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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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

1. Introduction

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

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

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

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

2. Material and methods

2.1.   Study site

The population of *I. echinospora* Durieu has inhabited Plešné Lake situated in the Bohemian Forest, the Czech Republic (48°47′N,13°52′E; 1,087 m a.s.l.; Fig. 1) since the end of the last Glacial (~ 10 kyr BP; Jankovská 2006). The lake is of glacial origin, dimictic, mesotrophic (area of 7.5 ha, volume of 617,000 m^3, maximum depth of 18 m), with small catchment area

(66.6 ha) on granitic bedrock forested by Norway spruce (*Picea abies*) (Kopáček *et al.* 2007). The lake has been acidified by atmospheric deposition of sulphur (S) and nitrogen (N) compounds since the late 1960s. Acidification progressed until the middle 1980s, when pH ranged between 4.4 and 4.7, carbonate buffering system was entirely depleted, and total aluminium (Al_t) and ionic aluminium (Al_i) concentrations reached 1.1 and 0.8 mg l^{-1}, respectively (Kopáček *et al.* 2009). Since the 1990s, the lake chemistry has been recovering, with a temporary renewal of carbonate buffering capacity and increase of mean pH values to approximately 5 during summer stratification in the early 2000s. A partial lake water re-acidification has occurred since 2004 due to forest dieback in the Plešné catchment due to bark-beetle infestation (Kaňa, Tahovská & Kopáček 2013; Oulehle *et al.* 2013).

Isoëtes echinospora forms a monospecific plant stand in the inshore area of approximately 0.03 ha, at depths of 0.3–0.5 m (max. 1.0 m; Čtvrtlíková *et al.* 2009). The lake shore is grown by Bottle Sedge (*Carex rostrata*); its dense stand forms a dynamic inshore border of the quillwort population. The sediment at the quillwort stand is an aqueous sapropel with a high proportion of organic matter. The lake is usually ice-covered from December to April.

2.2. Population dynamics

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

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

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

change of plant abundance) were evaluated from the census data to describe population dynamics. The abundance of adult plants before and after the plant stand disturbance in autumn 2013 was assessed in particular transect sections (not quadrates) grown by distinct plant assemblages and it was expressed per square meter and classified within intervals: 1; 10–100; 100–200, and > 200 individuals per 1 m^2.

2.3. Time lags

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

2.4. Environmental conditions

Lake water temperature (°C) and photosynthetically active radiation (PAR, μmol m^2 s$^{–1}$) in the quillwort plant stand was recorded with the Minikin QT smart sensor (Environmental Measuring Systems Company, Brno, Czech Republic) in 30-minute intervals from June 2003 to July 2014 (parallel to the plant observation period). Temperature and PAR effects on the quillwort growth were analysed from these measured data. Lake water temperature data for hindcast modelling of quillworts growth in 1997–2003 were reconstructed by a mathematical temperature lake model (CE-QUAL-W2; Cole & Wells 2015), calibrated on the 2004–2013 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, NO_3-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 NO_3-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 $$Y = b_0 + \sum_1^8 b_i X_i \qquad\qquad [1]$$

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 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 924–1361°C (average of 1086 $\pm$

272 111°C) and 1693–2474°C (2121 $\pm$ 220°C), respectively, during the past 15 years. The spring

273 and growing periods began in May and lasted 60–80 days (67 $\pm$ 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 NO_3-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.

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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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 (NO_3-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**
					G_{t-1}	W_t	S_t
Juveniles	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 (NO_3-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.
517

|	FA				PLSR						
	Factor loadings			Commun.		w	w	w	Regression coefficients		
X	F1	F2	F3		X	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
NO_3–N	**0.96**	–0.16	0.18	0.99	NO_3–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

