

Lake water acidification and temperature have a lagged effect on the population dynamics of Isoëtes echinospora via offspring recruitment

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1 **Lake water acidification and temperature have a lagged effect on the population**
2 **dynamics of *Isoëtes echinospora* via offspring recruitment**

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19 **Abstract**

20 The aquatic quillwort, *Isoëtes echinospora*, survived the strong water acidification during
21 1960s–1990s in Plešné Lake (Bohemian Forest, Central Europe), but failed to reproduce. We
22 studied the relationships between a recent population recovery and an improvement of lake
23 water quality. We used correlation analysis to evaluate lagged seasonal effects of lake water
24 quality on population dynamics during the past decade, and factor analysis to determine the
25 independent factors responsible for population recovery. We also provided a water-quality-
26 based reconstruction of population growth from the beginning of the lake recovery two
27 decades ago, using a partial least squares regression (PLSR) model of population growth. We
28 identified three independent controlling factors: nutrients (nitrate, phosphorus, calcium,
29 potassium, magnesium), stressors (pH, ionic aluminium) and temperature. Of these, nutrient
30 availability did not limit the quillwort growth, but annual mean pH and winter mean
31 concentrations of toxic ionic aluminium influenced population growth through negative
32 effects on sporeling establishment until the age of one year, while cumulative temperature in
33 spring and summer controlled the later plant growth. Thus, water quality in the acidified
34 Plešné Lake mainly controls recruitment success rather than adult survival of *Isoëtes*
35 *echinospora*. This study provides the first *in situ* evidence that the recruitment success,
36 namely the annual increment in the adult quillwort population, indicates the degree of
37 recovery from acidification, however further extensive investigation is required to more
38 accurately quantify, and therefore understand, the relationships between recruitment, water
39 quality and other factors.

40 **Key words:** aluminium toxicity; aquatic plant; bioindication; herbivory; nutrients; plant life-
41 history traits; population dynamics; reproductive ecology; stress

42 1. Introduction

43 Isoetid species are small, slow-growing, evergreen water plants that are highly specialised for
44 life in carbonate poor (weakly buffered) and nutrient poor (oligotrophic) lakes (Hutchinson
45 1975; Smolders, Lucassen & Roelofs 2002). During the last century, isoetid vegetation in
46 lakes of the Northern Hemisphere declined or became endangered due to anthropogenic
47 acidification and eutrophication (Arts 2002; Brouwer, Bobbink & Roelofs 2002; Smolders,
48 Lucassen & Roelofs 2002). Many of the atmospherically acidified lakes have been chemically
49 and biologically recovering since the 1980s (e.g., Stoddard *et al.* 1999; Graham *et al.* 2007;
50 Gray & Arnott 2009; Garmo *et al.* 2014), allowing for unique ecological studies on the
51 ecosystem functioning along rapidly changing temporal gradients of water chemistry. In
52 contrast to fish, plankton and benthos, current knowledge on the environmental stress
53 affecting isoetids in acidified lakes remains fragmented, since long-term quantitative data on
54 their growth under *in situ* conditions has been missing. The successional changes in plant
55 communities ascribed to lake water acidification are quite common in the literature (for
56 review see Arts 2002), supported by inconsistently surveyed presence-absence data on
57 ‘sensitive’ species. Only a few transplant (Brandrud & Johansen 1994) or germination
58 (Čtvrtlíková *et al.* 2009; Čtvrtlíková, Znachor & Vrba 2014) experiments focused on the
59 symptoms of plant sensitivity to particular stressors including low pH and toxic ionic
60 aluminium (Al₃). There is, however, no compelling evidence that these stressors within other
61 physico-chemical conditions *in situ* play a significant role in decline of the original flora in
62 acidified lakes (Arts 2002).

63 Isoetids obtain both nutrients and carbon from relatively rich sediments through a large root
64 system (for review see Smolders, Lucassen & Roelofs 2002), while the green aboveground
65 parts of isoetid leaves are covered by thick cuticle, thus, only responsible for light absorbance
66 and photosynthesis but not for nutrient uptake from lake water (Madsen, Olesen & Bagger
67 2002). Isoetid vulnerability to changes in ambient water quality has been generally described
68 in eutrophic lakes, where they are eventually outcompeted by taller rooting macrophytes,
69 epiphytes, floating macrophytes, or phytoplankton (Arts 2002; Brouwer, Bobbink & Roelofs
70 2002), all better competitors for nutrients dissolved in the water column and ultimately for
71 light. By analogy, the shading effect of expanding acidotolerant macrophytes has also been
72 suspected to be responsible for a large decline of isoetids in acidified lakes (Arts 2002;
73 Brouwer, Bobbink & Roelofs 2002). Since lake water acidification does not affect deeper
74 sediment horizons (Herlihy & Mills 1986; Kopáček *et al.* 2001), it remains unclear why the

75 isoetids - with impermeable leaves and rooted in those favourable sediment horizons -
76 decline in acidified lakes, even if competitive plants are absent and light conditions are
77 satisfactory for growth (Murphy 2002; Čtvrtlíková *et al.* 2009).

78 Quillworts (*Isoëtes*) are representatives of isoetid growth and typically are the dominant
79 species in European unproductive lakes (Rørslett & Brettum 1989; Murphy 2002). Sole,
80 monospecific population of *Isoëtes echinospora* persists as a glacial relic in Plešné Lake
81 (Bohemian Forest), which has been strongly acidified since the 1960s (Majer *et al.* 2003). Our
82 laboratory experiments on the effects of strong acidity and Al_i toxicity of lake water on spore
83 germination of *I. echinospora* showed that both stressors significantly damaged the fine root
84 system of the quillwort sporelings (Čtvrtlíková *et al.* 2009). Therefore, we hypothesised that
85 the extreme stress during spring germination of *I. echinospora* has been responsible for the
86 quillwort reproduction failure over the 30 years of severe acidification of Plešné Lake.
87 Nonetheless, there is no observed weakness in deeply rooted adult plants in the lake
88 population. In fact, there were approximately 1000–5000 adult plants forming the population
89 until the 2000s (Husák, Vöge & Weilner 2000). The resistance of long living adults to lake
90 water stressors most likely allowed for long-term survival of *I. echinospora* in acidified
91 Plešné Lake. Recently we have witnessed remarkable reproduction recovery of the quillwort
92 following improvement of the Plešné Lake water quality due to reduced sulphur and nitrogen
93 deposition (Oulehle *et al.* 2013). In this study, we aim to elucidate the controlling role of the
94 presumed environmental stressors that affect *I. echinospora* recruitment in Plešné Lake during
95 its recovery from atmospheric acidification. To achieve this aim, the study has four
96 objectives: (1) to assess relationships between population dynamics of *I. echinospora* and
97 ambient lake water conditions in Plešné Lake, (2) to verify controlling roles of still seasonally
98 high acidity and Al_i toxicity in the quillwort renewal *in situ*, (3) to confirm that the early
99 ontogenetic stages of *I. echinospora* are sensitive to environmental stresses associated with
100 lake water acidification, and (4) to reconstruct the population growth from the beginning of
101 the lake recovery two decades ago.

102 **2. Material and methods**

103 **2.1. Study site**

104 The population of *I. echinospora* Durieu has inhabited Plešné Lake situated in the Bohemian
105 Forest, the Czech Republic (48°47'N, 13°52'E; 1,087 m a.s.l.; Fig. 1) since the end of the last
106 Glacial (~ 10 kyr BP; Jankovská 2006). The lake is of glacial origin, dimictic, mesotrophic
107 (area of 7.5 ha, volume of 617,000 m³, maximum depth of 18 m), with small catchment area

108 (66.6 ha) on granitic bedrock forested by Norway spruce (*Picea abies*) (Kopáček *et al.* 2007).
109 The lake has been acidified by atmospheric deposition of sulphur (S) and nitrogen (N)
110 compounds since the late 1960s. Acidification progressed until the middle 1980s, when pH
111 ranged between 4.4 and 4.7, carbonate buffering system was entirely depleted, and total
112 aluminium (Al_t) and ionic aluminium (Al_i) concentrations reached 1.1 and 0.8 $mg\ l^{-1}$,
113 respectively (Kopáček *et al.* 2009). Since the 1990s, the lake chemistry has been recovering,
114 with a temporary renewal of carbonate buffering capacity and increase of mean pH values to
115 approximately 5 during summer stratification in the early 2000s. A partial lake water re-
116 acidification has occurred since 2004 due to forest dieback in the Plešné catchment due to
117 bark-beetle infestation (Kaňa, Tahovská & Kopáček 2013; Oulehle *et al.* 2013).
118 *Isoëtes echinospora* forms a monospecific plant stand in the inshore area of approximately
119 0.03 ha, at depths of 0.3–0.5 m (max. 1.0 m; Čtvrtlíková *et al.* 2009). The lake shore is grown
120 by Bottle Sedge (*Carex rostrata*); its dense stand forms a dynamic inshore border of the
121 quillwort population. The sediment at the quillwort stand is an aqueous sapropel with a high
122 proportion of organic matter. The lake is usually ice-covered from December to April.

