AQUATIC PLANT DISTRIBUTION IS DRIVEN BY PHYSICOCHEMISTRY AND HYDROPERIOD IN A MEDITERRANEAN TEMPORARY POND NETWORK

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ABSTRACT

The aim of this study was to assess aquatic plant distribution patterns in a Mediterranean temporary pond network (Doñana National Park, SW Spain). We analyzed differences in species composition employing multivariate ordination techniques; we specifically examined the importance of hydroperiod and physicochemistry in the five geomorphological areas across which the pond network is spread.

The ponds significantly segregated along a north-south gradient, matching the segregation pattern of the aquatic plant assemblages. Ponds in the three northernmost areas showed higher levels of species richness than ponds in the two southernmost areas. In the north, ponds were present at higher densities, spanned a broader hydroperiod range, and had lower conductivity levels; these features were associated with the presence of wet-meadow species and larger numbers of submerged and emergent species. In the south, alkaline waters were mainly associated with different charophyte species, and the predominance of long-hydroperiod ponds helped increase the number of floating species.

Managed ponds, which had been artificially deepened, contributed to increase pond heterogeneity and hydroperiod breadth across the entire network. At present, such ponds are key to the preservation of Doñana's rich and unique aquatic plant community.

KEYWORDS

Mediterranean temporary ponds; aquatic plants; environmental segregation; pond management; hydroperiod.

INTRODUCTION

Temporary ponds are singular ecosystems that alternate between wet and dry phases. These habitats retain high levels of biodiversity (Grillas et al., 2004; Rhazi et al., 2006; Bagella et al., 2010), which include aquatic species that are able to resist dry conditions and terrestrial species that tolerate wet conditions, shaping interesting plant communities whose species composition demonstrates significant spatio-temporal heterogeneity (Médail, 2004). Such ponds therefore contain specialist species that are not normally present in permanent waters, many of which are rare or threatened (Médail 2004; Rhazi et al., 2006).

The characteristic unpredictability of Mediterranean climate, with their high interannual variability in rainfall, means that these aquatic habitats demonstrate dramatically fluctuating inundation patterns (Rhazi et al., 2009; Díaz-Paniagua et al., 2010). This variability constrains the composition of species assemblages found in Mediterranean temporary ponds (Casanova & Brock, 2000; Warwick & Brock, 2003; de Meester et al., 2005); and because of their high conservation value are recognized as priority habitats in Europe (EU Habitats Directive, Natura code 3170). Plant species in temporary ponds cope with changing conditions using diverse morphological and physiological adaptations (Brock & Casanova, 1997; Humphries & Baldwin, 2003). Many typical temporary pond species are fast growing with short life cycles (i.e., annuals) and are able to complete their annual life cycle within the seasonal inundated period of the ponds (della Bella et al., 2008; Bagella & Caria, 2012), either as submerged or floating biotypes. These habitats also host inundation-resistant emergent species from surrounding areas (Rhazi et al., 2009; Bagella et al., 2010). These three biotypes have distinct temporal dynamics; the abundance or proportion of both groups fluctuates in response to annual rainfall variation and/or the alternation of wet/dry periods (Rhazi et al., 2009).

The geomorphological origin of an area determines the physical and chemical characteristics of its soils, and thus also determines the features of the ponds found atop these soils (Keddy, 2000). The chemical characteristics of aquatic habitats strongly influence species composition, especially that of plants (van der Valk, 2006). In temporary ponds, the spatial and temporal distribution of plants is also determined by water-depth gradients and the length of the inundation phase, or hydroperiod (Gauthier et al., 2004), which are, in turn, also affected by differences in soil characteristics (Morgan & Stolt, 2006). As plants are key elements of aquatic ecosystems (Bornette & Puijalon, 2011), they are an ideal group with which to examine the effects of both inundation patterns and geomorphological characteristics on pond networks.

