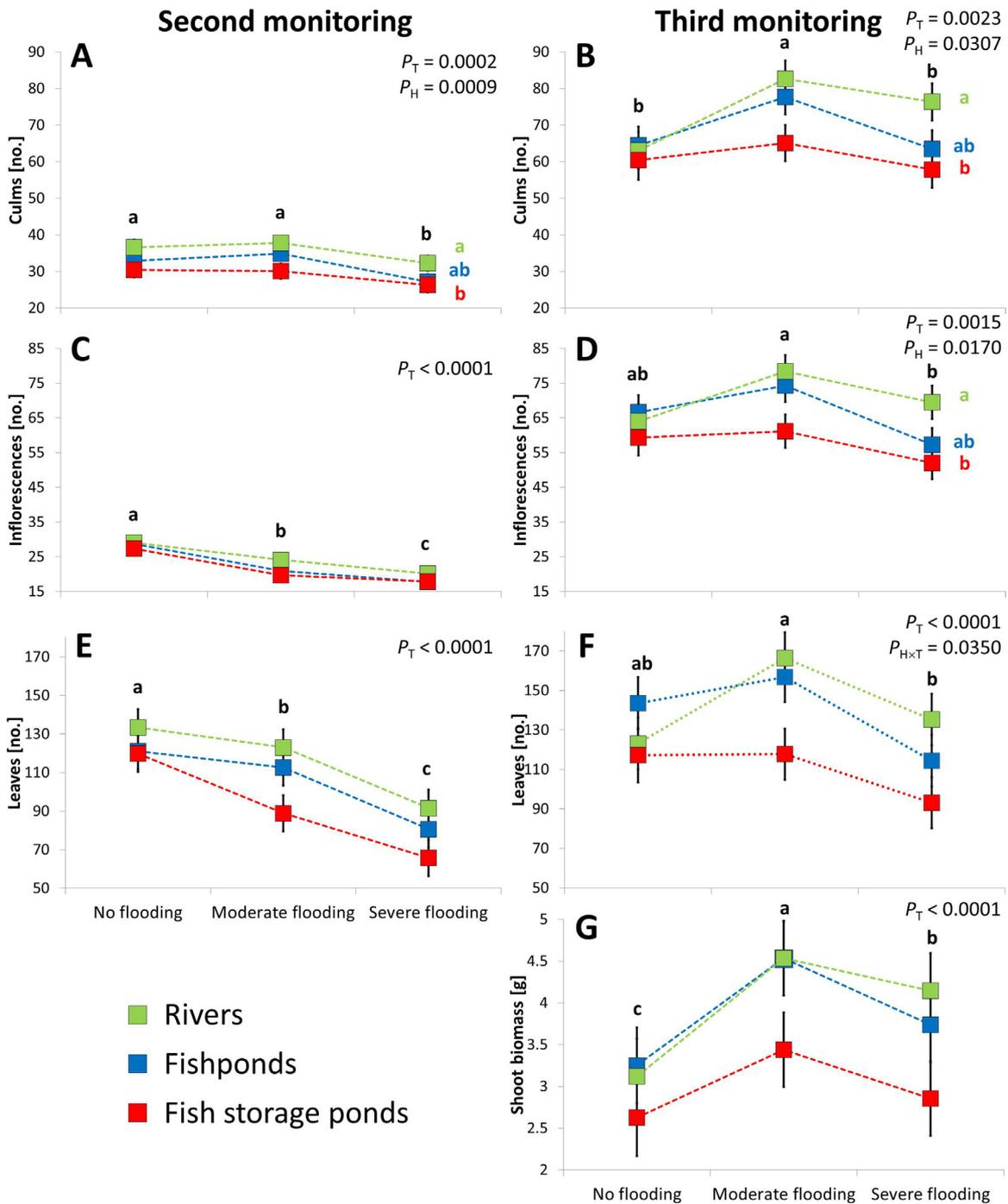
**4. Discussion**

**4.1. Plastic response to flooding**

*Cyperus fuscus* followed a low-oxygen escape strategy to avoid the negative effects of partial submergence by growing taller when submerged (Voeselek et al., 2004; Bailey-Serres and Voeselek, 2008). The more severe the flooding, the taller the plants grew.

