

1 **Interannual variation in filling season affects zooplankton diversity in**
2 **Mediterranean temporary ponds**

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13

14 **ABSTRACT**

15 In Mediterranean temporary ponds, the timing of annual flooding is highly variable and
16 depends on heavy seasonal rains. Ponds can flood in the autumn, winter, or spring and
17 thus the environmental conditions faced by emerging zooplankton can be very different.

18 We performed an experiment in a climatic chamber where we simulated annual
19 variation in natural temperature and light conditions to study how differences in pond-
20 filling season affected zooplankton assemblage composition. We sampled sediments
21 from a temporary pond and placed them in aquariums that were filled with water during
22 three different seasons: autumn (October), winter (January), and spring (March).

23 Zooplankton abundance, species richness, diversity, and assemblage composition
24 differed significantly among treatments, and post-inundation temperature and pH
25 appeared to be the main drivers of these differences. Diversity was highest in the winter

26 treatment. It was lower in the autumn treatment and the spring treatment, and no
27 indicator species were present in the latter. Our results suggest that interannual
28 variability in initial inundation conditions favours the emergence of different species
29 and thus contributes to high species richness in the egg bank. However, climate change
30 and/or groundwater drawdown could delay pond flooding, impoverishing the
31 zooplankton assemblage in the long term.

32 **Keywords:** cladocerans, copepods, hydroperiod, lentic systems, microcrustaceans,
33 rotifers

34

35 **Introduction**

36 Temporary ponds display highly variable abiotic and biotic conditions over time. In
37 these freshwater ecosystems, the inundation period (i.e., hydroperiod) is followed by a
38 dry period whose duration changes from year to year (Williams, 2006). In the
39 Mediterranean, the start of the annual inundation period in temporary ponds is highly
40 unpredictable; it occurs after the summer but will only be triggered when ponds have
41 accumulated a sufficient amount of rainfall. In some years, ponds are filled by heavy
42 rains in autumn, while, in other years, filling occurs in winter or spring (Díaz-Paniagua
43 et al., 2010). Consequently, these ponds display dramatic interannual variability, not
44 only in their hydroperiod, but also in their post-inundation environmental conditions.
45 Aquatic organisms inhabiting temporary waters need strategies for surviving dry
46 periods so that they can re-establish themselves when their habitats are flooded again .
47 Most zooplankton species have short life cycles and form dormant/resistant stages that
48 will hatch during a future wet cycle, depending on pond environmental conditions in
49 subsequent years (Williams, 2006). These resistant stages enable the long-term
50 persistence of species, allowing them to avoid unfavourable or intense competitive

51 conditions (Chesson & Huntly, 1989). As a result of this storage effect, competing
52 species with short generation times can coexist via dormant eggs or cysts that will give
53 rise to new populations when conditions are favourable (Cáceres, 1997). When
54 conditions are unfavourable (e.g., the hydroperiod is short or inundation occurs late),
55 some zooplankton species display lower egg hatching rates, a strategy that may prevent
56 total reproductive failure (Kneitel, 2014; Pinceel et al., 2017). Because only a fraction
57 of the egg bank hatches at a given time, the remaining eggs may increase population
58 resilience in future inundation cycles (Brendonck et al., 2017). These traits make
59 crustacean and rotifer species good models for examining the consequences of the
60 frequent environmental changes that characterise temporary aquatic ecosystems, such as
61 Mediterranean temporary ponds. The egg bank that remains dormant during dry phases
62 allows the re-establishment of the ponds' zooplankton communities, therefore
63 contributing to their resilience or ability to recover after drying (Brock et al., 2003).
64 Dormant zooplankton eggs often hatch shortly after ponds flood (Brendonck & De
65 Meester, 2003). However, the unpredictable timing of flooding results in dramatic
66 interannual differences in initial abiotic conditions, thus influencing hatching dynamics
67 (e.g., de Eyto & Irvine, 2001). Furthermore, since species often respond differently to
68 environmental conditions—every inundation cycle, conditions will be optimal for a
69 specific set of species—zooplankton assemblage composition can vary greatly.
70 Hydroperiod can act as a major determinant of zooplankton assemblage diversity and
71 composition in temporary ponds because it influences seasonal variation in
72 environmental conditions (e.g., pH, levels of chlorophyll a, and temperature); these
73 conditions, in turn, greatly affect zooplankton phenology (see Tavernini et al., 2005;
74 Seminara et al., 2008).

75 In this study, we experimentally examined seasonal variation in zooplankton
76 assemblage composition using sediments from a Mediterranean temporary pond
77 located in Doñana National Park, Spain. Zooplankton assemblages in this area have
78 been described in previous studies and are particularly species rich: a total of 78
79 crustacean species (Fahd et al., 2009) and 47 rotifer species (Serrano & Fahd, 2005)
80 have been observed across different temporary ponds, and 72 rotifer species have been
81 seen in a seasonal marsh (Fahd et al., 2009). This regional diversity is associated with
82 heterogeneity in pond hydroperiod (Serrano & Fahd, 2005; Fahd et al., 2009; Frisch et
83 al., 2006), and seasonal variation in zooplankton communities can lead to different
84 patterns of beta diversity among ponds with different hydroperiods (Florencio et al.,
85 2016).

