

Newly created ponds complement natural waterbodies for restoration of macroinvertebrate assemblages

KEYWORDS: restoration, wetlands, macroinvertebrate, nestedness, Doñana

AUTHORS: Cristina Coccia*^{1,2}, Bram Vanschoenwinkel^{3,4}, Luc Brendonck³, Luz Boyero^{1,5,6,7}, Andy J. Green¹

¹Department of Wetland Ecology, Doñana Biological Station, EBD-CSIC, C/ Américo Vespucio s/n, 41092 Sevilla, Spain

²Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago CP 8331150, Chile

³Laboratory of Aquatic Ecology, Evolution and Conservation, KU Leuven, Charles Deberiostraat, 32, 3000 Leuven Belgium

⁴Department of Biology, Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Brussels, Belgium

⁵Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain

⁶PIKERBASQUE, Basque Foundation for Science, Bilbao, Spain

⁷College of Sciences and Engineering, James Cook Univ., Townsville, Australia

Correspondance: Cristina Coccia, Departamento de Ecología, Pontificia Universidad Católica de Chile

Email: ccoccia@uc.cl

Author Posting: This is the author's version of the work. It is posted here for personal use not for redistribution. The definitive version was published in *Freshwater Biology* 61: 1640-1654. (2016: doi: 101111./fwb 12804)

SUMMARY

1. Ecological restoration is becoming increasingly widespread to compensate for wetland loss worldwide. However, most post-restoration studies fail to establish whether the restored wetlands replace or complement natural wetlands for communities of aquatic organisms such as macroinvertebrates.
2. During two consecutive hydroperiods (*ca* 6 months each), we studied the macroinvertebrate communities in 32 new temporary ponds created during a restoration 6 to 7 years previously in Doñana, SW Spain, and compared them with ten natural temporary sites nearby. We compared results for two dominant groups of active dispersers (Coleoptera and Hemiptera) and for the whole aquatic macroinvertebrate community (a mix of active and passive dispersers) to shed light on the role of dispersal constraints during ecosystem recovery. We also compared the ranks of new ponds and reference sites in nested matrices to assess whether communities in new ponds are impoverished subsets of communities in reference sites.
3. Because of their young age, newly created ponds were predicted to have less stable communities over the two study years than reference sites, and to have lower species diversity for the whole community but not for active dispersers. On the other hand, communities in new ponds were predicted to approach the taxonomic composition of reference sites as time went on.
4. New ponds differed in environmental conditions (particularly less emergent vegetation cover and lower chlorophyll concentration) from reference sites, but their invertebrate richness and diversity matched those in reference sites and invertebrate abundance was even higher. Richness and diversity increased in the second hydroperiod in new ponds, but not in reference sites. Significant differences in community composition occurred between new ponds and reference sites, but were largely explained by their environmental

differences. As succession progressed within a hydroperiod, communities in new ponds were first dominated by large branchiopods, then by active dispersers such as Chironomidae and Coleoptera, then finally by halotolerant taxa such as the beetle *Ochthebius viridis fallaciosus*.

5. Communities in new ponds were not impoverished subsets of those in reference sites, and communities in new and reference ponds diverged towards the end of the hydroperiods. We conclude that new temporary ponds can provide diverse and complementary habitats important for maintaining macroinvertebrate diversity at the regional scale.

INTRODUCTION

Wetlands are dynamic ecosystems that support unique biodiversity and provide important ecosystem services (Millennium Ecosystem Assessment, 2005; Ramsar Convention Secretariat, 2006). However, most of the world's wetland surface area has been lost since 1900 ([Davidson, 2014](#)), and human activities such as land conversion and introduction of alien species continue to cause further degradation and loss (Millennium Ecosystem Assessment, 2005). On the other hand, there are increasing numbers of wetland restoration projects that aim to re-establish ecosystem functions and reverse biodiversity loss ([Nakamura, Tockner & Amano, 2006](#); [Palmer, 2009](#); [Bullock *et al.*, 2011](#)).

Post-restoration monitoring is essential to evaluate the effectiveness of restoration projects and to facilitate adaptive management, but in many restoration projects monitoring is absent or inadequate ([Ruiz-Jaen & Aide, 2005](#)). Where monitoring exists, the overall restoration success is typically evaluated based on similarities between the restored sites and reference sites ([Moseman *et al.*, 2004](#); [Matthews & Spyreas, 2010](#); [Meyer, Whiles & Baer, 2010](#)).

In general, new ponds are rapidly colonized by species from nearby water bodies ([Williams, Heeg & Magnusson 2007](#)), which may serve as reference sites. If common species inhabit both new ponds and reference sites, whereas rarer species occur only in reference sites, species assemblages in new ponds will be impoverished and nested within those of reference sites ([Patterson, 1987](#)). This may result from differences in habitat heterogeneity (e.g. plant richness and structure) between new and reference sites, especially during the early years after wetland restoration ([Ruhí *et al.*, 2013](#)). Nestedness analyses can thus help to evaluate the value of restored sites for biodiversity conservation.

Monitoring macroinvertebrates provides different insights compared to waterbirds, plants and zooplankton, which are more frequently used in monitoring programs, but can be less sensitive to ecological change ([Guareschi *et al.*, 2015](#)). However, macroinvertebrates have a broad range of dispersal abilities. The arrival of passive dispersers to new habitats depends on vectors such as water, wind and/or birds, therefore it can be slowed by spatial constraints even if habitat conditions are suitable. In contrast, flying active dispersers are independent from such vectors and should actively select habitats suitable to them. Thus, they should be affected more by environmental control than by spatial processes ([Heino, 2013](#)). As a consequence, active dispersers may be better than the whole community for evaluating short-term ecosystem recovery.

The present study is centred in the “Caracoles estate” within Doñana National Park (South West Spain), where a large complex of new, temporary ponds was created during marsh restoration. We investigated the value of these new ponds for aquatic macroinvertebrates 6 to 7 years after restoration. We compared species diversity and composition between new ponds and reference sites throughout flooding-drying cycles (hereafter hydroperiods, *ca* 6 months each) in consecutive years, to compare the assemblages in new ponds and reference sites, and investigate how community composition could be

explained by environmental variables. We compared patterns for the entire macroinvertebrate community with those for the speciose Coleoptera and Hemiptera, which are active dispersers. The Coleoptera include particularly sensitive taxa ([Bloechl *et al.*, 2010](#); [Van den Broeck *et al.*, 2015](#)), which may be good indicators of environmental differences between new and restored ponds. We examined nestedness between new ponds and reference sites to investigate whether new ponds held nested assemblages relative to those in reference sites, or if they provide complementary habitats for macroinvertebrates by supporting unique taxa.

Given their younger age, new ponds were predicted to provide a simpler environment (e.g. with less vegetation structure) and to lack some passive dispersers owing to dispersal constraints. This would translate into lower species diversity for the whole community, but less so for active dispersers such as Coleoptera and Hemiptera. New ponds were predicted to have greater differences in community structure between hydroperiods, with greater diversity in the second hydroperiod as the ponds matured and more microhabitats become available for the colonization of new species. Thus, we predicted that communities in new ponds would become more similar to reference sites during the second hydroperiod. Finally, we predicted that new ponds would have higher nested ranks than reference sites in the combined, nested matrices, owing to lower habitat complexity and species richness than reference sites.

MATERIAL AND METHODS

Study area and climate

The study was conducted within and around the Caracoles estate in the northern edge of Doñana National Park, where a large complex of new ponds was created during marsh restoration (Fig. 1). During the 1960s, this estate of 2700 ha was hydrologically disconnected from the surrounding marshes to prevent flooding and was converted into arable farmland.

During 2004-2005, restoration was carried out in the estate, with the aim of restoring connectivity with surrounding marshes. This involved the complete removal of dykes along the western and southern borders, the filling-in of drainage channels, and excavating a set of 96 new temporary ponds. These ponds were of similar elliptical shape but of three sizes (long axes 60, 125 and 250 m) and two depths of excavation (30 and 60 cm). Two clusters of 44 ponds each were combined with 8 medium-sized ponds positioned in greater isolation (Fig. 1). The colonization of these ponds by zooplankton and by water birds has been studied previously ([Badosa *et al.*, 2010](#); [Frisch *et al.*, 2012](#); [Sebastián-González & Green, 2014](#)).

Doñana has a Mediterranean climate with rainfall concentrated between October and March (wet season) and little precipitation from April to September (dry season). Caracoles ponds and surrounding water-bodies are usually flooded during the wet season and dry out in May, June or July. Dates of flooding and drying vary among years, as a result of different rainfall and evaporation patterns. Total precipitation (between the months of september and august) was 784 mm for our first study hydroperiod (2009-2010) and 712 mm for our second hydroperiod (2010-2011), with much higher rainfall from December to February during the first hydroperiod than the second. See [Frisch *et al.* \(2012\)](#) and [Sebastian-Gonzalez & Green \(2014\)](#) for more details of the study area.

