

**Rescue of the critically endangered long-stalked pondweed  
(*Potamogeton praelongus*) in the Czech Republic**

Ohranjanje močno ogroženega podaljšanega dristavca (*Potamogeton praelongus*)  
na Češkem

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**Abstract:** *Potamogeton praelongus* occurs in the Czech Republic at only one natural site. As part of a rescue programme, micropopulations were introduced into new locations. The aim of the paper is to describe the realised measures: monitoring micropopulations and habitat factors at *P. praelongus* sites, investigation of seed germination, and preparation of a sterile *in-vitro* culture for plant propagation and conservation purposes. Between 2008–2010, both micropopulations in the Orlice floodplain increased their size by three times annually. In 2010, 1461 shoots occurred at the natural site and 199 at the artificial one. In 2010, stand areas varied between 12–50 m<sup>2</sup>. The most effective treatment to break seed dormancy involved 2-h surface sterilisation in 5 % or 2.5 % NaClO solution preceded by a period of desiccation, temperature variation, hypoxic conditions, and mechanical abrasion of the seed coat. Using similar methods of NaClO sterilisation, an *in-vitro* culture of *P. praelongus* was prepared and 30 clones with an expected genetic variability are available.

**Keywords:** long-stalked pondweed, conservation programme, monitoring, seed germination tests, sterile culture *in vitro*

**Izvleček:** Vrsta *Potamogeton praelongus* se na Češkem v naravi pojavlja le na eni lokaciji. V okviru programa ohranjanja vrste smo vnesli rastline na nove lokacije. V članku predstavljamo celoten postopek: spremljanje stanja mikropopulacije in habitatne parametre na območjih uspevanja vrste *P. praelongus*, raziskave kalitve semen ter postopek priprave sterilnih *in-vitro* kultur za razširjanje. V času od 2008–2010 sta se obe mikropopulaciji na poplavni ravnici reke Orlice povečali za 3-krat letno. Leta 2010 je bilo 1461 poganjkov na naravni in 199 na novi lokaciji. Leta 2010 so bili sestoji veliki od 12 do 50 m<sup>2</sup>. Za najbolj učinkovit način prekinitve dormance se je izkazala 2-h površinska sterilizacija s 5 % oz. z 2.5 % raztopino NaClO, ki ji je sledilo obdobje izsuševanja, izpostavljanja različnim temperaturam, anoksičnim razmeram in abrazija semenske lupine. S sterilizacijo z NaClO smo pripravili *in-vitro* kulturo vrste *P. praelongus*; 30 klonov z ustrezno gensko variabilnostjo.

**Ključne besede:** podaljšani dristavec, načrt ohranjanja, spremljanje stanja, kalitev semen, sterilna kultura *in vitro*

## Introduction

The perennial aquatic plant *Potamogeton praelongus* Wulfen, listed among the critically endangered plant taxa of the Czech flora, is restricted to just a single natural location in the Czech Republic, near the town of Hradec Králové in E Bohemia (Prausová and Janová 2010), where it grows in an oxbow tributary of the Orlice river. Globally, *P. praelongus* is a rare species of a nordic, partly suboceanic, and circumpolar distribution, spread predominantly throughout northern Europe (Vöge 1992). Within the same latitudes, it is also spread throughout Asia and North America, however is rare throughout its entire distribution. The species occurs in unpolluted mesotrophic waters in humic or sandy soils, clayish, muddy, or peaty beds (Casper and Krausch 1981). It is a distinctly stenotopic species for which characteristic growth is in clean, deeper, hard lowland waters on calcareous sediments (Husák and Adamec 1998). Ellenberg (1991) categorised *P. praelongus* as a photophilous plant growing only exceptionally at a relative irradiance below 40 %. The fruit of *P. praelongus*, morphologically an achene and herein referred to as “seed”, germinate very poorly (Janová 2010, Prausová et al. 2010). Recent molecular-genetic analyses have revealed only minimal genetic variability of *P. praelongus* micropopulations in the Czech Republic (Kitner et al. unpubl.), suggesting that the species propagates predominantly vegetatively. Recent studies have however noted methods of markedly increasing seed germination through breaking physical seed dormancy (Janová 2010, Prausová et al. 2010). Propagation in this manner could be used in rescuing the species in the Czech Republic.

The last native Czech population of *P. praelongus* is endangered by sedimentation due to a nutrient-rich sediment in the standing oxbow reach, covering leaf surfaces in fine sedimentary particles (Prausová and Janová 2010). High concentrations of mineral nutrients in the water also lead to the growth of detrimental filamentous algae, and the disturbance of plants and micropopulations during flood events is of potential concern. To support the last micropopulation in the Transient Protected Area, several principal measures have so far been conducted (Prausová et al. 2010). Muddy sediment was excavated from the standing oxbow

reach in 2001, and inshore tree stands were cut in 2002 to clear the water surface. In 2003, the Czech Ministry of Environment approved a rescue programme for *P. praelongus*, involving the clearing of surplus mud using a suction dredge from a part of the oxbow inhabited by *P. praelongus* near the estuary of the Orlice river. Since 2005, regular monitoring of all Czech *P. praelongus* micropopulations has occurred, in conjunction with monitoring habitat factors including water and sediment chemistry. Simultaneously, re-patriation of the species to the Orlice river floodplain has been conducted using plants raised in an outdoor rescue culture at the Institute of Botany at Třeboň, Czech Republic (Husák and Adamec 1998, Prausová et al. 2010).

