The effect of temperature on the phenology of germination of *Isoëtes lacustris*

Vliv teploty na fenologii klíčení druhu *Isoëtes lacustris*

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Isoëtes lacustris (quillwort) is an aquatic macrophyte commonly dominating oligotrophic softwater lakes in Europe. Reproductive ecology of a relic population of quillwort based on spore germination was studied in an acidified mountain lake in the Czech Republic. In a four-year experiment, we recorded temperature-related temporal changes in micro- and macrospore germination and sporeling establishment in (i) natural in situ conditions in Černé jezero lake and (ii) in the laboratory at various temperatures. Germination of both micro- and macrospores increased gradually over four consecutive growth seasons. Several annual cohorts of germinating macrospores born together in a sporangium indicate that spores remain viable for up to several years and the formation of a spore bank. Minimum temperature necessary for germination was lower for microspores (6 °C) than macrospores (12 °C) and this ensured the availability of spermatozoids for the fertilization of the long-living macrogamethophytes whenever they produced archegonia during growing season(s). Macrogametophyte development started between July and October and sporeling development always followed in the next or subsequent springs. Long germination and embryo development may limit reproduction in *I. lacustris* by making it sensitive to both episodic and chronic changes in the environment. The relatively high minimum temperature for macrospore germination may set general limits for the reproduction of *I. lacustris* in lakes, by constraining its distribution along latitudinal and altitudinal gradients, and to particular depths. The mean length of time when the temperature (≥ 12 °C) was high enough for the germination of *I. lacustris* in Černé jezero lake was 119 days and occurred during the period June to September in 2004–2011.

K e y w o r d s: aquatic macrophyte, early ontogeny, lycopod, reproduction, spore bank, spore viability

Introduction

Isoëtes lacustris is a heterosporous aquatic lycopod, which characteristically occurs in oligotrophic soft water lakes in the boreal region of Europe. This quillwort is also a rare glacial relict persisting in a few lowland and mountain lakes in temperate central Europe. Though its dispersal relies almost exclusively on generative reproduction by spores, *I. lacustris* often dominates the vegetation in lakes forming monospecific stands in the deepest part of the littoral zone. Although its reproductive success depends on the germination of spores little is known about this process. A few laboratory studies indicate that temperature controls dormancy of quillwort spores (Kott & Britton 1982) and when they

start to germinate (Bennert & Danzebrink 1996, Čtvrtlíková et al. 2012). The effect of temperature on the phenology of germination of *I. lacustris*, however, is unclear. A laboratory study on a German relic population of *I. lacustris* (Bennert & Danzebrink 1996) revealed that the percentage germination of macrospores is as low as 0–19% when kept at 12 to 24 °C and several times lower than that of microspores. This low reproductive success was ascribed to the weakness of the relic population studied (Bennert & Danzebrink 1996). Another study on American *I. macrospora* (Kott & Britton 1982), which is considered to be equivalent to European *I. lacustris* (Taylor & Hickey 1992), reports far more successful germination of macrospores up to ~80% at 23 °C. As fertilization success and sporeling yield were not evaluated, the potential of this species for regeneration remains unclear. According to Szmeja (1994), high in situ mortality of sporelings (70–80%) reduces population recruitment in *I. lacustris*. The proportion of juveniles and adults in native populations of *I. lacustris* is very variable (Gacia & Ballesteros 1993).

This study focused on the sole relic population of *I. lacustris* in the Czech Republic. The quillwort inhabits Černé jezero lake, which became chronically acidic during the second half of the 20th century (Kopáček & Veselý 2005). Although it is known that atmospheric acidification caused a severe reduction in species diversity in the lake, e.g. extinction of fish and some invertebrates (Vrba et al. 2003), there is little information on the quillwort population before 2001 (for review, see Husák et al. 2000). Our preliminary survey revealed an absence of juveniles, despite of the fact that the population was composed of healthy adult specimens bearing many spores. We suggested that high acidity and aluminium (Al) toxicity of the water in the lake might be severely impairing spore germination and sporeling development of *I. lacustris* as recorded for another relic population of *I. echinospora* in the Bohemian Forest (Čtvrtlíková et al. 2009). The improvement in the water chemistry of the lake during summer stratification in recent years is responsible for the recent increase in the reproduction of *I. echinospora* (Čtvrtlíková et al. 2012), the same effect, however, was not recorded for *I. lacustris*. We therefore hypothesize that *I. lacustris* is unable to benefit from summer periods of low acidity and low aluminium toxicity of the water in Černé jezero lake due to its germination phenology.

In this study, we evaluated the effects of temperature on the phenology of the germination of *I. lacustris* over four consecutive years. Our objectives were to determine the time course of micro- and macrospore germination and sporeling establishment (i) in situ in Černé jezero lake and (ii) in the laboratory at various temperatures.

Material and methods

Sampling site

This study was conducted on a population of *Isoëtes lacustris* inhabiting the 1–4 m depth of water in Černé jezero lake (49°11' N, 13°11' E; 1008 m a.s.l.; surface of 18.8 ha; maximum depth of 40 m; Nedbalová et al. 2006). The lake is of glacial origin, oligotrophic and dimictic with a covering of ice during winter from November to April. There is a small hydroelectric power plant on the outflow from Černé jezero lake, which keeps the level of water in the lake within 0.5 m (usually in spring or autumn).