Site selection and data collection

We sampled 32 new ponds representative of all size and depth classes, of which 24 were within the two clusters, and 8 medium-sized ponds were outside these clusters and relatively isolated (Fig. 1). Most of the selected ponds had already been studied for zooplankton by [Badosa *et al.* \(2010\)](#). We also sampled 10 older temporary sites that were located nearby within the same marshland area and served as reference sites (Fig. 1). These

reference sites shared a common hydrology and geomorphology with the new ponds, as part of the seasonal “marismas” marsh system based on clay and fed by rainwater in the absence of groundwater influence ([Espinár & Serrano 2009](#)). New ponds and reference sites share similar timing of flooding in response to rainfall and drying in response to the dry summers. Occasional heavy floods can establish temporary connections between some new ponds and reference sites, and this occurred during our study, especially during the first hydroperiod (Fig. S1). Owing to the limited choice for reference sites and the design of the new ponds, the former included a greater range in size and depth (as measured in the middle of a hydroperiod).

Sampling was conducted during two consecutive hydroperiods (2009 - 2010 and 2010 – 2011, hereafter referred to as 2010 and as 2011 respectively). It started approximately 2 months after initial pond filling, i.e. the early phase of the hydroperiod (February), and was then repeated every 45 days, twice during the middle phase of the hydroperiod (March and May, respectively) and once in the drying phase of the hydroperiod (June), resulting in a total of 4 sampling events along each hydroperiod. During the first hydroperiod, the connections between ponds in clusters and some reference sites (Fig. S1) inhibited access to the southern part of the estate, preventing sampling of some ponds during some months.

Environmental variables measured in ponds

During each visit we visually estimated the percentage of each pond area that was inundated, and water depth profiles were recorded with a measuring stick in five locations evenly distributed in the water body. We measured *in situ* pH, salinity and temperature with a WTW 340i multiprobe. We also collected water samples for laboratory analyses of chlorophyll-a, nutrient concentrations (total phosphorous and nitrogen) and turbidity. Chlorophyll *a* concentration ($\mu\text{g l}^{-1}$) was determined using methanol extraction (Talling &

Driver, 1963). Total phosphorus was determined by colorimetry after acid hydrolysis (APHA, 1980). Total nitrogen was determined by measuring absorbance at 220 nm after digestion with alkaline potassium persulphate (D'Elia, Steudler & Corwin, 1977). The presence of fish was determined by visual inspection of the sweep net contents after invertebrate sampling (see below). The presence/absence of submerged vegetation and total cover (%) of emergent vegetation was estimated visually.

Sampling and processing of macroinvertebrates

In each pond on each sampling occasion, 3 samples of macroinvertebrates were collected using a D-framed pond net (500 µm mesh; 16 × 16 cm) by sweeping at 5 m intervals (at 0, 5 and 10 m from the shore) along a transect. At each of the three points, a sweep was carried out over 1m during 30 s. Invertebrate samples were preserved in plastic containers filled with 70% ethanol. Data from the three samples were pooled before analysis.

Macroinvertebrates were identified in the laboratory under a stereo-microscope. The Coleoptera, Hemiptera and Crustacea were identified mostly to species level (after [Jansson, 1986](#); [Friday, 1988](#); [Vondel, 1991](#); [Alonso, 1996](#); [Nieser *et al.*, 1994](#)); Odonata, Gastropoda and Ephemeroptera to genus level ([Carchini, 1983](#); [Gerken & Sternberg, 1999](#); [Tachet *et al.*, 2000](#)); and Diptera, Trichoptera and Lepidoptera to family level ([Tachet *et al.*, 2000](#)). We also counted the Collembola, Hydracarina, Coelenterata, Turbellaria, Oligochaeta and Hirudinea, but did not identify them further. For Coleoptera and Hemiptera, some juveniles could not be identified beyond genus level and were assigned to species according to the proportions of congeneric adults in the same pond. If adults were not present, juveniles were left at genus level. Some Hemiptera instars could not be classified to genus, and were left at family level.

Statistical analyses

All analyses were conducted in the statistical programming environment R version 2.15.3 (R Development Core Team, 2009), including functions in the Vegan (adonis, cor, diversity, metaMDS, nestednodf, oecosim, rarefy, raupcrick, simper, speaccum), Bipartite (nestedrank) and Coin (Wilcox_test, permutational test) packages.

Environmental variables

To investigate changes between hydroperiods in abiotic conditions in either new ponds or reference sites, and differences between new ponds and reference sites in a given sampling event, we used a Permutational Multivariate Analysis of Variance with distance matrices (PERMANOVA; “ADONIS” in R, see [Oksanen *et al.*, 2012](#)). Analyses were conducted on $\log(x+1)$ transformed data (with the exception of pH and variables expressed as presence/absence), and dissimilarities were calculated using Euclidean distances. Differences between years were tested using samples from May, as this was the month when the number of sites sampled was highest (Table 1).

When ADONIS revealed significant differences between hydroperiods in new ponds or in reference sites, or significant differences between reference sites and new ponds in a given sampling month, we performed a Multivariate Homogeneity of Group Dispersion (SIMPER) analysis to identify the influential explanatory variables. Environmental variables were reported according to their average contribution to dissimilarities between hydroperiods or between new ponds and reference sites. Variable lists were cut off when the cumulative contribution to dissimilarity reached ~ 50%. P values were calculated using Wilcoxon paired tests (for differences between hydroperiods) and Mann-Whitney U tests (for differences between new ponds and reference sites), respectively. We also calculated Pearson correlations

between the environmental variables found to contribute to the ~ 50% of cumulative dissimilarities by SIMPER.

Changes in macroinvertebrate abundance and diversity between hydroperiods

Richness and diversity were calculated using the lowest possible taxonomic level. Estimates of richness were thus conservative. However, since richness can be expected to increase as the number of individuals in a sample increases, and as this number varied between samples, we used rarefied richness to compare differences between hydroperiods in new or reference sites. Comparison was performed using the “rarefy” function in R after standardising the data to the lowest number of invertebrates collected in any single site over the two hydroperiods. Changes between hydroperiods in rarefied richness, the Shannon-Wiener diversity index and relative abundance (individuals/m²) were performed separately for May samples from new ponds and reference sites using Wilcoxon matched pairs tests. As an alternative method, we also estimated richness using the Chao 2 estimator, obtaining very similar results. We used sample-based rarefaction curves based on the Chao 2 estimator to compare completeness of our sampling between years.

Similarity in composition between hydroperiods for new ponds or reference sites was tested using ADONIS based on the Hellinger-transformed Euclidean distance matrix of invertebrate abundance (i.e. Hellinger distances, see [Legendre & Gallagher, 2001](#)). Rare species that occurred in only one pond were excluded to avoid potential bias. If significant differences were found, we performed a SIMPER analysis to identify the taxa that were responsible. Taxa were reported depending on their average contribution to dissimilarities between new ponds and reference sites. Species lists were cut off when the cumulative contribution to dissimilarity reached ~ 50%. All analyses were conducted separately for the total community, for Coleoptera and for Hemiptera.

Differences in community between new ponds and reference sites within hydroperiods

We first visualized the differences in community structure between new ponds and reference sites for each month during each hydroperiod using non-metric multidimensional scaling (NMDS) based on the Hellinger distance matrices. We then compared rarefied richness (standardized to the lowest number of invertebrates collected in any sampling event in any single site), Shannon-Wiener diversity indices and relative abundance (individuals/m²) between new and reference ponds for each sampled month by means of a Mann-Whitney U test. As an alternative method, we also estimated rarefied richness using the Chao 2 estimator, obtaining equivalent results.

We also tested the similarity in community composition between new and reference ponds using a month-by-month ADONIS analysis based on Hellinger distances. SIMPER analysis was used to identify the taxa that contributed most to significant differences. Analyses were conducted separately for the total community, for Coleoptera and for Hemiptera.

Finally, we investigated the compositional similarity between new and reference ponds while controlling for the environmental variables that were found to contribute to ~70% of cumulative dissimilarities between them each month in the SIMPER, using ADONIS. As the order of incorporation of non-orthogonal variables can influence the outcome of significance testing in this procedure, the explanatory descriptors were introduced as the first predictors in the analysis, adding water body types (new ponds and reference sites) as the last variable. This procedure allowed measurement of the pure effect of the difference between “new” and “restored” sites after controlling for environmental variability, which was largely responsible for their differences in community composition.

Temporal trajectories in community assembly within hydroperiods

To assess whether communities in new ponds became more similar to reference sites as time passed and their age increased, we used a modified version of the Raup-Crick dissimilarity index (β_{RC} Chase *et al.*, 2011), which is robust to variation in species richness. This index uses presence/absence data to express the dissimilarity between two observed communities relative to the null expectation under a random assembly. The null expectation was generated using 9999 randomizations of a null model. This index was calculated using one single matrix containing data from both new ponds and reference sites.

