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Zooplankton changes at six reservoirs in the Ebro watershed, Spain

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ABSTRACT

Zooplankton changes at six reservoirs in the Ebro watershed, Spain

In the present study, six reservoirs of the Ebro watershed were sampled during summer and autumn of 2016, with the objective of recognizing the zooplankton community, the environmental variables that are correlated with them and update the species checklist. We identify 40 zooplankton species among reservoirs: 21 rotifer species, 10 cladocerans, 8 copepods, and the veliger larvae of the invasive zebra mussel. Species that had higher abundances and biomass were: the rotifer *Polyarthra dolichoptera* (up to 278 ind/L), the cladoceran genera *Daphnia* spp. and *Ceriodaphnia* spp., the copepods *Copidodiaptomus numidicus* (83 ind/L), *Acanthocyclops americanus* (72 ind/L), *Eudiaptomus vulgaris* (62 ind/L) and zebra mussel *Dreissena polymorpha* (540 ind/L). In general, the smaller species were dominant during the summer while the larger species were dominant in the autumn. The density and biomass of zooplankton in four out of six reservoirs during the summer were double that in autumn. The main physicochemical variables correlated with the zooplankton species through a Canonical Correspondences Analysis (CCA) were: chlorophyll a, Secchi disk, total phosphorus, pH and depth. We report for the first time the species presented at the Ullibarri-Gamboa reservoir, and present new species registered in the reservoirs compared with those reported in previous studies. For the first time, the presence of the zebra mussel was detected at La Sotonera reservoir. This indicate its expansion throughout the watershed, suggesting that knowledge of zooplankton species and the changes that occur through time can be a tool for reservoirs and watershed management.

Key words: zooplankton community, reservoirs, Ebro watershed, physicochemical parameters, species seasonal variation

RESUMEN

Cambios en el zooplancton en seis embalses en la cuenca del Ebro, España

En el presente estudio, seis embalses de la cuenca del Ebro fueron muestreados durante las estaciones de verano y otoño de 2016, con los objetivos de conocer la estructura de la comunidad de zooplancton, las variables ambientales con las que se correlación y actualizar los listados de especies. Se identificaron 40 especies de zooplancton entre todos los embalses; 21 especies de rotíferos, 10 de cladóceros, 8 de copépodos y las larvas veligeras del invasivo mejillón cebra. Algunas de las especies que tuvieron mayores abundancias y biomasa fueron: el rotífero Polyarthra dolichoptera *(hasta 278 ind/L), los géneros de cladóceros* Daphnia *spp, y* Ceriodaphnia *spp., los copépodos* Copidodiaptomus numidicus *(83 ind/L),* Acanthocyclops americanus *(72 ind/L),* Eudiaptomus vulgaris *(62 ind/L) y el mejillón cebra* Dreissena polymorpha *(540 ind/L). En general, durante el verano las especies de menor tamaño fueron las dominantes mientras que en el otoño tuvieron una mayor dominancia las especies de mayor tamaño. La densidad y biomasas del zooplancton en cuatro de los seis embalses durante el verano fue el doble que en otoño. Las principales variables fisicoquímicas correlacionadas a las especies de zooplancton a través de un Análisis de Correspondencias Canónicas (ACC) fueron: clorofila a, disco de Secchi, fosforo total, pH y la profundidad. Se reportan por primera vez las especies presentes en el embalse de Ullibarri-Gamboa, además de que se presentan nuevas especies registradas en el resto de los embalses contra aquellas reportadas en estudios anteriores. Se detecto por primera vez la presencia del mejillón cebra en el embalse de La Sotonera, indicando su expansión a través de la cuenca, esto nos sugiere que el conocimiento de las especies del zooplancton y los cambios que presentan a través del tiempo puede ser una herramienta para el manejo de los embalses y la cuenca.*

Palabras clave: *comunidad del zooplancton, embalses, cuenca del Ebro, parámetros fisicoquímicos, variación estacional de especies*

INTRODUCTION

Zooplankton is, an important component inside the freshwater ecosystem, playings a big role in the transfer of energy in the aquatic food web between primary producers and higher consumers, while significantly contributing to the recycling of nutrients (Lampert & Sommer, 1997).