Experimental setup

The experimental design of this study followed that of Čtvrtlíková et al. (2012). The effect of temperature on spore germination of *I. lacustris* was studied in situ and under laboratory conditions from 2007–2011. In October 2007, spores were obtained from outer sporophylls (leaves) collected from 50 adult quillwort plants. Macrospores were obtained from 105 intact or broken (but complete) macrosporangia, cleaned of debris and arranged in sets of 60–130 spores, with each set originating from the same complete macrosporangium. This allowed us to evaluate the variation in germination of macrospores from a single macrosporangium. Microspores were obtained from more than 50 intact or broken microsporangia and pooled. To study macrospore germination both in the laboratory and in situ, each set of macrospores together with a pool of microspores was placed in a Petri dish (55 mm in diameter) with distilled water, or in a modified Eppendorf tube (1.5 ml, Čtvrtlíková et al. 2012) with lake water. Microspore germination was studied in sole microspore culture using the same experimental procedure. A week after collection, spores were inspected and the percentage that had germinated determined (see below) and then placed back in the plant stand in the lake (2.5 m depth) or in growth boxes in the laboratory (Samsung RW33EBSS, Samsung Electronics Czech and Slovak, Ltd., Prague, Czech Republic), which were kept at 4 °C to simulate winter conditions.

Lake survey and in situ experiment

During four growing seasons between 2008 and 2011, in situ sporeling growth of the native quillwort population was assessed by SCUBA diving. The annual course of the temperature of the lake water in stands of plants was recorded continuously using a Minikin QT smart sensor (Environmental Measuring Systems Company, Brno, Czech Republic). To evaluate spore germination under naturally fluctuating lake water temperature, an in situ experiment was performed in Eppendorf tubes placed within the plant stand and removed only for inspection. In total, there were 12 inspections during the course of this study (October 2007, June and September in 2008, June, July and September in 2009 and 2010, May, June and July in 2011). Evaluation of macrospore germination and sporeling development was based on 30 replicates (i.e. single macrosporangia), microspore germination on 3 replicates.

The temperature regimes used in the in vitro experiment (see below) enabled us to determine the lower temperature threshold for *I. lacustris* germination. The period when the mean daily temperature of the water in the lake exceeded the lower temperature threshold was used to define the thermal germination season.

Laboratory experiment

To investigate the effect of temperature on spore germination and sporeling development, both types of spores were grown in Petri dishes in growth boxes kept at five different temperatures (6, 8, 10, 12 and 17 °C) and a 14:10 light:dark period (PAR ≤ 50 μmol m^{-2} s^{-1}). The experiment ran for three consecutive years from 2007–2010. To evaluate the success of fertilization of macrogametophytes during 2010, additional determinations of sporeling development at 12 °C were conducted in 2011. From November to mid-May (200±5 days), all treatments were kept in the dark at 4 °C to simulate the winter season. Samples were inspected at least monthly during 2008 and 2009, and less frequently in 2010. There

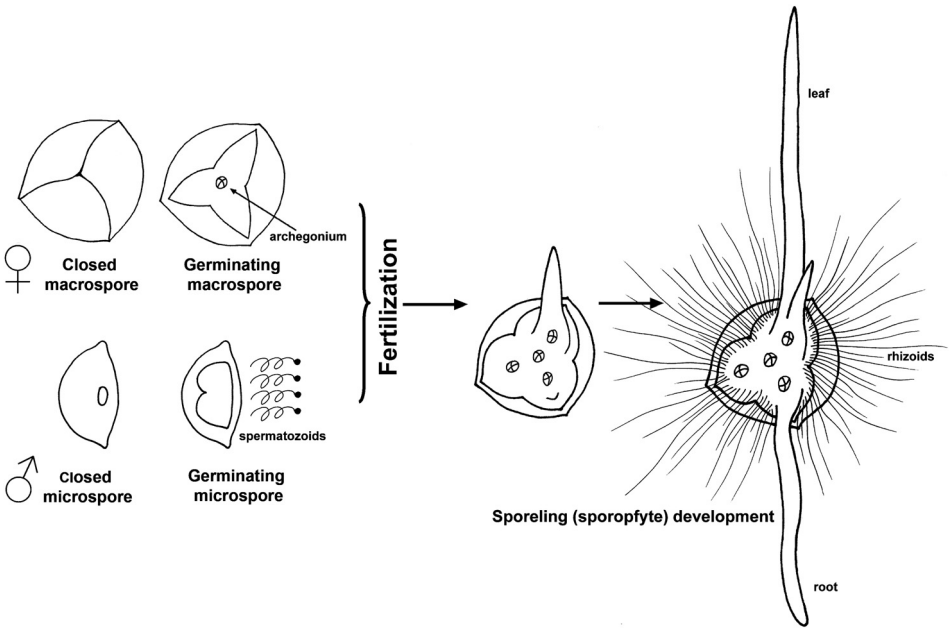

Fig. 1. – Early ontogeny of *Isoëtes lacustris* – a heterosporous species with macro- (female) and microspores (male). Macrospore germination starts when it splits along the triradiate ridges. Later, archegonia develop on the exposed macrogametophyte. When the microgametophyte matures inside a microspore, the spore splits and four spermatozoids are released. After fertilization, a sporophyte remains attached to the rich storage tissue of the macrogametophyte for weeks or even months until 2–4 leaves develop. In *I. lacustris*, rhizoids are not present on the surface of the macrogametophyte prior to the first leaf and root protruding from its surface.

were two additional inspections of macrospore germination at 12 °C in May and August 2011. The numbers of macrospores and sporelings were counted in 15 replicates (i.e. single macrosporangia) and of microspores in 3 replicates.

