

1 **Relationships between hydroperiod length, and seasonal and spatial patterns of**
2 **beta-diversity of the microcrustacean assemblages in Mediterranean ponds**

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21 intra-annual variation, seasonal variation

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26 **Abstract**

27 Temporary ponds are unpredictable habitats that exhibit inundation periods of variable
28 duration (hydroperiods). We hypothesised that changes in microcrustacean assemblages,
29 namely among ponds (spatial variation) and within ponds at a monthly scale (intra-
30 annual variation), would differ according to hydroperiod length. We found that the
31 microcrustacean assemblages of the study ponds demonstrated distinctive individual
32 patterns that were dependent on monthly variation. During pond filling, species turnover
33 was particularly high in short-hydroperiod ponds, probably due to the fast hatching of
34 the initial pool of species, which seemed to determine subsequent species succession.
35 Some of the long-hydroperiod ponds were able to hold water during the summer, which
36 meant that they could harbour adult microcrustaceans in the early autumn, in contrast to
37 the recently filled ponds; species turnover could thus increase at the onset of the
38 inundation cycle. In the summer, species turnover decreased (while the contribution of
39 nestedness to β -diversity increased) because only a few tolerant species were able to
40 withstand the poor water quality found in drying ponds. We suggest that preserving the
41 broader hydroperiod gradient, by protecting ponds with different environmental
42 conditions within each hydroperiod category, would safeguard the ecological dynamics
43 of the microcrustacean assemblages found in temporary ponds.

44

45 **Introduction**

46 The extent to which spatio-temporal heterogeneity influences diversity remains a
47 challenging problem in community ecology (Jurasinski et al., 2009; Anderson & Walsh,
48 2013). Species diversity comprises three components: α -, β -, and Υ -diversity. Local
49 and regional species richness determine α - and Υ -diversity, respectively, while variation
50 in species composition among sites within a geographical area often defines β -diversity
51 (Legendre et al., 2005; but see e.g., Tuomisto, 2010; Anderson et al., 2011). However,
52 β -diversity can also express temporal variation in species assemblages (Chalcraft et al.,
53 2004; La Sorte & Boecklen, 2005; Korhonen et al., 2010). Both spatial and temporal
54 variation in species turnover (or species replacement) and the nestedness components of
55 β -diversity can be assessed with the additive process of β -diversity partitioning
56 (Baselga, 2010). Nestedness is a type of biodiversity pattern; it occurs when species-
57 poor areas contain species-rich subareas, and the degree of nestedness describes the
58 species shared between high- and low- diversity areas (Patterson, 1990; Atmar &
59 Patterson, 1993; Ulrich et al., 2009). Environmental heterogeneity and species dispersal
60 limitations are considered to be the main factors that affect species biodiversity at local
61 and regional spatial scales, thus favouring β -diversity (Anderson et al., 2006; Verleyen
62 et al., 2009; Steiner, 2014). Consequently, temporal variation in biodiversity can be
63 expected to track seasonal changes in species environmental conditions and life cycles.
64 Temporal variation in β -diversity can also be estimated at both short time scales (intra-
65 annual, e.g., Siokou-Frangou, 1996; Tackx et al., 2004) and long time scales
66 (interannual, e.g., Romo et al., 2005; Fahd et al., 2009).
67 Invertebrate and plankton communities have often been used to explore the multiple
68 interlinked factors that drive diversity across different spatial scales, including
69 connectivity, disturbance (droughts), and dispersal (Pedruski & Arnott, 2011; Lopes et

70 al., 2014; Vanschoenwinkel et al., 2011); nutrient enrichment and predation (Pinel-
71 Alloul & Mimouni, 2013; Beisner & Peres-Neto, 2009); and, more generally, stochastic
72 vs. deterministic assembly processes (Freestone & Inouye, 2015). Lakes and wetlands
73 have been the classical settings for studies of invertebrate community structure (Brooks
74 & Dodson, 1965; Wellborn et al., 1996). Temporary water bodies, in particular, offer
75 clear environmental gradients that can be used to examine the combined effect of
76 predation and life-history adaptations on diversity. Temporary ponds experience
77 recurrent phases of desiccation of unpredictable duration (Williams, 1997). The
78 microcrustacean species found in temporary ponds have consequently adjusted their life
79 cycles to cope with the unpredictability of their habitats (Williams, 2006). For instance,
80 they produce an egg bank of propagules that remains in the sediment, a strategy to
81 guarantee long-term species persistence even as the environment changes (Hairston,
82 1996). As ponds dry up, the deterioration of environmental conditions (e.g.,
83 deoxygenation, high temperatures, drastic reduction in the size of the pond surface, high
84 concentration of nutrients and solutes) and the strong pressure exerted by biotic
85 interactions may cause invertebrate species to produce drought-resistant forms that can
86 survive desiccation (Florencio et al., 2013). In Doñana National Park (SW Spain), the
87 timing of the onset of the wet period is highly variable, and the duration of the
88 inundation period (hydroperiod) also varies widely among ponds (Diaz-Paniagua et al.,
89 2010). In previous studies, Doñana's temporary ponds have been found to harbour rich
90 and dynamic zooplankton communities (Fahd et al., 2000) that are structured by
91 episodic predation (Fahd et al., 2007). Although some differences in species
92 composition have been observed along the hydroperiod gradient in long-term studies
93 (Serrano & Fahd, 2005; Fahd et al., 2009), no clear seasonal patterns have been found
94 so far. However, small organisms living in highly unpredictable habitats are expected to

95 have high turnover rates, according to a multi-taxon study performed across different
96 aquatic systems and biogeographical areas (Korhonen et al., 2010). Therefore, the
97 microcrustacean assemblages found in Doñana's temporary ponds are an excellent
98 model with which to analyse spatial and temporal patterns of species turnover,
99 especially when fine-scale monthly sampling is carried out across a wide hydroperiod
100 gradient. We analysed the contributions of species turnover and nestedness to β -
101 diversity among ponds along the hydroperiod gradient (spatial scale) and across months
102 (temporal scale). We hypothesised that intra-annual variation in microcrustacean β -
103 diversity would be high at both spatial and temporal scales.