Besides their essential role in trophic levels of aquatic environments, this group can also provide valuable information that other groups cannot. For example, changes in certain metrics such as, size, proportion of large and small zooplankton, mean of body weight and proportion of resting eggs together with the zooplankton:phytoplankton biomass ratio, which can indicate a "top-down" process (Jeppesen *et al.*, 2011). Top-down control is one of the main attributes of zooplankton. This occurs when zooplankton consumes high quantities of phytoplankton and becomes a pressure factor, this pressure can determine the composition of phytoplankton assemblage and decrease their abundances and biomass (Naselli-Flores & Rossetti, 2010). On the other hand, the zooplankton community can respond quickly to phytoplankton blooms during the bottom-up control (Carpenter *et al.*, 1985), such as, changes in the biomass, the proportion of calanoids copepods and numbers of rotifers could indicate this process (Jeppesen *et al.*, 2011). Due to their pivotal position in the transfer of nutrients and energy in aquatic food webs and the valuable data they can provide, it is essential to have a wide knowledge of zooplankton composition and the factors related to this group (Caroni & Irvine, 2010).

All the species and individuals that make up the zooplankton community exhibit diverse responses to changes (Stemberger *et al.*, 2001). These changes can be done by biotic (e.g. food availability, predation and competition) and abiotic (physical and chemical habitat conditions: temperature, dissolved oxygen, pH, etc.) factors, as both can affect the species richness, increasing or decreasing their abundances and biomass, and promoting shifts in their diversity (Jeppesen *et al.*, 2000; Wetzel, 2001; Dodson *et al.*, 2009; Bonecker *et al.*, 2013). Hence, studies focused on such factors can provide useful information to manage natural resources (Gulati *et al.*, 1990) as well as the understanding of how its community structure (species richness, density and abundances) varies with time and in different aquatic systems (Dodson *et al.*, 2009; Boix *et al.*, 2008).

Seasonal variation also has an important role in waterbodies. Over the course of a year many environmental variables can suffer big changes depending on the season (Margalef, 1983). On a regional and local scale, these seasonal changes in natural components, in addition to anthropogenic pollution, can impact on aquatic communities and affect the zooplankton groups in different ways (Tavernini *et al.*, 2009).

Many studies have correlated density, species richness and the presence or absence of zooplankton, for example, rotifers (Sladečeck, 1983; Ejsmont-Karabin, 1995, 2012; May & O'Hare, 2005) and micro-crustaceans (Pinto-Coelho *et al.*, 2005) to the trophic gradient. Moreover, zooplankton, can be an element in evaluating the trophic state of reservoirs and lakes (Haberman *et al.*, 2007; Haberman & Haldna, 2014) and a good indicator of the different trophic states related to natural processes, man-made activities and climate changes (Jeppesen *et al.*, 2011). Recently, in man-made reservoirs have proved that even zooplankton density can be a tool to determinate the trophic state of a large watershed in Spain (Garcia-Chicote *et al.*, 2018). Although the Water Framework Directive has the aim of evaluating the European waters through several Biological Quality Indicators, zooplankton and its valuable data is not included as one of these indicators.

Despite the Ebro watershed being the second large watershed in Spain, studies related to zooplankton presence in the reservoirs are few and focus principally on rotifera phylum´s description or distribution (De Manuel & Armengol, 1993; De Manuel, 2000). In the present study we focus on zooplankton communities of six reservoirs located across the watershed. These were chosen due to the fact that existing data is more than 30 years old (De Manuel & Jaumel, 1993) or no previous data was available. The lack of information of these reservoirs throughout the last three decades could significate changes in species composition and non-detected invaders. Therefore, it´s important to update the current knowledge on zooplanktonic fauna in this watershed and know how environmental variables can affect the composition of communities through seasons. Thus, all this information could be a helpful tool for reservoirs management.

