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Assessing the patterns of the invertebrate community in the marshes of Doñana National Park (SW Spain) in relation to environmental factors

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ABSTRACT

Assessing the patterns of the invertebrate community in the marshes of Doñana National Park (SW Spain) in relation to environmental factors

The marshes of Doñana (SW, Spain) are some of the largest and best preserved Mediterranean marsh areas represented in Western Europe. They are considered a hotspot of biodiversity, and as such receive protected-area status under two different systems of protected-area management. The importance of submerged macrophytes in the functioning of marsh ecosystems has been addressed in several studies. However, most of the animal biodiversity studies have been developed for vertebrates. Thus, the aims of this study are i) to assess the composition of the invertebrate community in the marsh of Doñana (zooplankton and zoobenthos) in a set of sites representing the different habitats of the marsh in both *clear* and *turbid water* states; ii) to compare diversity among patches in different states (clear *vs* turbid water); and iii) to address the main environmental factors that have influenced their community structure and diversity. A total of 102 taxa were recorded. The highest abundance values were attained by cladocerans and ostracods, both microcrustaceans, and by dipteran insects. It was possible to distinguish different marsh environments characterised by patches of *clear water*, where the macrophyte beds contribute to an increase of the structural heterogeneity of the marsh, providing the invertebrates with shelter and food resources and subsequently influencing different invertebrate assemblages. Conductivity, soluble reactive phosphorus (SRP) and chlorophyll-*a* concentration were the environmental variables that influenced the presence of turbid patches, which showed lower macrophyte cover, diversity and richness values than those seen in the clear water patches. The relationship between diversity (H′) and richness (S), suggested that processes related to species migration (i.e., hydrologic connection with other water bodies, flood duration, and dissemination of propagules) are the main constraints influencing the invertebrate community structure in the Doñana marshes.

Key words: Invertebrates, marsh, diversity, evenness, CCA.

RESUMEN

Evaluación de los patrones de la comunidad de invertebrados en las marismas del Parque Nacional de Doñana (SO España) en relación con los factores ambientales

Las marismas de Doñana (SO España) constituyen una de las áreas de marismas de la región mediterránea más grandes y mejor conservadas de Europa y son consideradas uno de los humedales más importantes de la Europa occidental. Constituyen un punto caliente de biodiversidad y poseen diferentes figuras de protección. La importancia de los macrófitos sumergidos en el funcionamiento de la marisma ha sido documentada en numerosos estudios. Por otro lado, la mayor parte de los estudios de descripción de la biodiversidad animal se han focalizado en los vertebrados. De modo que los objetivos de este estudio son: i) describir la composición de la comunidad de invertebrados acuáticos de la marisma de Doñana (zooplancton y zoobentos) en un conjunto de estaciones de muestreo representativas de los diferentes ambientes de la marisma que estén en aguas claras y en aguas turbias; ii) comparar la diversidad entre los parches en distintos estados (claras vs turbias); y iii) caracterizar los principales factores ambientales que influyen en la estructura de la comunidad y su diversidad. Se registraron un total de 102 taxa (las abundancias más altas fueron alcanzadas por los microcrustáceos, cladóceros y ostrácodos, y los dípteros). En la marisma se distinguieron diferentes ambientes durante el período de aguas claras, donde las praderas de macrófitos contribuyeron a aumentar la heterogeneidad estructural de la marisma proporcionando refugio y recursos

*alimenticios a los invertebrados. Las variables ambientales que más contribuyeron a caracterizar las estaciones en aguas turbias fueron la conductividad, el fósforo reactivo soluble (PRS) y la clorofila-*a*, con valores más bajos de cobertura de macrófitos sumergidos, diversidad y riqueza que los parches en aguas claras. La relación entre diversidad (H′) y riqueza (S) sugirió que las principales fuerzas que limitan la estructura de los invertebrados de las marismas de Doñana están relacionadas con procesos como la migración y dispersión de las especies (p. ej. la conexión hidrológica con otros cuerpos de agua, la duración de la inundación, y la diseminación de los propágulos).*

Palabras clave: *Invertebrados, marisma, diversidad, equitatividad, CCA.*

INTRODUCTION

Marshes are wetlands dominated by herbaceous plants emerging through the water and rooted in hydric soils constrained by periods of flooding, periods of disturbance, periods of drought and periods of competition (Keddy, 2000). There are two main types of marshes: *tidal salt marshes*, which are brackish and influenced by ocean tides characterised by wet and dry cycles, salinity and large temperature fluctuations that limit diversity, and *freshwater tidal marshes*, which, although influenced by tides, are not saline and thus often have higher biodiversity and production than their salt marsh counterparts (Dodds & Whiles, 2010). The latter have a high ecological value because they are repositories for high levels of biodiversity and provide substantial ecological services and functions to society (Skinner & Zalewski, 1995; Zedler & Kercher, 2005). They are also one of the most remarkable wetland environments while also being the most threatened ecosystems in the Mediterranean world (Grillas *et al.*, 2004).

One of the largest and best preserved Mediterranean marsh areas represented in Europe is located in Doñana (SW Spain). This area is considered to be one of the most important wetland areas in western Europe, being a UNESCO Biosphere Reserve (1980), a Ramsar Site (1980), included in the Montreux Record (a register of Ramsar sites where changes in ecological character have occurred, are occurring or are likely to occur) in 1990 and a Natural World Heritage Site (1994). The marshes of Doñana are also integrated into the Natura 2000 network (García-Novo & Marín, 2005). In fact, natural and transformed marshes occupy nearly half of the areal extent of Doñana Natural Space (DNS), a protected area resulting from the merger of the former National Park (1978) and Natural Park (1989), covering a total of 113,644 ha (Bravo-Utrera, 2010). These marshes are vast wetland areas in which estuarine and tidal ecosystems co-exist with freshwater ecosystems located in a floodplain, which is principally fed by rain in autumn and also by water entering the area through a number of small streams (e.g., La Rocina, el Partido). In summer, the area dries out for several months, promoting the spatial and temporal heterogeneity of this system and allowing the existence of multiple habitats and high species diversity (García-Novo & Marín, 2005; Serrano *et al.*, 2006). Nonetheless, the Doñana marshes, similar to other wetlands in Spain and elsewhere, have been subjected to very large human pressures (e.g., agriculture, desiccation, and water extraction for urbanisation). These pressures cause major changes, such as the reduction of the marsh surface by almost 80 %, so that today, the untransformed marsh area occupies just over 27,000 ha (Ojeda-Rivera, 1987; Ojeda-Rivera & Moral, 2004; Serrano & Zunzunegui, 2008; Urdiales *et al.*, 2010).