Evaluation of spore germination

Macrospores were considered to have germinated when they opened and the macrogametophyte surface was recognizable; sporeling is a particular ontogenetic stage in which the first leaf protrudes from the macro-gametophytic tissue (Fig. 1). Microspores were considered to have germinated when there were either four cells with spermatozoids inside a microspore, or when it was empty. The germination of macrospores and sporeling development was studied under a stereomicroscope (Olympus SZ61; magnification 5–45×) and microspore germination using an inverted microscope (Olympus IMT 1; magnification 60×). To quantify germination, the percentage of macrospores germinating or sporelings growing was determined based on results for complete sets of macrospores, while the percentage of microspores germinating was determined from five random fields of view per Petri dish or an Eppendorf tube (~200 spores per field). In the lake experiment, the contents of each Eppendorf tube was placed in a Petri dish for a complete inspection and then returned.

Statistical analyses

Statistical analyses of the data were performed using Prism 5 (GraphPad Software Inc., La Jolla, USA). The percentages of micro- and macrospore germination and sporeling development under various temperature regimes over three consecutive years was evaluated using repeated-measures ANOVA, with temperature as a fixed factor and time (year) as a repeated measure. The data on macrospore germination and sporeling development were arcsine transformed to fit ANOVA assumptions for normality. Tukey's HSD test was used for multiple comparisons.

Results

Lake survey and in situ experiment

Ice and snow covered Černé jezero lake each winter, but did not affect the stand of *Isoëtes lacustris*. Water temperature around the plants dropped to 2–4 °C during winter (November to April). The growing season lasted approximately six months (April to October) and the maximum water temperature in the plant stand increased to ~18 °C in summer.

The in situ observations of adult plants revealed that leaf fall accompanied by spore release occurred throughout the year. The numerous sporelings that were present from July to August; however, did not reach the juvenile stage and died during autumn.

Both types of spores germinated immediately after they were collected in October (2007). Thus, 3.1% of the microspores had already germinated at the beginning of the in situ experiment. Of the 30 macrosporangia examined, nine included 0.9–66.3% (mean 17.1) macrospores that had germinated (early-germinating, EG), whereas none of the macrospores in the 21 macrosporangia had germinated (late-germinating, LG; Fig. 2). Even though the EG and LG macrosporangia differed only in when the spores germinated, they were examined separately in order to clearly demonstrate their analogous phenology. Moreover, it was important to be able to distinguish EG and LG macrosporangia in order to accurately compare the germination of spores in the lake and laboratory experiments. In the latter, the macrosporangia did not initially include germinating macrospores and therefore were analogous to the LG macrosporangia that developed in the lake.

In the lake, germination of both macro- and microspores increased gradually over three or four consecutive growing seasons (Fig. 2). In one exceptional case, macrospores (EG) did not germinate at all in 2010, but they did germinate in the following season. Seasonal maxima of both types of spores occurred at the end of the season, except in the last growing season when the experiment was terminated in July (Fig. 2). Seasonal maxima of macro- and microspore germination increased significantly ($P < 0.0001$; Fig. 3, Table 1) over two and three consecutive seasons, respectively, when most of the macro- (95%) and microspores (100%) germinated. There was no significant difference between the seasonal maxima of macrospores germinating in EG and LG macrosporangia (2008: $P = 0.12$, 2009: $P = 0.98$, 2010: $P = 1.00$; Fig. 3, Table 1).

In general, the percentage of both germinating macro- and microspores increased suddenly from July to September, while few or no spores germinated in autumn, winter and spring, when the water temperature dropped below ~15 °C (Fig. 2). The stepwise increase in the germination of spores did not allow the modelling of continuous time courses by