The main objectives of this study were; first, report and compare the zooplankton composition (species richness, density and biomass) during two studied seasons (summer and autumn) in six reservoirs at the Ebro watershed. Second, determine the environmental variables related to the zooplankton groups structure (density and biomass). Third, update information on the zooplanktonic fauna and verify if new species are present in the reservoirs compared with available data of previous studies.

MATERIAL AND METHODS

Study area

The data presented in this study was obtained from six reservoirs, located in different areas and altitudes along the Ebro watershed (Fig. 1). Each reservoir was sampled at the beginning of two different seasons in 2016: summer (last week of June) and autumn (last week of September). One sampling point was established at each reservoir in the deepest part of the reservoir at 300-500 meters from the dam.

Environmental Variables

For each reservoir the following variables were measured along the water vertical profile, temperature, conductivity, dissolved oxygen, pH, turbidity and chlorophyll *a*, all *in situ* measurements, by

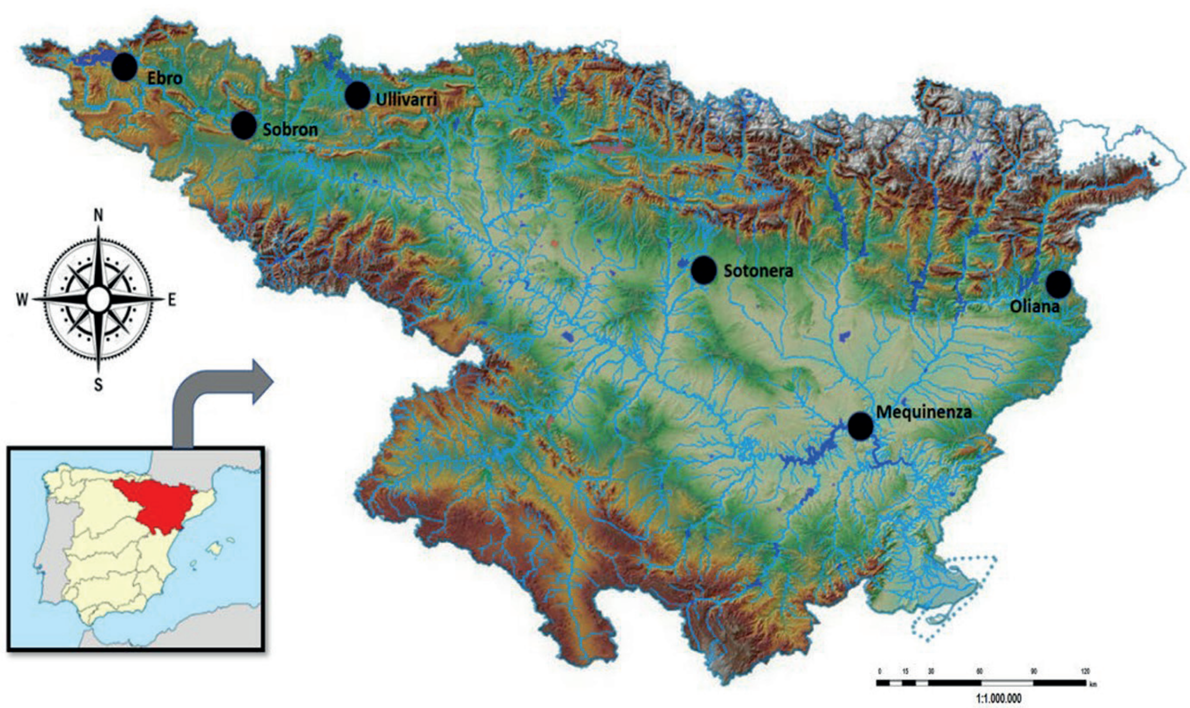

Figure 1. Location of the Ebro Watershed with the sampled reservoirs. *Localización de la Cuenca del Ebro con los embalses muestreados.*