Bearing in mind the ecological importance of the site since its initial protected area designation in the 1970s, a number of research groups have completed several studies in the area. These studies have aimed to understand the structure, dynamics and functioning of this complex system but have focused principally on vegetation and terrestrial vertebrate fauna, often excluding aquatic ecosystems altogether (Millán *et al.*, 2005 and the following references of the Introduction are listed in Appendix 1, www.limnetica.net/internet). This is particularly

true for the aquatic invertebrates present in the marsh. It is important to note that a small number of studies characterising aquatic invertebrate communities have been carried out; however, these were mainly completed in dune ponds outside the marshland area. With respect to the class Insecta, the order Coleoptera (beetles) is possibly the best-known group in the Doñana marsh (Montes *et al.*, 1982a, 1982b; Montes & Soler, 1986; Garrido *et al.*, 1997; Castro *et al.*, 2003; Millán *et al.*, 2005). Other insect clades that have been reviewed include the families *Culicidae* (Jordá *et al.*, 1993) and *Chirono-midae* (Jordá *et al.*, 1996) within Diptera and the orders Odonata (Ferreras *et al.*, 2005) and Heteroptera (Nieser & Montes 1984). There are also studies, such as those of Montes *et al.* (1982c) and Montes & Ramirez-Díaz (1983) that have looked at groups of Odonata, Heteroptera and water beetles. Regarding the abundant micro-invertebrate groups, Boix *et al.* (2007) compared the biodiversity of different coastal wetlands, including data of crustacean species from 18 scientific publications completed in Doñana, while Fahd *et al.* (2009) reviewed 19 scientific publications and 4 unpublished studies that studied crustaceans in the Doñana marsh from 1962 to 2007 (see also Badosa *et al.* 2010; Frisch *et al.* 2012). It is also worth mentioning that particular attention has been paid to the alien invasive American crayfish (*Procambarus clarkii*) in the marsh, with many publications written about its biology, physiology, trophic ecology, impact on the environment and assessment as a bioindicator (Gutiérrez-Yurrita, 1998; Alcorlo *et al.*, 2004, Geiger *et al.*, 2005; Crehuet *et al.*, 2007; Cruz & Rebelo, 2007; Alcorlo *et al.*, 2008; Vioque-Fernández *et al.*, 2007, 2009; Crehuet *et al.*, 2009; Noguerales *et al.*, 2010; Tablado *et al.*, 2010; Alcorlo & Baltanás, 2013).

With the exception of the crayfish research, most of the studies mentioned above are descriptions of the composition of particular invertebrate taxa; therefore, there is a scarcity of information related to the functional role of these organisms within the ecosystem and the environmental factors influencing the marshland invertebrate biodiversity, although conductivity, surface area, and vegetation cover have been found to be the main factors that explain the structure of the microcrustacean communities, as described in Frisch *et al.*, (2006). Indeed, there are several studies that demonstrate the importance of submerged macrophytes in marsh-ecosystem functioning (Duarte *et al.*, 1990; Murillo *et al.*, 2006, Reina *et al.*, 2006), while others describe the importance of the coexistence of patches of the marsh in a *clear-water* state (i.e., patches with well-developed submerged macrophyte beds, high water column transparency and high biodiversity) with patches in a *turbid-water* state (i.e., high turbidity due to sediment resuspension and/or phytoplankton biomass and low biodiversity) (Gómez-Baggethun *et al.,* 2011). Thus, the aims of this study were 1) to assess the composition of the invertebrate community in the marsh of Doñana (zooplankton and zoobenthos) in a set of sites representing the different habitats of the marsh in both *clear* and *turbid water* states, 2) to compare diversity among patches in different states (clear *vs* turbid water), and 3) to elucidate the main environmental factors that have influenced their community structure and diversity.

MATERIALS AND METHODS

Study area

The Doñana marsh, found in the lower Guadalquivir River basin (371°N, 61°W) in southwestern Spain, is located in a floodplain with silty-clayey, calcareous and saline materials. The marshland is seasonally flooded by freshwater supplied by direct rainfall, two streams (El Partido stream and La Rocina brook) and groundwater flows (Serrano *et al.*, 2006). The average annual rainfall in the region is approximately 600 mm, and real evapotranspiration is estimated to be between 400 and 500 mm/y. The water level may vary by several centimetres as wind force and intensity change, although the prevailing winds are from the west (Bayán, 2005). The marsh follows a marked annual cycle. It fills up in October-November, reaching an average depth of 20 cm, and remains flooded until

Figure 1. Locations of the studied sampling sites in the Doñana marsh. White circles represent sites with *clear waters*, and black circles represent the *turbid* ones. *Localización de las estaciones de muestreo estudiadas en la marisma de Doñana. Los círculos blancos representan estaciones en aguas claras, y los negros en aguas turbias.*

March or April, forming an inundated surface area of 27,000 ha that falls within the boundaries of Doñana National Park (53 835 ha). Af-

terwards, net water losses through evapotranspiration gradually dry out the marsh. By the end of the summer, only the deepest depressions re-

Table 1. Locations of the sites and summary of their marsh's habitat type (*Marisma de Hinojos*: typical flat marsh; *Lucio* and *Hondura*: depressions that hold the water for a longer time; *Veta*: small sandy elevations; *Caño*: river branches) and their ecological state (*clear waters*: predominance of submerged vegetation, *turbid waters*: predominance of phytoplankton or resuspended sediments). *Localización de las estaciones de muestreo y del tipo de hábitat de la marisma que representa (Marisma de Hinojos: típica marisma somera y plana; Lucio y Hondura: depresiones que retienen agua durante más tiempo; Veta: pequeñas elevaciones arenosas; Caño: brazos fluviales); y su estado ecológico (aguas claras: predominio de vegetación sumergida, aguas turbias: predominio de fitoplanctyon y/o sedimentos resuspendidos).*

Site	Location UTM (29S)		Habitat type	Ecological state
	X	Y		
M01	197533	4103678	Marisma de Hinojos	Turbid
M02	197323	4103822	Marisma de Hinojos	Clear
M03	194987	4104401	Lucio	Clear
M04	196608	4098079	Water-filled depressions near Veta	Clear
M05	196668	4097912	Water-filled depressions near Veta	Turbid
M06	196913	4098179	Water-filled depressions near Veta	Clear
M07	191982	4112542	Caño	Turbid
M08	195308	4104413	Lucio	Clear
M09	194598	4097354	Water-filled depressions near Veta	Turbid
M10	194265	4096693	Lucio	Clear
M11	207750	4107948	Caño	Turbid
M12	197425	4103449	Marisma de Hinojos	Turbid
M13	196975	4103651	Marisma de Hinojos	Clear
M14	192291	4111921	Caño	Turbid
M15	194500	4096760	Lucio	Turbid
M16	194007	4096111	Lucio	Clear
M17	194291	4103719	Caño	Turbid
M18	194473	4103218	Lucio	Clear
M19	197046	4097895	Hondura	Clear
M20	207314	4101007	Lucio	Turbid
M21	207005	4099961	Lucio	Clear
M22	207438	4101586	Lucio	Clear waters
M23	207588	4102383	Caño	Turbid
M24	205360	4102176	Caño	Turbid
M25	205468	4102955	Caño	Turbid
M26	203605	4103370	Caño	Turbid
M27	203560	4103263	Lucio	Clear

tain highly saline waters (shallow ponds, gullies and some channel stretches) (Montes *et al.*, 1993, Bayán, 2005, Geertz-Hansen *et al*., 2010).

Sampling methods

This study was performed at 27 sites distributed throughout the Doñana marsh, selected to repre-

sent the variety of conditions that occur in such a heterogeneous system. The study was conducted in May 2007 (from the 1st to the 14th) during the flooded period, when there are lush macrophyte beds and associated invertebrate communities (Fig. 1, Table 1). All sampling sites were assigned to one of the two following ecological states: *clear water,* those sites with transparent

water and extensive macrophyte cover, and *turbid water,* those lacking or with very scarce macrophyte cover and with low visibility of the bottom. This procedure was formerly used by Bayley & Prather (2003) and Bayley *et al.* (2007) in other wetlands and in previous work carried out by our group in the Doñana marshland, as described by Gómez-Baggethun *et al.* (2011).