During 2009–2010, the dominant part of the rescue programme within the project “Rescue of long-stalked pondweed (*Potamogeton praelongus*) in the Czech Republic”, raised by the Czech Ministry of Environment, was carried out. The aim of this paper is to describe the main biological measures which have been realised within the programme so far: monitoring plant fitness and habitat factors at natural and artificial *P. praelongus* sites, an investigation of seed germination, and preparation of a sterile tissue culture for plant propagation and consequent re-patriation purposes.

## Material and methods

### *Recent distribution of Potamogeton praelongus in the Czech Republic*

#### *Orlice river floodplain near Hradec Králové*

Recently, *P. praelongus* occurs natively only in the Transient Protected Area “Rameno u Stříbrného rybníka” at Malšova Lhota in the suburb of Hradec Králové (50°12'35"N, 15°53'17"E) in E Bohemia (Prausová and Janová 2010). The site, a standing oxbow, is situated at an altitude of 232 m a.s.l. in the left-bank part of the Orlice river floodplain and its lower reach has its estuary into the river. The oxbow sustains considerable deposition by organic sediments, the main source of which is tree litter originating from bank stands. Water chemistry at the site is weakly eutrophic and stagnant (Prausová et al. 2010). As a result of re-patriation efforts as part

of a rescue programme for this species, a new micropopulation has been successfully established in the Lake Kašparovo jezero (50°12'47"N, 15°52'19"E). It is an oxbow in the Orlice river floodplain on the right river bank, with similar ecological characteristics as the existing native site. Both oxbows are inhabited by dense stands of *Nuphar lutea* and loose stands of *Potamogeton crispus*, *Ranunculus trichophyllus* and *R. circinatus*.

#### *The Protected Landscape Area Kokořínsko*

Introduced *P. praelongus* populations have been growing in restored backwater pools in the Protected Landscape Area Kokořínsko in Central Bohemia (50°28' N, 14°31' E), since 2001–2005. Four populations exist, including pools below Plešivec, pools above the fishpond Harasov, pools near Štampach, and backwater pools in the Liběchovka rivulet floodplain (Prausová et al. 2010). The source plants for these introductions originated from the last native site.

#### *Rescue cultivation at the Institute of Botany at Třeboň, Czech Republic*

A rescue culture of *P. praelongus*, using plants sourced from the last native site, has been maintained at the Institute of Botany at Třeboň (IBT) since 1988 (see Husák and Adamec 1998). One plastic container (volume 1.5 m<sup>3</sup>), which is used for aquatic plant collection and is never emptied, houses *P. praelongus* plants potted in 26x26x26 cm plastic pots. Several further plastic containers of the same size, which are regularly emptied over winter, are maintained as *ex-situ* cultures, with *P. praelongus* planted directly into a shallow soil layer. For both methods a mixture of sand, garden loam, fishpond clay (mud), fen soil, and milled limestone fertilised by organic compost provides substrate. Over the warm summer season, all containers are partly shaded due to the growth of filamentous algae as surface water temperatures increase, with algal mats removed regularly. The plants overwinter either inundated (collection container) or in moist substrate (rescue culture) under a 15-cm layer of dry leaves covered by plastic plates (Husák and Adamec 1998). The water depth in both types of cultures is 40–50 cm.

#### *Micropopulation monitoring*

Regular monitoring of all Czech *P. praelongus* micropopulations was carried out, as part of the rescue programme, since 2005 (Prausová and Janová 2010, Prausová et al. 2010). At both sites in the Orlice river floodplain (Rameno u Stříbrného rybníka and Kašparovo jezero), individual shoots occurring in distinct bunches or visually distinguishable groups were counted using a boat. Observations were made in mid-July during the flowering or fruiting period, at a time of high water transparency and normal water level (Prausová et al. 2010). Both total number and proportion of flowering shoots were counted. Individual shoots could not be counted at sites within the Protected Landscape Kokořínsko (PLK), and the method of assessment of the plant stand area (in m<sup>2</sup>) was used during monitoring in the 2005 and 2007–2010 seasons. At all sites, plant occurrence was recorded in geographical coordinates using the GARMIN eTrex H (resolution ±4 m) GPS-instrument.

#### *Monitoring of site-specific habitat factors*

For monitoring habitat factors at *P. praelongus* sites, at all sites water samples were collected twice a year for water chemistry analyses (July, September). Electrical conductivity and pH were measured using portable instruments (Multimetric water Quality Sonde YSI600XLM) directly in the field. No pre-treatment of the water sample was used for chemical oxygen demand (COD) determination using the KMnO<sub>4</sub> titratory method. For all other analyses, water samples were filtered using a membrane filter (mesh size 0.45 µm). Concentrations of NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub> were determined (Flow injection ion analyzer Alliance) colorimetrically, while those of Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> using atomic absorption spectrometry (Spectrometer ICP/OES GBC Integra). See Pekárková and Lischke (1974) for all analytical details. Measured factors are presented for 11 individual measurements at the natural site between 2005–2010, and for 4 sites at Kašparovo jezero and the Protected Landscape Kokořínsko during 2009–2010. This excludes the pools near Štampach, where only two measurements were conducted in 2010.

