

The influence of geomorphology on the composition of aquatic flora and fauna within a temporary pond network

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ABSTRACT

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Geomorphological and hydrological features can provide a sound basis for global wetland classification. Temporary ponds located on the sandy area of Doñana can be classified into five different geomorphological areas. We hypothesised that these ponds would differ in soil characteristics, which may also explain differences in the composition of their macroinvertebrate, amphibian, and aquatic plant species assemblages. The study ponds were significantly segregated into southern and northern geomorphological areas based on the depth to the water table. The results of the multivariate ordination of soil variables were consistent with a north-south segregation, which was explained by the carbonate and other main ion concentrations in the pond basins. The ponds located in ecotones (marsh-sand and stable-mobile dunes) were particularly rich in amphibians and macroinvertebrates. This result may be explained because the ponds with the longest permanence of water were located in these areas, which represented the only flooded ponds during droughts. In addition, they also contained a high number of temporary ponds, thus favouring connectivity and environmental heterogeneity in these areas.

Key words: Temporary ponds, geomorphology, Doñana, aquatic assemblages, macroinvertebrates, amphibians, aquatic plants.

RESUMEN

Influencia de la geomorfología sobre la composición de la fauna y flora acuáticas de un sistema de lagunas temporales

Las características hidrológicas y geomorfológicas pueden ser de gran utilidad para la clasificación de humedales. Las lagunas temporales del manto eólico de Doñana se pueden clasificar en función de las cinco áreas geomorfológicas en que se encuentran. Nuestra hipótesis se basa en que las lagunas de estas áreas difieren en las características de sus suelos, lo cual puede explicar también las diferente composición de las comunidades de macroinvertebrados, anfibios y plantas que las habitan. Las lagunas del sur de Doñana se diferenciaron de las del norte principalmente en la distancia a la capa freática. Se realizó una ordenación de las lagunas a partir de un análisis multivariante de las características del suelo que resultó consistente con la segregación norte-sur. Se observó una mayor segregación en las comunidades de plantas acuáticas en este gradiente norte-sur, que se explica en función de las concentraciones de carbonatos y de otros iones principales en el suelo de las lagunas. Las lagunas localizadas en áreas de ecotono (marisma-arenas y arenas estabilizadas-arenas móviles) resultaron ser las de mayor riqueza de anfibios y macroinvertebrados. Esto podría deberse a que estas áreas contienen las lagunas de mayor duración, siendo los únicos medios inundados en épocas de sequía, y al alto número de lagunas temporales que presentan, favoreciendo la conectividad y heterogeneidad de ambientes.

Palabras clave: Lagunas temporales, comunidades acuáticas, geomorfología, Doñana, macroinvertebrados, anfibios, plantas acuáticas.

INTRODUCTION

The length of the wet phase has long been regarded as a fundamental factor that influences all other features in temporary aquatic systems (Rzóska, 1961). The hydrological regimes of temporary ponds may differ due to many factors such as the pond basin soil, which plays an important role in temporary pond ecosystems. Over long-term periods, an area's inundation frequency and duration affect the physical and chemical processes involved in the development of hydric soils (Rabenhorst, 2004). Hydrogeomorphic-based approaches are preferentially applied in wetland functional assessments (Cole *et al.*, 1997). These approaches are particularly useful in the classification of temporary wetlands in semi-arid regions, where the unpredictability of the climate results in a wide range of hydrological conditions in a single water body between years.

Mediterranean temporary ponds are found in many parts of the world that are characterized by Mediterranean climates. Most of these ponds form in depressions in soils overlying an impermeable substrate and result in a perched water table (Keeley & Zedler, 1998), as described in California, Chile, South Africa, and western Australia. By contrast, in the Mediterranean basin temporary ponds also occur in floodplains that drain relatively large watershed systems (Keeley & Zedler, 1998) and are not necessarily associated with impermeable fine sediments. They are common in areas with a Mediterranean climate, in which the desiccation period generally occurs in the summer, and the flooding period follows heavy winter or autumn rainfall (Zedler, 1987; Grillas *et al.*, 2004; Gómez-Rodríguez *et al.*, 2009; Díaz-Paniagua *et al.*, 2010). Despite the worldwide distribution of temporary wetlands, there are few studies addressing the classification of both flora and fauna based on the geomorphological characteristics of their habitats. However, such an approach has been common in wetland plant classifications (Haslam, 2003). The ability of hydrogeomorphic classes to discriminate among other biological communities has been

occasionally tested in several wetland areas, with soil organic matter and moisture as the strongest drivers for the typification of macroinvertebrate assemblages (USEPA, 2002). In the Iberian Peninsula, Alonso (1998) developed a typology of crustacean assemblages closely related to substrate, climate and geographic attributes. Recently, Sahuquillo & Miracle (2013) provided a typology for Mediterranean ponds using crustacean assemblages based on water permanence, climatic and orographic features.

However, pond soil characterisation appears to provide valuable information about the main characteristics of an area, which is related to the composition of aquatic species, and may provide a novel perspective for the classification of aquatic communities.

