

Spatio-temporal nested patterns in macroinvertebrate assemblages across a pond network with a wide hydroperiod range

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Abstract Nestedness has been widely used to measure the structure of biological communities and occurs when species-poor sites contain subsets of species-rich ones. Here, we examine nested patterns across the macroinvertebrate assemblages of 91 ponds in Doñana National Park, Spain, and explore temporal variation of nestedness and species richness in 19 temporary ponds over 2 years with differing rainfall. Macroinvertebrate assemblages were significantly nested; both pond spatial arrangement and environmental variation being important in driving nested patterns. Despite the nested structure observed, a number of taxa and ponds deviate from this pattern (termed idiosyncratic), by occurring more frequently than expected in species-poor sites, or having assemblages dominated by species largely absent from species-rich sites. Aquatic adults of winged insects, capable of dispersal, were more highly nested than non-dispersing taxa and life-history stages. Idiosyncratic taxa were found in ponds spanning a wide range of hydroperiods, although nestedness was higher in more permanent waterbodies. Monthly sampling

demonstrated a gradual increase of species richness and nestedness from pond filling to April–May, when the most temporary ponds started to dry. Although the degree of nestedness of individual pond assemblages varied from month to month, the overall degree of nestedness in the two study years was practically identical despite marked differences in hydroperiod. Our results suggest that differential colonization and environmental variation are key processes driving the nested structure of Doñana ponds, that macroinvertebrate assemblages change in a predictable manner each year in response to cycles of pond wetting and drying, and that connectivity and environmental variability maintain biodiversity in pond networks.

Keywords Colonization · Community composition · Dispersal · Nestedness · Temporary ponds

Introduction

Nested systems occur when species-poor sites contain subsets of the assemblages found in species-rich sites, and the degree of nestedness thus quantifies the overlap in species composition between high and low diversity areas (McAbendroth et al. 2005). Nestedness has been widely used to measure the structure of biological communities (Fleishman and Murphy 1999; Hylander et al. 2005; Meyer and Kalko 2008; Elmendorf and Harrison 2009), including lentic freshwaters (Baber et al. 2004; McAbendroth et al. 2005; Angeler et al. 2008; Wissinger et al. 2009), although few studies have explored the factors which may drive this pattern. Factors that lead to consistent differences amongst species in immigration or extinction rates cause strong patterns of nestedness across species assemblages (Wright and Reeves 1992). Although, originally, historical

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extinction was assumed to be the main cause of nestedness (Atmar and Patterson 1993), local immigration through the differential dispersal abilities of species can also be very important in generating nestedness under particular conditions (Cook and Quinn 1995). Whilst extinction is important in structuring biological communities over long timescales (Patterson 1990; Hausdorf and Hennig 2003), differential colonization can be important over shorter-term scales (Patterson 1990; Atmar and Patterson 1993), particularly when colonization drives community assembly in originally empty sites such as new and ephemeral habitat patches (Norton et al. 2004). In addition to selective immigration and extinction, variation in environmental conditions, including habitable area, across sites can also generate nested patterns (Lomolino 1996; Baber et al. 2004; McAbendroth et al. 2005; Heino et al. 2009), although studies of nestedness which assess the importance of immigration–extinction dynamics and environmental heterogeneity are rare in any habitat.

Temporary ponds are fluctuating waterbodies with recurrent seasonal phases of flooding and desiccation in most years (Grillas et al. 2004; Brönmark and Hansson 2005; Williams 2006). Although some macroinvertebrates persist in ephemeral habitats as resting stages in dry sediment (see Batzer and Wissinger 1996), dispersal to more permanent waterbodies is the main strategy through which they survive dry phases in temporary aquatic habitats (Wiggins et al. 1980; Bilton et al. 2001; Garrido and Munilla 2008). As a consequence, much of the invertebrate biota of temporary ponds is assembled through colonization on each re-wetting cycle, making these an ideal system in which to explore the role of colonization in generating nestedness. Such a dispersal-driven system can have important implications for nestedness patterns, and isolated ponds would show lower nestedness if the regional species pool is dominated by poor dispersers (Cook and Quinn 1995; McAbendroth et al. 2005). In lentic freshwaters, hydroperiod duration is well established as a key factor driving assemblage structure (e.g., Baber et al. 2004; Urban 2004; Waterkeyn et al. 2008). As nestedness can be related to assemblage stability (Atmar and Patterson 1993), this could be expected to increase with hydroperiod length due to the presence of species which require more time to complete their life cycles which are not able to survive even occasional dry phases. The temporal variability of nested patterns in biological communities can shed light on the role of colonization and extinction in generating nestedness (Patterson 1990; Loo et al. 2002; Bloch et al. 2007), and the temporal cycle of wetting and drying in ephemeral waters makes them an ideal system in which to explore such processes. Despite this, no studies to date have explored temporal variation in nestedness in these systems over their wetting/drying cycles.

Idiosyncratic species and sites are those that depart from the general nested pattern, reducing the value of matrix nestedness (Atmar and Patterson 1993). Idiosyncratic species are those which occur more frequently than one would expect in species-poor sites, and/or less frequently in species-rich ones, something which could result through habitat specialization or competition avoidance, as well as being a locally infrequent habitat generalist (see McAbendroth et al. 2005). Individual sites are considered idiosyncratic when they contain a higher proportion of idiosyncratic species than one would predict from their species richness—in other words, their assemblages contain a number of species which are largely absent from the most species-rich sites. The detection of idiosyncratic species and sites is of potential interest for conservation (McAbendroth et al. 2005; Heino et al. 2009) as idiosyncratic sites may harbor specialist taxa not present in species-rich localities, and idiosyncratic species may require specialized conditions, present in relatively few sites in a region (Atmar and Patterson 1993).

Here we examine nestedness and its temporal variation, across a natural pond network in southern Spain, spanning a wide range of hydroperiods. We determine the degree of nestedness across the pond network as a whole by analyzing data from close to 100 ponds, collected over a single season. We use this dataset to determine whether nestedness increases with: (1) the dispersal ability of macroinvertebrates; (2) the degree of isolation of ponds; and (3) the length of hydroperiod. We go on to examine whether variation in environmental conditions across sites may contribute to nestedness, and evaluate the relative importance of colonization and environmental variation in generating observed patterns. Additionally, we explore whether species richness and nestedness vary in a predictable manner over the hydrological cycle in a subset of ponds sampled monthly across 2 years with differing rainfall regimes. Finally, we consider the possible implications of our findings for the conservation of lentic biodiversity in the region.