Doñana National Park contains one of the most important natural temporary pond networks in Europe. This complex network provides an excellent wetland study system: pond creation and inundation dynamics are intimately related to local geomorphology, which has generated substrates resulting from different historic sand erosion and deposition events. All these factors have favored a high environmental heterogeneity within the pond system; in which the density and extension of the water bodies are strongly influenced by geomorphology and phreatic depth (Díaz-Paniagua et al., 2010).

In Doñana, previous studies have found a direct relationship between the water availability in the park's different geomorphological areas and the composition of terrestrial plant communities (Zunzunegui et al., 1998). The aquatic flora has also been described; compared to other parts of the Mediterranean, the park contains unique and diverse species of aquatic macrophytes, including large populations of singular and endangered species (García-Murillo et al., 2006).

In this study, we analyzed variation in aquatic plant composition over a highly heterogeneous pond network to 1) assess the relationship between plant composition and the physical and chemical characteristics of the ponds; 2) determine whether different aquatic plant compositions are found in different geomorphological areas; and 3) explore the link between hydroperiod and aquatic plant composition.

MATERIAL AND METHODS

Study area

The Doñana National Park encompasses an area of approximately 54,000 ha located at the estuary of the Guadalquivir River on the Atlantic Coast of southwestern Spain (Figure 1). Half the park is composed of an extensive marshland in the Guadalquivir's floodplain, while the other half is a sandy area that contains both mobile and stable dunes. Doñana harbors one of the most important natural wetlands in Europe and is characterized by a large diversity of aquatic ecosystems; most have been classified as temporary aquatic habitats (García-Novo & Marín, 2006). This region has an Atlantic-influenced Mediterranean climate: the summers are warm and dry and the winters are mild. Rainfall averages 545 mm annually and occurs mainly in the autumn and/or winter. It is highly variable across years (Díaz-Paniagua et al., 2010).

Figure 1

The pond network is located in the sandy area of the park and includes a large number of temporary ponds that vary in their water persistence. The annual inundation period (hydroperiod) of the ponds also varies from year to year as it is strongly related to the quantity and timing of annual rainfall. The sandy part of the park has been broken up into five different geomorphological areas (Siljestrom et al., 1994), throughout which the ponds are unevenly distributed. The northern area includes an ancient stable dune system and a high density of temporary ponds. The southern area also comprises stable dunes with a more recent geomorphological origin; its pond density is low. An area containing mobile dunes runs parallel to the coast and contains a large number of short hydroperiod ponds that occur in interdune valleys in wet years. Between the northern stable dunes and the marsh, there is an ecotone area, which locals have named *La Vera*. It is characterized by the presence of small, intermittent streams of run-off that discharge water towards the marsh, forming ephemeral water bodies, mainly in periods of heavy rainfall. A second ecotone, located between the mobile dunes and the stable dunes of the northern area, has been named the peridune area. It includes the park's most permanent ponds. The pond network further contains about 160 semiartificial ponds, which are scattered throughout the entire sandy area; they are locally referred to as *zacallones*. In the northern half of the park, these ponds are usually natural water bodies that have been deepened in order to provide water for wild mammals or cattle during dry periods. In the southern half of the park, most of these artificially deepened waterholes are isolated and frequently disconnected from natural ponds (Florencio et al., 2014). The approximate number of ponds found in the park's different geomorphological areas is provided in Table 1.

The number of temporary ponds varies annually with rainfall. In wet years, more than 3,000 ponds may be formed (Gómez-Rodríguez et al., 2011), while, in dry years, fewer than 200 ponds are present (and most are *zacallones*). Temporary ponds are generally flooded after the first heavy rains (in autumn or winter), when the groundwater level rises, filling the smooth depressions that act as discharge areas. Usually, most ponds are dry in summer

Sampling procedure

During the spring of 2007, we performed an extensive survey of the aquatic vegetation found in 77 ponds located across the five geomorphological areas described above. We also quantified their relative species abundance after the occurrence of each species within a 1m^2 sampling square. The number of sampling squares used for each pond depended on pond area (ranging from 1 sampling square in the smallest ponds of approximately 6.5 m^2 to 10 squares in the largest pond of 80105 m^2), so as to standardize sampling effort per pond and to obtain a better description of species richness and community composition for any given sampling point (Gotelli & Colwell, 2001). Relative abundance of each species were given a score ranging from 0 (absence of the species within the sampling square) to 5 (100% plant cover within sampling square). We used the highest value obtained from the total sampling scores to define the relative abundance of each species in each pond.