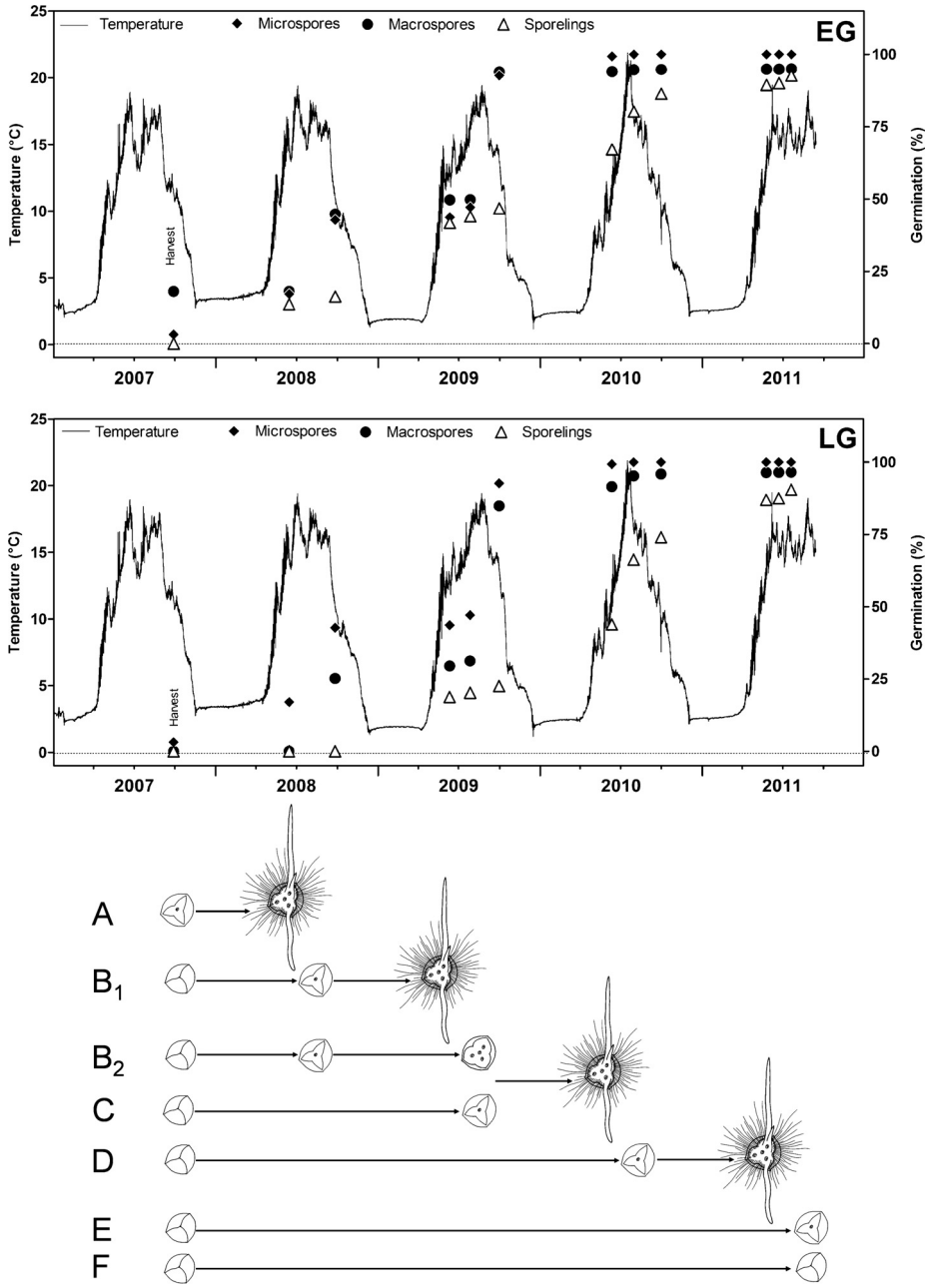

Fig. 2. – In situ germination of micro- and macrospores of *Isoëtes lacustris* and sporeling development recorded in Eppendorf tubes along with the ambient lake water temperature. Top panel – early germination (EG) includes the time course of macrospore germination in 9 macrosporangia, which contained germinating macrospores when they were collected. Bottom panel – late germination (LG) of spores in 21 macrosporangia, which contained only closed macrospores at the beginning of the in situ experiment. Note identical microspore germination and same lake water temperatures in both panels. Symbols are means of cumulative data. Both types of spores germinated gradually over several growth seasons as illustrated schematically for macrospores (A–F, same time axis in the plots). In the clutches of spores of macrosporangia studied, we distinguished several cohorts (A–E) of macrospores, which started to germinate in different years. We further determined a phenological principle (see cohort B as an example) that a macrospore can germinate over one (subscript 1) or two (subscript 2) growth seasons prior to the sporelings completing their development after winter. Some macrospores remained closed for the whole period of the study (F) in the macrosporangia studied.

