



Seed germination ecology of common bladderwort (*Utricularia vulgaris* L.)

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ABSTRACT

Generative reproduction of the carnivorous aquatic plant *Utricularia vulgaris* (Lentibulariaceae) from seeds may be a critical process in the recovery of natural populations following temporary drying of habitat, and in the colonisation of new potential sites through dispersal of seeds by water birds. However, little is presently known about the seed ecology and germination biology of this species. We tested the germination response of seeds under various temperature and seed storage regimes, to examine the processes required for seed dormancy alleviation and the effects of different germination solution and temperature on germination probability. Seeds likely possess non-deep simple morphophysiological dormancy alleviated by warm stratification. Highest germination success was recorded for warm-stratified seeds and seeds exposed to ethylene. Seeds were photophilous, with germination more successful at 21 °C than at 25 °C and greatest in slightly alkaline (pH 8) germination solution containing KHCO₃, CaCl₂ and MgSO₄ mimicking the mesotrophic humic waters in which the species naturally occurs. In the alkaline solution, 97 % of seeds rose to the surface prior to germination. In natural habitats, this effect may facilitate seedlings reaching the warmer and irradiated water surface. As seed germination success appears linked to light availability, water chemistry, and seed position in the water column, careful management and ecological restoration of remnant habitats harbouring this species may need to ensure positive conservation outcomes.

1. Introduction

Utricularia vulgaris L. (Lentibulariaceae) is a perennial, rootless, free-floating aquatic carnivorous plant producing linear, poorly-branched shoots ranging from 0.5 to 2.5 m long (Taylor, 1989). The species is widespread in Europe, North Africa and Asia (Taylor, 1989), where it occurs in shallow standing, mesotrophic to mildly eutrophic humic waters (e.g., lakes, backwater pools and oxbows, fishponds, dam reservoirs, fens) that are rich in carbonates and calcium and neutral to slightly alkaline (Koshiba, 1992a,b, 2004; Fleischmann and Schlauer, 2014). The filamentous, pinnately-divided leaves are arranged in leaf nodes and bear abundant bladder-shaped traps of foliar origin 1–5 mm long (Taylor, 1989; Fleischmann and Schlauer, 2014). Over the growing season, *U. vulgaris* rapidly reproduces vegetatively through frequent branching of shoots, and single shoots overwinter as turions (winter buds) similar to other closely-related *Utricularia* species (Adamec,

2018a).

Utricularia vulgaris forms an emergent racemose inflorescence with golden-yellow flowers on an erect flower scape 30–50 cm high under favourable conditions from June to September. Although flowers can be pollinated by dipterans (Piachno et al., 2018), *U. vulgaris* is one of three European *Utricularia* (with *U. intermedia* and *U. minor*) capable of self-pollination to form fertile fruit and viable seeds (Taylor, 1989; Fleischmann and Schlauer, 2014). Mature fruit are globose, 3–5 mm long, open by an upper lid and contain about 50–150 small (mean seed mass is 0.052 mg; L. Adamec, unpubl. data), cubic to prismatic, 4–6 angled, polygonal brown-black seeds (Figs. 1 and 2; Taylor, 1989; Cross et al., 2018a). Seeds usually ripen in fruit above the surface of the water, and upon dehiscence are released into the water column where they sink to bottom sediments (L. Adamec, unpublished) and form a persistent sediment seed bank (Gálová and Hájková, 2014).

At least some species of *Utricularia* produce seeds with an

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undifferentiated dwarf embryo occupying most of the seed volume, and the seeds of these species possess either morphological dormancy (MD) or morphophysiological dormancy (MPD; Cross et al., 2018a). Seeds with underdeveloped embryos require a period of embryo development prior to germination, and seeds with MPD also require a physiological cue following this period of embryo maturation (Baskin and Baskin, 2014; Kildisheva et al., 2020). Seeds of *Utricularia* spp. that germinate shortly after dehiscence (within several weeks) likely have MD (Swamy, Ram, H.Y., 1969), while those requiring additional environmental cues before germinating likely have MPD (Cross et al., 2018a).

Little is known about the seed germination ecology of *U. vulgaris* and other aquatic *Utricularia* species. Seed dormancy is apparently alleviated by cold stratification in *Utricularia* from temperate regions (Baskin and Baskin, 2014), and it has been reported that seed germination in *U. vulgaris* can be stimulated by exposure to ethylene (Cross et al., 2018a). The seeds of numerous aquatic plants exhibit a germination response to ethylene exposure, including the aquatic carnivorous plant *Aldrovanda vesiculosa* (Droseraceae) which commonly co-occurs, often sympatrically, with *U. vulgaris* throughout its European natural range and produces seeds with physiological dormancy (PD; Cross, 2012; Cross et al., 2016). Many species producing small seeds are photophilous (light-requiring for germination; Baskin and Baskin, 2014), and it appears likely that the seeds of *U. vulgaris* are light-requiring as they commonly rise to the surface prior to germinating (L. Adamec, unpublished). In a field experiment, 95–97 % of *U. vulgaris* seeds enclosed in nylon bags persisted through an 11-month period shallowly buried in or exposed on the surface of sediment in a shallow sand-pit pool, with ca. 41 % germinating in outdoor culture over the following 15 months (Adamec, 2018b). A similar germination response was reported for *U. vulgaris* seeds aged in wetland sediments for 70–100 years in South Moravia (Czech Republic) after exposure to light (Gálová and Hájková, 2014), indicating that the seeds of *U. vulgaris* are long-lived under suitable conditions and likely form large and long-lived sediment seed banks. However, the type of seed dormancy in *U. vulgaris*, as well as the specific environmental cues responsible for alleviating seed dormancy and stimulating seed germination, remain unresolved.

The aim of this study was to elucidate the seed germination ecology of *U. vulgaris*, by (i) examining the germination response of *U. vulgaris* seeds to different pH of germination media, to variable temperature and light regimes, to ethylene as a germination stimulus, and to dormancy alleviation treatments including cold and warm stratification; (ii) determining whether seeds displayed orthodox seed storage behaviour, by testing seed germination of freshly-collected and dry-stored seeds and following sub-zero temperature storage.