123 **2.2. Population dynamics**

124 Population dynamics of *I. echinospora* in Plešné Lake were investigated at 3 adjacent study
125 plots ($3 \times 10 \times 15$ m) covering most of the lake population (95% in July 2013). The plant
126 stands outside the study plots were not taken into account as they had been gradually
127 overgrown by the Bottle Sedge. Underwater visual censuses were performed in transitory strip
128 transects (0.5×10 m) using snorkelling every June–July from 2004 to 2014 and additionally
129 in October 2013, when a plant stand disturbance occurred. All plant individuals in transects
130 were recorded by the same observer throughout this study.

131 Two phenological stages of juvenile and adult plants were defined to determine the life
132 history stages most sensitive to lagged environmental stresses (the quillwort phenology is
133 illustrated in Fig. 2 according to Čtvrtlíková *et al.* 2012). A juvenile plant was a plant of more
134 than four leaves and linear symmetry of the leaf rosette that survived the first winter. An adult
135 plant was a plant with leaves (usually > 10) clutched in a rosette of radial symmetry that
136 survived more than two winter seasons. Sporelings, the (juvenile) plantlets established from
137 germinating spores between May and June and forming less than four leaves until July (time
138 of inspections), were not included in censuses of juvenile plants.

139 Plant abundance (number of individuals at the transects within three study plots), age
140 structure (proportion of juvenile individuals), and population annual growth (year-to-year

141 change of plant abundance) were evaluated from the census data to describe population
142 dynamics. The abundance of adult plants before and after the plant stand disturbance in
143 autumn 2013 was assessed in particular transect sections (not quadrates) grown by distinct
144 plant assemblages and it was expressed per square meter and classified within intervals: 1;
145 10–100; 100–200, and > 200 individuals per 1 m².

146 **2.3. Time lags**

147 Our previous findings showed that juvenile and adult population recruits from sporelings
148 within one and two years, respectively (Čtvrtlíková *et al.* 2009; Fig. 2). Therefore, water
149 quality effects on juvenile and adult population growth were tested with three and six time
150 lags, respectively, which had been assumed to be biologically relevant. The time lags were
151 related to the growing (G), winter (W) and spring (S) periods up to two years before (t, t–1, t–
152 2) the current plant stage. The periods were defined by the water temperature threshold of
153 12°C for *I. echinospora* germination (Čtvrtlíková *et al.* 2012; Fig. 2). The growing and winter
154 periods distinguished between the daily mean temperature above and below 12°C,
155 respectively. The spring period began when the water temperature first time surpassed the
156 12°C threshold and included both the next 60 days when daily mean temperature was > 12°C
157 (according to Čtvrtlíková *et al.* 2012) and any intermittent day when the temperature was <
158 12°C (if present). We suppose that the offspring recruited to the adult population (i.e.
159 censused ‘new’ adults) during current spring (S_t) had been at its juvenile age within the last
160 winter (W_{t–1}), the last growing (G_{t–1}) period and the last spring (S_{t–1}), and at its sporeling age
161 within the first winter (W_{t–1}) and the first growing period (G_{t–2}), when born (Fig. 2). The
162 sporelings recruited to the juvenile population (i.e. censused juveniles) during current spring
163 (S_t) overwintered only once, during the last winter (W_{t–1}), since they had originated during the
164 last growing (G_{t–1}) period (Fig. 2).

165 **2.4. Environmental conditions**

166 Lake water temperature (°C) and photosynthetically active radiation (PAR, μmol m² s^{–1}) in
167 the quillwort plant stand was recorded with the Minikin QT smart sensor (Environmental
168 Measuring Systems Company, Brno, Czech Republic) in 30-minute intervals from June 2003
169 to July 2014 (parallel to the plant observation period). Temperature and PAR effects on the
170 quillwort growth were analysed from these measured data. Lake water temperature data for
171 hindcast modelling of quillworts growth in 1997–2003 were reconstructed by a mathematical
172 temperature lake model (CE-QUAL-W2; Cole & Wells 2015), calibrated on the 2004–2013
173 period with climatic data from a nearby meteorological station of the Czech

174 Hydrometeorological Institute (Churáňov; 49°04'N,13°37'E; 1118 m a.s.l.). The agreement of
175 daily means from the measured and simulated water temperature data was good (mean error,
176 0.0°C; mean absolute error, 0.8°C; root mean square error, 1.1°C; R^2 , 0.98). Aquatic
177 quillworts are well adapted to grow at low ambient light (e.g. Keeley 1998) and light
178 conditions in the shallow stands of *I. echinospora* in Plešné Lake might comply with the
179 species preferences (Gacia & Ballesteros 1994) during the past decades, when water level was
180 stable (with maximum oscillations ± 10 cm during high flow events or rare occasions of dry
181 and hot weather) and water transparency satisfactory (> 1.5 m in summer; Vrba et al. 2000).
182 Daily mean water temperature was used to identify the spring, growing and winter periods in
183 1998–2013. To study the temperature effect on population dynamics, we used the following
184 characteristics: (i) seasonal mean temperature (average of daily mean temperature, T_m), (ii)
185 cumulative temperature (sum of daily mean temperature, T_c), (iii) cumulative days (sum of
186 days within a period, D_c), and (iv) temperature variation during a period (standard deviation,
187 T_v) that were calculated for the spring, growing and winter periods. To study the effects of
188 lake water chemistry on population dynamics, mean values of pH, $\text{NO}_3\text{-N}$, total phosphorus
189 (TP), Ca, K, Mg, Al_t , and Al_i concentrations in an epilimnetic outflow of Plešné Lake were
190 calculated for the respective periods. These characteristic were selected from a wider range of
191 long-term data on water chemistry with respect to the nutrients preferred by quillworts
192 (Smolders, Lucassen & Roelofs 2002) and the species-specific adverse effects of acidity and
193 Al_i toxicity (Čtvrtlíková *et al.* 2009). Lake water was sampled from the depth of 0.05–0.3 m at
194 the outflow of Plešné Lake several times (1–8 samplings) a year in summer-to-autumn period
195 between 1990 and 1997 and in 1- to 3-week intervals between 1998 and 2014. TP was
196 determined by perchloric acid digestion and the molybdate method according to Kopáček &
197 Hejzlar (1993). Concentrations of $\text{NO}_3\text{-N}$ and other ions (Ca^{2+} , Mg^{2+} , K^+) were determined by
198 ion chromatography (Dionex IC25, USA). Al_t and Al_i were analysed according to Driscoll
199 (1984) using the spectrophotometric method by Dougan & Wilson (1974) and pH was
200 measured using a glass electrode (Radiometer PHC2401).

201

202 **2.5. Statistical analyses**

203 The Pearson correlation analysis was performed to identify candidates for both response and
204 predictor variables explaining the quillwort population recovery which was visually observed
205 between 2004 and 2013. The response (Y) variables in the analysis included plant abundance
206 and population growth, each specified for juvenile and adult stage. The predictor variables (X)
207 included the 12 above mentioned physicochemical lake water characteristics, each lagged by

208 age at different periods. The best scores of correlation coefficients for explanatory response
209 and predictor variables were assessed, using the software package STATISTICA 12
210 (product/module STATISTICA Base; StatSoft, Inc., Tulsa, OK, USA).

211 The factor analysis was performed to help to find an explanation for the quillwort recovery, in
212 particular to identify a reduced number of independent factors from the correlated observed
213 predictors influencing the population growth. Factor analysis was applied to the most
214 important time lag at which eight of the lake water characteristics, including lake water
215 chemistry and temperature, operated. The meanings and the true explanatory magnitudes of
216 latent factors were evaluated from the factor loadings of the rotated matrix Varimax, the
217 variability explained by each factor (R^2), and the communalities for each variable, using the
218 software package STATISTICA12 (product STATISTICA advanced, module STATISTICA
219 Multivariate Exploratory Techniques).

220 The partial least squares regression (PLSR) model was used for a water-quality-based
221 reconstruction of population growth in 1998–2003. Data on the adult population growth
222 (response variable, Y ; 2005–2013) and the same (eight) lake water characteristics (predictor
223 variables, X_i ; 1998–2013) re-inserted from the factor analysis were used in PLSR, designed as
224 a multiple linear regression model. The meaning and the true explanatory magnitude of latent
225 components was estimated from the weights of the original predictor variables and the total
226 explanatory capacity (R^2 of Y and R^2 of X_i) of extracted components. The following regression
227 equation was used to explore the growth reconstruction:

$$228 \quad Y = b_0 + \sum_{i=1}^8 b_i X_i \quad [1]$$

229 where b_0 is the regression coefficient for the intercept and the b_i values are the regression
230 coefficients (for the predictor variables 1 through 8) computed from the data. The abundance
231 reconstruction was also provided using data on modelled growth (referring to inter-annual
232 difference in abundance). The regression algorithm was performed by the Statistica 12
233 (product STATISTICA advanced, module STATISTICA Advanced Linear/Nonlinear
234 Models). No model was used for predicting the quillwort improvement in the ongoing decade
235 as the population was largely reduced at the end of our study (in 2013).

236 **Results**

237 *Environmental conditions*

238 Long-term monitoring of water chemistry in Plešné Lake (Fig. 3) demonstrated a steady
239 decrease in sulphate since 1990 in response to the reduced nitrogen and sulphur deposition.