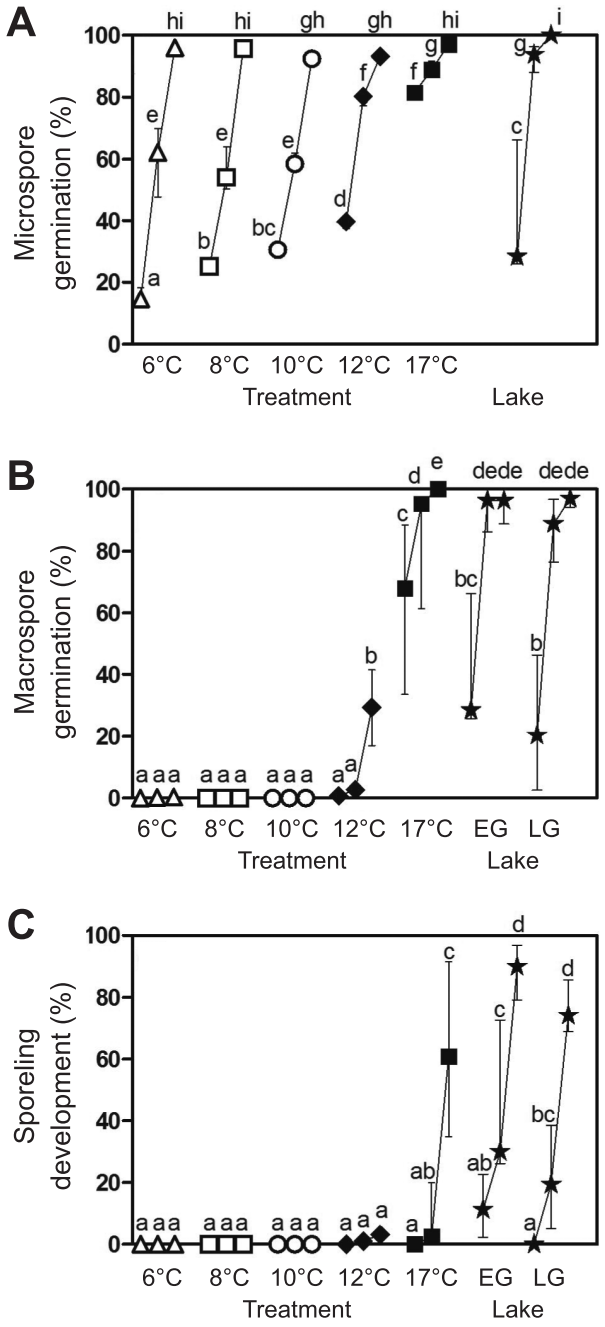

Fig. 3. – Effect of various temperature regimes on maximum germination of both types of spores of *Isoëtes lacustris* in the laboratory and in situ in the field. Microspore (A) and macrospore (B) germination and sporeling development (C) was evaluated at the end (October) of each growth season (2008, 2009 and 2010). For each temperature, cumulative data for all growth seasons are shown as neighbouring points connected by a line. Symbols are medians and error bars represent inter-quartiles. Letters above the symbols (a–i) indicate significant differences among treatments (Tukey's HSD test).

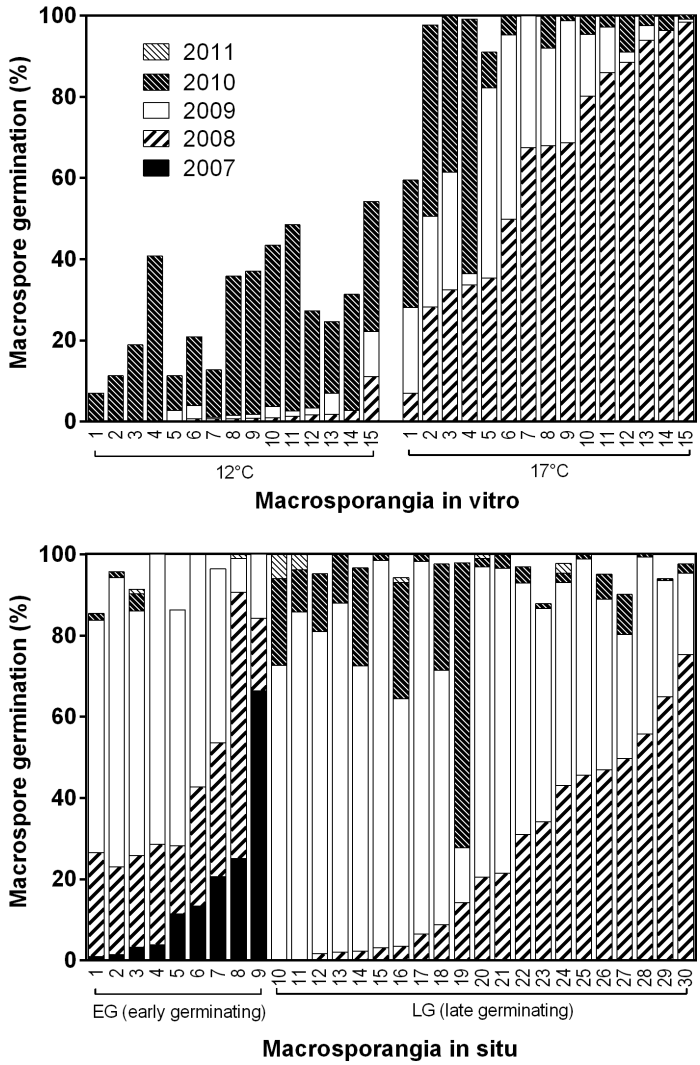

Fig. 4. – Annual cohorts of macrospores of *Isoëtes lacustris* germinating within single macrosporangia kept at 12 °C and 17 °C in the laboratory or exposed to a natural lake water temperature regime from 2007 to 2011. Each column represents a single macrosporangium with its annual cohorts of germinating macrospores; both the early (EG) and late (LG) germinating macrosporangia in the lake are distinguished.

curve fitting. Therefore, we defined three points on the timescale, when the percentage germination recorded was 5% (T_{G5}), 50% (T_{G50}) and maximum (T_{Gmax}), for evaluating the timing of spore germination in situ (Table 2). Germination of macrospores in EG macrosporangia and microspores started earlier (T_{G5}) and increased faster than that of macrospores in LG macrosporangia. However, when spore germination reached ~50% (T_{G50}), the percentage of germinating macrospores in both EG and LG macrosporangia was similar to that of microspores (Fig. 2).

On germinating the macrospores formed 1–4 archegonia during the course of the growing season and then overwintered as macrogametophytes. Sporelings continued to develop in the next growth season as documented by the different sporeling development in EG and LG macrosporangia in 2008 (Fig. 2). In several instances, sporelings developed from macrospores that took longer to germinate than one growth season. The percentage of sporelings in both EG and LG macrosporangia increased significantly ($P < 0.0001$; Fig. 3, Table 1) over two consecutive growth seasons (2009–2010), increasing rapidly every May–June (usually before the onset of germination of macro- and microspores) and achieving the seasonal maximum at the end of that particular growth season (Fig. 2). Sporeling production of each macrosporangium was the result of distinct timing of germination of the macrospores, which resulted in several annual cohorts (Fig. 2, cohorts A–F, Fig. 4). Macrosporangia most frequently contained 3–4 macrospore cohorts and 2 or 5 cohorts were rare. Macrosporangia in which 100% of the macrospores germinated produced sporelings gradually over three or even four seasons.