Figures
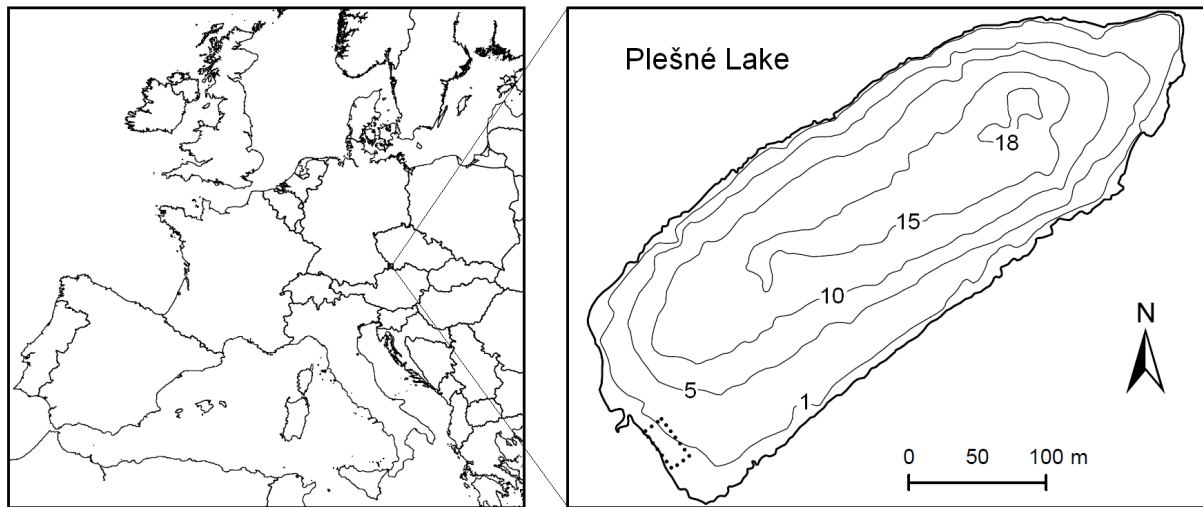
521   **Fig. 1** Situation map of Plešné Lake and study plots (dotted rectangle) of *Isoëtes echinospora*
522   population along the south-west shore.
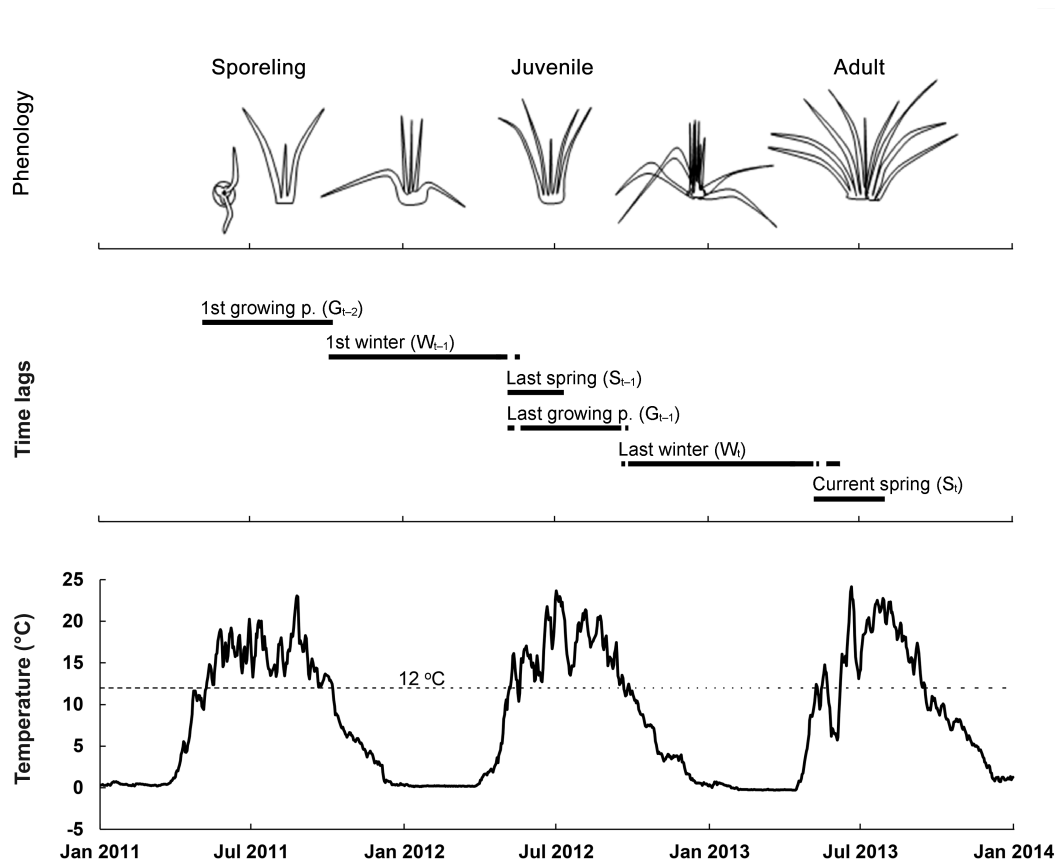
Fig.
2 Illustration of *Isoëtes echinospora* phenology related to time lags of lake water effects. The time lags are based on lake water temperature in Plešné Lake and the species specific temperature threshold (12°C) for germination. The periods below and above the threshold are winter (W) and growing (G) periods, respectively. The spring (S) period includes both the next 60 days when daily mean temperature rises above the threshold and all intermittent colder days if present. Lake water conditions are lagged by up to two years (t, t−1, t−2) of plant early ontogeny.

