

Recovery of zooplankton diversity in a restored Mediterranean temporary marsh in Doñana National Park (SW Spain)

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Abstract Over 30 years after drainage for agriculture, a 2700 ha temporary marshland was recently restored in Doñana National Park. We describe the recovery of zooplankton communities (copepods, cladocerans and rotifers) in 47 new temporary ponds excavated as part of the restoration project during the first two hydroperiods (April 2006 and 2007), and compare them to those of eight reference sites in the surrounding marshland. Major changes in the species composition and abundance occurred in new ponds between years. While rotifers and cyclopoid copepods dominated in terms of number of individuals in 2006, calanoid copepods and cladocerans were the most abundant groups in 2007. Rotifer species richness was significantly lower in 2007, but there was an increase in Simpson and β -diversity in 2007 owing largely to a dramatic decline in the abundance of *Hexarthra* cf. *fennica* (rare in reference sites) from 93% of all rotifer individuals in new ponds in 2006 to

only 32% in 2007. In contrast, species richness of copepods and cladocerans was significantly higher in new ponds in 2007, but there were no changes in Simpson diversity. β -Diversity of cladocerans was also significantly higher in 2007. In 2006, the species richness of cladocera and copepods was significantly lower in new ponds than in reference sites, but by 2007 there were no differences in richness or Simpson diversity. Overall, 7 copepod, 13 cladoceran and 26 rotifer taxa were recorded in new ponds, including 80% of taxa recorded in reference sites. These results indicate that zooplankton communities can be rapidly restored in Mediterranean temporary wetlands, at least when large source populations in the surrounding area reduce dispersal limitation. They also illustrate the importance of comparing different metrics of richness and diversity in studies of zooplankton restoration.

Keywords Wetland restoration · Mediterranean temporary ponds · Zooplankton · Species richness · α - and β -diversity · Colonization

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Introduction

In recent decades, freshwater biodiversity has declined faster than terrestrial or marine biodiversity, and increasing demands on freshwater resources, climate change and the impact of alien species will

lead to a continuing loss of biodiversity in the future (Jenkins, 2003; García-Berthou et al., 2007; Moss et al., 2009). Restoration of degraded wetlands or creation of new ones are now common measures for conservation of aquatic biodiversity (Cooke et al., 2005; Williams et al., 2008).

Zooplankton communities can provide an excellent model for assessing the success of restoration and increasing our understanding of restoration and community ecology (Dodson & Lillie, 2001; De Meester et al., 2005). To date, studies of restoration of zooplankton communities have concentrated mainly on lake communities in northern-temperate regions. For example, in lakes degraded by eutrophication and fish introductions, reduction of nutrient loading and control of fish stocks can lead to recovery of many cladoceran taxa present before degradation within 3 years (Louette et al., 2008), which in turn can facilitate top-down control of phytoplankton abundance and restoration of clear water states that favour high biodiversity in general (Jeppesen et al., 2007). Dodson et al. (2007) found new lakes in Wisconsin (average age of 14 years) to have similar zooplankton species richness than much older lakes, although it is unclear how long it took to reach such levels of richness. In Canadian mountain lakes, zooplankton communities took about 19 years to recover fully after removal of introduced sports fish (Donald et al., 2001). Zooplankton recovery after restoration of acidified lakes can be highly successful, at least for some taxa (Yan et al., 2004; Gray & Arnott, 2009). After an experimental acidification of an 18 ha lake during 6 years, the zooplankton community returned to reference levels after another 6 years (Frost et al., 2006). Factors determining the recovery speed of individual taxa include the length of the degradation period prior to restoration, and the life-history of the zooplankton (Knapp & Sarnelle, 2008). In cases where dispersal limitation prevents recolonization by natural means, some taxa have been successfully reintroduced (Kohout & Fott, 2006).

Wetland loss has been particularly intense in the Mediterranean region (Green et al., 2002; De Stefano, 2004), and Mediterranean environments are a priority for biodiversity conservation at a global scale (Brooks et al., 2006). Temporary ponds in Mediterranean regions are biodiversity hotspots highly threatened by habitat degradation (Grillas et al., 2004; Oertli et al., 2009) and are among the most

protected European aquatic environments (EU Habitat Directive 92/43/EEC). The temporary ponds and marshes in Doñana National Park in South-West Spain (Serrano et al., 2006) are particularly famous and are protected as a UNESCO World Heritage Site, owing largely to their great international importance for waterbirds (Rendón et al., 2008). Studies of zooplankton since 1980 have focused mainly on dune ponds outside the main marshland, which host an endemic rotifer species (Fahd et al., 2000, 2009). After mine waste contaminated the area surrounding the National Park in 1998 (Taggart et al., 2006), a major restoration project was launched to restore some areas of temporary marsh previously transformed for agriculture (Enggass, 1968; García-Novo & Marín, 2006).

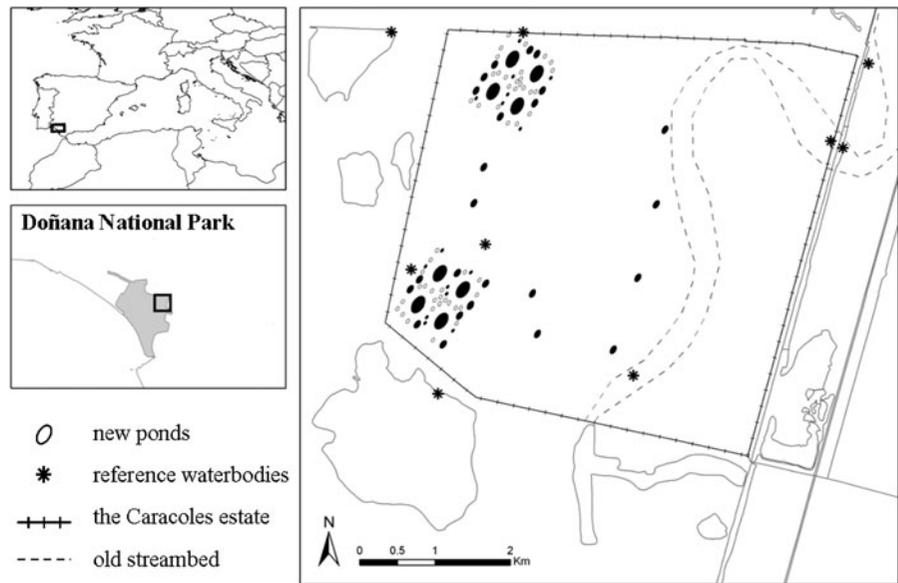
In this study, we describe the zooplankton communities that have developed in the largest restoration area in Doñana, including the copepods, cladocerans and rotifers. As recommended for studies of restoration of zooplankton communities (Gray & Arnott, 2009), given the absence of historical data for our study area we combined temporal sampling and the use of reference sites to assess the success of recovery. Furthermore, unlike most restoration studies (Dodson & Lillie, 2001; Gray & Arnott, 2009), we did not focus exclusively on species richness, but used and compared various metrics of diversity and richness as distinct indicators of recovery.