Materials and methods

Study area

Our study was performed in Doñana National Park, located between the mouth of the Guadalquivir River and the Atlantic Ocean in south-western Spain (see Siljeström et al. 1994). The climate of this area is Mediterranean sub-humid, with most rainfall in autumn and winter, hot and dry summers, and mild winters. In this area, two main regions can be differentiated: an extensive marsh and an adjacent sandy area where numerous temporary waters

form in natural depressions; more than 3,000 occurring in wet years. Ponds in the park differ widely in surface area and hydroperiod, and constitute a heterogeneous network of aquatic habitats which has been listed under the RAMSAR Convention since 1982. Temporary ponds in Doñana are seasonally flooded after heavy rains, usually filling in autumn or winter, and persisting until late spring or early summer. There are also two large semi-permanent ponds which only occasionally dry in years of severe drought, and some natural ponds have been artificially deepened to supply water for cattle and wild mammals, making them permanent in most years. A detailed description of the environmental characteristic of these ponds is given in Gómez-Rodríguez et al. (2009), Espinar and Serrano (2009) and Díaz-Paniagua et al. (2010).

Sampling surveys

Two linked sampling surveys were carried out, and these provide the data which form the basis of this study.

Extensive macroinvertebrate survey In order to assess the degree of nestedness across the pond network as a whole, we sampled a total of 91 ponds which ranged from 24 to 122,672 m² in area between end-March and mid-June 2007. Pond assemblages in Doñana were relatively stable over this time period (no consistent change in species richness between March and June; linear regression: $R^2 = 0.017$, $p = 0.22$), which was the minimum window over which all sites could be visited. Ponds were chosen to span the full range of hydroperiod variation present within the study area, and cover all areas of the park where discrete lentic waterbodies occur (Fig. 1). Based on their

depth, area, and previous observations (see Florencio et al. 2009), ponds were assigned to one of three categories based on their degree of permanence: short hydroperiod ($n = 28$), intermediate hydroperiod ($n = 22$), and long hydroperiod (including permanent ponds; $n = 41$). Pond surface area varied considerably within each hydroperiod category, and did not increase with pond duration. Although short and intermediate hydroperiod categories did not differ in area, long hydroperiod ponds were actually significantly smaller (one-way ANOVA, $F_{(2,88)} = 10.919$, $p \searrow 0.0001$; Tukey HSD, $p \searrow 0.0001$; see Table 1), often being relatively small pools which had been artificially deepened (see above).

Monthly macroinvertebrate survey To analyse temporal variation in nestedness over wet–dry cycles, we sampled aquatic macroinvertebrates in 19 temporary ponds during their wet phases, from February 2006 to August 2007. All these ponds were distributed within the central area of the Park (Fig. 1). The first study year was relatively dry (468 mm rainfall; hereafter referred to as the dry year), and most ponds were only wet from February to May. The second study year was wetter (716.9 mm rainfall; hereafter referred to as the wet year), and most ponds were flooded from November to July. Due to the increase in the number of ponds formed in the second year (as a result of higher rainfall), we sampled three additional ponds replacing three ponds only sampled in the dry year in order to include ponds spanning the highest range of hydroperiod observed in the area. For the monthly survey, we calculated hydroperiod as the number of months ponds were flooded in a given year, and classified this in relation to the longest hydroperiod seen in each year. In the dry year, hydroperiod

Fig. 1 Location of the ponds sampled in Doñana National Park, identified by a hydroperiod categories and b idiosyncratic or nested character of their macroinvertebrate assemblages. The 19 ponds sampled for the monthly survey are within the Doñana Biological Reserve area marked by a dashed line. Marsh, where discrete ponds are largely absent during flooding periods, is indicated by light shading



Table 1 Environmental characteristics of ponds in the extensive survey, showing the mean, minimum (min.) and maximum (max.) values

Environmental variables	Mean (min–max)
Pools (%)	3.8 (0–80)
Maximum depth (cm)	74.2 (9–126)
Pond area (m ²)	4,103 (24–122,672)
Short hydroperiod	3,427 (25–23,097)
Intermediate hydroperiod	4,517 (25–37,544)
Long hydroperiod	4,370 (24–122,672)
Pond number	4.4 (0–16)
NH ₄ ⁺ (mg l ⁻¹)	1.34 (0.04–3.59)
i-P (mg l ⁻¹)	0.11 (0.01–0.75)
[O ₂] (mg l ⁻¹)	2.2 (0.2–16.8)
EC (IS cm ⁻¹)	962.8 (82–8,800)
Turbidity (NTU)	35.0 (1.1–975.5)
Organic matter (%)	5.43 (0.17–27.74)
SO ₄ ²⁻ (meq l ⁻¹)	0.82 (0.04–23.24)

Pond number The number of ponds ≤ 150 m² within 200 m of each pond, i-P dissolved inorganic phosphate, [O₂] dissolved oxygen concentration, EC electrical conductivity

categories were short (≤ 2.5 months), intermediate (2.5–3.5 months) and long (≥ 3.5 months). In the wet year, short hydroperiod was ≤ 7 months, intermediate 7–8 months and long ≥ 8 months (for further details on hydroperiod of these ponds, see Florencio et al. 2009).

Macroinvertebrate sampling and taxon identification

We sampled macroinvertebrates with a dip-net (39 \times 21 cm, 1 mm mesh), netting a stretch of water of approximately 1.5 m length in each sampling unit. In each pond, we sampled all different available microhabitats, based largely on differences in aquatic plant cover and depth (Heyer et al. 1994). As the efficiency of dip-netting increases in small ponds (Heyer et al. 1994), we took more samples in larger ponds, which also typically contained a higher number of microhabitats, in order to achieve comparable effort in detecting rare species. In all ponds, sampling was concentrated amongst vegetation, where most macroinvertebrate species are located. In most cases, a minimum of three samples were taken (maximum 13), with the exception of five small ponds sampled at the end of their dry phase, which were so small that three discrete 1.5-m sampling areas could not be identified. Most macroinvertebrates were identified in situ and then returned to the pond, with those taxa which could not be determined in the field preserved in 70% ethanol for examination in the laboratory. Identifications were performed to the lowest taxonomic level possible, in general to species (most adults) or genus (most larvae), although Basomatophora,

Diptera, Oligochaeta and saldid bugs were identified only to family. We kept the different taxonomic levels reached for adults and larvae, using these stages of taxa separately (hereafter referred to as taxa/stages), to assess the role of each stage in generating nested patterns.

Environmental variables

In order to determine whether pond characteristics influence macroinvertebrate nestedness, we measured a range of environmental variables across the ponds (see Table 1). In the field, we measured the proportion of the pond surface divided into separate pools, maximum water depth (with a graduated pole at the deepest point of the pond), conductivity (on bed using HI 9033), dissolved oxygen concentration (on bed using YSI 550A) and turbidity (in water column using HANNA HI93703). Surface water (500 ml) was also collected to determine nutrient [dissolved inorganic phosphate and ammonium—using an Auto Analyzer (Bran $\&$ Luebbe)] and SO₄²⁻ anion concentrations [using inductively coupled plasma mass spectrophotometer (ICP)]. Surface sediment samples (5 cm depth) were collected and organic matter measured in the laboratory (mean of three replicates via loss on ignition, 450°C, 5 h). Pond area and the number of ponds ≤ 150 m² within 200 m of each pond were extracted from a GIS-based map constructed at the time of maximum inundation (see Gómez-Rodríguez et al. 2008).