531

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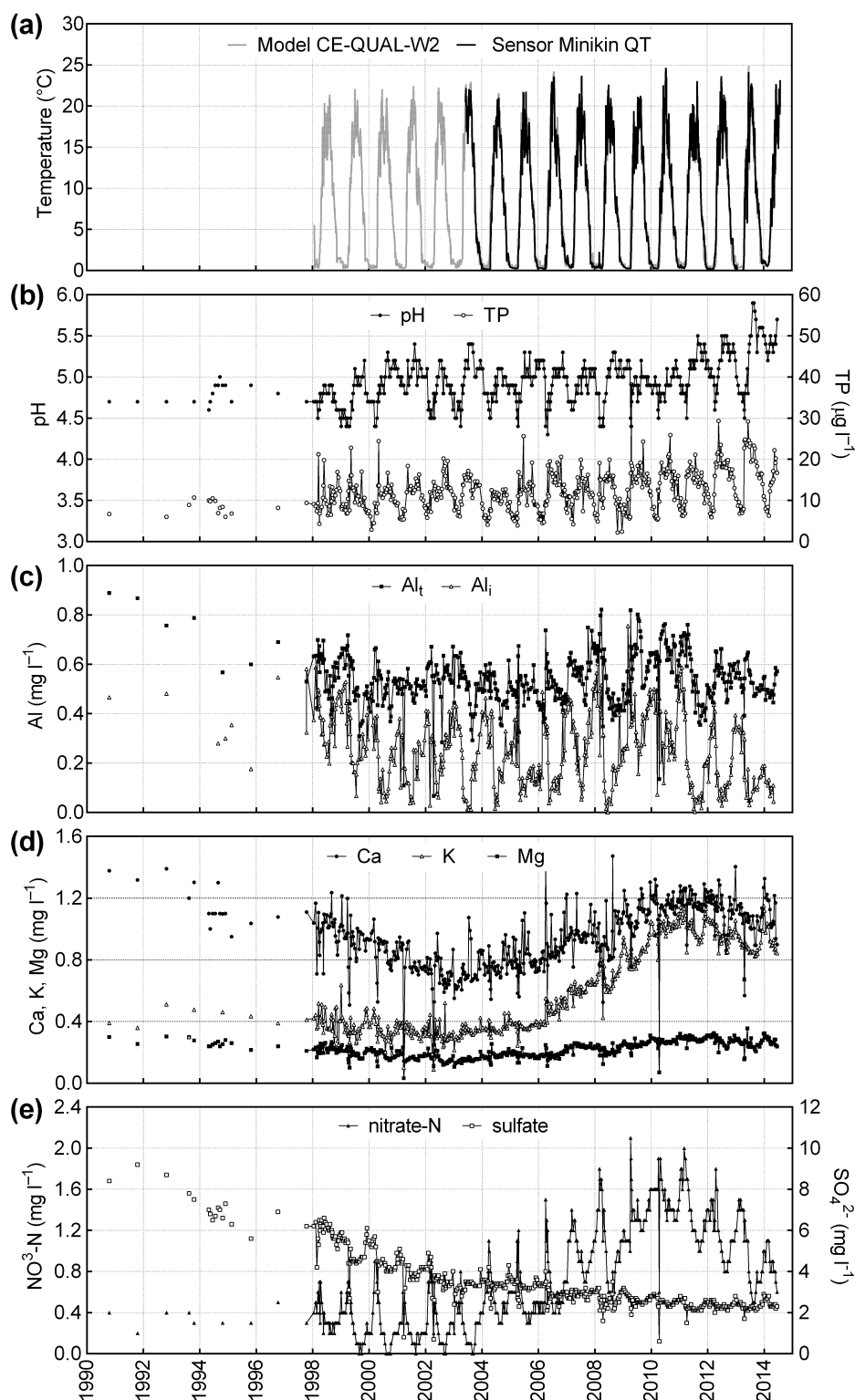
Fig. 3 Trends in daily mean water temperature and water chemistry in Plešné Lake during 1990–2014: **a)** lake water temperature recorded at the quillwort plant level with the Minikin QT sensor in 2003–2014 and reconstructed with model CE-QUAL-W2 in 1998–2003 (calibration period 2004–2013), **b)** pH