86 The aim of our study was to analyse how post-inundation environmental
87 conditions could influence zooplankton assemblage composition in Mediterranean
88 temporary ponds. First, we hypothesised that the common interannual variation in pond-
89 filling season that characterises temporary ponds would have a major effect on
90 assemblage composition, given that egg hatching rates can decline if pond filling occurs
91 very late in the annual inundation cycle. Second, we hypothesised that such differences
92 in zooplankton emergence would affect species abundance, richness, and diversity.
93 Third, we hypothesised that pond-filling season affects zooplankton assemblage
94 composition because the timing of inundation determines the environmental conditions
95 experienced by zooplankton. To test these hypotheses, we performed an experiment in
96 which variation in the seasonal timing of pond filling was simulated. Pond sediments
97 were collected in the field and placed in laboratory microcosms. We then “flooded”
98 these microcosms and subjected them to temperature and photoperiod regimes typical of
99 field conditions during one of three filling seasons: autumn, winter, or spring. We

100 subsequently measured the abundance, species richness, and species diversity of the
101 microcrustaceans and rotifers in each treatment group.

102

103 **Material and Methods**

104 We collected sediment from a temporary pond in Doñana National Park, which is
105 located in southwestern Spain (36°59'10" N, 6°29'21" W). This area contains a pond
106 network with a large number and high density of Mediterranean temporary ponds that
107 are located on sandy soil and usually dry out during the summer (Díaz-Paniagua et al.,
108 2015). Every year, a new inundation cycle starts when post-summer rains accumulate
109 and recharge the aquifer, producing a rise in groundwater level for the pond basins. The
110 temporary pond that we selected had a sandy bottom, a maximum depth of 70 cm, and
111 an intermediate hydroperiod (relative to the known hydroperiod range in the study area
112 [Díaz-Paniagua et al., 2015]). We chose a pond with an intermediate hydroperiod
113 because it provided a better representation of the study area. Such ponds usually harbour
114 eurytopic invertebrate faunas, which also occur in long- and short-hydroperiod ponds
115 (Florencio et al., 2011). Furthermore, for most of the ponds in the study area, filling
116 month varies interannually and depends on rainfall accumulation dynamics; usually,
117 most ponds dry up by June or July. We gathered dry sediment from the upper soil layer
118 (1–10 cm) in different parts of the target pond's basin (i.e., from the pond's border to its
119 point of maximum depth) and mixed all the samples together. We put six small pots (6.5
120 cm × 6.5 cm × 7 cm) filled with sediment in 39 aquariums (22 cm × 22 cm × 37 cm)
121 and placed the aquariums in a climatic chamber (ARALAB, Fitoclima 10000EH). We
122 programmed the chamber to simulate natural temperature and photoperiod regimes
123 across an annual inundation cycle. More specifically, there were weekly changes in
124 photoperiod and temperature, and the daily temperature range was based on air and

125 water temperature values measured in the field (mean data from 1979–2007 and 2006–
126 2007, respectively).

127 We simulated variation in annual inundation cycles by filling the aquariums with
128 17 L of dechlorinated tap water during one of three different seasons: autumn, winter, or
129 spring; we randomly assigned 13 aquariums to each treatment group. On October 25,
130 2011, we began the experiment by placing all 39 aquariums in the climatic chamber and
131 simulating flooding in the autumn treatment group. Flooding was simulated in the
132 winter and spring treatment groups on January 24, 2012 and March 27, 2012,
133 respectively. Once an aquarium had been filled, its water level was checked every week
134 and topped off as necessary. In the autumn treatment, the mean daily air temperature
135 started at 20.1°C and ended at 12.1°C. In the winter treatment, it started at 11.7°C and
136 ended at 16°C. In the spring treatment, it started at 17.4°C and ended at 22.8°C (Fig. 1a).
137 Each treatment was run for 50 days: during this time, aquatic plants were allowed to
138 grow, and zooplankton species were left to hatch and develop. We then removed the
139 water from the aquariums and filtered it through a nylon fiber net (100- μ m mesh size).
140 The resulting zooplankton sample was preserved in 70% ethanol until it could be
141 analysed in the lab. We were able to identify most of the zooplankton taxa to species,
142 notably the cladocerans, copepods, and the rotifers that were more than 100 μ m in
143 length. The exceptions were the rotifers *Asplanchna*, *Cephalodella*, and *Proales*, which
144 we could only identify to genus (although we will refer to them as species hereafter for
145 simplicity's sake). Taxonomic identification was carried out using Alonso (1996) for
146 the cladocerans, Dussart (1967, 1969) for the copepods, and Koste (1978) for the
147 rotifers. We also determined species abundance (i.e., number of individuals) per
148 aquarium. We measured electrical conductivity (using an HI 9033, Hanna Instruments),

149 pH and temperature (using a Multi 340i, WTW), and turbidity (using an HI 93703,
150 Hanna Instruments) in the aquariums right before collecting the zooplankton.

