Interannual variation in filling season affects zooplankton diversity in Mediterranean temporary ponds

Florencio, M.[1,2,3*], Fernández-Zamudio, R.[1], Mayca Lozano[1], Díaz-Paniagua, C.[1]

[1]Estación Biológica de Doñana, Avda. Américo Vespucio 26, Sevilla, E-41092

[2]Department of Life Sciences, University of Alcalá, 28805 Alcalá de Henares, Madrid, Spain

[3]Present address: Centro de Investigación en Biodiversidad y Cambio Global (CIBC-UAM) / Departamento de Ecología, Universidad Autónoma de Madrid, Madrid, Spain.

[*]Corresponding author: mflorenciodiaz@gmail.com; margarita@ebd.csic.es, telephone number: 91 497 8007,

fax number: 91 497 8001.

ABSTRACT

In Mediterranean temporary ponds, the timing of annual flooding is highly variable and depends on heavy seasonal rains. Ponds can flood in the autumn, winter, or spring and thus the environmental conditions faced by emerging zooplankton can be very different. We performed an experiment in a climatic chamber where we simulated annual variation in natural temperature and light conditions to study how differences in pond-filling season affected zooplankton assemblage composition. We sampled sediments from a temporary pond and placed them in aquariums that were filled with water during three different seasons: autumn (October), winter (January), and spring (March). Zooplankton abundance, species richness, diversity, and assemblage composition differed significantly among treatments, and post-inundation temperature and pH appeared to be the main drivers of these differences. Diversity was highest in the winter

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

Keywords: cladocerans, copepods, hydroperiod, lentic systems, microcrustaceans, rotifers

Introduction

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

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

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

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

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

Material and Methods

We collected sediment from a temporary pond in Doñana National Park, which is located in southwestern Spain (36º59’10” N, 6º29’21” W). This area contains a pond network with a large number and high density of Mediterranean temporary ponds that are located on sandy soil and usually dry out during the summer (Díaz-Paniagua et al., 2015). Every year, a new inundation cycle starts when post-summer rains accumulate and recharge the aquifer, producing a rise in groundwater level for the pond basins. The temporary pond that we selected had a sandy bottom, a maximum depth of 70 cm, and an intermediate hydroperiod (relative to the known hydroperiod range in the study area [Díaz-Paniagua et al., 2015]). We chose a pond with an intermediate hydroperiod because it provided a better representation of the study area. Such ponds usually harbour eurytopic invertebrate faunas, which also occur in long- and short-hydroperiod ponds (Florencio et al., 2011). Furthermore, for most of the ponds in the study area, filling month varies interannually and depends on rainfall accumulation dynamics; usually, most ponds dry up by June or July. We gathered dry sediment from the upper soil layer (1–10 cm) in different parts of the target pond’s basin (i.e., from the pond’s border to its point of maximum depth) and mixed all the samples together. We put six small pots (6.5 cm × 6.5 cm × 7 cm) filled with sediment in 39 aquariums (22 cm × 22 cm × 37 cm) and placed the aquariums in a climatic chamber (ARALAB, Fitoclima 10000EH). We programmed the chamber to simulate natural temperature and photoperiod regimes across an annual inundation cycle. More specifically, there were weekly changes in photoperiod and temperature, and the daily temperature range was based on air and

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

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

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

Data analyses

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

We analysed differences in assemblage structure among treatments using Primer software version 6 and the add-on package PERMANOVA+ (Anderson et al., 2008). The abundance matrix of all the observed species was log (x+1) transformed to create a triangular resemblance matrix based on Bray-Curtis similarity (the inverse of the Bray–Curtis index of dissimilarity). This new matrix was then used to perform a one-way permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). We