*Seed dormancy and germination biology*

Germination tests investigated seed dormancy type and alleviation in *P. praelongus*, as well as assessing the species capacity for long-term hermetic storage (Janová 2010, Prausová et al. 2010). Seed was sequentially harvested from plants in cultivation at IBT or collected from the PLK, and stored in darkness either dry at room temperature ( $21\pm 2$  °C) or in water ( $8\pm 1$  °C) for up to six months before beginning experimental work. To assess natural cycles of desiccation and rewetting on germination, some treatments involved direct rehydration of seed and subsequent storage in water at room temperature for one month prior to germination testing. In all treatments seed were placed in 10 cm Petri dishes and allowed to germinate in dark conditions on moistened filter paper, incubated either at a slightly variable temperature of  $21\pm 2$  °C or in a thermostatted chamber at  $23\pm 0.5$  °C or  $28\pm 0.5$  °C. The filtered water collected from the natural site was used to wet the seeds. Where microbial contamination of replicates occurred, seeds were immediately removed, thoroughly washed in distilled water, and transferred to new Petri dishes. Replicates placed in 1–6 parallel Petri dishes varied in number due to the sequential availability of seed, and ranged from 34 to 294 per treatment. To assess the effect of a chemical agent in alleviating dormancy, SAVO Prim (Bochemie, Bohumín, Czech Republic), containing 5 % NaClO and saponate, was applied in varying concentration and for varying duration (see below; Janová 2010). After chemical treatment, seeds were thoroughly washed in distilled water before placing in Petri dishes. Germination was determined by development of the cotyledon, as radicle emergence in the species occurs at a later developmental stage (Janová 2010), with the tests running for four months. Germinants with 1–2 leaves were transferred to 4L aquaria to allow continued growth. In total, 27 variants of seed germination tests were conducted (Janová 2010, Prausová et al. 2010).

1. Controls: 34 seeds collected in the IBT, kept in water at  $8\pm 1$  °C, no treatment of seeds, germination at ca.  $21\pm 2$  °C;
2. Controls: 68 seeds collected in the IBT, kept in water at  $8\pm 1$  °C, no treatment of seeds, germination at  $23\pm 0.5$  °C;

3. Freeze tolerance: 34 seeds collected in the IBT, kept in water at  $8\pm 1$  °C, exposed at  $-18$  °C in a refrigerator for 36 h before the germination test, germination at ca.  $21\pm 2$  °C;
4. Freeze tolerance: 68 seeds collected in the IBT, kept in water at  $8\pm 1$  °C, exposed at  $-18$  °C in a freezer for 36 h before the germination test, germination at  $23\pm 0.5$  °C;
5. Treatment with gibberellic acid (GA<sub>3</sub>): 50 seeds collected from the PLK, kept in dry state at room temperature for 3 months till the experiment, washed in distilled water, addition of GA<sub>3</sub> to the germination solution (final concentration 15 mg.l<sup>-1</sup>), germination at  $28\pm 0.5$  °C;
6. Treatment with GA<sub>3</sub>: 50 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then kept in the water for one month till the experiment, washed in distilled water, addition of GA<sub>3</sub> to the germination solution (final concentration 15 mg.l<sup>-1</sup>), germination at  $28\pm 0.5$  °C;
7. Scarification: 100 seeds collected from the PLK, kept in dry state at room temperature for 3 months till the experiment, seed testa abraded thoroughly by an abrasive paper, germination at  $28\pm 0.5$  °C;
8. Scarification: 50 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then kept in the water for one month till the experiment, seed testa abraded thoroughly by an abrasive paper, germination at  $28\pm 0.5$  °C;
9. Hypoxic conditions: 100 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then kept in the water for one month till the experiment, ethanol droplets added to the germination water to cause hypoxia, Petri dishes thoroughly sealed by 3 layers of sealing tape, germination at  $28\pm 0.5$  °C;
10. Long cold stratification: 50 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then kept in dry state in a refrigerator at 8 °C for 2.5 months followed by ca.  $21\pm 2$  °C for 14 d, then wetting and germination at  $28\pm 0.5$  °C;
11. Long cold stratification: 50 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then kept in the water