151

152 *Data analyses*

153 We used R software version 3.2.5 (R Development Core Team, 2018) to compare the
154 variables that differed among the three inundation treatments. We compared the
155 physical and chemical conditions associated with the treatments using the permutation
156 tests for linear models in the *lmPerm* package (Wheeler, 2016). We estimated species
157 richness and abundance overall (i.e., encompassing all the zooplankton species) and for
158 the separate taxonomic groups (i.e., the cladocerans, copepods, and rotifers). We used a
159 generalised linear model (GLM) with a negative binomial distribution (due to the
160 overdispersion that resulted when the Poisson error distribution was employed) to
161 compare abundance among treatments; for the richness data, a GLM with a
162 quasipoisson error distribution was utilised. We also estimated the Shannon diversity
163 index for the cladocerans, copepods, and rotifers and compared the index value among
164 treatments using a GLM with a Gaussian error distribution. However, because copepod
165 diversity did not meet the assumption of normality, it was analysed using the
166 permutation tests. All post-hoc comparisons between treatments were carried out with
167 Tukey tests implemented via the *multcomp* R package (Hothorn et al., 2008).

168 We analysed differences in assemblage structure among treatments using Primer
169 software version 6 and the add-on package PERMANOVA+ (Anderson et al., 2008).

170 The abundance matrix of all the observed species was $\log(x+1)$ transformed to create a
171 triangular resemblance matrix based on Bray-Curtis similarity (the inverse of the Bray-
172 Curtis index of dissimilarity). This new matrix was then used to perform a one-way
173 permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). We

174 analysed the ability of the environmental variables to explain the Bray-Curtis similarity
175 values using a distance-based linear model (DistLM) and a distance-based redundancy
176 analysis (dbRDA; McArdle & Anderson, 2001). The dbRDA is a multivariate multiple
177 regression of principal coordinates analysis (PCA) axes on predictor variables, which is
178 constrained to find linear combinations of the predictor variables. All the environmental
179 variables were square root transformed beforehand, with the exception of pH. The
180 DistLM allowed us to estimate the significance of the individual environmental
181 variables by partitioning the total explained variance via forward stepwise multiple
182 regression. The variance explained was taken to be the value of each variable's adjusted
183 R^2 conditioned on the presence of the variables previously sequentially added to the
184 model. These relationships were visualised using a dbRDA.

185 We calculated the IndVal index (Dufrêne & Legendre, 1997), using the R
186 package *indicpecies* (De Cáceres et al., 2010), to determine which species were
187 associated with the different inundation treatments (hereafter referred to as indicator
188 species). The results of this analysis revealed how the treatments affected species
189 occurrence and abundance. This index is the product of two components: specificity
190 (probability that a species belongs to the site group [i.e., inundation treatment] in which
191 it was found) and fidelity (probability of finding the species in sites [i.e., aquariums]
192 that belong to the site group [i.e., inundation treatment]). The significance of the IndVal
193 index was calculated using permutation tests. We only considered the abundance of
194 nauplii and immature copepods when examining differences in assemblage structure
195 among the inundation treatments using PERMANOVA.

196

197 **Results**

198 *Seasonal differences in environmental conditions*

199 Fifty days after flooding, environmental conditions significantly differed among
200 inundation treatments (pH: $F_{2,36} = 48.61$; $P < 0.0001$; turbidity: $F_{2,36} = 12.01$, $P <$
201 0.0001 ; conductivity: $F_{2,36} = 6.74$, $P = 0.003$; water temperature: $F_{2,36} = 669.90$, $P <$
202 0.0001), and the values of all these variables were lowest in the autumn treatment
203 (Fig. 1b).

204

205 *Composition of the zooplankton assemblages*

206 We observed a total of 14 crustacean and 12 rotifer species across the three treatments.
207 Maximum total richness was reached in the autumn treatment (11 crustacean and 10
208 rotifer species). Five cladoceran species, one copepod species, and two rotifer species
209 were present in all three treatments (Table 1). The total number of species differed
210 significantly among treatments ($F_{2,36} = 10.06$, $P < 0.0005$). The same was true for the
211 separate taxonomic groups (rotifers: $F_{2,36} = 12.15$, $P < 0.0001$; cladocerans: $F_{2,36} = 4.50$,
212 $P = 0.018$; copepods: $F_{2,36} = 5.40$, $P = 0.009$). On average, significantly more species
213 were present in the winter treatment; the rotifers were an exception because their
214 species richness was also high in the autumn treatment (Fig. 2a). Two cladoceran
215 species, two copepod species, and six rotifer species were exclusively seen in the
216 autumn treatment, and two copepod species and two rotifer species were only observed
217 in the spring treatment. In contrast, there were no species that were exclusive to the
218 winter treatment. *Macothrix rosea* (which occurred in the winter and spring) and the
219 species that were exclusive to the spring treatment were the only species that did not
220 appear in the autumn treatment (Table 1).

221 Total abundance differed significantly among the treatments ($\chi^2_{2,36} = 38.88$,
222 $P < 0.0001$), and it was lowest in the winter treatment (Fig. 2b). Significant differences
223 were observed in cladoceran abundance ($\chi^2_{2,36} = 71.36$, $P < 0.0001$), which was lowest

224 in the winter treatment, in rotifer abundance ($\chi^2_{2,36} = 13.87, P = 0.001$), which was
225 lowest in the spring treatment, and in copepod abundance ($\chi^2_{2,36} = 11.68, P = 0.003$),
226 which was highest in the spring treatment (Fig. 2b).