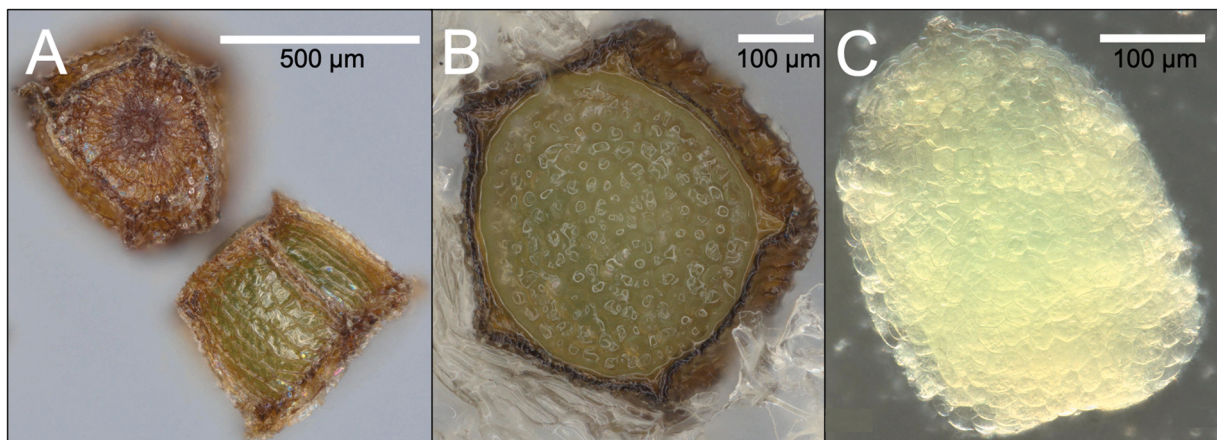


Fig. 1. Representative seeds of *Utricularia vulgaris* showing prismatic shape and multiple faces (A), longitudinally sectioned seed illustrating testa thickness and central position of the embryo within the endosperm (B), and extracted embryo (C). White bars indicate scale. Photos by R. Prausová.

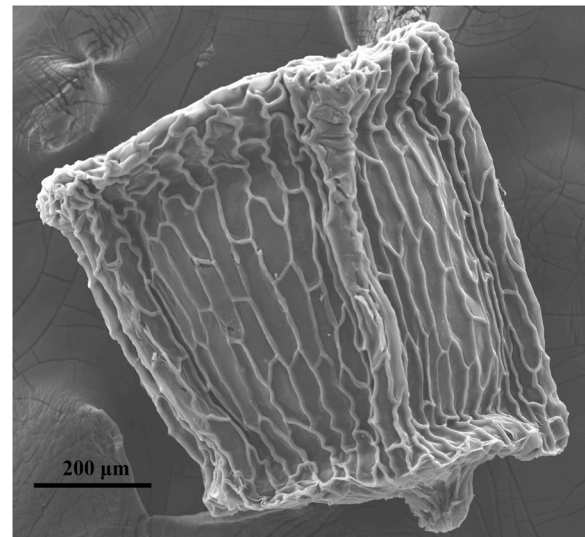


Fig. 2. Seed surface of *Utricularia vulgaris* in an electronic microscope. Photo by M. Vojta (HITACHI FlexSEM 1000).

2. Materials and methods

2.1. Seed collection and storage

Mature seeds (dark brown and dehiscing) of *U. vulgaris* L. were collected from plants in outdoor cultivation at the Institute of Botany of the Czech Academy of Sciences at Třeboň, Czech Republic (Sirová et al., 2003). Cultivated plants originate from South Moravia, Czech Republic (ca. 250 km far away; Gálová and Hájková, 2014). Experiments were undertaken using seeds collected in 2018 and 2020. Germination experiments were conducted on freshly collected seeds where possible, but where seed storage prior to experimental use was necessary seeds were stored depending upon treatment under either dry (at 22–24 °C and relative humidity of 55–60 %) or wet (in tap water at 4 ± 1 °C) conditions at the University of Hradec Králové, Czech Republic.

To provide representative temperature ranges experienced by seeds of *U. vulgaris* under natural conditions, temperature data loggers (Minikin Tie, EMS Brno, Czech Republic) were established at two natural *U. vulgaris* populations in Central and East Bohemia. The first, Hrabanovská černava fen (50° 12' 53.7"N, 14° 49' 41.2"E, 187 m above sea level), comprises a central pool harbouring numerous macrophytes surrounded by wetlands, fens and *Molinia caerulea* meadows. The second, Bohdanečský fishpond (50° 5' 48.6"N, 15° 40' 56.1"E, 212 m above

sea level), comprises a complex of fens and *M. caerulea* meadows surrounding the Matka and Bohdanečský fishponds. Average water depth during the growing season where *U. vulgaris* occurs is 50–60 cm at Hrabanovská černava fen (electrical conductivity 440–1790 $\mu\text{S}\cdot\text{cm}^{-1}$ and pH 7.1–8.8) and 50–70 cm at Bohdanečský fishpond (electrical conductivity 150–440 $\mu\text{S}\cdot\text{cm}^{-1}$ and pH 6.5–9.7; Jará and Prausová, 2021). Data loggers were installed in the water column among *U. vulgaris* individuals at 30 cm depth at both sites, and measured water temperature at 1 h intervals from January to December 2019.

2.2. Seed morphometric measurements

Before undertaking germination tests, each seed collection was examined at 10 \times magnification using a stereomicroscope (SZP 1102 – T ZOOM s, Arsenal, Doubrovčice, Czech Republic), and underdeveloped, damaged or immature seeds were removed. Seeds from each collection were photo-documented using a 3-D microscope (KEYENCE VHX-500, Keyence Corporation, Osaka, Japan; Fig. 1). To determine seed size, 100 seeds from each collection were randomly selected. Seed width and height of them were measured at 100 \times magnification. As the seeds of *U. vulgaris* are cubic to prismatic (Cross et al., 2018b), their width was measured on the upper and lower parts of seeds, and the number of surfaces on each seed was also counted. Additionally, a scanning electron microscope HITACHI FlexSEM 1000 (Hitachi, Japan) was used to show their shape and surface features (Fig. 2).

