Aquatic ecology: richness and distribution of microcrustaceans in Doñana.
Ecología acuática: riqueza y distribución de microcrustáceos en Doñana.

Species richness and distribution of copepods and cladocerans and their relation to hydroperiod and other environmental variables in Doñana, south-west Spain

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Abstract

Twenty-five sites located in five wetland zones within Doñana, south-west Spain were studied for copepod and cladoceran species richness and composition in relation to habitat characteristics from January to March 2004. The γ-diversity of copepods and cladocerans combined varied between wetland zones, which differed significantly in conductivity, surface area and abundance of vegetation. However, there were no significant differences between zones in local species richness of either copepods or cladocerans or the two combined. Species richness was significantly higher in sites with intermediate hydroperiods (duration between 3 and 5 months) than in sites with shorter or longer hydroperiods. CCA analyses performed separately for copepods and cladocerans extracted conductivity, surface area, and vegetation cover as the main factors structuring species composition of both groups. Ours is one of the first studies of zooplankton in the natural marshes of the Doñana World Heritage Site, and we recorded five species new to the Doñana area.

Introduction

In temporary ponds of riverine and coastal habitats and in smaller isolated temporary ponds, both community composition of aquatic invertebrates (Wellborn et al., 1996) and zooplankton species richness (Frisch et al., 2005; Serrano & Fahd, 2005) are affected by hydroperiod. Hydroperiod duration and the presence or absence of hydrological connections between ponds were better predictors for copepod richness than pH, chlorophyll *a*, or temperature in floodplain ponds (Frisch et al., 2005). The duration of the aquatic period may also affect the set of aquatic invertebrates present in temporary ponds. Temporary waterbodies are inhabited by species with a life-cycle that allows survival through the terrestrial period or recolonisation after adverse periods (Wiggins et al., 1980; Williams, 1996). However, the impact of hydrological factors may differ between taxa. For example, Eitam et al. (2004b) found that microturbellarian diversity in temporary ponds was closely related to surface area, but not to pond permanence. In contrast, they reported microcrustacean richness to be related to pond permanence but not to surface area (Eitam et al., 2004a).

Various environmental (physico–chemical) gradients have been identified as determinants of the structure of aquatic invertebrate communities, including temperature and oxygen (Armengol et al., 1998), pH (Schartau et al., 2001), salinity

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(Wollheim & Lovvorn, 1995; Drake et al., 2002; Vieira et al., 2003), trophic state (Duggan et al., 2002), or altitude (Jersabek et al., 2001). However, these studies are commonly performed in permanent ponds, and information on environmental factors structuring zooplankton assemblages in temporary waterbodies remains very limited.

The various wetland zones within Doñana in the south-west of Spain offer an excellent opportunity to study determinants of zooplankton richness and composition. This region contains a large number of temporary waterbodies spread over an area of 180 000 ha. However, with the exception of the dune lagoons in the Doñana National Park (Fahd et al., 2000; Serrano & Fahd, 2005), the species composition, diversity, and distribution of crustacean zooplankton of Doñana are largely unknown. Ours is one of the first studies of zooplankton in the natural marshes within the Doñana World Heritage Site.

The main objective of this study was to examine the impact of hydroperiod duration and other environmental variables on species richness and distribution of cladocerans and copepods in temporary ponds in the Doñana region.

Methods

Study area

Doñana is a Mediterranean wetland complex in the Guadalquivir delta in Andalusia, south-west Spain (Fig. 1). The area has very dry and hot summers and wet and cool winters. Annual rainfall ranges from 158 to 1062 mm year^{-1} with a mean of 562 mm year^{-1} (Castroviejo, 1993). The study was performed in five spatially and hydrologically separated wetland zones of Doñana: Veta la Palma, natural marshes (Marismas) of the

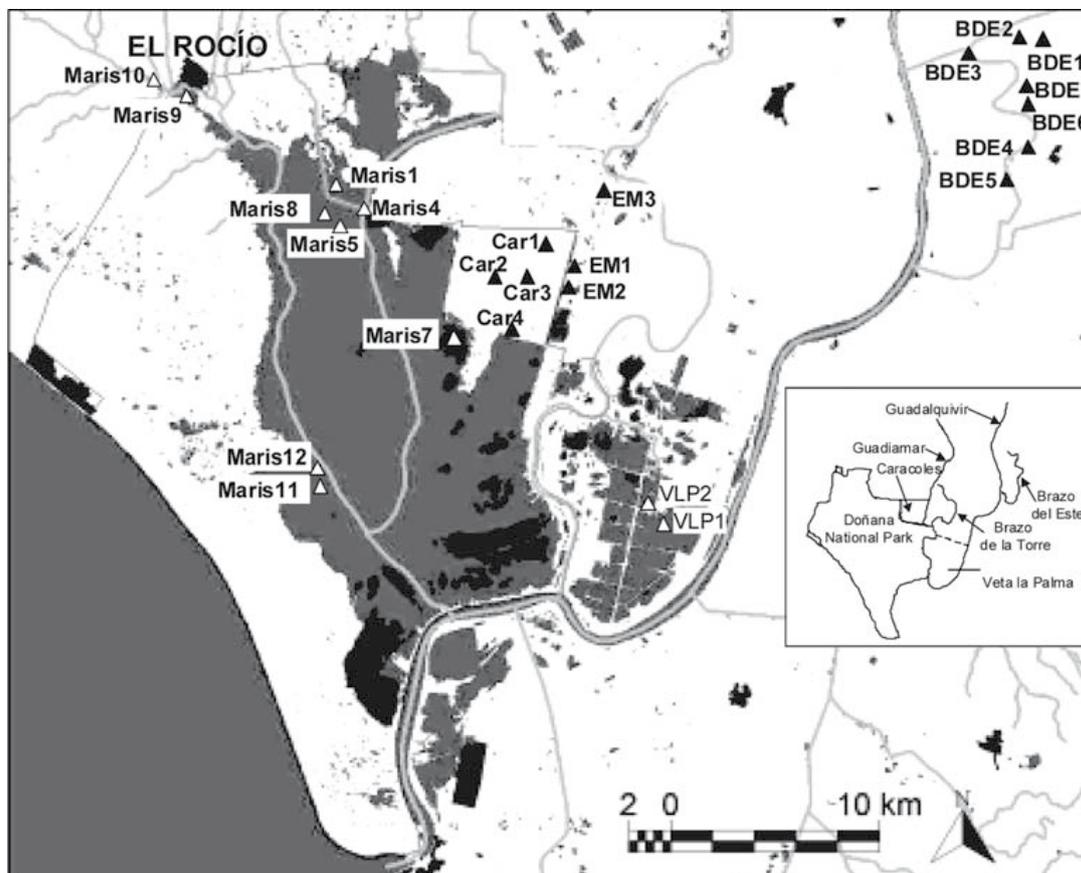*Figure 1.* Map of Doñana, with overview of all sampled zones (small map insert) and site names corresponding to Table 2 (large map). The town of El Rocío (37° 07′ 50″ N, 6° 29′ 06″ W) is located at the northern boundary of the National Park, of which the outline is marked in the map insert. The flooded area of the Marismas (natural marshes) is depicted in grey and black, the latter referring to deeper areas with a longer hydroperiod.