227 Shannon diversity followed the opposite trend from abundance. Total species
228 diversity differed significantly among treatments ($F_{2,36} = 24.98, P < 0.0001$) and reached
229 its highest value in the winter treatment (Fig. 2c). We also observed significant
230 differences when we examined the diversity of cladocerans ($F_{2,36} = 4.79, P = 0.014$),
231 copepods ($F_{2,36} = 3.70, P < 0.05$), and rotifers ($F_{2,36} = 3.792, P = 0.032$) separately (Fig.
232 2c).

233 There were significant differences in assemblage structure among treatments
234 (PERMANOVA, explained variance = 36.0%, Pseudo- $F_{2,36} = 8.32, P < 0.001$), and all
235 the pairwise comparisons were significant as well (all $P < 0.01$). The environmental
236 variables explained 26.7% of the total variance: temperature, pH, and conductivity had a
237 significant effect (Table 2). In the variance partitioning analyses, water temperature
238 contributed the most to explaining the observed differences (15.5%); pH (7.4%) was
239 second in importance; and conductivity made a minor contribution (3%) (Table 2, Fig.
240 3).

241 Only five species were indicator species. The rotifer *Proales* sp. was associated
242 with the autumn treatment, although it did not appear in all the aquariums (10 out of
243 13). The indicator species for the winter treatment were the rotifer *Keratella quadrata*
244 and the copepod *Hemidiaptomus roubaoui*. The cladoceran *Alona iberica* was an
245 indicator species for both the spring and autumn treatments. Finally, the rotifer
246 *Euchlanis dilatata* was an indicator species for both the autumn and winter treatments
247 (Table 3).

248

249 **Discussion**

250 Mediterranean temporary ponds are known to display major interannual
251 variation in filling season. In this study, we experimentally simulated three different
252 pond-filling seasons (autumn, winter, and spring) and demonstrated that the timing of
253 pond inundation can affect zooplankton assemblage composition. Differences existed in
254 pond environmental conditions and thus different assemblages of microcrustacean and
255 rotifer species arose. Post-inundation environmental conditions play a key role in
256 mediating the emergence of zooplankton from the egg bank, and they determine the
257 early species composition of the assemblages (Brendonck & De Meester, 2003).

258 Among the environmental variables known to affect zooplankton assemblage
259 composition in lentic ecosystems, temperature is one of the most influential factors in
260 the activation of dormant zooplankton eggs (Hairston et al., 2000; Vanderkherkove et
261 al., 2005). Here, we found that differences in water temperature range among the
262 different treatments had the greatest structuring influence on zooplankton assemblages.
263 The richness of cladoceran and copepod species was highest in the winter treatment,
264 whose air temperature range encompassed the optimal temperatures (10–15°C)
265 described for the hatching of resting eggs in many cladocerans (Vandekerkhove et al.,
266 2005). In contrast, the autumn and spring treatments had higher temperatures. Rotifer
267 species richness was highest in the autumn treatment, perhaps because of the lower
268 predation pressure exerted by copepods therein (compared to that in the other
269 treatments).

270 Furthermore, pH played an important role in structuring zooplankton
271 assemblages in this study. We observed an increase in pH across treatments (from
272 earliest to latest filling season), which may have been related to the greater
273 photosynthetic activity of the aquatic vegetation growing in the aquariums. More

274 specifically, the variation in pH values largely remained within the optimal range for
275 zooplankton species (7.5–9); lower or higher values would have had sublethal effects on
276 species survival, growth, and egg production (Locke & Sprules, 2001; Ghazy et al.,
277 2011). Conductivity varied very little—it remained low after inundation (335–411 μS
278 cm^{-1})—and thus only minimally explained differences in assemblage composition. It
279 has been suggested that the ability to hatch at low levels of conductivity is an adaptation
280 in zooplankton species to conditions in temporary ponds (Brendonck, 1998). In the
281 temporary ponds in our study area, the lowest levels of conductivity typically occur
282 after pond inundation (Florencio et al., 2013). Such conditions may trigger zooplankton
283 hatching and thus allow zooplankton to synchronise their life cycles with hydroperiod
284 length (Brendonck et al., 1998). It is important to note that most of the copepod species
285 that emerged at these low conductivity levels are seen as typical of the study area’s
286 temporary ponds (*Diaptomus kenitraensis*, *D. cyaneus*, *H. roubaoui*, and *D. baeticus*),
287 and they rarely occur in nearby marshes characterised by higher conductivity (Fahd et
288 al., 2009). Although we did not analyse light exposure, this factor is key in initiating
289 hatching in most branchiopod species (Brendonck et al., 1998). Levels of light energy,
290 the timing of light exposure, and egg pigmentation patterns should be considered in
291 future studies (see Pinceel et al., 2013).