A large network of temporary ponds is located in Doñana National Park (SW Spain), where more than 3 000 ponds may be inundated during years of heavy rainfall (Díaz-Paniagua *et al.*, 2010). These ponds constitute important habitats for amphibians, macroinvertebrates, microcrustaceans, and aquatic vegetation (Díaz-Paniagua *et al.*, 2010). To manage and conserve these habitats, it is of crucial importance to assess their water regime and evaluate the main factors sustaining their wet phase. The several attempts by short-scale studies to classify Doñana ponds into different hydrological categories have produced contradictory results (García-Novo *et al.*, 1991; Bravo & Montes, 1993; Manzano, 2001). However, the characteristics of the soil of their pond basins could provide a geomorphological framework upon which to base a classification system.

In this study, we analysed the characteristics of the soils of temporary ponds located in the different geomorphological areas of Doñana National Park. We hypothesised that the geomorphological classification of ponds was robust and that ponds in different geomorphological areas would have different compositions of species assemblages (macroinvertebrates, amphibians, and aquatic plants). We were particularly interested in determining if idiosyncratic species were exclusively associated with particular geomorphological areas.

METHODS

Geomorphology of the Doñana pond network

Doñana National Park is a coastal dune area that is located over a large sedimentary aquifer system of approximately 3000 km². The Doñana region was formed in the Quaternary age when the estuary of the Guadalquivir River was enlarged and reshaped by the formation of sandy spits after the last postglacial transgression (Siljestrom *et al.*, 1994). One half of the park includes an extensive marsh, and the other half is composed of ae-

olian sands that form a permeable layer through which waters drain to an unconfined aquifer. The water table is generally shallow in this area and intersects with smooth depressions in the soil surface, forming both permanent and temporary ponds (Manzano & Custodio, 2006). This aeolian unit has been separated into the following five geomorphological areas that differ in their dynamics, parent material, and age: northern stabilised dunes, southern stabilised dunes, mobile dunes, and two contact areas, or ecotones, which include the peridune ponds and the marsh-dune border (Siljestrom *et al.*, 1994) (Fig. 1).

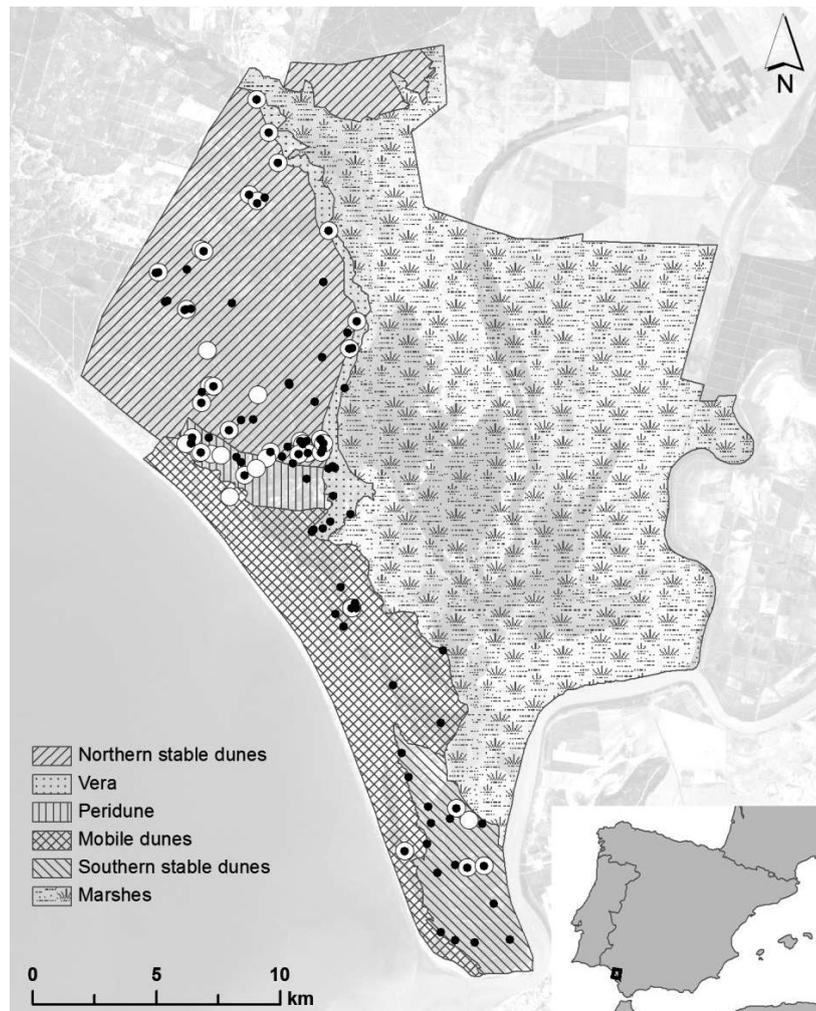


Figure 1. Location of the pond sample soils (○) and the flora and fauna pond samples (●) in the five geomorphological areas described for Doñana National Park. *Localización de las lagunas muestreadas para el análisis de los suelos (○), y para la determinación de su flora y fauna acuáticas (●) en las cinco áreas geomorfológicas descritas en el Parque Nacional de Doñana.*