2.3. Germination biology

To determine the seed germination response of *U. vulgaris* to temperature, light, pH and germination stimulants, seeds were surface sterilised in a 2.5 % NaClO solution for two minutes before being rinsed thoroughly with sterile distilled water and placed in 20 mL sealed plastic tubes containing 15 mL of either acidic (Solution 1), neutral (Solution 2) or alkaline (Solution 3) germination media solution. Solution 1 comprised 0.5 mM KCl with 0.1 mM CaCl_2 and 0.1 mM MgSO_4 adjusted to pH 5.5, Solution 2 comprised 0.5 mM KHCO_3 with 0.1 mM CaCl_2 and 0.1 mM MgSO_4 adjusted to 6, and Solution 3 comprised 0.5 mM KHCO_3 with 0.1 mM CaCl_2 and 0.1 mM MgSO_4 adjusted to pH 8, (all pH adjustments made using HCl). Five replicates of 20 seeds for each treatment were placed in incubators at a constant temperature of either 21 or 25 °C (± 1 °C), on a 15-h photoperiod (fluorescent light intensity 2–2.5 klx equal to 35–40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation) or in constant darkness (prepared in darkness and placed in light-exclusion boxes). Germination (protrusion of the first filamentous “cotyledonoids” from the seed, *sensu* Cross et al., 2016) was scored weekly for six weeks in light treatments, but only once after six weeks in dark treatments. Germinating seeds (Fig. S1) were transferred to a 4 L container containing water collected from natural sites of *U. vulgaris* with dry litter of *Carex* spp. for further cultivation.

To investigate the potential role of ethylene as a germination stimulus, additional replicates of 20 seeds were prepared as previously described but imbibed in distilled water for 24 h and exposed to an 80 $\text{mg}\cdot\text{L}^{-1}$ Ethephon (Sigma Aldrich, Germany) for a further 24 h prior to being placed in 20 mL sealed plastic tubes containing 15 mL of either acidic, neutral or alkaline germination media solution. Ethephon is known to gradually release ethylene gas in solution, and ethylene is a known a germination stimulant for many aquatic and wetland plant species (e.g., Baskin and Baskin, 2014; Cross et al., 2014, 2015, 2018b; Prausová et al., 2014). Seeds were then incubated at 21 or 25 °C on a 15-h photoperiod or in constant darkness for six weeks as previously described. Germination was scored weekly for six weeks in light treatments, but only once after six weeks in dark treatments.

To investigate the impact of cold and warm stratification on the alleviation of seed dormancy in *U. vulgaris*, additional replicates of 20 seeds were prepared as described previously for acidic, neutral or alkaline germination media solution treatments. For stratification, seeds

were incubated in sealed plastic tubes at 4 ± 1 °C (cold stratification) or at 30 ± 1 °C (warm stratification) in constant darkness for four weeks, then incubated at 21 °C for a further two weeks on a 15-h photoperiod or in constant darkness. *Utricularia vulgaris* seeds regularly experience sub-zero temperatures in the sediment seed bank throughout the species natural range in winter. Additionally, to investigate the impact of sub-zero stratification temperatures on the alleviation of seed dormancy, additional replicates of dry seeds were incubated in sealed plastic tubes at -18 ± 1 °C (frost stratification) in constant darkness for four weeks, or at -18 ± 1 °C for two weeks and then at 4 ± 1 °C for two weeks (varying stratification), and then incubated at 21 °C for further two weeks on a 15-h photoperiod or in constant darkness. Germination at 21 °C and 25 °C was scored weekly for six weeks in light treatments, but only once after six weeks in dark treatments.

2.4. Deep-frost seed storage

To provide an assessment of seed response to deep-frost seed storage, the germination response of seeds after three or fifteen months of dry storage at 4 ± 1 °C, was tested after either eight or sixteen weeks of dry storage in liquid nitrogen at -80 °C. Following dry storage, seeds were placed in 20 mL sealed plastic tubes containing 15 mL of either acidic or neutral germination media solution and incubated at 21 °C on a 15-h photoperiod or in constant darkness for six weeks as previously described. Germination was scored weekly for six weeks in light treatments, but only once after six weeks in dark treatments.

2.5. Seed rising before germination

The relationship between seed rising (buoyancy) and seed germination was investigated for 800 seeds using Solution 3 in 20 mL test tubes. Three treatments each comprising five replicates of 20 seeds were established, including (1) controls; (2) light regime (15 h light/ 9 h dark) or continuous darkness; (3) temperature of seed germination of 21 ± 1 or 8 ± 1 °C. Germination was scored weekly for six weeks in all replicates, with seeds scored either as germinating without rising (e.g., remaining on the bottom), germinating after rising (i.e., germinated on the solution surface), rising without germinating, or neither germinating nor rising.

2.6. Statistical analysis

Binary logistic regression (SPSS Statistics 28; IBM, United States) was used to assess the main and interaction effects of light exposure, incubation temperature, treatment (ethylene exposure, stratification treatments and seed storage treatments) on seed germination success. Three-way ANOVA with Tukey post-hoc tests were used to determine the main and interaction effects of temperature, light exposure and seeds rising on the number of seeds from each replicate that germinated. All statistical tests were conducted using the 95 % confidence interval (CI), with significance determined by $P < 0.05$. Data are presented as mean \pm SE of the raw data, unless stated otherwise.

3. Results

3.1. Monitoring of temperatures from natural sites

Daily water temperature trends were similar for the two *U. vulgaris* populations over the January to December period monitored (Fig. 3), with daily water temperature at 30 cm depth ranging from 2.3 to 22.9 °C at Hrabanovská černava fen and -2.6 – 26.5 °C at Bohdanečský fishpond. Water remained cold (<10 °C) from October following seed production and dehiscence through to March–April, before warming markedly (18 – 28 °C) prior to observation of natural seed germination in June–July.