- for one month, kept in a refrigerator at 8 °C for 2.5 months followed by ca. 21±2 °C for 14 d, then germination at 28±0.5 °C;
12. Short cold stratification: 50 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then kept in the water for one month, kept in a refrigerator at 8 °C for 1 month, in a freezer at -20 °C for 1 month, again in a refrigerator at 8 °C for 14 d followed by ca. 21±2 °C for 14 d, then germination at 28±0.5 °C;
  13. Short cold stratification: 100 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then kept in the water for one month, kept in a refrigerator at 8 °C for 1 month followed by ca. 21±2 °C for 14 d, then germination at 28±0.5 °C;
  14. Long desiccation: 50 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then germination at 28±0.5 °C;
  15. Short desiccation: 34 seeds collected in the IBT, kept in water at 8±1 °C till the experiment, dried out at ca. 21±2 °C for 36 h, then wetting and germination at ca. 21±2 °C;
  16. Short desiccation: 68 seeds collected in the IBT, kept in water at 8±1 °C till the experiment, dried out at ca. 21±2 °C for 36 h, then wetting and germination at 23±0.5 °C;
  17. Long desiccation: 100 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then germination at ca. 21±2 °C;
  18. Transient long desiccation: 218 seeds collected from the PLK, kept in water at 8±1 °C for 3 months, then kept at dry state at room temperature for 3 months, germination at 28±0.5 °C;
  19. One-fourth strength SAVO for 2 h: 74 seeds collected from the PLK, kept in dry state at room temperature for 3 months, treated by one-fourth strength SAVO for 2 h, then germination at 28±0.5 °C;
  20. Half strength SAVO for 2 h: 75 seeds collected from the PLK, kept in dry state at room temperature for 3 months, treated by half strength SAVO for 2 h, then germination at 28±0.5 °C;
  21. Half strength SAVO for 2 h: 50 seeds collected from the IBT, kept in water at 8±1 °C till the experiment, treated by half strength SAVO for 2 h, then germination at 28±0.5 °C;
  22. Half strength SAVO for 2 h: 50 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then kept in the water for one month, treated by half strength SAVO for 2 h, then germination at 28±0.5 °C;
  23. Full strength SAVO for 36 h: 34 seeds collected from the IBT, kept in water at 8±1 °C till the experiment, treated by full strength SAVO for 36 h, then germination at ca. 21±2 °C;
  24. Full strength SAVO for 36 h: 68 seeds collected from the IBT, kept in water at 8±1 °C till the experiment, treated by full strength SAVO for 36 h, then germination at 23±0.5 °C;
  25. Full strength SAVO for 2 h: 132 seeds collected from the PLK, kept in dry state at room temperature for 3 months, treated by full strength SAVO for 2 h, then germination at ca. 21±2 °C;
  26. Full strength SAVO for 2 h: 294 seeds collected from the PLK, kept in dry state at room temperature for 3 months, treated by full strength SAVO for 2 h, then germination at 28±0.5 °C;
  27. Full strength SAVO for 2 h: 50 seeds collected from the PLK, kept in dry state at room temperature for 3 months, then kept in the water for one month, treated by full strength SAVO for 2 h, then germination at 28±0.5 °C.
- We are aware of the fact that all seeds within one treatment are pseudoreplicates and no statistical treatment of the results is possible. Thus, the results basically show the qualitative effects.

#### *Preparation of a sterile in-vitro culture*

Seeds harvested at the IBT in September 2009 were used for the preparation of a sterile *in-vitro* culture. All seeds were kept in a refrigerator at 3 °C in darkness, with some in a dry state and others in tap water. Before sterilisation, both dry and wet seeds were shortly shaken in a diluted saponate solution (v/v 1:200), washed in streaming tap water for around 30 h, and the remains of the pericarp were mechanically removed. Seeds were surface sterilised using half or full strength SAVO Prim for 4, 8 or 16 h. The seeds were then thoroughly washed in three bottles filled with sterile tap or distilled water. A tenth strength Murashige-Skoog medium (Murashige and Skoog

1962) supplemented with 2 % sucrose was used as a germination medium. The medium was either liquid or solidified with 0.6 % gellite (natural anionic polysaccharide, Duchefa, Haarlem, The Netherlands). The pH of both solutions was 6.5 before autoclaving. Test-tubes (3 cm diameter) were filled each with either 10 ml of the solid or 20 ml of the liquid medium. One seed was placed in each test-tube and all seeds were spread evenly between each media type. In total, 238 seeds were used. Seed germination took place in darkness, at 14 to 18 °C during the first month and then at 18±0.5 °C. Upon germination test tubes were transferred to fluorescently illuminated boxes (PAR irradiance of ca. 30–40  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and 12-h photoperiod) kept at 21±1 °C.

After two months of post-germination growth, seedlings were transplanted into a fresh liquid culture of higher concentration (half strength Gamborg B5 medium with 500  $\text{mg}\cdot\text{l}^{-1}$   $\text{KNO}_3$ , with 2.5 % sucrose, pH 5.7 or 6.5; Adamec and Pásek 2000). The volume of renewed sterile culture medium was 50–70 ml in 350 ml flasks or 300 ml in 0.5 l flasks. Seedlings rapidly resumed vigorous growth after transferral to new medium. Plants outgrew

flasks in 2–3 month cycles (ca. 8–15 shoot apices produced), exhausted the medium (the final pH of media was 4.65–6.25), and at this point 1–3 apical shoot segments were transplanted into new flasks. Excess plants were planted outdoors in rescue culture (IBT) to mature.

## Results

### *Monitoring of populations and habitat factors*

The remaining natural *P. praelongus* micro-population at Rameno u Stříbrného rybníka has increased since 2005, with marked increase since 2008 (Fig. 1). Original shoot numbers rose from 32 in 2005 to 1461 growing in 115 colonies in 2010; of these 199 shoots were fertile. Though the micropopulation is still concentrated in the oxbow reach in front of the estuary to the Orlice river, the re-patriation of plants in 2008 has led to spread along both oxbow banks further away from the main estuary. New colonies originate both from the original and also from re-patriated shoots. At the artificial site Kašparovo jezero in the Orlice river floodplain, where a successful introduction