The northern stable dune area is an ancient dune system (6000-12000 BP) where temporary ponds form in the lower areas after the rise of the water table by rainfall infiltration. The southern stable dune area, which is of more recent origin (2000-4000 BP), has a smaller extension, and the soils differ in the physico-chemical and mineralogical characteristics (rich in CaCO₃ and other salts) from those found in the northern stable dunes, which are mostly siliceous (Siljestrom *et al.*, 1994). In the mobile dunes, discharges from a local dune aquifer form temporary ponds in depressions in interdune valleys (or slacks). The two interfaces between these geomorphological areas play a special role in the formation of ponds because they are characterised by phreatic discharges of groundwater. The peridune ecotone is the area in which mobile dunes and northern stable dunes come in contact. It includes the largest and most permanent ponds of the park that are mainly fed by the dune aquifer. The marsh-stable dune interface is locally known as “La Vera”. In this ecotone, the discharges of deep water levels originating from the marsh and stable dunes coincide, often resulting in water running off in shallow and temporary streams after heavy rainfall (Clemente *et al.*, 1998).

Doñana ponds are fed by freshwater (rainfall, runoff, and groundwater discharge) and have no surface or groundwater connection to the sea,

although they do receive salts of marine origin through airborne deposition (Serrano *et al.*, 2006). Temporary ponds are flooded after heavy rains, which in this area may occur in autumn, winter, or less frequently, in the spring. The dry phase coincides with summer; it starts in the most ephemeral ponds in the spring, whereas those with the longest hydroperiods (i.e., duration of the flooded period) may retain water until the end of summer (Díaz-Paniagua *et al.*, 2010).

Soil sampling

To relate the soil geomorphological characteristics of ponds with their aquatic assemblages, soil samples of 43 pond basins located across the five different geomorphological areas were obtained by performing borehole sampling during pond dry periods in 2007 ($n = 34$) and 2012 ($n = 9$). We collected samples from each horizon in all profiles for laboratory analyses. Chemical analyses (pH, electrical conductivity, cation exchange capacity (CEC), phosphorus extraction, organic matter characterisation, assimilable cations, and carbonates) were conducted following Sparks & Bartels (1996). Soil texture was determined using a Boyoucos densimeter (Day, 1986); the percentages of silt and clay were summed to provide the overall percentage of fine sediments. Heavy metals were obtained using humid digestion and

Table 1. Percentages of ponds with different water regimes for which macroinvertebrate, amphibian, and aquatic plant species composition was characterised in 2007. Ponds are categorised into one of the five geomorphological areas described for Doñana National Park. Water regime was classified as follows: temporary ponds (natural temporary ponds with an annual dry phase), excavated ponds (isolated ponds that have been artificially excavated to maintain water in summer), temporary+excavated (temporary ponds that include an excavated pond in their basin to retain water in summer), permanent ponds (large natural ponds that do not dry up in summer) and streams (running waters after heavy rains than then persist as temporary ponds). For amphibians, we sampled an additional pond; the modified percentage is included in brackets. *Porcentajes de lagunas con diferente régimen hídrico sobre el total de lagunas en las que se mostraron macroinvertebrados, anfibios y plantas acuáticas en 2007. Las lagunas se han clasificado dentro de cada una de las cinco áreas geomorfológicas descritas en el Parque Nacional de Doñana. En los muestreos se anfibios se muestreó una laguna más que en los otros grupos, por lo que el porcentaje modificado para este grupo se muestra entre paréntesis.*

	Northern stable dunes	Southern stable dunes	Mobile dunes	Peridune ecotone	La Vera ecotone
Temporary ponds	61.3 (62.5)	11.8	57.1	87.5	65.4
Excavated ponds	19.4	88.2	42.9	0	11.5
Temporary+excavated ponds	19.4	0	0	0	0
Permanent ponds	0	0	0	12.5	0
Streams	0	0	0	0	23.1
N ponds sampled	31(32)	17	7	8	26

ICP/OES lectures (Williamson, 1994). To assess the differences in the groundwater levels of the geomorphological areas, we also measured the depth to the groundwater table in the sampled ponds and 36 additional ponds ($n = 79$ total sites) throughout the different areas of the park.

Ponds, fauna, and flora

To relate our data on pond soils to the presence of particular species of aquatic fauna and flora, we used presence/absence data on the vegetation, macroinvertebrates, and amphibians of temporary ponds across the entire park. Data from 89 ponds were collected from March-June 2007 (an additional pond was sampled to gain more information on amphibians: $n = 90$ ponds for amphibian data). The ponds' geomorphological locations and water regimes are indicated in Table 1. The water regimes were first classified as follows: temporary, permanent, stream, or

artificially excavated. Streams refer to intermittent running waters that only flow towards the marsh after heavy rains and then form lentic ponds. Artificially excavated ponds refer to holes excavated in the deeper parts of natural ponds to reach the phreatic level during dry periods and may be considered as permanent water bodies; they were mainly built to provide water for cattle or wild fauna during the summer (temporary+excavated ponds). These excavated ponds sometimes occupy the entire pond basin, are isolated from natural temporary ponds, and occur mainly in the southern stable dunes.