240 Summer values of pH increased above 5 and the seasonal re-establishment of carbonate
241 buffering system has occurred in the epilimnion since 1999 (Fig. 3a). In parallel, the seasonal
242 summer-to-autumn minima of Al_i concentration decreased to $< 0.1 \text{ mg l}^{-1}$, although its spring
243 peaks remained high (up to 0.4 mg l^{-1} ; Fig. 3b). Nitrate concentrations increased markedly
244 after the large-scale forest dieback in the Plešné catchment during 2006–2009 and peaked two
245 years later (Fig. 3d) due to elevated terrestrial export, resulting from increased mineralization
246 of fresh litter and ceased uptake after the forest dieback (Kaňa, Tahovská & Kopáček 2013).
247 The elevated leaching of nitrate was compensated for by increased leaching of cations,
248 especially Al_i , K, H, Ca and Mg (Fig. 3a–c). The forest dieback also resulted in elevated TP
249 leaching (Fig. 3a).

250 *Population dynamics*

251 Plant abundance of *I. echinospora* in Plešné Lake observed in 2004 (Fig. 4a) was similar to
252 that reported before 1998 (Husák, Vöge & Weilner 2000). A recovery of the quillwort
253 population was observed between 2006 and 2013, when the total plant abundance increased
254 steadily, showing a proportion of more than 20% of juvenile plants every year (Fig. 4a).
255 Population growth (Fig. 4b) reflected recruitment success rather than adult survival, since low
256 mortality of adults has been observed *in situ*.

257 *Pearson correlation analysis*

258 Population growth in general and adult growth in particular showed significant ($p < 0.05$)
259 correlations with lake water pH, Al_i , and temperature (Table 1, Fig. 2, 5). Seasonally high
260 water acidity (low pH) and Al_i concentrations affected adult growth through negative (toxic)
261 effects on establishment of early ontogenetic stages. The only time lag at which Al_i operated
262 was age at the first wintering (W_{t-1} ; Table 1, Fig. 2), whereas responsiveness to pH was
263 determined also at two subsequent time lags corresponding to a plantlet age ≤ 1 year (between
264 W_{t-1} and G_{t-1} ; Table 1, Fig. 2). Clear signs of the positive relationships between adult growth
265 and spring-to-summer temperature (T_c , T_{var} , or D_c) were determined at the time lags
266 corresponding to age ≥ 1 year (G_{t-1} and S_t ; Table 1, Fig. 2). In addition, significant ($p < 0.05$)
267 positive relationship between adult growth and total phosphorus (TP) lagged at the last (S_{t-1})
268 and current (S_t) spring was shown (Table 1, Fig. 2).

269 Cumulative rather than mean thermal characteristics have profound effects on the population
270 dynamics (Table 1, Fig. 5). The cumulative water temperature available for the *I. echinospora*
271 growth in Plešné Lake in the spring and growing periods was $924\text{--}1361^\circ\text{C}$ (average of $1086 \pm$
272 111°C) and $1693\text{--}2474^\circ\text{C}$ ($2121 \pm 220^\circ\text{C}$), respectively, during the past 15 years. The spring
273 and growing periods began in May and lasted 60–80 days (67 ± 6 days) and 98–151 days (127

274 ± 15 days), respectively. Water temperature was significantly controlled by atmospheric PAR
275 (Pearson $r > 0.85$, $p < 0.05$; data not shown) showing analogous effects on adult growth.
276 Therefore, atmospheric radiation was excluded from further analyses and only temperature
277 was used, representing the underwater thermal conditions.

278 *Factor analysis*

279 Three independent factors explained 98% of the total variability of eight lake water
280 characteristics lagged by age at the first wintering (W_{t-1}), which are clearly separated into
281 four clusters using Factor Analysis (Table 2, Fig. 6). The first factor is marked by high
282 nutrient concentrations including $\text{NO}_3\text{-N}$, TP, Ca, K, and Mg, which all are correlated with
283 each other. The second factor is marked by the biotoxic stressors accompanied with the lake
284 water acidification, including negatively intercorrelated pH and Al_i (two opposite clusters).
285 The third factor is marked by water temperature, represented by T_c .

286 *Partial least squares regression model*

287 The results of the Partial least squares regression analysis demonstrated that three components
288 explain 85% of the original variance in the adult growth (Table 2, Fig. 7a). The first
289 component accounts for a major proportion (58%) of the explained variance and associates
290 negatively correlated pH and Al_i . The second and the third component account for almost the
291 same proportion (14% and 13%) of the explained variance and are dominated by Al_i
292 associated to temperature and pH (positive correlation between Al_i and pH), respectively. The
293 2- and 3-component PLSR models rather than the 1-component model fit the data for the
294 whole period 2005–2013, as well as for the extreme events in 2006 (high growth *in situ*) and
295 2011 (low growth *in situ*). The two higher-component models, however, diverge substantially,
296 when extrapolating beyond the observation period in 2000 and 2003. Nevertheless, the results
297 from all the three models show that recovery of *I. echinospora* in Plešné Lake is
298 predominantly controlled by the reduction in winter Al_i concentrations and acidity of the lake
299 water. It is evident that intermittent mitigations of both stressors have enabled a discontinuous
300 growth of the population since 2001. From the three growth models, relevant adult plant
301 abundances were reconstructed, explaining each of about 90% of variance in the observed
302 abundances between 2004 and 2013 (Fig. 7b). Nonetheless, only the abundance provided
303 from the 2-component model shows the most realistic feature, as it fits well the former field
304 observations (Husák, Vöge & Weilner 2000), when extrapolated between 1999 and 2004.

305 *Plant disturbance*

306 Highly dense stands of the quillwort population formed during recovery from acidification
307 (Fig. 8) were suddenly grazed by Mallards (*Anas platyrhynchos*), which damaged 90% of all

308 plants in the population within a month between August and October 2013. The field
309 observations showed a consumptive grazing of a female with seven grown ducklings on
310 stunted stems of adult plants. Because some of the damaged plants regenerated, the abundance
311 of adult and juvenile plants in summer 2014 was reduced by 73% and 83%, respectively,
312 when compared with that in July 2013 (Fig. 8). The proportion of juveniles in 2014 was in
313 line with the recovery trend prior to the population disturbance (Fig. 4a).

314 **3. Discussion**

315 This study brings compelling evidence that water quality controls *I. echinospora* recruitment
316 in the acidified Plešné Lake. Low pH and high Al_i concentrations, both known as root-
317 inhibitors, are clearly distinguished from the other lake water compounds as synergistic
318 acidification stressors that prevent development of the early ontogenetic stages of the
319 quillwort (Tables 1 and 2, Fig. 5, 6). The sporelings and juveniles with short roots developing
320 at the sediment surface are affected by the acidic lake water, but it is not detrimental to the
321 deep rooted adults. Survival of the population, which lost sporelings due to the harsh
322 conditions, depends entirely on the adult perennials, since quillworts cannot reproduce
323 clonally. There is missing information, whether the plantlets establishing on ramets of the
324 other isoetids that are capable of clonal reproduction would be also affected by lake water
325 acidity or they would benefit from a physiological integrity. A renewal failure due to lake
326 water pollution and gradual senescence of an isoetid plant stand may result in a decline in
327 population size, even if the adults grow under satisfactory conditions.

328 In Plešné Lake recovering from acidification, *I. echinospora* recruitment has still been
329 inhibited by the seasonal or episodic extremes in acidity and elevated Al_i concentrations,
330 caused by snow melting or high precipitation events (Fig. 3a, b, 5). We identified the early
331 ontogenetic stages of the quillwort until the age of ~1 year to be sensitive to these
332 environmental stresses, when surpassing certain thresholds (Table 1, Fig. 2, 5). The thresholds
333 of Al_i (0.3 mg l^{-1}) and pH (5.0) for sporeling establishment of *I. echinospora* derived from
334 laboratory experiments (Čtvrtlíková *et al.* 2009) are clearly effective under the lake conditions
335 as well (Fig. 5). The Al_i concentrations have still exceeded the threshold in winter, but they
336 could prevent any sporeling recruitment only in synergy with the extreme acidity (pH < 4.9;
337 Fig. 5). In addition, the juveniles may be further impaired by the extreme acidity itself during
338 spring and/or summer, when Al-toxicity is not critical (periods S_{t-1} and G_{t-1} ; Table 1).
339 Interestingly, no stress from any of the lake water condition was observed within the very first
340 few months of a sporeling life (period G_{t-2} , Table 1), when the plantlet is still attached to
341 macrogametophyte storage tissue (Eames 1936; Foster & Gifford 1959), and a supplemental

342 nutrition may temporarily offset the nutrient deficiency caused by a dysfunction of sporeling
343 roots under the harsh conditions.

344 Population growth of *I. echinospora* in Plešné Lake was most likely absent over 30 years
345 between 1970 and 2000 due to all-year-long extreme acidity and high Al_i concentrations in
346 the lake water (Majer *et al.* 2003; Fig. 3a, b) exceeding the thresholds for the offspring
347 survival. Our reconstruction model (Table 2, Fig. 7a, b) supports the presence of numerous
348 adult survivors during that time reported by Husák, Vöge & Weilner (2000). Such a long
349 survival despite the severe chemical conditions indicates the high resistance of adult plants to
350 the stressors in lake water, as well as the long life span of *I. echinospora*. The model further
351 showed the discontinuous population recovery during the past 15 years (Fig. 7b), when
352 relatively favourable conditions in growing period (Fig. 3, 5) allowed for spore germination
353 and sporeling development, as well as for regeneration of grown juveniles after being exposed
354 to the still occasionally harmful conditions in winter.