and concentrations of total phosphorus (TP), **c)** total (Al_t) and ionic (Al_i) aluminium, **d)** calcium (Ca), potassium (K) and magnesium (Mg) cations, **e)** sulphate (SO_4^{2-}) and nitrate (NO_3-N).
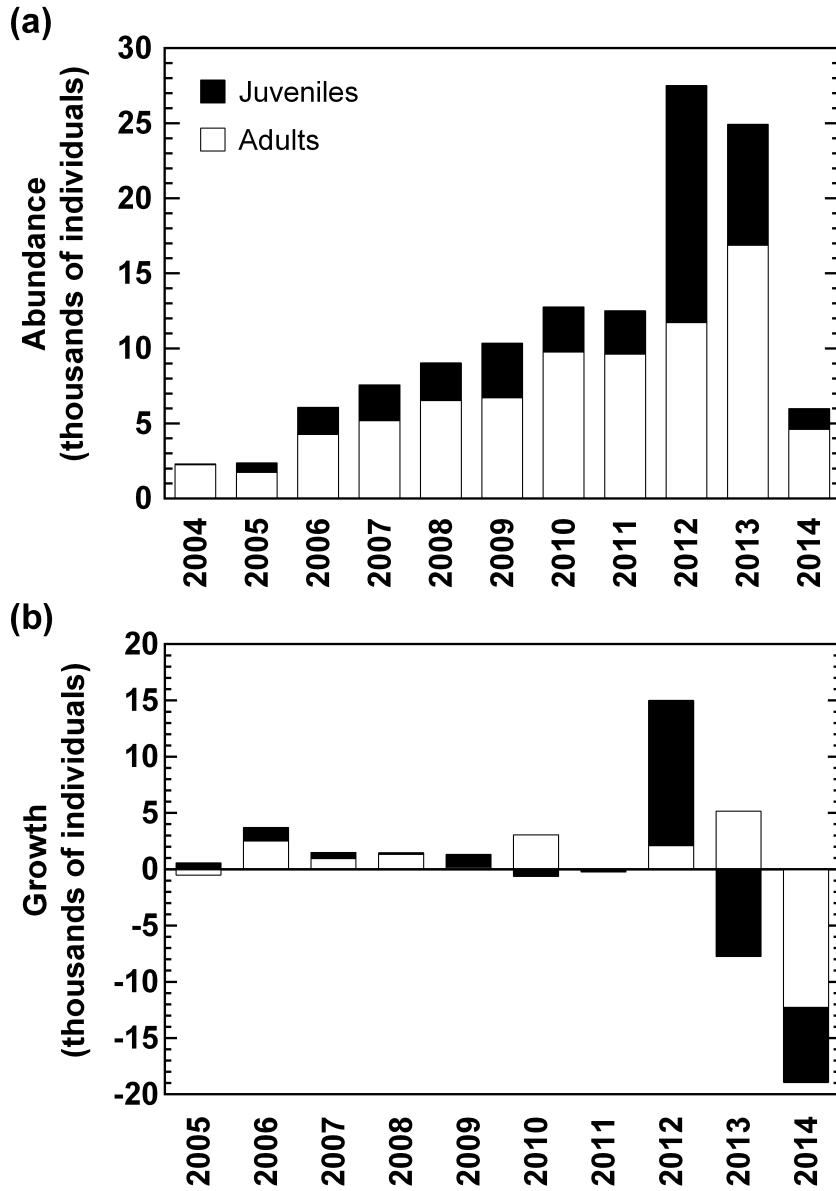
Fig. 4 Plant abundance (**a**) and population growth (**b**) of *Isoëtes echinospora* in Plešné Lake between 2004 and 2014. Growth of