means of a multiparametric devise Sea-Bird 19 plus V2. The depth of the photic zone was calculated by measuring the light penetration using a quantometer. The water transparency was determined measuring the Secchi disk depth (SD). An integrative water sample was collected from the photic zone of each reservoir using a 25 mm ballasted tube technique for *ex-situ* analyses (Vicente *et al.*, 2005). For measurements of the following variables, we used the standard methodology (APHA, 1998) described for suspended solids (APHA 2540D), turbidity (ISO7027-1999), total nitrogen (TN) (APHA method 4500-N C), total phosphorus (TP) (4500-P B/APHA 4500-P C), and chlorophyll *a* (Shoaf & Lium, 1976). The complete data set of environmental variables can be found at C.H.E. (2016).

To estimate the reservoir´s trophic conditions, we used the criteria of the trophic state index (TSI) (Carlson, 1977). The TSI´ values of each reservoir were obtained with the following formulae (Carlson & Simpson, 1996):

Total phosphorus; $TSI (TP) = 14.42 \ln (TP) + 4.15$
Chlorophyll *a*, $TSI (Chl\text{-}a) = 9.81 \ln (Chl\text{-}a) + 30.6$
Secchi disk, $TSI (SD) = 60 - 14.41 \ln (SD)$

Total phosphorus and Chl-*a* are measured in micrograms per liter (µg/L) and Secchi disk depth is expressed in meters. TSI is the average value of the three above mentioned variables.

$$TSI' = [(TSI(TP) + TSI (Chl\text{-}a) + TSI (SD))/3]$$

Zooplankton samples

The zooplankton samples were collected using a Ruttner bottle with a capacity of 2.7 L. For each reservoir were taken two Ruttner bottles to obtain 5.4 liters of water sample, then the sample was filtered through 30 µm mesh size Nytal, fixed with formaldehyde at 4 % final concentration and stored in a hermetic glass vial. The sample depth was established in each reservoir at the beginning of oxygen decline, where has been reported as the richest zone of zooplankton fauna (Miracle & Vicente, 1983). Also, a zooplankton vertical tow net of 50 µm mesh size Nytal was towed from 30 m deep to the surface, collected and fixed with formalin. These vertical tow net samples were taken mainly for taxonomic purposes.

Zooplankton species were identified using the following guides: Ruttner-Kolisko (1974), Koste (1978), Nogrady *et al.*, (1995) and Nogrady & Segers (2002) for rotifers, Alonso (1996) for cladocerans, and Dussart (1967, 1969) for copepods.

For quantitative results, we used the samples taken from the Ruttner bottles, all individuals were counted using a Sedgewick Rafter-type counting chamber under inverted microscopy. After individuals were counted and densities were obtained, we calculate the biomass, to determine it, a minimum of 30 specimens of all species were measured and using the formulas that relate the total length with the dry weight of the specimens were obtained the corresponding conversion factors (Ruttner-Kolisko, 1977; Dumont *et al.*, 1975; Culver *et al.*, 1985). The Shannon–Wiener diversity index (H’) (Shannon & Weaver, 1963) was calculated from data on the abundance of zooplankton for each reservoir at both seasons.

Statistical analysis

The correlation coefficients between zooplankton data and the environmental factors were calculated by linear Pearson correlations. Analysis of similarity (ANOSIM) tests were performed on the zooplankton data to determine which, if any, reservoirs showed significant differences in zooplankton community structure between the two seasons. ANOSIM is a nonparametric analogue to analysis of variance and tests for multivariate differences between groups based on Bray-Curtis distance and rank dissimilarity. Also, we ran a similarity percentage routine (SIMPER), to test which zooplankton species were contributing to the community changes. The SIMPER routine uses average Bray-Curtis dissimilarities between all pairs of sites to produce a percent contribution from each species, identifying the species most responsible for the dissimilarity (Clarke & Warwick, 2001).