Table 1. – Results of the repeated-measures ANOVA comparing seasonal maxima of spore germination and sporeling development of *Isoëtes lacustris* under various temperature regimes in the three consecutive growing seasons in which temperature was a fixed factor and time (season) a repeated-measures factor.

Factor	Microspore germination			Macrospore germination			Sporeling development		
	df	F	P	df	F	P	df	F	P
Temperature	5	242.0	< 0.0001	6	212.9	< 0.0001	6	67.5	< 0.0001
Time	2	3136.0	< 0.0001	2	185.6	< 0.0001	2	233.6	< 0.0001
Temperature × Time	10	112.0	< 0.0001	12	37.7	< 0.0001	12	53.1	< 0.0001

Laboratory experiment

There were no germinating macrospores in macrosporangia at the beginning of the laboratory experiment, but there was a low percentage of germinating microspores. Germination of micro- and macrospores, as well as sporeling development differed significantly and depended on temperature, time and their interaction (Fig. 3, Table 1). Microspores germinated more successfully than macrospores in all treatments. A high percentage germination of macrospores was recorded at 12 and 17 °C, whereas it was negligible at ≥ 10 °C. In all treatments, both types of spores germinated gradually over the course of three consecutive growth seasons with the seasonal maxima occurring at the end of each season (Fig. 5). The seasonal maxima of the percentage of microspores that had germinated were significantly greater at ≥ 6 °C and for macrospores at ≥ 12 °C ($P < 0.0001$; Fig. 3, Table 1). Similar to the lake experiment, size of annual cohorts of germinating macrospores varied both between and within individual macrosporangia in vitro (Fig. 4)

The effect of temperature and its interaction with time on both the germination of spores and sporeling development was ambiguous (Fig. 5, Table 2). The higher the temperature, the earlier the spores germinated and sporelings developed (T_{G5}), or achieved 50% (T_{G50}). Surprisingly, at the end of the third year, the maximum percentage of microspores that germinated was the same regardless of temperature (Fig. 3, 5; Table 1, 2). At

the lowest temperature (4 °C) during winter, spore germination ceased, whereas sporelings continued to develop, which was apparent from the huge increase that occurred immediately after winter (Fig. 5).

In all treatments, sporelings only developed in the second growth season and their phenology confirmed the results of the lake experiment that the sporelings never develop in the same growth season as the corresponding macrogametophytes (Fig. 5). In all treatments, sporelings started developing during winter or at the very beginning of the growth season. The maximum percentage of sporelings occurred in the first few weeks of the season. At 17 °C, there was a significant inter-seasonal increase in the percentage sporelings in the third growth season ($P < 0.0001$; Fig. 3, Table 1), whereas, at 12 °C, it occurred in the fourth season ($P < 0.0001$; data not shown) when the sporeling seasonal maximum was equal to that of the macrospores that germinated in the previous season ($P = 0.48$; Fig. 3), indicating a high percentage fertilization.

High percentage fertilization was evident from the high percentage of spores that germinated at the most favourable temperature conditions, 12 and 17 °C. Based on the numbers of sporelings that developed from the macrogametophytes present in the previous growth season, sporeling development was more successful at 12 °C than at 17 °C. However, it was still possible for the macrogametophytes kept at 17 °C to be fertilized in the following season, which resulted in a delay in sporeling development (Fig. 5).

Assuming 12 °C is the lower temperature threshold for germination in *I. lacustris*, the thermal germination season (i.e. period when the mean daily temperature was ≥ 12 °C) in Černé jezero lake lasted 98–146 days, from June to September, or an average of 119 ± 15 days over the period 2004–2011.

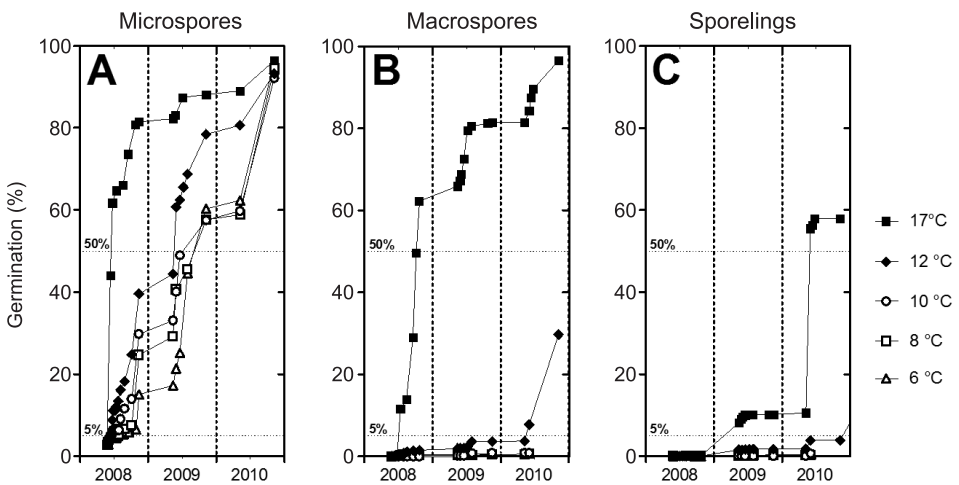

Fig. 5. – Temporal changes in microspore (A) and macrospore (B) germination and sporeling development (C) of *Isoëtes lacustris* recorded in the laboratory (2008–2010). Means of cumulative data are shown. Note that in order to simulate the natural 6-month over wintering period the different temperature treatments were all kept at 4 °C for this period.

