

## Effects of temperature on the phenology of germination of *Isoëtes echinospora*

Vliv teploty na fenologii klíčení druhu *Isoëtes echinospora*

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*Isoëtes echinospora*, a submerged aquatic quillwort, is native in northern latitudes and a rare glacial relict in mountain lakes in temperate Central Europe. A relic population of this quillwort in the Plešné jezero lake has recovered recently from a 30-year period of failure to reproduce caused by acidification. Early ontogenetic stages of the quillwort are considered to be the most vulnerable to environmental changes. Therefore, the objective of this study was to investigate the phenology of germination of *I. echinospora*. In a two-year experiment, we examined the time course of germination of micro- and macrospores and establishment of sporelings under (i) natural in situ conditions in the Plešné jezero lake and (ii) at various temperatures (6–17 °C) in the laboratory. We developed a mathematical model that describes the temperature-specific temporal changes in the early ontogeny of *I. echinospora*. Our experiments clearly show that spores do not germinate at once but gradually over time if exposed to favourable temperatures. Generally, percentage germination tended to increase during the course of a season under most temperature regimes but was inhibited at the lowest temperature. With increasing temperature, microspores germinated earlier and more successfully than macrospores, as described by the model. Sporelings also developed faster at the higher temperature. However, the highest temperature used in the experiments (17 °C) desynchronized the phenology of germination in *I. echinospora* as it resulted in the two types of spore not being available for fertilization at the same time. Thus, climate change might affect interactions between temperature and the phenology of quillwort reproduction and threaten the survival of this species in Central Europe.

**Key words:** aquatic macrophyte, quillwort, reproduction, ontogeny, germination

### Introduction

The quillwort, *Isoëtes echinospora* Durieu, is a characteristic aquatic macrophyte lycopsid of soft-water lakes in Northern Europe (e.g. Hultén 1958, Jalas & Suominen 1972, Rørslett & Brettum 1989, Murphy 2002). In Central Europe, this species has survived as a glacial relict in several mountain lakes, where environmental conditions are similar to those in cold lakes at northern latitudes. One such relic population of *I. echinospora* occurs in the Plešné jezero lake (Bohemian Forest, Czech Republic). Recently, we witnessed the population recover from a 30-year period of severe acidification during which sexual reproduction in quillwort was seriously impaired (Husák et al. 2000, Čtvrtlíková et al. 2009).

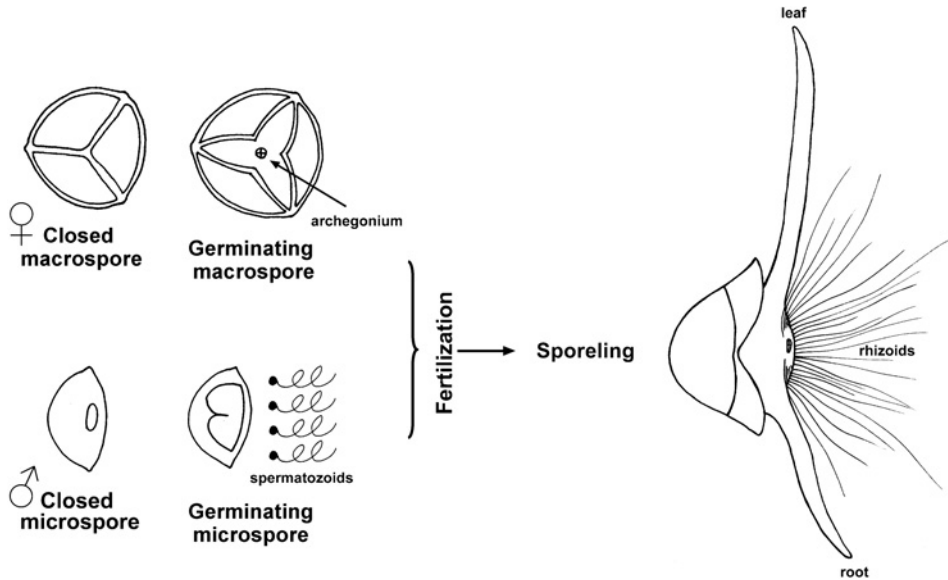


Fig. 1. – Early ontogeny of *Isoetes echinospora*. As a heterosporous species, it has both macro- (female) and microspores (male). Macrospore germination starts when its coat splits along the triradiate ridges. Later, archegonia develop on the exposed macrogametophyte. When the micro-gametophyte matures inside a microspore its coat splits and four spermatozooids are released from mother cells. After fertilization, a sporophyte is dependent, for weeks or even months, on nutrients stored in the macrogametophyte until it develops 2–4 leaves (Eames 1936, Foster & Gifford, 1959).