To determine the influence of different factors on zooplankton we performed two canonical correspondence analysis (CCA). For the first, we

Table 1. Complete data of physicochemical variable measurements during both seasons, data modified from C.H.E (2016). *Datos completos de los parámetros fisicoquímicos medidos durante las dos sesiones. Datos modificados de C.H.E. (2016).*

		Summer						Autumn					
Parameter	**unit**	ULL	MEQ	EBR	OLI	SOB	SOT	ULL	MEQ	EBR	OLI	SOB	SOT
Temperature	**°C**	20.80	23.62	18.15	18.87	21.59	23.50	19.49	24.64	18.41	22.99	17.77	23.29
Disolved oxygen	**mg/L**	7.16	6.79	7.84	8.70	7.31	8.04	5.85	3.52	7.95	7.45	6.87	7.29
Conductivity	**µS/cm**	244	815	188	187	324	331	219	1288	195	270	255	318
pH		8.43	8.24	8.18	8.45	8.31	8.34	8.09	7.94	7.87	8.35	7.89	8.38
Depth	**m**	23	57	20	50	27	20	21	53	14	37	27	12
Secchi	**m**	3.50	3.70	4.50	2.90	1.70	2.25	7.75	3.80	1.40	2.70	2.00	1.10
Suspended solids	**mg/L**	1.12	2.13	1.29	4.32	4.47	3.40	0.79	2.02	7.41	4.11	3.39	7.56
Turbidity	**NTU**	1.88	1.88	2.89	3.80	4.37	1.61	1.55	1.22	6.99	3.86	4.47	4.74
Alkalinity	**Meq/L**	2.28	3.12	1.24	1.52	2.32	2.52	2.00	2.85	1.24	1.92	1.88	2.24
Chl-*a*	**µg/L**	2.26	3.66	2.26	6.73	11.13	3.38	1.37	3.69	5.00	21.14	3.03	3.69
TN	**µg/L**	660	217	450	680	810	450	324	1692	427	622	484	276
TP	**µg/L**	9.41	9.12	13.54	80.78	25.50	8.39	22.44	13.00	26.48	25.00	19.29	16.00

analyzed those variables that are corresponded to the principal zooplankton species. For the second, we performed an analysis using the zooplankton groups (rotifers, copepods, cladocerans and zebra mussels). For each CCA we included the densities and or biomass of zooplankton and the following environmental variables (temperature, dissolved oxygen, conductivity, turbidity, pH, Secchi disk, depth, nutrients (TP and TN) and Chl-*a*. In order to normalize the data, they were transformed logarithmically Log(x + 1), except for pH. The models were tested using Monte Carlo permutation (n = 499). Nauplius, copepodites and bdelloid rotifers were excluded since they were not identified to species level. Both CCAs were executed using the Canoco 4.5 for Windows computer program (Ter Braak & Šmilauer, 2002).

RESULTS

Environmental parameters

During the two seasons of this study the physical and chemical parameters varied at the different reservoirs, complete data is reported in Table 1. The water temperature on average was higher during summer in all waterbodies, except at Oliana, where it was higher in autumn. In general, the dissolved oxygen presents higher values during summer than those in autumn. The pH values do not show an important difference between seasons because the buffer effect of the bicarbonate in the waters and the conductivity values were stable (with exception of Mequinenza during autumn with a peak of 1288 µS/cm). Suspended solids in both seasons were similar in four of the six reservoirs, however, data from the Ebro and La Sotonera reservoirs during the autumn were double compared to the summer data. The Secchi disk visibility presented a wide variability among reservoirs and seasons: Ebro, Oliana and La Sotonera had higher values in summer, nevertheless, Ullibarri-Gamboa, Mequinenza and Sobrón were higher during autumn.