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Doñana National Park, Caracoles, Entre Muros and Brazo del Este (see map in Fig. 1 and details of sample sites in Table 1). Most sample sites contained fish, except for the smallest ponds and drainage ditches. Although data on fish abundance is unavailable, we recorded presence and absence of fish (Table 1).

Veta la Palma (VLP) is a private estate containing 37 artificial ponds created between 1990 and 1993 (total 3125 ha), which are used for extensive fish culture. These ponds are shallow (average depth 40 cm) and connected to deeper canals used to transport water to and from the Guadalquivir estuary. All VLP ponds are connected to each other by an artificial water exchange system with pumps. Because of this, the surface area of ponds sampled in this zone is listed in Table 1 as the combined surface area of all ponds of VLP.

The 'Marismas' or natural marshes of Doñana National Park contain shallow lakes and other temporary waterbodies that are regularly connected with each other during floods (Montes et al., 1998). Waterbodies in the natural marshes are dry during summer and refill with water during the wet season in late autumn to spring (Amat, 1981; Montes et al., 1982). During this study the water level was especially high and all sites were part of a single large flooded area of 30 000 ha (Table 1).

Caracoles is an area of 2700 ha of former marsh that has been cultivated (mainly with cereals) for over 20 years. It was hydrologically disconnected from the Marismas to prevent flooding.

Table 1. Environmental variables of the sites sampled for copepods and cladocerans

Pond	Type	Temp. (°C)	Conduct.(μS cm^{-1})	Size (sqm)	Depth (m)	Veg. Cover	Hydro-period	Fish	Date
VLP1	P	13.3	17 500	3.2×10^7	0.50	0	4	+	21.1.04
VLP2	P	13.3	14 000	3.2×10^7	0.50	1	4	+	21.1.04
EM1	O	13.1	1012	2000	0.20	0	2	+	21.1.04
EM2	O	13.3	1005	2000	0.20	1	2	+	21.1.04
EM3	P	16.0	1017	20	0.20	1	1	−	29.1.04
Maris1	TM	12.9	1490	3.0×10^8	0.40	2	3	+	21.1.04
Maris4	TM	12.5	1560	3.0×10^8	0.30	2	3	+	21.1.04
Maris5	TM	14.1	1580	3.0×10^8	0.30	2	2	+	21.1.04
Maris7	TM	13.3	1610	3.0×10^8	1.10	2	3	+	29.1.04
Maris8	TM	14.5	1037	3.0×10^8	0.50	0	2	+	9.3.04
Maris9	TM	17.3	859	3.0×10^8	0.30	2	3	+	9.3.04
Maris10	TM	17.0	362	3.0×10^8	0.40	2	3	+	9.3.04
Maris11	TM	19.8	482	3.0×10^8	0.40	3	2	+	9.3.04
Maris12	TM	21.8	664	3.0×10^8	0.40	3	2	+	9.3.04
BDE1	P	10.6	128	24	0.20	2	1	−	3.3.04
BDE2	P	14.6	236	30	0.15	1	1	−	3.3.04
BDE3	O	20.0	1804	30 000	0.50	1	4	+	3.3.04
BDE4	O	13.6	2850	30 000	0.50	2	4	+	3.3.04
BDE5	O	17.5	2550	25 000	0.30	3	4	+	3.3.04
BDE6	P	18.8	1026	2400	0.20	3	2	−	3.3.04
BDE7	P	18.1	1911	1600	0.20	1	2	−	3.3.04
Carac1	D	27.6	28 100	2000	0.05	0	2	−	17.3.04
Carac2	P	22.6	3630	50	0.20	1	1	−	17.3.04
Carac3	D	21.2	14 810	8000	1.50	0	2	−	17.3.04
Carac4	P	28.0	7090	20	0.05	0	1	−	17.3.04

Abbreviations used for type are P = pond, O = oxbow, TM = temporary marsh and D = drainage ditch. The categories used for vegetation cover are 0 = no vegetation present; 1 = low, < 20% cover; 2 = intermediate, between 20 and 40% cover; 3 = high, more than 40% cover. Categories of hydroperiod are < 3 months (1); 3–5 months (2); 5–12 months (3); > 12 months (4). Presence of fish = +, no fish observed = −. For further details see Methods.

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Waterbodies in this area were either drainage ditches or ponds formed after heavy rainfall in small surface depressions. This area was incorporated into Doñana National Park in February 2004, and will be restored into marsh over the next few years.