355 An annual increment in the adult quillwort population is the most important life-history
356 parameter for reliable estimates of stress responsiveness lagged by age at early ontogeny
357 (Table 1). Alternatively, abundance or annual growth of the juvenile population is a poor
358 indicator for stress tolerance during a plant life history (Table 1). Neither adults nor juveniles
359 of *I. echinospora* were influenced by water column nutrient concentrations (Table 1, Fig. 5),
360 what is in conformity with the known fact that nutrient availability does not limit the growth
361 of quillworts adapted to oligotrophic conditions (Smolders, Lucassen & Roelofs 2002). The
362 only significant positive correlation between the juvenile stage and total phosphorus in spring
363 (S_{t-1} ; Table 1) is likely an artefact caused by the increasing trend in TP.

364 The *I. echinospora* plantlets of the age of ~1 year are no more influenced by the acidification
365 stressors, but their growth is controlled by lake water temperature in Plešné Lake (Table 1,
366 Fig. 2 and 5). The change in environmental drivers during the second summer of a plantlet life
367 probably indicates that its root system reaches relatively deep sediment, where the acidic Al
368 rich lake water does not penetrate. At that time, only the heat transmitted from aboveground
369 starts to control the intrinsic slow growth of the quillwort, so that the prolonged spring with
370 altering warm and cold spells has a clear positive effect of on the establishment of adult plants
371 (Table 1). Our analyses showed that the minimum water temperature threshold of 12°C for *I.*
372 *echinospora* germination derived from laboratory experiments by Čtvrtlíková *et al.* (2012) fits
373 well for the growth of the lake plants too. The cumulative thermal characteristics (T_c and D_c)
374 rather than mean temperature are suitable for assessing a thermal response of the quillwort
375 (Table 1) and may provide useful predictors of the quillwort phenophases and distribution in

376 analogy to widely used effective temperature sums (e.g., McMaster & Wilhelm 1997),
377 growing degree-days (e.g., Lindgren & Walker 2012), or season length.

378 To our best knowledge, this is the first long term (> 14 years) study of an isoetid population
379 dynamics based on intensive repeated measurements *in situ*. We observed strongly reductive
380 effects of an anthropogenic as well as natural disturbance on the glacial relic population of *I.*
381 *echinospora* in Plešné Lake. In addition to acidification effects, we bring (accidental but
382 unique) details on mallards grazing on aquatic quillwort *I. echinospora* (Fig. 8). Grazing by
383 Mallards (*Anas platyrhynchos*), Canada Geese (*Branta canadensis*), or Common Loon (*Gavia*
384 *immer*) have been briefly noted for several quillwort species including *I. echinospora*
385 (Brunton & Britton 1999). The Mallards grazing preference of dense plant stands might be the
386 reason why the quillwort population in Plešné Lake was untouched during its recovery until
387 reaching the high density in 2013 (Fig. 8). The leaves clipped (uneaten) from the damaged
388 plants in autumn were observed to spread along the entire shoreline, where maturing spores
389 might be released from their sporangia; yet no sporelings have been observed there since the
390 next spring. This indicates a large loss of individuals reducing a genetic variability of the
391 population, which already might be low due to its decline during acidification period, or some
392 other disturbances in the shallow littoral. Given the responsiveness of *I. echinospora*
393 recruitment to the recent trends in chemistry of Plešné Lake (Majer *et al.* 2003), the current
394 growth rate of the resting population is anticipated to continue or even increase.

395 **3.1. Conclusions**

396 As hypothesized, lake water quality controls *Isoëtes echinospora* recruitment in Plešné Lake
397 recovering from acid stress. In particular, seasonal extremes in acidity and aluminium-toxicity
398 have lagged effects on population dynamics, allowing for water-quality-based reconstruction
399 of population growth since the beginning of lake recovery two decades ago. Our study
400 revealed an important life-history parameter for reliable estimates of stress responsiveness of
401 *Isoëtes echinospora* lagged by age at its early ontogeny. *Isoëtes echinospora* is an iconic
402 macrophyte of oligotrophic softwater lakes that have been suffering from environmental
403 degradation. This study brings compelling evidence that the quillwort possesses an indicatory
404 capacity for determining the status of recovering lake ecosystems.

405

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411 temperature and light conditions in Plešné Lake.

412

413 **References**

414 Arts, G.H.P., 2002. Deterioration of Atlantic soft water macrophyte communities by
415 acidification, eutrophication, and alkalisation. *Aquat. Bot.* 73, 373–393.

416 Brandrud, T.E., Johansen, S.W., 1994. Effects of acidification on macrophyte growth in the
417 humex lake Skjervatjern, with special emphasis on *Sphagnum auriculatum*. *Environ. Int.* 20,
418 329–342.

419 Brouwer, E., Bobbink, R., Roelofs, J.G.M., 2002. Restoration of aquatic macrophyte
420 vegetation in acidified and eutrophied softwater lakes: an overview. *Aquat. Bot.* 73, 405–431.

421 Brunton, D.F., Britton, D.M., 1999. Maritime quillwort, *Isoetes maritime* (*Isoëtaceae*), in the
422 Yukon Territory. *Can. Field-Naturalist.* 113 (4), 641–645.

423 Cole, T.M., Wells, S.A., 2015. CE-QUAL-W2: A Two-Dimensional, Laterally Averaged,
424 Hydrodynamic and Water Quality Model, Version 3.72. User Manual. Department of Civil
425 and Environmental Engineering, Portland State University, USA, Portland.

426 Čtvrtlíková, M., Znachor, P., Vrba, J., 2014. The effect of temperature on the phenology of
427 germination of *Isoetes lacustris*. *Preslia.* 86, 279–292.

428 Čtvrtlíková, M., Znachor, P., Nedoma, J., Vrba, J., 2012. Effects of temperature on the
429 phenology of germination of *Isoetes echinospora*. *Preslia.* 84, 141–153.

430 Čtvrtlíková, M., Vrba, J., Znachor, P., Hekera, P., 2009. The effects of aluminium toxicity
431 and low pH on the early development of *Isoetes echinospora*. *Preslia.* 81, 135–149.

432 Dougan, W.K., & Wilson, A.L., 1974. The absorptiometric determination of aluminium in
433 water. A comparison of some chromogenic reagents and the development of an improved
434 method. *Analyst.* 99, 413–430.

435 Driscoll, C.T., 1984. A procedure for the fractionation of aqueous aluminum in dilute acidic
436 waters. *Int. J. Environ. Analytical Chem.* 16, 267–284.

- 437 Eames, A.J., 1936. Morphology of vascular plants. Lower groups (*Psilophytales to Filicales*),
438 McGraw-Hill Book Company, New York.
- 439 Foster, S., Gifford, E.M., 1959. Comparative Morphology of Vascular Plants, W. H. Freeman
440 and Company, San Francisco.
- 441 Garmo, O.A., Skjelkvale, B.L., de Wit, H.A., Colombo, L., Curtis, C., Folster, J., Hoffmann,
442 A., Hruška, J., Høgåsen, T., Jeffries, D.S., Keller, W.B., Krám, P., Majer, V., Monteith, D.T.,
443 Paterson, A.M., Rogora, M., Rzychon, D., Steingruber, S., Stoddard, J.L., Vuorenmaa, J. &
444 Worsztynowicz, A., 2014. Trends in surface water chemistry in acidified areas in Europe and
445 North America from 1990 to 2008. *Water, Air, & Soil Pollut.* 225: 1880.
- 446 Graham, M.D., Vinebrooke, R.D., Keller, B., Heneberry, J., Nicholls, K.H., Findlay, D.L.,
447 2007. Comparative responses of phytoplankton during chemical recovery in atmospherically
448 and experimentally acidified lakes. *J. Phycol.* 43, 908–923.
- 449 Gray, D.K., Arnott, S.E., 2009. Recovery of acid damaged zooplankton communities:
450 measurement, extent, and limiting factors. *Environ. Rev.* 17, 81–99.
- 451 Herlihy, A.T., Mills, A.L., 1986. The pH regime of sediments underlying acidified waters.
452 *Biogeochem.* 2, 95–99.
- 453 Husák, Š., Vöge, M., Weilner, C., 2000. *Isoëtes echinospora* and *I. lacustris* in the Bohemian
454 Forest lakes in comparison with other European sites. *Silva Gabreta.* 4, 245–252.
- 455 Hutchinson, G.E., 1975. A Treatise on Limnology: Limnological Botany, Wiley, New York.
- 456 Jankovská, V., 2006. Late Glacial and Holocene history of Plešné Lake and its surrounding
457 landscape based on pollen and palaeoalgalogical analyses. *Biologia.* 61, 371–385.
- 458 Kaňa, J., Tahovská, K., Kopáček, J., 2013. Response of soil chemistry to forest dieback after
459 bark beetle infestation. *Biogeochem.* 113 (1–3), 369–383.
- 460 Kopáček, J., Hejzlar, J., 1993. Semi-micro determination of total phosphorus in fresh waters
461 with perchloric acid digestion. *Int. J. Environ. Analytical Chem.* 53, 173–183.
- 462 Kopáček, J., Hejzlar, J., Kaňa, J., Norton, S.A., Porcal, P., Turek, J., 2009. Trends in
463 aluminium export from a mountainous area to surface waters, from deglaciation to the recent:

464 Effects of vegetation and soil development, atmospheric acidification, and nitrogen-
465 saturation. *J. Inorg. Biochem.* 103, 1439–1448.