Amphibians and macroinvertebrates were sampled using a dip-net with a 1-mm mesh size. We performed three successive sweep-nets across a stretch of water of *c.* 1.5 m in each sampling unit to sample the heterogeneous environments mainly based on depth and aquatic plant cover within each pond; we followed the same procedure for both groups of organisms

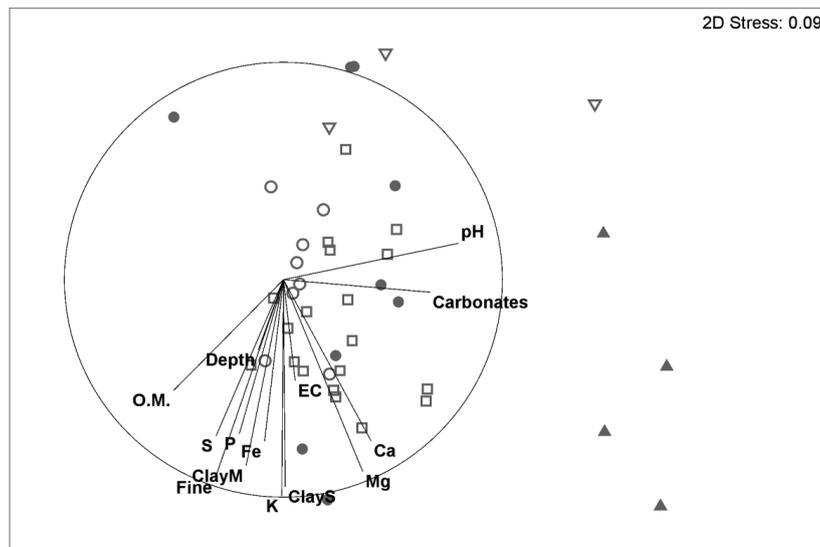


Figure 2. Non-metric multi-dimensional scaling ordination performed on the similarity matrix of pond soil samples according to pond location in the different geomorphological areas of the Doñana aeolian sands: northern stable dunes (□), southern stable dunes (▲), peridune (stable-mobile dunes) ecotone (○), mobile dunes (▽), and La Vera (marsh-sand) ecotone (●). The relative magnitude of Spearman's correlation values between each soil variable and the pond ordination is indicated by the line size (Depth: phreatic depth; Fine: fine fraction; O.M.: organic matter; EC: electrical conductivity; ClayM: % of clay in the clay-rich horizon; ClayS: % of clay in the surface horizon). *Ordenación multidimensional no métrica realizada sobre la matriz de similitud de las muestras de suelo de las lagunas localizadas en las diferentes áreas geomorfológicas descritas en el manto eólico de Doñana: Arenas estabilizadas del norte (□), arenas estabilizadas del sur (▲), ecotono peridunar (arenas estabilizadas-dunas móviles (○), dunas móviles (▽), y ecotono de La Vera (marisma-arenas) (●). Se indica la magnitud de los valores de la correlación de Spearman entre los tipos de suelos y la ordenación de las lagunas. (Depth: nivel freático, Fine: fracción fina, O.M.: materia orgánica; EC: conductividad eléctrica, ClayM: % de arcilla en el horizonte con mayor proporción de arcillas, ClayS: % de arcillas en el horizonte superficial).*

(see, e.g., Florencio *et al.* 2009a for a detailed description of the process). Aquatic plants were identified at the same locations. In our analyses, we examined adult and larval macroinvertebrates separately (hereafter referred to as “taxa” for simplicity). Macroinvertebrates were identified to species in the case of most adults or genus in the case of most larvae, although most members of the orders Bassomatophora, Diptera, Haptotaxida, and Lumbricula, as well as of the family Saldidae (Heteroptera), were only identified to the family level. Amphibians and aquatic plants were identified to the species level.

Data analyses

A multivariate ordination analysis (non-metric MDS) was performed on the similarity matrix of the 14 physico-chemical soil variables using Primer 6.0. These variables were measured in the 43 pond basins that included the deep clayey layer or the topsoil layer when clay was not present. The variables included in this analysis were all log transformed ($X + 1$), with the exception of the non-transformed pH; electrical conductivity, organic matter content, fine sediments (clay and silt), carbonates, Tot-P, TotCa, Mg, S, Mg, K, and Fe in the surface horizon; clay and total fine sediment contents in the clay-rich horizon; and distance to the groundwater table. We then obtained a triangular similarity matrix using Euclidean distances. A multivariate ANOVA analogue (one-way ANOSIM) was used to test for multivariate differences in the soil characteristics of samples from ponds located in different areas, i.e., using the geomorphological areas as grouping factors in the one-way ANOSIM analyses (Primer v6). The ANOSIM statistic R is close to one when groups are completely different (Clarke & Warwick, 2001). The ANOSIM analyses also allowed for pair-wise comparisons between the different geomorphological areas. Spearman’s rank order correlations were also performed on the untransformed variables. In addition, differences in the depth to the groundwater table among the geomorphological areas were tested using ANOVA and Tukey’s *post hoc* test (STATISTICA 6.0).