The Brazo del Este (BDE) was originally one of three arms of the Guadalquivir River, but the river bed has been divided into 10 isolated oxbows during reclamation for agriculture. These oxbows are flooded mainly by outflow from ricefields, and are protected within a Natural Area of 1336 ha. Waterbodies sampled were three oxbows and four rain-fed ponds.

Entre Muros ('between walls') is a channelised section of the Guadiamar River and Brazo de la Torre stream (2700 ha) that was affected by a spill of waste from the Aznalcollar mine in 1998 (Grimalt et al., 1999). Waterbodies sampled were two sections of seasonal streams and a rain-fed pond.

Sampling and identification of crustacean zooplankton

A total of 25 temporary waterbodies was sampled within the five zones between 21 January and 17 March 2004. Each pond was visited once and sampled using five horizontal hauls with a 150 μm plankton net. The hauls were made in different areas of the study sites with the attempt to equally sample the different microhabitats (e.g. between macrophyte stands or in the open water). One sample per site was rinsed into a screwcap plastic bottle (125 ml) and preserved in formalin for identification.

Copepods and cladocerans were identified to species level in the laboratory and their presence/absence recorded in each sample. In order not to miss rare species, we examined the whole sample. Copepods were identified according to Brylinski (1981), Einsle (1993, 1996) Dussart (1967, 1969) and Stella (1982), and cladocerans according to Margaritora (1985), Scourfield & Harding (1994), and Alonso (1996).

Environmental variables

Temperature and conductivity were measured *in situ* with WTW Multi 340I/Set. Waterbody depth and vegetation cover in each site was recorded in the field. Vegetation cover of the emergent and submerged plants growing within the waterbodies was estimated at 4 levels (0 = no vegetation present; 1 = low, < 20% cover; 2 = intermediate, between 20 and 40% cover; 3 = high, more than 40% cover). Vegetation consisted of *Arthrocnemun macrostachyum* (only in the Marismas), grasses, small-leaved herbal terrestrial vegetation and *Ranunculus aquaticus* in the smaller ponds of the BDE and Entre Muros zones, reed stands in the more permanent sites of all zones, and filamentous algae. The surface area of sites (from hereon referred to as size) was estimated using measurements made in the field and with maps.

According to data obtained from our own observations in the field and from monthly maps of the flooded area in the Marismas and Entre Muros (*C. Urdiales*, Ministerio del Medio Ambiente) we assigned the hydroperiod duration of each sample site to 4 levels: < 3 months (1), 3–5 months (2), 5–12 months (3), > 12 months (4).

Statistical analysis

Prior to statistical tests, we log-transformed the environmental variables temperature, conductivity and size, and squareroot-transformed the variable depth to approach normality. The two environmental variables hydroperiod duration and vegetation remained untransformed. All statistical analyses except for the CCA were done with StatSoft Statistica 6.0.

To test for differences of environmental variables between zones we used Multivariate Analysis of Variance (MANOVA) with hydroperiod duration, vegetation cover, temperature, conductivity, size and depth as dependent variables and zone as predictor. Differences of species richness between zones and the two zooplankton groups were tested by MANOVA, with cladoceran and copepod richness as dependent variables and zones as predictor. Significant MANOVAs were followed by *post hoc* tests (Tukey's test for unequal N).

The difference in frequencies (percentage of ponds in which a given species was present) between copepods and cladocerans was tested using a Mann–Whitney U test. The relationship between hydroperiod duration and species richness was tested by one-way ANOVA. Correlation analyses (Spearman's correlation) were performed

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between (1) species richness of cladocerans and copepods, (2) total richness (cladocerans and copepods combined) and number of sample sites in a given zone, and (3) total richness and total surface area of sampled waterbodies in a given zone.

We used Canonical Correspondence Analysis (CCA, Ter Braak, 1986) to examine possible relationships between six environmental variables (temperature, conductivity, depth, size, hydroperiod and vegetation cover) and species distribution, analysing cladoceran and copepod taxa separately. Three sites (VLP1, BDE1, BDE2) were excluded from the CCA of cladocerans, because they did not contain individuals of this group. The ordination was performed using the program PC-Ord for Windows, Version 3.14. Axis scores were centred and standardised to unit variance, and axes were scaled to optimise representation of species. As recommended by Palmer (1993), we used linear combination scores (LC scores) for plotting CCA results. Graphs of the CCA ordination depict the first two axes, which had the highest eigenvalues (Axis 1 and 2), and thus showed the highest amount of variation explained by CCA ordination.

Monte-Carlo permutation tests were performed (1000 runs) to test the statistical strength of eigenvalues of the ordination axes and of the species-environment correlations (Intraset correlations of Ter Braak, 1986). The p-value was calculated as the proportion of randomised runs with eigenvalues, or species-environment correlations greater than or equal to the observed eigenvalue or species-environment correlation respectively. The null hypothesis for the Monte-Carlo test was that there is no relation between the environmental and species matrices.

Results

Environmental variables

The five zones differed significantly in the environmental variables we measured (MANOVA, Wilks test, $F_{(24,54)} = 12.05$, $p < 0.001$). Conductivity did not differ between VLP and Caracoles sites ($p > 0.98$), but both zones had significantly higher conductivity than any of the other zones (Table 1, $p < 0.03$). Temperature was significantly higher in

the Caracoles sites than in any of the other zones (Table 1, $p \leq 0.03$). Vegetation cover was higher in the Marismas and the BDE sites than in the other zones. However, this difference was only significant between the sites of the Marismas and the Caracoles sites (Table 1, $p = 0.04$). The size of sites sampled in VLP and the Marismas was larger than that of other sites (Table 1, $p < 0.005$). Depth and hydroperiod did not differ significantly between zones.