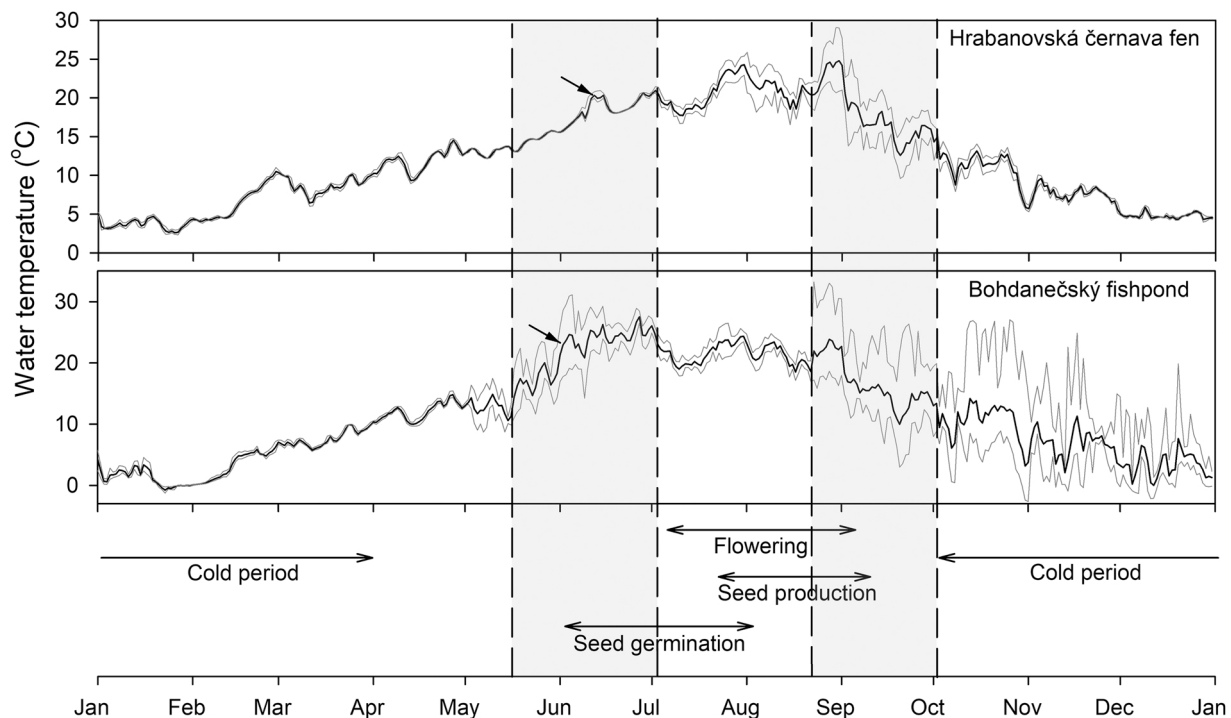


Fig. 3. Average daily temperature (bold lines) and daily minimum and maximum temperature (grey lines) at two *Utricularia vulgaris* populations in East Bohemia, Czech Republic, with key ecological periods relating to seed-based reproduction in *U. vulgaris* annotated (L. Adamec and R. Prausová, unpubl.). Grey columns indicate periods where temperatures may be conducive to warm stratification of seeds in certain areas of *U. vulgaris* habitat, while arrows indicate the point at which average daily water temperature reached 21°C for the first time over the seasonal monitoring period.

3.2. Seed morphometric characteristics

Measured *U. vulgaris* seeds were prismatic (Fig. 2), mostly pentagonal with seven faces (86 % of studied seeds), occasionally with eight (13 %) or, rarely, six faces (1 %) (Figs. 1 and 2). Mature, dehiscent seeds were dark grey to dark green-black in colour, the width of the upper part ranged from 0.4 to 1.9 mm (mean 0.9 ± 0.3 mm), of the bottom part from 0.6 to 2.0 mm (mean 1.2 ± 0.3 mm) and the height from 0.3 to 1.7 mm (mean 1.0 ± 0.22 mm). The embryo appears undifferentiated at seed maturity (Fig. 1).

3.3. Germination biology

Germination success for freshly-collected, untreated control seeds ranged from 0.21 to 0.39 at 21 °C and 0.07–0.31 at 25 °C on a 15-h photoperiod, and 0.09–0.29 at 21 °C and 0.04–0.27 at 25 °C in constant darkness (Table S1, Fig. 4). Numerous interaction effects on germination success were present among light exposure, incubation temperature, storage method and treatment, with strongest effects from the interaction of Light exposure \times Incubation temperature, Germination solution \times Incubation temperature, Germination solution \times Treatment, and Light exposure \times Treatment (Table 1).

Germination success across all treatments was greater for seeds incubated on a 15-h photoperiod than in constant darkness (Fig. 4), and this effect was more pronounced for seeds incubated at 25 °C (0.14 ± 0.005 in light and 0.05 ± 0.003 in darkness) than for seeds at 21 °C (0.19 ± 0.006 in light, 0.09 ± 0.004 in darkness). Similarly, the suppression of germination at 25 °C resulted in comparable germination success across all treatments among the three germination solutions (0.11 ± 0.005 at pH 5.5, 0.08 ± 0.005 at pH 6, 0.10 ± 0.005 at pH 8), while germination was notably more successful at pH 8 (0.17 ± 0.007) and pH 6 (0.16 ± 0.007) compared with pH 5.5 (0.10 ± 0.005) when seeds were incubated at 21 °C.

Compared with controls, germination success was markedly

increased following warm stratification and exposure to ethylene (up to 0.75–0.90; Fig. 4). This effect was most pronounced for wet-stored seeds in pH 8 and pH 6 solutions incubated on a 15-h photoperiod. Although the effects were partly suppressed at both temperatures in dry-stored seeds and markedly in seeds incubated in constant darkness, both warm stratification and ethylene exposure did still generally improve germination success under these conditions compared with controls. Cold stratification, varying stratification, and frost stratification had no consistent or pronounced effect on germination success except for the pH 6 solution (Fig. 4). The storage of *U. vulgaris* seeds in liquid nitrogen did not appear detrimental to germination success, and, indeed, seeds incubated at 21 °C and pH 8 following liquid nitrogen storage germinated much better than in control treatments in both light and constant darkness (0.6–0.7; Fig. 4).

3.4. Relationship between seed rising and germination

Of 461 seeds that germinated, 448 (97 %) rose to the surface of alkaline solution prior to germination while only 13 (3 %) germinated without rising. The effect of seeds rising on germination numbers was highly significant ($F(2108) = 275.1$, $P < 0.001$). Neither temperature ($F(1108) = 2.974$, $P = 0.087$), nor light exposure ($F(1108) = 275.05$, $P = 0.052$) significantly influenced germination in this trial (Fig. 5), and no significant interaction effects were evident.