Leaves responded in a similar way, but leaf length did not further increase under severe flooding in comparison to moderate flooding, as it was the case for the culms, possibly due to reduced carbohydrate reserves to invest in longer leaves.

The energy investment for growing taller was potentially compensated by the production of fewer culms (evident when comparing the moderate and severe flooding treatments), as also described for the perennial wetland plant *Carex secta* (Sorrell et al.,



**Fig. 6.** Interaction of habitat with water treatment in the flooding experiment at the second and third monitoring (harvest). Least square means and standard errors of the linear mixed models are shown for traits related to fitness: number of culms (A,B), number of culms with inflorescences (C,D), number of leaves (E,F), and shoot biomass (G). Letters denote significant differences in least square means (Tukey-Kramer adjustment for multiple comparisons:  $P < 0.05$ ;  $P_T$ ,  $P$ -value of treatment;  $P_H$ ,  $P$ -value of habitat;  $P_{H \times T}$ ,  $P$ -value of the interaction of habitat and treatment).

2012). The production of leaves and leaf width were reduced under flooding as well. Phenotypic plasticity can be costly if it requires investment into the organ responding plastically to its environment (Huber et al., 2012). Our results suggest that the submerged plants allocated resources to increase the height of their culms, at the expense of producing more culms and, most notably, leaves. This reallocation makes sense in the light of energy management and reproductive success. We hypothesize that the green culms are similarly well capable of photoautotrophic nutrition as leaves, facilitating the reallocation of resources from leaves to culms. Further,

the terminal flowers producing seeds project beyond the water surface with this strategy.

The non-flooded, but daily watered plants performed better than the flooded plants in most traits related to fitness at the second monitoring, supposedly because the flooded plants needed resources to respond to submergence. At the harvest, however, this was no longer the case. The non-flooded plants even had the lowest biomass. We suspect that the hot summer days in the last phase of the experiment have led to superficial drying out of the soil in the treatment without flooding in the afternoon. This indicates that

even a minor drought stress is more critical for the growth of *C. fuscus* than moderate flooding, which can be expected for wetland plants (e.g., Kirkman and Sharitz, 1993).

In the genus *Cyperus*, only the perennial *C. rotundus*, a major weed of crops and vegetables, has been investigated for its response to flooding so far (Fuentes et al., 2010). Along with larger air spaces in the culms and larger tubers with higher carbohydrate content, a steady activity of alcohol dehydrogenase in the roots as a measure of sustained anaerobic respiration under hypoxia characterizes the flood-tolerant ecotype of *C. rotundus*. Such additional possible responses described for various perennial wetland plants (e.g., rice; Voeselek and Bailey-Serres, 2015) were not in the focus of our study. Nevertheless, our results confirm that *C. fuscus* as an annual wetland plant is able to modify its morphology to cope with flooding like perennials, at least in nutrient-rich environments such as in our experiment (see also Voeselek et al., 2004; Song et al., 2015).

## 4.2. Soil seed bank

Mudflat species are known for their long-term persistent soil seed banks, where the seeds stay viable for decades or even more than hundred years (Salisbury, 1970; von Lampe, 1996; Thompson et al., 1997; Leck and Brock, 2000; Deil, 2005; Šumberová et al., 2012). The relative abundance of seeds of *C. fuscus* buried in the deep fraction of the soil seed bank as well as the occurrence of viable seeds of *C. fuscus* buried in soil on the bottom of ponds not drained for many years as observed in the fishpond Novozámecký (First fishpond in Table 1), suggest that this species possesses a long-term persistent seed bank as well.