Species richness and frequency of crustacean zooplankton

Zonal species richness of copepods and cladocerans was positively related to number of sites sampled in a given zone (Spearman's $R = 0.90$, $p = 0.04$) but not to the combined surface size of sites sampled in a given zone (Spearman's $R = 0.30$, $p = 0.62$). Average local cladoceran and copepod richness (i.e. mean per site) did not differ between zones (MANOVA, Wilks test, $F_{(8,38)} = 1.10$, $p = 0.38$), nor did average local species richness of the two groups combined differ between zones (ANOVA, $F_{(4,20)} = 1.95$, $p = 0.14$).

Using hydroperiod as a predictor variable across zones, we did not find significant differences when examining copepod and cladoceran richness separately (MANOVA, Wilks test, $F_{(6,40)} = 1.94$, $p = 0.09$). However, richness of copepods was positively correlated to that of cladocerans (Spearman's $R = 0.42$, $p = 0.038$) and the combined species richness of both groups differed significantly between sites with different hydroperiods (one-way ANOVA, $F_{(3,21)} = 3.26$, $p = 0.04$). Sites with a hydroperiod of 3–5 months duration (mean richness 7.9) had significantly higher species richness than sites with either the shortest (< 3 months, mean richness 3.2) or the longest hydroperiod (> 12 months, mean richness 4.0, $p = 0.01$ and $p = 0.03$ respectively, Fisher LSD post hoc tests). The species richness of sites with a hydroperiod between 5 and 12 months did not differ from that of sites with other hydroperiods (Fisher-LSD post hoc test, $p > 0.16$).

Frequency (percentage of ponds in which a species was present) of cladocerans and copepods throughout the Doñana study area did not differ between the two taxa (Mann–Whitney U test, $p = 0.55$).

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Copepods

A total of 18 copepod species were recorded in Doñana, with eight Calanoida, nine Cyclopoida and one Harpacticoida (Table 2). The VLP zone had the lowest richness (three species), followed by Caracoles and Entre Muros (four species each), and BDE area (six species). The highest richness was recorded in the Marismas (12 species).

The CCA ordination of copepods (Fig. 2) explained a total of 34.3% of the variance in the species data (Table 3a). Axis 1 was positively related to vegetation cover and size of the sites, and negatively related to conductivity. Axis 2 was strongly positively related to size and hydroperiod, and negatively to temperature (Table 3b).

Most of the copepod assemblages of the Marismas sites were strongly positively related to Axis 1 and 2, indicating their positive association with hydroperiod, size and vegetation cover (Fig. 2a, group A). Species typical of the plant rich and low conductivity sites of the Marismas were *Mesocyclops leuckarti*, *Eucyclops serrulatus*, *Macrocyclops albidus*, *Copidodiaptomus numidicus* and *Dussartius baeticus* (Fig. 2b, group A). CCA ordination positioned the assemblages of the permanent BDE oxbows (BDE 3–5) scattered among those of the Marismas, although they contained only one or two species (*Acanthocyclops robustus* and *Metacyclops minutus*, Table 2). The assemblages of the small ponds in the BDE area (BDE 1, 2, 6, 7) were associated with small size, higher temperatures and slightly increased conductivity (Fig. 2a, group B). Species found in these assemblages were *Arctodiaptomus wierzejski*, *Megacyclops viridis* and *Metacyclops minutus* (Fig. 2b, group B). Caracoles assemblages and one of the Entre Muros assemblages (EM 3) were strongly negatively associated to either of the first two axes and thus related closest to higher temperature and/or conductivity, and their smaller size and short hydroperiod (Fig. 2a, group C). Typical species of these assemblages were *Diacyclops bisetosus*, *Metacyclops minutus* and *Cletocamptus* cf. *retrogressus* (Fig. 2b, group C). Veta la Palma sites were separated from all other sites, with a strongly negative relation to Axis 1 and a strongly positive relation to Axis 2, reflecting their high conductivity and long hydroperiod (Fig. 2a, group D). The species associated with these environmental properties were *Acartia tonsa*, *Calanipeda aquaedulcis* and *Halicyclops rotundipes* (Fig. 2b, group D).

The two copepods with the highest frequency were cyclopoids. *Metacyclops minutus* was present in 44% of the sites studied. However, it was missing in almost all of the sites of the Marismas and VLP. *Acanthocyclops robustus* was found in 68% of all sites, missing only in the zones with highest conductivity (VLP and Caracoles).

Cladocerans

A total of 21 cladoceran species were recorded (Table 2). The lowest richness was recorded in VLP (1 species), followed by Caracoles and Entre Muros (three and four species respectively). The BDE zone contained 10 species and the Marismas 20 species.

The CCA ordination of cladocerans and environmental variables explained 28.4% of the variation in the cladoceran species data (Table 3a). Axis 1 was strongly positively correlated with size and vegetation (Fig. 3), and strongly negatively with conductivity. Axis 2 was positively correlated with vegetation and hydroperiod (intraset correlations > 0.35, Table 3b).