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

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

Results

Seasonal differences in environmental conditions

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

Composition of the zooplankton assemblages

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

Conclusion

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

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

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

Treatment	**Autumn**	**Winter**	**Spring**

CLADOCERA			
Alona affinis	**0.31±1.11**		
Alona iberica	**58.92±65.28**	**1.38±1.12**	**113.15±177.71**
*Chydorus sphaericus**	**522.46±621.40**	**77.77±79.28**	**2463.23±1737.13**
*Ceriodaphnia quadrangula**	**581.84±865.62**	**177.31±203.76**	**220.31±362.02**
*Simocephalus vetulus**	**584.31±879.46**	**24.77±36.09**	**121.00±197.01**
Dunhevedia crassa	**0.31±1.11**		
Oxyurella tenicaudis	**2.15±5.00**	**0.54±1.13**	**0.31±1.11**
Macrothrix rosea		**0.62±0.65**	**6.46±15.01**
COPEPODA			
Diaptomus cyaneus	**4.62±16.64**		
Diaptomus kenitraensis			**1.08±3.88**
*Dussartius baeticus**	**19.77±17.81**	**36.31±20.86**	**117.08±172.85**
Hemidiaptomus maroccanus			**0.08±0.28**
Hemidiaptomus roubaui	**0.62±2.22**	**2.00±2.45**	
*Naupli and copepodits**	**42.00±43.42**	**134.31±113.96**	**158.00±313.32**
ROTIFERA			
Asplachna spp.			**0.31±1.11**
Brachionus quadridentatus	**2.31±8.32**		
Cephalodella spp.	**4.92±13.68**	**3.07±8.83**	
Euchlanis dilatata	**45.92±68.11**	**135.92±168.79**	**11.23±27.39**
Keratella quadrata	**1.85±4.79**	**48.00±95.91**	
Lecane luna	**0.08±0.28**		
Lecane quadridentatus	**3.38±7.09**	**0.38±1.12**	**0.15±0.55**
Lepadella patella	**0.08±0.28**		

Platyias quadricornis	**0.62±1.50**	
Testudinella patina	**0.62±2.22**	
Tetrocephala ambigua		**5.23±18.86**
Proales spp.	**306.15±395.00**	

Table 2 Variance partitioning (adjusted R^2) of the distance-based linear models (DistLM). The multivariate multiple correlation between each environmental variable and the Bray-Curtis similarity matrix was sequentially tested. $AdjR^2$ is the adjusted R^2 and indicates the cumulative variance explained by the sequential addition of each variable during multiple forward stepwise regression.