292 The pattern for zooplankton abundance was the opposite of that for species
293 richness. Notably, it increased in the spring for cladocerans, mainly because of the
294 predominance of a particular species, *Chydorus sphaericus*. This species was highly
295 abundant in the spring treatment ($4.7 \times$ more abundant than in the autumn treatment and
296 $31.7 \times$ more abundant than in the winter treatment). Because the treatments ran for 50
297 days, it is possible that our samples captured not only the emergence phase, but also a
298 few generations of zooplankton species. It has been observed that species with short

299 generation times can exponentially increase in abundance when conditions are optimal.
300 In zooplankton, generation time is related to body size. It is small-bodied species rather
301 than large-bodied species that tend to rapidly increase in abundance when temperatures
302 are within optimal ranges (Gillooly, 2000). The predominance of *C. sphaericus* in the
303 warmer treatment fits with this hypothesis. It is a small-bodied species with a short life
304 span, whose generation time may drop from 38 days at 5°C to 8.9 days at 20°C (Bottrell,
305 1975). *Chydorus sphaericus* can also tolerate a wide range of conditions (Fryer, 1968).
306 This species frequently becomes highly abundant under particular conditions, likely
307 because it grows faster than other species (e.g., Verbitsky & Verbitskaya, 2011). It is
308 also important to consider the presence of the copepods, which can prey on a wide
309 variety of zooplankton species (Anderson, 1970; Brandl, 2005); as a result, both
310 hydroperiod and interspecific interactions may have shaped zooplankton assemblage
311 composition in this study. Similarly, the conditions associated with the autumn
312 treatment could have favoured the emergence and rapid reproduction of other species,
313 notably *Simocephalus vetulus*, *Ceriodaphnia quadrangula*, *C. sphaericus*, and *Proales*
314 *sp.*

315 The overall patterns of species richness and abundance revealed that Shannon
316 diversity was highest in the winter treatment. The low average species richness and the
317 dominance of certain species (as mentioned above) seemed to reduce Shannon diversity
318 in the autumn and spring treatments. This result was reflected in the fact that
319 assemblage composition in the winter was clearly distinct from that in the autumn and
320 spring. The winter treatment also had the highest number of indicator species. One
321 indicator species (*Proales sp.*) was unique to the autumn treatment. However, the
322 autumn treatment did share other indicator species with the winter (*Euchlanis dilatata*)
323 and spring (*Alona iberica*) treatments, probably because there was overlap in their

324 temperature ranges. Total species richness was highest in the autumn treatment,
325 indicating that the individual aquariums often harboured different species. There were
326 no indicator species for the spring treatment, which also had low diversity. This result
327 could be explained by the spring treatment's higher temperatures, which can cause
328 increased evaporation and faster pond desiccation, thus leaving emergent species
329 without enough time to complete their life cycles (Williams, 2006). In our study, the
330 low number of species that emerged in the spring treatment suggests that most species
331 may avoid emerging in years in which pond filling occurs very late, as a strategy for
332 avoiding reproductive failure (i.e., there is no time to reproduce before pond
333 desiccation; see Kneitel, 2014; Pinceel et al., 2017). Consequently, the species richness
334 and egg abundance in the egg bank may be depleted if spring filling increases in
335 frequency over time.

336 When examining patterns of zooplankton emergence from the egg bank,
337 experimental results and field observations do not always match up (Cáceres &
338 Schwalbach, 2001). Our study area, Doñana National Park, contains a large diversity of
339 zooplankton species, a phenomenon that has been attributed to the high local abundance
340 of water bodies that display pronounced differences in hydroperiod as well as to
341 broadscale connectivity that favours colonisation (Frisch et al., 2006; Fahd et al., 2009;
342 Serrano & Toja, 1998; Serrano & Fahd, 2005; Florencio et al., 2016). However, no
343 research has been done on the relationship between filling season and interannual
344 variation in zooplankton assemblages in Doñana's temporary ponds. Consequently, our
345 experimental results should be interpreted with caution until we know more about
346 dynamics in the field.

347 **Conclusion**

348 Environmental variability, and especially temperature variability, enhances the richness
349 of zooplankton assemblages (Shurin et al., 2010). The results of this study reveal how
350 temporary ponds may vary interannually: in different years, the same pond may be
351 flooded in different seasons and thus display different environmental conditions that
352 favour the hatching and persistence of different species. Over the long term, pronounced
353 interannual variability in filling season in these aquatic habitats could serve to increase
354 species number, where zooplankton occur either as emerged individuals or as eggs in
355 the egg bank. As a result of differences among years in rainfall dynamics, zooplankton
356 species may be differentially added to the regional pool of species in the egg bank,
357 which could help explain the high richness and diversity of zooplankton species in our
358 study area. However, ecosystem-level threats may put this diversity at risk. These
359 threats include the drawdown of groundwater levels due to the overexploitation of the
360 aquifer (Manzano & Custodio, 2006; Custodio et al., 2009), which may be exacerbated
361 by the increased temperatures and altered precipitation regimes associated with climate
362 change (Green et al., 2017). Such factors increase the amount of rain needed to fill the
363 ponds, which can delay pond filling and reduce the length of the wet phase (Díaz-
364 Paniagua *et al.*, 2015), thereby impoverishing the ponds' zooplankton assemblages. The
365 indicator species that we observed in the autumn and winter treatments may be the first
366 to disappear if both the phenomena cited above occur for several years in a row.
367 *Hemidiaptomus roubaoui*, an indicator species in the winter treatment, might be
368 particularly affected, given that it is only found along the northwestern boundary of the
369 Mediterranean Basin (Marrone & Naselli-Flores, 2004, and references therein).
370 Biodiversity may function as a form of insurance when ecosystems face environmental
371 perturbations (Loreau et al., 2001). The interannual variability in filling season
372 experienced by Mediterranean temporary ponds helps guarantee that their egg banks