Most of the assemblages of the Marismas sites were positively related with Axis 1 or 2, indicating their positive association with vegetation and size (Fig. 3a, group A). These sites included Maris 11 and 12 with the highest cladoceran species richness (12 and 9 species respectively). Species of these assemblages were *Alona affinis*, *Alonella excisa*, *Ceriodaphnia quadrangula*, *Chydorus sphaericus*, *Daphnia atkinsonii*, *D. longispina*, *D. pulex*, *Dunhevedia crassa*, *Megafenestra aurita*, *Simocephalus expinosus* and *S. mucronata* (Fig. 3b, group A). The second group was positively associated with conductivity (Fig. 3a, group B). This group included the Caracoles assemblages and the only VLP site where cladocerans were found. Species present in these assemblages were *Daphnia magna*, *Moina brachiata* and *M. salina* (Fig. 3b, group B). Other, more permanent sites in the Marismas (Maris 9 and 10) with less abundant vegetation were negatively associated with Axis 2, and positively with Axis 1. Other sites ordinated in a similar way were EM 1 and 2, and BDE 3. Most of these assemblages contained only 1 or 2 species, among them *Ilyocryptus sordidus*, *Leydigia acanthocercoides*, *Ceriodaphnia laticaudata* and

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Table 2. List of copepod and cladoceran species and their abbreviations used in the figures

Species	Abbrev.	VLP 1	VLP 2	BDE 1	BDE 2	BDE 3	BDE 4	BDE 5	BDE 6	BDE 7	EM 1	EM 2	EM 3	Maris 1	Maris 4	Maris 5	Maris 7	Maris 8	Maris 9	Maris 10	Maris 11	Maris 12	Carac 1	Carac 2	Carac 3	Carac 4
COPEPODA																										
Calanoida																										
Acartia tonsa (Dana 1849)	Atonsa	x	x																							
Arctodiaptomus wierzejski (Richard) 1888	Awierz											x	x				x						x			
Calanipeda aquaedulcis Kritchagin 1873	Caquae	x	x						x																	
Copidodiaptomus numidicus (Gurney) 1909	Cnum																			x		x				
Dussartius baeticus (Dussart) 1967	Dbaet																				x					
Hemidiaptomus maroccanus Kiefer 1954	Hmaroc																x									
Mixodiaptomus incrassatus (SarsGO) 1903	Mincras											x	x	x	x	x	x	x								
Neolovenula alluaudi (Guerne & Richard) 1890	Nallu																x									
Cyclopoida																										
Acanthocyclops robustus (Sars, 1863)	Arob			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x					
Diacyclops bisetosus (Rehberg) 1880	Dbiset																								x	
Eucyclops serrulatus (Fischer) 1851	Eserr																		x	x		x				
Halicyclops rotundipes Kiefer 1935	Hrotun	x																								
Macrocyclops albidus (Jurine) 1820	Malbi																		x	x						
Megacyclops viridis (Jurine) 1820	Mviri			x																						
Metacyclops minutus (Claus) 1863	Mminut			x	x		x	x	x	x			x					x					x	x	x	x
Metacyclops planus (Gurney) 1909	Mplan			x												x	x	x								
Mesocyclops leuckarti (Claus) 1857	Mleuck																		x							
Harpacticoida																										
Cletocamptus cf. retrogressus Shmankevich 1875	Cretro																						x			
CLADOCERA																										
Alona affinis (Leydig 1860)	Aaffin																				x					
Alona rectangula (Sars, 1862)	Arect							x	x	x	x	x				x				x	x					
Alonella excisa (Fischer, 1854)	Aexc										x	x									x					
Ceriodaphnia laticaudata (Müller, 1867)	Clati										x									x	x					
Ceriodaphnia quadrangula (Müller, 1785)	Cquad						x		x	x	x		x							x	x	x				
Ceriodaphnia reticulata (Jurine, 1820)	Cretic												x				x			x	x	x				
Chydorus sphaericus (Müller, 1776)	Cshpae							x	x												x					
Daphnia atkinsoni (Baird, 1859)	Datk																x									
Daphnia longispina (Müller, 1776)	Dlongi						x										x				x					

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Table 2. (Continued)

Species	Abbrev.	VLP 1	VLP 2	BDE 1	BDE 2	BDE 3	BDE 4	BDE 5	BDE 6	BDE 7	EM 1	EM 2	EM 3	Maris 1	Maris 4	Maris 5	Maris 7	Maris 8	Maris 9	Maris 10	Maris 11	Maris 12	Carac 1	Carac 2	Carac 3	Carac 4
Daphnia magna (Straus, 1820)	Dmag					x	x	x		x								x			x				x	x
Daphnia pulex (Leydig, 1860)	Dpul							x									x				x	x				
Dunhevedia crassa (King, 1853)	Dcras								x	x				x							x	x				
Ilyocryptus sordidus (Liévin, 1848)	Isord			x		x													x							
Leydigia acanthocercoides (Fischer, 1854)	Lacant			x		x						x						x								
Megafenestra aurita (Fischer, 1849)	Maur								x													x				
Moina brachiata (Jurine, 1820)	Mbrach		x							x				x		x					x	x		x	x	x
Moina salina (Daday, 1888)	Msal																						x		x	
Pleuroxus letourneuxi (Richard, 1888)	Pletou												x			x		x								
Scapholebris mucronata (Müller, 1776)	Smucro																			x	x					
Simocephalus exspinosus (DeGeer, 1778)	Simex													x								x				
Simocephalus vetulus (Müller, 1776)	Simvet																	x				x				
Number copepod species		3	2	2	3	1	2	1	5	3	1	3	3	2	2	2	3	6	4	2	4	4	3	1	2	1
Number cladoceran species		0	1	0	0	3	3	3	5	5	1	2	2	3	1	4	4	4	1	2	12	9	1	2	3	2
Total species number		3	3	2	3	4	5	4	10	8	2	5	5	5	3	6	7	10	5	4	16	13	4	3	5	3

Details on species distribution in five zones of the Doñana wetlands are given as presence (x) and absence (blank). VLP = Veta la Palma, BDE = Brazo del Este, EM = Entre Muros, Maris = Marismas, Carac = Caracoles.

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C. reticulata. Of these, the latter two species were restricted to the fresher sites of the Marismas (Maris 8, 9 and 10).

The three cladocerans with the highest frequencies were *Daphnia magna* (in 32% of the sites), *Moina brachiata* and *Alona rectangula* (both in 28% of the sites). The first two of these were associated with higher conductivity, whereas *A. rectangula* was located close to the centroid of environmental variables, and was missing in the high conductivity zones of VLP and Caracoles.