We first tested for differences in the levels of similarity between new ponds and reference sites by means of Mann Whitney U tests on the averaged pairwise dissimilarity of all pairwise comparisons within the group (i.e. on the one hand comparing each new pond P_1 with all other new ponds P_2 - P_{32} , on the other hand comparing each reference site R_1 with all other reference sites R_2 - R_{10}). We then tested if the dissimilarity index within each taxonomical group was affected by water body type [i.e., comparing new ponds with each other (PP) on the one hand, and new ponds with reference sites (PR) on the other]. If water body type affected community composition, the pairwise dissimilarity indices between communities in new ponds and reference sites (PR) should be greater than those among individual new ponds (PP). Because the pairwise dissimilarity indices calculated either among different new ponds (PP) or between new ponds and reference sites (PR) were not independent, the significance of this comparison was tested via Permutational Multivariate Analyses (ADONIS).

Nestedness

To evaluate whether assemblages in new ponds were effectively nested within those of reference sites, we carried out nestedness analyses based on nested overlap and decreasing

filling (NODF, see Almeida-Neto *et al.*, 2008). NODF can assume values ranging from 0 to 100, with higher values indicating higher nestedness of communities. Nestedness analyses were calculated on presence/absence data, for a maximally nested matrix (i.e. sites ranked in decreasing order of species richness, and species ranked in decreasing order of incidence, so that the most diverse assemblage will occupy the first row and have a nested rank of 1). The significance of nestedness was evaluated by comparing observed values with those generated by 999 null models randomized according to a quasi swap algorithm (Gotelli & Entsminger, 2001). The quasi swap method creates independent matrices that maintain both row and column frequencies. This method is less vulnerable to Type I error (Gotelli, 2000). When significant nested patterns were detected, we tested for significant differences between the nested rank of reference sites and new ponds using a Mann-Whitney U test with 999 permutations. All analyses were repeated for each sampled month, each hydroperiod and for each taxonomic group.

RESULTS

Environmental variables

Environmental conditions varied from 2010 to 2011 in both new ponds and reference sites (ADONIS, $P < 0.05$). Submerged vegetation, pH and turbidity explained ~50 % of cumulative differences between hydroperiods for new ponds (according to SIMPER analysis). During the second hydroperiod, turbidity was significantly lower (82.5 ± 10.3 vs. 47.8 ± 5.8 , mean \pm SE Wilcoxon paired test, $V = 454.5$, $P < 0.001$), but pH was significantly higher (9.0 ± 0.0 vs. 9.4 ± 0.1 , $V = 35.5$, $P < 0.001$) and submergent vegetation was detected in a higher number of new ponds (14 vs. 29). There was a strong negative correlation between pH and turbidity (Pearson's $R = -0.75$). Submergent vegetation, pH and chlorophyll-a explained ~50

% of cumulative differences between hydroperiods for reference sites (according to SIMPER analysis). During the second hydroperiod, pH and chlorophyll-a were not significantly different (8.4 ± 0.0 vs. 8.5 ± 0.2 and 16.0 ± 5.6 vs. $15.7 \pm 3.2 \mu\text{g l}^{-1}$) but submergent vegetation was detected in a higher number of reference sites (2 vs. 8).

There were significant differences in environmental parameters between new ponds and reference sites (ADONIS, $P < 0.05$) in each month, for each hydroperiod (see Table 1 and Fig. S3 for mean monthly values). Overall, SIMPER analysis revealed that 6 variables explained ~50 % of cumulative monthly dissimilarity between new ponds and reference sites, but they had different contributions in each month (Table 1 and Table S1). New ponds consistently had higher pH and presence of submerged vegetation, and lower chlorophyll-a concentration and presence of emergent vegetation and fish (Table 1). pH correlated negatively with emergent vegetation in February 2011 (Pearson's $R = -0.61$) and with chlorophyll-a concentration in March 2011 (Pearson's $R = -0.56$). Dissimilarity between new ponds and reference sites was higher in June during both hydroperiods (ADONIS, ~11%), when new ponds also exhibited significantly higher salinity (Mann Whitney U test, $W_{2010}=60$ $P = 0.003$; $W_{2011}= 116$, $P = 0.007$). See Table S2 for detailed information on vegetation and fish recorded.

Changes in macroinvertebrate abundance and diversity between hydroperiods

Over the two hydroperiods, we identified a total of 109 taxa (55 identified to species level) belonging to 22 major taxonomical groups dominated by insects (Table S3). During the first hydroperiod we found 77 taxa, of which 13% were recorded exclusively in new ponds, 35% in reference sites and 48% were shared between new ponds and reference sites (Table S3). During the second hydroperiod we found 93 taxa, of which 15% were recorded exclusively in new ponds, 20% in reference sites and 62% were shared (Table S3). Not all the

taxa consistently occurred during each hydroperiod, both in the case of new ponds and of reference sites (e.g. the Coleoptera *Laccophilus minutus* and *Enochrus* spp, see Table S3). In new ponds, rarefied richness and diversity showed a significant increase in the second hydroperiod for all groups ($P < 0.001$, Wilcoxon paired- tests, Fig. 2), whereas in reference sites no significant differences between hydroperiods were recorded for any group (Fig. 2). Equivalent results were obtained with the Chao 2 estimator of richness (details not shown).

Differences between hydroperiods in community composition were found in new ponds for the whole community (ADONIS; $R^2 = 0.07$, $P = 0.001$), for Coleoptera ($R^2 = 0.04$, $P = 0.03$) and for Hemiptera ($R^2 = 0.11$, $P = 0.001$). In reference sites such differences were only significant for Hemiptera ($R^2 = 0.14$, $P = 0.006$). Decisive changes identified with SIMPER analysis were higher abundance of *Berosus affinis* in new ponds in 2011 and of *Sigara lateralis* in reference sites in 2010.

Species accumulation curves approached an asymptotic trend for new and reference ponds for each group in each hydroperiod (Fig. S4). Therefore, considering that we analysed rarefied richness, any differences in number of ponds sampled between hydroperiods were unlikely to affect the above results.

Differences between new ponds and reference sites in the invertebrate communities within hydroperiods

Non-metric multidimensional scaling ordination showed that the extent to which new ponds were different in community structure from reference sites depended on the taxonomic group, the month and the hydroperiod (Fig. 3). New ponds had significantly lower rarefied richness ($P < 0.001$, Mann-Whitney U test) and diversity ($P < 0.001$) for the whole community than reference sites during February 2011 (Fig. 2). New ponds also had significantly higher Coleoptera abundance than reference sites in May ($P < 0.05$) and June (P

< 0.05) for each hydroperiod (Fig. 2), higher Hemiptera abundance in June 2011 ($P = 0.01$), and higher Hemiptera richness in May 2011 ($P = 0.05$, Fig. 2). Equivalent results were obtained with the Chao 2 estimator.

New ponds and reference sites differed in community composition in 7 out of 8 sampled months for the whole community and for Coleoptera (ADONIS; $P < 0.05$; see Tables S4 and S5 for the main differences in taxa according to SIMPER analysis), and in every month of the second hydroperiod for Hemiptera (ADONIS; $P < 0.05$; see Table S6 for the main differences in taxa according to SIMPER).

Most of the variation in community composition between new ponds and reference sites was explained by their environmental differences, and most of the above differences between them disappeared after adjusting for their environmental dissimilarities with ADONIS. However, differences between new ponds and reference sites remained significant for the whole community in March 2011 (ADONIS; $R^2 = 0.04$, $P = 0.032$), for Coleoptera in June 2010 ($R^2 = 0.15$, $P = 0.041$) and for Hemiptera in February 2011 ($R^2 = 0.07$, $P = 0.012$).

Temporal trajectories in community assembly within hydroperiods

For the whole macroinvertebrate community, dissimilarity between new ponds was significantly lower compared to reference sites in each month (i.e. comparing PP and RR in Fig. 4), during both hydroperiods (Mann-Whitney U test, $P < 0.05$). Differences were not significant in March 2010 for Coleoptera ($W = 32.5$; $P = 0.06$) and Hemiptera ($W = 62.5$, $P = 1$), or in June 2010 for Hemiptera ($W = 14$; $P = 0.06$). When comparing differences within new ponds with those between new ponds and reference sites (i.e. PP and PR in Fig. 4), dissimilarity was higher for PR than for PP (i.e. water body type effected community composition) every month for the whole community, and for all months other than March for Coleoptera (ADONIS; $P < 0.05$). For Hemiptera, dissimilarity was higher for PR than for PP

in May 2010 ($R^2 = 0.05$, $P = 0.05$) and all months in 2011 except March (ADONIS, $P < 0.05$).

Nestedness

During the first hydroperiod, there was a trend of species-poor sites being nested within species-rich sites for Coleoptera in May (NODF= 36.49, $P= 0.08$). During the second hydroperiod 2011, the whole community was significantly nested in March (NODF = 36.41, $P = 0.021$), whereas Coleoptera were nested in March (NODF = 35.51, $P = 0.03$) and June (NODF = 50.28, $P = 0.037$). Also in 2011, Hemiptera were nested in May (NODF = 49.56, $P = 0.021$) and June (NODF = 65.70, $P = 0.037$). In no case did new ponds have a significantly higher nestedness rank than reference sites.