We collected physical and chemical data for the 77 ponds sampled in 2007. We recorded *in situ* maximum depth (cm), pH (on bed; using HANNA 991000), dissolved oxygen (mg L^{-1}, on bed; using YSI 550A), electrical conductivity at 20ºC (µS cm^{-1}, on bed; using HI 9033), and turbidity (NTU; using HANNA HI93703). Surface water (500 ml) was collected to determine nutrient concentrations (dissolved inorganic phosphate [µM], nitrate [µM], nitrite [µM], and ammonium [µM)]) and alkalinity (meq L^{-1}). Concentrations of the main cations (Na^+, Ca^{2+}, K^+, and Mg^{2+} [meq L^{-1}] and Fe^{2+} [mol L^{-1}]) and anions (Cl^-, HCO_3^-, CO_3^{2-}, and SO_4^{2-} [meq L^{-1}]) were also determined. Ion concentrations were measured using an inductively coupled plasma (ICP) mass spectrometer, while nutrient concentrations were measured colorimetrically using an AutoAnalyzer (Bran + Luebbe). Alkalinity was measured with the titration method described in APHA (1998). Furthermore, several cation ratios were calculated: Na^+/ Ca^{2+}, Na^+/ Mg^{2+}, and $Na^++K^+/ Ca^{2+}+Mg^{2+}$.

Additional visits to these and different ponds were made in 2011, 2012, and 2013 to collect complementary data on aquatic plants. We exclusively recorded species presence to increase the sampling effort in the different geomorphological areas. The species observed were classified into three biotypes (floating, submerged, and emergent). The total number of ponds with information on plant species composition was increased to 234. All ponds visited were classified in relation to their geomorphological area and hydroperiod (Table 1). Hydroperiod was divided into five general categories: t: temporary ponds (those drying in the summer), L: large, long-hydroperiod ponds (large and deep ponds that usually dry out in the summer but that may persist in very rainy years), tz: temporary ponds including *zacallones* (temporary ponds in which a small extension of the basin has been deepened to achieve water persistence in summer); z: artificially deepened *zacallones* (small deepened holes, unconnected to natural ponds, in which water persists all year round); s: temporary streams (the mouth of small temporary flowing waters, where water may persist as a pond during the spring and summer).

Since the density and distribution of the different pond types differed greatly across areas (Table 1), pond sampling was uneven; for instance, more temporary ponds were sampled in the northern area and more artificially deepened water holes were sampled in the southern area (Table 1).

Table 1

Statistical analyses

Physical and chemical variables from ponds sampled in 2007 were analyzed using principal component analysis (PCA) to identify any environmental gradients. We grouped the sites on both geomorphological areas and hydroperiod classification. To meet normality assumptions, we log-transformed all the variables. We excluded the correlated variables with Pearson's correlation coefficient > 0.7 and thus generated an environmental data matrix containing 13 variables.

Using the 2007 species abundance data, we generated three abundance matrices (for emergent, submerged, and floating species) and estimated the Bray-Curtis index to obtain abundance resemblance matrices. We performed a similarity percentage analysis (SIMPER) using the resemblance matrices to assess the contribution of emergent, submerged, and floating species to mean similarity between areas and to identify each area's characteristic species. All analyses were performed using Primer v.6 (Clarke & Warwick, 2001).

Using the plant species presence/absence data from 234 ponds sampled in 2007, 2011-2013, we first constructed a complete vegetation matrix; we then obtained a resemblance matrix after applying the Sorensen similarity index. Non-metric multidimensional scaling (NMDS) was performed to visualize the spatial variability in plant composition, grouping on both geomorphology and hydroperiod classification.