4. Discussion

Utricularia vulgaris produces minute (<1 mm) endospermous seeds with a small, undifferentiated embryo (Fig. 1). Seed germination appears photophilous (or, at least, germination was markedly suppressed in seeds incubated under constant darkness), and was most successful in an alkaline (pH 8) germination solution reflective of the water chemistry of sites harbouring natural *U. vulgaris* populations (noting that the pH 6 solution may be more reflective of the pH of the sediment). The seeds of

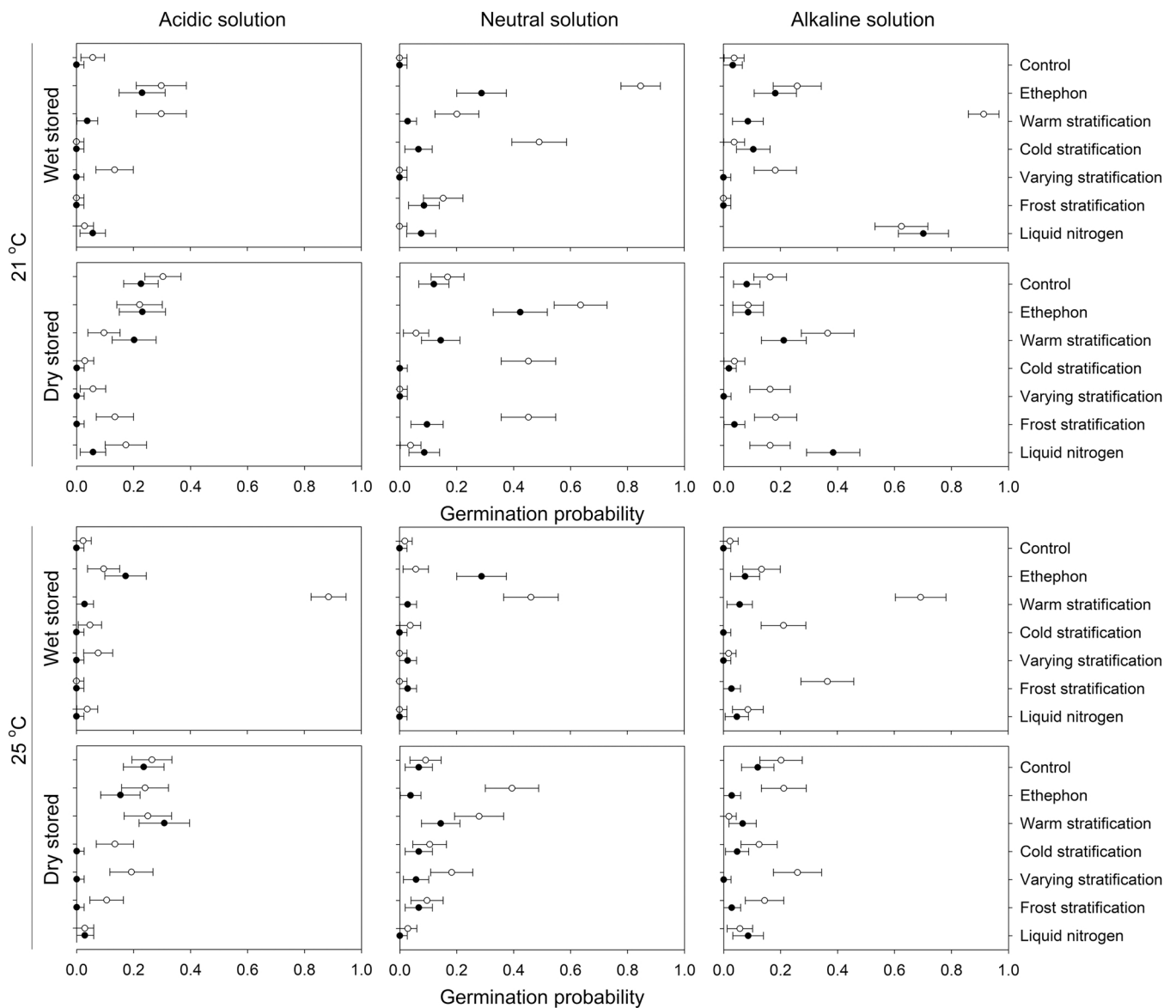


Fig. 4. Germination probability $\pm 95\%$ CI of *Utricularia vulgaris* seeds incubated in light (open symbols) or constant darkness (closed symbols) under different temperature conditions in acidic, neutral, or alkaline germination media following either wet or dry storage and in response to different stratification, germination stimulation and storage treatments.

Table 1

Statistical outputs (interaction effects) from testing the influence of experimental factors (light exposure, incubation temperature, germination solution, treatment, and storage method) on *Utricularia vulgaris* seed germination success. B indicates unstandardised regression weight (i.e., slope of the regression line).

Factor	B	χ^2	P
Light exposure \times Incubation temperature	0.129	157.5	<0.001
Germination solution \times Incubation temperature	-0.126	95.8	<0.001
Germination solution \times Treatment	-0.132	88.3	<0.001
Light exposure \times Treatment	-0.210	70.7	<0.001
Light exposure \times Storage method	-0.540	27.0	<0.001
Incubation temperature \times Storage method	0.086	20.1	<0.001
Incubation temperature \times Treatment	-0.014	11.1	0.001
Germination solution \times Storage method	0.199	10.5	0.001
Treatment \times Storage method	-0.077	10.0	0.002

U. vulgaris appear to possess non-deep simple morphophysiological dormancy (MPD) on the basis of an undifferentiated embryo at seed maturity and an apparent physiological component to dormancy as

indicated by a strong positive effect of warm stratification for light-exposed seeds. Exposure to ethylene appears to act as a germination stimulant in *U. vulgaris*, as has been reported for other, often co-occurring aquatic carnivorous plants including *Aldrovanda vesiculosa* (Cross et al., 2012, 2016). Indeed, a stimulatory effect of ethylene on seed germination has also been reported for other carnivorous plants from wet habitats, such as species of *Byblis* (Cross et al., 2018a).