the adult or juvenile population is defined as a year-to-year change in an appropriate plant abundance observed in July.
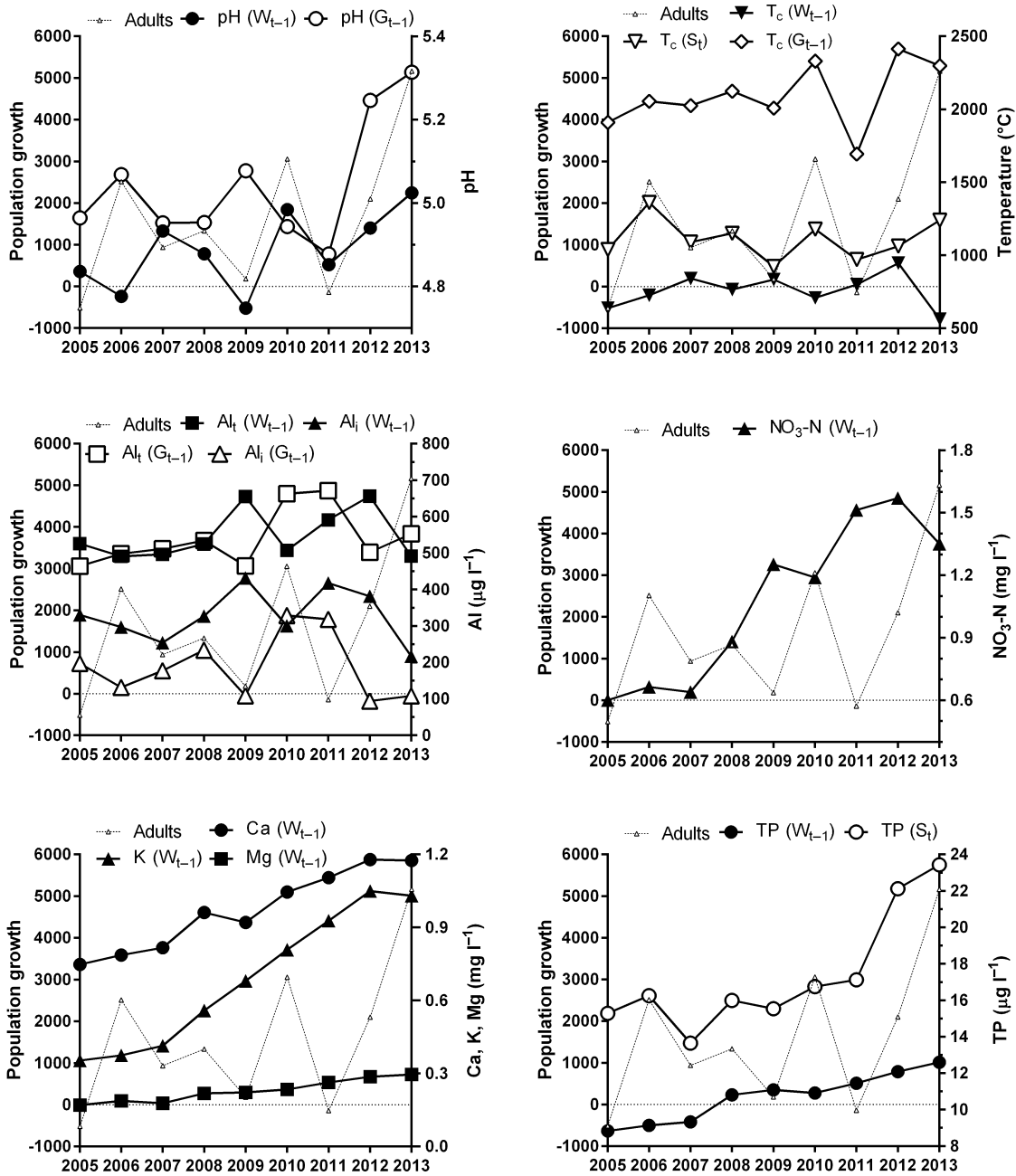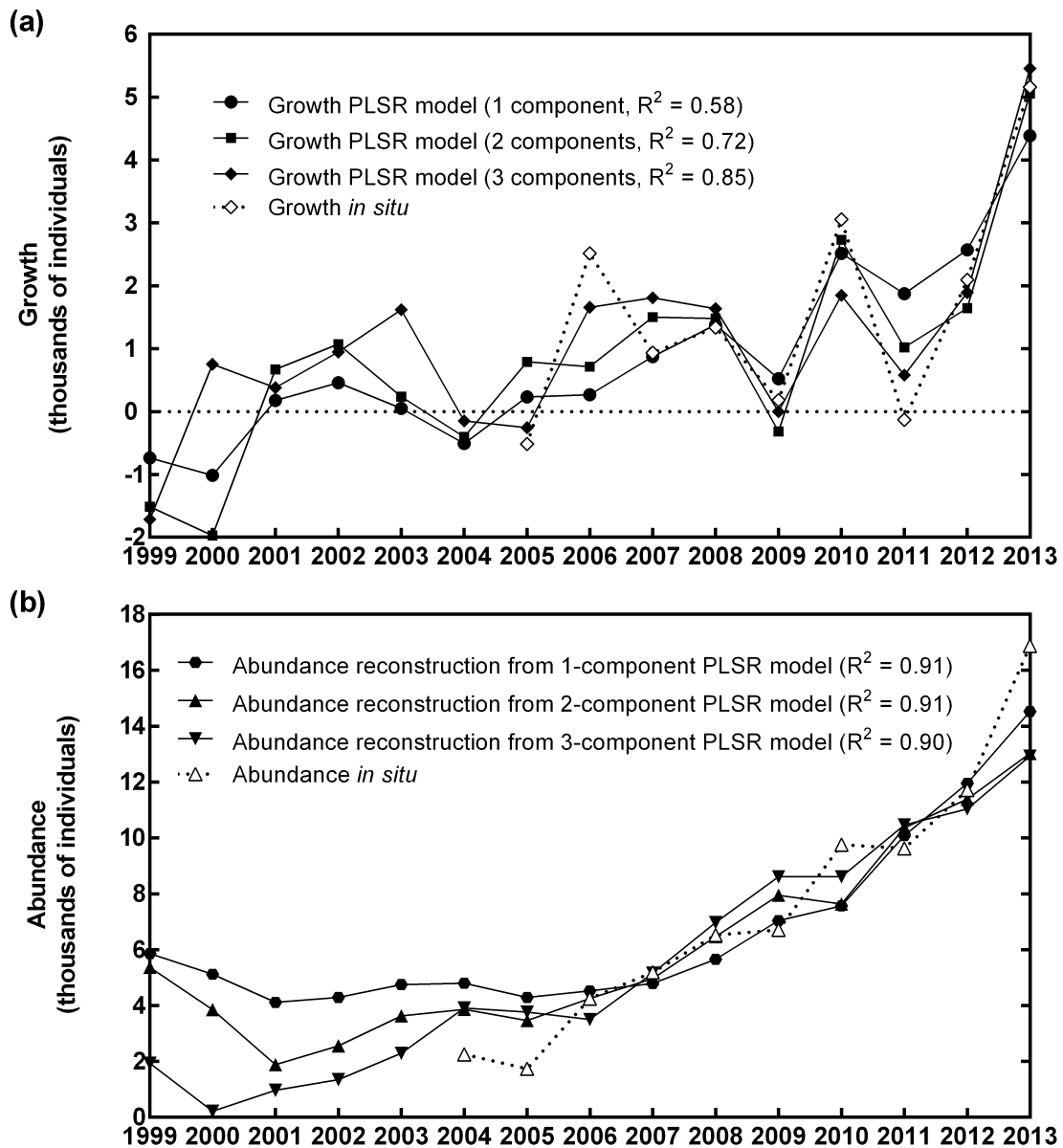
Fig. 5 Population growth of *Isoëtes echinospora* in Plešné Lake plotted with lake water characteristics that are lagged at distinct periods. The growth is defined as a year-to-year change in number of adult plants observed in July. Mean values of pH, nitrate (NO_3-N), total phosphorus (TP), calcium (Ca), potassium (K), magnesium (Mg), total (Al_t) and ionic (Al_i) aluminium, and cumulative temperature (T_c) in spring (S), growing (G), or winter (W) periods were lagged by up to one year (t, t−1). For the period settings and the respective phenophases see methods and Fig. 2.