Finally, to identify the main variables affecting aquatic plant abundance in ponds, we used STATISTICA v.7 to we perform a logistic regression analysis (forward stepwise approach) on the presence/absence data for the SIMPER-selected species within subset of 77 ponds sampled in 2007. We included eight predictor variables: pH, log-transformed depth, alkalinity, turbidity, electrical conductivity, Fe^{2+}, ammonium, and dissolved inorganic phosphates.

RESULTS

Physical and chemical characteristics of the ponds

Ponds from the five geomorphological areas were segregated into two main contrasting groups based on water physical and chemichal characteristics (Figure 2A). The first two principal components (59.9 % of the variance) revealed the existence of a first broad group that encompassed ponds from the three northernmost areas (northern stable dunes, *La Vera,* and the peridune area); these ponds had low pH, conductivity, and alkalinity levels and high concentrations of Fe^{2+} ,which is associated with poor carbonate content. A second group was also present that encompassed ponds from the southern and mobile dune areas; these ponds had higher conductivity and carbonate concentrations (Figure 2A). When the sites were grouped according to hydroperiod (Figure 2B) a separated group appeared along the PC1, associated with southern area ponds that predominantly included semiartificial permanent ponds (*zacallones*).

Figure 2

Aquatic plant distribution according to physical and chemical features and hydroperiod

A total of 116 plant species were present in Doñana ponds. The number of ponds and the geomorphological areas in which they were found are shown in Table S1 in the Supplementary Material. We found seven floating species, 32 submerged species and 77 emergent species (Table S1).

A total of 104 species were found in the northern area (89.7%), 76 (65.5%) in the peridune area, 88 (75.9%) in *La Vera*, 58 (50.0%) in the mobile dunes, and 44 (37.9%) in the southern area. Twenty-six species occurred in all five areas.

The NMDS analysis revealed spatial segregation in the occurrence of aquatic plants across sampling sites. Two groups were formed. The northern area, *La Vera*, and the peridune area had similar plant assemblages, as did the southern area and the mobile dunes (Figure 3). Plant assemblages also varied in relation to hydroperiod: artificial ponds (*zacallones*) harbored distinct vegetation compared to the other pond types (Figure 3).

Figure 3

Floating species were the least common (n=7) and mainly occurred in permanent ponds. The highest number of floating species was found in ponds in the mobile dunes and the southern area, mainly appearing in *zacallones*. The highest number (total richness) of submerged and emergent species was found in ponds from the northern area, the peridune area, and *La Vera*. Total plant richness was highest in natural and deepened temporary ponds in all these three areas. However, average richness per pond was highest in peridune ponds (compared to the northern and *La Vera* ponds) because peridune ponds are large and have long hydroperiods, which means that they harbor more species than most of the temporary ponds found in the other areas. In general, the northern area made the largest contribution to total aquatic plant richness in the park, while the peridune area and *La Vera* contributed a few additional species. The mobile dunes contributed only one unique submerged species (Figure 4).

Figure 4

Characteristic aquatic plant species in the different geomorphological areas

We estimated the similarity in plant species compositions in ponds for each geomorphological area and also distinguished the most abundant and commonly occurring species characterizing their plant assemblages (Table 2).

The most characteristic emergent species in the northern area, *La Vera*, and the peridune area were those typically found in wet meadows (*Panicum repens* L., *Cynodon dactylon* (L.) Pers. and *Baldellia ranunculoides* (L.) Parl.), which were mainly associated with short hydroperiod ponds. Of these, *P. repens* was the only emergent species that was also typical of the mobile dunes and the southern area (Figure 5 and Table 2).

Figure 5: Table 2

The most characteristic submerged species varied for the different geomorphological areas. Species such as *Juncus heterophyllus* Dufour and *Myriophyllum alterniflorum* DC. were common in ponds in the northern and peridune areas, whereas *Ranunculus peltatus* Schrank and *Callitriche stagnalis* L. were representative of ponds in *La Vera* (Figure 2). In ponds in the southern area and mobile dunes, charophytes such as *Chara fragilis* Desv. and *Chara vulgaris* L. predominated (Figure 5 and Table 2).