At each site, an array of environmental variables (water depth, water temperature, pH, electric conductivity, dissolved oxygen concentration) was recorded with a WTW Multi350i multiprobe, while turbidity was measured using a HANNA HI91703 turbidity meter. As proposed by Scheffer (2009), water samples were also taken for later determination of the descriptors more tightly linked to the existence of clear/turbid water regimes, i.e., turbidity, soluble reactive phosphorus (SRP), and total phosphorus (total-P) in both water and the sediments. SRP (µg/l) was measured following APHA protocols and then filtered in the field with a Whatman GF/C filter (1.2 µm nominal pore size), stored in dark glass bottles and kept refrigerated until being processed in the laboratory. Samples for total-P were stored in dark glass bottles and kept refrigerated until being processed in the lab using the APHA methodology (ascorbic acid method after persulfate digestion) (Clesceri *et al.*, 1999). Sediment samples of the upper 3 cm layer were obtained using a cut-down syringe (3 cm diameter), stored in plastic bags and kept frozen until analysis. The content of total-P in sediments, hereafter P_{sed}, was estimated using the ignition method (Andersen, 1976). The organic matter content of the sediments (measured as loss of ignition, LOI) was determined by the combustion of samples in porcelain crucibles at 550 °C for 4.5 h in a muffle furnace. The final product of the combustion was expressed as the ash content of the sediment (Clesceri *et al.*, 1999). Samples for the estimation of chlorophyll-*a* concentration (µg/l), here used as a proxy for phytoplankton biomass, were filtered *in situ* (GF/C filter) and kept dark and cool (4 °C) until being processed in the lab (acetone extraction, 90 % v/v) (Jeffrey & Humphrey, 1975). Following Prodon (1988),

macrophyte abundance was estimated as the percentage of plant cover observed in three sampling quadrats (50 cm^2) randomly placed at each site. Macrophyte richness was recorded simultaneously in all sampling quadrats. The same procedure was applied to estimate helophyte coverage and richness. Identification of aquatic plants was done following descriptions offered by García-Murillo *et al.* (1993, 2006, 2009).

Invertebrate community samples were obtained by sweeping a 250 µm mesh size hand net along 20 m transects with a width of 1 m, covering a sampling area of 20 m^2. The organisms were stored in 4 % formalin until being identified and counted in the lab using binocular dissecting microscopes (Leica MZ6 and MZ12) and a microscope (Olympus BH-2).

Identification of invertebrates

Most invertebrate taxa were recognised as species, though many were not given a species name due to the difficulties in performing a correct identification using the specialised determination keys (i.e., Copepoda, Diptera) available in the lab and detailed below. Nematodes and worms (Oligochaeta) were the exception to that rule and were identified only to class. Rotifera and ciliates were occasionally found in the samples, but they were excluded from the analysis because their abundance was severely underestimated due to the mesh size used in sampling. Several specific invertebrate keys were used (including Froglia, 1978; Argano, 1979; Girod *et al.,* 1980; Conesa, 1985; Larranz & Equisoain, 1993; Nieser *et al.*, 1994; Alonso, 1996; Barrientos, 2004; Tachet *et al.*, 2000, references cited in Appendix 1, www.limnetica.net/internet).

The following community parameters were calculated using quantitative data of the pooled invertebrate community: species richness (S), diversity (H′), calculated with the Shannon index (Shannon & Weaver, 1963), and evenness (J′) (Pielou, 1969), both based on the numerical abundance of each taxon and expressed as $\log_2$. Nematoda and Oligochaeta were excluded because they were not identified at the species level. Further assessment of the relationships between

invertebrate distribution and environmental variables were performed using the abundances of the identified taxa as explained above.

Data analyses

Prior to any statistical analysis, the normality of environmental and biological variables was examined, and appropriate transformations [$\log(x)$, $\log(x) + 1$ or $\text{arcsine}(x)$] were used when necessary (Zar, 1999).

For all the analyses, a species with a maximum abundance less than 10 % of the total abundance of its faunal group in a sample was considered to be a rare species and excluded from the analysis. Those sites where there were no animals were also excluded. Linear regression analysis was used to explore the relationship between the measured diversity and richness ($\log_2 S$). A one-way ANOVA was performed to analyse the relation of the estimated diversity (H') to the clear-*vs* turbid-water state of the sites, and a Mann-Whitney U test was used to compare S and J' for clear and turbid patches.

A Canonical Correspondence Analysis (CCA) was carried out to analyse the relationships between taxon abundance and the main environmental variables. A Monte Carlo test (9999 permutations, $p < 0.05$) was performed to test the significance of the canonical axes. All CCA were carried out using CANOCO 4.5.

Further explorations of the relationships between environmental variables retained in CCA with H and $\log_2 S$ were performed through general linear models (GLM) and multiple regression analysis using H and $\log_2 S$ as dependent variables. All analyses, except CCA, were done using Statistica 8.0.

RESULTS

The location of sampling sites was distributed to capture the representativeness of the different habitat types (e.g., *vetas, lucios,* and *caños*) placing a similar number of sites within each of them (Fig. 1, Table 1). Most of the recorded descriptor variables showed a wide range of variation (represented by the high coefficient of variation) and reflected the heterogeneity of the environments surrounding each site. Thirteen of the sampled sites were in a clear-water state, and fourteen were in turbid states (Table 2). Finally, four sites were excluded from analyses due to the

Table 2. Summary of the averages, ranges and coefficients of variation (%) of the environmental variables recorded at sites with clear waters and with turbid waters. *Resumen de los valores medios, rango y coeficiente de variación (%) de las variables ambientales medidas en las estaciones en estado de aguas claras, y de aguas turbias.*

	CLEAR WATERS (*n* = 13)			TURBID WATERS (*n* =14)		
	average	range	V(%)	average	range	CV (%)
Conductivity EC$_{25}$ (µS/cm)	3906	1392-8100	56.6	4610	211-8550	71.2
Dissolved oxygen (%)	99.7	28.8-220.0	55.2	133.3	52.6-184.6	30.8
Dissolved oxygen (mg/l)	8.2	2.4-17.3	51.4	10.9	4.4-16.2	32.9
pH	8.3	7.3-9.9	10.1	8.7	7.3-10.4	11.5
T (°C)	24.4	20.4-29.5	10.9	25.4	19.1-30.1	13.8
Water level (Z, cm)	23	12-30	27	28	7-43	37
Turbididity (FTU)	25	6-53	52	47	3-67	78
SRP (µg/l)	99	23-212	47	157	13-424	81
Total P (µg/l)	295	84-748	59	301	33-795	81
Total P sed (µg/l/g)	5014	3371-8317	35	5223	640-11366	67
Organic Matter content (%)	57	46-65	11	55	48-62	8
Submerged macrophytes coverage (%)	60	20-90	35	0	0-30	76
Helophytes coverage (%)	20	0-70	131	0	0-80	275
Chlorophyll-*a* (µg/L)	9.6	1.0-46.7	90.9	23.2	1.1-44.8	138.2

absence of all animals except ostracod's valves (M03, M11, M14 and M26).

Invertebrate community structure

A total of 102 taxa were described in the samples: Nematoda and Oligochaeta, with all the species pooled together in both cases; 4 species of Gastropoda; 4 of Copepoda; 12 of Branchiopoda; 20 of Ostracoda; 3 of Malacostraca; and 57 of Insecta (Appendix 2, www.limnetica.net/internet). After a review of the species matrix, 31 were excluded because they were only present at one site. No records of the invasive heteropteran *Trichocorixa verticalis* were obtained despite the fact that it has been found abundantly in subsequent samplings (Rodríguez-Pérez *et al.,* 2009;

van de Meutter *et al.*, 2010). The most common species, present at 70 % of the sites, were the microcrustaceans *Plesiocypridopsis newtonii* and *Cyclopoida* sp2. and the insects *Anisops debilis*, *Berosus* sp. and *Orthocladiinae* sp2. The highest value of richness (S) was 31 species, recorded at site M05 (*clear water* state), while the minimum was 5 species at site M17 (*turbid water* state), with an average value of 20.43 (± SD= 8.35). Indeed, clear water patches had higher values of S than the turbid ones ($U = 23$, $Z = 2.64$, $p < 0.05$).