104

105 **Methods**

106 *Study site*

107 The study was carried out in 19 ponds located within the Doñana Biological Reserve
108 (Doñana National Park, SW Spain). Doñana National Park is one of the most pristine
109 wetlands in Europe; it was included in the RAMSAR convention in 1982 and later
110 designated as a World Heritage Site by UNESCO, in 1995. The study site was located
111 in a coastal dune system, where several dune generations have been deposited over a
112 large sedimentary aquifer system (ca. 3400 km² in surface area) located along the
113 Atlantic coast by the mouth of the Guadalquivir River. The area has a Mediterranean
114 subhumid climate, which is characterised by hot and dry summers, mild winters, and
115 rainfall that falls mainly in the autumn and winter. The park is divided up into a marsh
116 and a sandy area, where more than 3000 temporary ponds may be formed following
117 heavy autumn or winter rainfall during wet years, while these ponds usually dry up in
118 summer (except for a couple of ponds that retain water during normal summers). These
119 ponds are mainly fed by freshwater (rainfall, runoff, and a shallow water table) and have

120 no surface or groundwater connection to the sea, although they can receive salts of
121 marine origin through airborne deposition (Serrano et al., 2006). The length of the
122 inundation period, or hydroperiod, is highly variable and depends on the timing of the
123 start of the drying phase for each pond. The most ephemeral ponds usually dry up in the
124 spring; in contrast, the ponds with the longest hydroperiods may hold water until the
125 end of the summer during years with normal rainfall and may even avoid desiccation
126 during the summers of very wet years (Díaz-Paniagua et al., 2010).

127

128 *Sampling procedure*

129 Our study, which took place from November 2006 to September 2007, covered a
130 complete annual cycle—from the inundation to the desiccation of most of the temporary
131 ponds in the study area. Annual rainfall during the study was 716.9 mm, which is above
132 average for the area (mean = 544.6 mm; see Diaz-Paniagua et al., 2010). In years of
133 high rainfall, some long-hydroperiod ponds do not dry out, as was the case for two of
134 our study ponds. The 19 temporary ponds selected for this study spanned a wide
135 hydroperiod gradient and were grouped into three categories: short- (<7 months),
136 intermediate- (7–7.5 months), and long-hydroperiod ponds (>7.5 months) (for further
137 details on the hydroperiods of these ponds, see Florencio et al., 2009). The number of
138 ponds in each category that were sampled changed over the course of the study because
139 of variation in pond inundation related to: i) the onset of inundation (i.e. only three of
140 the seven short-hydroperiod ponds and six of the seven intermediate-hydroperiod ponds
141 were inundated in November 2006); ii) the early desiccation of two short-hydroperiod
142 ponds in January 2007 that were, later, re-inundated in February; and iii) the start of the
143 drying phase of the ponds. With regards to the latter, pond desiccation was a gradual
144 process that started with the desiccation of the short-hydroperiod ponds in May. The

145 intermediate-hydroperiod ponds dried out in June, and some water persisted in two of
146 the long-hydroperiod ponds up until when we stopped sampling, in September.
147 Microcrustacean assemblages (i.e. planktonic copepods and cladocerans) were sampled
148 using a nylon filter net (100- μ m mesh size). We filtered a known volume of water,
149 which ranged from 5 to 21 litres per pond, depending on pond size (more water was
150 sampled from larger ponds) and water turbidity (smaller volumes were sampled if the
151 filter clogged more quickly). We sampled the water column of each pond along a
152 transect, which ran from the littoral zone to the open water: a 1-l sample jar was lifted
153 from the pond's bottom to its surface. Ponds could not be sampled when the water
154 column was < 15 cm, which occurred in one pond in February, March, and April. To
155 ensure that we had a complete inventory of the crustaceans present, including the large
156 branchiopods (i.e. Anostraca, Notostraca and Spinicaudata), we collected additional
157 samples using a protocol that targets macroinvertebrates. With a dip net (1-mm mesh
158 size), we netted a stretch of water of about 1.5 m in length. For each pond, samples were
159 taken at several different points along one or two transects spanning from the littoral
160 zone to the open water; the number of sampling points was proportional to pond size
161 (see Florencio et al., 2009 for more details). From these additional dip-net samples of
162 large branchiopods, we only examined incidence per pond and month; these data were
163 included in presence-absence matrices. For simplicity's sake, we use here the term
164 "microcrustaceans" to refer to all cladocerans, copepods, and large branchiopods.
165 All the samples were preserved in 70% ethanol and identified in the laboratory. The
166 different morphological species were separated out under a stereoscopic microscope,
167 and the specimens were placed on a glass slide so they could be identified to the species
168 level using an optical microscope. Taxonomic identification was performed using
169 Alonso (1996) for the cladocerans and large branchiopods and Dussart (1967; 1969) for

170 the copepods. In the case of *Triops baeticus*, we followed the nomenclature proposed in
171 Korn et al. (2010). Then, the abundance of each species was determined using an
172 inverted microscope. Juveniles were separated in copepod nauplii, immature
173 diaptomids, immature cyclopoids, immature harpacticoids, immature cladocerans, and
174 large branchiopod larval stages. These juvenile stages were only used in the
175 PERMANOVA analysis, which utilised averaged abundances (see below). They were
176 excluded from the β -diversity analyses.

177 We averaged the number of individuals per litre and per pond and then constructed a
178 matrix including these standardised values per species (columns) for the whole ponds
179 and the full months (rows). These averaged abundance data were transformed in a
180 species presence-absence matrix that was used in the analyses of β -diversity, which
181 included the supplementary data on the large branchiopods. From these presence-
182 absence matrices, we extracted two further types of matrices: i) per-month matrices that
183 included all the ponds sampled for a given month and ii) per-pond matrices that
184 included all the months that a given pond was sampled.

185

186 *Data analyses*

187 *Variation across ponds and months*

188 We built triangular similarity matrices using the inverse of the Bray–Curtis index of
189 dissimilarity on the matrix of the averaged abundances of the microcrustaceans.

190 Differences in microcrustacean assemblage similarity across ponds and months, as well
191 as the pond-by-month interaction, were analysed using a two-way crossed permutational
192 multivariate analysis of variance (PERMANOVA; Anderson, 2001) on the matrix of
193 averaged abundances. This non-parametric procedure allowed us to analyse differences
194 between two or more groups or factors based on any distance or similarity measure.