Seeds of mother plants originating from the soil seed bank germinated faster than seeds of mother plants originating from ripe plants collected in the field. Because the seeds used in the experiment had the same age and experienced the same storage conditions, we can exclude differences in their state of dormancy as underlying reason. The maternal environment is known to influence seed fitness and the early stages of plant development (Galloway, 2005; Bischoff and Müller-Schärer, 2010; Baskin and Baskin, 2014). Here, however, we can exclude effects of the parental (but not of the grandparental) generation on germination, because it grew under similar conditions in the glasshouse. We therefore suggest that the observed differences in germination are mainly under genetic control.

Many studies on annual wetland plants suggest that even after a germination event, a much higher amount of seeds than the number of seedlings germinated usually remains in the soil (Leck and Brock, 2000; Deil, 2005; Bernhardt et al., 2008). In our experiment, the amount of viable, but not germinated seeds in the soil remained unclear. Moreover, the use of the seedling emergence method to obtain *C. fuscus* plants from the soil may have led to a bias towards genotypes that are best adapted to germinate under the specific glasshouse conditions (e.g., temperature, air moisture). Similarly, the specific conditions on the site in the given period including the weather situation (e.g., Fernández-Pascual et al., 2013) could support the germination of particular genotypes of *C. fuscus* and suppress the germination of other genotypes, and thus function as an ecological filter. This supports the view that the soil seed bank stores genetic variability, as a result of the accumulation of seeds of the yearly cohorts (established plants), to ensure germination and establishment of above-ground populations under various environmental conditions and thus plays an important role not only as a way to survive unsuitable conditions (Leck, 1989; Mandák et al., 2012). Selection of the plants established every year is thus mediated by on site conditions during germination. This possible short-term filtering of genotypes by fluctuating environmental factors has not yet been studied in mudflat species and deserves further attention.

## 4.3. Adaptation to habitat

We found significant differences in growth between the habitat types not just in the field, but also — at least for culm-related traits — under standardized growing conditions in the experiment. This suggests that some differences are caused by the growing conditions at the localities (e.g., height) and some are genetically fixed, i.e. local adaptations (e.g., number of culms with inflorescences; Erfmeier et al., 2011; Rice et al., 2013; Anderson et al., 2014). The different number of sites included might explain why the differences between the habitat types are not as clear-cut in the experiment (with only five sites per habitat type) as in the field (10–12 sites per habitat type; Leyer and Wesche, 2007). Transgenerational effects (TEs), where the phenotype of an individual is affected by the environment of its parents and grandparents, could underlie some of the results, but we controlled for parental effects via one generation of selfing (Latzel, 2015). Transgenerational effects should therefore be negligible compared to local adaptation to growing conditions in the different habitat types as underlying mechanism of the observed differences between them in the experiment.

The significant habitat × treatment effect found for some traits related to fitness in the experiment (vitality, number of leaves) could be the result of a reduced growth rate under stressful environmental conditions, which is a pre-requisite for local adaptation (Pigliucci, 2001; Kawecki and Ebert, 2004). Plants from rivers were superior in both flooding treatments, whereas plants from fishponds were superior in the treatment without flooding at the third monitoring (i.e. a treatment that might have implied a minor drought stress). This supports the hypothesis that the populations have adapted to their local conditions, i.e. water-logged or flooded soil in river habitats and superficially dry soil in fishpond habitats. Plants from fish storage ponds also performed best in the treatment without flooding (e.g., with respect to vitality), though on a lower overall level, suggesting that they can cope well with a minor drought stress too. This may imply a higher cost of flooding-induced shoot elongation for plants from anthropogenic habitats than from river habitats or — vice versa — a higher cost of drought-induced plastic responses (e.g., intensified root growth) for plants from river habitats than from anthropogenic habitats. Alternatively — if we interpret the traits showing a habitat × treatment effect not just as indicators of fitness, but also as potentially adaptive traits — the habitat × treatment effect may also suggest that divergent selection on flooding induced plasticity has taken place in the different habitats. According to that, even within the same populations of *Veronica peregrina* in vernal pools, significant different adaptations to inter- and intraspecific competition of plants originating from the center and periphery of populations, could be proved in glasshouse experiments (Linhart, 1988). To definitively assess local adaptation, however, reciprocal transplant experiments as a complement to our experiment are essential (Pigliucci, 2001; Kawecki and Ebert, 2004; Leimu and Fischer, 2008).