Data from the present study suggests the free-floating aquatic *U. vulgaris* produces photophilous seeds, with light exposure a requirement for seed germination. Seeds of aquatic plants typically require light for seed germination to occur (Frankland et al., 1987; Baskin et Baskin, 1998). This prevents seeds from germinating underwater in conditions unsuitable for further growth of seedlings; for example, in anaerobic environments in muddy substrate or at low temperatures (Bibbey, 1948; Holm, 1972; Baskin et Baskin, 1998). In seed-rising experiments, the vast majority of seeds surfaced prior to germination, though a few also germinated without rising (Fig. 5). This same trend has also been commonly observed for *U. vulgaris* seeds germinating outdoors in containers under experimental culture (L. Adamec, unpublished). We

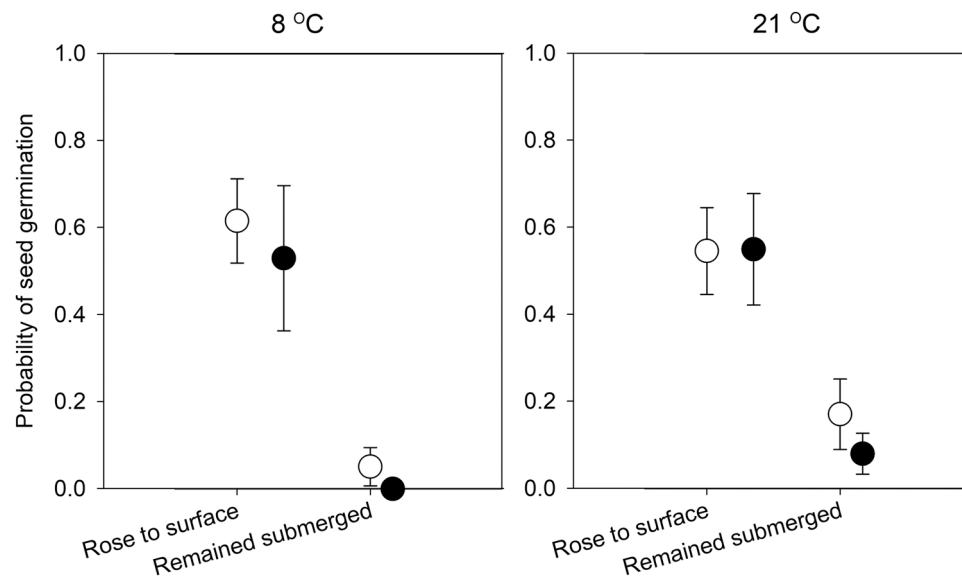


Fig. 5. Germination probability $\pm 95\%$ CI of *Utricularia vulgaris* seeds between those that rose to the surface of germination media and those that remained fully submerged, incubated in light (open symbols) or constant darkness (closed symbols) under different temperature conditions.

therefore suggest that the rising of seeds to the water surface is (broadly) a necessary precursor to seed germination, likely facilitating light exposure. The rising of *U. vulgaris* seeds in this manner is probably an important ecological process, as otherwise the tiny seedlings (Fig. S1) may become entangled at depth and at low temperatures, potentially in low-light conditions and under anoxia. To the best of our knowledge, this process has not been described and published so far. It seems likely that gas accumulation (possibly CO_2) inside or within the seed testa influencing seed buoyancy, in a similar fashion proposed for *A. vesiculosa* (Cross, 2012), may facilitate this process; future studies should examine this interesting and potentially important ecological process in greater detail.

During experiments, greatest seed germination in *U. vulgaris* occurred in a germination solution of pH 8. This treatment most closely replicated the pH conditions of the natural habitat at Hrabanovská černava fen, where water pH during the growing season ranges from pH 7–8. This may be expected given *U. vulgaris* most commonly occurs in pH-neutral water in fens (Kaplan et al., 2017), and suggests the conditions required for seed germination in the aquatic *U. vulgaris* may differ from those of terrestrial *Utricularia* species. For example, Kondo (1971) employed four different pH solutions (pH 4.5, 4.7, 5.1, 7.2) in germinating the seeds of terrestrial *U. juncea* and *U. cornuta*, and reported highest germination probability for both species at pH 4.5–5.1.

Seed dormancy with a physiological component must be alleviated by exposure to periods of suitable environmental conditions (Baskin and Baskin, 1998). Previous authors have proposed that seed dormancy in *U. vulgaris*, at least in temperate regions, is likely alleviated by a period of cold stratification over the cold winter months prior to germination in spring, as indicated by high germination success reported for seeds incubated under indicative spring temperatures following cold stratification (Baskin et Baskin, 1998). Similar germination responses following cold stratification have been reported for species co-occurring with *U. vulgaris*, including *Potamogeton praelongus* (Prausová et al., 2013, 2014). However, data from the present study suggests that cold stratification was poorly effective at alleviating seed dormancy in *U. vulgaris*, and that MPD was instead alleviated by a period of warm stratification (Fig. 4). Response to warm stratification in this manner may facilitate seed germination in shallow wetland pools where the water column warms in temperature more rapidly following the onset of spring conditions. However, such shallow wetland habitats may experience more pronounced drying during late spring and early summer without

consistent rainfall; for example, complete desiccation of *U. vulgaris* habitat was observed in 2018 at both study sites in the Czech Republic (Bohdanečský fishpond and Hrabanovská černava fen). As warm stratification occurs when seeds are exposed to periods of warm, moist storage (Kaplan et al., 2017), it is also possible that seed dormancy is alleviated in late summer as water levels begin rising but ambient temperatures remain warm. Further study is required to determine the seasonal timing of warm stratification in *U. vulgaris*.

It is plausible that the apparent stimulatory effect of ethylene on seed germination in *U. vulgaris* represents a bet hedging mechanism, with biogenic ethylene release from sediment signaling suitable sediment moisture levels for seed germination as has been reported for other shallow, highly ephemeral wetlands (Cross et al., 2014). The light requirement can be somewhat alleviated by ethylene exposure. According to our results, an impact of Ethephon releasing ethylene stimulates seed germination in both the light and dark conditions (the most successful variant of all tests in dark conditions, germination rate 17.3%). The presence of ethylene probably compensates the lack of light during germination. Positive effect of Ethephon on germination was proved for other aquatic plants, for example up to 5% *Potamogeton praelongus* seeds germinated with Ethephon (Prausová et al., 2014). Germination rate was high (>75%) in ethylene-exposed seeds incubated at 21 °C, on a 15-h photoperiod in alkaline germination solution. This is almost an identical mechanism to what was found for *Aldrovanda vesiculosa* (Cross et al., 2016), and very similar to other carnivorous plants from wet habitats such as *Byblis* (Cross et al., 2018a).