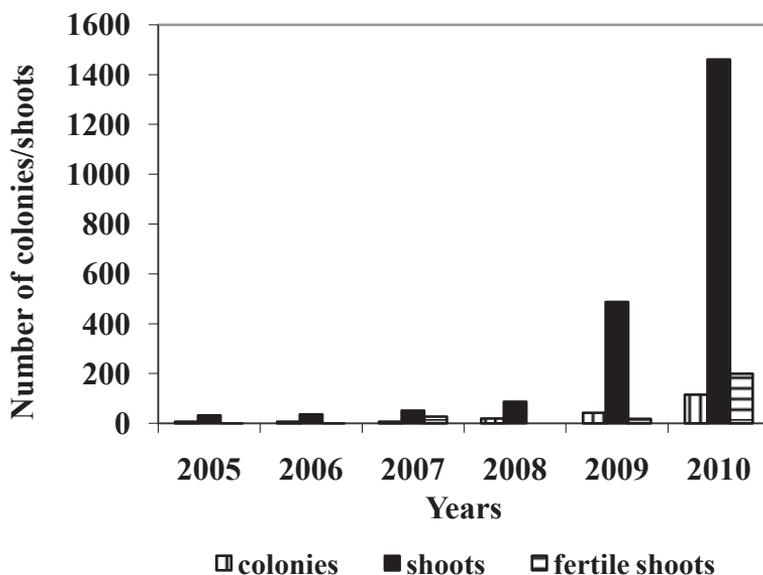


Figure 1: Development of the natural micropopulation of *P. praelongus* at the site Rameno u Stříbrného rybníka in the Orlice river floodplain (2005–2010).

Slika 1: Spremljanje naravnih mikropopulacij vrste *P. praelongus* na lokacijah Rameno u Stříbrného rybníka na poplavni ravnici reke Orlice v letih 2005–2010.

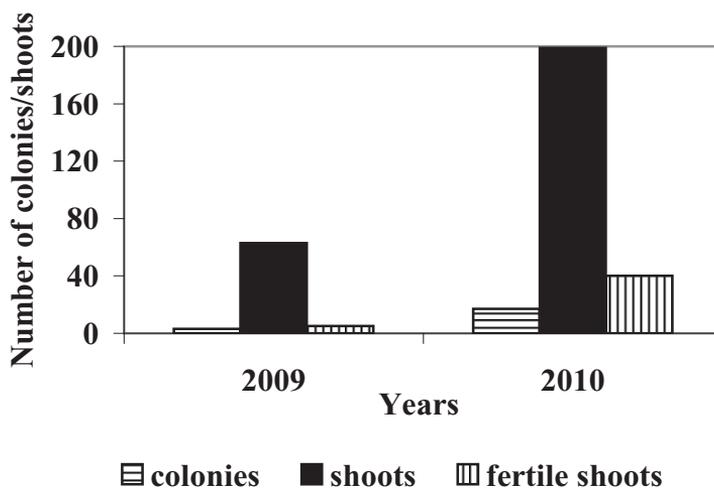


Figure 2: Development of the introduced micropopulation of *P. praelongus* at the site Kašparovo jezero in the Orlice river floodplain (2009–2010).

Slika 2: Spremljanje vnešenih mikropopulacij vrste *P. praelongus* na lokaciji Kašparovo jezero na poplavni ravnici reke Orlice v letih 2009–2010.

was conducted in 2008, 199 shoots (40 fertile) in 17 colonies were recorded in 2010 (Fig. 2).

The total population area of all four micropopulations in the PLK was rather variable between 2005–2010 (Fig. 3). All but the Štampach pools

partly stabilised between 2007–2010, but area still varied in relation to intensity of overgrowing (infilling) of the relatively small, shallow pools by a littoral emergent vegetation. In 2010, the stand areas were 12–50 m<sup>2</sup>.

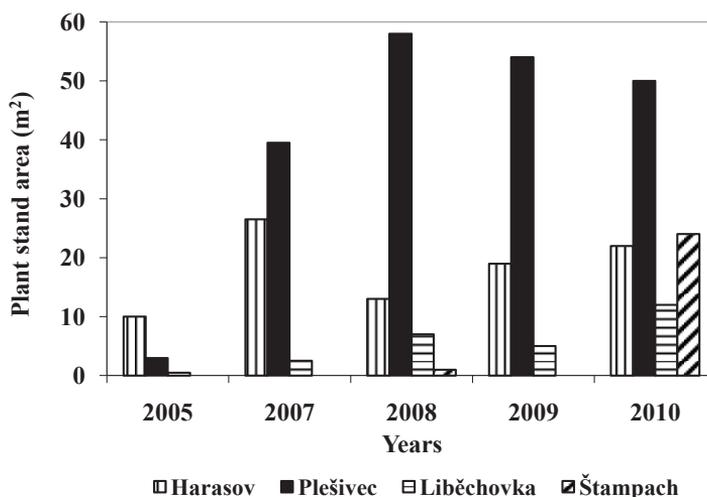


Figure 3: Comparison of plant stand area of the four introduced micropopulations of *P. praelongus* in the Kokořínsko region (2005–2010).

Slika 3: Spremljanje površine sestojev štirih vnešenih mikropopulacij vrste *P. praelongus* na območju Kokořínsko v letih 2005–2010.

Table 1: Water chemistry at *P. praelongus* sites monitored during 2005-2010. Ranges of values and medians (in parentheses) are shown.

Tabela 1: Kemizem vode na lokacijah z vrsto *P. praelongus* v obdobju 2005-2010. Predstavljeni so rangi in mediane izmerjenih vrednosti (v oklepaju).