Materials and methods

Study site

The main study area was the Caracoles estate (Fig. 1), a former seasonally inundated marshland area of 2700 ha that was isolated from surrounding marshes (also known as the “marismas del Guadalquivir”) and turned into arable farmland in the 1960s. It is located on the northern edge of Doñana National Park (Fig. 1) and situated in the Guadalquivir estuary at 1–2 m a.s.l., but free from direct tidal influence. During the “Doñana 2005” restoration plan, the estate was incorporated into the National Park, the surrounding dykes and agricultural drainage system were removed and a set of 96 experimental temporary ponds were created between summer 2004 and spring 2005 (Santamaría et al., 2006; Frisch & Green, 2007).

Fig. 1 Map of the study area. The subset of new ponds included in the present study is in *black*. Note the old streambed (Caño Travieso, *dotted line*), whose connection to natural marshes to the south was restored at the same time as the new ponds were excavated



All the ponds have the same elliptical shape but with three different sizes (surface area of 0.18, 0.74 and 2.95 ha), and an excavated depth of 30 or 60 cm. Ponds are distributed in two major blocks of 44 ponds, plus 8 relatively isolated ponds distributed throughout the estate (Fig. 1). The main blocks were situated in relatively low-lying areas that were regularly inundated prior to the agricultural transformation (Santamaría et al., 2006). The ponds are primarily filled by precipitation and local surface run-off from within the estate, with variation in microtopography causing differences in depth and hydroperiod. During this study, some ponds temporarily overflowed and connected to flooded grassland areas during and after major rainfall events. During the summer, all new ponds and reference sites (see below) dry out completely, as does almost all the natural marsh system within the National Park (see Serrano et al., 2006; Espinar & Serrano, 2009 for details). During the wettest periods, movement within the Caracoles estate and the rest of the marshes is impossible by vehicle, making research difficult.

Doñana has a Mediterranean climate with Atlantic influence, with dry, hot summers and short, mild winters. Rainfall is variable and concentrated mainly between the end of September and the beginning of April, with high temperatures causing rapid evaporation in the summer months (Espinar & Serrano, 2009 and references therein). The autumn–winter following pond construction was exceptionally dry, with

only 167 mm of rainfall from September 2004 to March 2005 (compared to a mean of 455 mm for 1979–2009, data from Palacio de Doñana), and only puddles were formed in the bed of a few of the ponds for a maximum of 20 days (Frisch & Green, 2007). Thus, the basins of the new ponds were completely inundated for the first time in January 2006 following heavy rainfall (136 mm in January, followed by 150 mm from February to April). Hydroperiod length varied, with some ponds beginning to dry out in early May and others persisting until late June 2006. The next hydroperiod started much earlier in late October 2006 owing to above-average precipitation between September and December 2006 (445 mm, compared to 149 mm for the same months in 2005). The first ponds dried out in early May and some persisted until July 2007. Water temperatures became high from May onwards and already reached maxima of over 35°C in the first week of May.

Zooplankton egg banks were almost undetectable in the new ponds after their excavation and prior to first hydroperiod. Only two rotifer individuals hatched from a total of 72 sediment samples (Frisch & Green, 2007). Just before the restoration project, the egg bank in an old streambed that crosses the estate near the ponds (Fig. 1) was low in diversity and abundance, despite being the only part of the estate that still flooded in winter during the agricultural phase (Frisch et al., 2009). Cattle were eliminated from the estate before the new ponds filled with

water, but hares and fallow deer were present throughout the study and acted as potential agents of zooplankton dispersal, together with waterbirds (Frisch et al., 2007; Rendón et al., 2008).

Sampling

A subset of 47 new ponds representative of all ponds, in terms of size, depth and connectivity was selected for sampling, including ponds from both major blocks as well as isolated ponds (Fig. 1). Nearby natural and semi-natural temporary water bodies present around the Caracoles estate were selected as reference sites to be sampled simultaneously (Fig. 1). These included eight sites which were a potential source of colonists for the new ponds and varied in hydrogeomorphology (e.g. ditches, an old streambed, small marsh depressions and shallow ponds), and aquatic communities (e.g. presence or absence of fish, extent of aquatic vegetation).

In this paper we present data from two sampling campaigns that were carried out from 17–26 April 2006 and 16–24 April 2007 to compare the zooplankton community present during the first two hydroperiods at a standard time of year. April was selected as an optimal month for long-term monitoring, because access to the area is complicated during earlier, wetter months, and because the ponds and reference sites begin to dry out completely from May onwards. Sampling was carried out between 10:00 and 18:00 h, avoiding periods of high winds or rainfall.

In each new pond and reference site, zooplankton was sampled by filtering a total of 20 L of water through a 64 μm mesh-size net, and preserved in 70% ethanol. The smallest rotifers are likely to have passed through this mesh. Forty subsamples (each 500 ml) were combined to overcome the expected patchy distribution of zooplankton. The water was collected with a 500 ml plastic jar while walking along a transect from the shore to the centre of the pond. Every subsample was taken in front or to the side of the person sampling, taking care to avoid areas where sediment had been disturbed. To avoid spreading zooplankton between ponds, the sampling equipment was thoroughly rinsed with tap water and 70% ethanol in between consecutive ponds. In addition, boots were covered with plastic bags which were replaced for each pond to avoid sediment and propagule transfer from one pond to another.

All zooplankton individuals (including rotifers, copepods and cladocerans) were counted, and identified to species level (whenever possible), using Utermöhl chambers under a Zeiss inverted microscope, mainly at magnifications from 50 \times to 400 \times . For the observation of some morphological details, involving individual dissection, a light microscope with magnifications from 400 \times to 1000 \times was used. Taxa were identified according to Dussart (1967, 1969) and Einsle (1993) for copepods, to Alonso (1996) for cladocerans, and to Koste (1978) for rotifers. Bdelloid rotifers and juvenile stages of copepods and some cladocerans could not be identified to species level. Samples with less than 400 individuals were counted without subsampling. At higher densities, subsamples representing at least 1/16th of the total sample, and containing at least 200 individuals of the most frequent taxon, were counted. The complete sample was then screened microscopically to find and identify rare taxa.

Depth (taken as the average from five points), temperature, pH and electrical conductivity were measured in situ (Table 1). Environmental conditions were similar between years for both new ponds and reference sites (Table 1), the only exception being that pH was higher in the new ponds in 2007 (Wilcoxon paired test, $P < 0.001$). In 2007, chlorophyll-a (mean \pm SE = $3.84 \pm 0.67 \mu\text{g l}^{-1}$ for new ponds, 9.60 ± 4.84 for reference sites), total phosphorus ($59.81 \pm 5.15 \mu\text{g l}^{-1}$ for new ponds, 132.23 ± 29.04 for reference sites), and turbidity (17.60 ± 3.12 NTU for new ponds, 63.78 ± 41.08 for reference sites) were also measured. pH was negatively correlated with chlorophyll-a concentration (Pearson's $R = 0.353$, $P = 0.012$).