Statistical analyses

We constructed several matrices using presence–absence data of macroinvertebrate occurrence by pond:

For the extensive survey we built: (1) the taxon matrix, pooling adults and larvae of individual taxa, to the highest taxonomic resolution possible; (2) the stage matrix, with adults and larvae separated; (3) the disperser matrix, including only adults of taxa capable of flight; and (4) the non-disperser matrix, including only larvae and taxa with non-flying adults. To evaluate whether longer hydroperiod increased nestedness, we built three sub-matrices of the stage matrix which included the ponds catalogued as short, intermediate or long hydroperiod for each matrix. A χ^2 test was used to evaluate whether idiosyncratic taxa or stages were associated with ponds of any of the hydroperiod categories. A χ^2 test was also used to evaluate whether idiosyncratic ponds were more common in some hydroperiod categories than others.

In order to evaluate how the degree of nestedness observed across ponds changed through their wetting–drying cycle, we built a stage matrix as described above for each month when ≥ 2 ponds were wet (4 months in the dry year; 9 months in the wet year). To compare nestedness

between the two study years, we also built two annual presence–absence matrices with the occurrence of taxa/stages pooled across months (one matrix per year). The size of monthly matrices varied between sampling months with the gradual desiccation of ponds, as not all ponds were flooded in all months. Also, different numbers of taxa/stages were recorded each month.

For nestedness analysis, we used ANINHADO (see <http://www.guimaraes.bio.br>; Guimaraes and Guimaraes 2006), a recent package with advantages over the more widely used nestedness temperature calculator (Atmar and Patterson 1995). We considered two nestedness metrics: (1) temperature (T), widely used to analyse nested patterns (Atmar and Patterson 1993), and (2) the recently developed NODF, which differs from T in being independent of both matrix size and shape (Almeida-Neto et al. 2008). Atmar and Patterson (1993) interpret T as a thermodynamic measure of disorder which ranges from 0 to 100°, being low in ordered (i.e., nested) systems and high in disordered systems. The procedure to calculate both T and NODF requires a presence–absence matrix ordered by species presences (top-to-bottom), automatically generated in ANINHADO (Guimaraes and Guimaraes 2006). Using this ordered matrix, T is calculated through the number of departures from a perfectly nested matrix (Atmar and Patterson 1993). In order to scale the degree of nestedness, or order, to values between 0 and 1 (maximum nestedness), we used N instead T, calculated as $N = (100 - T)/100$ (Bascompte et al. 2003). As we compared nestedness between matrices with different sizes, and N is dependent on matrix size (Atmar and Patterson 1993; Almeida-Neto et al. 2008), we calculated the relative nestedness [$N^* = (N - N_R)/N_R$] where N is the degree of nestedness of the original matrix and N_R is the average nestedness of 1,000 null model matrices (Bascompte et al. 2003). NODF, the recently developed metric to calculate nestedness, varies from 0 to 100 (maximum nestedness). NODF is obtained through the percentage overlap of presences for each pair of columns and for each pair of rows in an ordered matrix (Almeida-Neto et al. 2008). We include reference to NODF and N here to allow comparison with previous studies, most of which have employed variations of the latter metric.

ANINHADO allows the degree of nestedness present within a system to be assessed against four separate null models, all of which were tested here: (1) presences assigned at random across the matrix; (2) a fixed–fixed null model, with both column sums and row sums fixed; (3) only column sums fixed; and (4) only row sums fixed (see <http://www.guimaraes.bio.br>). The significance of observed values of nestedness was assessed using 1,000 permutations of each null model. Using N and NODF, whenever significant nestedness was obtained with the

fixed–fixed null model, it was also obtained using the other three, and we therefore used the fixed–fixed null model (the most restrictive; incurring fewer Type I errors; Gotelli 2000; Ulrich and Gotelli 2007a, b; Almeida-Neto et al. 2008) to assess the degree of nestedness. To detect idiosyncratic ponds and taxa we calculated N for each pond and taxon/stage, and considered as idiosyncratic those taxa/stages whose N values were lower than the value of the original matrix (Atmar and Patterson 1993). Since the degree of nestedness can be affected by sample size, and the number of samples taken differed between ponds, we explored the effect of sample number on nestedness via sample-based rarefaction (Gotelli and Colwell 2001) with the minimum number of samples per pond and month using EcoSim Version 7 (Gotelli and Entsminger 2004). In all cases, we obtained similar nested patterns to those produced with raw data, and consequently present only the latter here.

Pond spatial isolation was used to assess the contribution of differential colonization to nestedness patterns, following Lomolino (1996). We evaluated whether the degree to which a pond departed from nestedness (N) was affected by the spatial arrangement of sampled ponds (i.e., whether the level of nestedness observed depended on relative isolation), using distance-based Moran's eigenvector maps (MEM; see Dray et al. 2006), a general framework of principal coordinates of neighbour matrices (PCNM; see Borcard and Legendre 2002). We also compared the results for disperser and for non-disperser matrices in order to evaluate the role of dispersal in generating the nested pattern. We used the Delaunay triangulation criterion (see Legendre and Legendre 1998) which was appropriated to connect large distances, but did not consider edge effects since the Park boundaries are not the limit of the aquatic systems in the region. The number of eigenvectors was reduced using the 'ortho.AIC' command in R software ('spacemaker' package; Dray et al. 2006). Only significant eigenvectors were used directly as explanatory variables in a multiple forward stepwise regression with the nestedness of ponds (in STATISTICA 6.0). Forward stepwise regression was performed following Blanchet et al. (2008), in which all eigenvectors were included to analyze global significance (preselected $\alpha = 0.05$) considering the adjusted coefficient of multiple determination ($\text{Adj. } R^2$). This complete procedure is an effective way of controlling for Type I error (Peres-Neto and Legendre 2010).

To explore the role of inter-pond environmental variation in driving nestedness in the macroinvertebrate community, we used the Monte Carlo approach of Lomolino (1996). Analyses here were restricted to a subset of 80 ponds for which full environmental variables were available. The ponds of the stage matrix were ranked by each

environmental variable, and the number of unexpected absences followed by a presence quantified as the departure (D) from perfect nestedness (Honnay et al. 1999). Statistical significance was estimated as the number of randomizations of the fixed–fixed null model giving $D \setminus \text{observed}$ (see Lomolino and Davis 1997). Environmental variables with identical values in two or more ponds (proportion of pools, maximum depth, nutrient concentrations and the number of ponds $\leq 150 \text{ m}^2$ within 200 m) were not analyzed, as the approach requires unique values for each site (Lomolino 1996).