466 Kopáček, J., Marešová, M., Hejzlar, J., Norton, S.A., 2007. Natural inactivation of
467 phosphorus by aluminium in preindustrial lake sediments. *Limnology and Oceanogr.* 52 (3),
468 1147–1155.

469 Kopáček, J., Ulrich, K.U., Hejzlar, J., Borovec, J., Stuchlík, E., 2001. Natural inactivation of
470 phosphorus by aluminium in atmospherically acidified water bodies. *Water Res.* 35, 3783–
471 3790.

472 Lindgren, C.J., Walker, D., 2012. Growth Rate, Seed Production, and Assessing the Spatial
473 Risk of *Lythrum salicaria* using Growing Degree-Days. *Wetlands.* 32, 885–893.

474 Madsen, T.V., Olesen, B., Bagger, J., 2002. Carbon acquisition and carbon dynamics by
475 aquatic isoetids. *Aquat. Bot.* 73, 351–371.

476 Majer, V., Cosby, B.J., Kopáček, J., Veselý, J., 2003. Modelling reversibility of Central
477 European mountain lakes from acidification: Part I – the Bohemian Forest. *Hydrol. and Earth
478 System sciences.* 7, 494–509.

479 McMaster, G.S., Wilhelm, W.W., 1997. Growing degree-days: one equation, two
480 interpretations. *Agric. and Forest Meteorol.* 87(4), 291–300.

481 Murphy, K.J., 2002. Plant communities and plant diversity in softwater lakes of northern
482 Europe. *Aquat. Bot.* 73, 287–324.

483 Oulehle, F., Chuman, T., Majer, V., Hruška, J., 2013. Chemical recovery of acidified
484 Bohemian lakes between 1984 and 2012: the role of acid deposition and bark beetle induced
485 forest disturbance. *Biogeochem.* 116 (1–3), 83–101.

486 Rørslett, B., Brettum, P., 1989. The genus *Isoëtes* in Scandinavia: an ecological review and
487 perspectives. *Aquat. Bot.* 35, 223–261.

488 Smolders, A.J.P., Lucassen, E.C.H.E.T., Roelofs, J.G.M., 2002. The isoetid environment:
489 biochemistry and threats. *Aquat. Bot.* 73, 325–350.

490 Stoddard, J.L., Jeffries, D.S., Lükewille, A., Clair, T.A., Dillon, P.J., Driscoll, C.T., Forsius,
491 M., Johannessen, M., Kahl, J.S., Kellogg, J.H., Kemp, A., Mannio, J., Monteith, D., Murdoch,

492 P.S., Patrick, S., Rebsdorf, A., Skjelkvåle, B.L., Stainton, M.P., Traaen, T., van Dam, H.,
 493 Webster, K.E., Wieting J., Wilander, A., 1999. Regional trends in aquatic recovery from
 494 acidification in North America and Europe. *Nat.* 401, 575–578.

495 **Table 1** Pearson correlation coefficients (r) between population growth (Y) of *Isoëtes*
 496 *echinospora* in Plešné Lake and the lake water characteristics (X) lagged at spring (S),
 497 growing (G) or winter (W) periods up to two years backward (t , $t-1$, $t-2$); for the period
 498 settings and the respective phenophases see methods and Fig. 2. The growth (Y) is defined as
 499 a year-to-year change in number of adult or juvenile plants observed in July. The lake water
 500 characteristics are pH, nitrate (NO₃-N), total phosphorus (TP), calcium (Ca), potassium (K),
 501 magnesium (Mg), total (Al_t) and ionic (Al_i) aluminium, mean temperature (T_m), cumulative
 502 temperature (T_c), temperature variation during a period (T_v), and cumulative days (D_c). The
 503 significant ($p < 0.05$) correlations are highlighted in bold.

504

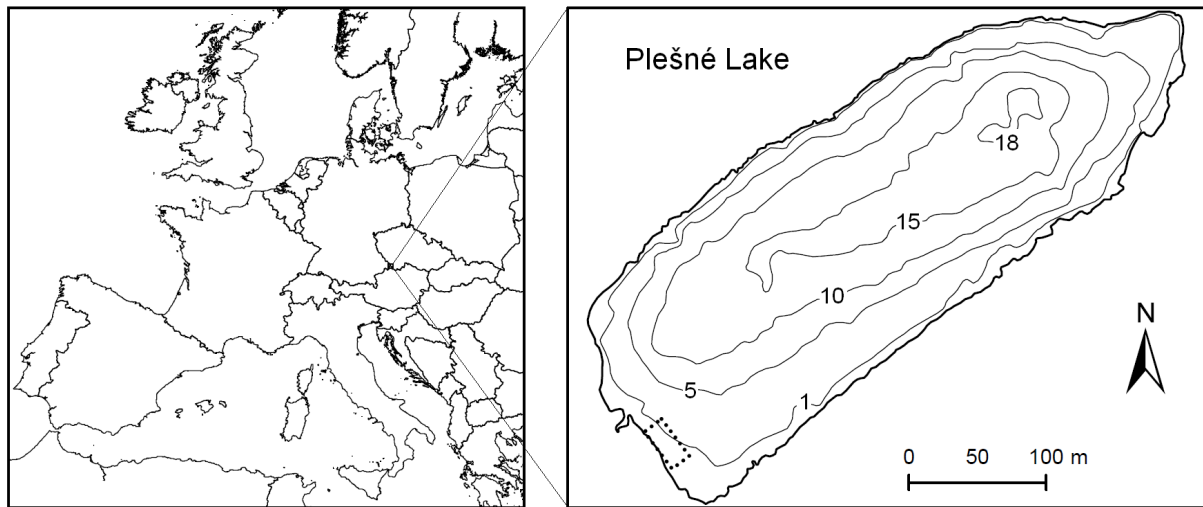
Y	X	Pearson r between Y and lagged X					
		G _{t-2}	W _{t-1}	S _{t-1}	G _{t-1}	W _t	S _t
Adults	pH	0.3	0.7	0.7	0.7	0.3	0.3
	NO ₃ -N	0.3	0.2	0.3	0.2	0.2	0.0
	TP	0.1	0.5	0.7	0.6	0.5	0.7
	Ca	0.4	0.5	0.6	0.4	0.2	0.1
	K	0.5	0.4	0.3	0.3	0.3	0.3
	Mg	0.4	0.5	0.4	0.4	0.2	0.1
	Al _t	-0.2	-0.4	0.2	0.2	0.0	0.2
	Al _i	-0.4	-0.7	-0.2	-0.2	-0.3	-0.1
	T _m	-0.2	-0.3	-0.2	-0.3	-0.2	0.3
	T _c	-0.2	-0.4	-0.4	0.8	-0.1	0.8
	T _v	-0.3	-0.3	-0.3	0.1	0.1	0.9
	D _c	0.0	-0.6	-0.5	0.7	0.2	0.8
Juveniles					G _{t-1}	W _t	S _t
	pH				0.1	0.2	0.0
	NO ₃ -N				0.1	0.0	0.1
	TP				-0.3	-0.1	0.1
	Ca				0.2	0.2	0.3
	K				0.1	0.1	0.1
	Mg				0.2	0.2	0.0
	Al _t				-0.3	-0.3	0.1
	Al _i				-0.2	-0.3	0.0
	T _m				-0.4	-0.5	0.2
	T _c				0.2	-0.6	-0.3
	T _v				-0.3	-0.7	-0.2
D _c				0.3	-0.6	-0.5	

505

506 **Table 2** Results of the factor analysis (FA) and partial least squares multiple regression
507 (PLSR). Predictor (X) variables lagged at age of the first winter (W_{t-1} ; for the period settings
508 see methods and Fig. 2.) included pH, nitrate ($\text{NO}_3\text{-N}$), total phosphorus (TP), calcium (Ca),
509 potassium (K), magnesium (Mg), ionic aluminium (Al_i), and cumulative temperature (T_c).
510 Response (Y) variable analysed in PLSR refers to the adult population growth. FA results: the
511 factor (F1–3) loadings rotated in the manner of Varimax rotation; the variability (R^2 of X)
512 explained by each factor; the communalities from three factors (Commun.). PLSR results: the
513 weights (w) of three components (COMP 1–3) extracted; the explained variability of response
514 (R^2 of Y) and predictor (R^2 of X) variables; the regression coefficients for the multiple linear
515 regression models based on one (1C), two (2C) and three (3C) components. The significant (p
516 < 0.05) correlations are highlighted in bold.