No fish were present in the new ponds during the study, but both fish and cattle were present in some of the reference sites outside the Caracoles estate (Fig. 1). In new ponds, there was a notable increase from 2006 to 2007 in the proportion of the bottom covered with submerged vegetation, especially charophytes, *Ruppia drepanensis* and *Ranunculus peltatus*. Unlike new ponds, reference sites had emergent vegetation (e.g. *Scirpus maritimus* and *Juncus subulatus*).

Zooplankton diversity and community similarity

α -Diversity was quantified by two measures: local species richness as the number of all identified taxa

Table 1 Mean, standard error (in parentheses) and range for environmental variables recorded in the new ponds and reference sites

	April 2006		April 2007	
	Mean (SE)	Range	Mean (SE)	Range
New ponds	<i>N</i> = 47		<i>N</i> = 47	
Temperature (°C)	20.31 (0.36)	16–27.30	21.02 (0.41)	16.80–28.30
pH	8.50 (0.07)	7.09–9.21	9.05 (0.05)	8.50–10.18
Electrical conductivity (mS cm ⁻¹)	6.88 (0.50)	2.03–17.04	6.94 (0.33)	2.64–15.22
Water column depth (cm)	19.06 (1.44)	5.00–49.40	21.32 (1.42)	6.80–48.40
Reference sites	<i>N</i> = 8		<i>N</i> = 6	
Temperature (°C)	21.96 (0.98)	19.8–27.8	22.18 (1.63)	16.30–25.70
pH	8.62 (0.13)	7.96–8.96	8.99 (0.26)	8.16–9.65
Electrical Conductivity (mS cm ⁻¹)	4.55 (0.52)	2.16–6.64	5.37 (0.74)	4.09–9.00
Water column depth (cm)	12.92 (2.75)	4.4–29.6	18.80 (3.21)	10.02–33.40

Significant differences between years were only found for the mean pH value in the new ponds (Wilcoxon paired test, $P < 0.001$)

per pond and year, and the Simpson index, which takes into account the relative abundances of the identified taxa. All these measures were computed for each new pond and reference site for the whole zooplankton community, as well as for rotifer, cladoceran and copepod assemblages separately. Between-year differences for measures in a given new pond were tested with non-parametric Wilcoxon matched-paired tests. Differences between new ponds and the reference sites were tested for both years using non-parametric Mann–Whitney tests.

β -Diversity as a measure of community differentiation among sites can be estimated by averaging the dissimilarity values among sites (Legendre et al., 2005). Dissimilarities among ponds were estimated using the complement-to-one of the abundance-based Chao–Sørensen similarity index (computed using EstimateS v.8.0 software, Colwell, 2005). This index is recommended for assessing compositional similarity between samples that differ in size and are likely to contain numerous rare species (Chao et al., 2005). For a given year, the β -diversity was estimated by averaging the dissimilarity values obtained from pairwise comparisons among ponds. It was also estimated for rotifer, cladoceran and copepod assemblages. Between-year differences of the frequency distributions of pairwise comparisons of β -diversity were tested with Wilcoxon matched-paired tests (i.e. comparing the values for a given pond in 2006 and 2007).

A redundancy analysis (RDA) was carried out to examine community composition patterns in new

ponds and their relations with the environmental variables measured (Lepš & Šmilauer, 2003). Prior to analysis, water depth, conductivity and temperature were log ($x + 1$) transformed. The year of sampling (2006 or 2007) was added also as a predictor variable in the RDA (nominal variable). Abundances of juvenile stages of copepods and cladocerans were included to perform this analysis. The abundance data matrix was subjected to the Hellinger transformation following Legendre & Gallagher (2001).

Differences between years in the frequency of occurrence (i.e. number of ponds where a given taxa was found), and in the frequency of dominance (i.e. number of ponds where a given taxon was the single most abundant taxon) were tested by means of Fisher exact tests.

Results

Changes in diversity and abundance between years in new ponds

In the new ponds, there was a significant decrease in the total abundance of zooplankton from 2006 to 2007 (means of 709 and 164 individuals per litre, respectively, Wilcoxon paired tests, $P < 0.001$). This reflects a drastic reduction in the mean abundance of rotifers (from 459 to 22 individuals per litre, $P < 0.001$) as well as a significant reduction in the mean abundance of copepods (from 240 to 117 individuals per litre, $P = 0.044$, Fig. 2). In contrast, the absolute abundance

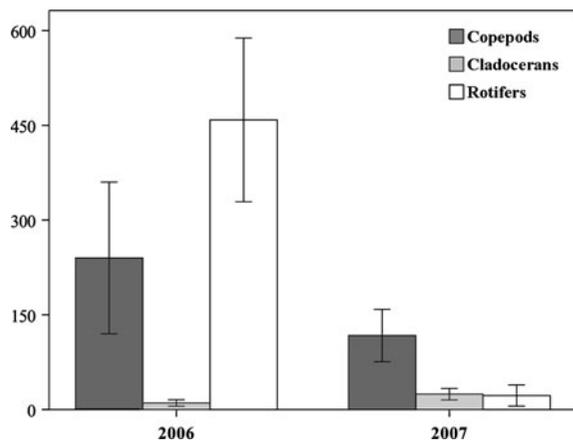


Fig. 2 Bar plots showing the mean total abundance (no. of individuals per litre) of the copepod, cladoceran and rotifer assemblages recorded in new ponds for both years. Error bars show 95% confidence intervals

of cladocerans increased from a mean of 10 individuals per litre in 2006 to 24 in 2007 ($P = 0.002$). In terms of relative abundance, both cladocerans and copepods increased in 2007 at the expense of rotifers (Wilcoxon paired tests, $P < 0.001$). The mean percentage of rotifers dropped from 65% in 2006 to 14% in 2007, whereas for copepods and cladocerans it increased from 34 to 72 and from 2 to 15, respectively.