Although strictly floating species were scarce, *Lemna minor* L. frequently occurred in the northern area, *La Vera*, and the peridune area. In contrast, in the southern area, *Lemna gibba* L. was more common. No one species was characteristic of the mobile dunes. It is worth noting that the invasive fern, *Azolla filiculoides* Lam., is distinctively present in *La Vera*. This is the only area in which this species appeared, likely due to its proximity to invaded marshes (Figure 5 and Table 2).

The logistic models revealed the physical and chemical conditions associated with the presence of the most characteristic species (Table 3). The occurrence of emergent species largely found in the northern area, *La Vera*, and the peridune area - i.e., *B. ranunculoides, Juncus maritimus* Lam., and *P. repens* - was related to Fe^{2+} content and turbidity. In the case of *P. repens*, conductivity and phosphates were also important.

Table 3

For submerged species, we found clear differences in physicochemical requirements across the different areas. Typical species in the northern area, *La Vera*, and the peridune area - i.e., *J. heterophyllus, M. alterniflorum*, and *R. peltatus* - were significantly more common in ponds with low conductivity, low turbidity, and high Fe^{2+} content. In contrast, characteristic species in the southern area and the mobile dunes - i.e., *C. fragilis* and *Chara connivens* Salzm. ex A. Braun - mainly occurred in high pH, alkaline ponds.

Only the occurrence of one floating species, *L. gibba*, could be analyzed. It was present mainly in low alkalinity ponds.

DISCUSSION

Mediterranean temporary ponds are aquatic habitats that support rich plant communities that include rare and threatened species (Médail, 2004). Our study found similar levels of hydrophyte richness as reported in temporary ponds in different Mediterranean locations (Table 4). Doñana's pond network harbors several species of high conservation status (i.e.:*Avellara fistulosa* Blanca & C. Díaz, *Caropsis verticillato-inundata* (Thore) Rauschert, *Ricciocarpos natans* (L.) Corda, *Wolffia arrhiza* (L.) Horkel ex Wimm., or *Callitriche lusitanica* Schotsman).

The great richness of aquatic plants in this area was favored by the increase in heterogeneity associated with differences among geomorphological areas and by the availability of ponds of different hydroperiod. In general, with regard to plant species composition, the five geomorphological areas can be divided into two groups; ponds from northern areas (the northern area, *La Vera*, and the peridune area) which had characteristic species typically found in slightly mineralized temporary ponds (i.e M. *alterniflorum* and *C. stagnalis*); and ponds from the two areas to the south (the southern area and the mobile dunes), which were characterized by several species that require water with high levels of carbonates (i.e., *C. fragilis* and *C. vulgaris).*

The availability of ponds within a wide range of hydroperiod also varied, notably from north to south. In the north, the ponds varied greatly in size and depth (Díaz-Paniagua et al., 2010; Gómez-Rodríguez et al., 2011), and had the greatest number of species with different requirements. In contrast, in the south, the hydroperiod range was narrower and the number of temporary ponds was low, which translated to a lower number of species.

The Doñana ponds contain seven of the 10 floating species found in the Mediterranean (Euro+Med, 2006-2015). Floating biotype is rarely present in these types of aquatic habitats (Rhazi et al., 2006; Fraga, 2008; Pinto-Cruz et al., 2009; del Pozo, 2012). Doñana temporary pond network therefore acts as a refuge of this type of species, mostly linked to the most permanent ponds. Indeed, they were most common in the southern area, where they usually colonized the isolated, permanent *zacallones*. These specific water bodies play an important role in the preservation of floating species, as some of them are the only places where threatened species, such as *Lemna trisulca* L., *Wolffia arrhiza*, *Spirodela polyrrhiza* (L.) Sleichd., and *R. natans*, occur. For many of these species, the Doñana ponds represent their southernmost occurrence in Europe; in some cases, they are separated by hundreds of kilometers from the nearest subpopulation to the north.