DISCUSSION

In this study we found that, 6-7 years after restoration, new ponds provided different environmental conditions to reference sites while still supporting diverse communities overlapping with those of reference sites and representative of natural systems. As a whole, newly created ponds increased the habitat heterogeneity of the area, which in turn benefited species with different and/or more specific requirements than those found in reference sites.

Patterns of community change between hydroperiods

Between the two consecutive hydroperiods studied, richness and diversity increased in new ponds for all invertebrate groups, whereas they were almost stable in reference sites. This result supports our prediction that communities in new ponds would be less stable between hydroperiods than those in reference sites. As ponds age, they are likely to be colonized by an

increasing number of new species over time. The colonisation of new ponds occurs when passive dispersers are transported via vectors, or when actively flying insects (e.g. Coleoptera and Hemiptera) arrive from nearby water bodies ([Williams *et al.*, 2007](#)). Passive dispersers with dormant propagules (e.g. branchiopods or turbellarians; [Brendonck & De Meester 2003](#)) can recolonise temporary ponds from the egg bank in subsequent years when they are re-flooded. Hence, one reason why richness increased in the second hydroperiod could be an increase in diversity of the propagule bank in the sediments. Newly created habitats have unoccupied niches that become rapidly colonized by agile, generalist taxa. As communities develop, more niches become available (e.g. through an increase in submerged vegetation as we recorded in the second hydroperiod) and species with specialized requirements can colonize progressively ([Townsend & Hildrew, 1994](#)). The development of vegetation is a prime driver of macroinvertebrate diversity in freshwater systems ([Thomaz & Cunha 2010](#)). This is supported by the colonization or increased abundance of taxa typically associated with macrophytes in the second hydroperiod (e.g. *Laccophilus minutus*; *Enochrus* spp., Libellulidae, *Berosus affinis*, see Table S3). However, the difference between study hydroperiods may also partly have been a consequence of the dynamic hydrology. Reduced connectivity and increased isolation between new ponds in the second hydroperiod (Fig. S1) could in itself cause increases in beta and gamma diversity. Different communities can develop in similar but disconnected ponds because, particularly in passive dispersers with restrictive dispersal abilities, beta diversity can be promoted by differences in stochastic colonisation events among ponds and maintained via priority effects and monopolization effects ([De Meester *et al.*, 2016](#)).

Macroinvertebrate recovery and dispersal limitation

At a given point in time within each hydroperiod, new ponds and reference sites often differed in community composition, richness and diversity. One major reason for this may be that some taxa had not yet managed to colonize new ponds. The absence of some taxa such as Gastropoda from new ponds and the presence of others such as Oligochaeta in low abundance suggest that populations of these species had not yet fully established. Lack of planktonic dispersal stages and development of juveniles in cocoons delay colonization of new habitats by Gastropoda and Oligochaeta ([Brady *et al.*, 2002](#)). These results support our prediction that dispersal limitation will be more apparent for the whole community than for Coleoptera and Hemiptera, which are active dispersers.

On several occasions, new ponds and reference sites differed in community composition but not in richness or diversity, suggesting that some taxa showed site-specific preferences, largely due to variation in environmental conditions, as observed in the middle of hydroperiods. New ponds were usually fishless, which enabled the dominance of the large branchiopods *Streptocephalus torvicornis* and *Chirocephalus diaphanus* that hatch from dormant egg banks. These taxa did not emerge in hatching experiments using sediments taken from new ponds when they were first created ([Frisch & Green, 2007](#)), suggesting that their eggs were rapidly dispersed from surrounding areas either through water connections, wind, or by the waterbirds abundant in the area ([Brochet *et al.*, 2010](#); [Sebastián-González & Green, 2014](#); [Horvát, Vad & Ptacnik, 2015](#)). The dominance of chironomids in the new ponds was to be expected ([Layton & Voshell, 1991](#)) and as with the large branchiopods, this might reflect their lack of dependence on emergent plants. New ponds generally had a higher presence of submergent vegetation than reference sites. This difference could partly explain the higher abundances of adult *Berosus* (Hydrophilidae) in new ponds than reference sites in May and June each hydroperiod ([Bloechl *et al.*, 2010](#); [Touaylia, Garrido & Boumaiza, 2013](#)). Lower predation pressure exerted by fish in new ponds may have also played a role. On the other

hand, the lower abundance of Odonata may reflect the lack of emergent vegetation in new ponds.

As summer begins and temporary ponds begin to dry out, salinity increases cause osmotic stress to macroinvertebrates. In this drying phase, only the most halotolerant species could persist in the new ponds that reached highest salinities, such as the Coleoptera *Ochthebius viridis fallaciosus* (Garrido & Munilla, 2008; [Millán *et al.*, 2011](#)) or the Hemiptera *Sigara stagnalis* and the alien *Trichocorixa verticalis* (Van de Meutter, [Trekels & Green, 2010](#)). Higher Hemiptera richness recorded in new ponds in May and June also reflects their acquisition of some widespread taxa absent from reference sites (e.g. *Notonecta glauca*). Among Hemiptera, the families of Notonectidae and Corixidae include large taxa that are particularly vulnerable to fish predation, especially because they swim to the water surface to breathe ([Schilling, Loftin & Huryn, 2009](#)). Hence, lower fish predation in new ponds may have influenced the distribution of several taxa from these families.

Our finding that richness and diversity in new ponds matched the levels in reference sites 6-7 years after restoration, and that invertebrate abundance even surpassed the reference levels, is not unusual in restored wetlands. Several studies have found a rapid recovery of species richness and diversity in macroinvertebrates within a few years ([Meyer & Whiles, 2008](#)), and this was previously observed for zooplankton in our new ponds ([Badosa *et al.*, 2010](#)). In contrast, the recovery of natural community composition generally takes more time ([Levin & Talley, 2002](#)). We recorded 19 taxa exclusive to new ponds and 40 Coleoptera taxa in new ponds (Table S3), including specialist herbivores (e.g. Curculionidae), indicating that the new ponds are healthy ecosystems of conservation value (Bameul, 1994).

Community similarity and the effect of water body type

Overall, different individual new ponds supported communities that were more similar to each other (including ponds within and outside the clusters) than recorded for different reference sites. This probably reflects greater habitat heterogeneity between individual reference sites, which include a broader range of waterbody shape, depth and spatial distribution (Fig. 1). The slow recovery for emergent plants in new ponds might have contributed to the high levels of community dissimilarity between new and reference ponds. Emergent plants appear to be relatively poor at colonizing by seeds compared to submerged ones, and were mainly expanding their cover in new ponds slowly by clonal horizontal expansion. A recent meta-analysis ([Moreno-Mateos *et al.*, 2012](#)) showed slow recovery rates for vegetation in restored wetlands.

In general, our results did not support the prediction that community composition between new and reference ponds becomes more similar during the second hydroperiod, when new ponds become more mature and the development of new habitats may allow the colonization of new species. Instead, communities were shaped mainly by environmental differences between new ponds and reference sites, which were sufficiently strong to prevent convergence in community composition. This is especially true towards the end of the hydroperiod, when the environmental dissimilarity between new ponds and reference sites increased and the new ponds became more saline through evaporation. During the first hydroperiod, differences between new ponds and reference sites in levels of dissimilarity within each class (i.e. comparing PP with RR in Fig. 4) were weakest early on in March, and became weak again later in June in the case of the Hemiptera, probably because of their broad niches ([Bloechl *et al.*, 2010](#)). Typically, early colonists are the most generalist, and should therefore be the least affected by specific habitat properties ([Vanschoenwinkel *et al.*, 2010](#)). The lack of differences between water body types for Coleoptera and Hemiptera in March 2010 (comparing PP with PR in Fig. 4) suggests that pioneer taxa colonized both new and

reference ponds indiscriminately, irrespective of their environmental differences. This is probably due to the high connectivity in the early phase of the first hydroperiod, when there was extensive flooding (Fig. S1), allowing high exchange of Coleoptera and Hemiptera between water body types that may have decreased the inter-pond dissimilarity.

Differences in the abundance of invasive species between new ponds and reference sites and between hydroperiods may also have had strong effects. This concerns invasive fish (Table S2) but also the alien corixid *T. verticalis* whose impact on other macroinvertebrates requires more detailed investigation (Van de Meutter *et al.*, 2010; Coccia *et al.*, 2013).

Contribution of new ponds to nestedness and macroinvertebrate assemblages

A nested species assemblage occurs when taxa in sites with lower species richness are a proper subset of those in richer sites (Patterson, 1987). Nestedness is the result of a non-random distribution of species between sites that differ in characteristics such as area, isolation or habitat diversity (Patterson & Brown, 1991; Wright *et al.*, 1998). We did not find support for our initial hypothesis that reference sites would be more complex owing to greater maturity and would contain more habitat specialists, so that new pond communities would be ranked higher in the maximally nested matrices than reference sites.