Parameter	Natural site (Rameno u Stříbrného rybníka)	Kašparovo jezero near the Orlice river	Pools in the Liběchovka river floodplain	Pools above Harasov fishpond	Pools below Plešivec	Pools near Štampach
pH	7.2–7.7 (7.4)	7.5–8.3 (8.0)	7.5–8.4 (8.0)	7.5–8.3 (7.7)	7.4–7.7 (7.7)	7.7–7.9 (7.8)
El. Cond. (mS m <sup>-1</sup> )	11.9–49.1 (36.7)	15.0–36.7 (31.1)	10.4–51.8 (28.1)	6.7–28.9 (13.3)	10.6–36.9 (27.3)	33.5–42.9 (38.2)
COD (mg l <sup>-1</sup> )	5.1–8.1 (5.5)	3.4–6.7 (4.9)	4.1–6.4 (5.1)	8.8–16.0 (10.8)	4.8–11.0 (7.2)	6.7–6.8 (6.8)
NO <sub>3</sub> <sup>-</sup> -N (mg l <sup>-1</sup> )	2.6–12.8 (7.4)	6.4–14.0 (12.5)	<0.5 (<0.5)	<0.5 (<0.5)	<0.5 (<0.5)	<0.5 (<0.5)
NH <sub>4</sub> <sup>+</sup> -N (μg l <sup>-1</sup> )	40–230 (110)	40–270 (120)	30–50 (37)	30–80 (43)	7–50 (34)	20–60 (40)
PO <sub>4</sub> <sup>-</sup> -P (μg l <sup>-1</sup> )	140–400 (260)	200–370 (245)	30–70 (37)	30–1100 (173)	30–80 (37)	30–60 (45)
Ca <sup>2+</sup> (mg l <sup>-1</sup> )	48.6–77.0 (60.8)	44.5–63.3 (56.8)	35.0–94.9 (49.2)	18.6–61.1 (37.4)	40.7–75.5 (52.3)	50.9–70.6 (60.8)
Mg <sup>2+</sup> (mg l <sup>-1</sup> )	4.6–7.4 (5.5)	3.7–4.9 (4.1)	3.5–5.7 (4.8)	1.8–4.6 (2.6)	3.4–5.8 (4.4)	9.9–11.3 (10.6)
K <sup>+</sup> (mg l <sup>-1</sup> )	3.0–4.5 (4.0)	3.0–4.2 (3.6)	0.23–3.5 (0.38)	0.96–5.8 (2.1)	3.0–4.1 (1.5)	2.1–2.2 (2.2)

At all sites, *P. praelongus* grows in neutral to slightly alkaline waters (Tab. 1). The waters can be considered meso- to eutrophic as determined by N and P concentrations. NO<sub>2</sub><sup>-</sup>-N concentration ranged between 0 and 0.28 mg.L<sup>-1</sup> between sites (data not shown). Despite relatively high Ca<sup>2+</sup> concentrations across sites, K<sup>+</sup> concentrations at some PLK sites were <1 mg.L<sup>-1</sup> and may act to co-limit plant growth.

#### Testing of seed germination

No germination was observed in control seeds stored in water (Fig. 4). Highest germination percentage was noted among SAVO treatments (germination between 5–98 %). While full-strength SAVO treatment for 36 h proved highly effective in promoting seed germination, the treatment appeared to injure seed embryos, resulting in non-normal growth of the first leaves. This growth inhibition was not noted at 2-h SAVO treatment (Fig. 5). Germination does not appear to be enhanced by periods of freezing or cold

stratification, and seed do not appear capable of surviving hypoxic storage at –18°C for 36 hours. Seed germination was also stimulated by transient desiccation (1–35 % germination), hypoxia (14 % germination), and scarification (4–8 % germination). Overall, germination occurred during the day 4 to 101. No seeds germinated after exposure to 15 mg.l<sup>-1</sup> GA<sub>3</sub> for the next 4 months.

#### Preparation of a sterile in-vitro culture

Out of the total 238 sterilised seeds, 49 (28 %) germinated; 25 on solid and 24 in liquid medium. This would imply that media type has little effect on germination. Germination of desiccated seeds was 42 %, compared with 24 % for seeds stored in water. High concentrations of SAVO slightly increased germination. Longer exposure to SAVO also increased germination (37 % at 16 h; 19 % at 8 h). Contamination was noted in all seeds sterilised for only four hours. Germination frequency was greatest during the first two months (data not shown).

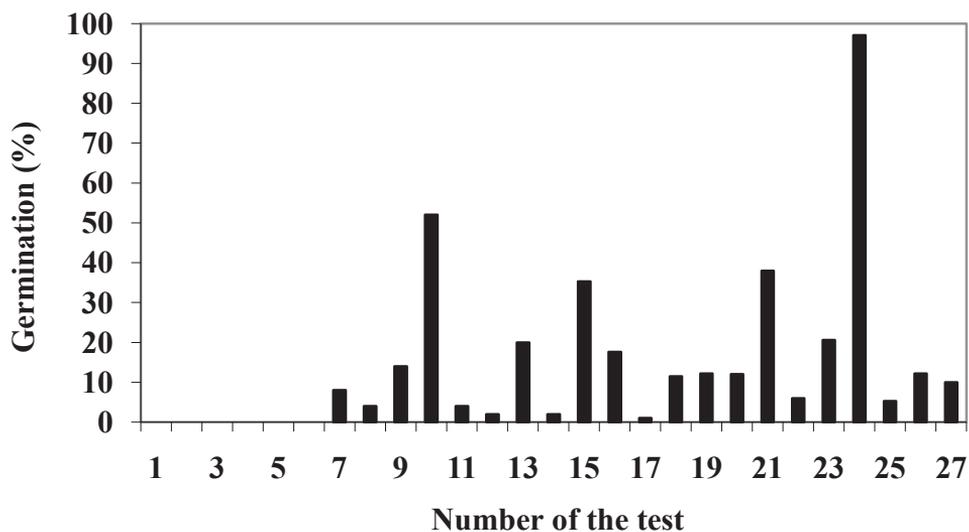


Figure 4: Results of germination tests with *P. praelongus* seeds. For the description of experimental conditions (the numbers 1–27) see the text.