It appears that *U. vulgaris* produces a long-lived sediment seed bank, as seeds discovered in 70–100-year-old sediments in South Moravia have been subsequently germinated under laboratory experiments (Gálová and Hájková, 2014). This may imply that *U. vulgaris* seeds cycle in and out of dormancy in response to seasonal conditions, as has been shown for other species producing seeds with a physiological component to dormancy (e.g., Merritt et al., 2007). *Utricularia vulgaris* seeds were stored under both dry and wet conditions before seed germination experiments, and we observed no decline in germinability following even extended periods of dry storage prior to germination experiments. Baskin and Baskin (1998) report that the seeds of some aquatic plants both exit and re-enter dormancy in response to seasonal drought, and Arts and van der Heijden (1990) confirmed that drought or warm stratification for 28 days at about 20/8 °C temperature alleviated or broken seed dormancy in *Littorella uniflora* (Plantaginaceae). Muenscher (1936)

reported that air-dried *Potamogeton* seeds stored dry for 2–3 months did not germinate, while Hay et al. (2008) suggested that *Potamogeton* seeds survived drying and germinated upon subsequent rewetting. Seeds of *P. praelongus* survived drying and germinated after waterlogging (Prausová et al., 2013, 2014). Warm stratification or dry storage broke seed dormancy of mediterranean species *P. schweinfurthii* (Spence et al., 1971). Seeds of *Aldrovanda vesiculosa* also appear tolerant of desiccation and may survive across seasons in a persistent sediment seed bank (Cross et al., 2015). Future studies should examine the persistence of the seed bank in *U. vulgaris* and other aquatic carnivorous plants, and the degree to which seeds are tolerant of extended periods of seasonal desiccation.

The storage of seeds at low temperature is a possible method to prevent physiological changes within seeds responsible for seed dormancy alleviation or the stimulation of germination (Baskin and Baskin, 1998). Cryopreservation at ultra-low temperatures (liquid nitrogen, $-196\text{ }^{\circ}\text{C}$) allows for the long-term storage of plant materials, including the seeds of at least some species of carnivorous plants (North et al., 2021). Standard cryopreservation techniques are based on dehydration caused by freezing (Engelmann, 2004), and data from the present study suggests that storage of *U. vulgaris* seeds in liquid nitrogen was not deleterious to seed germination success. Indeed, *U. vulgaris* seeds stored at $-18\text{ }^{\circ}\text{C}$ or in liquid nitrogen at $-80\text{ }^{\circ}\text{C}$ germinated more readily than seeds stored for a comparable period at room temperature (Fig. 4). Future studies should examine the efficacy and viability of cryopreservation as an *ex situ* conservation tool in *U. vulgaris* and other aquatic carnivorous plants in greater detail.

5. Conclusions

Apparent morphophysiological seed dormancy in *U. vulgaris* appears alleviated by warm stratification, with seed germination stimulated by exposure to ethylene gas under suitable temperature and moisture conditions. However, the success of seed germination appears linked to light availability, water chemistry, and, thus, seed position in the water column, which indicates careful management of remnant habitats harbouring this species may be needed to ensure positive conservation outcomes. As *U. vulgaris* has been assessed as Critically Endangered in the Czech Republic (Grulich, 2017), threatened primarily by successional degradation of sites, eutrophication, grounding of pools and ponds, and hydrological changes (Šumberová, 2011), management activities should focus on the maintenance of hydrological regimes, water quality and control of invasive macrophytes. Management measures should maintain the shallow, open, mesotrophic habitats enabling vigorous growth of *U. vulgaris* (Kaplan et al., 2017), ensuring that conditions are optimal for both asexual and seed-based reproduction.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