Table 2. – Time schedule of micro- and macrospore germination and sporeling development of *Isoëtes lacustris* in various temperature regimes. Germination started on day 1 (1 November 2007). In some samples of both types of spores (in the microspore mixture and EG macrosporangia) germination started before day 1, therefore the beginning of spore germination was defined as time T_{G5} (days) when 5% of the spores had germinated. T_{G50} and T_{Gmax} denote points (days) in time when 50% and the maximum number of spores had germinated, respectively. G_{max} is maximum germination achieved (mean and range of percentages); empty cells indicate that germination was not achieved.

	Microspore germination			
Treatment	T_{G5}	T_{G50}	T_{Gmax}	G_{max}
6 °C	258–268	671–692		96.1 (95.1–98.0)
8 °C				94.6 (92.9–96.6)
10 °C	223–227	630–645	1136	92.2 (83.2–97.3)
12 °C	215–219	593–610		93.3 (91.1–95.4)
17 °C	200–215	215–227		96.4 (90.7–100)
Lake EG	1–226	634–699	1064	100
Lake LG				
	Macrospore germination			
Treatment	T_{G5}	T_{G50}	T_{Gmax}	G_{max}
6 °C				0.9 (0.0–4.5)
8 °C			978	0.6 (0.0–2.7)
10 °C				0.9 (0.0–3.5)
12 °C	952–978		1136	29.8 (7.0–54.2)
17 °C	228–237	330–348		96.5 (59.5–100)
Lake EG	< 1	634–699	1357	95.0 (85.5–100)
Lake LG	226–330		1301	96.5 (87.8–100)
	Sporeling development			
Treatment	T_{G5}	T_{G50}	T_{Gmax}	G_{max}
6 °C				0.5 (0.0–3.0)
8 °C			978	0.2 (0.0–1.3)
10 °C				0.6 (0.0–2.9)
12 °C	978–1342		1421	27.0 (7.0–54.2)
17 °C	389–593	952–977	1136	57.9 (4.6–100)
Lake EG	1–226	699–953	1357	92.7 (78.6–100)
Lake LG	330–588	953–1002		90.5 (63.2–100)

Discussion

This study revealed novel information about the prolonged period of time over which germination occurs in *Isoëtes lacustris* in the field, which is determined by both the narrow range of temperatures over which macrospores germinate and the long-term viability of spores and macrogametophytes. Based on the inhibitory effect of low temperature, we conclude that natural fluctuations in the temperature of the water in the lake substantially prolonged quillwort germination. We therefore believe that, if mature spores were not exposed to the low winter temperatures, they would probably continue to germinate. Cold treatment is probably not necessary for breaking spore dormancy, as in the related *I. macrospora* (Kott & Britton 1982). Quillwort germination may also be constrained during the growth season by its relatively high minimum temperature (≥ 12 °C) threshold for macrospore germination, which may set the general limits for the reproduction of *I. lacustris* in lakes.

In Scandinavian lowland lakes inhabited by dense quillwort populations, the suitable thermal conditions for germination (≥ 12 °C) in summer last 2–3 months (Dale 1986, Rørslett & Johansen 1995). Data on temperature in extreme habitats at the altitudinal and latitudinal margins, or edges of the depths at which this species occurs would reveal whether the distribution of *I. lacustris* is determined by its narrow temperature range for germination or there is some other environmental constraint. Gacia & Ballesteros (1994) report the occurrence of stands of *I. lacustris* at a depth of 0.6–2.3 m in the high-mountain Lake Baciver (2120 m a.s.l., Pyrenees), where water temperature exceeded 12 °C in July and August. Another *I. lacustris* population occurring at a depth of 3 m in the mountain Lake Atnsjøen (61°53' N, 10°11' E; 701 m a.s.l., Norway) experienced an even shorter germination season of 42 days in 2009 (M. Čtvrtlíková, unpublished results).

The prolonged development of early ontogenetic stages of *I. lacustris* seems to compensate for the narrow temperature range (12–24 °C) of macrospore germination recorded in this study (the lower limit) and by Bennert & Danzebrink (1996; the upper limit). Both traits allow quillwort to germinate whenever temperature is favourable and synchronize sporeling emergence in spring. The minimum temperature necessary for germination was lower for microspores (6 °C) than for macrospores (12 °C) and this ensured that spermatozoids were available for fertilization of the long-lived macrogamethophytes whenever they develop archegonia during the growth season. We were unable to determine by microscopic examination whether a macrogametophyte was fertilized or not, because rhizoids, i.e. trichomes on the surface of macrogametophytes, do not emerge immediately after fertilization (Fig. 1) as they do in *I. echinospora* (Čtvrtlíková et al. 2012). Hence, we could not determine for how long an embryo had been developing inside a macrogametophyte until it produced its first leaf. Fertilization supposedly did not occur in winter, when microspore germination (release of spermatozoids) was inhibited by low temperature. We suggest that the period for embryo development is probably longer than the growth season, which shifts the onset of sporeling development to winter and allows their simultaneous appearance in spring.