To assess the relative role of pond spatial arrangement and local environmental variation in generating nested patterns, we used a variance partitioning approach. Urodele predators (*Triturus pygmaeus*, *Lissotriton boscai* and *Pleurodeles waltl*) and exotic fish (*Gambusia holbrooki*), were ubiquitous and highly infrequent, respectively, had no influence on macroinvertebrate nestedness, and were therefore excluded from further analyses. As a measure of the spatial arrangement of ponds, we used spatial descriptors extracted from MEMs; the effect of environmental variation being summarized using the first axis scores from a principal components analysis (PCA) of all environmental variables (Primer v.6; Clarke and Warwick 2001). Variance partitioning was performed using the ‘varpart’ command in R software (‘vegan’ package; Oksanen et al. 2008), which obtains an adjusted multiple coefficient of determination (Adj. R^2 , 0–1) in order to compare explanatory variables (Peres-Neto et al. 2006). Significance was tested using the complete procedure described above, through a multiple forward stepwise regression for the spatial descriptors, and a linear regression for the PC1 scores.

Results

Nestedness in the Doñana ponds network

The macroinvertebrate assemblages of Doñana ponds were highly nested overall (stage matrix: $N = 0.81$, 16.43% fill;

taxon matrix: $N = 0.83$, 17.65% fill; Table 2). The taxon matrix was more highly nested than the stage matrix (including adults and larvae separately), in which we detected a higher number of idiosyncratic taxa and stages (Table 2). The disperser matrix exhibited higher nestedness than the non-disperser matrix (Table 2).

Out of the 135 taxa/stages recorded, 59 were idiosyncratic (Table 3), occurring in 1–53 ponds, across all hydroperiod classes. Some idiosyncratic beetles [*Yola bicarinata* (Latreille, 1804), *Hygrobia hermanni* (Fabricius, 1775), *Hygrotus confluens* (Fabricius, 1787)] and damselflies [*Lestes virens* (Charpentier, 1825)] were significantly more abundant in long hydroperiod ponds, whereas some idiosyncratic flies (Culicidae and *Chaoborus* spp.) were significantly more frequent in short hydroperiod ponds. Sympetrid dragonflies [*Sympetrum fonscolombei* (Selys, 1841), *S. meridionale* (Selys, 1841), *S. striolatum* (Charpentier, 1840) and *S. sanguineum* (Müller, 1764)] were significantly more frequent in short and intermediate hydroperiod ponds rather than those with long hydroperiods (Table 3). Of the 91 studied ponds, 34 were idiosyncratic at the assemblage level (Table 4). These idiosyncratic ponds tended to cluster in both northern and southern areas of the park, suggesting an effect of isolation, but did not correspond to a particular hydroperiod category (Fig. 1).

Four significant spatial descriptors extracted from MEMs analyses were strongly correlated with the degree of nestedness of the ponds (N) of the stage matrix (Adj. $R^2 = 0.35$, $F_{(24,66)} = 2.987$, $p \setminus 0.001$); i.e., the spatial arrangement of ponds, based on their inter-pond distances (pond isolation), can partially drive the macroinvertebrate nested structure. This effect was much higher in the disperser matrix with six significant spatial descriptors (Adj. $R^2 = 0.40$, $F_{(21,68)} = 3.849$, $p \setminus 0.0001$) than in the non-disperser matrix with only a single significant spatial descriptor (Adj. $R^2 = 0.13$, $F_{(10,79)} = 2.354$, $p \setminus 0.05$). This means dispersers were much more affected by the spatial structure and isolation of ponds than non-dispersers. We detected significant differences in the proportion of idiosyncratic ponds in each hydroperiod category ($v^2 = 11.99$, $df = 2$, $p \setminus 0.01$).

Table 2 Degree of nestedness (N, N* and NODF) detected in the extensive macroinvertebrate survey for different matrices: the taxon matrix, the stage matrix (which included adult and larval stage

separately), matrix of dispersers (adults with capability of flight) and non-dispersers (larvae and non-flying adults)

	N	N*	NODF	Columns (taxa)	Idio-ponds	Idio-taxa
Stage matrix	0.81	0.43	34.52	135	34	59
Taxon matrix	0.83	0.46	45.20	102	37	32
Disperser matrix	0.82	0.48	42.32	66	40	24
Non-disperser matrix	0.88	0.34	41.55	68	30	24

Columns are the number of taxa/stages; idio-taxa the number of idiosyncratic taxa/stages; idio-ponds the number of idiosyncratic ponds. All nested values were significant at $p \setminus 0.001$

Table 3 Idiosyncratic taxa/stages (adults or larvae) with their degree of nestedness (N), the number of ponds in which they occurred (Pond occurrence) and hydroperiod category of these ponds: Short, Intermediate (Interm) and Long