23
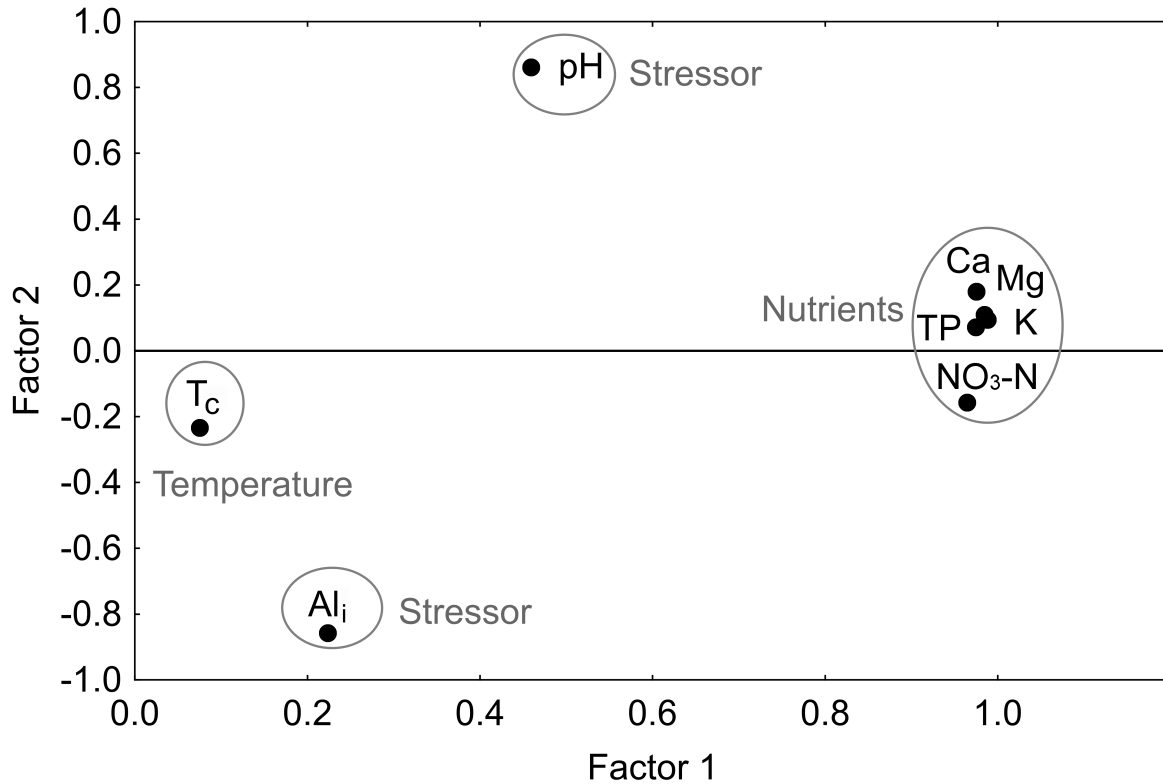
Fig. 6 Results of factor analysis – 2D scatterplot of factor loadings rotated in the manner of Varimax rotation. Mean values of pH, nitrate (NO_3-N), total phosphorus (TP), calcium (Ca), potassium (K), magnesium (Mg), ionic aluminium (Al_i), and cumulative temperature (T_c) of Plešné Lake water lagged at age of the first wintering (W_{t-1}) were analysed. The three factors extracted were intrepreted as nutrients (Factor 1), stressors (Factor 2) and temperature (Factor 3; axis not shown). The summary of factor loadings and communalities is shown in Table 2.