In the case of Chl-*a*, higher values were presented during the summer at Ullibarri-Gamboa and Sobrón, and during the autumn at Ebro and Oliana. The Oliana reservoir (autumn) had the biggest Chl-*a* concentration of all the study (21.14 µg/L). Finally, Mequinenza and La Sotonera had similar values during both seasons (average of 3.3 µg/L and 3.5 µg/L respectively). Total Nitrogen (TN) values at 5 of the reservoirs were higher during summer, only Mequinenza presented a high peak in autumn (1692 µg/L). The higher values of total phosphorus (TP) were reported in autumn, except at Oliana (80.78 µg/L) and Sobrón (25.5 µg/L), where the higher values were during summer.

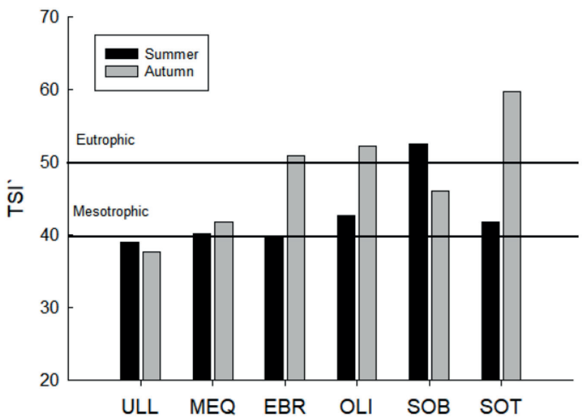

Figure 2. Reservoir TSI´ values, black charts (summer) and gray charts (autumn). *Valores de TSI´ de los embalses, barras en negro (verano) y gris (otoño).*

Trophic state

With the data obtained, we calculated the Trophic State Index (TSI) for every reservoir and season, the waterbodies were ordinated according to their TSI from lower to higher values. Ullibarri-Gamboa shows the lower trophic value in both seasons (39.01, summer and 37.70, autumn) and is classifiedd as oligotrophic. While Sobrón had the higher value during the summer (52.5) and La Sotonera in autumn (59.8). The reservoirs during the summer generally presented values between 30 to 50 and during autumn the values increase from 40 to 60. According to Carlson (1996), most reservoirs are classified as mesotrophic, however, Ebro, Oliana and La Sotonera are eutrophic during the autumn and Sobrón during the summer (Fig. 2).

Zooplankton

We registered a total of 40 zooplankton species during both seasons in the six reservoirs (Table 2). The rotifers were the group with more species reported (21), followed by cladocerans (10) and copepods (8). Since the veliger larvae of the zebra mussel invader (*Dreissena polymorpha*) were found at 4 reservoirs, they were considered a separate group inside this study and both abundance and biomass were counted. La Sotonera presented the highest number of species with, 13 in each season, followed by Sobrón with 12 in the summer, Oliana and Mequinenza with 11 during summer. This same species richness was present in Ebro and Ullibarri-Gamboa in the autumn. The lowest number of species was in Sobrón with only 6 during the autumn (Table 2). On average, each reservoir presented 10 zooplankton species per season. The rotifer *Polyarthra dolichoptera* was presented on all reservoirs in at least one season, followed by the zebra mussel, which was detected on four reservoirs during both seasons. The two copepod species *Cyclops vicinus* and *Cyclops* sp., and some rotifers were only presented in one reservoir during one season (Table 2).

The only previous study on these reservoirs was performed during 1987-1988 and reports data of summer and winter seasons. However, to compare species composition per season we only used the summer data from both studies, since the other season is not the same and cannot be compared equally (winter from the previous study and fall in the current). To indicate new registers for each reservoir we verified that species were not present in the data of both seasons from the previous study. The complete list of species present of the previous study can be found in De Manuel & Jaume (1993).