- Adamec, L., 2018a. Ecophysiology of aquatic carnivorous plants. In: Ellison, A.M., Adamec, L. (Eds.), *Carnivorous Plants: Physiology, Ecology, and Evolution*. Oxford University Press, Oxford, U.K., pp. 256–269.
- Adamec, L., 2018b. Germination rate and longevity of seeds of *Aldrovanda vesiculosa* and *Utricularia vulgaris*. *Carniv. Plant Newslett* 47, 64–69.
- Arts, G.H.P., van der Heijden, R.A.J.M., 1990. Germination ecology of *Littorella uniflora* (L.) Aschers. *Aquat. Bot.* 37, 139–151.
- Baskin, C.C., Baskin, J.M., 1998. *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination*. Academic Press, San Diego, USA.
- Baskin, C.C., Baskin, J.M., 2014. *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination*, 2nd edition. Academic Press, San Diego, USA.
- Bibbey, R.O., 1948. Physiological studies of weed seed germination. *Plant Physiol.* 23, 467–484.
- Cross, A.T., 2012. *Aldrovanda: The Waterwheel Plant*. Redfern Natural History Productions. Dorset, UK.
- Cross, A.T., Cawthray, G.R., Merritt, D.J., Turner, S.R., Renton, M., Dixon, K.W., 2014. Biogenic ethylene promotes seedling emergence from the sediment seed bank in an ephemeral tropical rock pool habitat. *Plant Soil* 380, 73–87.
- Cross, A.T., Turner, S.R., Renton, M., Baskin, J., Dixon, K.W., Merritt, D.J., 2015. Seed dormancy and persistent sediment seed banks of ephemeral freshwater rock pools in the Australian Monsoon Tropics. *Ann. Bot.* 115, 847–859.
- Cross, A.T., Adamec, L., Turner, S.R., Dixon, K.W., Merritt, D.J., 2016. Seed reproductive biology of the rare aquatic carnivorous plant *Aldrovanda vesiculosa* L. (Droseraceae). *Bot. J. Linn. Soc.* 180, 515–529.
- Cross, A.T., Davis, A., Fleischmann, A., Horner, J.D., Jürgens, A., Merritt, D.J., Murza, G. L., Turner, S.R., 2018a. Reproductive biology and prey-pollinator conflicts. In: Ellison, A.M., Adamec, L. (Eds.), *Carnivorous Plants: Physiology, Ecology, and Evolution*. Oxford University Press, Oxford, U.K., pp. 294–313.
- Cross, A.T., Barrett, M.D., Turner, S.R., Dixon, K.W., Merritt, D.J., 2018b. Seed-dormancy depth is partitioned more strongly among habitats than among species in tropical ephemerals. *Aust. J. Bot.* 66, 230–242.
- Engelmann, F., 2004. Plant cryopreservation: progress and prospect. *In Vitro Cell. Develop. Biol.* 40, 427–433.
- Fleischmann, A., Schlauer, J., 2014. Die Gattung *Utricularia* in Bayern. *Ber. Bayer. Bot. Ges.* 84, 65–90.
- Frankland, B., Bartley, M.R., Spence, D.H., 1987. Germination under water. In: Crawford, R.M.M. (Ed.), *Plant Life in Aquatic and Amphibious Habitats*. Blackwell Scientific Publications, Oxford, U.K., pp. 167–177.
- Gálová, A., Hájková, P., 2014. [*Utricularia vulgaris* in Hodonínská Důbrava.] In *Czech. Zprávy České Bot. Spol.* 49, 261–271.
- Grulich, V., 2017. [The Red List of vascular plants of the Czech Republic.] In *Czech. In: Grulich, V., Chobot, K. (Eds.), Červený seznam ohrožených druhů České republiky, 35. Cévnaté rostliny, Příroda (Prague)*, pp. 75–132.
- Hay, F., Probert, R., Dawson, M., 2008. Laboratory germination of seeds from 10 British species of *Potamogeton*. *Aquat. Bot.* 88, 353–357.
- Holm, R.E., 1972. Volatile metabolites controlling germination in buried weed seeds. *Plant Physiol.* 50, 293–297.
- Jará, N., Prausová, R., 2021. [The comparison of two wetland sites in the Elbe region as regards to their suitability for survival of mixotrophic species of *Utricularia vulgaris* and *Utricularia australis*.] In *Czech. Vě. Sb. pírř. – Práce a Stud.* 27, 49–75.
- Distributions of vascular plants in the Czech Republic. In: Kaplan, Z., Danihelka, J., Šumberová, K., Chrtek, J., Rotreklová, O. (Eds.), 2017, Part 5. *Preslia*, 89, pp. 333–439.
- Kildisheva, O.A., Dixon, K.W., Silveira, F.A.O., Chapman, T., Di Sacco, A., Mondoni, A., Turner, S.R., Cross, A.T., 2020. Dormancy and germination: making every seed count in restoration. *Restor. Ecol.* 28, S256–S265.
- Kondo, K., 1971. Germination and developmental morphology of seeds in *Utricularia cornuta* Michx. and *Utricularia juncea* Vahl. *Rhodora* 73, 541–547.
- Koshiha, P., 1992a. Studies on the ecology of *Utricularia vulgaris* L. I: ecological differentiation of *Utricularia vulgaris* L. population affected by chemical factors of the habitat. *Ekol. Pol.* 40 (2), 147–192.
- Koshiha, P., 1992b. Studies on the ecology of *Utricularia vulgaris* L. II: physical, chemical, and biotic factors and the growth of *Utricularia vulgaris* L. in cultures in vitro. *Ekol. Pol.* 40 (2), 193–212.
- Koshiha, P., 2004. Chemical properties and similarity of habitats of *Utricularia* species in Lower Silesia, Poland. *Acta Soc. Bot. Pol.* 74 (4), 335–341.
- Merritt, D.J., Turner, S.R., Clarke, S., Dixon, K.W., 2007. Seed dormancy and germination stimulation syndromes for Australian temperate species. *Aust. J. Bot.* 55, 336–344.
- Muenschler, W.C., 1936. The germination of seeds of *Potamogeton*. *Ann. Bot.* 50, 805–821.
- North, T., Chong, C., Cross, A.T., van der Walt, K., Ballesteros, D., 2021. Special collections and under-represented taxa in Australasian *ex situ* conservation programs, 2021. In: Martyn Yenson, A.J., Offord, C.A., Meagher, P.F., Auld, T., Bush, D., Coates, D.J., Commander, L.E., Guja, L.K., Norton, S.L., Makinson, R.O., Stanley, R., Walsh, N., Wrigley, D., Broadhurst, L. (Eds.), *Plant Germplasm Conservation in Australia: Strategies and Guidelines for Developing, Managing and Utilising Ex Situ Collections*. Third edition. Australian Network for Plant Conservation, Canberra, pp. 403–439, 2021.
- Plachno, B.J., Spiczynska, M., Adamec, L., Miranda, V.F.O., Świątek, P., 2018. Nectar trichome structure of aquatic bladderworts from the section *Utricularia* (Lentibulariaceae) with observation of flower visitors and pollinators. *Protoplasma* 255, 1053–1064.
- Prausová, R., Janová, J., Safárová, L., 2013. Testing achene germination of *Potamogeton praelongus* Wulfen. *Cent. Eur. J. Biol.* 8, 78–86.

- Prausová, R., Sikorová, P., Šafářová, L., 2014. Generative reproduction of long stalked pondweed (*Potamogeton praelongus* Wulfen) in the laboratory. *Aquat. Bot.* 120, 268–274.
- Sirová, D., Adamec, L., Vrba, J., 2003. Enzymatic activities in traps of four aquatic species of the carnivorous genus *Utricularia*. *N. Phytol.* 159, 669–675.
- Spence, D.H.N., Milburn, T.R., Ndawula-Senyimba, M., Roberts, E., 1971. Fruit biology and germination of two tropical *Potamogeton* species. *N. Phytol.* 70, 197–212.
- Swamy, R.D., Ram, H.Y., Mohan, 1969. Studies on growth and flowering in axenic cultures of insectivorous plants, I. Seed germination and establishment of cultures of *Utricularia inflexa*. *Phytomorphology* 19, 363–371.
- Šumberová, K., 2011. [Vegetation of free-floating aquatic plants.] In Czech. In: Chytrý, M. (Ed.), *Vegetace České republiky. 3. Vodní a mokřadní*. Academia, Prague.
- Taylor, P., 1989. The Genus *Utricularia* - A Taxonomic Monograph. *Kew Bull. Ser. XIV.* HMSO, London.