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Adaptation and growth performance of four endangered amphibious freshwater species

Oliver Mørk*, Theis Kragh, Emil Kristensen, Kaj Sand-Jensen

Freshwater Biological Laboratory, University of Copenhagen, 3. floor, DK-2100, Copenhagen, Denmark

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ABSTRACT

Small amphibious plants have decreased extensively in Europe over the last century. owing to eutrophication, formation of organic sediments and improvements for strong competitors. To deepen our understanding of this rarity we tested adaptation and growth of four species exposed in air and under water. All species saved costs by having the same leaf type in air and water, but *Baldellia repens* and *Isoëtes echinospora* maintained leaf porosity and cuticle gas resistance, while *Pilularia globulifera* reduced porosity and *Crassula aquatica* increased resistance in air. The relative growth rate in leaf number on shoots was higher for *P. globulifera* than *I. echinospora* and *C. aquatica* and higher in air than under water for *C. aquatica* and *B. repens.* The vegetative expansion by new lateral shoots was negligible for *C. aquatica* and *I. echinospora*, but high for *B. repens* and *P. globulifera* doubling areal cover within only 9–18 days. All species survived mild frost exposure for one day, but died after one week exposure to hard frost in air. Only *B. repens* and *P. globulifera* survived deep-frozen in ice at −9 °C. We conclude that ability to grow in air and water and tolerate mild, not hard, frost is a common ability of the small amphibious species. Slow growth rate is not a general feature. Low stature represents the main common threat to their survival in competition with tall, overtopping plants benefitting from nutrient enrichment and less physical disturbance that reduce the formation of exposed soils suitable for small amphibious species.

1. Introduction

Small amphibious freshwater species are critically endangered by eutrophication and land use changes in densely populated and intensely cultivated European countries (Arts, 2002; Sundberg, 2014; Baastrup-Spohr et al., 2017). Higher nutrient availability, cessation of grazing and haymaking as well as reduced water level fluctuations on lake banks during the last 100 years due to lake management (Swedish Board of Agriculture, 2011; Hartvig, 2015) have stimulated growth of tall emergent plants at the cost of small, oligotrophic plant species (Maad et al., 2009; Sand-Jensen et al., 2018). This development has led to the decline, and even loss, of a series of small, competitively inferior species on local, regional and national scales (Sand-Jensen et al., 2018). Among sixteen small amphibious species in Denmark, six species have totally disappeared from the eastern part of the country during the last 100 years and three species have been confined to just one single locality (Schou et al., 2017). A similar decline has been observed in many other countries in Europe emphasizing the extinction risk of a large contingent of small amphibious species from a wide part of their distribution range (Arts, 2002; Sundberg, 2014; Bolpagni et al., 2018). This critical situation motivated us to examine the adaptations and growth performance of four small, rare species in order to define the critical steps in their life cycle and their potential for recovery under specific environmental conditions.

We selected *Baldellia repens*, *Crassula aquatica*, *Isoëtes echinospora* and *Pilularia globulifera* for the study. All four species are small, oligotrophic amphibious species that are rare or red-listed and declining in occurrence in European countries (www.gbif.org, Schou et al., 2017). *Crassula aquatica*, *I. echinospora* and *P. globulifera* are still relatively widespread in nutrient-poor regions of Norway, Sweden and Finland (www.artsportalen.dk artsdatabanken.no; artsportalen.se; Atlas Flora Europaea). In contrast, *C. aquatica* has not been observed in Denmark for the last 20 years (Schou et al., 2017), and the species has supposedly gone extinct in Germany and the Baltic countries (www.gbif.org). *Baldellia repens* has disappeared in Germany, one population is left in Denmark, three in Norway and two populations are verified in Sweden (Lindblad and Stahl, 1990; Schou et al., 2017). The species has declined in six European countries for which data is available (Kozlowski et al., 2009).

Although the European Union and several countries focus on securing the survival of the small, amphibious species through pollution control and the establishment of Natura 2000 areas, these initiatives

* Corresponding author.
E-mail addresses: shj644@alumni.ku.dk, oliver.mork@gmail.com (O. Mørk).

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Table 1
Four small amphibious species. Longevity, morphology, occupied grid cells (5 × 5 km) in Denmark and Ellenberg-N category.
Sources: Ellenberg (1988); Hartvig (2015) and Schou et al. (2017).

Species	Longevity	Lateral shoots	Max height (cm)	Grid cells in Denmark		Ellenberg-N
				Number	Percentage	
Baldellia repens	Perennial	Many	15	1	0.1	2
Crassula aquatica	Annual	Few	5	1 (or 0)	0.1 (or 0)	1
Isoëtes echinospora	Perennial	None	15	7	0.5	1
Pilularia globulifera	Perennial	Many	30	26	1.8	1