Discussion

Species richness

Previous zooplankton studies in Doñana have concentrated on dune ponds in Doñana National Park (Serrano & Toja, 1998; Fahd et al., 2000; Serrano & Fahd, 2005). Furest & Toja (1981), who included sites within the Marismas, reported 9 calanoids, 9 cyclopoids, 5 harpacticoids and 28 cladocerans from a one-year study with monthly sampling. Considering that in the present study only one sample was taken in each waterbody, the total species richness reported here was relatively high (8 calanoids, 9 cyclopoids, 1 harpacticoid, 21 cladocerans). To our knowledge, five species recorded in the present study are new to Doñana. These are two cyclopoids (*Metacyclops planus*, *Mesocyclops leuckarti*) and three cladocerans (*Ilyocryptus sordidus*, *Moina salina*, *Pleuroxus letourneuxi*).

Across the zones of Doñana, we found a significant relationship between local species richness and hydroperiod, the richest assemblages being those of the ponds with a 3–5 month hydroperiod. Fahd et al. (2000) made a similar observation for the Doñana dune ponds, although their results were not significant. Ponds with shorter hydroperiods (< 3 months) in our study also had the smallest surface area, thus reducing the likelihood of propagules arriving at a given site. In addition, smaller wetlands are less likely to be visited by waterfowl (Green et al., 2002), which play a major role in the dispersal of aquatic organisms in Doñana (Figuerola et al., 2003). Ponds with longer hydroperiods, on the other hand, are more likely to contain invertebrate predators (Schneider

& Frost, 1996) or fish predators (Hobaek et al., 2002), which are important factors controlling zooplankton richness.

A successive general increase in zonal species richness (γ-diversity) was observed from Veta la Palma (VLP), Caracoles and Entre Muros (EM), Brazo del Este (BDE) to the Marismas of the Doñana National Park in both copepods and cladocerans. The number of sites sampled in the respective wetland zones was positively related to γ-diversity of zones. This can partly be attributed to higher sampling effort increasing the probability of sampling a larger habitat variety with a more heterogeneous habitat structure, which is likely to have a richer fauna (Amoros & Bornette, 2002). In addition, interconnectivity between temporary sites can significantly enhance dispersal probability and zooplankton diversity, as seen for copepods in floodplain habitats (Frisch et al., 2005). Larger areas contain a higher number of species, according to species–area relationships reported in other zooplankton studies (Dodson, 1992; Shurin et al., 2000). In the species-rich Marismas, regular flooding leads to interconnection between the ponds (Montes et al., 1998), creating a unified and large waterbody likely to enhance microcrustacean diversity. In contrast, the artificial VLP ponds contained only few species despite high interconnectivity and this appears to be related to a variety of other factors discussed below.

Overall, the presence of fish seemed to play a minor role in shaping species richness in Doñana wetlands. Fish were observed in almost all of the sites sampled, with the exception of all Caracoles sites and only the smallest ponds of other zones. Of these, only the BDE ponds were species-rich, indicating that other factors apart from fish are important in promoting species diversity. The Marisma sites with the highest richness, such as Maris 7, 11, and 12 contained fish but also had abundant vegetation, which provides shelter from vertebrate predation (Jeppesen et al. 1997; Timms & Moss, 1984). This is in sharp contrast to the VLP ponds, where vegetation was scarce or absent. As these ponds are used for fish farming, the predation pressure by fish is likely to be higher than in other parts of Doñana, and this in combination with low abundance of vegetation is also likely to reduce zooplankton richness (Hobaek et al., 2002).

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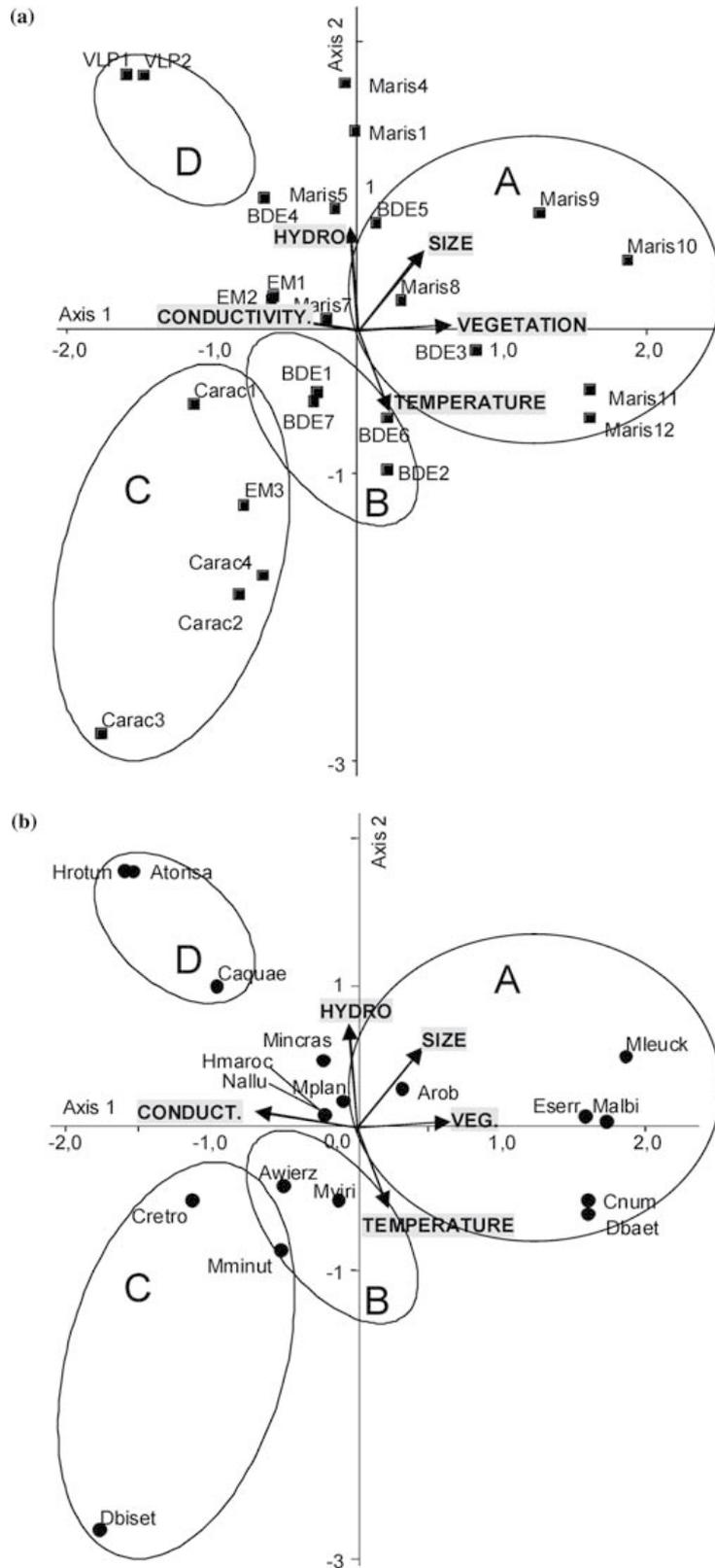Ecología acuática: riqueza y distribución de microcrustáceos en Doñana.