*Isoetes echinospora* inhabits littoral zones in lakes, penetrating to depths of 2–3 m (Rørslett & Brettum 1989). This species is perennial, slow-growing and heterosporous. Macro- and microsporangia, borne on separate leaf bases, produce haploid macrospores and microspores, respectively, which germinate into a female (egg-cell-producing) macrogametophyte and male (spermatozoid-producing) microgametophyte. Both gametophytes are incapable of independent growth and die after the nutrient supply in the spore is exhausted (Campbell 1891). Early ontogeny of aquatic quillworts, including spore germination, embryogenesis and sporeling development has been extensively studied (Fig. 1; Engelmann 1886, Campbell 1891, Eames 1936, La Motte 1937, Foster & Gifford 1959). However, little research has focused on the phenology of germination (Kott & Britton 1982, Taylor & Luebke 1986) and the environmental factors that control spore germination and sporeling growth.

Even though quillworts occupy nutrient-poor habitats (Rørslett & Brettum 1989, Smolders et al. 2002), the ambient nutrient pool has little effect on germination as there are relatively rich food resources in the macrospores (Eames 1936). The resources in the storage tissue support macrogametophyte development and growth of the embryo and sporeling (Campbell 1891, Eames 1936, Foster & Gifford 1959). Since quillworts are adapted to grow at low light intensities (Sand-Jensen & Søndergaard 1978, Gacia & Ballesteros 1994, Rørslett & Johansen 1995) the effects of ambient light intensity on spore germination are not considered to be important.

Temperature is the most important environmental factor affecting spore germination in aquatic quillworts (Kott & Britton 1982, Taylor & Luebke 1986). Kott & Britton (1982) demonstrate experimentally that macrospores of *I. echinospora* need to experience a period of at least 12 weeks of cold to germinate, while microspores germinate successfully without any cold treatment. The ecological consequences of this difference in the behaviour of the spores remain to be addressed. There is only a limited amount of experimental data on the phenology of germination for *I. echinospora*. At 20–23 °C, macrospores germinate within 30–50 days with a germinability of only 15% (Kott & Britton 1982). In contrast, on average 50% of microspores germinate within 30 days and germination continues to increase at a slower rate for at least 70 days. However, it is unclear whether the poor germination of macrospores of *I. echinospora* results from the inherent behaviour of the spores or whether the macrospores of this species simply require a longer period to achieve maximum germination, as suggested by Kott & Britton (1982).

In this study, we evaluate the effect of temperature on the phenology of germination of *Isoëtes echinospora*. Our objectives were to: (i) determine the time course of spore germination and sporeling establishment under natural temperature conditions in the Plešné jezero lake; (ii) evaluate the effect of various temperature treatments on early ontogeny in the context of the seasonal variation in in situ temperatures; and (iii) develop a mathematical model that describes the temporal changes in germination of micro- and macrospores, and sporeling establishment at various temperatures.

## Material and methods

### *Sampling site*

This study was conducted on the population of *Isoëtes echinospora* in the Plešné jezero lake, Czech Republic (48°47' N, 13°52' E; 1087 m a.s.l.; surface 7.48 ha; maximum depth 18.3 m; volume  $0.62 \times 10^6 \text{ m}^3$ , catchment area  $0.67 \text{ km}^2$ ), which covers the inshore area at depths of 0.3 to 0.75 m (Čtvrtlíková et al. 2009). This dimictic lake is of glacial origin and is covered with ice from November to April (Kopáček et al. 2006).

### *Experimental setup*

The effect of temperature on the early ontogeny of quillwort was studied both in situ and under laboratory conditions. In October 2008, micro- and macrospores were obtained from outer sporophylls (leaves) collected from 50 adult plants of quillwort. In the laboratory, the spores were released from the sporangia and cleaned of debris by rinsing them with distilled water. Macrospores were removed from 105 intact macrosporangia. In contrast to the common practice of using a mixture of macrospores from several macrosporangia (Kott & Britton 1982, Taylor & Luebke 1986) we analyzed separately sets of 60 to 300 macrospores per macrosporangium. Microspores were obtained from more than 30 intact or broken microsporangia and then pooled. To study macrospore germination and sporeling development in both the laboratory and in situ in the field, each set of macrospores together with a mixture of microspores was placed in a Petri dish (55 mm in diameter) with distilled water or an Eppendorf tube (1.5 ml) with lake water, respectively. The Eppendorf tubes were modified by replacing the cap with a 200 µm mesh. Microspore

germination was studied separately using the same experimental design. A week after collection, spores were placed back in the plant stand in the lake (0.4 m depth) or kept in darkness in laboratory grow boxes (Samsung RW33EBSS, Samsung Electronics Czech and Slovak Ltd., Prague, Czech Republic) at a temperature of 4 °C (day/night), which simulate winter conditions.