Order	Taxa/stages	N	Pond occurrence	Short	Interm	Long
	Adults					
Bassomatophora	Physa spp.	0.42	33	10	6	17
Coleoptera	Gyrinus dejeani Brullé, 1832	0.47	15	4	3	8
Heteroptera	Trichocorixa verticalis (Fieber, 1851)	0.50	13	5	4	4
Coleoptera	Hydrobius fuscipes (Linnaeus, 1758)/Limnoxenus niger (Zschach, 1788)	0.54	36	12	13	11
Coleoptera	Hydroglyphus geminus (Fabricius, 1792)	0.54	28	5	5	18
Coleoptera	Rhantus hispanicus Sharp, 1882	0.62	31	15	6	10
Heteroptera	Notonecta meridionalis Poisson, 1926	0.63	25	8	6	11
Coleoptera	Ochthebius dilatatus Stephens, 1829	0.64	5	3	0	2
Coleoptera	Helochares lividus (Forster, 1771)	0.66	10	4	1	5
Heteroptera	Notonecta viridis Delcourt, 1909	0.67	23	4	5	14
Coleoptera	Agabus nebulosus (Forster, 1771)	0.67	19	8	3	8
Coleoptera	Hydroporus lucasi Reiche, 1866	0.67	41	12	13	16
Coleoptera	Yola bicarinata (Latreille, 1804)	0.67	6*	0	0	6
Coleoptera	Hygrotus confluens (Fabricius, 1787)	0.70	21*	3	3	15
Coleoptera	Hygrobia hermanni (Fabricius, 1775)	0.71	29*	5	4	20
Heteroptera	Gerris thoracicus Schummel, 1832	0.71	55	18	16	21
Coleoptera	Berosus signaticollis (Charpentier, 1825)	0.71	15	4	6	5
Heteroptera	Notonecta glauca Linnaeus, 1758	0.72	18	6	6	6
Coleoptera	Enochrus fuscipennis (Thomson, 1884)	0.73	32	7	11	14
Coleoptera	Hygrotus lagari (Fery, 1992)	0.73	34	5	9	20
Bassomatophora	Planorbidae	0.74	13	2	3	8
Heteroptera	Sigara scripta (Rambur, 1840)	0.74	12	3	2	7
Heteroptera	Plea minutissima Leach, 1817	0.75	26	4	6	16
Heteroptera	Nepa cinerea Linnaeus, 1798	0.75	8	2	2	4
Coleoptera	Anacaena lutescens (Stephens, 1829)	0.75	49	16	12	21
Heteroptera	Micronecta scholzi (Fieber, 1860)	0.77	6	1	0	5
Coleoptera	Helophorus spp.	0.78	36	10	9	17
Coleoptera	Noterus laevis Sturm, 1834	0.78	6	1	1	4
Coleoptera	Ilybius montanus (Stephens, 1828)	0.78	11	1	4	6
Coleoptera	Laccobius revelierei Perris, 1864	0.79	1	1	0	0
Heteroptera	Sigara lateralis (Leach, 1817)	0.79	39	11	8	20
Coleoptera	Berosus affinis Brullé, 1835	0.79	17	4	6	7
Heteroptera	Notonecta maculata Fabricius, 1794	0.80	14	3	4	7
Coleoptera	Laccophilus minutus (Linnaeus, 1758)	0.80	29	6	9	14
Heteroptera	Sigara stagnallis (Leach, 1817)	0.80	15	4	2	9
Coleoptera	Colymbetes fuscus (Linnaeus, 1758)	0.80	35	10	10	15
	Larvae					
Coleoptera	Hydrobius fuscipes (Linnaeus, 1758)/Limnoxenus niger (Zschach, 1788)	0.44	23	5	10	8
Diptera	Culicidae	0.49	27*	15	6	6
Coleoptera	Hyphydrus aubei Ganglbauer, 1892	0.55	22	4	4	14
Diptera	Chironomus plumosus (Linnaeus, 1758)	0.61	53	11	12	30
Odonata	Sympetrum fonscolombei (Selys, 1841)	0.61	46*	17	16	13
Odonata	Ishnura pumilio (Charp., 1825)	0.62	33	9	12	12
Diptera	Chaoborus spp.	0.64	5*	2	3	0
Heteroptera	Corixidae	0.72	52	16	13	23
Coleoptera	Cybister lateralimarginalis (De Geer, 1774)	0.73	21	7	6	8
Coleoptera	Halipplus spp.	0.73	7	2	4	1

Table 3 continued

Order	Taxa/stages	N	Pond occurrence	Short	Interm	Long
Coleoptera	Laccophilus minutus	0.73	47	15	10	22
Coleoptera	Colymbetes fuscus/Rhantus spp.	0.75	17	7	6	4
Odonata	Sympetrum meridionale (Selys, 1841)	0.77	16*	8	6	2
Odonata	Sympetrum striolatum (Charpentier, 1840)	0.78	21*	10	7	4
Odonata	Aeshna mixta Latreille, 1805	0.78	7	2	2	3
Diptera	Tanypodinae	0.78	8	2	2	4
Odonata	Lestes virens (Charpentier, 1825)	0.78	8*	0	0	8
Odonata	Lestes barbarus (Fabricius, 1798)	0.79	13	3	5	4
Coleoptera	Agabus spp.	0.79	9	1	3	5
Heteroptera	Nepa cinerea	0.79	8	1	1	6
Odonata	Sympetrum sanguineum (Müller, 1764)	0.79	20*	7	9	4
Coleoptera	Berosus spp.	0.80	22	8	7	7
Odonata	Ischnura elegans (Vander Linden, 1820)	0.80	24	4	10	10

* Significant over-representation in one pond hydroperiod category at level $p \leq 0.05$, χ^2 test

Fifty-four percent of short hydroperiod ponds were idiosyncratic, 23% of intermediate and 34% of long hydroperiod ponds (Table 4). Although the macroinvertebrate assemblages of ponds with different hydroperiod categories were similarly nested using NODF, with N^* we observed the highest nestedness in the macroinvertebrate assemblages of long hydroperiod ponds ($N^* = 0.41$, $NODF = 33.66$, both indices $p \leq 0.001$) whilst short and intermediate hydroperiod ponds showed similar degrees of nestedness ($N^* = 0.38$, $NODF = 32.81$, both indices $p \leq 0.001$; and $N^* = 0.34$, $NODF = 35.17$, both indices $p \leq 0.001$, respectively).

All measured environmental variables made a significant contribution to the nested pattern according to the number of Lomolino's departures (D) from perfect nestedness (Table 5). Pond area produced the lowest D, making the greatest contribution to the macroinvertebrate nestedness (Table 5). Despite the contribution of environmental variables to the nested pattern, variance partitioning showed that spatial descriptors were more strongly associated with nestedness (pure explained variation $Adj. R^2 = 0.117$, $p \leq 0.001$) than environmental variables (pure explained variation $Adj. R^2 = 0.041$, $p \leq 0.01$).

Monthly variation in species richness and nestedness

Most of the ponds which were sampled monthly differed in hydroperiod between dry and wet years (Table 6). In the dry year, the monthly variation in taxon/stage richness per pond in each of the three hydroperiod categories reached a maximum in April, except for short hydroperiod ponds which had already dried up (Fig. 2). In the wet year,

maximum richness occurred in March–April for short hydroperiod ponds, but in May for those with intermediate and long hydroperiods (Fig. 2). The maximum taxon/stage number per pond occurred in long hydroperiod ponds with up to 30 taxa and/or stages (Fig. 2). In ponds of all three hydroperiod categories, we observed an initial increase in the proportion of adult dispersers with respect to larvae just after ponds filled. From then on, adult dispersers and larvae remained in similar proportions until April, when the relative proportion of dispersers increased again (Fig. 2). A higher total number of taxa and/or stages were recorded in the wet year ($n = 132$) than in the dry year ($n = 108$).

In both years, we observed a similar seasonal shift in the degree of nestedness, with a gradual increase from the time at which ponds first became wet (November and February in the wet and dry years, respectively) to April–May in both years when nestedness decreased or disappeared (Fig. 3). Significantly nested assemblages were found in more months using N^* than NODF (Fig. 3). The nested pattern observed was similar using N^* and NODF, these differing only when NODF was non-significant (Fig. 3). We obtained a highly similar degree of nestedness for the pool of macroinvertebrate taxa and stages recorded every year (dry year: $N^* = 0.65$, $NODF = 61.62$, both indices $p \leq 0.001$; wet year: $N^* = 0.64$, $NODF = 60.15$, both indices $p \leq 0.001$). With one exception, the idiosyncratic character of ponds varied between months as well as between study years (Table 6). While a lower number of ponds were detected as idiosyncratic in the wet year (Table 6), a higher number of taxa/stages were detected as idiosyncratic in the wet year ($n = 53$) than in the dry year ($n = 38$).