The groups with the highest abundance values were the microcrustaceans Anomopoda (*Ceriodaphnia quadrangula* 162.7 ind/m², *Macrothrix rosea* 86.2 ind/m², *Simocephalus exspinosus* 79.15 ind/m²), Ostracoda (*Plesiocypridopsis new-*
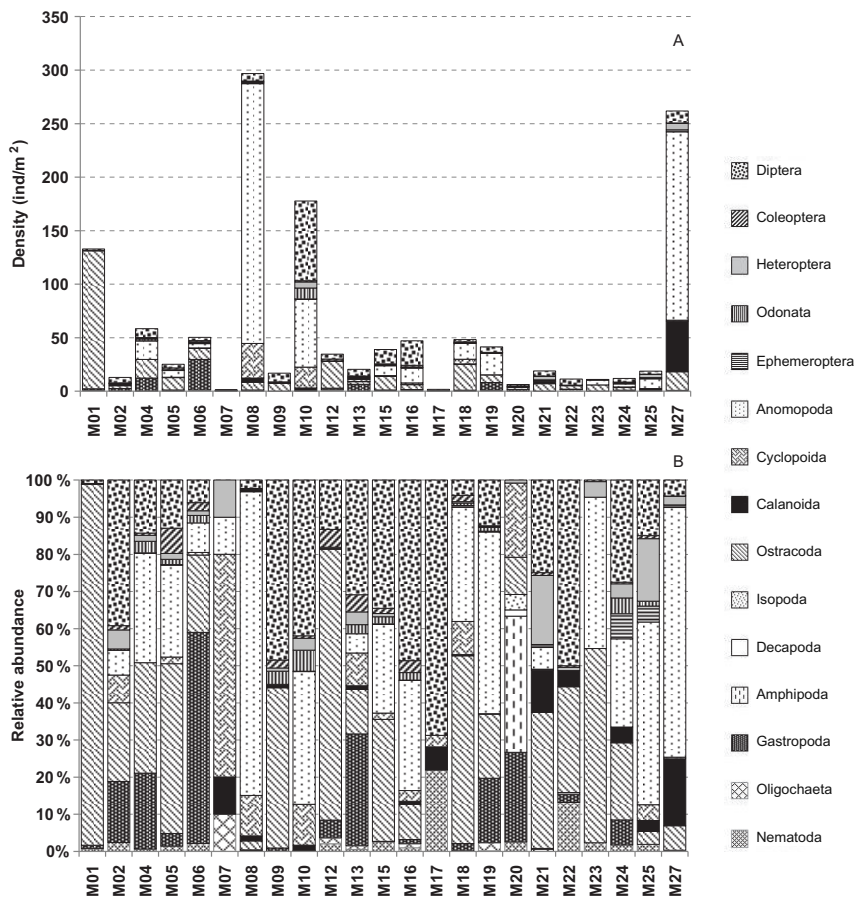

Figure 2. Density (ind/m²) (A) of the taxonomic groups recorded at each sampling site, and their relative abundance (%) (B). *Densidad (ind/m²) (A) de los grupos taxonómicos registrados en cada estación de muestreo; y su abundancia relativa (%) (B).*

tonii, *Potamocypris unicaudata*, *Ilyocypris getica*, all with maximum abundances of 43 ind/m^2), and dipterans (*Orthocladiinae* spp., 41.7 ind/m^2) (Fig. 2A). The sites that exhibited the highest invertebrate density values were almost all in the clear-water state (M04: 58.45 ind/m^2, M08: 296.7 ind/m^2, M10: 177.7 ind/m^2, M18: 48.15 ind/m^2, M27: 261.8 ind/m^2), while two were in the turbid state (M01: 132.8 ind/m^2, M06: 50.2 ind/m^2) (Fig. 2A). Conversely, the relative abundances of the different invertebrate groups considered showed a more even distribution throughout the sites ($U = 45.5$, $Z = 1.26$, $p > 0.05$) (Fig. 2B), a result that agrees with the estimated evenness (J′), which displayed an average value of 0.57 ($\pm$ SD = 0.13) and a range of 0.31 to 0.77 (Fig. 3).

Relationships between diversity and the clear/turbid water state

The estimated diversity (H′) showed a positive and direct relationship with richness, expressed as $\log_2(S)$ ($r^2 = 0.54$, $p < 0.05$) (Fig. 3). Maximum H′ values were recorded in sites in a clear-water state (M21: 3.74 bits/ind, M13: 3.65 bits/ind, M02: 3.6 bits/ind), and the minimum was recorded in the turbid-water state site M17 (1.54 bits/ind). In addition, the average values of H′ estimated in sites with clear water were greater than those registered at sites with turbid water ($F_{(1,21)} = 4.2589$, $p < 0.1$) (Fig. 4).

Relationships between invertebrate abundances and environmental variables

Seventy-one species and 8 environmental variables (oxygen concentration, electric conductivity, pH, depth, SRP, chlorophyll-*a*, and helophyte and submerged macrophyte coverage) were retained for the CCA. The first two axes showed a total variance of 48.5 %, with both being significant results (F-ratio = 2.111, $p < 0.01$ for the first axis; $F = 1.361$, $p < 0.01$ for the second one). The first axis separated the sampled sites according to their pH and electric conductivity (inversely correlated) and macrophyte coverage (directly and strongly correlated). The second axis was inversely correlated with submerged macrophyte coverage and directly correlated with
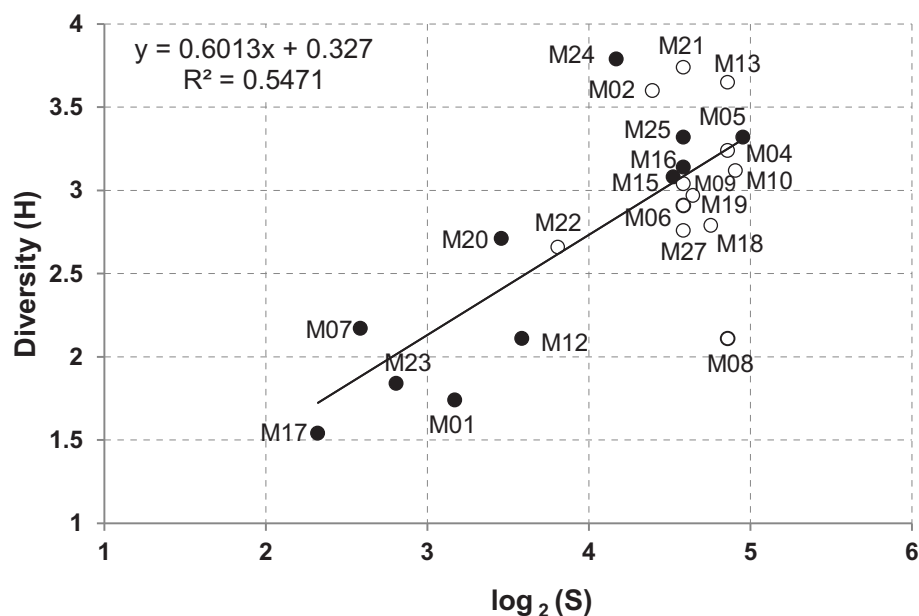

Figure 3. Linear relationship between diversity (H′) and $\log_2$S. White circles represent sites with *clear waters*, and black circles represent the *turbid* ones. *Relación lineal entre la diversidad (H′) y log$_2$S. Los círculos blancos representan estaciones en aguas claras, y los negros en aguas turbias.*

chlorophyll-*a*. For instance, it is possible to distinguish sites in a *clear water* state with high macrophyte coverage, low values of conductivity and the lowest pH values from those sites with the highest chlorophyll-*a* and SRP values (Fig. 5A).