α -Diversity measured as local species richness (richness per pond) of the zooplankton community did not differ significantly between 2006 and 2007 (Table 2). In contrast, α -diversity measured by the Simpson index was significantly higher in 2007. When analyzing copepods, cladocerans and rotifers separately, the species richness for each group differed significantly between years (Table 2). While the local richness increased in 2007 for copepods and

Table 2 Mean, standard error and range for local species richness and Simpson diversity index in new ponds ($N = 47$) and the reference sites (only those sampled in both years,

$N = 6$) for the whole zooplankton community, and for copepods, cladocerans and rotifers separately

	April 2006		April 2007		<i>P</i> value
	Mean (SE)	Range	Mean (SE)	Range	
<i>New ponds</i>					
Species richness					
Total	8.72 (0.41)	4–16	8.62 (0.36)	4–14	0.909
Copepods	2.28 (0.14)	1–4	2.70 (0.12)	1–4	0.031
Cladocerans	2.38 (0.18)	1–6	3.15 (0.18)	1–6	0.012
Rotifers	4.06 (0.28)	1–8	2.77 (0.27)	0–7	0.002
Simpson diversity					
Total	0.25 (0.04)	0–0.82	0.47 (0.03)	0.03–0.79	0.000
Copepods	0.26 (0.04)	0–0.69	0.24 (0.04)	0–0.83	0.829
Cladocerans	0.17 (0.03)	0–0.60	0.23 (0.03)	0–0.79	0.300
Rotifers	0.13 (0.03)	0–0.55	0.25 (0.04)	0–0.69	0.029
<i>Reference sites</i>					
Species richness					
Total	12.20 (1.8)	7–17	10.60 (1.66)	8–17	0.461
Copepods	3.40 (0.51)	2–5	2.40 (0.51)	1–4	0.285
Cladocerans	4.80 (0.37)	4–6	3.60 (0.93)	2–7	0.194
Rotifers	4.00 (1.67)	1–9	4.60 (0.51)	2–6	0.586
Simpson diversity					
Total	0.46 (0.09)	0.15–0.65	0.56 (0.09)	0.25–0.78	0.686
Copepods	0.36 (0.09)	0.15–0.60	0.23 (0.13)	0–0.62	0.345
Cladocerans	0.33 (0.14)	0.04–0.79	0.31 (0.07)	0.08–0.51	0.500
Rotifers	0.13 (0.08)	0–0.37	0.41 (0.13)	0.10–0.78	0.345

Differences were analyzed with Wilcoxon paired tests

cladocerans, it decreased for rotifers. Between-year differences in the Simpson index was found only for rotifers, with a significant increase in 2007 (Table 2). When comparing the frequency distributions of pairwise comparisons of β -diversity between 2006 and 2007 (Fig. 3), significant differences were found (Wilcoxon paired tests, $P < 0.001$), indicating a greater among-pond differentiation or higher β -diversity in 2007 for the whole zooplankton community, and also separately for cladoceran and rotifer assemblages. The β -diversity of the copepod assemblages was also higher in 2007, but not significantly so (Wilcoxon paired test, $P = 0.067$).

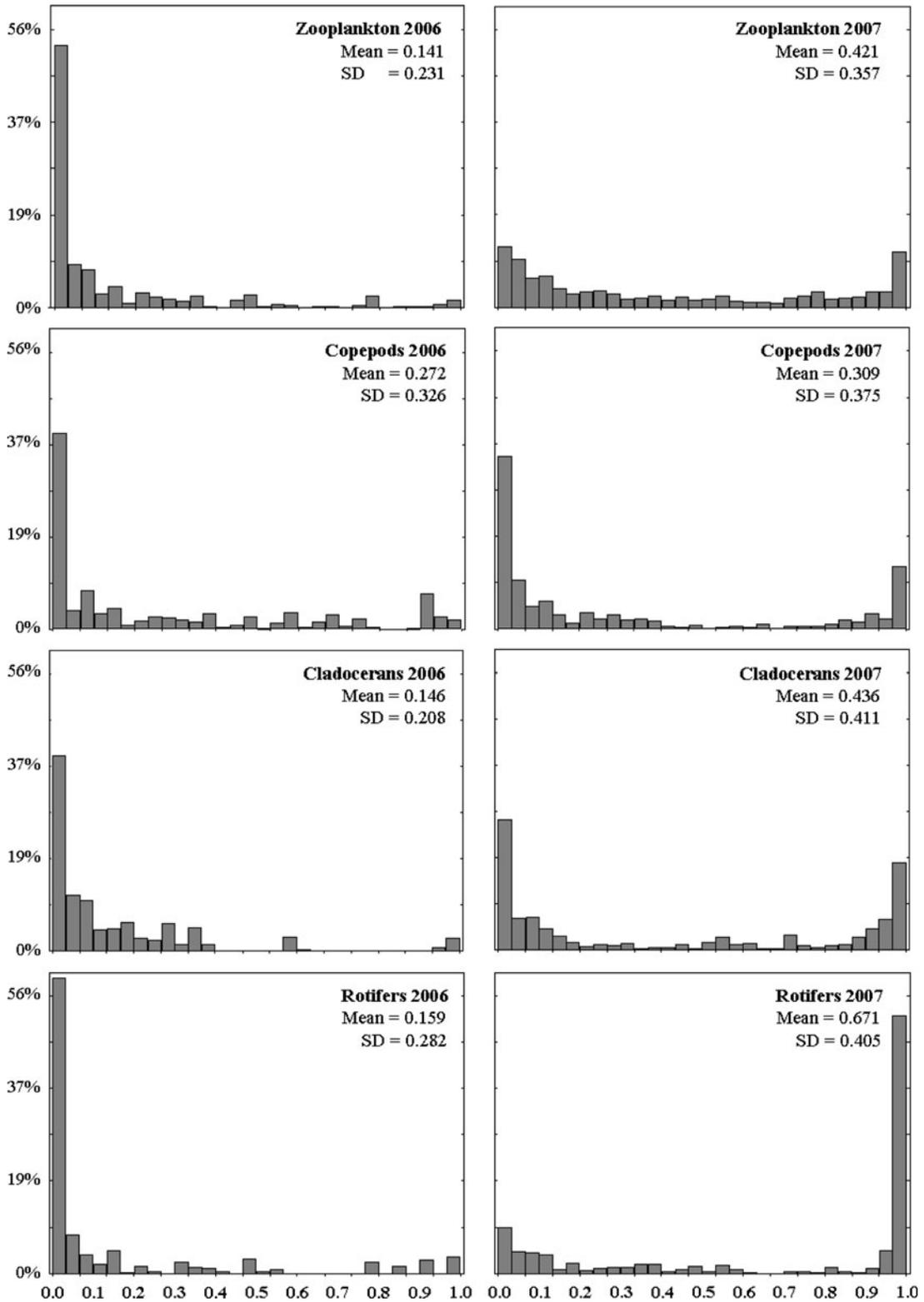
Changes in taxonomic composition in new ponds

Up to 37.9% of the total variation of the species data was explained by the predictor variables. The first RDA axis alone accounted for 31.3% of the variability whereas the second one only accounted for 4.1%. The effect of the two first axes was significant ($P = 0.002$ with 499 permutations) but the effect of the third canonical axis was not ($P = 0.268$). The first RDA axis was highly related to the year of sampling ($r = -0.870$). pH was the second variable most related with this axis ($r = -0.490$). Conductivity and water depth were the variables most related with the second axis ($r = 0.565$ and $r = -0.449$, respectively). The year of sampling was clearly the most important variable in explaining the variation observed in the species data since communities from both years were perfectly separated along this axis with positive scores for the 2006 communities and negative scores for those of 2007 (Fig. 4). This indicates that zooplankton composition differed greatly between years, with major differences in the most abundant species. The rotifer *Hexarthra* cf. *fennica* was highly abundant in 2006, whereas calanoid nauplii, adults of the calanoid *Neolovenula alluaudi* and the cladoceran *Moina brachiata* were highly abundant in 2007. When removing the effect of the year of sampling as a predictor in a second RDA (results not shown), the environmental variables only explained 6.9% of the species variation, although their effect was also significant ($P = 0.002$ with 499 permutations), and conductivity and water depth were the more important variables. High abundances of the rotifer *Brachionus plicatilis* were related to higher conductivity, whereas

high abundances of calanoid copepodids were related to greater depth (Fig. 4).