#### *Lake survey and in situ experiment*

In 2009 and 2010, sporeling growth was investigated in the field quillwort population by snorkelling. The annual course of lake water temperature in plant stands was recorded continuously using a Minikin QT smart sensor (Environmental Measuring Systems Company, Brno, Czech Republic). An in situ experiment on quillwort germination was done in Eppendorf tubes placed in an open 250 ml PET bottle anchored on the bottom of the lake in a stand of quillworts. During each inspection, Eppendorf tubes with spores were removed from the plant stand for an hour and then placed back in the lake. In total, six inspections were performed during the study period (June 2009, July 2009, October 2009, May 2010, July 2010 and September 2010). Macrospore germination and sporeling development was evaluated using 30 replicates, microspore germination using 3 replicates.

#### *Laboratory experiment*

To investigate the effect of temperature on spore germination and sporeling development, both types of spores were kept in Petri dishes in grow boxes at either 6, 8, 10, 12 or 17 °C day/night and under a 14:10 light:dark photoperiod ( $PAR \leq 100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Experiments ran for two consecutive years (2009–2010). From November to mid-May, all treatments were subjected to a 4 °C day/night temperature and continuous darkness, simulating the winter period. From the beginning of the growing season until July, samples were inspected at weekly intervals and then less frequently (monthly). Spores kept at 17 °C were inspected at monthly intervals in both 2009 and 2010. During an inspection, each sample was removed from a grow box for 15 minutes at most. The numbers of macrospores and sporelings were counted in 15 replicates and microspores in 3 replicates.

#### *Germination*

Macrospores were recorded as germinated when they were open and the surface of the macrogametophyte was visible. A sporeling is the ontogenetic stage when the first leaf protrudes from the macrogametophyte. Microspores were recorded as germinated when there were either four cells with spermatozoids inside a microspore or the spore was empty. Germination of macro- and microspores and sporeling development was determined by examining them under a stereomicroscope (Olympus SZ61; magnification 5–45×) or an inverted microscope (Olympus IMT 1; magnification 60×), respectively. To make a quantitative estimate of germination, the percentage of macrospores that germinated or established sporelings was determined from complete sets of macrospores, while percentage of germinating microspores was determined from five fields of view per Petri dish or an Eppendorf tube (~200 spores per field). For the lake experiment, the contents of each Eppendorf tube were placed in a Petri dish, examined under a microscope and after enumeration returned to the field.

### Statistical analyses

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

### Modelling of the time course of germination

We developed a mathematical model describing the time course of micro- and macrospore germination and sporeling development under various temperatures. The model assumes that the percentage of specimens undergoing germination ( $G_t$ ) is constant from the beginning of the experiment up to the start of germination (time  $t_0$ ) and subsequently increases following a decelerating exponential function characterized by a half time and plateau (Fig. 2). The line fitting the individual data on percentage germination recorded at given times was fitted using the following two-segment function with three parameters,  $t_0$  (start of germination; days),  $T_{1/2}$  (half time; days) and  $G_{\max}$  (maximum germination or plateau; %):

SEGMENT 1, for  $t < t_0$ :  $G_t = G_0$ ,

$$\text{SEGMENT 2, for } t \geq t_0: G_t = G_0 + (G_{\max} - G_0) \times \left( 1 - \exp\left(\frac{-\ln 2(t - t_0)}{T_{1/2}}\right) \right),$$

where  $t$  (days) is time,  $G_t$  (%) is germination at time  $t$ , and  $G_0$  (%) is initial germination at the beginning of experiment (set as a constant). The routine for segmental non-linear regression for estimating the parameters  $t_0$ ,  $G_{\max}$  and  $T_{1/2}$ , was programmed in the GraphPad PRISM version 5 statistical package as described by Motulsky (1999). Using this procedure both segments of the function were fitted in parallel and the value of  $t_0$  defining the breakpoint of the function (i.e. the boundary between the two segments) was estimated as a true parameter.