195 Statistically significant differences were tested using permutations of group
196 membership; we performed 9999 replicates using the software PRIMER v.6 (Anderson
197 et al., 2008). In these analyses, we excluded a short-hydroperiod pond in November and
198 two long-hydroperiod ponds, one in June and the other in July, because they contained
199 no crustaceans. To analyse the effect of hydroperiod type on microcrustacean richness,
200 we performed a nested ANOVA (Statistica v.8) on total richness per pond and month;
201 hydroperiod (three levels) was nested within month (eleven levels) to avoid monthly
202 variation when assessing hydroperiod differences. Separate nested ANOVAs were
203 performed for cladoceran, copepod, and large branchiopod species richness; within the
204 copepods, separate tests were performed for cyclopoid, diaptomid, and harpacticoid
205 richness. We also performed a Tukey HSD post-hoc test between all the combinations
206 of hydroperiod types and months (Online Resource). The unequal levels of the different
207 factors meant that we could not look at the interaction between hydroperiod type and
208 month—there were unequal levels because of the gradual desiccation of the ponds.

209

210 *Changes in β -diversity across ponds and months*

211 We built triangular similarity matrices using the Sørensen index and the presence-
212 absence data. To assess spatial differences between ponds, we constructed per-month
213 triangular matrices, which comprised the dissimilarity values for all the pond pairs for a
214 given month. To assess temporal differences, we constructed one matrix per pond and
215 dissimilarity values were calculated between pairs of successive months.

216 We used the β -diversity partitioning approach proposed by Baselga (2010): i) per-
217 month spatial dissimilarities between ponds were calculated—the three hydroperiod
218 types were considered separately and ii) per-pond temporal dissimilarities were
219 calculated between pairs of successive months.

220 To partition β -diversity, we used two approaches that took into account the fact that the
221 number of ponds sampled varied across months within each hydroperiod type. First, to
222 determine spatial β -diversity partitioning and obtain comparable values of β -diversity
223 for different matrix sizes (which included different numbers of ponds), we used
224 multiple-site dissimilarity patterns. Second, to determine β -diversity partitioning for
225 each pond between pairs of successive months, we used a pairwise dissimilarity
226 procedure (Baselga, 2012; 2013). To calculate the multiple-site β -diversity partitioning
227 using different matrix sizes, we used the minimum number of ponds sampled in a month
228 (three) to resample the total number of ponds sampled per month (1000 random
229 samples). To determine multiple-site and pairwise β -diversity partitioning, we used the
230 functions “beta.sample” and “betapair”, respectively (betapart package, Baselga &
231 Orme, 2012), which were implemented in R software 2.14.2 (R Development Core
232 Team, 2012). Both multiple-site and pairwise β -diversity (β_{sor}) were partitioned into two
233 additive components that accounted for dissimilarity due to species replacement (β_{sim})
234 and dissimilarity due to nestedness (β_{sne}), respectively, in accordance with the formula
235 $\beta_{\text{sor}} = \beta_{\text{sim}} + \beta_{\text{sne}}$ (Baselga, 2010). To distinguish between the measures of β -diversity
236 derived from the multiple-site or pairwise calculations of dissimilarity, we hereafter
237 refer to the multiple-site dissimilarities using capital letters (i.e. β_{SOR} , β_{SIM} and β_{SNE}) and
238 the pairwise similarities using lowercase letters (i.e. β_{sor} , β_{sim} and β_{sne}) (see Baselga,
239 2012). Because juvenile stages were not included in these analyses, we could not
240 include July, August, and September in our comparisons because adult individuals were
241 absent from most ponds during those months.

242

243 **Results**

244 We collected 54 species, including 33 cladocerans, 14 copepods, and 7 large

245 branchiopods (Table 1). *Chydorus sphaericus* was present in all the ponds. Thirty
246 additional species (19 cladocerans, 10 copepods, and the notostracan *Triops baeticus*)
247 were found across the entire hydroperiod gradient, and 11 species (5 cladocerans, 2
248 copepods, and 4 large branchiopods) were found in two out of the three hydroperiod
249 categories. Twelve species were represented by just a few individuals, which occurred
250 in a few ponds of one hydroperiod type. One exception was *Daphnia magna*, which was
251 quite abundant even though it was present only in long-hydroperiod ponds (Table 1,
252 Online Resource ESM 1). Only seven species of cladocerans and five species of
253 copepods reached high levels of abundance (average per pond and month >10 ind L⁻¹;
254 Table 1, Online Resource ESM 1). At the beginning of the inundation phase, in
255 November, juvenile microcrustaceans were abundant in all the inundated ponds, while
256 numbers of adults were low; one exception was a long hydroperiod pond in which
257 adults were quite abundant (Fig. 1).

258 The nested ANOVA analyses revealed that species richness per pond and month was
259 significantly greater in intermediate-hydroperiod ponds than in short- and long-
260 hydroperiod ponds in the case of copepods, large branchiopods, cyclopoids, and
261 harpacticoids (Table 2). Increased richness was often observed at the beginning of the
262 pond inundation phase—around February–April in long-hydroperiod ponds, in May for
263 intermediate-hydroperiod ponds, and in March for short-hydroperiod ponds. Total
264 richness was considerably higher than average pond richness, which indicates that
265 different ponds in the same hydroperiod category harboured different microcrustacean
266 species (Fig. 1).

267

268 *Species patterns across ponds and months*

269 Two species had very wide temporal ranges—they were present from November to June

270 (*Chydorus sphaericus*) in short- and intermediate-hydroperiod ponds and from
271 November to July (*Copidodiaptomus numidicus*) in long-hydroperiod ponds. Of the 21
272 species observed following pond inundation in November, only *Diacyclops*
273 *bicuspidatus*, *Ceriodaphnia quadrangula*, *C. laticaudata*, and *Canthocamptus*
274 *staphylinus* were found in more than three ponds (Table 1). Some “early” species only
275 occurred in the first few months after pond inundation (i.e. *Metacyclops minutus*,
276 *Daphnia magna*, and *Moina brachiata*). From January to April, the microcrustacean
277 assemblages added an additional 12 species. In the summer, nine species persisted at
278 low densities in the ponds that remained flooded, while in September, the only two
279 species present were extremely abundant (i.e. *Acanthocyclops americanus* and *Moina*
280 *micrura*, Table 1, Online Resource ESM 1).

281

282 *Variation across ponds and months*

283 We detected significant effects of both spatial (ponds, explained variance = 25%, $P <$
284 0.0001) and temporal (months, explained variance = 22%, $P <$ 0.0001) factors on the
285 microcrustacean assemblages, as well as a significant interaction between them. By
286 itself, the interaction explained 53% of the variance ($P <$ 0.0001), which revealed that
287 pond assemblage composition varied across months. Notably, less than 1% of the
288 variance remained unexplained.