In 2006, juvenile cyclopoid stages (nauplii and copepodids) and adults of the cyclopoid *Metacyclops minutus* were the most abundant copepods in new ponds, representing 94% of copepods (Table 3). The most abundant cladocerans were *Moina brachiata* followed by *Ceriodaphnia quadrangula*, together constituting 92% of cladocerans. The most abundant rotifers were *Hexarthra* cf. *fennica*, followed by *B. plicatilis*, together representing 98% of rotifers (Table 3). In terms of frequency of occurrence, juvenile cyclopoid stages, *M. minutus*, *M. brachiata* and *H. cf. fennica* were the most widespread taxa in 2006, each one being present in at least 98% of ponds (Table 3). Of these taxa, *M. minutus* and *M. brachiata* did not dominate the community in any pond (i.e. they were never the most abundant taxon in a given pond), in contrast to cyclopoid nauplii and *H. cf. fennica* which were dominant in 13 and 30 of the 47 ponds, respectively (Table 3). The high abundance of these few taxa in a large number of ponds explained the very low dissimilarity indices found in 2006 for a large number of the pairwise comparisons (Fig. 3). Overall, 60% of the taxa recorded in 2006 occurred in less than 20% of the new ponds.

In 2007, calanoid juvenile stages were the most abundant copepods. Together with adults of the calanoid *Neolovenula alluaudi*, they represented 55% of copepod individuals. This species increased its presence from 4% of new ponds in 2006 to 85% in 2007 (Fisher exact, $P < 0.001$). Amongst cladocerans, the most abundant species were *M. brachiata* and *Daphnia magna*, together representing 93% of individuals. Compared to 2006, the former taxon increased markedly in the frequency of dominance, and the latter in frequency of occurrence (Table 3). *N. alluaudi*, *M. brachiata* and *D. magna* significantly increased their absolute abundance in 2007 (Wilcoxon paired tests, $P < 0.05$). The most abundant rotifers were *Keratella tropica* and *Hexarthra* cf. *fennica*, together representing 65% of individuals, although *Lecane luna* had the highest frequency of occurrence. However, *Hexarthra* underwent major declines in abundance ($P < 0.0001$) and frequency of occurrence ($P < 0.001$). It was the most abundant zooplankton in 30 ponds in 2006, but in none in 2007 ($P < 0.001$). In 2007, *L. luna* increased its total



◀ **Fig. 3** For 2006 and 2007 (*left and right* plots, respectively): frequency distributions of pairwise comparisons ($N = 1081$) of β -diversity (measured as complement-to-one of the Chao–Sørensen similarity index) in the new ponds for the whole zooplankton community and the copepods, cladocerans and rotifers separately

abundance ($P < 0.001$) and its frequency of occurrence ($P < 0.05$). The cyclopoid *Acanthocyclops* cf. *robustus* was not recorded in 2006, but was present in 23% of ponds in 2007 ($P < 0.001$). Also striking were significant reductions in absolute abundance ($P < 0.001$) and frequency of occurrence ($P < 0.001$) of the rotifers *B. plicatilis* and *B. quadridentatus*, and of the copepod *Mixodiaptomus incrassatus* in 2007 (Table 3). In 2007, 63% of the taxa recorded still had a frequency of occurrence below 20%.

Zooplankton communities in new and reference sites

In new ponds, a total of 46 taxa were recorded in 2006 and 2007 combined: 7 copepods, 13 cladocerans and 26 rotifers. From each group, 5, 9 and 14 species, respectively, were common to both years. Total species richness for copepods (6) and cladocerans (11) was the same for both years. For the rotifer assemblage, species richness increased from 19 in 2006 to 21 in 2007. Therefore, total species richness in new ponds was 36 in 2006 and 38 in 2007 (Table 3). Despite the small number of reference sites, the total species richness recorded in them was similar with a total of 44 taxa: 9 copepods, 14 cladocerans and 21 rotifers. From each group, 5, 6 and 7 species, respectively, were common to both years. Total cumulative species richness was also similar between 2006 and 2007 with 32 and 30 taxa, respectively (Table 3). Eighty percent of 44 taxa found in reference sites were also recorded in new ponds, only 3 copepods, 2 cladocerans and 4 rotifers being absent. On the other hand, 1 copepod, 1 cladoceran and 6 rotifer taxa found in new ponds were not found in reference sites (Table 3).

For the reference sites, there were no significant differences between 2006 and 2007 in local species richness and Simpson diversity of the whole zooplankton community or of the copepod, cladoceran or rotifer assemblages (Table 2). Similarly, no significant changes in the absolute abundance of zooplankton were recorded (Wilcoxon paired tests, $P > 0.05$).

When comparing new ponds with reference sites, significant differences in local species richness were recorded in 2006, with local species richness being lower in new ponds for the whole community, and also for copepods and cladocerans (Mann–Whitney test, $P < 0.05$, Fig. 5). For rotifers in 2006, and all comparisons in 2007, there were no significant differences between the new ponds and reference sites (Fig. 5). No significant differences in Simpson diversity index were found between new ponds and reference sites in either year (Mann–Whitney test, $P < 0.05$).