Table 2. Pair-wise comparisons obtained after a one-way ANOSIM analysis performed on pond soil variables between the five geomorphological areas (northern: northern stable dunes; southern: southern stable dunes; mobile dunes; peridune: peridune ecotone; and La Vera: La Vera ecotone) indicate significant differences ($p < 0.05$) between the pair-wise comparisons except for two cases ($p > 0.1$); ANOSIM statistic R is close to one when groups are completely different. *Comparaciones pareadas obtenidas en el análisis ANOSIM de una vía sobre las variables del suelo de las lagunas entre las áreas geomorfológicas (Northern: arenas estabilizadas del norte, Southern: arenas estabilizadas del sur, Mobile Dunes: dunas móviles, Peridune: ecotono peridunar y Vera: ecotono de La Vera) indicando diferencias significativas en todas las comparaciones pareadas ($p < 0.05$) excepto en dos ocasiones ($p > 0.1$); el estadístico R de ANOSIM se aproxima a 1 cuando los grupos son completamente diferentes.*

	R	p
Northern × Southern	0.99	0.0001
Northern × Mobile Dunes	0.86	0.0006
Northern × Peridune	0.12	0.11
Northern × Vera	0.26	0.012
Southern × Mobile Dunes	0.85	0.029
Southern × Peridune	0.99	0.002
Southern × Vera	0.70	0.001
Peridune × Mobile Dunes	0.87	0.006
Peridune × Vera	0.16	0.04
Mobile Dunes × Vera	0.16	0.177

We analysed the similarity in assemblage composition among the sampled ponds using species presence/absence data; separate analyses were performed for amphibians, aquatic plants, and macroinvertebrates. We obtained triangular similarity matrices for each group using the Sørensen similarity coefficient (i.e., the inverse of the Sørensen dissimilarity index). To examine differences in the species compositions of the ponds, we performed three additional one-way ANOSIM analyses using the corresponding triangular similarity matrices of amphibians, aquatic plants and macroinvertebrates; geomorphological area was the grouping factor in all three analyses.

RESULTS

Pond soil classification

A non-metric MDS ordination of the 14 pond soil variables showed that differences among the geomorphological areas were mainly attributable to a strong north-south spatial gradient

Table 3. Exclusive taxa found in particular geomorphological areas in Doñana National Park (presence/absence is indicated as 1/0). The total number of taxa recorded in each area is indicated for each general group (after Florencio *et al.* 2009a; Díaz-Paniagua *et al.*, 2006; Fernández-Zamudio, 2011). Taxa in bold were found exclusively in a particular area (Adult- A and Larvae- L were different for Coleoptera and Heteroptera; Odonata refers only to aquatic larvae). *Taxones que resultaron exclusivos o característicos de lagunas de determinadas áreas geomorfológicas del Parque Nacional de Doñana (la presencia/no detección se indica como 1/0). Se indica el número total de taxa registrados en cada área (según datos de Florencio et al. 2009; Díaz-Paniagua et al. 2006). Se muestran en negritas los taxa exclusivos de un área determinada (se diferencia entre Adultos -A y Larvas -L para Coleoptera y Heteroptera, Odonata se refiere sólo a larvas acuáticas).*

	Northern stable dunes	Southern stable dunes	Mobile dunes	Peridune ecotone	La Vera ecotone
Amphibians	8	6	6	10	10
<i>Bufo bufo</i> (Linnaeus, 1758)	0	0	0	1	1
<i>Pelodytes ibericus</i> Sánchez-Herráiz, Barbadillo, Machordom & Sanchiz, 2000	0	0	0	1	1
Coleoptera	42	39	28	32	40
<i>Yola bicarinata</i> (Latreille, 1804)-A	0	1	1	0	0
<i>Hydaticus leander</i> (Rossi, 1790) -L	0	0	0	0	1
<i>Hygrotus inaequalis</i> (Fabricius, 1777) -A	0	1	0	0	0
<i>Laccobius revelierei</i> Perris, 1864 -A	1	0	0	0	0
<i>Hydrophilus pistaceus</i> (Laporte, 1840) -L	1	0	0	0	1
<i>Hydrochara flavipes</i> (Steven, 1808) -L	0	0	0	0	1
<i>Limnebius furcatus</i> Baudi, 1872 -A	0	1	0	0	0
<i>Gyrinus</i> -L	1	0	0	1	0
Heteroptera	18	16	14	14	17
<i>Microvelia pygmaea</i> (Dufour, 1833) -A	1	0	0	1	0
Odonata	9	7	5	10	8
<i>Lestes macrostigma</i> (Eversmann, 1836)	0	0	0	1	0
Large Crustaceans	4	0	0	0	3
<i>Maghrebestheria maroccana</i> Thiéry, 1988	1	0	0	0	0
<i>Cyzicus grubei</i> Simon, 1886	0	0	0	0	1
<i>Branchipus cortesi</i> Alonso y Jaume, 1991	1	0	0	0	0
<i>Tanymastix stagnalis</i> (Linnaeus, 1758)	1	0	0	0	0
<i>Branchipus schafferi</i> Fischer de Waldheim, 1834	1	0	0	0	0
<i>Streptocephalus torvicornis</i> (Waga, 1842)	0	0	0	0	1
<i>Triops mauritanicus</i> (Ghigi, 1921)	0	0	0	0	1
Aquatic Plants	65	15	28	50	60
<i>Chara vulgaris</i> L.	0	1	1	0	0
<i>Chara fragilis</i> Desv.	0	1	1	0	0
<i>Isolepis fluitans</i> (L.) R. Br.	1	0	0	1	0
<i>Juncus heterophyllus</i> Dufour	1	0	0	1	0
<i>Nitella translucens</i> (Pers.) C. Agardh	1	0	0	0	1
<i>Potamogeton natans</i> L.	1	0	0	0	0
<i>Lemna trisulca</i> L.	0	1	1	0	0
<i>Wolffia arrhiza</i> (L.) Horkel ex Wimmer	0	1	1	0	0
Total number of species	146	83	81	116	138