Table 4 Idiosyncratic ponds in which macroinvertebrate assemblages departed from the nested pattern obtained with the stage matrix

Pond	N	Taxon/stage richness	Hydroperiod
3011	0.34	5	Short
Cry	0.36	8	Long
429	0.43	9	Short
1502	0.47	12	Short
3425	0.49	11	Long
Trp	0.50	32	Short
3411	0.57	7	Long
313	0.61	7	Long
308	0.62	12	Short
3431	0.64	12	Long
140	0.64	13	Intermediate
1300	0.65	13	Short
389	0.66	15	Short
3339	0.67	11	Short
Fre	0.69	16	Long
3362	0.70	14	Short
630	0.70	16	Short
3286	0.71	26	Long
3413	0.71	15	Short
3345	0.74	9	Intermediate
3432	0.74	18	Long
3315	0.74	26	Short
3410	0.75	7	Long
Trj	0.76	15	Long
Arm	0.76	2	Short
310	0.77	18	Intermediate
Acm	0.77	18	Intermediate
604	0.78	22	Intermediate
321	0.79	18	Short
311	0.79	7	Long
Orf	0.79	12	Long
3371	0.80	25	Long
299	0.80	16	Short
3398	0.80	19	Long

Degree of nestedness (N), taxon/stage richness and hydroperiod category of the ponds are also shown

Discussion

Nestedness in the macroinvertebrate assemblages of Doñana ponds

We detected a high degree of nestedness in the macroinvertebrate assemblages of Doñana ponds overall, when surveyed in a single season, despite this being a highly dynamic aquatic system (Gómez-Rodríguez et al. 2009; Florencio et al. 2009; Díaz-Paniagua et al. 2010). This

Table 5 Number of departures (D) by Lomolino's approach calculated with a subset of 80 ponds of the stage matrix

Ponds ranked by	D
Pond area	1,083
Richness	1,091
SO ₄ ²⁻	1,145
O ₂	1,146
EC	1,149
O.M.	1,155
Turbidity	1,165

Ponds were ranked by the value of each environmental variable. We did not find any randomization of the fixed-fixed null model giving D \leq observed (1,000 randomizations, all $p = 0.001$)

EC Electrical conductivity; O₂ the dissolved oxygen; O.M. the organic matter

finding is in line with some previous studies of lentic macroinvertebrate assemblages (e.g., McAbendroth et al. 2005; Baber et al. 2004), but such a pattern is not universal. Urban (2004), for example, did not detect nestedness in the macroinvertebrates of the temporary ponds of a 200-ha region. Nestedness of Doñana macroinvertebrate assemblages implies that a high proportion of taxa (including adult and larval stages) may be found in most of the ponds in the park, with the exception of those species and ponds which are idiosyncratic. The high degree of nestedness found contrasts with the high proportion of idiosyncratic taxa/stages (43%) and the presence of idiosyncratic assemblages in 37% of the sampled ponds. Soininen (2008) detected a similar proportion of idiosyncratic taxa amongst nested diatom assemblages, suggesting good dispersal amongst sites may be a major driver of nestedness. In our highly nested pond network, we also suggest that nestedness is partly driven by differential colonization amongst these temporary aquatic systems; whilst the high number of idiosyncratic taxa/stages and assemblages may be partly due to the presence of specialized environments in some sites. Consequently, high connectivity among sites (i.e., close enough proximity to facilitate dispersal) and high environmental variability are highlighted in this study as important factors maintaining biodiversity in nested systems.

Nestedness can be used as a measure of stable conditions in biological communities (Atmar and Patterson 1993), and in our study, such stability can be related to the degree of unpredictability in the composition of macroinvertebrate assemblages which is related to hydroperiod (Florencio et al. 2009). Hence, short duration ponds would be expected to generally exhibit lower nestedness, since only species adapted to sudden desiccation events could persist, than long hydroperiod ponds, where more stable environmental conditions allow more sensitive species to

Table 6 Ponds showing idiosyncratic macroinvertebrate assemblages which departed of the general nested pattern by month through the wet and dry years are marked with X

Pond	Dry year: idiosyncratic ponds						Wet year: idiosyncratic ponds										
	Hydroperiod	Feb	Mar	Apr	May	Total	Hydroperiod	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Total
Pol	Interm	X	X	X	D		Interm								D	D	
Acm	Interm	X			D	X	Interm	X		X	X	X		D	D	D	
Rp	Short				D	D	Interm								D	D	
Pg	Interm		X		D		Interm		X		X		X		D	D	
Jim	Short	X	X	D	D	X	Interm	X	X		X	X		X	D	D	
Cam	Interm		X	D	D		Interm		X						D	D	
Abe	Short	X	X	D	D		Interm	X	X		X				D	D	
Pp	Interm	X			D		Interm	X	X		X				D	D	
Tej	Interm			D	D	X	Interm	X			X	X			D	D	
Zah	Long	X	X	X	D	X	Long	X		X	X	X	X	X	X	X	X
Lve	Long	X			X	X	Long	X		X		X	X				
Dul	Long					X	Long	X	X							X	X
Bre	Interm		X	X	D	X	Long		X	X		X			X	D	
Orf	Long				X	X	Long						X				
Ant	Short	X	D	D	D	X	Short			X				X	D	D	
Wou	Interm		X	X	D		-	-	-	-	-	-	-	-	-	-	-
Mor	Interm				D		-	-	-	-	-	-	-	-	-	-	-
Tar	Long	X	X				-	-	-	-	-	-	-	-	-	-	-
Arm	-	-	-	-	-	-	Short		X					D	D	D	X
Vac	Short	D	X	D	D		Short							X	D	D	
Len	-	-	-	-	-	-	Short	X	X	X	X			X	D	D	X
Tps	-	-	-	-	-	-	Short		X				X		D	D	X

D Pond dry, - lack of sample

complete their life cycles (Baber et al. 2004). In our study, however, we detected only slightly higher nestedness in long hydroperiod ponds compared to those of short and intermediate hydroperiods. Similarly, Urban (2004) did not detect differences in nestedness related to hydroperiod, because although long hydroperiod ponds harbored higher numbers of species, temporary ponds in their study contained species which did not occur in long hydroperiod ponds, an observation which is also likely to apply in our system.