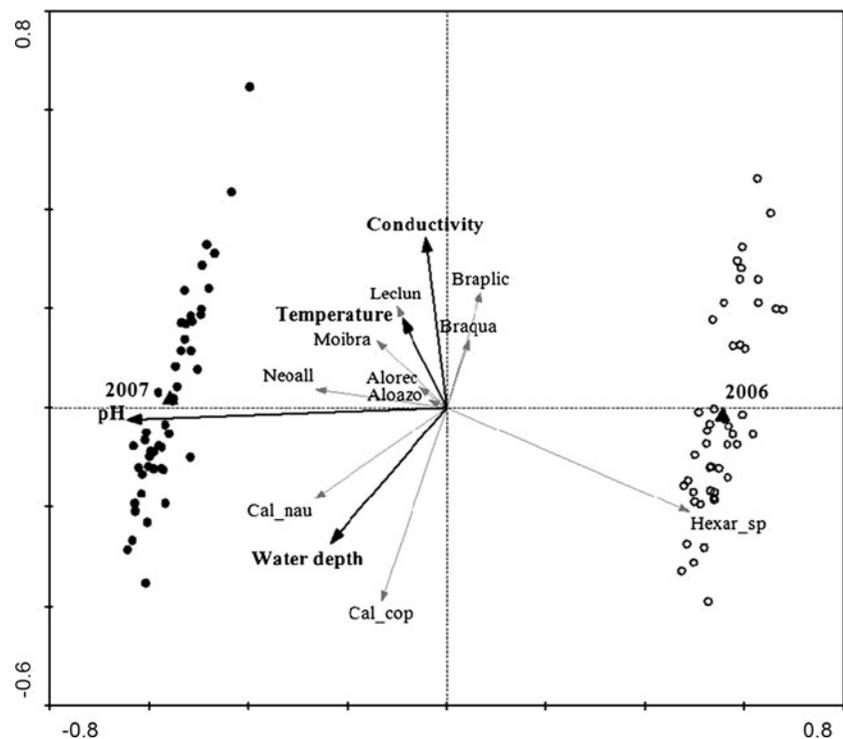
Discussion

To our knowledge, this is one of the most extensive studies to date of zooplankton in restored Mediterranean wetlands. The present study confirms the success of the Doñana restoration project and its new ponds, which have rapidly acquired diverse zooplankton communities with similarities to those found in the natural wetlands used as a reference sites. The much smaller number of reference sites than new ponds gave us reduced statistical power for comparisons with new ponds and to detect changes between years in the reference sites. However, we were restricted by the limited number of isolated and accessible waterbodies in the area surrounding the Caracoles estate suitable as reference sites (Fig. 1).

Snapshot samplings often fail to detect a substantial fraction of the annual species pool (e.g. Arnott et al., 1998; Fahd et al., 2007). Ideally samples should be taken more than one month a year, but comparison at several points in the annual cycle is difficult in temporary ponds with variable hydroperiods. The timing and extent of inundation is extremely variable in Doñana in response to rainfall fluctuations (Kloskowski et al., 2009), leading to major annual variation in hydroperiod and depth in the new ponds. The 30 year mean for precipitation between September and March inclusive at the Palacio de Doñana is 440 mm, and we studied one year that was slightly below average (402 mm in 2006) with a late start to the hydroperiod, and one that was well above average (599 mm in 2007).

A range of metrics indicated the recovery of zooplankton communities over time in the new ponds, although the results varied with the metric

Fig. 4 RDA ordination triplot summarizing the differences between years and the effects of explanatory variables on zooplankton communities. Species codes for the displayed species (only those with 17% of their variation explained) are: Aloazo: *Alona azorica*; Alore: *Alona rectangulara*; Braplic: *Brachionus plicatilis*; Braqua: *Brachionus quadridentatus*; Cal_nau: calanoida nauplii; Cal_cop: calanoida copepodids; Hexar_sp: *Hexarthra* sp.; Leclun: *Lecane luna*; Moibra: *Moina brachiata*; Neoall: *Neolovenula alluaudi*



used. Most studies of restoration of zooplankton have focused only on species richness (Gray & Arnott, 2009), but the inclusion of diversity metrics, as in the present study, can give different and complementary results. This is illustrated by rotifers in new ponds, which had lower richness per pond in the second year, but higher Simpson and beta diversity. In addition to this recovery of rotifer diversity, the total number of rotifer taxa found in all new ponds combined increased in the second year.

Changes between years in number of individuals of each group, and number of ponds dominated by each, indicated that rotifers and copepods colonized the new ponds faster than cladocerans (see also Frisch & Green, 2007). The colonization rates of these three groups in our new ponds will be compared in more detail elsewhere (Frisch et al., in preparation). Cladocerans also took the most time to colonize much smaller experimental ponds in Virginia (Jenkins & Buikema, 1998) and have often been the slowest group to recover in acidified lakes after pH has been restored (Yan et al., 2004, Gray & Arnott, 2009, but see Knapp & Sarnelle, 2008).

Higher community differentiation or β -diversity was recorded in new ponds in the second year. The

lower β -diversity found in 2006 may have been facilitated by a lack of environmental heterogeneity when the newly excavated ponds were first filled, and by the weakness of local regulatory processes (e.g. competition or predation) in the first stages of community assembly. Only a few species were successful in establishing widespread, large and dominant populations in 2006, constituting a “core-assembly” common to most of the ponds. The cyclopoid copepod *Metacyclops minutus*, the rotifer *Hexarthra* cf. *fennica* and the cladoceran *Moina brachiata* were prominent pioneers, and only the latter approached similar levels of abundance in reference sites and increased in abundance in 2007 (Table 3). *M. minutus* is able to recolonize temporary ponds within hours of inundation from diapausing stages in the sediment, and it was dominant in ephemeral rainpools formed in the new ponds in 2005 (Frisch & Green, 2007). Owing to its great abundance in 2006, the striking decline in the *Hexarthra* populations observed in 2007 had a major influence on the diversity patterns of the whole zooplankton community.

In well-established zooplankton communities, variation in predation intensity and habitat diversity have

Table 3 List of taxa found in new ponds ($N = 47$) and reference sites ($N = 8$ in 2006, $N = 6$ in 2007)