373 will remain diverse. In turn, egg bank diversity seems to favour zooplankton
374 recruitment, and, as a consequence, the resilience of zooplankton populations in the face
375 of variable environmental conditions.

376

377

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383

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539 **Table 1** Mean number of individuals (\pm SD) of each species seen in the different
540 inundation treatments (autumn, winter, and spring). Species that were not significantly
541 associated with any of the three treatments based on indicator species analysis are
542 indicated with an asterisk.

Treatment	Autumn	Winter	Spring
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CLADOCERA

<i>Alona affinis</i>	0.31±1.11		
<i>Alona iberica</i>	58.92±65.28	1.38±1.12	113.15±177.71
<i>Chydorus sphaericus</i> *	522.46±621.40	77.77±79.28	2463.23±1737.13
<i>Ceriodaphnia quadrangula</i> *	581.84±865.62	177.31±203.76	220.31±362.02
<i>Simocephalus vetulus</i> *	584.31±879.46	24.77±36.09	121.00±197.01
<i>Dunhevedia crassa</i>	0.31±1.11		
<i>Oxyurella tenicaudis</i>	2.15±5.00	0.54±1.13	0.31±1.11
<i>Macrothrix rosea</i>		0.62±0.65	6.46±15.01

COPEPODA

<i>Diaptomus cyaneus</i>	4.62±16.64		
<i>Diaptomus kenitraensis</i>			1.08±3.88
<i>Dussartius baeticus</i> *	19.77±17.81	36.31±20.86	117.08±172.85
<i>Hemidiaptomus maroccanus</i>			0.08±0.28
<i>Hemidiaptomus roubaui</i>	0.62±2.22	2.00±2.45	
<i>Naupli and copepodits</i> *	42.00±43.42	134.31±113.96	158.00±313.32

ROTIFERA

<i>Asplanchna</i> spp.			0.31±1.11
<i>Brachionus quadridentatus</i>	2.31±8.32		
<i>Cephalodella</i> spp.	4.92±13.68	3.07±8.83	
<i>Euchlanis dilatata</i>	45.92±68.11	135.92±168.79	11.23±27.39
<i>Keratella quadrata</i>	1.85±4.79	48.00±95.91	
<i>Lecane luna</i>	0.08±0.28		
<i>Lecane quadridentatus</i>	3.38±7.09	0.38±1.12	0.15±0.55
<i>Lepadella patella</i>	0.08±0.28		

<i>Platyias quadricornis</i>	0.62±1.50	
<i>Testudinella patina</i>	0.62±2.22	
<i>Tetrocephala ambigua</i>		5.23±18.86
<i>Proales</i> spp.	306.15±395.00	

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558 **Table 2** Variance partitioning (adjusted R²) of the distance-based linear models

559 (DistLM). The multivariate multiple correlation between each environmental variable

560 and the Bray-Curtis similarity matrix was sequentially tested. AdjR² is the adjusted R²

561 and indicates the cumulative variance explained by the sequential addition of each

562 variable during multiple forward stepwise regression.

AdjR² (in %)	Pseudo-F	P
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Temperature	15.5	7.966	< 0.001
pH	22.9	4.577	< 0.01
Conductivity	25.9	2.461	< 0.05
Turbidity	26.7	1.378	0.234

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578 **Table 3:** Results of the indicator species analyses for the crustaceans and rotifers for the
579 three inundation treatments. Specificity refers to the probability that a species belongs to
580 the inundation treatment in which it was found; fidelity refers to the probability of
581 finding the species in aquariums belonging to the inundation treatment indicated. We
582 only included species for which the results were statistically significant ($P < 0.05$).

583

Indicator species	Treatments	A(Specificity)	B(Fidelity)	IndVal	P
CLADOCERA					
<i>Alona iberica</i>	Spring+Autumn	0.992	0.846	0.916	0.008
COPEPODA					
<i>Hemidiaptomus roubaoui</i>	Winter	0.765	0.539	0.642	0.006
ROTIFERA					
<i>Proales</i> spp.	Autumn	1.000	0.769	0.877	0.001
<i>Keratella quadrata</i>	Winter	0.963	0.462	0.667	0.009
<i>Euchlanis dilatata</i>	Autumn+Winter	0.942	0.808	0.872	0.001