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Figure 2. (a, b) CCA ordination for copepods, showing the two main axes. Environmental vectors are inserted as arrows in the CCA ordination of both species and assemblages. (a) Ordination of copepod assemblages. (b) Ordination of copepod species. Labels for sites and species correspond to those used in Table 2. Circles refer to groups of assemblages or species as explained in the text.

Table 3. (a, b) Results of CCA analysis for copepods and cladocerans

	Copepods			Cladocerans		
	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
(a) Axis summary statistics and variance in species data						
Eigenvalue	0.716	0.510	0.384	0.629	0.408	0.329
p-value	0.001	0.002	0.001	0.003	0.014	0.006
% of variance explained	15.3	10.9	8.2	13.1	8.5	6.8
cumulative % explained	15.0	26.1	34.3	13.1	21.5	28.4
Pearson Corr., Spp-Envt	0.951	0.872	0.958	0.937	0.823	0.855
p-value	0.001	0.059	0.001	0.016	0.533	0.073
(b) Correlations of environmental variables and canonical axes (Intraset correlations of (ter Braak, 1987)						
Variable						
Temperature	0.215	−0.561	0.469	−0.197	0.101	−0.636
Conductivity	−0.704	0.112	0.156	−0.935	−0.051	−0.42
Size (surface area)	0.452	0.562	−0.476	0.576	0.142	−0.415
Depth	−0.042	0.051	−0.896	0.065	−0.023	−0.408
Vegetation cover	0.649	0.046	−0.135	0.709	0.578	−0.213
Hydroperiod	−0.061	0.727	−0.147	0.189	−0.366	−0.098

(a) Axis summary statistics of the three canonical axes extracted and percentage of variance explained by CCA ordination. (b) Correlation of environmental variables with ordination axes. These correlations represent intraset correlations of ter Braak (1986).

Species distribution and composition

The zones differed significantly in environmental properties. These differences involved most of the factors studied but were most obvious for conductivity, size and vegetation cover. Examining the results of the CCA ordination, these factors also appear to be related to differences in species distribution and composition. However, although species–environment correlations were high and significant, the CCAs explained only about 30% of the variability in both cladocerans and copepods, and thus have to be interpreted with some caution. These results suggest that the environmental variables used in the analyses are important in structuring species distribution and composition but that other variables not considered in our study have an important impact as well. These factors are likely to include pH or dissolved oxygen, trophic status, phytoplankton composition or presence of fish, as reported from other studies (e.g. Arnott & Vanni, 1993; Armengol et al., 1998; Cottenie et al., 2001; Hobaek et al., 2002).

For both copepods and cladocerans the main factors extracted by axis 1 of their respective CCA, which also explained the highest amount of vari-ation, were conductivity, vegetation and surface area. The extraction of these factors led to a well-defined separation of the two zones characterised by high conductivity (VLP and Caracoles), and of the vegetation rich sites of the Marismas, which had a generally lower conductivity. VLP had a distinctive species assembly with respect to both groups. The species recorded there were not present in any of the other zones studied in Doñana, with the exception of *Calanipeda aquaedulcis*, which was also present in one of the BDE ponds. Temperature was of some importance only in the second axis of the CCA for copepods, separating the clusters of the high conductivity zones Caracoles and VLP. However, faunal differences seen between these zones are unlikely to be directly caused by temperature, as the species recorded in either zone are not typical warm- or cold-water species. Temperature is likely to be a surrogate for depth and/or date (since date and temperature were correlated, Table 1). The distinct copepod assemblage found in VLP is likely to be due to the hydrological connection of these ponds to the Guadalquivir estuary, from which the ponds may receive estuarine fauna (Frisch et al., in press). It is likely that most species encountered in other