289

290 *Changes in β -diversity across ponds*

291 We observed high levels of species replacement (β_{SIM}) among ponds in the months
292 immediately after pond inundation; then, there was a gradual reduction in β_{SIM} until it
293 reached its minimum values in May–June (Fig. 2). Accordingly, the dissimilarity due to
294 nestedness (β_{NES}) remained low during the entire annual cycle, except in May and June

295 (Fig. 2). When we analysed these spatial differences separately for each hydroperiod
296 type, we observed that short-hydroperiod ponds reached their highest β_{SIM} immediately
297 after inundation, while β_{SNE} was highest in the long-hydroperiod ponds in the summer,
298 when the other ponds had already dried out (Fig. 3). Short-hydroperiod ponds attained
299 their minimum β_{SIM} values in January, when the ponds had low water levels and some
300 nearly became desiccated. Intermediate-hydroperiod ponds had more stable values of
301 β_{SIM} and β_{SNE} across the annual cycle. Long- and intermediate-hydroperiod ponds had
302 high levels of species replacement after pond inundation in November, while short-
303 hydroperiod ponds had high levels of replacement later on. This pattern contributed to
304 the generally high β_{SIM} at the beginning of the inundation period and high β_{SNE} just
305 before pond desiccation in long- and intermediate-hydroperiod ponds (Fig. 3).

306

307 *Monthly species turnover*

308 When microcrustacean β -diversity was calculated for each pond using pairs of
309 consecutive months, long-hydroperiod ponds had the highest β_{sim} (Fig. 4). Both long-
310 and short-hydroperiod ponds showed similar patterns for β_{sim} , which reached maximum
311 values after inundation (November–December). In contrast, intermediate-hydroperiod
312 ponds showed a different pattern. They attained maximum β_{sim} values in January–
313 March, while β_{sim} was low after pond inundation and when ponds were nearing
314 desiccation.

315 β_{sne} showed an opposite pattern to that of β_{sim} for intermediate-hydroperiod ponds: β_{sne}
316 peaked in November–December and April–May. In short-hydroperiod ponds, β_{sne} was
317 highest (ca. 0.3) during the months immediately following inundation (November–
318 January), while in long-hydroperiod ponds, β_{sne} was highest (ca. 0.15) in the summer
319 (May–June, June–July) (Fig. 4).

320

321 **Discussion**

322 To our knowledge, this is the first study about the temporal turnover of microcrustacean
323 assemblages in ponds across a hydroperiod gradient by assessing intra-annual variation
324 at a fine (monthly) scale. We found that microcrustacean assemblages differed greatly
325 across ponds and months. Notably, this pattern was largely driven by monthly variation
326 in species turnover that was specific to each hydroperiod type.

327 In a previous study, the microcrustacean assemblages of Doñana's ponds were found to
328 demonstrate strong interannual variation (Fahd et al., 2009). In our study, however, we
329 managed to collect a large percentage (66.7%) of Doñana's regional species pool (all
330 the species ever detected in the park) over the course of a single annual inundation-
331 desiccation cycle. This finding reveals that the microcrustacean assemblages of these
332 ponds vary greatly across months. We also collected two species not previously
333 observed in Doñana (*Alona iberica* and *Ephemeroporus phintonicus*), which brings the
334 park's total number of microcrustacean species to 80, when data from past studies are
335 included (Fahd et al., 2009). Among the microcrustacean species we collected were
336 some rare and relict species that are considered as "sentinels" in Mediterranean ponds:
337 they reflect the good conservation status of ponds (e.g., the copepod *Hemidiaptomus*
338 *roubau* and the large branchipods *Branchipus cortesi*, *Triops baeticus*, *Cyzicus grubei*,
339 and *Maghrebestheria maroccana*, which all have restricted distributions; see Sahuquillo
340 & Miracle, 2010; 2013). We discovered that most of the species tended to occur across
341 all three hydroperiod types. Some of these species are cosmopolitan, such as *Diacyclops*
342 *bicuspidatus*, *Megacyclops viridis*, and *Chydorus sphaericus* (Fahd et al., 2000; Serrano
343 & Fahd, 2005), and some have distributions in North Africa, such as *Diaptomus*
344 *kenitraensis* and *Copidodiaptomus numidicus* (Miracle, 1982). *Dussartius baeticus*, a

345 species endemic to the SW Iberian Peninsula, occurred across most sampling months
346 and hydroperiod types. *T. baeticus*, which is endemic to northwestern Africa and the
347 Ibero-Balearic region, was often found in all the pond types, except for in long-
348 hydroperiod ponds, which are thought to be out of their habitat suitability range.

349 The high level of microcrustacean species richness may result from high pond
350 heterogeneity providing a wide diversity of different habitat types, which has been
351 observed for macroinvertebrates in the same study ponds (Florencio et al., 2014). The
352 species richness of copepods and large branchiopods was highest in intermediate-
353 hydroperiod ponds, which harboured a large number of common species. In contrast,
354 the scarcest species in the study area tended to be found in short-hydroperiod ponds
355 (Fahd et al., 2000). For example, a previous study found that *Branchipus schaefferi* and
356 *C. grubei*, both uncommon species, only occurred in short-hydroperiod ponds exhibiting
357 waters with low conductivity ($< 200 \mu\text{S cm}^{-1}$) (see Florencio et al., 2013). Additionally,
358 the duration of inundation is less consistent in intermediate-hydroperiod ponds than in
359 long-hydroperiod ponds, which could favour the occurrence of a greater number of
360 species, in accordance with the intermediate disturbance hypothesis (see Lopes et al.,
361 2014).