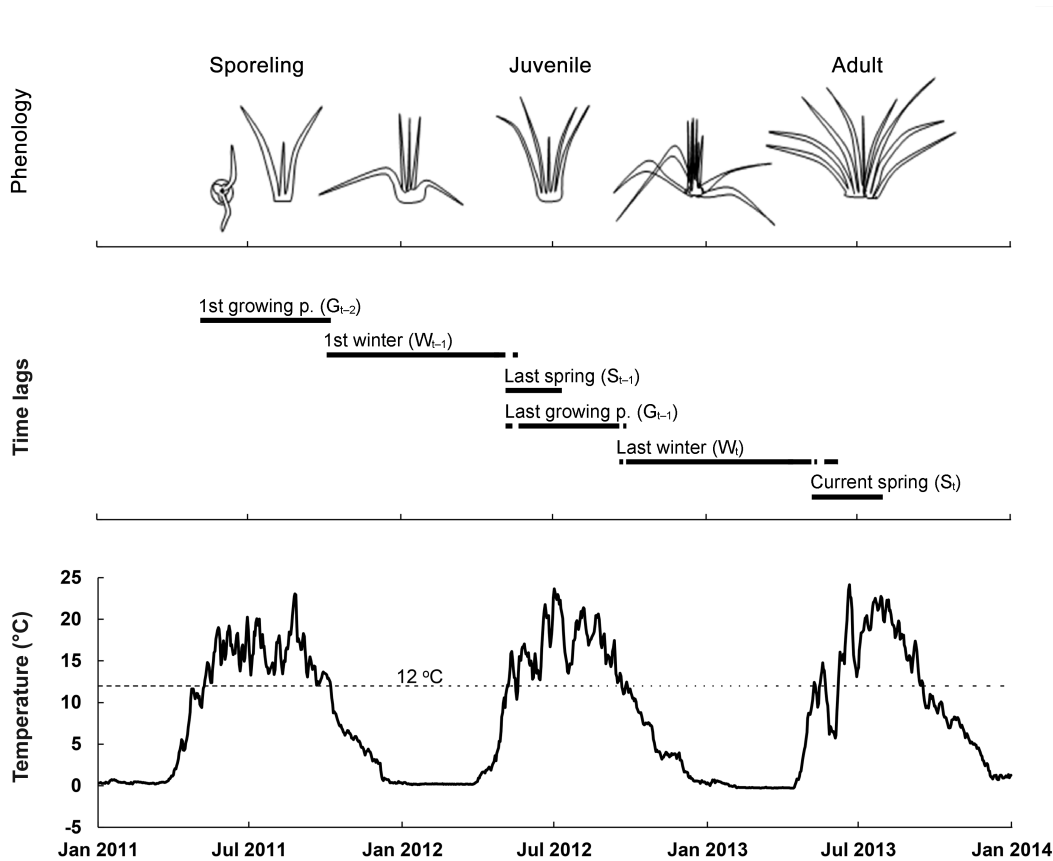
FA					PLSR						
X	Factor loadings			Commun.	X	w			Regression coefficients		
	F1	F2	F3			COMP1	COMP2	COMP3	1C	2C	3C
pH	0.46	0.86	-0.06	0.96	pH	0.46	0.23	-0.60	3583	4711	-7352
$\text{NO}_3\text{-N}$	0.96	-0.16	0.18	0.99	$\text{NO}_3\text{-N}$	0.17	-0.33	-0.04	315	-65.2	-262
TP	0.97	0.07	0.01	0.95	TP	0.34	-0.09	0.22	182	152	455
Ca	0.98	0.18	0.08	0.99	Ca	0.34	-0.11	0.00	1492	1182	1143
K	0.99	0.09	0.07	0.99	K	0.31	-0.16	-0.03	800	5402	339
Mg	0.98	0.11	0.00	0.98	Mg	0.36	-0.06	0.26	5624	5066	15576
Al_i	0.22	-0.86	0.41	0.95	Al_i	-0.47	-0.78	-0.65	-4.66	-9.51	-26.5
T_c	0.08	-0.23	0.97	0.99	T_c	-0.29	-0.43	0.31	-1.79	-3.46	1.57
R^2 of X	0.63	0.83	0.98		R^2 of X	0.54	0.90	0.92	Intercepts		
					R^2 of Y	0.58	0.72	0.85	-18559	-19877	35565

518 **Figures**
519



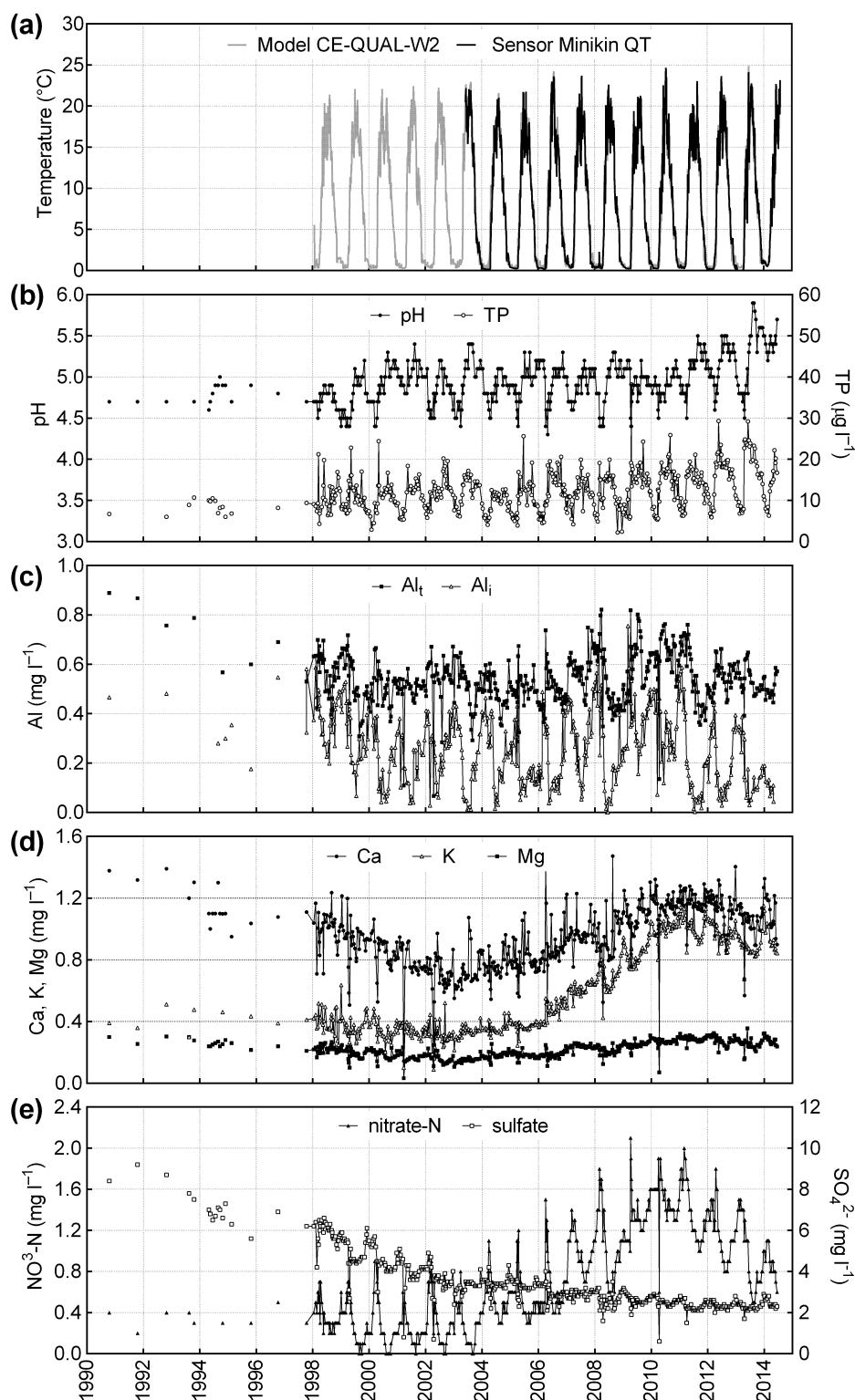
520

521 **Fig. 1** Situation map of Plešné Lake and study plots (dotted rectangle) of *Isoëtes echinospora*
522 population along the south-west shore.