For practical purposes, we derived an additional parameter,  $t_{\max}$  (end of germination; days), defined as  $t_{\max} = t_0 + 5T_{1/2}$ . This definition is based on the general assumption that exponential processes approach equilibrium after five half-times (or, more correctly, 97% of the exponential process is completed after five half-times). Therefore, we assume that the percentage of spores germinating drops to negligible values (i.e. <3% of initial rate) after  $t_{\max}$ , and, consequently, most spores germinate during the time period from  $t_0$  to  $t_{\max}$ . The model was used only for data collected in 2009, either because there was no further increase in spore germination or due to the low sampling frequency in 2010.

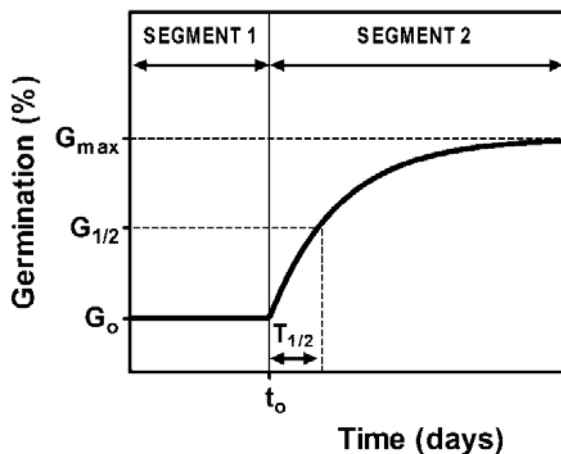


Fig. 2. – Graphical representation of the mathematical model's description of the temporal changes that occur in germination during the course of a season;  $t_o$  is the start of germination since the beginning of experiment (days),  $G_{max}$  maximum germination (%) and  $T_{1/2}$  50% germination (days).

## Results

### *Lake survey and in situ experiment*

Each winter, the Plešné jezero lake was covered with ice and snow and the water temperature in the stand of *Isoëtes echinospora* decreased to  $\sim 0$  °C (December–March, Fig. 3A). Overall, the cold period, when temperatures were below 4 °C, lasted six months (November–April) and the growing season another six months (May–October). In spring, water temperatures increased rapidly and achieved a maximum of  $\sim 20$  °C in the summer.

From in situ observations leaf abscission accompanied by spore release occurred regularly between October and April and sporelings developed in high numbers in July. In the Eppendorf tubes in the lake, germination of both types of spores was first recorded in June in 2009 (Fig. 3). The seasonal maximum germination of micro- and macrospores and sporeling establishment occurred in July (Fig. 3). A further significant increase in percentage germination of micro- and macrospores and sporeling development occurred in 2010 (Fig. 4, Table 1) indicating that spores germinate gradually over a period of time (Fig. 3A–D). Percentage germination of microspores increased significantly (Tukey's HSD test,  $P < 0.0001$ ) from 42 to 100% in the seasons studied (Fig. 4A). Similar results were obtained for macrospore germination and sporeling development but the percentage was generally lower than for microspore germination (Fig. 4). In 2010, macrospores germinated more successfully than in the previous year (Tukey's HSD test,  $P < 0.0001$ ) even so only 39% germinated. In addition, there was a significant inter-seasonal increase in sporeling development from 7.4% to 35.5% (Tukey's HSD test,  $P < 0.0001$ ). Similar values for macrospore germination and sporeling development in both seasons indicate that the *Isoëtes echinospora* in the lake is highly fertile.