### 4.3.1. Rivers

The experimental data confirmed our expectation that plants from rivers should be fitter than plants from anthropogenic habitats in the moderate and severe flooding treatments simulating conditions at rivers, where water levels can change fast and unpredictably (Colmer and Voeselek, 2009). The high shoot/root ratio of plants collected at rivers suggests that they are not limited by water or nutrient supply (Poorter and Nagel, 2000).

### 4.3.2. Fishponds

The main factor explaining the very good performance of *C. fuscus* at fishponds in the field seems to be the high nutrient availability in this habitat type. Since the intensification of fish production from the 1950s, the state of Czech fishponds in naturally

infertile regions changed from oligo- or mesotrophic to eutrophic (Květ et al., 2002; Šumberová et al., 2012; Wezel et al., 2014), whereas at least phosphorous concentrations in the water declined in European rivers in the last decades (Ludwig et al., 2009).

#### 4.3.3. Fish storage ponds

The experimental data did not confirm our initial hypothesis that plasticity to flooding should be disfavored in fish storage pond populations. Plants from fish storage ponds did follow the low-oxygen escape strategy typical for ephemeral mudflat species in their natural habitat (Voeselek et al., 2004). Similarly, flooding-induced plasticity did not vary among habitat types in *Rumex palustris* (Chen et al., 2009) and *Solanum dulcamara* (Zhang et al., 2016). The management (including the flooding) even within a single fish storage pond complex is very variable and, at times, less predictable than in river and fishpond habitats. Some of the fish storage ponds may be flooded suddenly for several days and then again become exposed. It remains to be tested whether the response to flooding is associated with higher costs in fish storage ponds than in the natural habitat (van Kleunen et al., 2000; Pigliucci, 2001).

The overall poor performance of plants from fish storage ponds could rest on the fact that we might have neglected some key environmental factors (such as management measures) in fish storage ponds in the experiment, which was primarily designed to test for the adaptation to flooding of plants at rivers (Kawecki and Ebert, 2004). Von Lampe (1996) demonstrated that plants facing unfavorable growing conditions at the beginning of their life adopt a “pessimistic strategy”, i.e. a plastic response resulting in an early flowering dwarf phenotype. Our results show that plants from fish storage ponds display a “dwarf” phenotype not just in the field, but also under experimental conditions, suggesting that dwarfism has become an adaptive strategy. Plants from fish storage ponds also started to flower earlier than the other plants in the experiment. By staying small and accelerating their life cycle, plants in fish storage ponds may be able to reproduce before drying out or being killed by management measures such as grazing, mowing, or application of herbicides (Šumberová et al., 2006, 2012). They thus seem to be optimally adapted to poor-quality habitats. The shift of biomass to the roots observed in plants from fish storage ponds also indicates that water and/or nutrients are limiting at the site (Poorter and Nagel, 2000). Along rivers, where the low water levels usually continue until late autumn, plants may have more time to reproduce, but in fish storage ponds, an even faster seed production seems to be favorable. A similar strategy has been observed in an annual sunflower species (Moyers and Rieseberg, 2016), where divergent selection in coastal barrier islands is thought to have led to a distinct short and early flowering life history syndrome.