The species distribution agrees with this ordination (Fig. 5B); Anomopoda, Calanoida and Cyclopoida species included in the analysis appeared mostly in the ordination space occupied by the sites with clear water, while ostracod species appeared and were associated with all the environmental conditions. Among insects, Odonata and most of the larvae of the Coleoptera species appeared and were associated with helophyte and submerged macrophyte coverage. Other species present in most of the sites, such as heteropterans or dipterans, appear in the centre of the figure.

The results of the multiple regression analysis of H′ on the environmental variables contributed more towards explaining the variance seen in the two most significant axes of the CCA (electric conductivity, SRP, chlorophyll-*a*, and submerged macrophyte coverage). Using this combination of predictors (multiple $R = 0.67$, $r^2 = 0.45$, $F_{(4,17)} = 3.56$, $p = 0.02$), 45 % of the variation of H′ was explained. Indeed, electric conductivity ($\beta = 0.70$, $r^2 = 0.20$, $p < 0.05$) and macrophyte coverage ($\beta = 0.70$, $r^2 = 0.33$, $p < 0.05$) were the explanatory variables. Moreover, no significant results were obtained for the same type of analysis between $\log_2(S)$ and the same predictors.

DISCUSSION

Our results reflect that the measured limnological variables showed a wide range of variation in the sampling sites of the marshland, although some of them showed a variation pattern that concurs with the environmental gradients described by Espinar *et al.* (2002) and causes the submerged macrophyte zonation in the marsh. The distribution of submerged macrophytes in the marsh of Doñana was found to be dependent on two independent environmental factors, a dominant gradient of flooding/salinity and a secondary gradient of nutrients related to the abundance of emergent macrophytes or helophytes (Espinar *et al.* 2002). In our work, the ordination space of the sites exhibited in the CCA showed how macrophyte coverage (i.e., helophyte plus submerged vegetation) and lower conductivity, pH and chlorophyll-*a* values were strongly correlated with the clear-water state. In addition, sites with clear water showed higher invertebrate abundance values, as well as richness and diversity values, than those seen in the turbid water sites (Fig. 5A). Conductivity (i.e., salinity) is one of the well-known environmental variables that negatively affects the species richness and influences the community structure in wetlands (e.g., Frisch *et al.*, 2006; Waterkeyn *et al.*, 2008; Gascón *et al.*, 2009; López-Flores *et al.*, 2014). In the marsh of Doñana, there is a salinity gradient from east to west during the flooding period (Llamas *et al.,* 1987), mainly due to the discharge of very low-salinity groundwater from the unconfined aquifer that surrounds the marsh and La Rocina stream basin, providing freshwater incoming flows in the Northeastern part of the marsh. In the Doñana marsh, salinity is a key environmental factor with a very strong seasonal variation that is spatially controlled by small differences in depth (Montes *et al.*, 1982 a, b), which in turn determines the community structure. This salinity gradient negatively influences the growth and production of macrophytes and consequently the floristic composition of their assemblages (Duarte *et al.,* 1986; Grillas *et al.*, 1993), as found in other Mediterranean marshes (Grillas, 1990) and in the structure of the clear-water patches sampled.

In addition, the influence of macrophyte cover in the invertebrate community structure has been addressed in experimental field studies that have analysed the influence of submerged macrophytes on the creation of structural heterogeneity through the provision of refuges and food resources (Taniguchi *et al.*, 2003). Thus the existence of patches with clear water containing submerged macrophytes acts as an important local factor influencing the invertebrate community structure.

The other variables explaining the CCA ordination, SRP and chlorophyll-*a*, were related
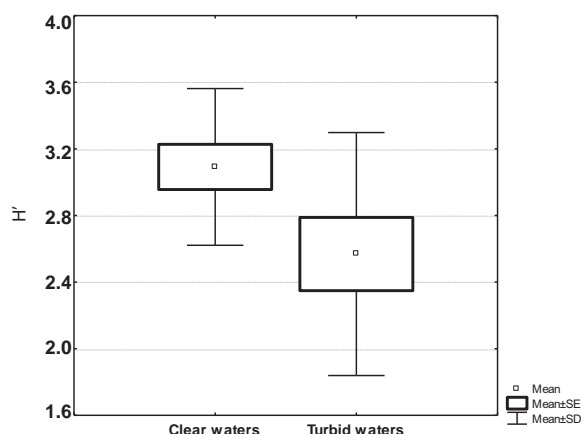
Figure 4. Comparison between average diversity (H′) values calculated using the Shannon index at sites with clear waters and sites with turbid waters. *Comparación de los valores medios de la diversidad (H′) calculada con el índice de Shannon entre las estaciones en aguas claras y las de aguas turbias.*

to trophic status and discriminate the sites with abundant phytoplankton concentration and high SRP concentrations (e.g., M06, M18, M17, M20) from the ones with lush macrophyte beds (e.g., M10, M13, M16, M19, M27). As the hydroperiod reaches an end in the marsh, the total depth of the water column decreases and phytoplankton and nutrients become concentrated. Additional nutrients (e.g., SRP) are liberated into the water when the macrophyte beds begin to die, thus providing the conditions that promote the occurrence of phytoplankton blooms (Espinar *et al.* 2002, García-Novo *et al*., 2007).

Average (± SD) invertebrate diversity (H′ = 2.84 ± 0.65) values were similar to those found in other freshwater marshes (Kang & King, 2013) and were larger in patches with clear water than in those with turbid water ($F_{(1,21)}$ = 4.2589, $p < 0.1$) (Fig. 4); this is also true for invertebrate abundance (Fig. 2A). Both can be explained by the increase in habitat complexity and heterogeneity due to the macrophyte beds, as previously mentioned. The average (± SD) evenness (J′ = 0.57 ± 0.13) showed that there was an equivalent contribution of the relative abundance of the different species at all of the sites (Fig. 2B), showing that the invertebrate community is being modulated by factors other than the presence of macrophytes (i.e., affecting their

life history traits), such as the flooding duration or the water depth (Serrano & Fahd, 2005; Kang & King, 2013).

The distribution of some taxa is largely explained by their physiological adaptations to salinity and trophic behaviour (Fig. 2B). Some Anomopoda species, such as *Macrothrix rosea* and *Tetrocephala ambigua*, live in clear water with abundant vegetation (Alonso, 1996), and they appeared in this part of the CCA ordination space. Others, such as *Leydigia acanthocercoides, Chydorus sphaericus* and *Ceriodaphnia quadrangula*, are considered littoral species (Alonso, 1996) and appeared separate from the former group. Ostracods and dipterans (*Chironominae* spp*., Orthocladiinae* spp.) are generalist species, most of which are detritivores, and they appeared in different positions in the ordination space of the CCA, reflecting their wide environmental tolerance. The same pattern was found for heteropterans, the coleopteran *Pomatinus substriatus,* and other detritivore taxa, such as the nematodes and oligochaetes. Scrapers, such as the gastropods (*Planorbidae* spp., *Physa acuta*) and the filter-feeding dipterans (*Dixidae* spp., *Rhagionidae* spp.), were associated with higher chlorophyll-*a* and SRP concentrations. Carnivores, such as Odonata (*Libellulidae* spp., *Coenagrionidae* spp., *Aeshnidae* spp.) and coleopterans (*Dytiscidae* spp.), appeared to be associated with macrophytes (submerged and helophytes) (Cummins, 1973, 1975), near which, it can be supposed, they find shelter and prey. The omnivorous beetle *Haliplus* sp. also appeared to be associated with macrophytes.