(a)

(b)

558

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

564
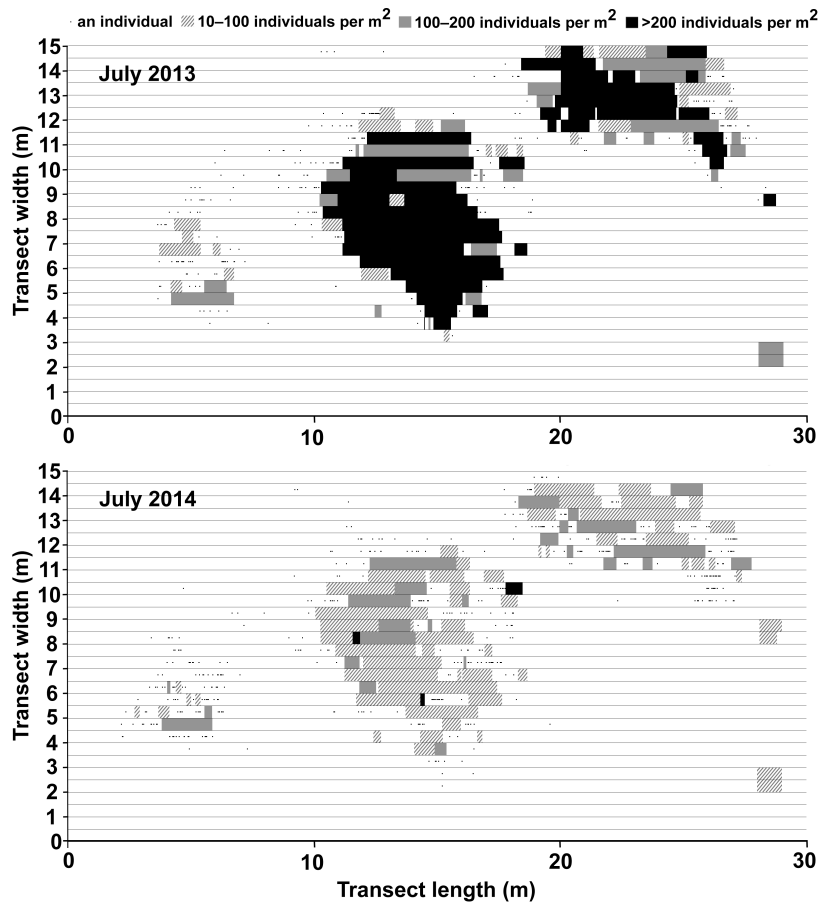
Fig. 8 Abundance of the adult population of *Isoëtes echinospora* in Plešné Lake before and after its grazing in October 2013. Underwater visual censuses of adult plants were performed in transitory strip transects (0.5 × 10 m) at 3 adjacent study plots (10 × 15 m) covering whole lake population in July 2013 and 2014. Transect length refer to cumulative length of three adjacent transects within the study plots.