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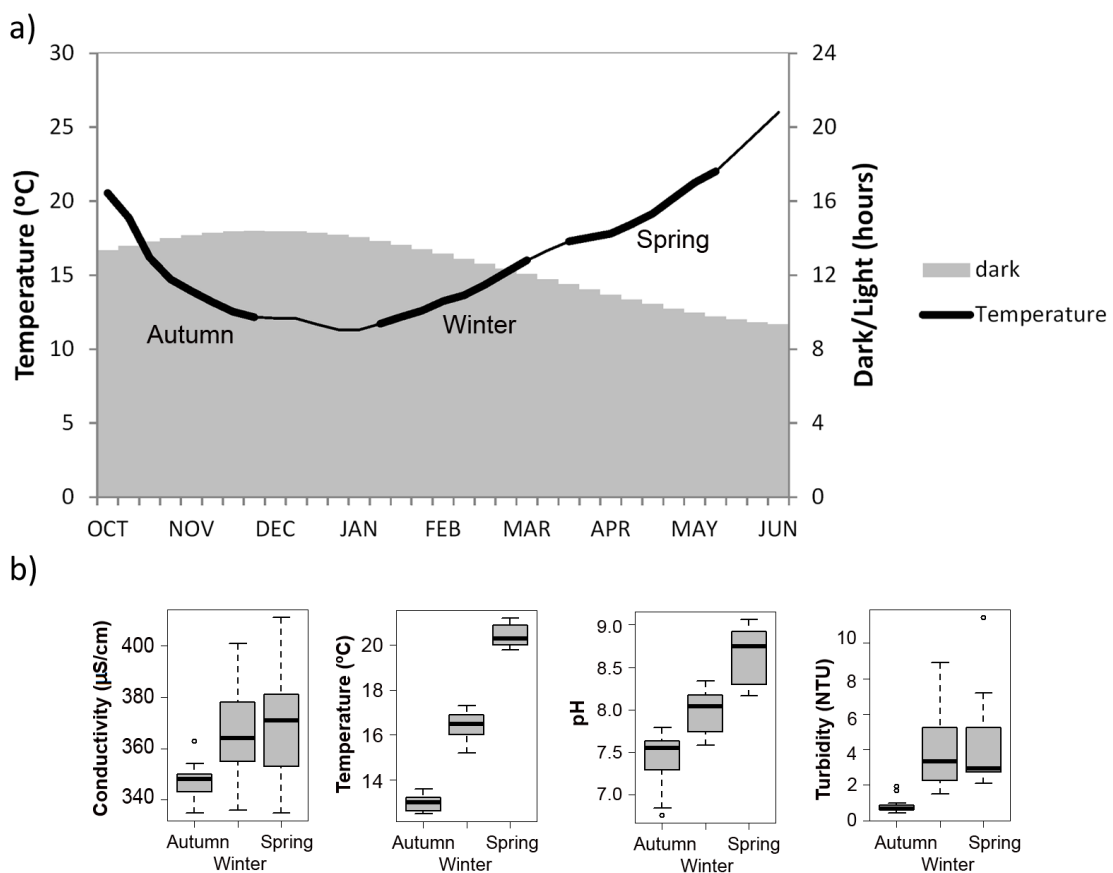
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595 **List of figures**

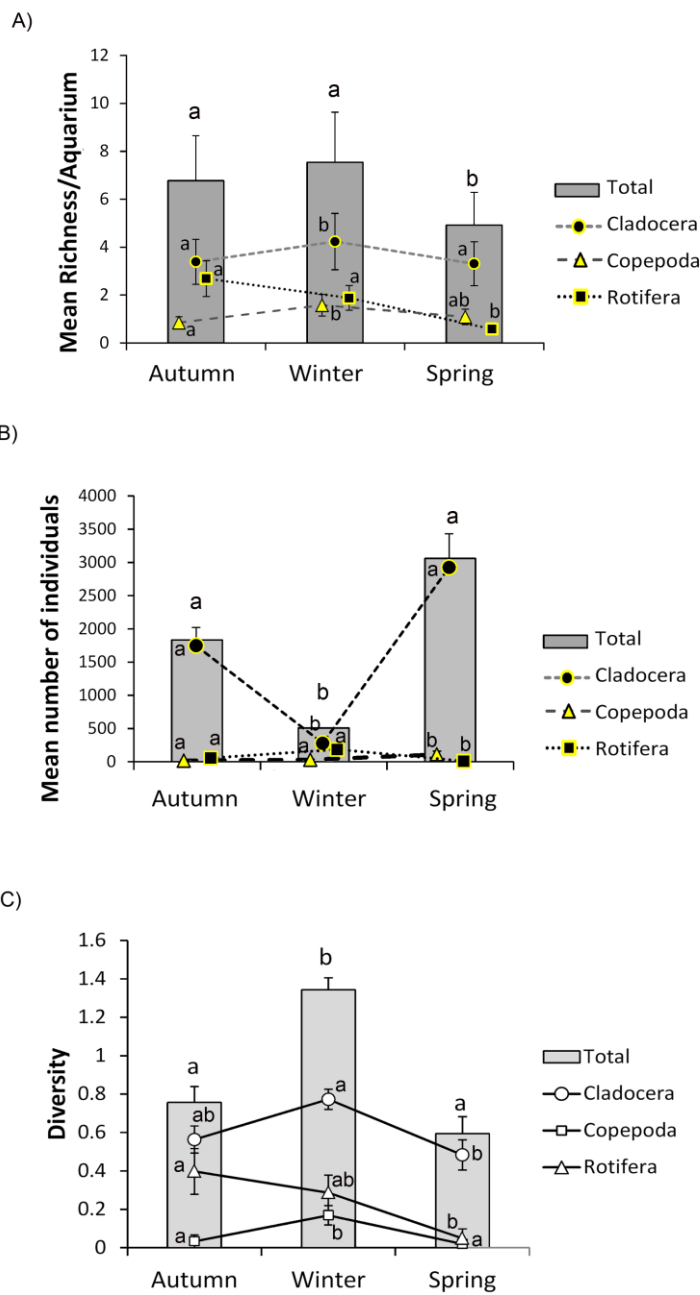
596 **Fig. 1** Differences in environmental conditions across the three inundation treatments
597 (autumn, winter, and spring). a) Weekly variation in the mean air temperature and the
598 dark/light cycle in the climatic chamber. The thicker sections of the temperature
599 line indicate the duration of each inundation treatment. b) Boxplot (median, P25, P75,
600 and range) of conductivity, pH, turbidity, and water temperature, which were recorded
601 in the aquariums at the end of the experiment.