The relationships of S, J′ and H′ have been explored and reviewed by several authors (Stirling & Wilsey, 2001; Wilsey & Sterling, 2007; Hillebrand *et al*., 2008; Isbell *et al*. 2009). The "neutral model" of Caswell (1976) estimated a slope of 0.75 for the relationship between H' and S and was highly influenced by migration (e.g., S), whereas scale (e.g., α, β and γ diversities) and biotic interactions (competition or predation) did not affect these variables. Stirling & Wilsey (2001) compiled data from 36 published papers and used univariate and multivariate regression analysis to explore the relationships among H′, J′
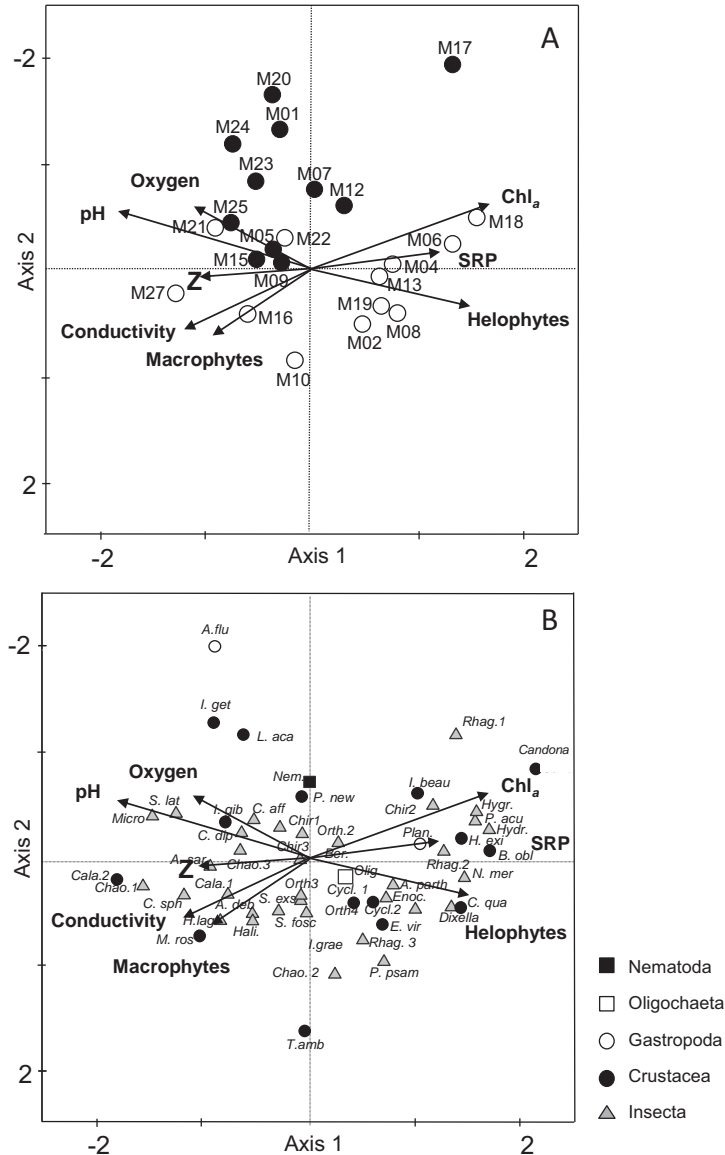
Figure 5. CCA ordination diagram showing the distribution of the sampling sites (A) and the loading of families (B) in relation to environmental variables in the space represented by the two first axes. White circles represent sites with clear waters, and black circles represent the turbid ones. Abbrevation taxa lists: Nematoda (Nem.); Oligochaeta (Olig.); *Physa acuta* (P. acu); *Planorbis (Plan.); Ancylus fluviatilis (A. flu); Candona (Cand.); Fabaeformiscandona fragilis (F. fra); Bradleycypris obliqua (B. obli); Plesiocypridopsis newtoni (P. new); Cypridopsis vidua (C. vid); Potamocypris cf unicaudata (P. uni); Heterocypris exigua (H. exi); Eucypris virens (E. vir); Isocypris beauchampi (I. beau); Ilyocypris gibba (I. gib); Ilyocypris getica (I.get); Paralimnocythere psammophila (P. sam); Calanoida sp. 1 (Cala 1); Calanoida sp. 2 (Cala 2); Cyclopoida sp. i (Cycl i); Simocephalus exspinosus (S. exs); Ceriodaphnia quadrangula (C. qua); Leydigia acanthocercoides (L. aca); Tretocephala ambigua (T. amb); Chyodorus sphaericus (C. sph); Macrothrix rosea (M. ros); Cloeon dipterum (C. dip); Ischnura graellsi (I. grae); Anax parthhenope (A. parth); Sympetrum foscolombii (S. fosc); Corixa affinis (C. aff); Sigara lateralis (S. lat); Micronecta (Micro); Anisops debilis perplexa (A. deb); Notonecta meridionalis (N. mer); Hygrotus (Hygr); Hygrotus lagari (H. lag); Haliplus (Hali.); Berosus (Ber.); Hydrobius (Hydr.); Enochrus (Enoc.); Chironominae i (Chir. i); Orthocladiinae i (Orth. i); Chaoboridae i (Chao. i); Dixella (Dixella); Rhagionidae i(Rhag. i). Diagrama de ordenación del ACC en el que se muestra la distribución de las estaciones de muestreo (A) y la posición de las familias (B) en relación con las variables ambientales en el espacio representado por los dos primeros ejes. Los círculos blancos representan estaciones en aguas claras, y los negros en aguas turbias.*

and S in different taxa (e.g., fungi, plants, invertebrates and vertebrates), comparing them to the "neutral model". They assumed that the mathematical relationships between these variables constrained them such that they were found to be strongly positively related. They found a slope of 0.6 for H′/log$_2$S, estimated for invertebrates, that was not significantly different from that of the "neutral model". The slope of the linear relationship between H′/log$_2$S estimated for the invertebrate community of the Doñana marsh showed a strong positive correlation between H′ and S ($\beta = 0.6$, $r^2 = 0.54$, $p < 0.05$) and had the same value as that estimated by Stirling & Wilsey (2001) for invertebrates. The slope we calculated to assess the bivariate relationship between H and S could be underestimated through the ordinary least square regression (OLS) method used, as has been reviewed in other biological and ecological relationships by Green (2001), Ardia (2005) and Schulte-Hostedde *et al.* (2005). Peig & Green (2009) proposed to use the reduced major axis regression model (RMA) instead of OLS for these types of relationships, and the conversion from an OLS regression's slope to a RMA regression's slope can be easily done by dividing the OLS regression's slope by the Pearson's correlation coefficient. In our case, after following this procedure, the estimated RMA regression's slope is 0.81, even more similar to the expectations of the "neutral model". If we interpret this slope in the same way as Stirling & Wilsey (2001), migration and dispersion (e.g., S) seemed to be the main biological factor regulating H′. This addresses the importance of lateral hydrologic connection between the marsh and other adjacent ponds, as found by Frisch *et al.* (2012) and Kang & King (2013), and propagule dispersion through wind and other faunal components, such as waterfowl, as found by Figuerola *et al.* (2003), Badosa *et al.* (2010). In this sense, the influence of hydroperiod length has been positively correlated with the accumulative richness of zooplankton species in the nearby ponds of the aeolian sands of Doñana by Serrano & Fahd (2005), while the macroinvertebrate's assemblages in these ponds seem to be determined by the high connectivity among ponds and the environmental variability (Florencio *et al.*, 2011).