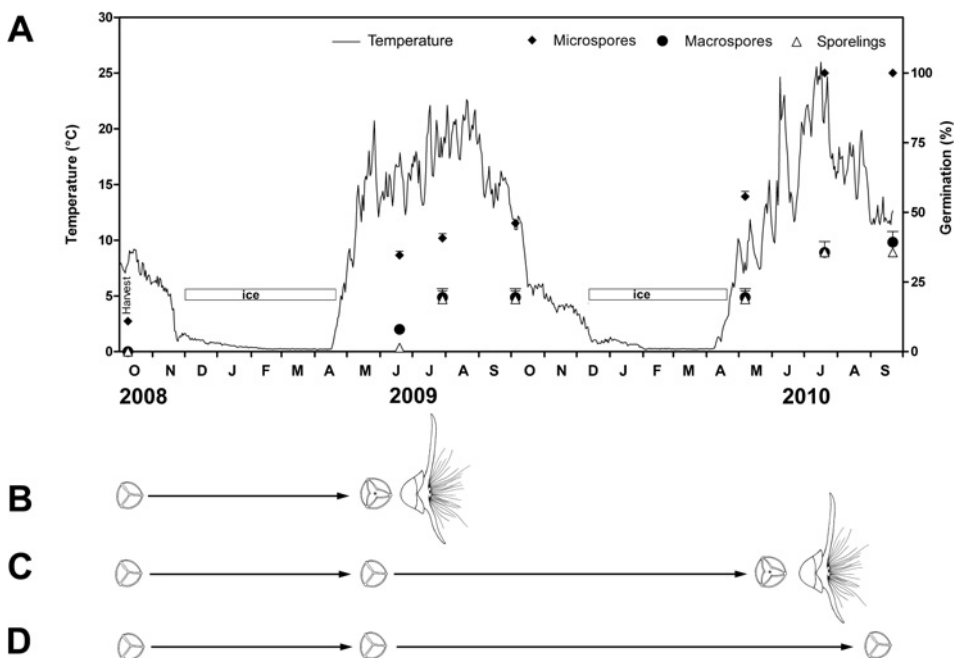


Fig. 3. – In situ germination of micro- and macrospores, sporeling development in Eppendorf tubes and ambient lake water temperature (A). Symbols are medians, error bars represent inter-quartiles. Both types of spore germinated continuously during the course of the two growing seasons as illustrated schematically for macrospores (B–D, same time axis as the graph). A macrosporangium containing macrospores that germinated in the first growing season (B), those that germinated in the second season (C) and those that did not germinate during the course of the study (D).

*Laboratory experiment*

Germination of micro- and macrospores, and sporeling development were significantly affected by temperature, time (season) and their interaction (Fig. 4, Table 1). Microspores germinated more successfully than macrospores in all treatments (Fig. 4). The maximum microspore germination at 17 °C was mainly due to a sudden increase in germination at the end of the first season in 2009 (Fig. 5A). In all treatments, both types of spores germinated in the first season (2009) except for macrospores at 17 °C, which continued germinating into 2010 (Tukey’s HSD test,  $P < 0.0001$ ; Fig. 4).

Table 1. – Results of the repeated-measures ANOVA comparing seasonal maxima of spore germination and sporeling development under various temperature regimes in the two consecutive seasons with temperature as the fixed factor and time (season) the repeated-measures factor.

	Microspore germination			Macrospore germination			Sporeling development		
	df	F	P	df	F	P	df	F	P
Temperature	5	279.0	< 0.0001	5	19.9	< 0.0001	5	21.9	< 0.0001
Time	1	60.7	< 0.0001	1	33.4	< 0.0001	1	12.0	0.0007
Temperature : Time	5	64.9	< 0.0001	5	18.5	< 0.0001	5	9.1	< 0.0001