have been insufficient to stop their decline. Encroachment of lake shores by tall emergent plants has continued owing to high atmospheric nitrogen deposition (European Environment Agency, 2014) and insufficient physical disturbance. Less disturbance comprises reduced water level fluctuations, haymaking and cattle grazing to keep down tall vegetation and generate open soil for establishment of the small, amphibious species (Tyler and Olsson, 1997; Rydberg et al., 2001). Lake restoration projects in the Netherlands aimed at removing the reed vegetation and organic sediments to expose the original mineral soils have been successful in the re-establishment of some small rosette species (Brouwer and Roelofs, 2001). Likewise, re-establishment of the large, wind-exposed, shallow Lake Filsø in Denmark has permitted the colonization of small rosette species (e.g. *B. repens* and *P. globulifera*) on sandy sediments kept open by cattle grazing and trampling (Baastrup-Spohr et al., 2016a; Sand-Jensen et al., 2017b). However, at the same time many lakes which used to support a rich vegetation of small rosette species have deteriorated because of severe pollution from dense populations of overwintering waterfowl and the runoff from fertilized fields (Pedersen et al., 2006, 2016). When pollution control and lake management have been established in order to benefit the recovery of small amphibious vegetation, species richness may, nonetheless, remain impoverished owing to insufficient dispersal capacity from few, small refugia (Sand-Jensen et al., 2000, 2017a).

Water level fluctuations in lakes were more common in the past than they are today (Andersson and Willen, 1999; Wantzen et al., 2008; Mjelde et al., 2013; Bejarano et al., 2017). Extensive water level fluctuations in European fish ponds used to expose a larger part of populations of small amphibious, annual species to air during spring and summer stimulating seed sprouting, vegetative growth and reproduction (Sumberová et al., 2012) and to submergence and protection against frost under winter ice. Nowadays, with more constant lake water levels, frost exposure above the water during winter may be more critical to species survival. Moreover, the advantage that amphibious species have in being tolerant to shifting emergence and submergence is lost when water level fluctuations disappear (Sumberová et al., 2012).

Our general objective was to measure the adaptation, survival and growth capability of the four selected amphibious species when submerged under water or exposed to air. Adaptation and growth were examined during winter in a greenhouse facility at temperatures and light regimes resembling late spring conditions (specifications in Materials and Methods). Furthermore, we studied the survival to frost exposure to test its detrimental effects and evaluate species differences that may contribute to their variable occurrence in relation to water level fluctuations. Individuals may acclimate to submergence, characterized by very low diffusion coefficients of CO_2, oxygen and other gases, by reducing the diffusion resistance across the leaf surface (e.g. through thinner and less permeable cuticle, Frost-Christensen et al., 2003). Individuals in air typically reduce evaporation rates through higher cuticle resistance (Nielsen et al., 1991; Pedersen and Sand-Jensen, 1992). Plants may also acclimate to the higher risk of anoxia underwater by increasing the internal oxygen transport capacity through formation of more aerenchyma (i.e. higher porosity) and shorter distance to apical root meristems in oxygen-depleted sediments (Møller and Sand-Jensen, 2011). We acknowledge that a complex of environmental conditions such as species traits, community interactions and evolutionary history influence commonness and rarity of plant species. We used an experimental approach in order to provide concise results on growth capacity and relevant stress factors in the life cycle over a two-month period, while not including very time-consuming quantifications of other possible bottle necks in the life cycle such as seed production, seed dispersal, seedling growth and competitive interactions that may influence species rarity as well.

Our three specific objectives were to measure: 1) Leaf evaporation, leaf porosity, root porosity and root length to test if the four small amphibious species acclimated to submergence *versus* air exposure, 2) Leaf and shoot formation and biomass production under water and in air to test if species differed in grow rates and particularly if slow growth could contribute to species rarity, and 3) Shoot frost survival to test if differences between species could account for their variable occurrence and geographical distribution. Our overall objective was to include these results in a general evaluation of the reasons for the decline of small, amphibious species in the contemporary European landscape.

2. Material and methods

2.1. Species characteristics

All four species are small, amphibious plants (Table 1). They primarily grow on oligotrophic sandy lake shores with fluctuating water levels and are classified as oligotrophic species with Ellenberg N categories of 1 or 2 (Ellenberg, 1988). *Crassula aquatica* also grows on shores of brackish waters. *Crassula aquatica* is annual and the shortest species on land (< 5 cm), while the other three are perennial and slightly taller with aerial leaves of 10–15 cm and submerged shoots of 10–30 cm (Table 1). Whereas *Baldellia repens* and *Pilularia globulifera* spread laterally by runners, *C. aquatica* and *Isoëtes echinospora* do not. All four species reproduce and spread generatively by seeds or macrospores.

Crassula aquatica and *I. echinospora* have the widest global distribution (Europe, North America, Russia, Japan), whereas *B. repens* is primarily restricted to W-Europe and N-Africa and *P. globulifera* to NW-Europe (Schou et al., 2017). *Isoëtes echinospora* and *C. aquatica* have a more northern distribution in Scandinavia than the other two species (Table 2). In Denmark, all four species have disappeared from the eastern parts, have become restricted to Jutland and are confined to few of the 1300 grid cells (5 × 5 km) examined in a recent nationwide botanical survey (Hartvig, 2015); i.e. *B. repens* (1 grid cell), *C. aquatica* (1 or 0), *I. echinospora* (7) and *P. globulifera* (27, Table 1).

2.2. Plant material and growth experiments

One individual of *B. repens* from a population of several hundreds and ten individuals of *P. globulifera* from a population of several thousands were collected in September 2017 on the banks of Lake Filsø (Baastrup-Spohr et al., 2016a). The plants were grown and multiplied by lateral spread in a greenhouse facility over a period of five months. Fifteen rosettes of *I. echinospora* and surface sediment from a lake site