362 Sahuquillo & Miracle (2010) defined three phases of inundation, which each influence
363 the seasonal variation in microcrustacean assemblages; they are the pond filling phase,
364 the intermediate phase, and the drying phase. In this study, we indeed observed that
365 different species occurred during these three different phases. During the pond filling
366 phase, “early” species appeared after the initial inundation of the ponds and even
367 persisted well into the intermediate phase; these species included diaptomids such as
368 *Arctodiaptomus wierzejskii* (occurred exclusively in long-hydroperiod ponds) and *H.*
369 *roubau*; large branchiopods such as *B. cortesi*, *Tanymastix stagnalis*, *M. maroccana*,

370 and *Branchipus schaefferi* (mainly occurred in short-hydroperiod ponds); copepods
371 such as the cyclopoid *Metacyclops minutus* (only occurred during the filling phase); and
372 cladocerans, such as *Daphnia hispanica*, *Ceriodaphnia laticaudata*, and *Macrothrix*
373 *rosea*. Most microcrustaceans and, especially cladocerans, were observed during the
374 intermediate phase (see Sahuquillo & Miracle, 2010). However, only *Ephemeroporus*
375 *margalefi* was abundant—in nine ponds—during this phase; it reached a density of 25
376 ind L⁻¹ in one pond in April and a density of 12 ind L⁻¹ in another pond in May, which
377 suggests that this species may thrive better later in the inundation-desiccation cycle.
378 Only the most opportunistic and tolerant species are able to make it through the
379 unfavourable conditions of the driest and warmest months of summer (Sahuquillo &
380 Miracle, 2010). In July–September, most of the ponds in our study area had dried out,
381 except some long-hydroperiod ponds, where a few species were found: *Alona*
382 *rectangular*, *Ceriodaphnia reticulata*, *Moina micrura*, *Acanthocyclops americanus*, and
383 *Copidodiaptomus numidicus*.

384 Assemblage dissimilarities were particularly dramatic among ponds just after the initial
385 filling phase, probably because of the restrictive requirements governing the hatching of
386 microcrustacean species, e.g., water level, osmotic pressure, oxygen concentration,
387 turbidity, pH, salinity, temperature, and light in the water column (Brendonck, 1996;
388 Waterkeyn et al., 2009; 2010). In short-hydroperiod ponds, assemblage differences in β -
389 diversity were accentuated among ponds during the filling phase, most likely because
390 more ephemeral sites usually have faster rates of turnover (Korhonen et al., 2010). In
391 these ponds, different species may hatch just after pond filling, and the newly emerged
392 species could condition the sequence of changes in assemblage composition by
393 facilitating or inhibiting the establishment of other species (Lopes et al., 2014 and
394 references therein). For example, different biotic interactions, such as predation or

395 competitive exclusion, may occur later in the annual cycle, resulting in high levels of
396 species replacement between successive months (see Florencio et al., 2013). In our
397 study, only two long-hydroperiod ponds still held water in September; they harboured
398 adults of *Moina micrura* and *Acanthocyclops americanus*. We thus suggest that, in wet
399 years, adult microcrustaceans of various species can persist through the summer in long-
400 hydroperiod ponds that do not dry up; their presence could condition the initial species
401 assemblages of the following inundation cycle. Assemblage differences among ponds
402 still flooded in the summer and ponds that flooded in November may favour a high level
403 of species replacement in long-hydroperiod ponds during the filling phase.

404 At local spatial scales, dispersal limitations have been found to have a negligible effect
405 on β -diversity because small aquatic organisms adapted to the recurrent disturbance that
406 is desiccation have good dispersal abilities (Lopes et al., 2014). Doñana's temporary
407 ponds have been revealed to be a robust and interconnected network when it comes to
408 the dispersal of macroinvertebrates and their recolonisation of ponds (Florencio et al.,
409 2011). Therefore, we consider it improbable that, in our study, dispersal limitations
410 would be driving changes in microcrustacean assemblages.

411 Starting in May, there was an increase in the assemblage dissimilarity attributable to
412 nestedness and a consequent reduction in species turnover, both in long-hydroperiod
413 ponds and in intermediate- and long-hydroperiod ponds between successive months.

414 Most of the short- and intermediate-hydroperiod ponds dried up in May and June,
415 respectively. Predator number usually increases along the inundation-desiccation cycle,
416 with large numbers of predators expected to occur during the drying phase (Schneider &
417 Frost, 1996; Spencer et al., 1999; Bilton et al., 2001). As they are nearing desiccation,
418 ponds exhibit non-favourable conditions because their water quality is poorer (Florencio
419 et al., 2013). Microcrustaceans without active dispersal abilities are not able to escape

420 such suboptimal conditions and are thus highly sensitive to seasonal environmental
421 changes (Siokou-Frangou, 1996; Tackx et al., 2004; Waterkeyn et al., 2010). Therefore,
422 pond desiccation generates stress, which may operate through predation and/or the
423 deterioration of environmental conditions, and thus may ultimately drive changes in
424 microcrustacean assemblages. Such changes could lead to differences in richness among
425 ponds, but still allow those ponds to harbour subsets of similar species (i.e. nestedness).
426 In our study, we found that the microcrustacean species of temporary ponds construct
427 dynamic assemblages at spatial and temporal scales. Given the distinctiveness of the
428 assemblages found across ponds, we suggest that assemblage dynamics could be best
429 preserved by protecting a wider hydroperiod gradient that includes ponds with different
430 environmental conditions within each hydroperiod type.

431

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440

441

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621 **Figure legends**

622

623 Figure 1. Monthly variation in the average number of individual microcrustaceans
624 (abundance) per pond; adults and juveniles were considered separately. Monthly
625 variation in the average richness and total richness per pond is also indicated, as well as
626 variation in the accumulated richness for the whole sampling period. Data are grouped
627 by hydroperiod type (short: < 7 months; intermediate: 7–7.5 months; long: > 7.5
628 months).

629

630 Figure 2. Monthly variation in the components of microcrustacean β -diversity across
631 ponds. The dissimilarity due to species replacement (β_{SIM} , filled circles) and the
632 dissimilarity due to nestedness (β_{SNE} , open circles) are indicated (SD values are
633 provided in Online Resource ESM 3).

634

635 Figure 3. Monthly variation in the components of microcrustacean β -diversity among
636 ponds of the same hydroperiod type (short, intermediate, and long). The dissimilarity
637 due to species replacement (β_{SIM} , filled circles) and the dissimilarity due to nestedness
638 (β_{SNE} , open circles) are indicated for each hydroperiod type (SD values are provided in
639 Online Resource ESM 3).

640

641 Figure 4. Boxplot indicating the median, maximum, minimum, and upper and lower
642 quartiles of the microcrustacean β -diversity components (the dissimilarity due to species
643 replacement is β_{sim} and the dissimilarity due to nestedness is β_{sne}) calculated between
644 pairs of two successive months for each pond and grouped by hydroperiod type.

645

646 **Online Resource legends**

647 ESM 1. Average abundance of each species in ponds of each hydroperiod type (average
648 number of individuals for all ponds of each hydroperiod type and for each month). N
649 indicates the number of ponds within each hydroperiod type in which the species was
650 observed. These averaged data do not include the additional large branchiopods
651 (presence-absence data) captured using a protocol that targets macroinvertebrates (see
652 Methods).