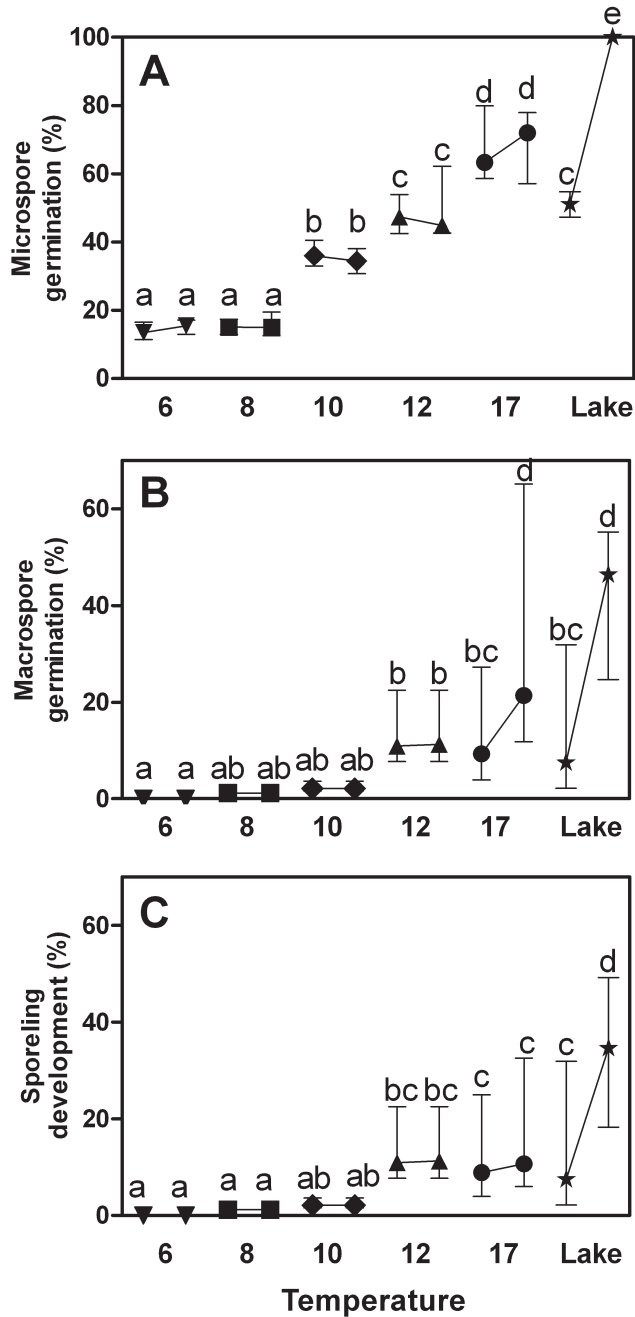


Fig. 4. – Effect of various temperature regimes on maximum germination of both types of spores in the laboratory and in situ in the lake. Microspore (A) and macrospore (B) germination and sporeling development (C) was evaluated at the end (October/November) of each growing season (2009 and 2010). For each temperature, cumulative data from both seasons are shown as neighbouring points connected by a line. Symbols are medians and error bars represent inter-quartiles. Letters above the symbols (a–e) indicate significant differences among treatments (Tukey’s HSD test).



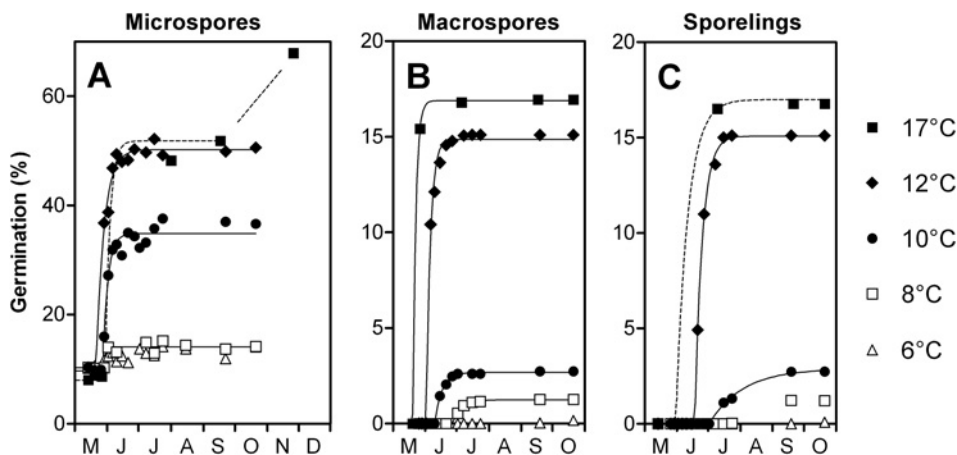


Fig. 5. – Temporal changes in mean microspore (A) and macrospore (B) germination and mean sporeling establishment (C) in 2009. Solid lines are the predictions of the model for various temperature treatments, dotted lines are the germination time courses at 17 °C (due to the low sampling frequency, these lines were fitted to the results by eye not predicted by the model). At the lowest temperature, germination was inhibited throughout the growing period and therefore not predicted by the model.

Temperature had a pronounced effect on the time course of spore germination and sporeling development and is well described by the model (Fig. 5, Table 2). Under most temperature regimes percentage germination of both types of spores tended to increase during the course of the season but was very low at the lowest temperature (6 °C, Fig. 5). The start of germination ( $t_0$ ) of both types of spores occurred earlier at the higher temperatures (Table 2), however, microspores appeared to germinate faster at 8 °C than at higher temperatures (Table 2). The beginning of macrospore germination was ~38 days earlier at 17 °C than 8 °C (Table 2). Sporeling development was also faster at the higher temperatures. Sporelings appeared 14 days earlier, developed much faster and a greater percentage developed at 12 °C than 10 °C (Table 2). At the same temperature, microspores germinated earlier than macrospores (Table 2). Delay in macrospore germination was more pronounced at low temperatures, ranging from 29 days at 8 °C to 10 days at 12 °C and the resultant low availability of macrogametophytes for fertilization reduced the chance of sporelings developing (Table 2).