in carbonates and an east-west gradient in clay (Fig. 2). An ANOSIM analysis showed significant differences between the soil characteristics of the five geomorphological areas (Global $R = 0.46$, $p = 0.0001$); all the pair-wise comparisons between these areas were significant ($p < 0.05$), except the comparisons between the peridune ecotone and the northern stable dunes and between *La Vera* ecotone and the mobile dunes ($p > 0.1$, Table 2).

The pond soils located in the southern stable dunes and the mobile dunes had significantly shallower phreatic depths than the rest of the ponds (ANOVA, $F_{4,73} = 8.7128$; $p = 0.0001$, and Tukey's test) (Fig. 3). We found larger percentages of excavated ponds in these areas (Table 1), which accounted for the shallower depth of the water table at these sites. Permanent ponds only occurred at sites located in the peridune area (Table 1), together with other extensive ponds with long hydroperiods.

Fauna and flora associated with particular geomorphological areas

The Doñana pond network provides aquatic habitats for a large number of species of flora and fauna (Table 3). For macroinvertebrates, the highest taxon richness was found in ponds located in the northern stable dunes and *La Vera* ecotone, with the exception of Odonata, which were richest in the peridune ecotone. We detected idiosyncratic taxa that were exclusively found in particular areas, such as Coleoptera, Odonata and particularly the group of large crustaceans. The highest amphibian species richness was found in the peridune and *La Vera* ecotones; two species were exclusive to these habitats. The gradient of conductivity largely contributed to a clear differentiation between aquatic plant compositions from the northern (northern stable dunes, Vera and peridune area) and southern areas (mobile dunes and southern stable dunes). The lowest richness of aquatic plants was found in the southern stable dunes and mobile dunes. We did not observe any plant species that exclusively occurred in one particular area, but several species were primarily associated

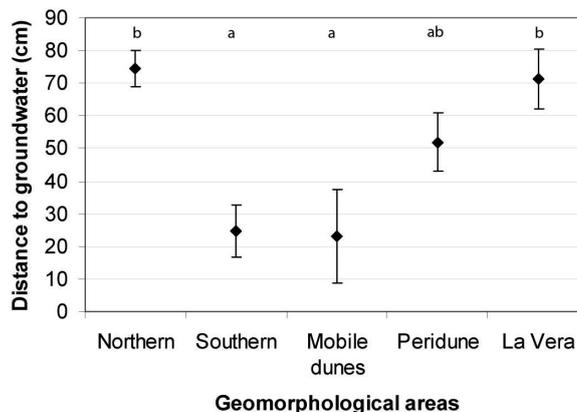


Figure 3. Mean \pm standard deviation of the depth to the groundwater table (phreatic level) of the ponds located in the five geomorphological areas described for Doñana National Park (northern: northern stable dunes; *La Vera*: *La Vera* ecotone-contact area between the northern stable dunes and marshes; peridune: peridune ecotone-contact area between the northern stable dunes and mobile dunes; mobile dunes; southern: southern stable dunes). Different letters indicate significant differences after Tukey's *post hoc* tests for the five geomorphological areas ($p < 0.05$). *Media y desviación típica de la distancia a la capa freática de las lagunas localizadas en las cinco áreas geomorfológicas descritas en el Parque Nacional de Doñana (Northern: arenas estabilizadas del norte; La Vera: Ecotono de la Vera-área de contacto entre las arenas estabilizadas del norte y las marismas; Peridune: ecotono peridunar-área de contacto entre las arenas estabilizadas del norte y las dunas móviles; Mobile dunes: Dunas móviles; Southern: Arenas estabilizadas del sur). Las diferentes letras en la figura indican diferencias significativas después de realizar el análisis *post hoc* de Tukey entre las cinco áreas geomorfológicas ($p < 0.05$).*

with two contiguous areas. Thus, particular plant species only appeared in areas with permanent waters, i.e., in the peridune ecotone that contained permanent ponds and in the southern stable dunes that mainly contained excavated ponds (Table 1 & Table 3).