Slika 4: Rezultati kalitvenih testov semen vrste *P. praelongus*. Opis poskusnih razmer je podan v tekstu (alineje 1–27).

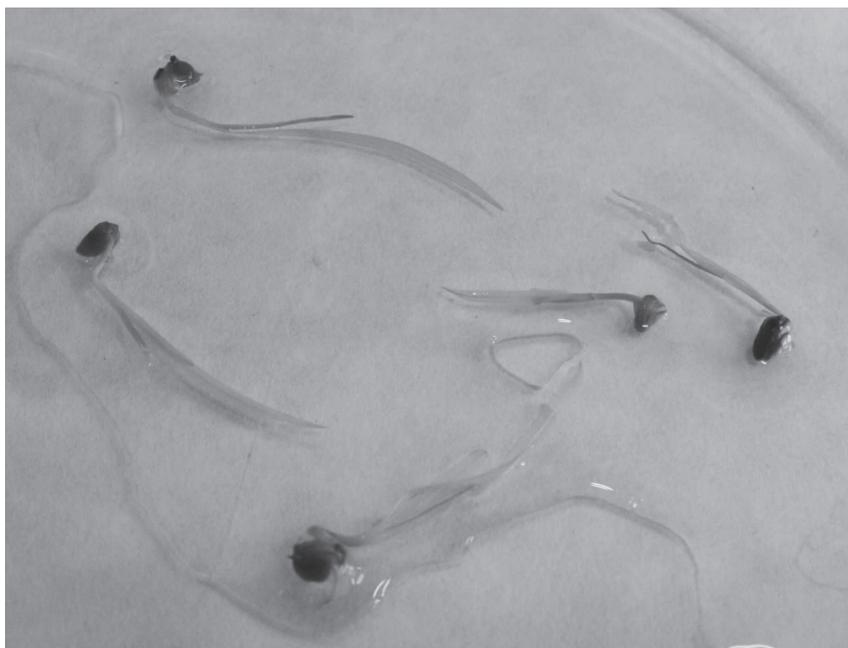


Figure 5: Small plantlets of *P. praelongus* after the test of seed germination to be transferred to the growth chamber (Photo R. Prausová).

Slika 5: Mlade rastlinice vrste *P. praelongus* po uspešni kalitvi, pred prenosom v rastne komore (foto R. Prausová).



Figure 6: Plants of *P. praelongus* raised in the *in vitro* culture before their transfer to the outdoor rescue culture (Photo L. Adamec).

Figure 6: Primerki vrste *P. praelongus* vzgojeni v *in vitro* kulturah pred prenosom v naravno okolje (foto L. Adamec).

Plants grew vigorously *in-vitro* in liquid half strength Gamborg B5 medium, outgrowing flasks within 2–3 months (Fig. 6). No growth malformations or disorders were apparent on the plants. To maintain continuous and vigorous growth, it was necessary to transplant individuals to new medium when flasks became outgrown. The minimal volume of medium (300 ml in a 0.5 l flask, 7 cm deep) appears to be a prerequisite for vigorous plant growth. A pH of 6.5 in the medium is preferred as it better meets ecological demands. Plants raised *in-vitro* resumed growth in the outdoor rescue culture at IBT around 2 weeks after transplanting.

## Discussion

Monitoring has revealed a marked increase of *P. praelongus* micropopulations at the last natural Czech site between 2008–2010, compared with

only slight increases during the 2005–2007 period (Fig. 1). Between 2008–2010, micropopulation size (both number of colonies and shoots) increased around threefold annually, with 14 % of all shoots producing flowers. New colonies were located in areas with markedly lower sediment depth, influenced indirectly by sediment dredging in 2003. Of all experimental introductions in the Hradec Králové region in the last decade, only those at the oxbow Kašparovo jezero have been successful so far (Fig. 2). From the 18 shoots introduced to this site between 2008–2009, in total 199 shoots (40 fertile) were scored in 2010. This new site is located in 1.5 km distance from the last known natural site, and the habitat characteristics and geomorphology of both sites are very similar (see Tab. 1): both micropopulations grow in oxbows of the Orlice river which are at least by one side connected with the river. Additionally, they grow near the oxbow estuary to the river within a riprap where the plants are protected during flood events but can favourably utilise the nutrient-rich sediment between boulders on the bottom and are positively influenced by the streaming river water (Prausová et al. 2010). Contrary to the Kašparovo jezero success, no backwater pools in the Orlice river floodplain, which are gradually filled in by sediments, have become suitable for introduced *P. praelongus* populations (Prausová et al. 2010).

A different situation occurs at PLK where micropopulations were introduced to newly built up backwater pools in 2003–2005 (Prausová et al. 2010). Here the small and shallow pools are prone to rapidly overgrowth by reeds, and succession followed by decline occurs in submerged species. High water temperature during the summer season is an important factor for the decline of *P. praelongus*, with upper parts of the shoots exposed to higher water temperature turning brown and senescing. Positive pool management maintains many of the pools in an early state of succession, which is favourable for *P. praelongus* and several other submerged macrophyte species (Prausová and Janová 2010, Prausová et al. 2010). It would appear from water chemistry at *P. praelongus* sites (Tab. 1) that the species is also capable of vigorous growth in eutrophic waters.