	$AdjR^2$ (in %)	**Pseudo-F**	***P***

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

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

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

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

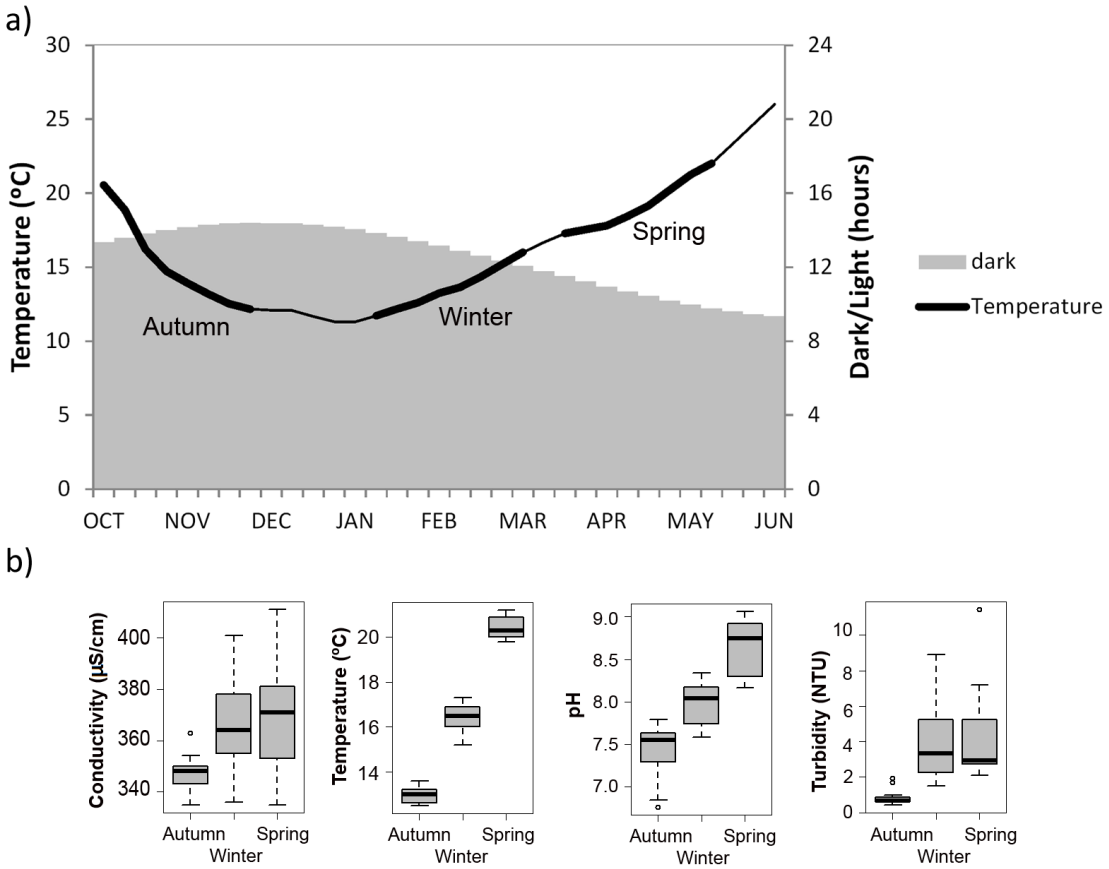

Fig. 2 Mean values and standard errors of a) species richness, b) abundance, and c) the Shannon diversity index for all the species together and for the separate taxonomic groups (cladocerans, copepods, and rotifers) for the three inundation treatments. For each group of species, different letters indicate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