The new registers of zooplankton species for each reservoir are: Sotonera (*Bosmina longirostris*, *Ceriodapnia dubia*, *Ceriodaphnia pulchella*, *Daphnia galeata*, *Diaphanosoma mongolianum*, *Acanthocyclops americanus*, *Anuraeopsis fissa*, *Polyarthra major*, *Ascomorpha ecaudis* and *D. polymorpha*). Ebro (*Eudiaptomus vulgaris*, *B. longirostris*, *D. mongolianum*, *Conochilus unicornis*, *Trichotria tetractis*, *P. major*, *Tricochercca cylindrica*). Mequinenza (*Copidodiaptomus numidicus*, *Thermocyclops dybowskii*, *A. ecaudis*). Sobron (*Daphnia cucullata*, *Cyclops vicinus*, *Asplanchna priodonta*, *P. major*, *A. fissa*) and Oliana (*A. americanus*, *E. vulgaris*, *C. sphaericus*, *D. mongolianum*, *Kellicotia longispina*). Since there is not previous data available for Ullibarri-Gamboa reservoir, all 14 species reported for this study are first register (Table 2).

Table 2. Complete list of zooplankton species found in the six reservoirs. *Listado completo de las especies de zooplancton presentes en los seis embalses.*

	Summer						Autumn					
	ULL	MEQ	EBR	OLI	SOB	SOT	ULL	MEQ	EBR	OLI	SOB	SOT
Cladocera												
Bosmina longirostris	X			X	X	X	X		X	X		X
Ceriodapnia dubia		X		X				X				
Ceriodaphnia pulchella					X	X	X		X	X	X	X
Chydorus sphaericus					X				X	X		
Daphnia cucullata	X				X		X				X	
Daphnia galeata				X		X				X		
Daphnia longispina							X		X			
Daphnia pulicaria			X									
Diaphanosoma mongolianum		X				X		X		X		
Pleuroxus sp.										X		
Copepoda												
Acanthocyclops americanus				X		X	X			X		X
Copidodiaptomus numidicus		X						X				
Cyclops abyssorum			X						X			
Cyclops vicinus					X							
Eudiaptomus vulgaris			X	X					X	X		
Neolovenula alluaudi						X						X
Thermocyclops dybowskii		X						X				
Cyclops sp.											X	
Rotifera												
Anuraeopsis fissa	X										X	X
Ascomorpha ecaudis		X				X		X				
Asplanchna priodonta		X		X		X						
Brachionus angularis	X											
Brachionus calcyflorus					X							
Brachionus havanaensis											X	
Brachionus quadridentatus					X							
Conochilus unicornis	X		X									
Filinia longiseta									X			
Hexarthra fennica								X				
Hexarthra oxyuris							X					
Kellicotia longispina longispina				X								
Keratella cochlearis	X	X	X	X		X	X	X				X
Keratella cochlearis tecta						X	X					X
Keratella quadrata				X								
Polyarthra dolichoptera	X	X	X	X	X	X	X	X	X	X		
Polyarthra major					X		X		X			X
Synchaeta pectinata	X				X	X						X
Synchaeta sp.			X							X		
Trichocerca cylindrica									X			
Trichotria tetractis			X									
Others												
Dreissena polymorpha	X	X			X	X	X	X			X	X

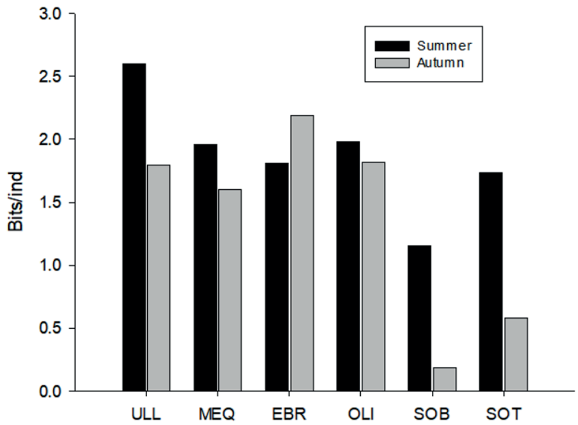

Figure 3. Shannon-Wiener diversity index (black bars represents summer, gray bars autumn). *Diversidad de Shannon-Wiener (barras negras representan verano, grises otoño).*