In conclusion, the data presented (Figs. 2–3) suggests that the selection of suitable sites for the stenotopic species *P. praelongus* in flood-

plain regions is possible. Creating new stable micropopulations presents an active method of conserving the species diversity. The prerequisite for successful introductions or re-patriations is to raise a sufficient number of plants, maintaining genetic variability using a sterile, seed-based tissue culture. Recent findings have revealed very low natural genetic variability within Czech *P. praelongus* micropopulations (Kitner et al. unpubl.), suggesting that vegetative propagation is dominant in natural populations. All existing Czech sites as well as the rescue culture in the IBT contain only the plants from the last natural site.

Testing of *P. praelongus* seeds using different treatments shows the seeds have a very high germination potential (to 98 %; Fig. 4). Results suggest that disturbance of the seed testa plays a part in breaking seed dormancy (Janová 2010, Prausová et al. 2010). Highest germination rates were noted in response to 2-h exposure in full- or half-strength SAVO (5 % or 2.5 % NaClO solution), desiccation, temperatures around 21–28°C, hypoxia, and scarification by abrasive paper (Fig. 4). It would appear thus that chemical disturbance to the seed testa provides better stimulation for seed germination than mechanical abrasion (Janová 2010). Similar results were reported for *Potamogeton* spp. seeds by Teltscherová and Hejny (1973), noting higher percentages of seed germination after a treatment with fouling water than nicking of the seed testa by a razor blade. Results from the present study (Fig. 4; Janová 2010) conform to similar studies on other *Potamogeton* species (Crocker 1907, Teltscherová and Hejny 1973). It is suggested that low germinability in *Potamogeton* seeds is not caused by embryo dormancy, but rather by mechanical restriction of growth by the seed testa (i.e., by physical dormancy).

Using the combined effect of NaClO on germination stimulation and seed sterilisation, sterile *in-vitro* cultures of *P. praelongus* have been prepared. Thirty new clones with expected genetic variability are available. Sterile growth of the plants *in-vitro* in a common liquid medium is rapid, and 5–15 cm long, healthy shoots (Fig. 6) can be cultured abundantly in this manner (over a 2–3 month period). Such plants are capable of surviving transplantation into an outdoor rescue culture, before subsequent introduction or re-patriation to new sites.

## Povzetek

Vrsta *Potamogeton praelongus* se je na Češkem v naravi pojavljala le na eni lokaciji; in sicer na poplavni ravnici reke Orlice, v bližini mesta Hradec Králové. V okviru programa ohranjanja vrste smo leta 2008 vnesli rastline na novo lokacijo v bližini, v mrtvem rokavu reke Orlice. Mikropopulacije so bile v letih 2003–2005 vnešene tudi v vodna telesa na zaščitenem območju Kokořínsko v osrednji Bohemji. Spremljanje stanja obeh mikropopulacij vrste *P. praelongus* na poplavni ravnici reke Orlice je pokazalo, da se je velikost populacij v letih med 2008 in 2010 povečala za tri-krat letno (število kolonij, vegetativnih in fertlnih poganjkov).

Septembra 2010 smo na naravni lokaciji našli 1461 in na novi lokaciji 199 poganjkov. Kar 14–20 % poganjkov je bilo fertlnih. Skupna površina sestojev na zaščitenem območju Kokořínsko je bila v letih 2005–2010 variabilna, vendar se je velikost večine sestojev v letih 2007–2010 ustalila. Septembra leta 2010 so bili sestoji veliki od 12 do 50 m<sup>2</sup>. Na vseh čeških lokacijah vrsta *P. praelongus* uspeva v nevtralnih do rahlo alkalnih, mezo do eutrofnih vodah. Za prekinitvev dormance je bila potrebno 2-h površinsko obravnavanje s 5 % oz. z 2.5 % raztopino NaClO, izsuševanje semen, stratifikacija, izpostavljanje anoksičnim razmeram in abrazija semenske lupine z grobim brusilnim papirjem. Vsi testi so pokazali, da je abrazija ključna za prekinitvev dormance. Kemična razgradnja semenske lupine se je izkazala za uspešnejšo kot mehanska. Z dvojnimi učinki delovanja NaClO na kalitev in sterilizacijo semen smo pripravili *in-vitro* kulturo vrste *P. praelongus* v obsegu 30 klonov z ustrežno gensko variabilnostjo. Rast rastlin *in-vitro* v običajnem mediju je zelo hitra, saj so od 5 do 15 cm dolgi, zdravi poganjki, zrasli v 3 mesecih.

## Acknowledgements

The authors are grateful to M. Kitner for conducting the genetic analyses of plant material, to K. Pásek for the preparation of the sterile *in-vitro* culture, and to J. Dvořák for his assistance with monitoring the field sites. Special thanks are due to A. Cross for critically reading the manuscript and

correction of the language. Thanks are also due to two anonymous reviewers for valuable comments. The study was supported from the funds awarded by EHP/Norway and the Ministry of Environment

of the Czech Republic. The study was also partly supported (to L.A.) from the Research Programme of the Academy of Sciences of the Czech Rep. (AV0Z 60050516).

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