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, cladoceros 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-\textit{a}, con valores más bajos de cobertura de macrofitos 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 \textit{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-Novó & 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-Novó & Marín, 2005; Serrano \textit{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 \textit{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 \textit{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 Chironomidae (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 & Ramírez-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 (37°1′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
March or April, forming an inundated surface area of 27,000 ha that falls within the boundaries of Doñana National Park (53 835 ha). Afterwards, 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 Honduras: 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 representan (Marisma de Hinojos: típica marisma somera y plana; Lucio y Honduras: 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).

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

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 represent 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 cm²) 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 m². 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 log₂. 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 ex-
amined, and appropriate transformations \([\log(x), \log(x) + 1 \text{ or } \arcsin(x)]\) were used when neces-
sary (Zar, 1999).

For all the analyses, a species with a maximum abundance less than 10% of the total abun-
dance of its faunal group in a sample was consid-
ered 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 \(2S\)). A one-
way ANOVA was performed to analyse the rela-
tion 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 be-
tween taxon abundance and the main environ-
mental variables. A Monte Carlo test (9999 per-
mutations, \(p < 0.05\)) was performed to test the
significance of the canonical axes. All CCA were
carried out using CANOCO 4.5.

Further explorations of the relationships be-
tween environmental variables retained in CCA
with \(H\) and log \(2S\) were performed through gen-
eral linear models (GLM) and multiple regres-
sion analysis using \(H\) and log \(2S\) 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 differ-
ent 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 de-
scriptor variables showed a wide range of varia-
tion (represented by the high coefficient of va-
riation) 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>
<thead>
<tr>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLEAR WATERS (n = 13)</strong></td>
</tr>
<tr>
<td>Conductivity EC_{25} (\mu\mu\mu S/cm)</td>
</tr>
<tr>
<td>average</td>
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<tr>
<td>range</td>
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<tr>
<td>Dissolved oxygen (%)</td>
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<td>average</td>
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<td>Dissolved oxygen (mg/l)</td>
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<td>pH</td>
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<td>T (°C)</td>
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<td>average</td>
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<td>range</td>
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<tr>
<td>Water level (Z, cm)</td>
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<td>average</td>
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<td>range</td>
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<tr>
<td>Turbidity (FTU)</td>
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<tr>
<td>average</td>
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<tr>
<td>range</td>
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<tr>
<td>SRP (\mu\mu\mu g/l)</td>
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<tr>
<td>average</td>
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<tr>
<td>range</td>
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<tr>
<td>Total P (\mu\mu\mu g/l)</td>
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<td>average</td>
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<td>range</td>
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<tr>
<td>Total P sed (\mu\mu\mu g/l/g)</td>
</tr>
<tr>
<td>average</td>
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<tr>
<td>range</td>
</tr>
<tr>
<td>Organic Matter content (%)</td>
</tr>
<tr>
<td>average</td>
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<tr>
<td>range</td>
</tr>
<tr>
<td>Submerged macrophytes coverage (%)</td>
</tr>
<tr>
<td>average</td>
</tr>
<tr>
<td>range</td>
</tr>
<tr>
<td>Helophytes coverage (%)</td>
</tr>
<tr>
<td>average</td>
</tr>
<tr>
<td>range</td>
</tr>
<tr>
<td>Chlorophyll-a (\mu\mu\mu g/L)</td>
</tr>
<tr>
<td>average</td>
</tr>
<tr>
<td>range</td>
</tr>
</tbody>
</table>
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 (Rodriguez-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 ($\pm$ 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/m$^2$, *Macrothrix rosea* 86.2 ind/m$^2$, *Simocephalus exspinosus* 79.15 ind/m$^2$), Ostracoda (*Plesiocypridopsis new-05*, 101.2 ind/m$^2$), Decapoda (*Depilperes* sp. 22.1 ind/m$^2$), and Insecta (Anisops debilis, Berosus sp. and Orthocladiinae sp2. 26.3 ind/m$^2$).

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**Figure 2.** Density (ind/m$^2$) (A) of the taxonomic groups recorded at each sampling site, and their relative abundance (%) (B). *Densidad (ind/m$^2$) (A) de los grupos taxonómicos registrados en cada estación de muestreo; y su abundancia relativa (%) (B).*
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...tonii, Potamocypris unicaudata, Ilyocypris getica, all with maximum abundances of 43 ind/m²), and dipterans (Orthocladiinae spp., 41.7 ind/m²) (Fig. 2A). The sites that exhibited the highest invertebrate density values were almost all in the clear-water state (M04: 58.45 ind/m², M08: 296.7 ind/m², M10: 177.7 ind/m², M18: 48.15 ind/m², M27: 261.8 ind/m²), while two were in the turbid state (M01: 132.8 ind/m², M06: 50.2 ind/m²) (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 ($± SD = 0.13$) 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 $\log_2S$. White circles represent sites with clear waters, and black circles represent the turbid ones. Relación lineal entre la diversidad ($H'$) y $\log_2S$. Los círculos blancos representan estaciones en aguas claras, y los negros en aguas turbias.](image-url)
chlorophyll-α. 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-α 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-α, 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-α 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-α, were related
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-Novó 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 ambiguа, live in clear water with abundant vegetation (Alonso, 1996), and they appeared in this part of the CCA ordination space. Others, such as Leydigia acanthoceroides, 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 Pomatius substratiуs, 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; Hilebrand 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., $\alpha$, $\beta$ and $\gamma$ 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. Abbreviation taxa lists: Nematoda (Nem.); Oligochaeta (Olig.); Physa acuta (P. acu); Planorbis (Plan.); Ancylus fluviatilis (A. flu); Candona (Cand.); Fabaformiscandona fragilis (F. fra); Bradleycypris obliqua (B. obli); Plesioscypridopsis 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 expsinus (S. exs); Cerdaphnia quadrangula (C. qua); Leydigia acanthoceroides (L. aca); Tretoccephala ambigu (T. amb); Chydomus sphaericus (C. sph); Macrobrachium rosea (M. ros); Cloeon dipterum (C. dip); Ischnura graei (I. grae); Anax parthenope (A. parth); Sympleurus fiscolumbit (S. fisc); Corixida affinis (C. aff); Sigara lateralis (S. lat); Micronecta (Micro); Anisops debilis perplexa (A. deb); Notonecta meridionalis (N. mer); Hygroitus (Hygr); Hygroitus 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 (Rha. 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.
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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 propagate 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.

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