Overall across the whole study area, the communities exhibited significant nested patterns. Previous studies have found macroinvertebrates to be highly nested in freshwater habitats (Florencio *et al.*, 2011; Ruhí *et al.*, 2013). There are several factors that may have contributed to the nestedness we observed, including differences in pond size or isolation (Kadmon, 1995; Heino, Mykrä & Rintala, 2010), whose effects may have been variable within and between hydroperiods. However, we did not find any significant differences in the nested ranks between new and reference ponds during each hydroperiod for any taxonomic group. Nestedness is a type of hierarchical organization of species, sites or both (Patterson &

Atmar, 2000), so that the rank order of site reflects the suitability gradient among them ([Azeria & Kolasa, 2008](#)). Similar ranking values between new and reference ponds suggest that new ponds, despite being less heterogeneous than reference sites, are now well integrated into our wetland complex. Furthermore, it seems likely that their ecological attributes increased the overall habitat complexity of the area. This in turn suggests that new ponds provide habitats that are rare in reference sites and have the capacity to accommodate different macroinvertebrate species, underlining the value of the new ponds for biodiversity conservation 6-7 years after restoration. New ponds, however, will not stay new and over time the unique conditions they house may be lost as their resemblance to reference sites increases. Future research is required to identify pioneer species that can only thrive in new ponds, even in such a dynamic wetland complex as Doñana.

CONCLUSION

Our study shows that 6-7 years after restoration new ponds constructed in Doñana hold different macroinvertebrate communities than do natural reference sites. This may indicate that new ponds are still supporting pioneer communities. A longer time is usually needed to reach a stable, mature macroinvertebrate community assemblage in restored wetlands ([Moreno-Mateos *et al.*, 2012](#)), which seems likely in our system given the slow recovery of emergent vegetation. However, communities in new ponds will not necessarily converge towards the composition of reference communities. Further studies will be needed to address this in the future. Environmental factors seem to be the main drivers of community assembly in this area, and the large number of new ponds (96) varying in size, depth, connectivity etc assures a high level of environmental heterogeneity that in turn translates into high beta and gamma diversity. The success of restoration is often evaluated based on similarities in species composition, diversity and density between the restored sites and target

reference sites. However, this study illustrates that there are high levels of variation within and between hydroperiods in temporary Mediterranean wetland systems that need to be considered. Although somewhat different to reference sites, newly created ponds are healthy ecosystems supporting diverse macroinvertebrate assemblages with considerable conservation value.

ACKNOWLEDGEMENTS

This research was funded by project P10- RNM-6262 financed by the Consejería de Innovación, Ciencia y Empresa, Junta de Andalucía, and by a JAE predoctoral grant from CSIC. We are grateful to Raquel López Luque, Anna Badosa, Carmen Diz-Salgado, Manuel-Lopez, Maria José Vargas and Melissa Rosati for laboratory and field assistance. We are also grateful to David Aragonés Borrego and Isabel Afan Asencio for preparing the maps of the study area, to Andrés Millán for help with identification of Coleoptera and Heteroptera, and to Miguel Clavero for help with identification of fish.

REFERENCES

- Almeida-Neto M., Guimaraes P., Guimaraes P. R., Loyola R. D. & Ulrich W. (2008). A consistent metric for nestedness analysis in ecological systems : reconciling concept and measurement. *Oikos*, **117** 1227–1239.
- Alonso M. (1996). Fauna Iberica. Crustacea Branchiopoda. Mus. Nac. Cien. Natur. Consejo Superior de Investigaciones Cientifica, Madrid.
- APHA (1980). *Standard methods for examination of water and wastewater 15th ed.* American Public Health Association, Washington, DC.
- Azeria E. T. & Kolasa J. (2008). Nestedness , niche metrics and temporal dynamics of a metacommunity in a dynamic natural model system. *Oikos*, **117**, 1006–1019.
- Badosa A., Frisch, D., Arechederra A., Serrano L. & Green A. J. (2010). Recovery of zooplankton diversity in a restored Mediterranean temporary marsh in Doñana National Park (SW Spain). *Hydrobiologia*, **654**, 67–82.
- Bameul F. (1994). Les Coléoptères aquatiques des Marais de La Perge (Gironde), témoins de la fin des temps glaciaires en Aquitaine. *Bulletin de La Société Entomologique de France*, **99**, 301–321.
- Bloechl A., Koenemann S., Philippi B. & Melber A. (2010). Abundance, diversity and succession of aquatic Coleoptera and Heteroptera in a cluster of artificial ponds in the North German Lowlands. *Limnologia - Ecology and Management of Inland Waters*, **40**, 215–225.
- Brady V. J., Cardinale B. J., Gathman, J. P. & Burton, T. M. (2002). Does facilitation of faunal recruitment benefit ecosystem restoration ? An experimental study of invertebrate assemblages in wetland mesocosms. *Restoration Ecology*, **10**, 617–626.
- Brendonck L. & De Meester L. (2003). Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. *Hydrobiologia*, **491**, 65-84.
- Brochet A. L., Gauthier-Clerc M., Guillemain M., Fritz H., Waterkeyn A., Baltanás A. & Green A. J. (2010). Field evidences of dispersal of brachiopods, ostracods and bryozoans by teal (*Anas crecca*) in the Camargue (southern France). *Hydrobiologia*, **637**, 255-261.
- Bullock J. M., Aronson J., Newton A. C., Pywell R. F. & Rey-Benayas J. M. (2011). Restoration of ecosystem services and biodiversity: conflicts and opportunities. *Trends in Ecology & Evolution*, **26**, 541–9.
- Carchini G. (1983). Odonata: guide per il riconoscimento delle species animali delle acque interne italiane. CNR Valdonega, Verona.

- Chase J. M., Kraft N. J. B., Smith K. G., Vellend M. & Inouye B. D. (2011). Using null models to disentangle variation in community dissimilarity from variation in α -diversity. *Ecosphere*, **2**(2), art24, 1-11.
- Coccia C., Calosi P., Boyero L., Green A. J. & Bilton D. T. (2013). Does ecophysiology determine invasion success? A comparison between the invasive boatman *Trichocorixa verticalis verticalis* and the native *Sigara lateralis* (Hemiptera, Corixidae) in South-West Spain. *PloS One*, **8**, e63105.
- Davidson N. C. (2014). How much wetland has the world lost? Long-term and recent trends in global wetland area. *Marine and Freshwater Research*, **65**, 934–941.
- De Meester L., Vanoverbeke J., Kilsdonk L. J. & Urban M. C. (2016). Evolving perspectives on monopolization and priority effects. *Trends in Ecology & Evolution*, **31**, 136-146.
- D'Elia C. F., Steudler P. A. & Corwin N. (1977). Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnology and Oceanography*, **22**, 760–764.
- Espinar J. L. & Serrano L. (2009) A quantitative hydrogeomorphic approach to the classification of temporary wetlands in the Doñana National Park (SW Spain). *Aquatic Ecology*, **43**, 323-334.
- Florencio M., Díaz-Paniagua C., Serrano L. & Bilton D. T. (2011). Spatio-temporal nested patterns in macroinvertebrate assemblages across a pond network with a wide hydroperiod range. *Oecologia*, **166**, 469–483.
- Friday L. E. (1988). A key to the adults of British water beetles. *Field Studies*, **7**, 1–151.
- Frisch D., Cottenie K., Badosa A. & Green A. J. (2012). Strong spatial influence on colonization rates in a pioneer zooplankton metacommunity. *PloS One*, **7**, e40205.
- Frisch D. & Green A. J. (2007). Copepods come in first: rapid colonization of new temporary ponds. *Fundamental and Applied Limnology / Archiv Für Hydrobiologie*, **168**, 289–297.
- Garrido J. & Munilla I. (2008). Aquatic Coleoptera and Hemiptera assemblages in three coastal lagoons of the NW Iberian Peninsula: assessment of conservation value and response to environmental factors. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **18**, 557–569.
- Gerken B. & Sternberg K. (1999). *Die Exuvien Europäischer Libellen (Insecta, Odonata)*. Huxuaria Druckerei GmbH, Höxter.
- Gotelli N. J. (2000). Null model analysis of species co-occurrence patterns. *Ecology*, **81**, 2606–2621.
- Gotelli N. J. & Entsminger G. (2001). Swap and fill algorithms in null model analysis: rethinking the knight's tour. *Oecologia*, **129**, 281–291.
- Guareschi S., Abellán P., Laini A., Green A. J., Sánchez-Zapata J. A., Velasco J. & Millán A. (2015). Cross-taxon congruence in wetlands: Assessing the value of waterbirds as