653

654 ESM 2. *P*-values obtained from Tukey HSD post-hoc tests for all the pairwise species-
655 richness comparisons across the three hydroperiod types (short-, intermediate- (Int), and
656 long-hydroperiod ponds) for all the months of the study. The highest (and significant)
657 levels of richness were found in intermediate-hydroperiod ponds and were attributable
658 to cladoceran, copepod, and large branchiopod richness, as well as to cyclopoid,
659 diaptomid, and harpacticoid richness.

660

661 ESM 3. SD values for the multiple-site microcrustacean β -diversity components,
662 following the resampling procedure (1000 samples in which a subset of three ponds
663 from all the ponds sampled each month was randomly selected), for the ponds overall
664 and for the ponds of each hydroperiod type (short, intermediate, and long; _ indicates
665 that analyses were not performed; the dissimilarity due to species replacement is β_{SIM} ,
666 and the dissimilarity due to nestedness is β_{SNE}).

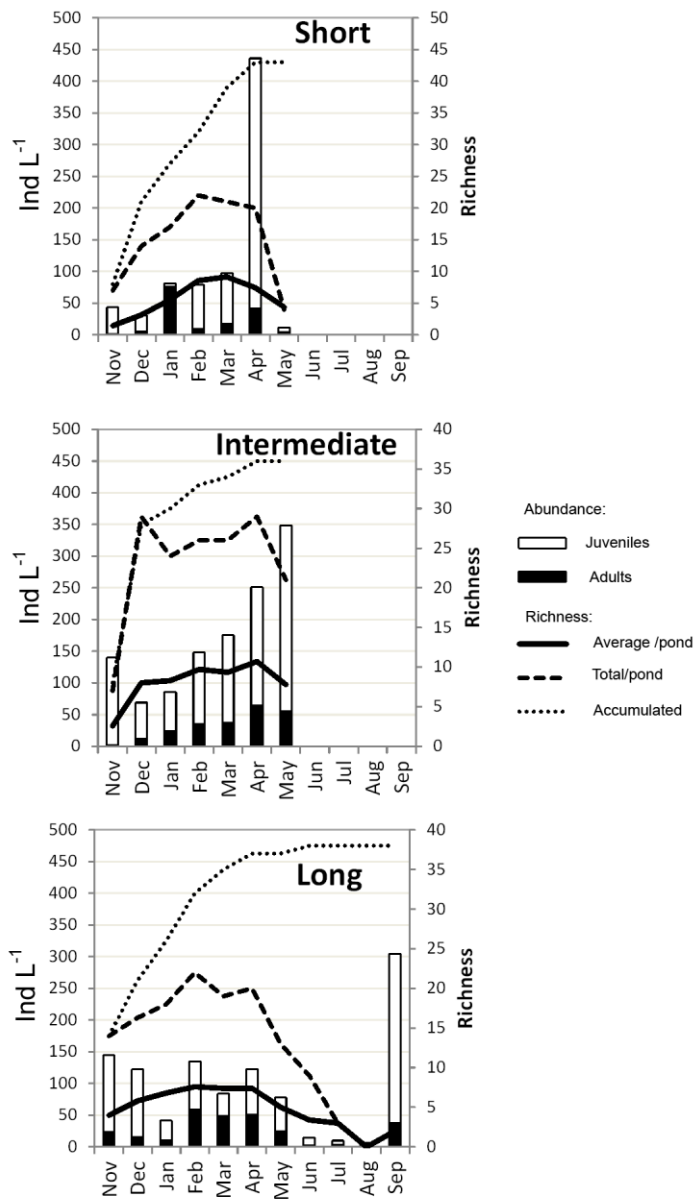
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671 Fig. 1



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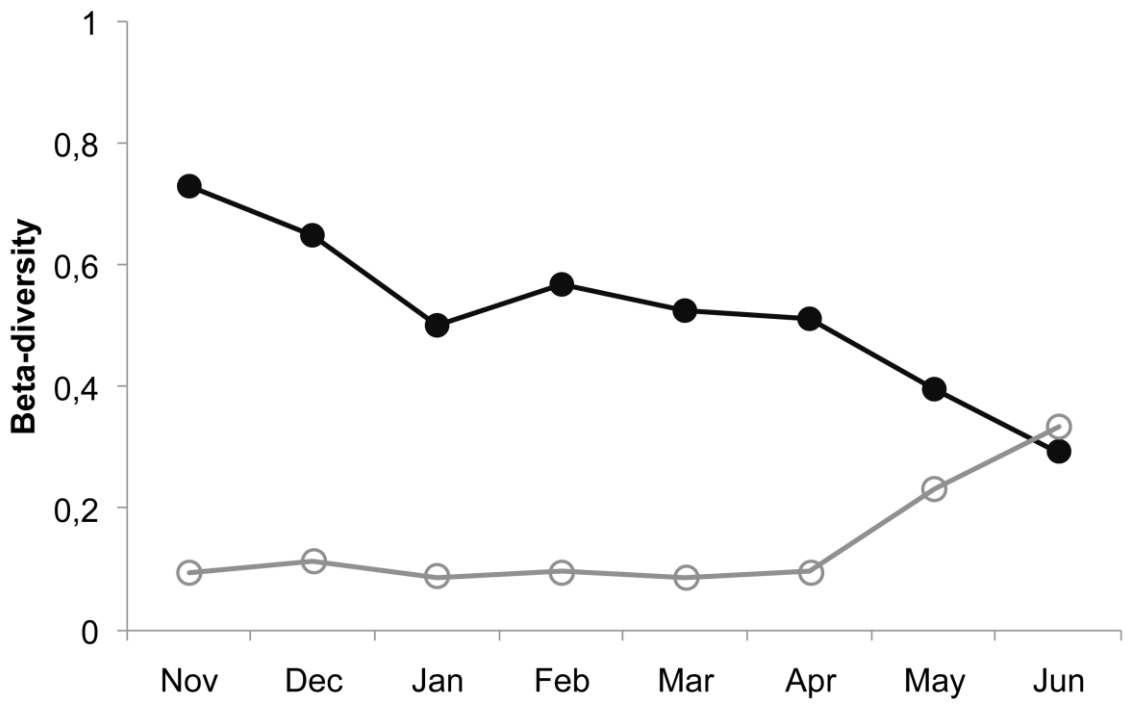
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680 Fig. 2