Among the submerged species, it is worth noting the presence of four *Potamogeton* species (*P. natans* L., *P. lucens* L., *P. polygonifolius* Pourr., and *P. pectinatus* L.), perennial herbs that require long-hydroperiod or permanent ponds to persist (Cirujano et al., 2014) that also restricted to *zacallones*. The distribution of these species was wider in the past (Castroviejo, 1980; Rivas -Martínez et al., 1980; Galiano & Cabezudo, 1976) and their currently restricted distribution clearly indicates that the entire pond network has deteriorated.

The inundated external edges of the ponds contained a large number of emergent species. They contribute to give a complex structure to these ponds' edge microhabitats. They are important refuges for animal communities, such as amphibians or aquatic insects (Díaz-Paniagua, 1987; Nilsson, 1996). In this group we find the most threatened plant species of the whole pond network, such as *A. fistulosa* or *C. verticillato-inundata*, both protected by several conservation laws (Habitat Directive 92/43/ECC and Spanish red list, RD 139/2011). Although many ponds were low species richness, the overall sum of emergent species resulted in high total richness in the north. In contrast, the number of emergent species was low in the south; their scarcity is mainly explained by the absence of ponds with short or intermediate hydroperiods, the most suitable habitats for them.

One major finding of this study is that strictly aquatic species are being lost, which evidenced that the entire pond network is deteriorating. Ponds in Doñana are mainly fed by groundwater (Sacks et al., 1992). During recent decades, groundwater abstraction in the urban and agricultural areas surrounding the park has severely affected the aquifer. In general, hydroperiods are getting shorter (Serrano & Serrano, 1996; Gómez-Rodríguez et al 2011) and some major ponds have been dessicated (Díaz-Paniagua & Aragonés, 2015).

At present, only the semiartificial *zacallones* help to preserve a significant percentage of the park's aquatic plant biodiversity. Our results reveal that these managed ponds serve an important role: they preserve aquatic biodiversity. They should be considered a complementary and efficient tool in the conservation of aquatic plant species that are currently threatened by the trend of shortened hydroperiods observed across the entire pond network.

The introduction of exotic species is another problem that affects plant composition in aquatic systems (Sakai et al., 2001). Exotic crayfish, *Procambarus clarkii* (Girard 1852), may have a great impact on aquatic vegetation in the Doñana pond network. This species can have a strong, negative effect on aquatic plants (Geiger et al., 2005; Arribas et al., 2014), but its populations do not survive in temporary ponds. However, the crayfish colonizes these habitats in years of high rainfall, when there is greater connectivity among the different water bodies (Díaz-Paniagua et al., 2014), and persists in the semiartificial *zacallones*, which remain inundated during the summer. In fact, isolated crayfish populations are currently present only in *zacallones* in the northernmost sandy areas; they have not reached ponds in the south, where the zacallones are isolated from other water bodies (Díaz-Paniagua et al., 2014). Although we consider that *zacallones* play an important role in the preservation of strictly aquatic plant species, this function is rendered void when exotic species invade these ponds. These ponds must therefore be managed so as to preserve appropriate water conditions and eradicate any exotic species that may be present.

We also occasionally observed the exotic fern *A. filiculoides,* a floating fern that has widely invaded the nearby marsh since 2001 (García-Murillo et al., 2007). However, only isolated individuals appeared in a few ponds close to the nearby invaded marshland in very rainy years. The moderate concentrations of nutrients in the pond network (Florencio et al., 2013) probably prevent this exotic species from sustaining viable populations.

In conclusion, the high degree of heterogeneity found in the Doñana pond network has helped maintain a unique and rich aquatic plant community. Plant richness is favored by the broad range of hydroperiod, the presence of managed permanent aquatic habitats, and the park’s geomorphological diversity. These are factors to be considered when preserving areas for aquatic plant conservation; the broader range in hydroperiod and in physical and chemical characteristics could help increase plant species richness. However, it is also important to preserve the flooding dynamics of the pond network. The continuous decline in groundwater levels, which reduces the range of pond hydroperiod, and the presence of exotic species that have a significant impact on aquatic plants are significant threats that should be addressed to guarantee the preservation of aquatic plant communities.