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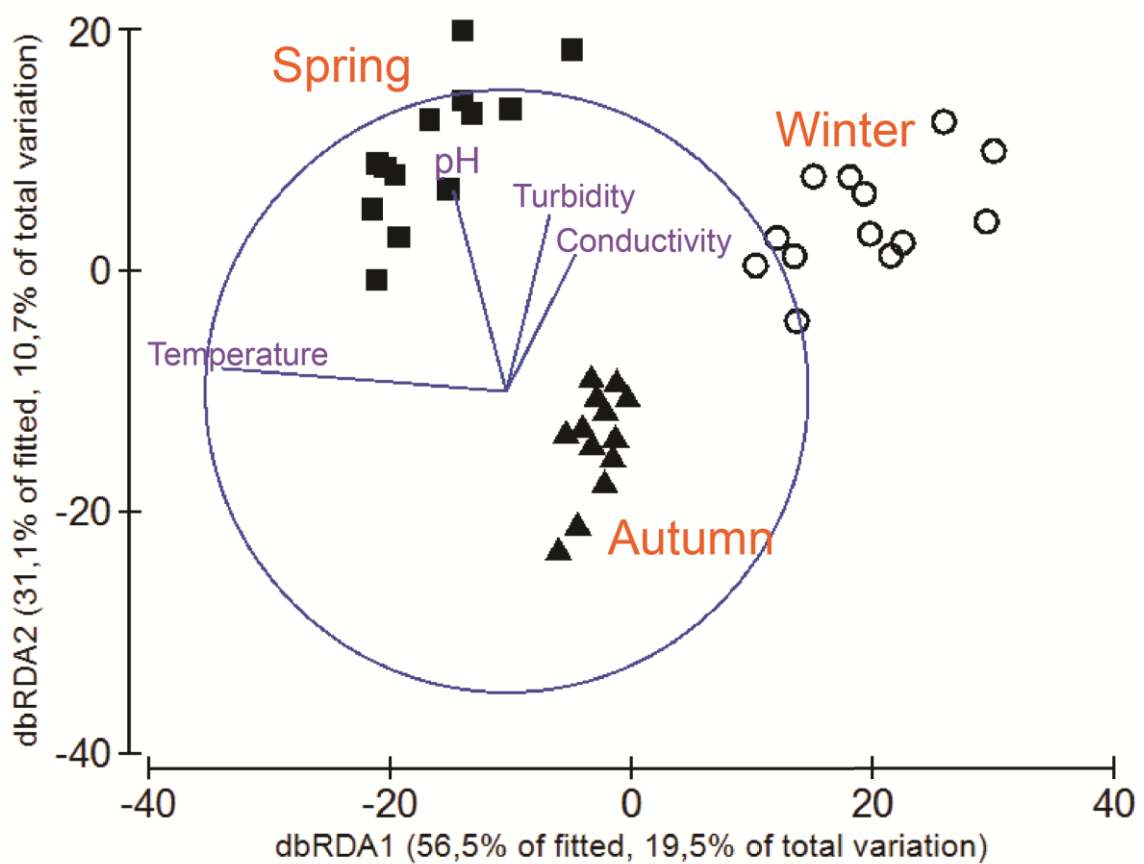
604 **Fig. 2** Mean values and standard errors of a) species richness, b) abundance, and c) the
 605 Shannon diversity index for all the species together and for the separate taxonomic
 606 groups (cladocerans, copepods, and rotifers) for the three inundation treatments. For
 607 each group of species, different letters indicate significant differences among treatments
 608 after Tukey's post hoc tests (all $P < 0.05$).



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611 **Fig. 3** Results of the distance-based redundancy analysis (dbRDA), which carried out a
612 multiple regression of each environmental variable on the Bray-Curtis similarity matrix
613 that included all the observed species (triangles: autumn treatment; circles: winter
614 treatment; squares: spring treatment). The percentages along the figure axes indicate the
615 percentage of variance explained and the percentage of the best-fit model represented by
616 the axes.



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