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Effects of hydrology on recruitment of *Pilularia minuta* Durieu (Marsileaceae), an endangered plant of Mediterranean temporary pools

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ABSTRACT

Hydrological requirements for germination and development of *P. minuta* were assessed through a 5-year field survey in Garâa Sejenane, Tunisia, and an experiment under controlled conditions. The spatial distribution of *P. minuta* was recorded in the field annually, while the experiment tested the effects of water levels and flooding dates on germination (the emergence of new individuals) and development. Water level was found to be the major factor affecting the germination and the development of *P. minuta* with flooding date as a secondary limiting factor. For germination to occur, the sediment must be completely waterlogged. Water depths of 5–10 cm are optimal for plant development. In addition, the plant needs a minimal flooding period of seven weeks to develop. Late-spring precipitation appears to be more critical than earlier rains.

1. Introduction

Acidic Mediterranean temporary pools are rich in species (Grillas et al., 2004) that make significant contributions to regional biodiversity while also helping to identify biodiversity hotspots (Blondel and Aronson, 1999; Médail and Quézel, 1999). The ephemeral nature, small size and shallow depth of Mediterranean temporary pools together with the extreme specialization of their plant communities could well make them highly susceptible to anthropogenic degradation, which is probably an important cause of their rapid, continual decline (*e.g.*, Rhazi et al., 2001, 2006; Dimitriou et al., 2006; Zacharias et al., 2007). The seasonal alternation of inundated and dry phases is an important ecological constraint for the flora of temporary wetlands (Santamaría et al., 1996; Grillas et al., 2004; Deil, 2005), most of which are annual, with the exception of the perennial amphibious species of genus *Isoetes*, whose bulbs are resistant to drought and whose underwater photosynthesis is enhanced by the Crassulacean acid metabolism (Pedersen et al., 2011).

In the Mediterranean Basin, hydroclimatic conditions are associated with significant inter-annual variability in rainfall (Bolle, 2003). The general effects of hydrology on the biology of Mediterranean wetland plants are well established (Webb et al., 2012). Flooding date, which combines the effects of several variables – temperature, solar irradiation, length of day – determines the germination rate (Arts and Van der Heijden, 1990; Bliss and Zedler, 1998; Warwick and Brock, 2003). Water depth and the duration of flooding influence the competition between aquatic and amphibious species, as the latter suffer from excessive water (Crosslé and Brock, 2002). Flooding frequency seems to affect neither richness nor biomass, even if the duration of individual flooding events may be important for segregating plant communities (Bornette et al.,

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1994; Casanova and Brock, 2000; Nicol et al., 2003; Drinkard et al., 2011). Lastly, the date and duration of drying appear to be critical for the seed production of certain species – for example, *Elatine brochonii* Clavaud (Rhazi et al., 2007) and *Littorella uniflora* (L.) Asch. (Arts and Van der Heijden, 1990) – but not for others such as *Ranunculus peltatus* Schrank (Volder et al., 1997). Such species-specific differences in adaptation (Rea and Ganf, 1994) could well enhance the community's resilience to hydrological disturbances.

In this paper, we present a study of the hydrological requirements of one of the rarest species of Mediterranean temporary pools (*Pilularia minuta* Durieu, Marsileaceae) whose largest populations are in Garâa Sejenane (northwest Tunisia; Daoud-Bouattour et al., 2009). For the long-term conservation of this species, we investigated how hydrology affects the biology of *P. minuta* using a combination of a field survey and an *ex situ* experiment.

P. minuta is a small, amphibious pteridophyte that only grows in Mediterranean temporary pools (Grillas et al., 2004). Usually considered to be perennial (rhizomatous geophyte or radicant hydrophyte; Prelli, 2001; Grillas et al., 2004), *P. minuta* is, in fact, an annual plant with an underground stem that dies together with the leaves during the dry period (Johnson, 1933; Ferchichi-Ben Jamaa, 2010). As an annual pteridophyte, this species combines short-lived individuals with the survival of spores during the dry phase and delayed fertilization of female gametes by motile antherozoids, triggered by flooding.

We hypothesized that late rainfall events (March–May), occurring during sporocarp maturation, are determinant for the development of *P. minuta* and verified this in the field. We tested the influence of water depth and hydroperiod experimentally on both germination and development. We hypothesized that moderate water depth and early flooding date were likely to control the success of fertilization and would enhance both the recruitment and development of *P. minuta*.