- surrogates of macroinvertebrate biodiversity in Mediterranean Ramsar sites. *Ecological Indicators*, **49**, 204–215.
- Heino J. (2013). Does dispersal ability affect the relative importance of environmental control and spatial structuring of littoral macroinvertebrate communities? *Oecologia*, **171**, 971–980.
- Heino J., Mykrä H. & Rintala J. (2010). Assessing patterns of nestedness in stream insect assemblages along environmental gradients. *Ecoscience*, **17**, 345–355.
- Horvát Z., Vad C. F. & Ptacnik R. (2015). Wind dispersal results in a gradient of dispersal limitation and environmental match among discrete aquatic habitats. *Ecography*, **38**, 001–007.
- Jansson A. (1986). The Corixidae (Heteroptera) of Europe and some adjacent regions. *Acta Entomologica Fennica*, **47**, 1–94.
- Kadmon R. (1995). Nested species subsets and geographic isolation : A case study. *Ecology*, **76**, 458–465.
- Layton R. J. & Voshell R. J. (1991). Colonization of new experimental ponds by benthic macroinvertebrates. *Environmental Entomology*, **20**, 110–118.
- Legendre P. & Gallagher E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia*, **129**, 271–280.
- Levin L. A. & Talley T. S. (2002). Natural and manipulated sources of heterogeneity controlling early faunal development of a salt marsh. *Ecological Applications*, **12**, 1785–1802.
- Matthews J. W. & Spyreas G. (2010). Convergence and divergence in plant community trajectories as a framework for monitoring wetland restoration progress. *Journal of Applied Ecology*, **47**, 1128–1136.
- Meyer C. K. & Whiles M. R. (2008). Macroinvertebrate communities in restored and natural Platte River slough wetlands. *Journal of the North American Benthological Society*, **27**, 626–639.
- Meyer C. K., Whiles M. R. & Baer S. G. (2010). Plant community recovery following restoration in temporally variable riparian wetlands. *Restoration Ecology*, **18**, 52–64.
- Millennium ecosystem Assessment (2005). *Ecosystems and Human Well-being: Synthesis* Island Press, Washington DC.

- Millán A., Velasco J., Gutiérrez-Cánovas C., Arribas P., Picazo F., Sánchez-Fernández D. & Abellán P. (2011). Mediterranean saline streams in southeast Spain: What do we know? *Journal of Arid Environments*, **75**, 1352–1359.
- Moreno-Mateos D., Power M. E., Comín F. A & Yockteng R. (2012). Structural and functional loss in restored wetland ecosystems. *PLoS Biology*, **10**(1), e1001247.
- Moseman S. M., Levin L. A., Currin C. & Forder C. (2004). Colonization, succession, and nutrition of macrobenthic assemblages in a restored wetland at Tijuana Estuary, California. *Estuarine, Coastal and Shelf Science*, **60**, 755–770.
- Nakamura K., Tockner K. & Amano K. (2006). River and wetland restoration : lessons from Japan. *BioScience*, **56**, 419–429.
- Nieser N., Baena M., Martínez-Avilés J. & Millán A. (1994). *Claves para la identificación de los heterópteros acuáticos (Nepomorpha y Gerromorpha) de la Península Ibérica- Con notas sobre las especies de las Islas Azores, Baleares, Canarias y Madeira*. Asociación Española de Limnología, Madrid.
- Oksanen J., Blanchet F. G., Kindt R., Legendre P., Minchin P. R., O’Hara R. G., ... Wagner, H. (2012). *Vegan: Community Ecology Package*. R package version 2.2-1. Available at: <http://cran.r-project.org/>.
- Palmer M. A. (2009). Reforming watershed restoration: science in need of application and applications in need of science. *Estuaries and Coasts*, **32**, 1–17.
- Patterson B. D. (1987). The Principle of nested subsets and its implications for biological conservation. *Conservation Biology*, **1**, 323–334.
- Patterson B. D. & Atmar W. (2000). Analyzing species composition in fragments. In: *Proceedings of the 4th international Symposium on Isolated Vertebrate Community in the Tropics*, Bonn (Ed.G. Rheinwald), Bonn: Bonn. zool. Monogr., 46. 9–2.
- Patterson B. D. & Brown J. H. (1991). Nested patterns of species composition in granivorous rodent assemblages. *Journal of Biogeography*, **18**, 395-402.
- Ruhí A., Boix D., Gáscon S., Sala J. & Quintana X. D. (2013). Nestedness and successional trajectories of macroinvertebrate assemblages in man-made wetlands. *Oecologia*, **171**, 545–556.
- Ruiz-Jaen M. C. & Aide T. M. (2005). Restoration success : how Is It being measured ? *Restoration Ecology*, **13**, 569–577.
- Schilling E. J., Loftin C. S. & Huryyn A. D. (2009). Macroinvertebrates as indicator of fish absence in naturally fishless lake. *Freshwater Biology*, **54**, 181-202.
- Sebastián-González E. & Green A. J. (2014). Habitat use by waterbirds in relation to pond size, water depth, and isolation: lessons from a restoration in southern Spain. *Restoration Ecology*, **22**, 311–318.

- Secretariat R. C. (2006). *The Ramsar Convention Manual: A Guide to the Convention on Wetlands (Ramsar, Iran, 1971)* 4 th. (Ed. Ramsar Convention Secretariat), pp.114, Gland, Switzerland.
- Tachet H., Richoux P., Bournaud M. & Usseglio-Polatera P. (2000). *Invertébrés d'eau douce: systématique, biologie, écologie (Eds CNRS)*. Paris, France.
- Talling J. F. & Driver D. (1963). Some problems in the estimation of chlorophyll a in phytoplankton. In: *Proceedings of the Conference on Primary Productivity Measurement, Marine, Freshwater* (Ed. M. Doty), pp.142-146. US Atomic Energy engineering Commission, Honolulu, Hi.
- Thomaz S. M. & Cunha E. R. da (2010). The role of macrophytes in habitat structuring in aquatic ecosystems: methods of measurement, causes and consequences on animal assemblages' composition and biodiversity. *Acta Limnologica Brasiliensia*, **22**, 218-236.
- Touaylia S., Garrido J. & Boumaiza M. (2013). Abundance and diversity of the aquatic beetles in a Mediterranean stream system (Northern Tunisia). *Annales de La Société Entomologique de France (N.S.): International Journal of Entomology*, **49**, 172–180.
- Townsend C. R. & Hildrew A. G. (1994). Species traits in relation to a habitat template for river systems. *Freshwater Biology*, **31**, 265-275.
- Van de Meutter F., Trekels H. & Green A. J. (2010). The impact of the North American waterbug *Trichocorixa verticalis* (Fieber) on aquatic macroinvertebrate communities in southern Europe. *Fundamental and Applied Limnology / Archiv Für Hydrobiologie*, **177**, 283–292.
- Van den Broeck M., Waterkeyn A., Rhazi A., Grillas P. & Brendonck L. (2015). Assessing the ecological integrity of endorheic wetlands, with focus on Mediterranean temporary ponds. *Ecological Indicators*, **54**, 1-11.
- Vanschoenwinkel B., Waterkeyn A., Jocqué M., Boven L., Seaman M. & Brendonck L. (2010). Species sorting in space and time—the impact of disturbance regime on community assembly in a temporary pool metacommunity. *Journal of the North American Benthological Society*, **29**, 1267–1278.
- Vondel B. V. (1991). Revision of the palaeartic species of *Haliphys* subgenus Guignot (Coleoptera: Haliplidae). *Tijdschrift Voor Entomologie*, **134**, 75–144.
- Williams D. D., Heeg, N. & Magnusson A. K. (2007). Habitat background selection by colonizing intermittent pond invertebrates. *Hydrobiologia*, **592**, 487–498.
- Wright D. H., Patterson B. D., Mikkelsen G. M., Cutler A. & Atmar W. (1998). A comparative analysis of nested subset patterns of species composition. *Oecologia*, **113**, 1–20.