Our findings suggest that fish storage ponds provide consistently different selection pressures when compared to rivers. Fish farming can thus serve as an excellent model system to study how specific selection pressures have led to phenotypic differentiation. Adaptation to anthropogenic environments has also been suggested for *C. rotundus*, where the recently evolved lowland ecotype is thought to have acquired numerous adaptive traits under the selection pressure of repeated management practices in flooded rice fields in the Philippines (Fuentes et al., 2010).

#### 4.4. Implications for conservation

During the last decades, many wetland plant species became threatened due to habitat destruction and management changes. The dwarf rush species are also considered to be rare and declining. However, they can grow in extreme as well as strongly and quickly changing conditions. A typical representative of this group of species is *Cyperus fuscus*, target species of our study. Surpris-

ingly, recent distribution mapping proved at least several hundreds of existing localities across various habitats on the territory of the Czech Republic (Kaplan et al., 2016). Our results agree with this finding and show the large potential of *C. fuscus* to grow in secondary habitats with multiple stresses such as floods, substrate desiccation, herbicide spraying, etc. Thus, we at least partly revealed the causes of the successful survival of *C. fuscus* in the current landscape, which is highly fragmented and markedly changed by man. However, our experiment showed that plants from rivers performed best under high and fluctuating water levels, whereas plants from anthropogenic habitats seem to have adapted to conditions offered by the fish farming. Historical fishpond systems providing a rich mosaic of wetland habitats are certainly worthy of protection in times of declining biodiversity, but our results also suggest that the plants growing in these substitute habitats may have lost some adaptations to their original habitat and gained divergent adaptations in return.

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#### Appendix A. Results of the generalized linear mixed models used to investigate the effect of habitat on plant traits in the field. For the fixed factor habitat, we report the $\chi^2$ -values and their associated P-values (in brackets). Z-values and their associated P-values are reported for random factors.

Source of variation	Plant height	No. of culms with infloresc.	Shoot biomass	Root biomass	Shoot/root ratio
Habitat (df=2)	<b>6.60</b> (0.0368)	<b>9.23</b> (0.0099)	<b>8.60</b> (0.0135)	<b>6.74</b> (0.0345)	<b>8.76</b> (0.0125)
Site (within habitat) (df=1)	<b>3.74</b> (<0.0001)	<b>3.71</b> (0.0001)	<b>3.73</b> (<0.0001)	<b>3.69</b> (0.0001)	<b>3.40</b> (0.0003)