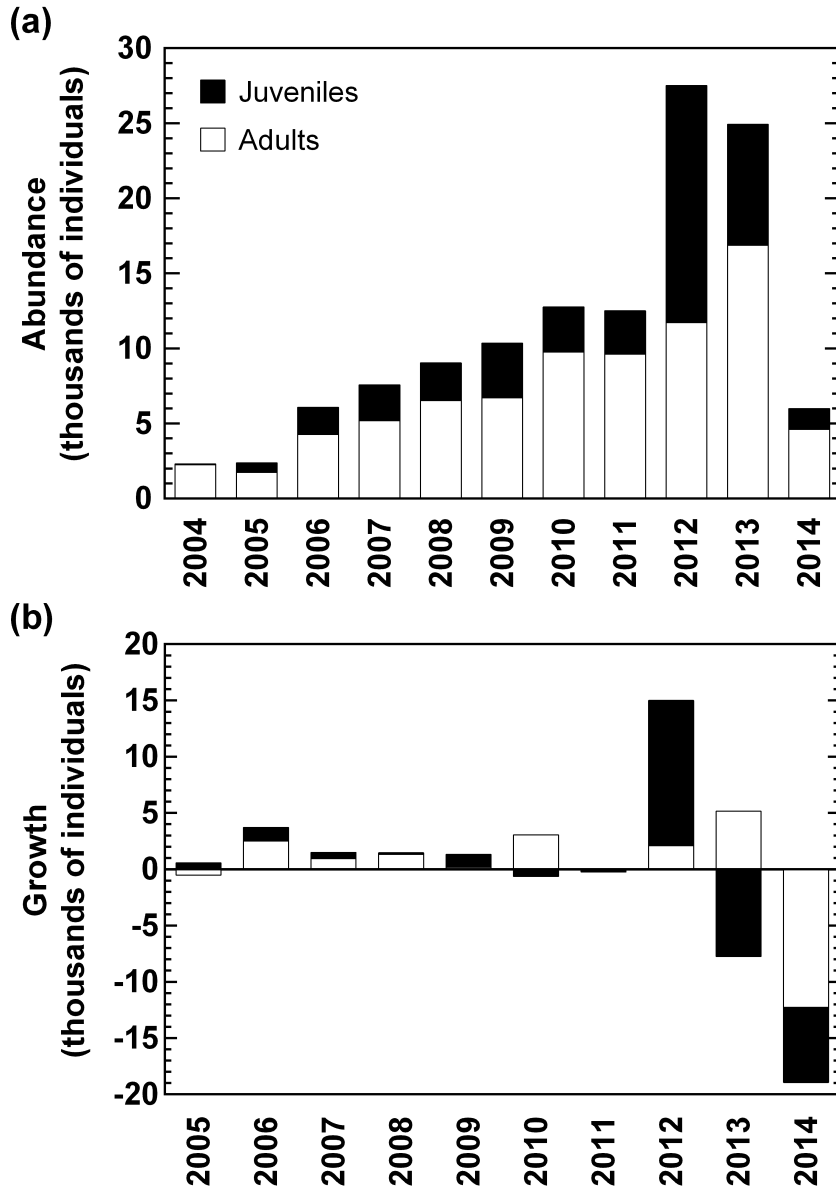
523 **Fig.**
 524 **2** Illustration of *Isoëtes echinospora* phenology related to time lags of lake water effects. The
 525 time lags are based on lake water temperature in Plešné Lake and the species specific
 526 temperature threshold (12°C) for germination. The periods below and above the threshold are
 527 winter (W) and growing (G) periods, respectively. The spring (S) period includes both the
 528 next 60 days when daily mean temperature rises above the threshold and all intermittent
 529 colder days if present. Lake water conditions are lagged by up to two years (t , $t-1$, $t-2$) of
 530 plant early ontogeny.

531



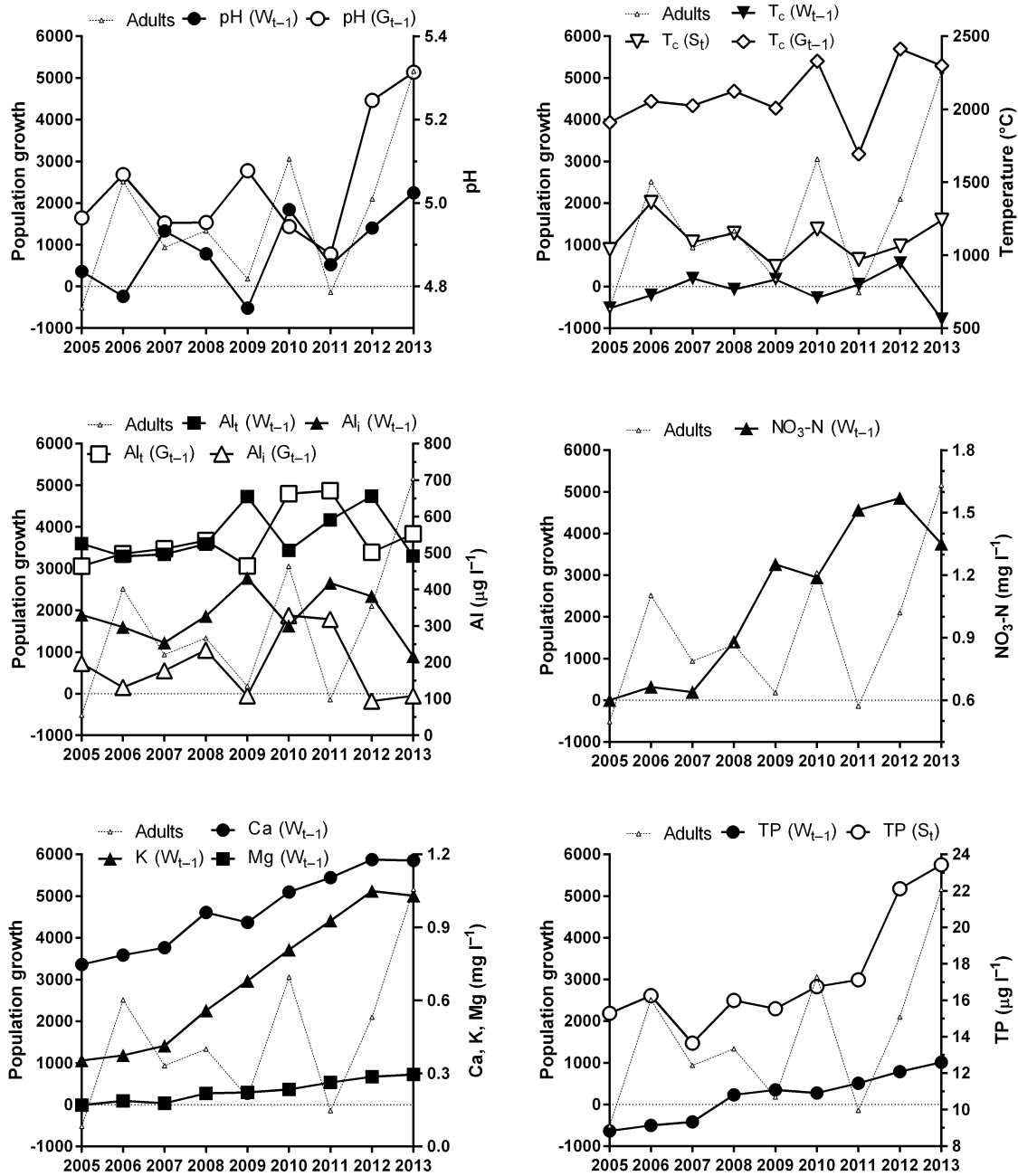
532

533 **Fig. 3** Trends in daily mean water temperature and water chemistry in Plešné Lake during
 534 1990–2014: **a)** lake water temperature recorded at the quillwort plant level with the Minikin
 535 QT sensor in 2003–2014 and reconstructed with model CE-QUAL-W2 in 1998–2003
 536 (calibration period 2004–2013), **b)** pH and concentrations of total phosphorus (TP), **c)**
 537 total (Al_t) and ionic (Al_i) aluminium, **d)** calcium (Ca), potassium (K) and magnesium (Mg) cations,
 538 **e)** sulphate (SO_4^{2-}) and nitrate (NO_3-N).



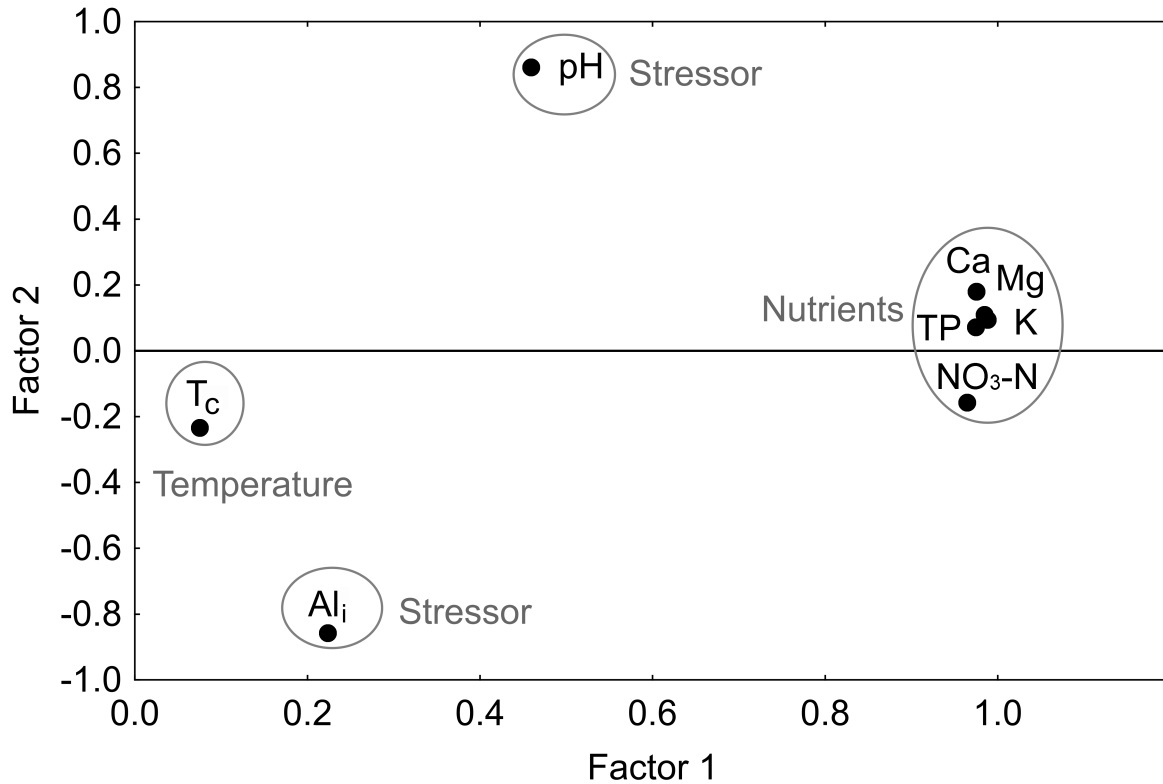
539

540 **Fig. 4** Plant abundance (a) and population growth (b) of *Isoetes echinospora* in Plešné Lake
 541 between 2004 and 2014. Growth of the adult or juvenile population is defined as a year-to-
 542 year change in an appropriate plant abundance observed in July.