FIGURE LEGENDS

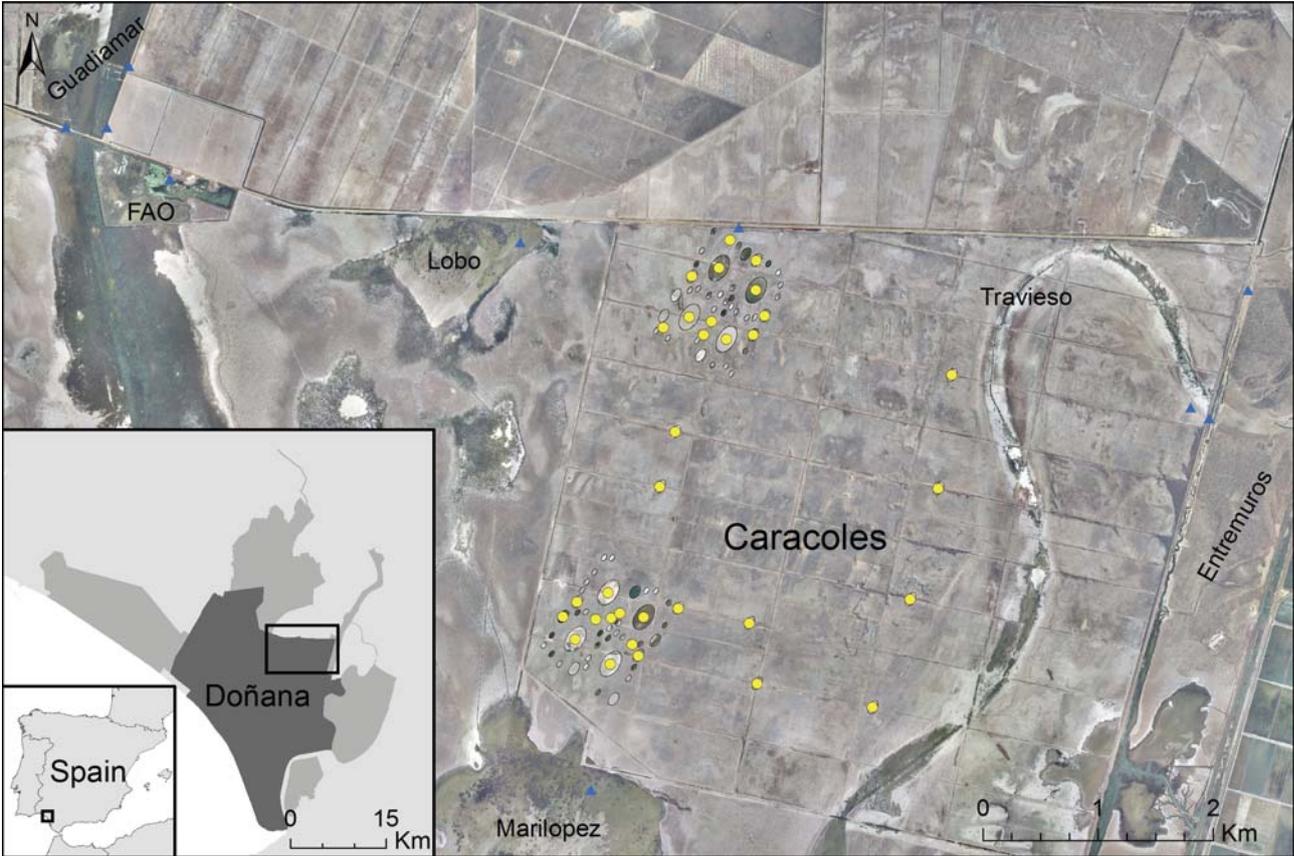


Figure 1. Map of the sampling sites in Doñana. The dark grey area in the bottom left figure indicates Doñana National Park and the light grey area indicates Doñana Natural Park. Circles identify sampled new ponds within the Caracoles estate, triangles identify reference sites.

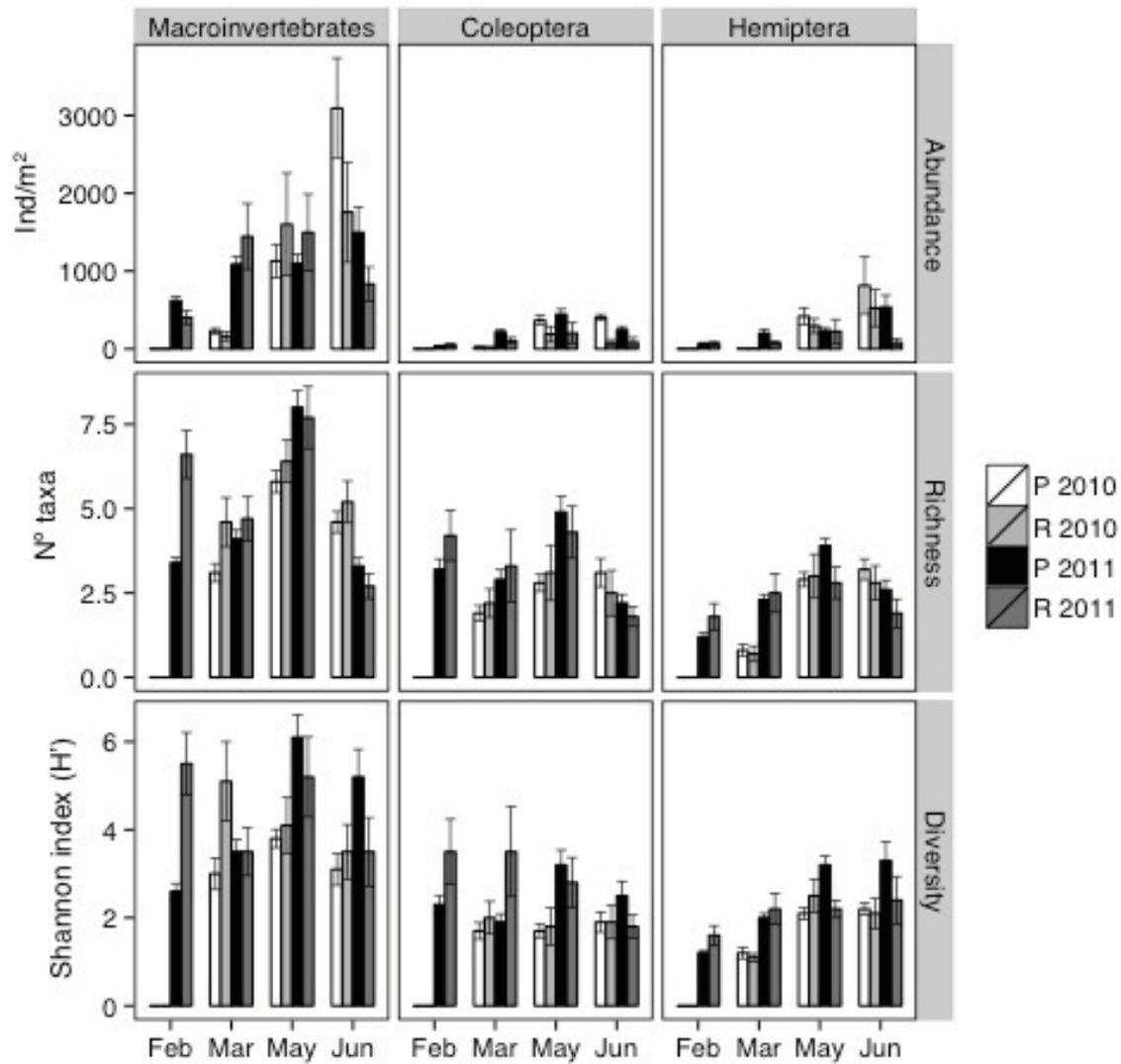


Figure 2. Mean (\pm SE) monthly abundance (individuals/m²), rarefied richness (N° taxa) and Shannon–Wiener diversity (H') in new ponds (P) and reference sites (R) for the total macroinvertebrate community (Macroinvertebrates), Coleoptera and Hemiptera during 2010 and 2011.