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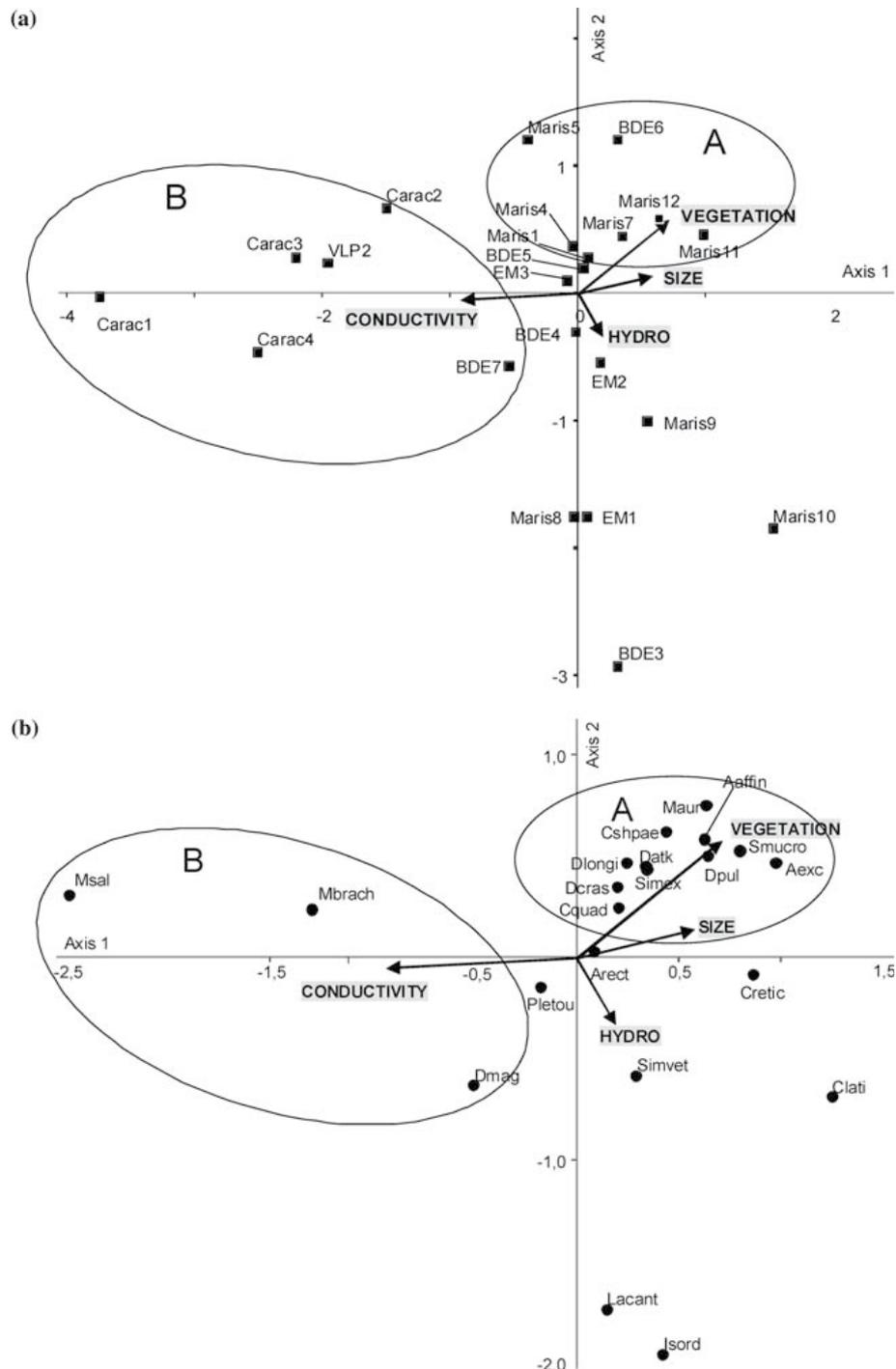Ecología acuática: riqueza y distribución de microcrustáceos en Doñana.

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Figure 3. (a, b) CCA ordination for cladocerans, showing the two main axes. Environmental vectors are inserted as arrows in the CCA ordination of both species and assemblages. (a) Ordination of cladoceran assemblages. (b) Ordination of cladoceran species. Labels for sites and species correspond to those used in Table 2. Circles refer to groups of assemblages or species as explained in the text. Note scaling differences of ordination axes between assemblage and species ordination.

wetlands of Doñana are excluded from colonizing these sites due to their relatively high salinities (> 14 mS cm^{-1}). The same might be true for the high conductivity sites in Caracoles. Two copepod species (*Diacyclops bisetosus*, *Cletocamptus* cf. *retrogressus*) and one cladoceran (*Moina salina*) were unique to this area. All of these are known for their tolerance of higher salinity (Alonso, 1990,

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1996). Other species present in Caracoles were *Metacyclops minutus*, *Arctodiaptomus wierzejski*, *Daphnia magna* and *Moina brachiata*, also previously reported as euryhaline (Alonso, 1990, 1998).

Sites sampled in BDE contained only one copepod species unique to this area (*Megacyclops viridis*). However, its presence in the National Park area was recorded in other studies (Serrano & Fahd, 2005; Fahd et al, 2000). While the CCA grouped several of the copepod assemblages of the small BDE ponds together, this was not the case for cladocerans. In fact, cladocerans were missing altogether from the two smallest BDE sites. Since fish were not present in these ponds, other factors must be responsible for the absence of cladocerans. Other equally small ponds with similarly short hydroperiods contained few cladocerans, probably related to their high degree of ephemerality. This finding parallels that of Mahoney et al. (1990), who found that cladoceran species richness increased with size and hydroperiod.

The Marismas contained the largest number of species unique to a given zone for both taxa. The association of these assemblages with abundant vegetation and large size is reflected in their position on the CCA axes. Some of the Marismas sites contained a variety of species typical of plant-rich freshwater assemblies, e.g., the copepods *Eucyclops serrulatus* and *Macrocyclops albidus* (Einsle, 1993), and the cladocerans *Alona affinis*, *Ceriodaphnia laticaudata*, or *Scapholeberis mucronata* (Alonso, 1996, 1998).

In conclusion, hydroperiod, conductivity, vegetation and wetland size all seem to be determinants of the microcrustacean fauna found from January to March in the Doñana wetlands. Further work is required to establish how these variables influence other invertebrate groups, and copepods and cladocerans at other times of the year.

Acknowledgements

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