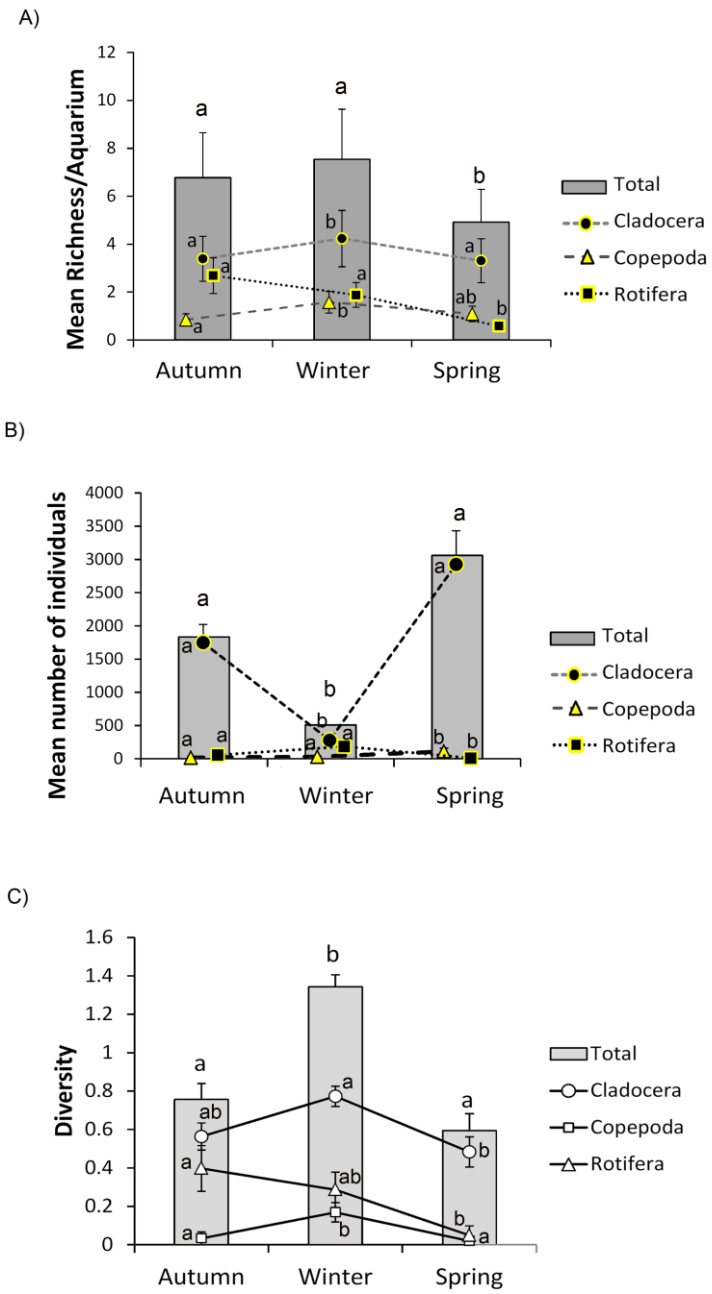
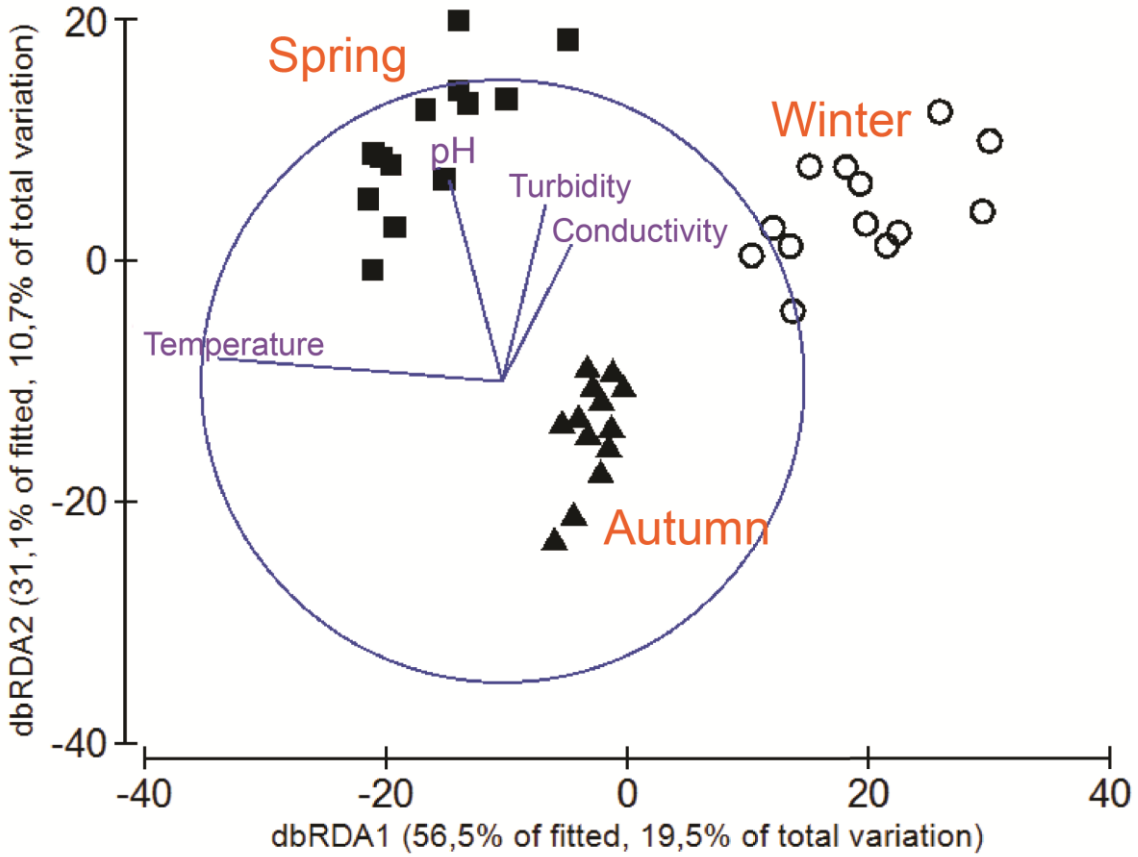
Fig. 3 Results of the distance-based redundancy analysis (dbRDA), which carried out a multiple regression of each environmental variable on the Bray-Curtis similarity matrix that included all the observed species (triangles: autumn treatment; circles: winter treatment; squares: spring treatment). The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