Table 2. – Time schedule of micro- and macrospore germination, and sporeling development under various temperature regimes predicted by the model. Germination started on day 133 (Julian days).  $t_0$  is the beginning of germination (Julian days),  $T_{1/2}$  is germination half time (days) and  $G_{max}$  is maximum germination (%). Mean numbers and S.E. are shown.

Treatment	Microspore germination				Macrospore germination				Sporeling development			
	$t_0$	$T_{1/2}$	$t_{max}$	$G_{max}$	$t_0$	$T_{1/2}$	$t_{max}$	$G_{max}$	$t_0$	$T_{1/2}$	$t_{max}$	$G_{max}$
6 °C	no fit				no fit				no fit			
8 °C	149±0.6	0.8±1.1	153	14.1±0.3	178±3.4	5.7±3.1	207	1.3±0.1	no fit			
10 °C	148±0.4	3.2±0.4	164	34.6±0.5	161±0.8	4.8±1.3	185	2.7±0.1	183±2.4	22.4±6.4	295	2.9±0.2
12 °C	143±1.7	4.1±0.9	164	48.8±0.5	153±0.6	2.7±1.1	167	14.9±0.8	169±2.1	4.9±2.5	193	15.1±0.9
17 °C	no fit				140±11	2.0±3.5	150	16.9±1.5	no fit			

## Discussion

The field and laboratory experiments in two consecutive growing seasons yielded a novel finding: the spores of *I. echinospora* do not germinate all at once but gradually if exposed to favourable temperatures for a sufficiently long period of time. The generally accepted notion that the germination success of spores of quillwort is low was inferred by Kott & Britton (1982), who recorded spore germination over a period of two months only. Germination of both types of spores at 23 °C over this short period (Kott & Britton 1982) was equal to that recorded in our study at 12 and 17 °C (Fig. 5A, B). Nevertheless, the prolonged period over which germination occurred in our experiments suggests that both a single macrosporangium and a random sample of microspores contain spores that differ in their rate of development.

The strong inhibiting effect of the low temperature on the germination of both macro- and microspores indicates the important role of a cold period in the reproductive cycle of *I. echinospora*. It is known that a cold period is needed to break macrospore dormancy (Kott & Britton 1982). Macrospores, unlike microspores, need a cold period of three months to break their dormancy (Kott & Britton 1982). The cold temperatures in our experiment lasted for longer and prevented both types of spores from germinating in winter. The results of the lake experiment further demonstrated that the timing of macro- and microspore germination is controlled by both cold and warm temperatures.

The effect of a prolonged warm period (17 °C) on reproduction in *I. echinospora* under laboratory conditions was the desynchronizing of the germination of macro- and microspores (Fig. 5A, B), which could have resulted in the low fertilization and poor sporeling development recorded in the next season (Fig. 4C). Close synchronization of the germination of macro- and microspores is reflected in successful fertilization and sporeling establishment. Both macro- and microspores kept at temperatures  $\geq 10$  °C were available for fertilization at the same time, as is well described by the model of the time course of germination (Table 2, Fig. 5). The germination periods of both micro and macrospores (Table 2) overlapped, which resulted in successful fertilization.

In the lake, spore germination gradually increased in both growing seasons similar to that observed at 17 °C in the laboratory. This indicates that in situ temperatures during the first growing season induced both spore germination and spore ripening. In the lake, temperatures favourable for germination ( $\geq 10$  °C) occurred continuously from May to September 2009 (Fig. 3). While the temperature of the water in the lake in summer was suitable for ripening macrospores, as when kept at 17 °C in the laboratory, it was not suitable for microspore germination. The effect of cold temperatures in the lake during winter mentioned above resulted in further synchronous germination of both spore types the following spring and an abundance of sporelings in the second season.

Since quillworts are slow growing plants (Gacia & Ballesteros 1994), early sporeling establishment is essential for their successful development and winter survival. Sporelings need to develop a root system before exhausting the food supply in the macrogametophyte (Campbell 1891). An extensive root system provides nutrients (Smolders et al. 2002) and carbon dioxide (Madsen et al. 2002) from the sediment and also anchors the plantlet to the lake bottom. To have well developed roots is of particular importance in shallow habitats frequently disturbed by wave action or ice scour (Szmeja 1994). Our recent findings indicate that sporelings in the Plešné jezero lake can survive the winter and achieve a juvenile

stage (Čtvrtlíková et al. 2009), and hence, current environmental conditions in the lake are favourable for quillwort reproduction. Until five years ago, however, the reproductive process of quillwort was impaired by both the strong acidity and aluminium (Al) toxicity of the lake water, which damaged the root systems of macrogametophytes and sporelings (Čtvrtlíková et al. 2009). The Al concentrations in the lake water fluctuate seasonally and exceed the critical threshold for quillwort survival, particularly in winter and spring (Kopáček et al. 2006, Čtvrtlíková et al. 2009). The recent recovery of quillwort reproduction apparently resulted from a decrease in Al concentrations (Kopáček et al. 2009). Our results indicate that the sensitive stage of quillwort reproduction (spore germination and sporeling development) occurs when the Al concentration is below the critical threshold.