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Table 1. Number of ponds with different water permanence regimes: A) Total number of sampled ponds; B) Ponds with physical and chemical data; C) Approximated number of ponds estimated in Doñana National Park (as per Díaz-Paniagua et al., 2010 and Gómez-Rodriguez et al., 2011). Classification of ponds: t: temporary ponds, L: large long-hydroperiod ponds or shallow lakes; tz: deepened natural temporary ponds; z: artificially deepened isolated ponds; s: temporary streams.

		t	**L**	**tz**	**z**	**s**
North	A	54	0	39	8	0
	B	12	0	7	5	0
	C	1355	0	50	15	3
Peridune	A	6	4	5	8	0
	B	3	2	4	0	0
	C	1147	7	5	9	0
Vera	A	19	1	11	5	9
	B	6	0	6	0	9
	C	182	1	13	16	17
Dunes	A	19	0	3	7	0
	B	3	0	0	4	0
	C	153	0	9	8	0
Southern	A	3	0	0	33	0
	B	1	0	0	15	0
	C	331	0	0	39	0

Table 2. Contribution of the most characteristic plant species found in each geomorphological area to between-pond similarity in the SIMPER analysis, separated by type (emergent, submerged, and floating).

	Northern	**Peridune**	**Vera**	**Mobile dunes**	**Southern**
Emergent	**39.9%**	**43.5%**	**25.6%**	**100.0%**	**2.6%**
Panicum repens	34.0	34.1	17.1	100.0	60.0
Cynodon dactylon	14.4	7.6	26.1	-	-
Baldellia ranunculoides	12.1	9.8	16.3	-	-
Eleocharis multicaulis	10.9	3.4	-	-	-
Eleocharis palustris	9.1	-	4.9	-	-
Illecebrum verticillatum	3.0	-	-	-	-
Juncus maritimus	2.8	19.6	3.3	-	-
Hypericum elodes	2.3	-	-	-	-
Mentha pulegium	2.0	-	1.8	-	-
Lythrum junceum	-	-	8.3	-	-
Cotula coronopifolia	-	-	6.4	-	-
Gliceria spicata	-	-	4.5	-	40.0
Cyperus longus	-	-	2.9	-	-
Agrostis stolonifera	-	5.2	-	-	-

Hydrocotile vulgaris	-	4.9	-	-	-
Galium palustre	-	6.2	-	-	-
Submerged	**40.6%**	**15.5%**	**15.5%**	**24.3%**	**27.8%**
Juncus heterophyllus	41.1	33.4	21.7	-	-
Myriophyllum alterniflorum	37.7	38.6	14.1	-	-
Ranunculus peltatus	8.3	11.3	26.1	6	-
Nitella translucens	4.4	-	-	-	-
Callitriche stagnalis	-	-	22.9	-	-
Callitriche obtusangula	-	-	10.0	-	-
Isolepis fluitans	-	7.9	-	-	-
Chara fragilis	-	-	-	54.5	33.0
Chara vulgaris	-	-	-	24.5	5.5
Potamogeton lucens	-	-	-	8.9	-
Chara connivens	-	-	-	-	48.3
Chara aspera	-	-	-	-	7.4
Floating	**6.7%**	**19.2%**	**11.49%**	**all similarities = 0**	**14.6%**
Lemna minor	100.0	100.0	85.1	-	31.6
Lemna gibba	-	-	-	-	68.4

Azolla filiculoides	-	-	14.9	-	-

Table 3. Results of the best-fit logistic regression models (*forward stepwise selection*) for species that had a greater than 50% contribution to the similarity of the general composition of aquatic plant assemblages within each geomorphological area. Eight different physical and chemical variables were used as predictors in the models. For each variable, the adjusted R^2 (Nagelkerke), the logistic coefficient ± SE, and the significance level according to the Wald statistic (**<0.01, *<0.05) are provided.