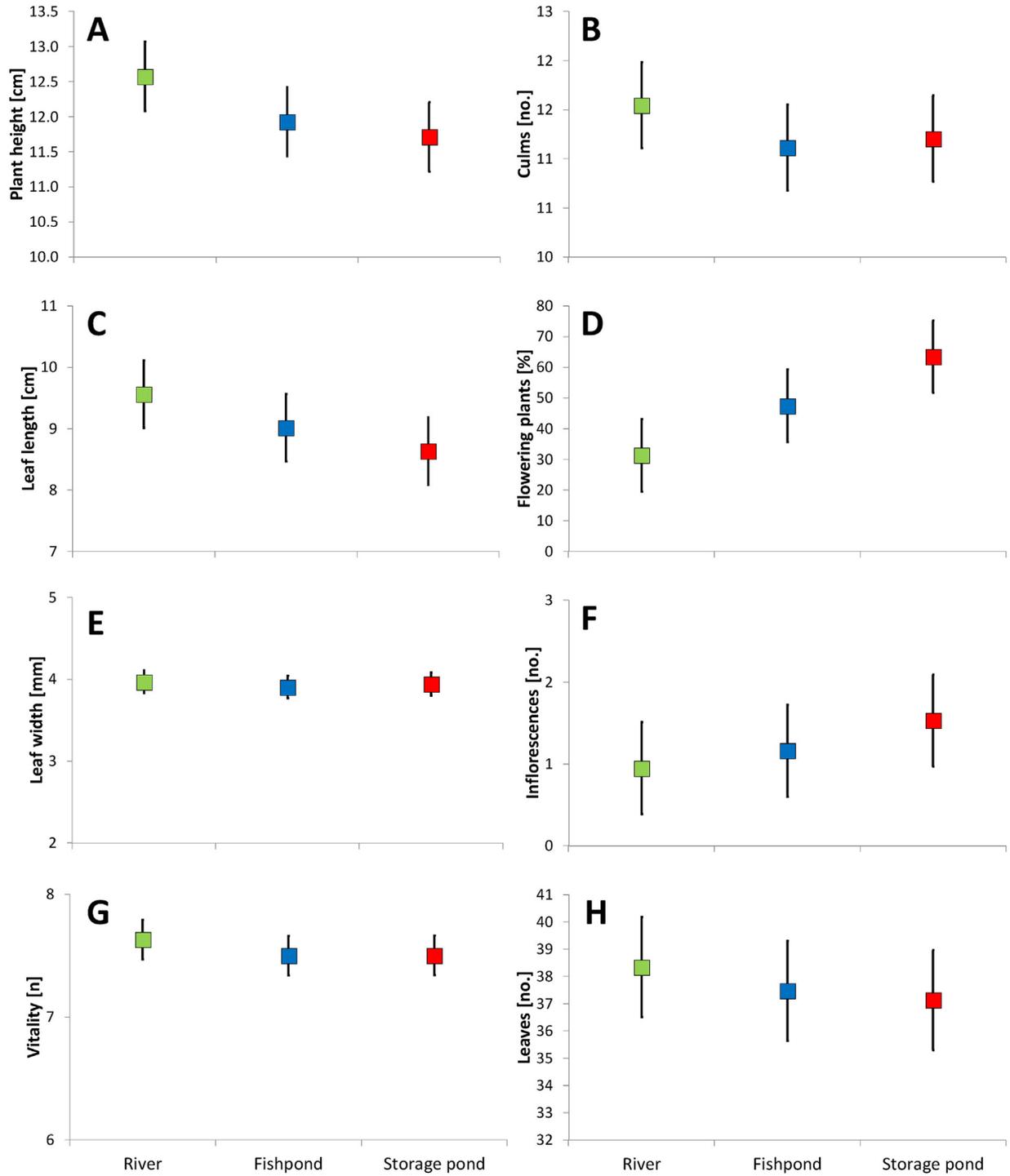
df = ° of freedom.

#### Appendix B. Results of the generalized linear mixed models used to investigate the effect of habitat, fraction and their interaction on germination traits. For each fixed factor, we report the $\chi^2$ -values and their associated P-values (in brackets). Z-values and their associated P-values are reported for random factors.