*Isoëtes echinospora* inhabits shallow water where the probability of ice-scour is high (Rørslett & Brettum 1989). In our study, we demonstrate that both types of spore do not germinate during winter. Therefore, the vulnerable stages present during germination are not subjected to ice-scour or freezing stress. Nevertheless, there are no data in the literature on the susceptibility of spores to freezing. Although each winter a thick layer of ice covers the Plešné jezero lake and the temperature in quillwort plant stands drop to 0 °C, there is no evidence that the water column freezes to the bottom, entrapping quillwort spores in ice. Our previous field observations indicate that there is a thin water layer above the sediment even under a thick ice cover. Interestingly, both types of spore of *I. echinospora*, accidentally frozen for two weeks during a six-month cold period in the laboratory, germinated and produced sporelings. Further experiments, however, are needed to determine the effect of a long period of winter-freezing on the survival of spores and vernalization of macrospores. Desiccation, which is analogous to freezing, seriously impairs germination of *I. echinospora* (Kott & Britton 1982). Obviously, further experiments on the susceptibility of quillwort spores to freezing are necessary to clarify how harsh winter conditions delimit quillwort distribution as theorized by Rørslett (1984, 1988) and Rørslett & Brettum (1989).

Our experiments clearly highlight important interactions between the temperature regime and the phenology of reproduction in *Isoëtes echinospora*. Low temperature during winter is essential for macrospore vernalization (Kott & Britton 1982) but clearly inhibits spore germination. In the growing season, moderate temperatures ( $\geq 10$  °C) are suitable for germination but not ripening of spores. High temperature (17 °C) are suitable for spore ripening but a long exposure to such temperatures disrupts the reproduction process, which results in the spores being unavailable for fertilization at the same time. Reproductive phenology is perhaps the simplest process in which one can track changes in species ecology in response to both historical and ongoing climate change (Walther et al. 2002). It is generally agreed that regional climate affects the distribution of species, often through species-specific physiological thresholds of temperature tolerance (Hoffman & Parsons 1997). The narrow temperature tolerance that defines reproduction success in quillwort certainly determined the distribution of this arctic species in Central Europe during the ice ages and post-glacial periods. The recent changes in climate are likely to result in a deterioration in the suitability of the habitats and so jeopardize the long term survival of relic quillwort populations in Central Europe.

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## Souhrn

*Isoetes echinospora* je submerzní vodní plavuň pocházející ze severních zeměpisných šířek, ve střední Evropě se však vyskytuje jako glaciální relikv v horských jezerech. Reliktní populace v Plešném jezeře se aktuálně zotavuje z třicetiletého období acidifikace, která bránila úspěšnému rozmnožování tohoto druhu. Protože acidifikací byla postižena nejvíce raná ontogenetická stádia, soustředili jsme se v naší studii na fenologii klíčení. V rámci dvouletých experimentů jsme sledovali klíčení mikro- a makrospor a tvorbu klíčnicích rostlin (i) v přirozeném prostředí Plešného jezera a (ii) v laboratorních podmínkách při různých teplotách (6–17 °C). Vliv teploty na průběh klíčení jsme vyhodnotili pomocí matematického modelu. Naše experimenty ukázaly, že za příznivých teplotních podmínek spory neklíčí všechny najednou, ale postupně během více let. Teplota měla pozitivní vliv na klíčení mikro- a makrospor a tvorbu klíčnicích rostlin. Pomocí modelu jsme spočítali, že s rostoucí teplotou mikrospory klíčí dříve a úspěšněji než makrospory. Při nejvyšší použité teplotě (17 °C) však došlo k desynchronizaci klíčení mikro- a makrospor, takže nemohlo dojít k úspěšnému oplození. Nepříznivý vliv vysoké teploty na reprodukci druhu jej činí zranitelným v období probíhajících klimatických změn.

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