	R^2	**Depth**	**pH**	**Alkalinity**	**Turbidity**	**Conductivity**	**Ammonium**	**Phosphate**	**Fe^{2+}**
Emergent									
Baldellia ranunculoides	0.22	-	-	-	-	-	-	-	-1.35±0.41**
Juncus maritimus	0.35	-	-	-	1.83±0.82**	-	-	-	-1.61±0.50**
Panicum repens	0.58	-	-	-	2.18±0.72**	2.35±0.99*	-	5.27±1.82**	-1.58±1.51**
Submerged									
Chara fragilis	0.56	4.11±2.1*	-	+2.75±1.25*	-	-	-	-	3.57±1.25*
Chara connivens	0.41	-	-1.71±0.46**	-	-	-	-	-	-
Juncus heterophyllus	0.56	-	-	-3.39±0.97**	1.93±0.68**	-	-	-	-1.14±0.48*
Myriophyllum alterniflorum	0.44	-	-	-	1.35±0.57*	2.27±0.94**	-	-	-1.45±0.50*
Ranunculus peltatus	0.29	-	-	-	1.88±0.56**	-	-	-	-0.94±0.39*
Floating									
Lemna gibba	0.38	-	-	-4.05±1.24*	-	-	-	-	-

Table 4. Number of plant species observed for Mediterranean temporary ponds in Europe and North Africa.

Country	Pond type	n ponds studied	n plant species	Reference
Algeria	Temporary ponds	26	136	de Bélair, 2005
Spain (Menorca)	Temporary ponds	63	108[1]	Fraga, 2008
Tunisia	Semipermanent and temporary	36	128[1]	Ferchichi-Ben Jamaa et al., 2010
Sardinia	Temporary ponds	50	138[1]	Bagella & Caria, 2012
Morocco	Temporary ponds	48	253[2]	Rhazi et al., 2012
W Tunisia	Temporary ponds + 1 semipermanent	6	79	Rouissi et al., 2014
France	Temporary ponds	-	83[3]	Medail, 2004
Portugal	Seasonal ponds and marshlands	13	129	Pinto-Cruz et al., 2009
Italy	Temporary ponds	20	171	Alfonso et al., 2011
Spain (Doñana)	Temporary + permanent ponds	234	116	This study

[1]: This number does not include terrestrial species occurring outside inundated areas.

[2]: This number includes terrestrial species found in -inundated areas.

[3]: Charophytes not included

Legends to the figures

Figure 1. Location of the sample sites in the five geomorphological areas described for Doñana National Park (SW Spain).

Figure 2. Principal component analysis (PCA) performed with the different physical andchemical variables.. The relative contribution of each is indicated by line length. A: ordination incorporating geomorphological areas - n: northern area; v: La Vera p: peridune area; s: southern area; d: mobile dunes. B: Ordination incorporating water permanence- t: temporary ponds, z: artificially deepened isolated ponds; s: temporary streams; tz: deepened natural temporary ponds; L: large, long-hydroperiod ponds or shallow lakes.

Figura 3. Non-metric multidimensional scaling (NMDS) performed on the total vegetation matrix. 3A. ordination incorporating geomorphological areas - n: northern area; v: La Vera p: peridune area; s: southern area; d: mobile dunes. 3B. Ordination incorporating water permanence- t: temporary ponds, z: artificially deepened isolated ponds; s: temporary streams; tz: deepened natural temporary ponds; L: large, long-hydroperiod ponds or shallow lakes.

Figure 4. Aquatic plant species richness in the ponds in the five geomorphological areas in Doñana National Park. Total species richness (Total) is a count of all the species observed in each area, which are summed (Accumulated) to obtain the total number of species in the park. Species richness was averaged for ponds in different areas (Average). The bars indicate the total species richness and average richness (solid colors and patterns respectively) estimated for ponds of different hydroperiods within each area.

Figure 5. Distribution maps for the main emergent, submerged and floating aquatic plants found in the study area.