The long period over which germination occurs increases the vulnerability of the early ontogenetic stages of *I. lacustris*. The species preference for deep littoral habitats protects the early ontogenetic stages from ice-scour and freezing in winter and desiccation in summer. The presence of germinating spores (Fig. 2, panel EG – time of collection) in sporangia on living leaves collected prior to natural leaf fall indicates that *I. lacustris* protects these spores by retaining them within the walls of the sporangium, which may also aid spore dispersal on leaves torn off by animals or wave action. Nevertheless, the early ontogenetic stages are exposed to the ambient environment until sporelings are well rooted and safe from both episodic and chronic environmental stress.

Spore viability i.e. the time for which spores retain their capacity to germinate, is of crucial importance in the formation and persistence of a soil spore bank. In pteridophytes with non-chlorophyllous spores, mean spore viability is ~3 years, with high inter- and intra-specific variation (Gabriel y Galán & Prada 2010). The variable time of the onset of germination (Fig. 2, cohorts A–E, Fig. 4) might result because the spores age at different rates (Gabriel y Galán & Prada 2010) and reflect that sporogenesis takes longer on the slow growing leaves of *I. lacustris* (leaf turnover 2–3 years; Kott & Britton 1983, Rørslett & Brettum 1989, Gacia & Ballesteros 1994). To the best of our knowledge, the phenology of sporogenesis in aquatic quillworts has not yet been studied, thus our hypothesis that

spores gradually mature and that it is this that results in the prolonged period over which spores germinate needs further study.

The importance of soil spore banks in the reproductive strategies of aquatic ferns remains generally poorly understood. It is well established that spore banks persisting in soil for at least one year are widespread in ferns. However, convincing evidence that they can survive for longer is scarce (for review see Dyer & Lindsay 1992, Sharpe & Mehltreter 2010) and mostly questionable due to either an indirect assessment of spore age based on their position in soil cores or the history of the sites sampled (Dyer & Lindsay 1992). Our long-term experiments on germination and available records on thermal conditions suggest that the soil spore bank of *I. lacustris* could persist for several years and up to a decade in cold lakes.

The phenology of reproduction and the range of temperatures over which the germination of deep-water *I. lacustris* occurred in this study sharply contrast with those of the sympatric, but shallow-water *I. echinospora* (Čtvrtlíková et al. 2012). Although the germination of the spores of both species extends over several years, germination in *I. echinospora* is restricted to a short period in early summer, while that of *I. lacustris* occurred throughout the year and even continued for several more years. The distinct recovery of populations of *I. lacustris* and *I. echinospora* that has occurred in Bohemian Forest lakes (Černé jezero and Plešné jezero, respectively) may have resulted from an improvement in lake water chemistry, which in these dimictic lakes is so far restricted to the summer epilimnion. While *I. echinospora* benefits from short periods of low acidity and aluminium toxicity in summer, the sensitive early ontogenetic stages of *I. lacustris* are exposed to harsh conditions in winter. A better understanding of the phenology of the early ontogenetic stages will provide deeper insights into the effects of adverse environmental conditions on the recovery of quillwort populations.

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Souhrn

Isoëtes lacustris je charakteristická a obvykle dominantní vodní rostlina oligotrofních jezer v Evropě. Výhradně pohlavní rozmnožování tohoto druhu prostřednictvím spor bylo zkoumáno na příkladu reliktní populace rostoucí v acidifikovaném horském jezeře na Šumavě. V rámci čtyřletých pokusů jsme sledovali klíčení mikro- a makrospor a tvorbu klíčních rostlin (i) v přirozeném prostředí Černého jezera a (ii) v laboratorních podmínkách při různých teplotách. Pokusy ukázaly, že mikro- i makrospory klíčí postupně v kohortách během tří nebo čtyř vegetačních sezón. V jednotlivých makrosporangiích bylo zjištěno zpravidla několik kohort makrospor, což ukazuje na možnost tvorby výtrusné banky v jezerním sedimentu. Minimální teplota pro klíčení mikrospor (6 °C) byla výrazně nižší než pro makrospory (12 °C). Tyto rozdílné požadavky zřejmě zajišťují dostupnost spermatozoidů pro oplození dlouho žijících makrogametofytů, kdykoliv vytvoří archegonia během jedné případně více vegetačních sezón. Vývoj makrogametofytu probíhal od července do října a vždy také následující zimu. Pokud nedošlo

k oplození, tvorba archegonií pokračovala v další vegetační sezóně. Klíční rostliny se nevyvíjely ve stejné vegetační sezóně jako jejich makrogametofyty, objevily se vždy hromadně na jaře následujícího roku, což naznačuje dlouhotrvající vývoj embrya. Vzhledem k tomu, že raný vývoj (ontogeneze) může trvat několik sezón, *I. lacustris* může být zranitelná krátkodobě i chronicky působícími nepříznivými vlivy. Poměrně vysoká minimální teplota pro klíčení makrospor zřejmě představuje limit pro rozmnožování *I. lacustris* v jezerech a může podmiňovat zeměpisné rozšíření druhu i jeho výskyt na hloubkovém gradientu. Průměrná klíční sezóna *I. lacustris* v Černém jezeře v letech 2004–2011 trvala 119 dní, vždy od července do září.

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