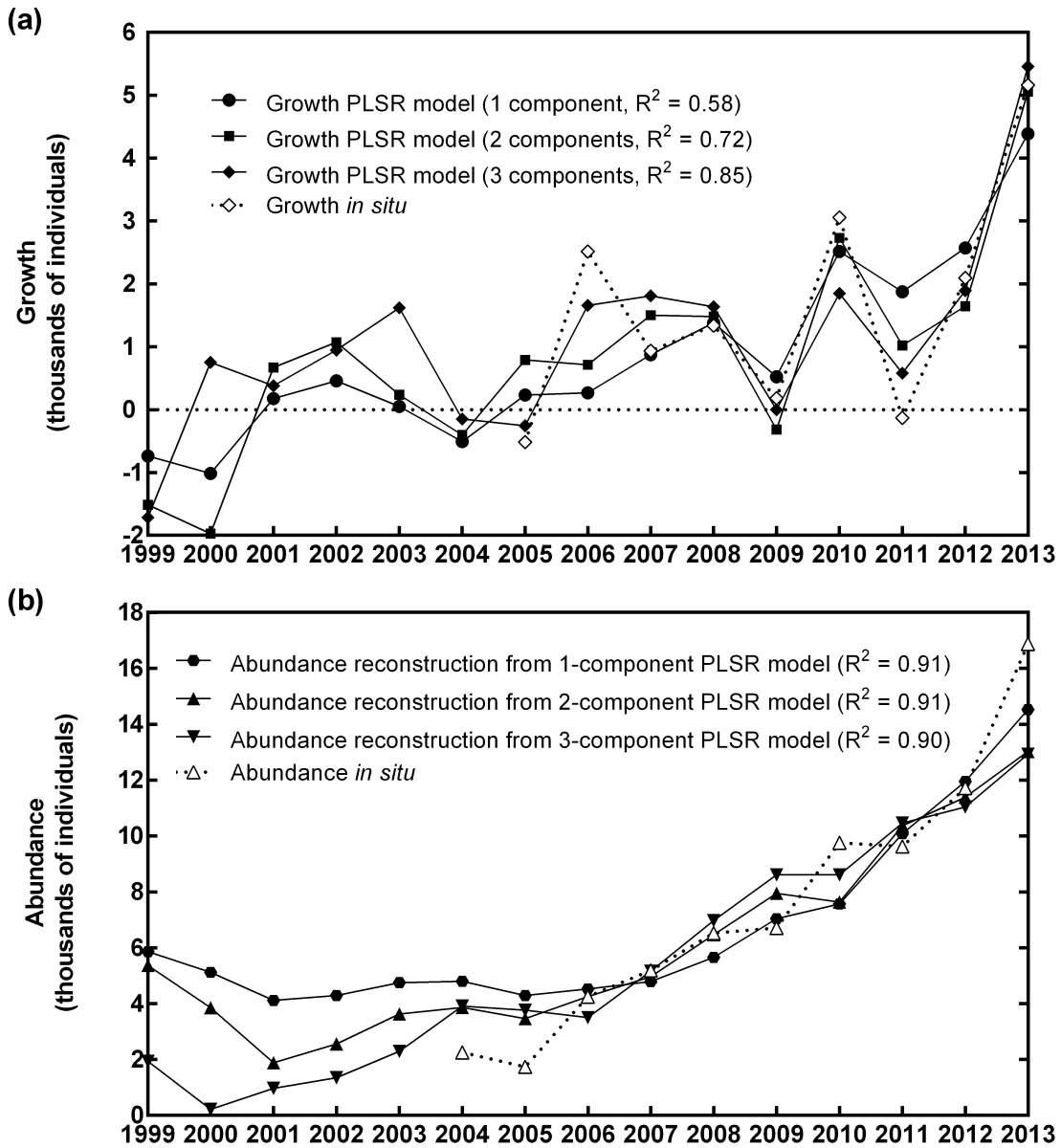
543

544 **Fig. 5** Population growth of *Isoetes echinospora* in Plešné Lake plotted with lake water
 545 characteristics that are lagged at distinct periods. The growth is defined as a year-to-year
 546 change in number of adult plants observed in July. Mean values of pH, nitrate (NO₃-N), total
 547 phosphorus (TP), calcium (Ca), potassium (K), magnesium (Mg), total (Al_t) and ionic (Al_i)
 548 aluminium, and cumulative temperature (T_c) in spring (S), growing (G), or winter (W)
 549 periods were lagged by up to one year (t, t-1). For the period settings and the respective phenophases
 550 see methods and Fig. 2.



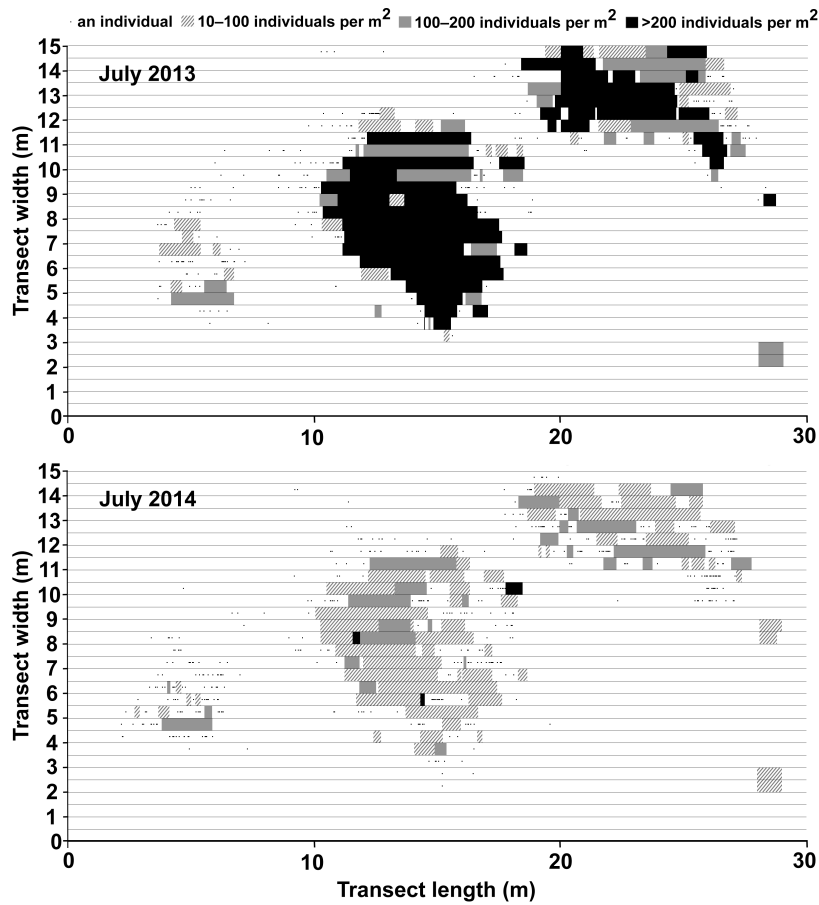
551

552 **Fig. 6** Results of factor analysis – 2D scatterplot of factor loadings rotated in the manner of
 553 Varimax rotation. Mean values of pH, nitrate (NO₃-N), total phosphorus (TP), calcium (Ca),
 554 potassium (K), magnesium (Mg), ionic aluminium (Al_i), and cumulative temperature (T_c) of
 555 Plešné Lake water lagged at age of the first wintering (W_{t-1}) were analysed. The three factors
 556 extracted were interpreted as nutrients (Factor 1), stressors (Factor 2) and temperature (Factor
 557 3; axis not shown). The summary of factor loadings and communalities is shown in Table 2.



558

559 **Fig. 7** Population growth (a) and abundance (b) of *Isoetes echinospora* in Plešné Lake
 560 observed between 2005 and 2013 and reconstructed by the Partial least squares regression
 561 (PLSR) model from 1999 to 2013. Annual growth is year-to-year change in number of adult
 562 plants (abundance) observed in July. The weights of the original predictor variables and the
 563 total explanatory capacity of extracted components see in Table 2.



564

565 **Fig. 8** Abundance of the adult population of *Isoëtes echinospora* in Plešné Lake before and
 566 after its grazing in October 2013. Underwater visual censuses of adult plants were performed
 567 in transitory strip transects (0.5 × 10 m) at 3 adjacent study plots (10 × 15 m) covering whole
 568 lake population in July 2013 and 2014. Transect length refer to cumulative length of three
 569 adjacent transects within the study plots.