In summary, in this study, we have described the structure of the invertebrate community of the marsh of Doñana, recording a total of 102 taxa. Even taking into account the limitations of our study (i.e., one sampling in May 2007) and the need to be cautious in the interpretation of our results, obtained for a narrow temporal scale, it was possible to distinguish different marsh environments characterised by patches of *clear water,* where the macrophyte beds increased the structural heterogeneity of the marsh. This provides shelter and food resources to invertebrates, which in turn influences different invertebrate assemblages. Conductivity, SRP and chlorophyll-*a* were the environmental variables that influenced the existence of the *turbid* patches, with lower macrophyte cover, diversity and richness values than in the clear water patches. The relationship between H′ and S suggested that those processes related to species' migration (i.e., hydrologic connection with other water bodies, flood duration, and dissemination of propagules) are the main constraints on the structure of the invertebrate community of the Doñana marsh.

REFERENCES

ALONSO, M. 1996. *Crustacea, Branchiopoda. Fauna Ibérica*, 7. Museo Nacional de Ciencias Naturales, CSIC. Madrid.

ANDERSEN, J. M., 1976. An ignition method for determination of total phosphorus in lake sediments. *Water Research,* 10: 329–331.

ARDIA, D. R. 2005. Super size me: an experimental test of the factors affecting lipid content and the ability of residual body mass to predict lipid stores in nestling European Starlings. *Functional Ecology*, 19(3): 414–420.

BADOSA, A., D. FRISCH, A. ARECHEDERRA, L. SERRANO & A. J., GREEN. 2010. Recovery of zooplankton diversity in a restored Mediterranean temporary marsh in Doñana National Park (SW Spain). *Hydrobiologia,* 654(1), 67–82.

BAYÁN, B. 2005. The paths of water in the marshes: changes in the hydrological network. In: *Water and Biosphere. Doñana.* García-Novo, F. & C. Marín (eds) Doñana:, Confederación Hidrográfica del Guadalquivir, Ministerio de Medio Ambiente, 143–147.

BAYLEY, S. E. & C. M. PRATHER. 2003. Do wetland lakes exhibit alternative stable states? Submersed aquatic vegetation and chlorophyll in western boreal shallow lakes. *Limnology and Oceanography,* 48 (6): 2335–2345.

BAYLEY, S. E., I. F. CREED, G. Z. SASS & A. S. WONG. 2007. Frequent regime shifts in trophic states in shallow lakes on the Boreal Plain: Alternative" unstable" states?. *Limnology and Oceanography*, 52(5): 2002–2012.

BRAVO-UTRERA, M. A. 2010. Monitoring aquatic ecosystems at Doñana Natural Space. In: *Conservation monitoring in freshwater habitats.* C. Hurdford, Schneider, M. & I. Cowx (eds)

CASWELL, H. 1976. Community structure: a neutral model analysis. *Ecological Monographs*, 46: 327–354.

CLESCERI, L. S., A. E. GREENBERG & A. D. EATON. 1999. *Standard methods for the examination of water and wastewater.* American Public Health Association and the Water Environment Federation.

CUMMINS, K. W. 1973. Trophic relations of aquatic insects. *Annual Review of Entomology,* 18: 183–206.

CUMMINS, K. W. 1975. *Macroinvertebrates*. River ecology. Blackwell Scientific Publication. London.

DODDS, W. & M. WHILES. 2010. *Freshwater ecology. Concepts & environmental applications of limnology.* 2nd edition; Academic Press, Elsevier, USA.

DUARTE, C. M., J. KALFF & R. H. PETERS. 1986. Patterns in the biomass and cover of aquatic macrophytes in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 43: 1900–1908.

ESPINAR, J. L., L. V. GARCÍA, P. GARCÍA MURILLO & J. TOJA. 2002. Submerged macrophyte zonation in a Mediterranean salt marsh: a facilitation effect from established helophytes?. *Journal of Vegetation Science*, 13(6): 831–840.

FIGUEROLA, J., A. J. GREEN & L. SANTAMARIA. 2003. Passive internal transport of aquatic organisms by waterfowl in Doñana, south-west Spain. *Global Ecology Biogeography* 12: 427–436.

FLORENCIO, M., C. DÍAZ-PANIAGUA, L. SERRANO & D. T. BILTON. 2011. Spatio-temporal nested patterns in macroinvertebrate assemblages across a pond network with a wide hydroperiod range. *Oecologia*, 166(2): 469–483.

FRISCH, D., E. MORENO-OSTOS & A. J. GREEN. 2006. Species richness and distribution of copepods and cladocerans and their relation to hydroperiod and other environmental variables in Doñana, south-west Spain. *Hydrobiologia*, 556 (1): 327–340.

FRISCH, D., K. COTTENIE, A. BADOSA & A. J. GREEN. 2012. Strong spatial influence on colonization rates in a pioneer zooplankton metacommunity. *PloS one*, 7(7).

GARCÍA-MURILLO, P., M. BERNUÉS. & C. MONTES 1993. Los macrófitos acuáticos del Parque Nacional de Doñana (SW España). Aspectos florísticos. *Actas VI Congreso Español de Limnología*: 261–267.

GARCÍA-MURILLO, P., R. FERNÁNDEZ-ZAMUDIO, S. CIRUJANO & A. SOUSA. 2006. Aquatic macrophytes in Doñana protected area (SW Spain): An overview. *Limnetica* 25 (1-2): 71–80.

GARCÍA-MURILLO, P. R. FERNÁNDEZ-ZAMUDIO & S. CIRUJANO. 2009. *Macrófitos. Habitantes del agua* (nº2). Agencia Andaluza del Agua, Consejería de Medio Ambiente, Junta de Andalucía.

GARCÍA-NOVO, F. & C. MARÍN. 2005. *Doñana, agua y biosfera*. Confederación Hidrográfica del Guadalquivir. Ministerio de Medio Ambiente.

GARCÍA-NOVO, F., A. MARTÍN-VICENTE & J. TOJA SANTILLANA. 2007. *La frontera de Doñana.* Universidad de Sevilla.

GASCÓN, S., D. BOIX, & J. SALA. 2009. Are different biodiversity metrics related to the same factors? A case study from Mediterranean wetlands. *Biological conservation*, 142 (11): 2602–2612.

GEERTZ-HANSEN, O., C. MONTES, C. M. DUARTE, K. SAND-JENSEN, MARBÁ, N. & P. GRILLAS. 2010. Ecosystem metabolism in a temporary Mediterranean marsh (Doñana National Park, SW Spain). *Biogeosciences Discussions*, 7: 6495–6521.

GÓMEZ-BAGGETHUN, E., P. ALCORLO & C. MONTES. 2011. Ecosystem services associated with a mosaic of alternative states in a Mediterranean wetland: case study of the Doñana marsh (southwestern Spain). *Hydrological Sciences Journal*, 56(8): 1374–1387.

GREEN, A. J. 2001. Mass/length residuals: measures of body condition or generators of spurious results?. *Ecology*, 82(5), 1473–1483.

GRILLAS, P. 1990. Distribution of submerged macrophytes in the Camargue in relation to environmental factors. *Journal of Vegetation Science,* 1(3): 393–402.

GRILLAS, P., P. GARCIA-MURILLO, O. GEERTZ-HANSEN, N. MARBÁ, C. MONTES, C. DUARTE, L. TAN HAM & A. GROSSMANN. 1993. Submerged macrophyte seed bank in a Mediterranean temporary marsh: abundance and relationship with established vegetation. *Oecologia*, 94(1): 1–6.