Consequently, ANOSIM analyses revealed that geomorphological areas differed significantly in the composition of their macroinvertebrate (Global $R = 0.12$, $p = 0.0002$), amphibian (Global $R = 0.14$, $p = 0.0001$), and aquatic plant assemblages (Global $R = 0.33$, $p = 0.0001$). Pair-wise comparisons revealed that the assemblage composition of the three organism groups significantly differed ($p < 0.05$) between the mobile dunes and northern stable dunes, northern and southern stable dunes, and southern stable dunes and *La Vera* ecotone (Table 4). Differences

Table 4. Pair-wise comparisons obtained after one-way ANOSIM analyses performed for assemblage composition among the five geomorphological areas (northern: northern stable dunes; southern: southern stable dunes; mobile dunes; peridune: peridune ecotone and La Vera: *La Vera* ecotone). A total of three one-way ANOSIM analyses were performed, one for each biotic assemblage: macroinvertebrates, amphibians, and aquatic plants. Only p-values of significant pair-wise comparisons at $p < 0.05$ between the biological assemblages of the geomorphological areas are shown for each group of organisms (ANOSIM statistic R is close to one when groups are completely different; all non-significant pair-wise comparisons at $p > 0.06$). *Comparaciones pareadas obtenidas en los análisis ANOSIM de una vía comparando la composición de las comunidades entre las áreas geomorfológicas (Northern: arenas estabilizadas del norte, Southern: arenas estabilizadas del sur; Mobile Dunes: dunas móviles, Peridune: ecotono peridunar y Vera: ecotono de La Vera). Se realizaron tres análisis ANOSIM de una vía, uno para cada grupo de especies: macroinvertebrados, anfibios y plantas acuáticas. Sólo se muestran los p-valores obtenidos en comparaciones pareadas significativas con $p < 0.05$ entre las composiciones biológicas de las diferentes áreas morfológicas (El estadístico R de ANOSIM se aproxima a 1 cuando los grupos son completamente diferentes; todas las comparaciones pareadas no fueron significativas con un valor de $p > 0.06$).*

	R	p
Macroinvertebrates		
Mobile Dunes × Northern	0.21	0.05
Mobile Dunes × Peridune	0.22	0.013
Northern × Southern	0.28	0.0001
Southern × Vera	0.17	0.002
Amphibians		
Mobile Dunes × Northern	0.31	0.015
Northern × Southern	0.37	0.0001
Northern × Vera	0.12	0.001
Southern × Vera	0.13	0.009
Macrophytes		
Mobile Dunes × Northern	0.43	0.001
Mobile Dunes × Southern	0.19	0.038
Mobile Dunes × Vera	0.21	0.041
Northern × Southern	0.68	0.0001
Northern × Vera	0.09	0.003
Peridune × Southern	0.58	0.0001
Southern × Vera	0.57	0.0001

among additional paired areas were also found for macroinvertebrates (1 paired comparison, Table 4), amphibians (1 paired comparison, Table 4) and aquatic plants (4 paired comparisons, Table 4).

DISCUSSION

The categorisation of ponds according to their geomorphological areas resulted in a useful and robust classification system that revealed important differences in the soil types and assemblages of aquatic flora and fauna. Because wetlands in any region develop according to geomorphological processes that are driven by the interaction of climate and geology (Gilman, 1994), geomorphological and hydrological features can provide a sound basis for a global wetland classification system in which biological variation is independently addressed (Semeniuk & Semeniuk, 1997). The Doñana wetlands have been traditionally grouped into dune ponds and marshes according to their location. Both types have been further classified by size, hydrological features, and littoral vegetation (as reviewed by Serrano *et al.*, 2006). A quantitative hydro-geomorphological approach established a threshold of 1.6 mS cm^{-1} to segregate dune ponds from marshes during heavy floods (Espinar & Serrano, 2009). García-Novo *et al.* (1991) separated dune ponds into two categories based on the $\text{Na}^+/\text{Mg}^{2+}$ ratio of their surface waters, which distinguished groundwater recharge and discharge areas during floods. Further discrimination among ponds has not been achieved, most likely due to the limited amount of information that can be obtained by measuring the concentrations of dissolved or suspended materials under water level regimes that show a high degree of fluctuation (but see Florencio *et al.*, 2009b). By contrast, we used ordination to determine that the patterns of pond soil variables agree with the distribution of the different geomorphological areas located within the Doñana aeolian sands and that the southern stable dune pond soils were segregated from the pond soils in the other areas.