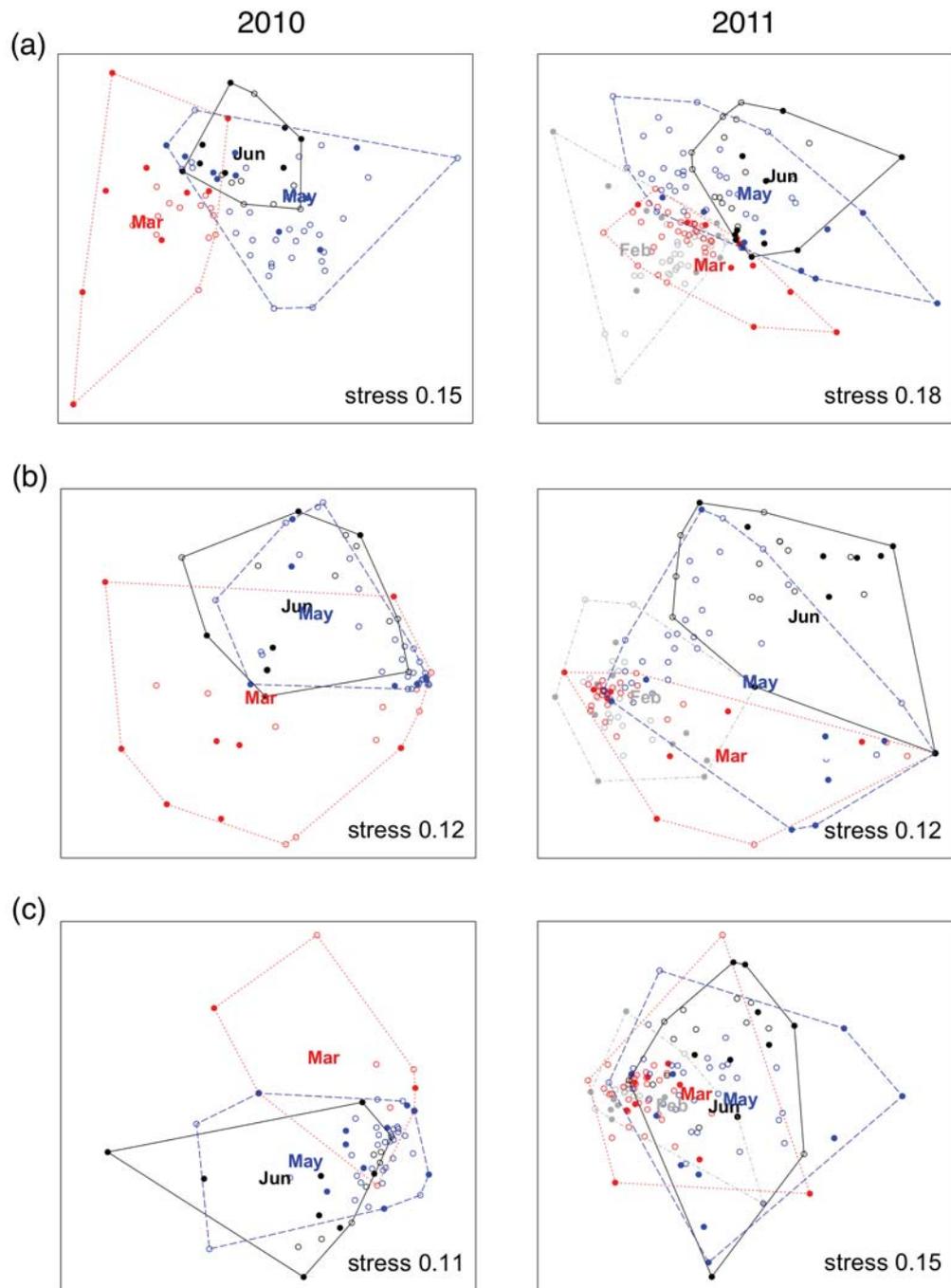


Figure 3. Non-metric multidimensional scaling ordination of (a) the whole macroinvertebrate community, (b) Coleoptera and (c) Hemiptera abundance showing the differences in community composition between new ponds (empty circles) and reference sites (filled circles) for each month. Polygons contain all the sites for a given month. Dot-dashed lines = Feb; dotted lines = Mar; long-dashed lines = May; solid lines = Jun.

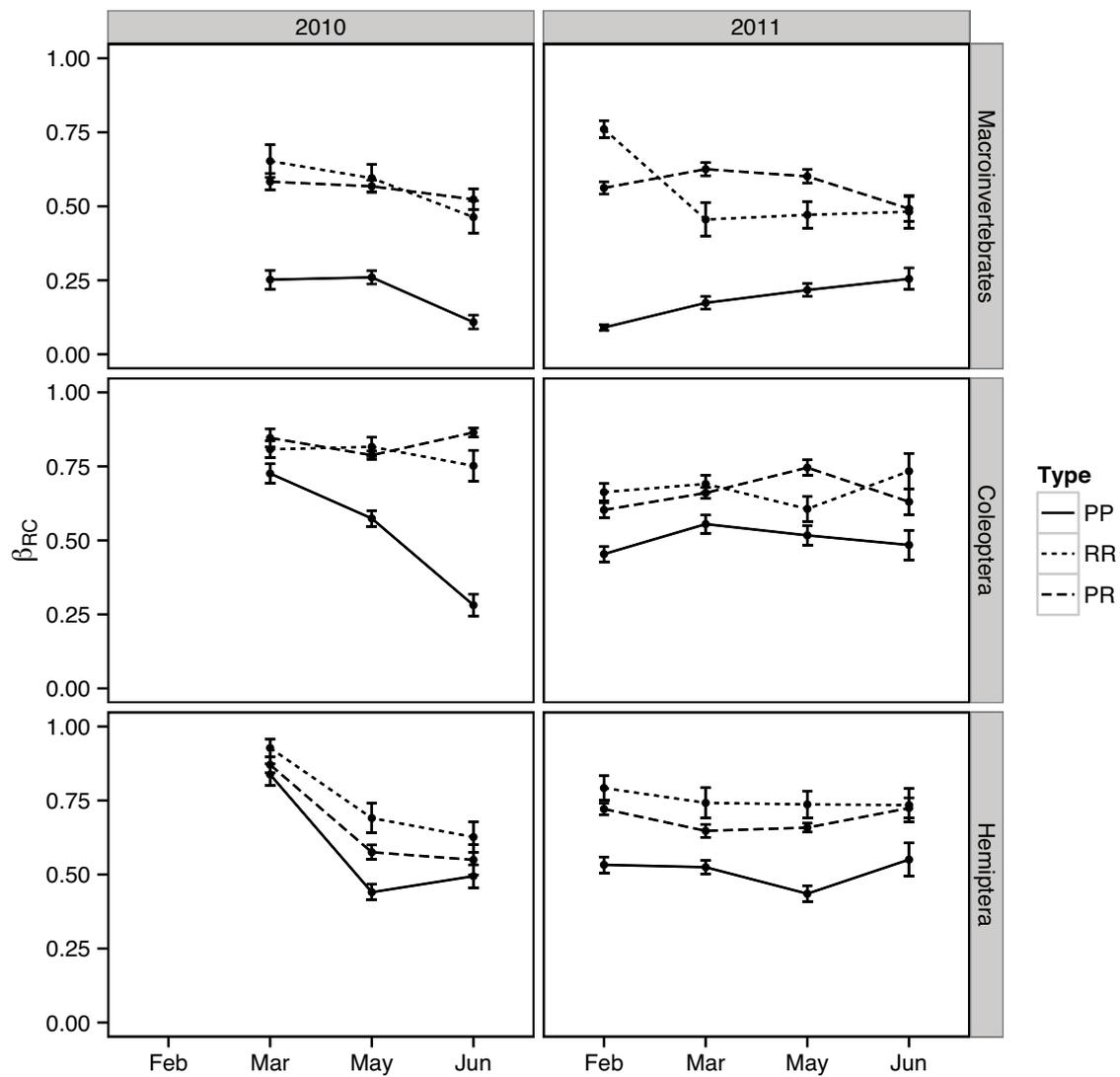


Figure 4. Mean pairwise Raup-Crick dissimilarity indices for the whole community (Macroinvertebrates), Coleoptera and Hemiptera, within new ponds (PP), within reference sites (RR) and between them (PR). February 2010 data are missing because extensive flooding prevented complete sampling of the area (Fig. S1).

Table 1. Mean monthly values (\pm SE) of environmental variables that most often explained the dissimilarity between new ponds and reference sites determined by the SIMPER analysis. In the case of fish and vegetation the values indicate the number of sites where they were detected. Emergent vegetation refers to the presence of *Juncus subulatus* and *Scirpus maritimus*.

	2010				2011		
	Mar	May	Jun	Feb	Mar	May	Jun
New Ponds							
<i>N</i>	14	32	8	30	32	32	14
pH	8.4 (0.1)	9.0 (0.0)	8.9(0.1)	8.9 (0.0)	9.2 (0.1)	9.4 (0.1)	9.1 (0.1)
Salinity (psu)	0.36 (0.0)	2.14 (0.2)	5.88 (0.85)	0.76 (0.1)	0.68 (0.1)	2.32 (0.1)	13.06 (2.0)
Turbidity (NTU)	226.4 (21.9)	82.5 (10.4)	94.5 (24.8)	64.2 (10.1)	50.9 (5.6)	47.8 (5.8)	77.0 (15.6)
Chla ($\mu\text{g l}^{-1}$)	9.3 (1.2)	18.9 (4.8)	74.3 (15.2)	5.7 (0.6)	3.3 (0.4)	8.5 (1.7)	42.1 (10.8)
Fish presence	3	4	2	1	9	7	9
Emergent Vegetation	1	25	4	5	28	31	13
Submerged vegetation	0	14	0	4	28	29	8
Reference sites							
<i>N</i>	9	10	8	10	10	10	10
pH	8.2 (0.1)	8.4 (0.1)	8.3 (0.2)	8.5 (0.0)	8.6 (0.2)	8.5 (0.2)	8.0 (0.2)
Salinity (psu)	0.21 (0.1)	1.33 (0.4)	2.15 (0.5)	0.52 (0.01)	0.53 (0.1)	1.58 (0.2)	5.27 (1.3)
Turbidity (NTU)	146.8 (32.6)	75.5 (11.0)	78.4 (16.5)	48.0 (7.7)	36.4 (13.1)	55.4 (13.8)	163.7 (44.9)
Chla ($\mu\text{g l}^{-1}$)	11.5 (3.4)	16.0 (3.2)	37.8 (9.6)	15.0 (4.1)	9.1 (2.2)	15.7 (5.6)	72.9 (9.9)
Fish presence	1	6	4	1	5	7	9
Emergent vegetation	9	10	8	9	10	10	10
Submerged vegetation	0	2	3	3	7	8	3