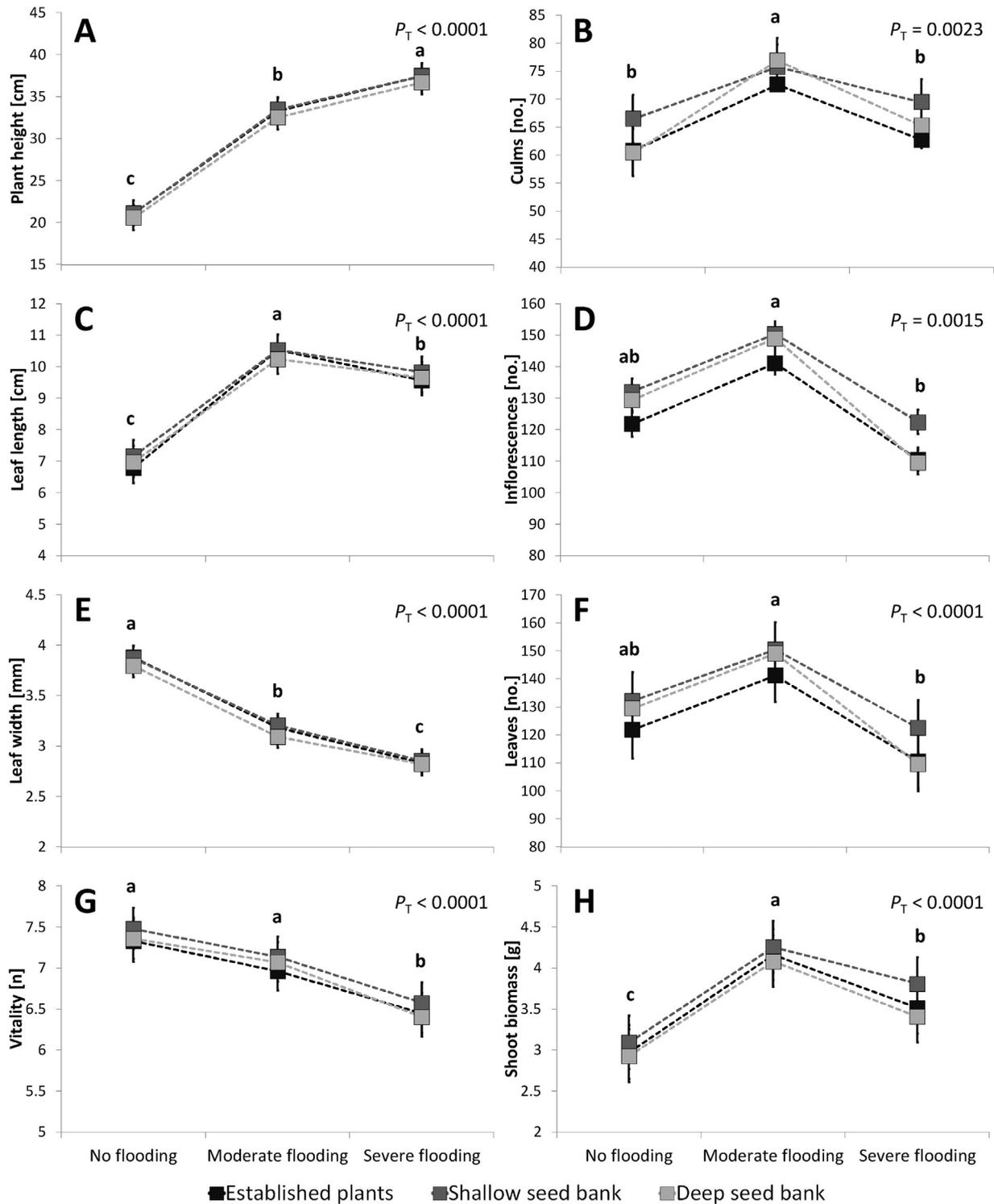
Source of variation	Germination time	Germination rate
Habitat (df=2)	4.34 (0.1141)	4.28 (0.1178)
Fraction (df=2)	<b>13.93</b> (0.0009)	5.09 (0.0784)
Habitat × fraction (df=4)	7.80 (0.0993)	7.57 (0.1088)
Site (within habitat) (df=1)	<b>2.34</b> (0.0096)	<b>2.31</b> (0.0103)

df = ° of freedom.

**Appendix C. Differences among habitat types in the flooding experiment at the first monitoring (before any treatment had taken place). Least square means and standard errors of the linear mixed models are shown: (A) plant height, (B) number of culms, (C) leaf length, (D) proportion of flowering plants, (E) leaf width, (F) number of culms with inflorescences, (G) vitality, and (H) number of leaves.**



**Appendix D. Interaction of fraction and water treatment in the flooding experiment at harvest (third monitoring). Least square means and standard errors of the linear mixed models are shown: (A) plant height, (B) number of culms, (C) leaf length, (D) number of culms with inflorescences, (E) leaf width, (F) number of leaves, (G) vitality, and (H) shoot biomass. Letters denote significant differences in least square means (Tukey-Kramer adjustment for multiple comparisons:  $P < 0.05$ ;  $P_T$ ,  $P$ -value of treatment)**



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