2. Materials and methods

2.1. Studied species

P. minuta Durieu (small pillwort, Marsileaceae) grows exclusively on non-calcareous substrates and is a pioneer light-demanding species with low competitive ability that benefits from disturbance-induced vegetation opening (Daoud-Bouattour et al., 2009). The very short life cycle of this amphibious species begins in very damp or flooded conditions in late February–early March. At this point, it develops creeping, filiform stems buried slightly below the surface, and 1–3 cm-long erect, slender green leaves which remain green at the beginning of the dry phase (April) but dry up and disappear quickly after May–June (Grillas et al., 2004). Sporocarps, the drought-resistant reproductive organs, form at each node in the inundated ground when the plant is submerged, after leaves have developed and are fully mature when drying out. A complete reproductive cycle of *P. minuta* requires a new flooding (Supplementary material 1). In the present paper, the term *germination* refers to the emergence of a new individual (sporophyte) identified by the appearance of the first leaf.

2.2. Field survey

The pool that we studied, Marchar2, is located in the Mogods Hills of northwest Tunisia (37°05′07″ N, 09°12′25″ E; 100 m a.s.l.; Fig. 1), at the eastern edge of Garâa Sejenane. Sheep, cattle, donkeys and mules graze here all year round. The marshy Garâa Sejenane plain of sandy-silty acidic soil includes a mosaic of cultivated/grazed fields, scattered marshes and temporary pools. The climate is Mediterranean humid with irregular rainfall (average of 880 mm year^{-1}) that is greatest during the winter months (Kassab, 1979). As measured in February 2010, the pool's maximal surface reaches an area of 620 m^2 with a maximum depth of 27 cm. The water pH, measured at diverse periods, was comprised between 6 and 7. The vegetation forms three concentric belts (Grillas et al., 2004). *P. minuta* covers about 100 m^2 in the pool's central and intermediate belts, together with *Callitriche brutia* Petagna, *Coronopus squamatus* (Forssk.) Asch., *Eleocharis palustris* (L.) Roem. & Schult., *Eryngium pusillum* L., *Illecebrum verticillatum* L., *Isoetes velata* A. Braun, *Lythrum borysthenicum* (Schrank) Litv., *L. hyssopifolia* L., *L. tribracteatum* Salzm. ex Spreng., *Myosotis sicula* Guss., and *Ranunculus baudotii* Godr. The peripheral belt is dominated by *Crassula tillaea* Lest.-Garl., *Isoetes histrix* Bory, *Ranunculus sardous* Crantz., *Rumex bucephalophorus* L. and *Sagina apetala* Ard.

Over a period of five years (2007–2011) we mapped the *P. minuta* population in late April–early May, at the end of the wet phase, recording the presence or absence of the species in a grid covering the entire pool, divided in 1934 cells of 0.25 m^2 (hereafter referred to as 'quadrats'). The grid was constituted of strings tightened between pegs that we repositioned annually in precisely the same place. Water depth was measured in each quadrat in February 2010 to reconstruct pool topography (Fig. 1). The Sejenane weather station provided precipitation data (*Source*: Bureau de l'Inventaire et des Ressources Hydrauliques, BIRH, Tunisia).

2.3. Experiment under controlled conditions

From December 15 2008 (W0) to June 1 2009 (W24), we conducted an experiment to identify the optimal hydrological conditions for the germination and development of *P. minuta*. We tested the effects of four flooding dates (December 15 (W0), January 15 (W5), February 15 (W10), March 15 (W14)) and five water levels (soil saturations of 70% and 100%, and water depths of 1 cm, 5 cm and 10 cm), and crossed these two factors with four replicates per combination of factors in a randomized design of containers (Supplementary material 2).

To avoid both disturbing the development of *P. minuta* in Maachar2 pool and impoverishing other populations of this rare species, we collected two kinds of surface sediments during the dry phase (July 2008): (1) 80 kg of sediments randomly collected at a depth of 5 cm near Maachar2 pool in an area without *P. minuta*, and sterilized for 48 h at 150 °C to remove any diaspore; (2) 5 kg of sediments collected at a depth of 2 cm in another pool near Maachar2, with dense populations of *P. minuta*. The presence of *P. minuta* sporocarps within these 5 kg of sediments was verified under binocular microscope after the sediment had been homogenized in 10 random samples of 1 cm^3 each (3–9 sporocarps per cm^3).

All sediments were conserved completely dry until the experimental flooding. The experiment was carried out *ex situ*, on the terrace of a villa at Tunis, under transparent shelter to prevent any rainwater being added. In December 2008, 84 plastic containers were sown with ~1443 cm^3 (thickness: 3 cm) of sterilized substrate covered with 100 cm^3 (thickness: 5 mm) of sediment containing *P. minuta* sporocarps. The containers were flooded on four dates (December 15 (W0), January 15 (W5), February 15 (W10), and March 15 (W14)) to different levels: 70% and 100% saturation, and 1 cm, 5 cm, and 10 cm deep, as per the protocol illustrated in Supplementary material 2. Four procedural control containers were filled exclusively with sterilized sediments and flooded on December15 (W0) to a depth of 5 cm.

During the course of the experiment, rainwater was regularly added to keep water levels constant. Containers with soil saturations of 70% and 100% were adjusted by weight. The arrangement