	New ponds						Reference sites					
	April 2006			April 2007			April 2006			April 2007		
	O	D	Total abund.	O	D	Total abund.	O	D	Total abund.	O	D	Total abund.
COPEPODS			11281			5508			1453			226
Juvenile stages												
<i>Cyclopoida nauplii</i>	47	13	10233	33	14	2252	8	2	652	3	0	101
<i>Cyclopoida copepodids</i>	47	1	264	34	0	80	8	0	93	5	0	9
<i>Calanoida nauplii</i>	38	1	275	45	10	1954	7	0	58	5	2	70
<i>Calanoida copepodids</i>	33	0	247	36	5	501	7	0	154	5	0	15
<i>Harpacticoida copepodids</i>	4	0	4	–	–	–	–	–	–	–	–	–
<i>Acanthocyclops cf. robustus</i>	–	–	–	11	0	1	1	0	0.05	3	0	3
<i>Metacyclops minutus</i>	46	0	148	32	0	35	8	0	62	2	0	0.10
<i>Metacyclops planus</i>	1	0	0.05	14	0	4	2	0	0.35	4	0	1
<i>Arctodiaptomus wierzejski</i>	31	0	77	25	0	87	6	1	348	1	0	1
<i>Copidodiaptomus numidicus</i>	–	–	–	–	–	–	1	0	0.05	–	–	–
<i>Mixodiaptomus incrassatus</i>	22	0	26	5	0	1	5	0	8	–	–	–
<i>Neolovenula alluaudi</i>	2	0	1	40	3	594	3	0	76	5	1	26
<i>Nitocra lacustris</i>	–	–	–	–	–	–	–	–	–	1	0	0.15
<i>Harpacticoida undet.</i>	5	0	6	–	–	–	–	–	–	–	–	–
<i>Harpacticoida undet.2</i>	–	–	–	–	–	–	1	0	0.20	–	–	–
CLADOCERANS			489			1143			1761			60
<i>Daphnia juveniles</i>	5	0	1	8	0	2	–	–	–	1	0	3
<i>Alona azorica</i>	2	0	0.20	21	0	18	1	0	1	2	0	1
<i>Alona rectangula</i>	20	0	3	30	0	38	6	0	8	3	0	4
<i>Bosmina longirostris</i>	–	–	–	–	–	–	2	0	0.20	–	–	–
<i>Ceriodaphnia quadrangula</i>	12	0	122	10	0	8	6	0	43	–	–	–
<i>Daphnia atkinsoni</i>	2	0	0.10	–	–	–	–	–	–	–	–	–
<i>Daphnia magna</i>	14	0	5	30	2	175	6	1	360	3	1	30
<i>Daphnia cf. similis</i>	1	0	0.05	–	–	–	–	–	–	2	0	11
<i>Dunhevedia crassa</i>	1	0	0.05	3	0	1	–	–	–	2	0	1
<i>Macrothrix hirsuticornis</i>	11	0	31	7	0	7	2	0	11	2	0	0.30
<i>Megafanestra aurita</i>	–	–	–	–	–	–	–	–	–	1	0	0.25
<i>Moina brachiata</i>	47	0	328	36	12	887	7	4	1337	4	0	5
<i>Oxyurella tenuicaudis</i>	–	–	–	1	0	0.05	–	–	–	1	0	0.05
<i>Pleuroxus letourneuxi</i>	1	0	0.05	4	0	2	2	0	0.25	–	–	–
<i>Scapholeberis ramneri</i>	1	0	0.05	1	0	0.05	–	–	–	1	0	2
<i>Simocephalus exspinosus</i>	–	–	–	5	0	4	1	0	0.10	3	0	2
ROTIFERS			21558			1041			872			463
<i>Ascomorpha</i> sp.	–	–	–	1	0	0.15	–	–	–	–	–	–
<i>Aplanchna</i> sp.	–	–	–	2	0	0.40	–	–	–	–	–	–
<i>Belloidea</i>	–	–	–	1	0	0.05	–	–	–	2	0	42
<i>Brachionus angularis</i>	–	–	–	–	–	–	2	0	100	–	–	–
<i>Brachionus plicatilis</i>	20	2	1277	2	0	4	1	0	1	1	1	254
<i>Brachionus quadridentatus</i>	30	0	169	14	0	9	6	0	5	3	0	1
<i>Brachionus urceolaris</i>	1	0	0.05	–	–	–	2	0	2	–	–	–

Table 3 continued

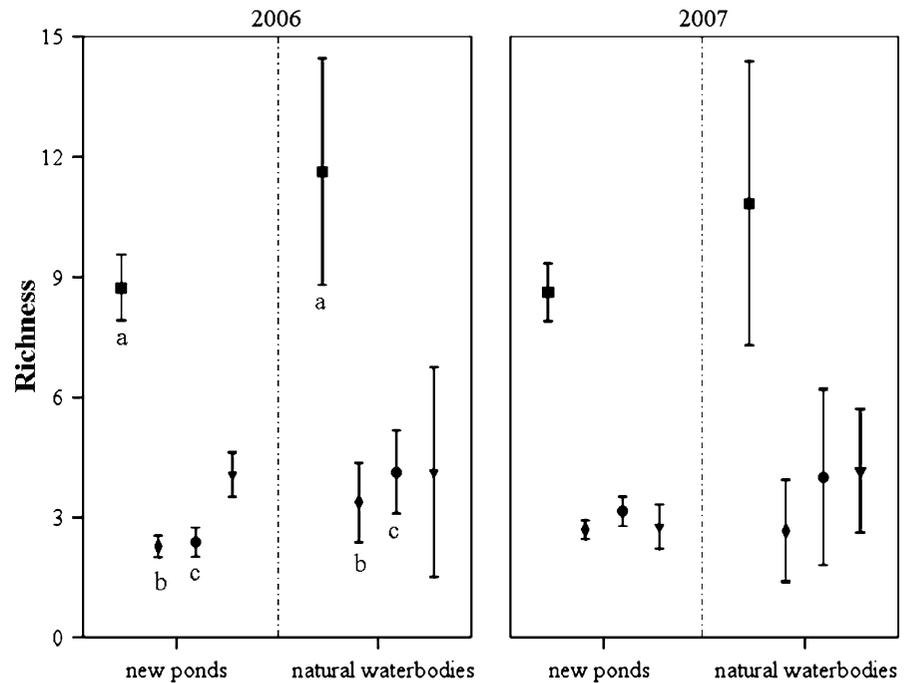
	New ponds						Reference sites					
	April 2006			April 2007			April 2006			April 2007		
	O	D	Total abund.	O	D	Total abund.	O	D	Total abund.	O	D	Total abund.
<i>Brachionus variabilis</i>	8	0	6	8	0	64	4	0	376	3	0	6
<i>Cephalodella gibba</i>	12	0	9	10	0	3	3	0	0.50	2	0	2
<i>Cephalodella</i> sp.	1	0	3	5	0	3	–	–	–	–	–	–
<i>Colurella</i> sp.	–	–	–	2	0	0.10	–	–	–	1	0	0.20
<i>Colurella uncinata</i>	–	–	–	2	0	0.10	–	–	–	–	–	–
<i>Filina longiseta</i>	–	–	–	–	–	–	1	0	330	–	–	–
<i>Gastropus hyptopus</i>	1	0	0.05	2	0	2	2	0	1	–	–	–
<i>Epiphanes</i> sp.	–	–	–	–	–	–	1	0	2	–	–	–
<i>Euchlanis</i> sp.	4	0	2	–	–	–	–	–	–	–	–	–
<i>Hexarthra</i> cf. <i>fennica</i>	46	30	19972	15	0	336	3	0	10	–	–	–
<i>Keratella quadrata</i>	1	0	8	1	0	0.20	2	0	1	–	–	–
<i>Keratella tropica</i>	9	0	12	3	0	342	1	0	2	–	–	–
<i>Lecane bulla</i>	–	–	–	1	0	0.05	–	–	–	–	–	–
<i>Lecane luna</i>	20	0	33	31	0	203	3	0	46	6	1	138
<i>Lecane lunaris</i>	–	–	–	3	0	0.50	–	–	–	1	0	0.10
<i>Lecane</i> sp.	18	0	9	–	–	–	1	0	0.05	1	0	10
<i>Lepadella patella</i>	1	0	0.05	1	0	0.05	–	–	–	–	–	–
<i>Notholca acuminata</i>	2	0	0.10	–	–	–	–	–	–	–	–	–
<i>Polyarthra</i> sp.	–	–	–	–	–	–	–	–	–	1	0	6
<i>Proales</i> sp.1	2	0	0.10	10	0	5	1	0	0.15	1	0	0.25
<i>Proales</i> sp.2	4	0	55	7	1	68	–	–	–	2	0	1
<i>Testudinella patina</i>	9	0	2	9	0	3	–	–	–	1	0	3
<i>Trichocerca</i> sp.	2	0	0.50	–	–	–	–	–	–	–	–	–