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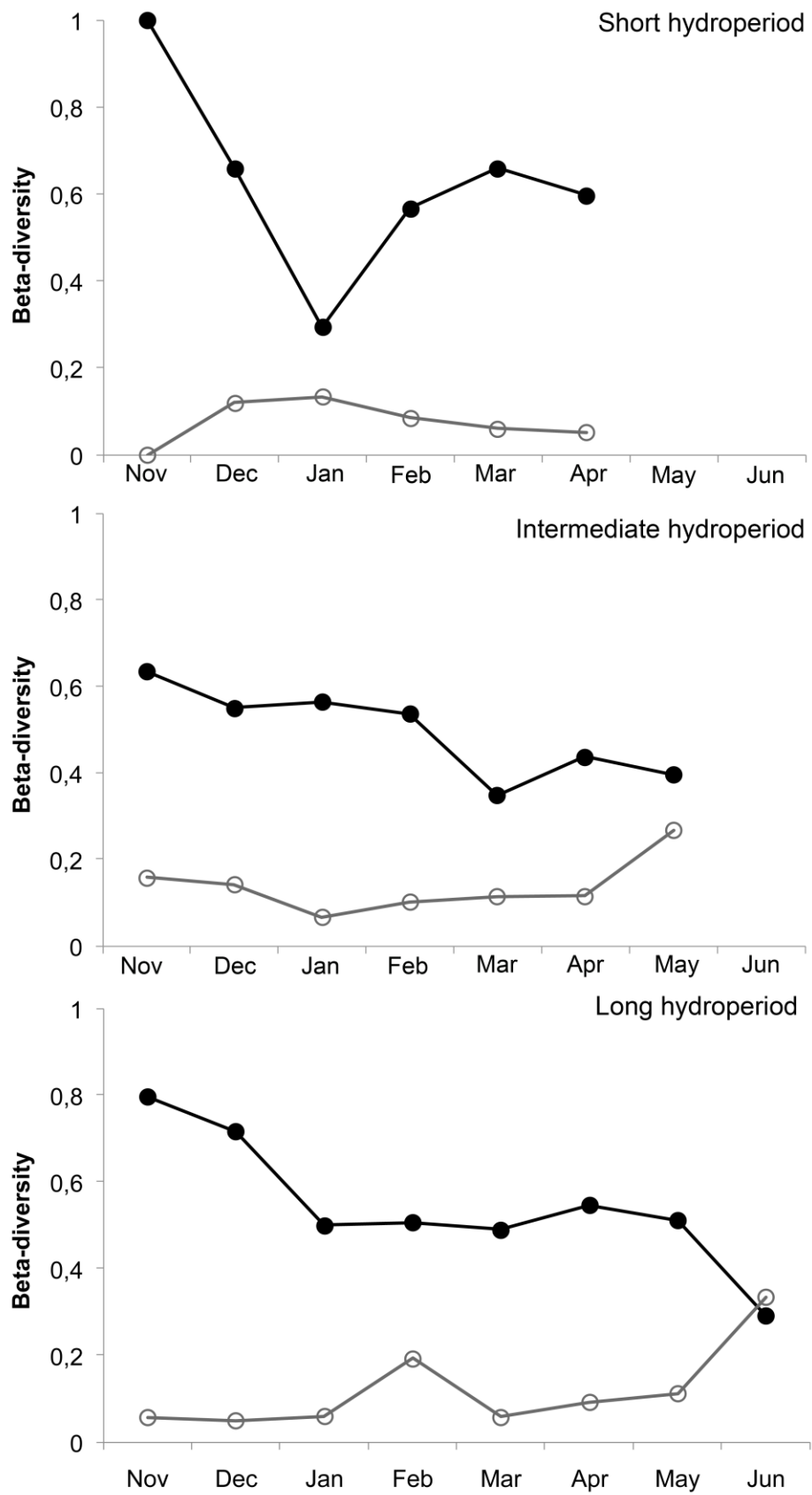
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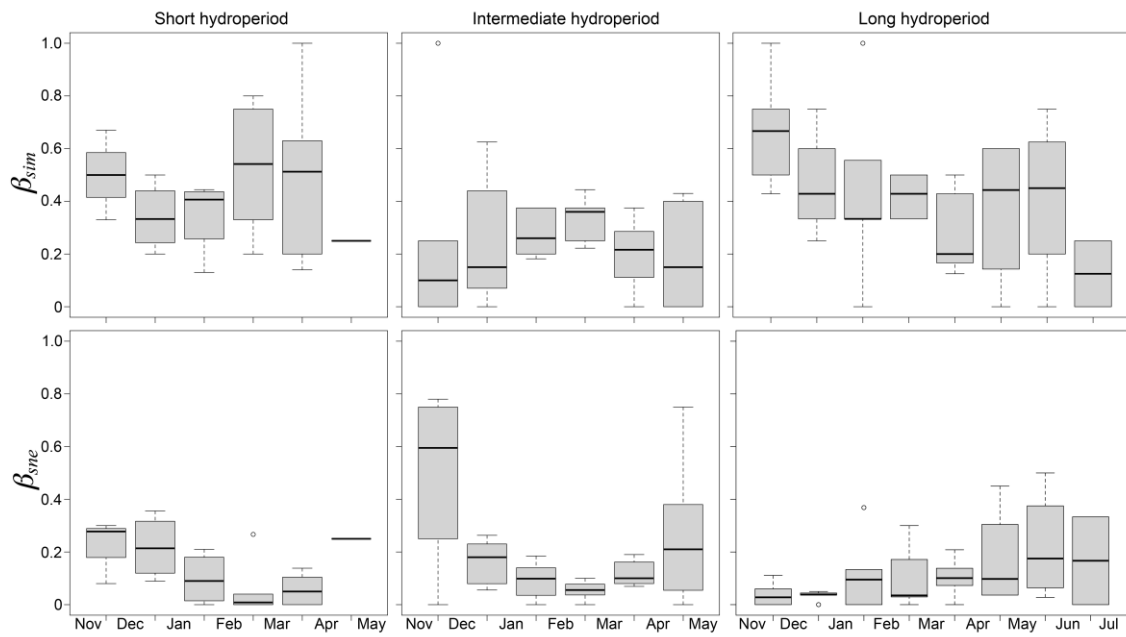
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696 Fig. 3



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716 Table 1. Species observed in the three types of ponds (S = short-hydroperiod ponds; I =
 717 intermediate-hydroperiod ponds; L = long-hydroperiod ponds). The number of ponds in
 718 which each species occurred is also indicated (species are ordered according to the
 719 chronology of their occurrence).