GRILLAS, P., P. GAUTHIER, N. YAVERCOVSKI & C. PERENNOU. 2004. *Mediterranean Temporary Pools; volume 1-Issues relating to conservation, functioning and management*. Arles: Station Biologique de la Tour du Valat.

HILLEBRAND, H., D. M. BENNETT & M. W. CADOTTE 2008. Consequences of dominance: a review of evenness effects on local and regional ecosystem processes. *Ecology,* 89: 1510–1520.

ISBELL, F. I., H. W. POLLEY & B. J. WILSEY. 2009. Biodiversity, productivity and the temporal stability of productivity: patterns and processes. *Ecology letters*, 12(5): 443–451.

JEFFREY, S. W. & G. F. HUMPHREY, 1975. New spectrophotometric equation for determining chlorophylls a, b, c1 and c2 in algal phytoplankton and higher plants. *Biochemie und Physiologie der Pflanzen*, 167: 191–194.

KANG, S. R. & S. L. KING. 2013. Effects of hydrologic connectivity on aquatic macroinvertebrate assemblages in different marsh types. *Aquatic Biology*, 18, 149–160.

KEDDY, P. A. 2000. *Wetland Ecology: Principles and Conservation.* Cambridge University Press.

LLAMAS, R., F. J. RODRÍGUEZ-ARÉVALO, J. TENAJAS & A. VELA. 1987. El Parque Nacional de Doñana: El Medio Físico. *Real Academia de Ciencias de Madrid. Bases Científicas para la protección de los humedales españoles:* 147–172.

LÓPEZ-FLORES, R., X. D. QUINTANA, A. M. ROMANÍ, L. BAÑERAS, O. RUIZ-RUEDA, J. COMPTE & J. J. EGOZCUE. 2014. A compositional analysis approach to phytoplankton composition in coastal Mediterranean wetlands: Influence of salinity and nutrient availability. *Estuarine, Coastal and Shelf Science,* 136: 72–81.

MONTES, C., J. A. AMAT & L. RAMÍREZ-DÍAZ. 1982a. Ecosistemas acuáticos del Bajo Guadalquivir (SW. España). Variación estacional de los componentes físico-químicos y biológicos de las aguas. *Studia Oecologica*, 3: 129–158.

MONTES, C., J.A. AMAT & L. RAMÍREZ-DÍAZ. 1982b. Distribución temporal de las características fisicoquímicas y biológicas de las aguas de algunos ecosistemas del Bajo Guadalquivir (SW España) a lo largo de un ciclo anual. *Anales Universidad Murcia,* 38 (1-4): 209–304.

MONTES, C., BRAVO-UTRERA, M. A., BALTANÁS, A., C. DUARTE & P. J. GUTIÉRREZ-YURRITA, 1993. Bases ecológicas para la gestión del cangrejo rojo de las marismas del Parque Nacional de Doñana, España. ICONA, España, 270 pp.

OJEDA-RIVERA, J. F. 1987. Organización del territorio en Doñana y su entorno próximo (Almonte). siglos XVIII-XX. Monografías-Instituto Nacional para la Conservación de la Naturaleza: 447–455.

OJEDA-RIVERA, J. F. & L. MORAL. 2004. Percepciones del agua y modelos de su gestión en las distintas fases de la configuración de Doñana. *Investigaciones Geográficas* 35: 25–44.

PAGGI, A. C. 2003. Los Quironómidos (Diptera) y su empleo como bioindicadores. *Biología Acuática*, 21: 50–57.

PEIG, J. & A. J. GREEN. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos*, 118(12): 1883–1891.

PIELOU, E. C. 1969. *An introduction to mathematical ecology*. Wiley-Interscience, New York.

PRODON, R., 1988. *Dynamique des systèmes avi-*

faune-végétation après déprise rurale et incendies dans les Pyrénées méditerranéennes siliceuses. Thèse de Doctorat d'Etat, Université Paris.

RODRÍGUEZ-PÉREZ, H., M. FLORENCIO, C. GÓMEZ-RODRÍGUEZ, GREEN, A. J., C. DÍAZ-PANIAGUA & L. SERRANO. 2009. Monitoring the invasion of the aquatic bug *Trichocorixa verticalis verticalis* (Hemiptera: Corixidae) in the wetlands of Doñana National Park (SW Spain). *Hydrobiologia*, 634(1): 209–217.

SCHEFFER, M. 2009. *Critical transitions in nature and society*. Princeton University Press.

SCHULTE-HOSTEDDE, A. I., B. ZINNER, J. S. MILLAR & G. J. HICKLING. 2005. Restitution of mass-size residuals: validating body condition indices. *Ecology*, 86(1): 155–163.

SERRANO, L. & K. FAHD. 2005. Zooplankton communities across a hydroperiod gradient of temporary ponds in the Doñana National Park (SW Spain). *Wetlands,* 25: 1101–111.

SERRANO, L., M. REINA, G. MARTÍN, I. REYES, A. ARECHEDERRA, D. LEÓN & J. TOJA. 2006. The aquatic systems of Doñana (SW Spain): watersheds and frontiers. *Limnetica,* 25: 11–32.

SERRANO, L. & M. ZUNZUNEGUI. 2008. The relevance of preserving temporary ponds during drought: hydrological and vegetation changes over a sixteen-year period in the Doñana National Park (SW Spain). *Aquatic conservation: marine and freshwater ecosystems,* 18: 261–279.

SHANNON, C. E. & W. WEAVER. 1963. The Mathematical theory of communication. University of Illinois Press. USA.

SKINNER, J. & S. ZALEWSKI. 1995. *Functions and values of Mediterranean wetlands*. MedWet Publication series. Tour de Valat, Le Sambuc, Arles. Francia.

STIRLING, G. & B. WILSEY. 2001. Empirical rela-

tionships between species richness, evenness, and proportional diversity. *The American Naturalist*, 158(3): 286–299.

TANIGUCHI, H., S. NAKANO & M. TOKESHI. 2003. Influences of habitat complexity on the diversity and abundance of epiphytic invertebrates on plants. *Freshwater Biology*, 48(4), 718–728.

URDIALES, C., D. GARCÍA, A. VALERO & J.M. FERNÁNDEZ-PALACIOS. 2010. Seguimiento de la inundación de la marisma de Doñana: Resultados del ciclo 2009/2010 y el efecto del dique de la montaña del río en el proceso de inundación. In: *Tecnologías de la información geográfica: La información geográfica al servicio de los ciudadanos* Ojeda. J., Pita, M. F. y Vallejo, I. (eds): 1146–1156. Secretaría de Publicaciones de la Universidad de Sevilla.

VAN DE MEUTTER, F., H. TREKELS & A. J. GREEN. 2010. The impact of the North American waterbug *Trichocorixa verticalis* (Fieber) on aquatic macroinvertebrate communities in southern Europe. *Fundamental and Applied Limnology/Archiv für Hydrobiologie,* 177(4): 283–292.

WATERKEYN, A., P. GRILLAS, B. VANSCHOEN-WINKEL & L. BRENDONCK. 2008. Invertebrate community patterns in Mediterranean temporary wetlands along hydroperiod and salinity gradients. *Freshwater Biology*, 53(9): 1808–1822.

WILSEY, B. & G. STIRLING. 2007. Species richness and evenness respond in a different manner to propagule density in developing prairie microcosm communities. *Plant Ecology*, 190(2): 259–273.

ZAR, J. H. 1999. *Biostatistical Analysis*. 4th ed. Prentice-Hall Inc., USA.

ZEDLER, J. B. & S. KERCHER. 2005. Wetland sources: status, trends, ecosystem services, and restorability. *Annual Review of Environment and Resources*, 30: 39–74.