The Doñana pond network is mainly comprised of temporary ponds, which are usually filled with autumn or winter rainfall and dry out in summer, when only a few permanent ponds retain water. The number of ponds and the filling date each year depend on the quantity of rainfall, which shows wide inter-annual variation, which plays an important role in driving unpredictability in the system (Serrano and Zunzunegui 2008; Díaz-Paniagua et al. 2010). Macroinvertebrates which are well adapted to temporary ponds adopt different physiological and behavioral mechanisms to survive desiccation (Williams 2006), with dispersal into permanent ponds being the most common strategy followed by dormant life-history stages such as

resistant eggs, larvae and adults burying into the mud (Wiggins et al. 1980; Higgins and Merritt 1999; Bilton et al. 2001). Dispersal also is favored by physical links between sites which can occur following heavy rainfall (Fahd et al. 2007). As high connectivity is one of the main drivers of nestedness in biological communities (Boecklen 1997; Cook and Quinn 1995; Wright et al. 1998; Higgins et al. 2006) and dispersal has been suggested as being important in driving nested patterns (Patterson 1990; Atmar and Patterson 1993; Loo et al. 2002; Hausdorf and Hennig 2003), we expected, and found, good dispersers to be more highly nested than poor and non-dispersing taxa (Cook and Quinn 1995). Isolation can affect the degree of nestedness observed across systems (Wright et al. 1998; McAbendroth et al. 2005; Monaghan et al. 2005), something that we have observed in distant ponds located in northern and southern areas of the park, which had more idiosyncratic assemblages. The fact that the spatial arrangement of ponds affected their degree of nestedness was much more evident for adult dispersers than for non-dispersing life history stages or taxa. As nested patterns may arise through inter-pond colonization processes operating on a more local scale within the pond network (McAbendroth et al. 2005;

Fig. 2 Monthly variation (mean \pm SD) in richness per pond, measured as the number of taxa and stages (crosses with dotted lines) for 19 temporary ponds with a, b short, c, d intermediate, or e, f long hydroperiods in the dry and the wet year. Adult dispersers (filled circles, solid lines) and larvae (open circles, solid lines) are shown as a percentage of the macroinvertebrate population per pond

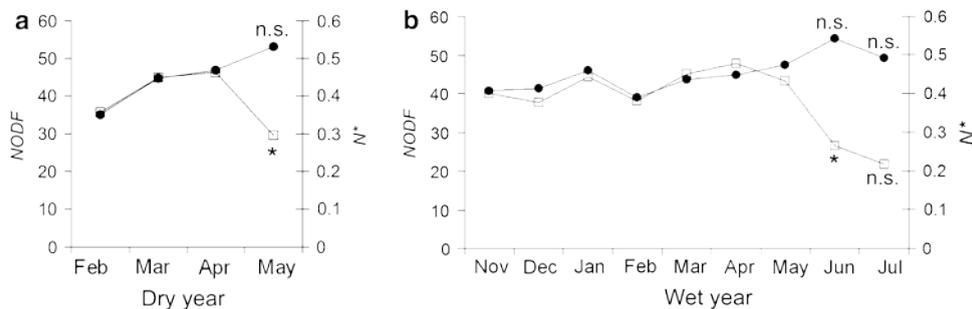
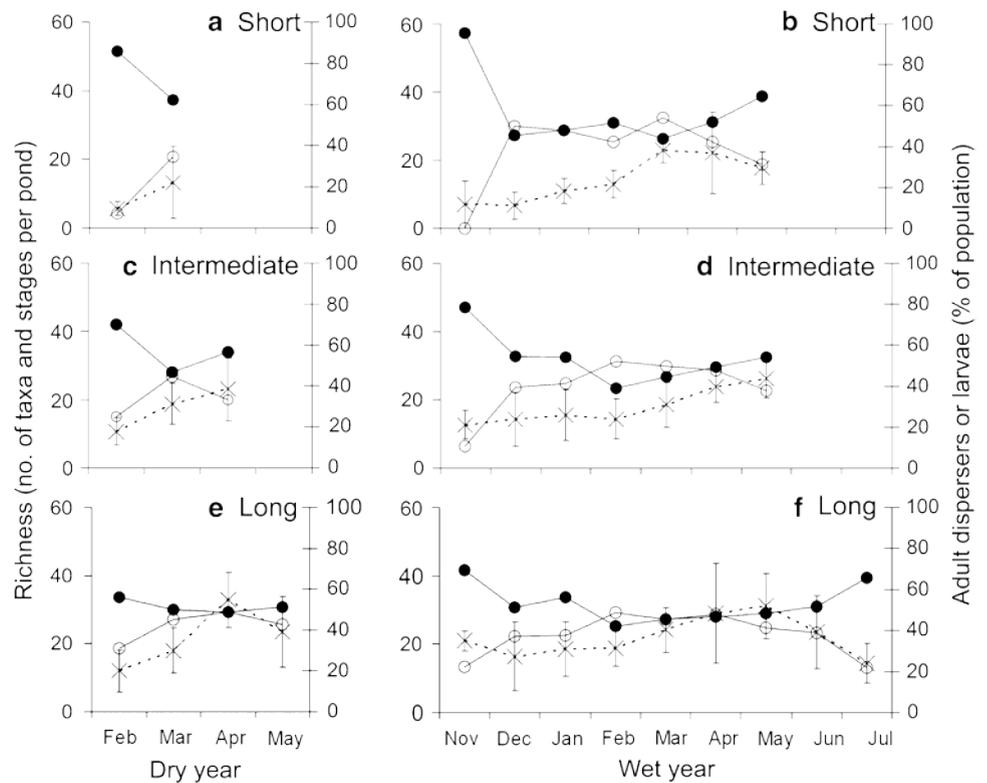


Fig. 3 Monthly variation in the nestedness of the macroinvertebrate assemblages of temporary ponds in a the dry and b the wet years, calculated with NODF (full circles) and relative nestedness N^* (open

squares). After 1,000 permutations of each null model, nestedness was significant at $p \leq 0.01$ level in all months, except as indicated: * $p \leq 0.05$, n.s. non-significant

Soininen 2008), our results suggest that isolation-driven differential colonization contributed to the observed nested pattern.

All environmental variables measured contributed significantly to the nested pattern, despite the wide physico-chemical variation observed across ponds, with area being identified as the most important factor. Pond area has been identified as an important cause of nestedness in macroinvertebrate assemblages of pond networks in other regions (Baber et al. 2004; McAbendroth et al. 2005), and patch area-dependent extinction is believed to be an important mechanism driving nested patterns (Lomolino 1996; Honnay et al. 1999). In our study, however, local extinction risk is not higher in small ponds, since the long hydroperiod

ponds, supporting higher numbers of taxa, were mainly artificially deepened small ponds. Instead, in our systems, the effect of pond area on macroinvertebrate nestedness may result from the fact that larger ponds have higher habitat heterogeneity.