The geomorphological areas differed significantly in the assemblage composition of the main groups of organisms that inhabited their ponds: macroinvertebrates, amphibians, and aquatic plants. We found that the southern stable dunes and mobile dunes in particular had distinct floral and faunal assemblages, likely as a result of the particular features described

above. Thus, organisms were either distributed along a north-south gradient in electrical conductivity detected in the park (Florencio *et al.*, 2014) or their occurrence was related to specific physico-chemical requirements, as in the case of particular plant species that can only survive in ponds in which water persists for long periods or that have suitable levels of carbonates and salts (Vestergaard & Sand-Jensen, 2000). Thus, species that required more permanent waters, such as *Potamogeton natans* L., *Lemna trisulca* L., or *Wolffia arrhiza* (L.) Horkel ex Wimmer, were associated with areas where ponds with long hydroperiods are frequent (i.e., peridune ecotone, southern stable dunes and mobile dunes). In the mobile dunes and southern stable dunes, we found characteristic species, such as *Chara vulgaris* L. and *Chara fragilis* Desv., that were associated with the characteristically high carbonated and saline waters in these areas, whereas other plant species, such as *Isolepis fluitans* (L.) R. Br., *Juncus heterophyllus* Dufour, and *Nitella translucens* (Pers.) C. Agardh, were associated with low levels of carbonates and salts typically found in the northern stable dunes and the peridune ecotone. It is also important to note that the large branchiopods, which were rare and specifically detected in shallow ephemeral and short-hydroperiod ponds, exhibited low conductivities and water column P concentrations (Florencio *et al.*, 2013). Particularly, those ponds located in northern stable dunes harboured species with a special biogeographical interest, such as *Maghrebestheria maroccana* Thiéry, 1988 and *Branchipus cortesi* Alonso and Jaume, 1991, which suggests the ancient undisturbed origin of these ponds (Sahuquillo & Miracle, 2013). These ponds mainly differed from ponds located in *La Vera* ecotone by harbouring particular species in their turbid waters that were associated with fine clay sediment; these areas presented the only records of *Triops mauritanicus* (Ghigi, 1921), *Cyzicus grubei* Simon, 1886 and *Streptocephalus torvicornis* (Waga, 1842) across all the study areas.

The most remarkable feature of the Doñana pond network is its capacity to support well-preserved communities of flora and fauna. Of

the three biotic groups we examined, aquatic plants were the most significantly associated with the pond geomorphological area, most likely due to the plant species' limited mobility and their strong relationships with the substrate (Sculthorpe, 1967). Doñana ponds provide refuge for several species of high conservation status (e.g., *W. arrhiza*, *L. trisulca*, *Hydrocharis morsus-ranae* L., and *Caropsis verticillatoinundata* (Thore) Rauschert) or with restricted distributions (*P. natans* and *Avellara fistulosa* (Brot.) Blanca & C. Díaz). In the case of animal species, temporary ponds are suitable habitats for specialised aquatic species with complex life cycles and that are vulnerable to the predators typically found in permanent waters (Wellborn *et al.*, 1996), although predators are not completely absent from these temporary habitats (Fahd *et al.*, 2007). These aquatic habitats are the reproductive sites of many species that do not breed successfully in permanent waters. This is the case for many macroinvertebrates (Collinson *et al.*, 1995; Williams 1997; Céréghino *et al.*, 2008), aquatic plants (Grillas *et al.*, 2004), and amphibian species that are specialised as pond breeders (Semlitsch, 2003). The particular characteristics of the soils found in these areas increased the pond heterogeneity in the park. Thus, in the southern areas of the park, pond persistence mainly depended on the water table fluctuations, and the ponds generally lasted longer. By contrast, in the northern stable dunes and the *La Vera* ecotone, the less permeable substrate contributed to increased pond density, and pond connectivity favoured an increase in species richness in these areas. The high inter- and intra-annual heterogeneity of Doñana ponds has been reported to sustain the turnover dynamics of amphibian (Gómez-Rodríguez *et al.*, 2010) and macroinvertebrate (Florencio *et al.*, 2014) taxa and support the high nestedness of the macroinvertebrate community (Florencio *et al.*, 2011). Every year after the dry summer, macroinvertebrates must recolonise these aquatic habitats from the small number of permanent ponds that have acted as refuges for the adults of many species (Florencio *et al.*, 2011). The traditional management practice of providing

drinking water for the park's fauna during dry periods, by making existing pond basins deeper or creating new watering holes, has also generated permanent water bodies that support the biodiversity of the entire system and are essential for the conservation of many aquatic plants.

The high degree of dependence of Doñana ponds on the groundwater table should be taken into account by environmental managers. The conservation of these particular habitats should be promoted by the management of groundwater dynamics. Despite Doñana National Park being assigned the highest protection status in Spain, several studies have reported a significant reduction in the length of the aquatic phase of long-hydroperiod ponds over the last decade due to groundwater extraction resulting from urban water use in the nearby tourist resort of Matalascañas (Serrano & Zunzunegui, 2008; Serrano *et al.*, 2008; Sousa *et al.*, 2009). In general, groundwater overexploitation has been reported in the northern stable dunes and adjacent areas due to improper water management by intensified agriculture in plastic greenhouses (Muñoz-Reinoso, 2001; Manzano & Custodio, 2006). If the severity of this situation is overlooked, the future of the largest network of temporary ponds in Western Europe will be at risk.

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