For each taxon the frequency of occurrence (O, number of ponds where a given taxa was found), and dominance (D, number of ponds where a given taxa was more abundant than any other), and the total abundance (sum of number of individuals/l for all ponds) are listed

been shown to produce strong differences in community composition, even in the presence of hydrological connections (Cottenie et al., 2001). New Caracoles ponds were already variable in their habitat characteristics by 2007 (Table 1). Diversification of aquatic vegetation and associated macroinvertebrate communities is likely to increase this variation in the future. Predation and competition are likely to have important effects on the zooplankton community composition in new ponds. It is possible that competition with species that established populations early on delayed colonization by other species present in nearby source populations, just as acid-tolerant species established in acidified lakes delay the recovery of acid-sensitive species after restoration

of normal pH (Binks et al., 2005; Frost et al., 2006). However, little is known about the interactions between the species recorded in this study. Although fish were absent in new ponds, macroinvertebrate predators such as Coleoptera and Hemiptera may have been important, although they have not been studied in detail to date (but see Rodriguez-Perez et al., 2009 for details of Corixidae). *Hexarthra* spp. populations can be partly controlled by predation by other zooplankters (Herzig & Koste, 1989; Starkweather, 2005), and the initial boom in abundance of *Hexarthra* in 2006 may have been associated with the low initial abundance and later arrival of cladocerans and calanoids. Although *Daphnia* are known to have a negative effect on rotifers, at least in lake systems

Fig. 5 For each year and class of waterbodies: mean values of richness for the whole zooplankton community (*square*) and the copepod (*rhombus*), cladoceran (*circle*) and rotifer (*triangle*) assemblages, separately. Error bars show 95% confidence intervals. Values sharing the *same letter* were significantly different. No significant differences were found in 2007 between new ponds and reference sites



(Fussmann, 1996), we found no correlation between ponds in the abundance of *Daphnia* and of rotifers (results not shown).

The higher cover of macrophytes in new ponds in 2007 is likely to have had an important influence on the zooplankton communities, and may have enhanced the relative abundance of large-bodied (e.g. *Daphnia*), and also vegetation-associated (e.g. *Alona*) cladocerans at the cost of rotifers and cyclopoids. Zooplankton species richness is generally positively related to the cover of submerged vegetation (Romo et al., 2004; Declerck et al., 2005). Cladocerans are associated with clear, macrophyte dominated shallow ponds, whereas rotifers and copepods often dominate in turbid, plant-free waters (Burks et al., 2006 and references therein). The higher pH in 2007 is also likely to be due to greater macrophyte biomass that year (Carpenter & Lodge, 1986).

The taxon richness of rotifers and copepods is negatively correlated with total phosphorus in lakes in southern Spain (Declerck et al., 2005), and phosphorus availability can influence zooplankton biomass (Conde-Porcuna et al., 2002). However, indirect effects owing to the influence of phosphorus concentration on submerged macrophytes may have more consequences for zooplankton diversity (Declerck et al., 2007). Although reference sites were

generally more eutrophic than new Caracoles ponds, this was not associated with a shift towards higher abundance of rotifers and cyclopoid copepods and lower abundance of large cladocerans in the more eutrophic and turbid sites (i.e. in reference sites), as might be expected when comparing ponds of similar age (Cottenie et al., 2001).

In Doñana as a whole, 41 copepod taxa, 48 cladocerans and 80 rotifers have been recorded to date (Arechederra et al., 2006; Fahd et al., 2009), and only 25% of these taxa were recorded in the new ponds. A similar percentage was found for the reference sites. All the taxa recorded in this study were already cited in these earlier studies. The vast majority of copepod and cladoceran taxa found in the new ponds inhabit a wide range of temporary habitats in Doñana, but tend to be more frequent in the marshland than in dune ponds (Fahd et al., 2009). The relatively high salinity in our study area is likely to limit the number of zooplankton species able to colonize (Schell et al., 2001; Frisch et al., 2006).

Despite the low density of any remaining egg bank after pond excavation, dispersal limitation is unlikely to have been a major factor for the new Doñana ponds, owing to their proximity to a large surface area of natural wetlands. Wind dispersal from nearby sources is likely to have been especially important for

smaller propagules such as rotifer eggs (Vanschoenwinkel et al., 2008). Waterbirds are likely to have been important vectors, especially for cladocerans (Frisch et al., 2007, Brochet et al., 2010), and 40 bird species were recorded in the new ponds during the study period (A.J. Green unpublished data). Within the major blocks of new ponds (Fig. 1), hydrological connections at peak flooding are likely to have facilitated the arrival of new species to some ponds, although others in the same blocks remained unconnected.

After the first year, no subsequent increases in local species richness or beta diversity were detected for cladocerans in new ponds with a permanent hydroperiod in Belgium (Louette et al., 2008). This contrasts with our own results, but this may be explained by the fact that our first sampling was carried out 4 months after new ponds were first filled, whereas the “first year” in the Belgian study including samples up to 15 months after pond filling. Indeed, a strong increase in cladoceran species richness was recorded between months 4 and 14 in the Belgian ponds. An average of 4.2 cladoceran species colonized within 15 months (Louette & De Meester, 2005).

These findings are consistent with our own in suggesting that restoration of zooplankton communities can be achieved in ponds in a short time period, well below the 10–20 years generally considered as necessary in lakes (Donald et al., 2001, Dodson et al., 2007). This is likely to be largely a consequence of the difference in volume of these ecosystems, resulting in time lags and allee effects that can inhibit the recovery of zooplankton in lakes (Knapp & Sarnelle, 2008). In Wisconsin wetlands varying in size from 0.4 to 4.5 ha (i.e. similar to our new ponds of 0.2–2.9 ha), agricultural use lowered the number of species of crustacean zooplankton present (Dodson & Lillie, 2001), but 6 years after restoration their zooplankton communities closely resembled those of reference sites.

Our study illustrates the value of zooplankton as indicators of successful restoration of aquatic communities in Mediterranean wetlands, and underlines the value of wetland restoration to mitigate for habitat loss in the Mediterranean region. More research is required in future to establish the long-term changes in zooplankton in the new ponds and how they respond to future development of vegetation and

changes in nutrient loading, salinity, etc., as well as to the annual variation in precipitation and expected climate change (Moss et al., 2009).

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