As colonization of more permanent sites is the main strategy many macroinvertebrates use to survive desiccation in temporary ponds (Wiggins et al. 1980; Bilton et al. 2001; Williams 2006), we expected colonization to be one of the main drivers of nestedness in our system. Indeed, our results suggest that colonization is important in driving nestedness across the Doñana pond network. Although dispersal events may be concentrated during the filling and desiccation of ponds, they are also likely to be occurring

continuously to avoid sub-optimal conditions, e.g., adverse conditions of temperature and food or in association with vital processes such as feeding and reproduction (Bilton et al. 2001; Williams 2006). Such inter-pond movements would help maintain the nested structure of the macroinvertebrate assemblages across the pond network.

Some of the idiosyncratic taxa were largely restricted to ponds with particular hydroperiods, although many had distributions which were not apparently driven by the pond hydroperiod. Taxa whose occurrence was influenced by hydroperiod included the diving beetles *Yola bicarinata* (Latreille, 1804) and *Hygrotus confluens* (Fabricius, 1787), the squeak beetle *Hygrobia hermanni* (Fabricius, 1775), and larvae of the damselfly *Lestes virens* (Charpentier, 1825), largely restricted to long hydroperiod sites, and some *Sympetrum* dragonfly larvae, which preferentially occurred in ponds with short and intermediate hydroperiods. Such taxa were apparently idiosyncratic through habitat specialization, something which may also apply to a number of taxa not restricted to ponds with a particular hydroperiod. Such taxa may have niches unrelated to the overall drivers of species diversity, or be distributed genuinely at random, reflecting stochastic colonization processes. Either way, this would result in them occurring in species-poor sites more often than expected, given their occupancy (McAbendroth et al. 2005). Snails such as *Physa* spp., as well as the whirligig beetle *Gyrinus dejeani* Brullé, 1832 and the invasive water boatman *Trichocorixa verticalis* (Fieber, 1851), apparently fall into this category. The number of idiosyncratic ponds in the three hydroperiod categories differed significantly. Amongst ponds detected as idiosyncratic, a similar proportion had short and long hydroperiods, whilst only five intermediate hydroperiod ponds were detected as idiosyncratic, probably due to their intermediate position in the hydroperiod gradient allowing them to support eurytopic taxa which also occur in ponds with long and short hydroperiods.

Temporal variation in species richness and nestedness

Since temporary ponds are characterized by an annual process of filling and desiccation, every year much of their fauna undergoes a colonization–establishment and dispersal–emigration cycle, reflecting the role of local immigration and local extinction, respectively, as drivers of the nested pattern. In the two study years, monthly nestedness increased from the month when ponds were first recorded as wet up until April–May, showing a similar pattern despite the longer inundation period during the wet year. Ponds began to dry up gradually from March onwards in the dry year and from May onwards in the wet year, short hydroperiod ponds drying first. We observed a high proportion of adult dispersers during the drying phase of ponds

in all hydroperiod categories. At the end of this drying phase, only permanent ponds retained water, these supporting all aquatic macroinvertebrates except those with resistant stages such as some beetle larvae, which we have observed burying into the mud of drying ponds. When ponds are filled again the next year, macroinvertebrates start to recolonize (Wiggins et al. 1980; Taylor et al. 1999; Grillas et al. 2004; Williams 2006), reflected by the high proportion of adult dispersers at the beginning of the hydroperiod. The temporary ponds in our study receive the majority of macroinvertebrates via dispersal after the dry phase, making colonization potentially important in generating nestedness. In contrast, resident fauna with resistant stages may reduce the degree of nestedness, and such taxa (e.g., large branchipods; Brendonck 1996; Brendonck et al. 2008) represent a small proportion of the macroinvertebrates present in our sites (Florescio et al. 2009).

The monthly change in the number of taxa per pond, following initial colonization, mirrored the monthly nestedness pattern, both increasing and decreasing together. Shurin (2007) suggested that higher invertebrate richness could be associated with more stable environmental conditions, and the increase of nestedness detected here along the annual hydroperiod may result from the tendency of macroinvertebrate assemblages to stabilize in composition, as a result of interspecific interactions, following initial, more stochastic assembly—a process which continues until ponds begin to dry out. As ponds dry, the degree of nestedness of the macroinvertebrate assemblages decreased or became insignificant. Whilst drying ponds may present more stressful environmental conditions for some taxa (e.g., lower oxygen content and increased temperature; Bazzanti et al. 1996), longer hydroperiod ponds retain optimal conditions for macroinvertebrates for longer. As a consequence, two distinct macroinvertebrate assemblages can be found in the study area: those with non-restrictive ecological traits in drying ponds, and those more specific to optimal conditions, contributing to the reduction of nestedness during the drying phase. The variability in the monthly nested pattern along the annual hydroperiod coincided with their changeable idiosyncratic character between months and study years. This variability in the idiosyncratic character of temporary ponds could be due to unpredictability in environmental conditions in different seasons and years (García Novo et al. 1991; Serrano and Toja 1995; Gómez-Rodríguez et al. 2009) or because ponds supported different macroinvertebrate stages through their hydroperiods, depending on the life-history traits of individual species (Florescio et al. 2009). Global nested patterns were very similar between the two study years, despite their hydroperiod differences. This reflects the fact that the macroinvertebrates of temporary ponds have strategies to deal with the characteristic unpredictability of these

systems, and suggest that variations in inter-annual nested patterns could indicate perturbation in such fluctuating systems. In a more general sense, increases in nestedness over time could be expected as systems assemble and deterministic processes become more important (Patterson 1990), mainly after initial colonization (e.g., Loo et al. 2002) or recurrent perturbations (e.g., Bloch et al. 2007).

Implications for conservation

In nested systems, the best conservation strategy may be to preserve a network of habitat patches within an area (Wright and Reeves 1992; Boecklen 1997). In systems where colonization plays an important role in creating nestedness, effective conservation of the regional biota must allow colonization and dispersal processes to take place (Cook and Quinn 1995; Monaghan et al. 2005). To allow the annual restructuring of the macroinvertebrate community in Doñana, it is therefore essential that the network of sites with differing hydroperiods are retained within the region. Nestedness, as detected in our study, is also an indicator of high quality habitats, with wide environmental gradients (Hylander et al. 2005), something which favors the conservation of natural temporary aquatic habitats, which have declined catastrophically elsewhere in Europe (Williams 1997; Zacharias et al. 2007). Alongside the general nested pattern observed across the ponds studied here, a relatively high number of idiosyncratic taxa and sites were detected. From a conservation perspective, this finding also emphasizes the need to maintain a diverse network of ponds to maintain regional biodiversity (Bilton et al. 2009), since even species-poor sites may contain specialist taxa, not found elsewhere within the region.

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