	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	S	I	L
Large Branchiopods														
<i>Triops baeticus</i>	IS	IS	IS	IS	S	S	LS					5	3	1
<i>Branchipus cortesi</i>	S	S	IS	S	S							4	1	0
<i>Maghrebestheria maroccana</i>		IS	IS	IS	IS	IS						3	1	0
<i>Tanymastix stagnalis</i>		IS	S	IS	S	S						4	3	0
<i>Branchipus schaefferi</i>		S				S						2	0	0
<i>Chirocephalus diaphanus</i>			I	S								1	1	0
<i>Cyzicus grubei</i>				S								1	0	0
Cladocerans														
<i>Chydorus sphaericus</i>	L	LI	ILS	I*LS	I*LS*	I*LS	ILS	L				7	7	5
<i>Ceriodaphnia quadrangula</i>	ILS	IL*S	LS*	IL*S	ILS	I*LS*	I*					6	7	4
<i>Daphnia hispanica</i>	L*	ILS	I*LS	IS	I							4	5	2
<i>Ceriodaphnia laticaudata</i>	IL	IL	IS	L	I							3	5	4
<i>Macrothrix rosea</i>	I	I	IL	ILS								4	2	2
<i>Estatheroporus gauthieri</i>	S	ILS		IL*S	IL	ILS	IL	L				5	2	3
<i>Daphnia magna</i>	L*	LS										1	0	3
<i>Ceriodaphnia reticulata</i>	S			LS	LSI	LI	LI		L			3	4	4
<i>Moina micrura</i>	L					I*LS					L*	1	1	2
<i>Alona rectangula</i>		I	I	IL	IS	IL	IL	L	L			1	6	3
<i>Dunhevedia crassa</i>		ILS	ILS	ILS	ILS	ILS	IL	L				3	4	3
<i>Alona quadrangularis</i>		I	S	ILS	ILS	ILS	IL					4	5	4
<i>Alona azorica</i>		I	I	I	ILS	ILS	IL					5	4	3
<i>Scapholeberis rammneri</i>		I	IS	IS	IS	IS	I					3	6	3
<i>Alonella excisa</i>		IL	L	L	ILS	IL	IL					1	6	4
<i>Simocephalus vetulus</i>		L	L	S	IS	IS	IL					4	4	2
<i>Simocephalus exspinosus</i>		I	I	IL	ILS	IS	I					3	3	1
<i>Alona iberica</i> ⁿ		S	I	LS	IL*	IL	L					2	2	1
<i>Oxyurella tenuicaudis</i>		L	I	IL	IL	IL		L				0	3	4
<i>Macrothrix hirsuticornis</i>		I	IS	IS	I	S						3	3	0
<i>Ephemeropus phintonicus</i> ⁿ		I	IL	IL	I	IS						1	3	2
<i>Alona affinis</i>		I		I								0	1	0
<i>Leydigia leydigii</i>		I				I						0	2	0
<i>Moina brachiata</i>		L										0	0	1
<i>Leydigia acanthocercoides</i>			L	L		L		L				0	0	4
<i>Alonella nana</i>			S		LS	I						2	1	1
<i>Ephemeropus margalefi</i>				IL	L	ILS	ILS					2	4	3

<i>Treptocephala ambigua</i>				I	I	IL						0	2	1
<i>Daphnia similis</i>				S								1	0	0
<i>Ilyocryptus silvaeducensis</i>					L							1	0	1
<i>Daphnia longispina</i>						L						0	0	1
<i>Scapholeberis mucronata</i>							I					0	1	0
<i>Diaphanosoma brachyura</i>								L				0	0	1
Copepods														
<i>Canthocamptus staphylinus</i>	ILS	ILS	ILS	ILS	IS	ILS	IS					7	6	2
<i>Copidodiaptomus numidicus</i>	L	IL	ILS	ILS	ILS	I*L*S*	I*L*S	L	L			5	6	5
<i>Diacyclops bicuspidatus</i>	ILS	ILS	ILS	ILS	ILS	ILS	I					6	6	5
<i>Dussartius baeticus</i> ^{End}	I	ILS	ILS	ILS	ILS	I*LS	I*L					5	6	5
<i>Megacyclops viridis</i>	IL	IS	ILS	ILS	IS	IS	IS					5	4	3
<i>Diaptomus kenitraensis</i>	L	ILS	ILS*	ILS	IS	IS						6	7	4
<i>Arctodiaptomus wierzejskii</i>	L	L	L	L								0	0	2
<i>Megacyclops gigas</i>	L	I	I		LS		I					1	3	2
<i>Acanthocyclops americanus</i>	L*	L		I	I	IS		L	L	L		1	2	3
<i>Metacyclops minutus</i>	ILS	I										1	3	1
<i>Hemidiaptomus roubaui</i>		IS	ILS	S	I	S						4	5	1
<i>Eucyclops serrulatus</i>		IS	I*	IS	I	I						2	1	0
<i>Attheyella trispinosa</i>		I		I		I	I					0	2	0
<i>Acanthocyclops robustus</i>			L		LS							1	0	2

720 * indicates that the number of individuals per pond was >10 ind L⁻¹; ^{End} indicates a species endemic to the
721 SW Iberian Peninsula; ⁿ indicates that the species was newly detected in Doñana in this study

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736 Table 2: Significant differences in species richness per pond and month among the three
 737 pond types (short-, intermediate-, and long-hydroperiod ponds) based on the results of a
 738 nested ANOVA. When significance was detected, the intermediate-hydroperiod ponds
 739 were always the most species rich (Online Resource ESM 2). The significance level
 740 (α) was set to 0.05; when a result was non-significant, the P value is provided.

	Month		Hydroperiod (Month)	
	F	<i>P</i>	F	<i>P</i>
Copepods	6.16	<0.00001	2.62	<0.01
Cladocerans	7.61	<0.00001	1.27	0.240
Cyclopoids	2.02	<0.05	2.1	<0.05
Diaptomids	6.66	<0.00001	1.37	0.179
Harpacticoids	1.84	0.063	3.26	<0.001
Large branchiopods	8.42	<0.00001	2.34	<0.01

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