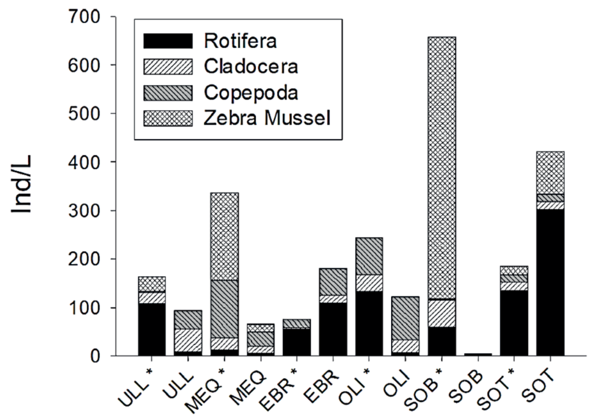

Figure 4. Abundances of zooplankton groups in the six reservoirs, (*) indicate summer values. *Abundancias de los grupos de zooplancton en los seis embalses, (*) indica valores de verano.*

Density and biomass

The zooplankton density varied in each reservoir and season, the average of individuals for all the reservoirs during summer was 277 ind/L, that was higher than in autumn with 148 ind/L. The higher densities in most of reservoir belong to rotifers and zebra mussels, except in Ullibarri-Gamboa, Mequinenza and Oliana during winter (Fig. 4).

In terms of biomass, microcrustaceans have a bigger role instead of rotifers, and each reservoir varied in quantity and group that dominates during both seasons. The reservoirs with major changes between biomass were La Sotonera (dominated during the summer for microcrustaceans to zebra mussels in fall), Sobrón (zebra mussels to cladocerans) and Ullivarri-Gamboa (cladocerans, rotifers and zebra mussels to microcrustaceans mainly) (Table 3). The Shannon-Wiener index indicated that diversity in the Ullibarri-Gamboa reservoir was the highest overall 2.59 bits/ind in summer. The lowest diversity was found in Sobrón during autumn with only 0.18 bits/ind (Fig. 3).

Data analysis

Through the linear Pearson correlations between environmental factors and zooplankton groups we found that pH was significantly correlated with the density of rotifer group (r^2 0.35, $p < 0.05$). Also, both zebra mussel density and biomass were positively correlated with Chl-*a* (r^2 0.60, $p < 0.05$ and r^2 0.62, $p < 0.05$ respectively). Besides, copepods density (r^2 0.39, $p < 0.05$) and biomass (r^2 0.34, $p < 0.05$) were correlated with the reservoir´s depth. Other correlations were not significant ($p > 0.05$). The Analysis of similarity (ANOSIM) doesn't show any difference between both seasons ($p > 0.05$). The contribution of the individual taxa in the dissimilarity of zooplankton was low (SIMPER values < 5 %), being *A. priodonta*, *C. pulchella*, *Synchaeta pectinata* and *D. mongolianum* the responsible for the cumulative of 20 % in the variance of dissimilarity between seasons.

The first CCA, related the physicochemical variables with the principal zooplankton species. The first two axes explains 45.2 % of the variance (*p* value 0.001 in the Monte Carlo permutation test). Temperature, conductivity and depth are strongly related to copepods (*C. numidicus*, *Cyclops* sp. and *T. dybowskii*) and the cladoceran *C. dubia*. Two of the most abundant rotifers are related with the pH (*S. pectinata* and *C. unicornis*) in addition to the cladoceran *D. cucullata*. A big group composed principally by cladocerans, few copepods and rotifers were related to dissolved oxygen (DO), turbidity, TP, Chl-*a* and suspended solids (SS) (Fig. 5). The rotifer *P. dolichoptera*

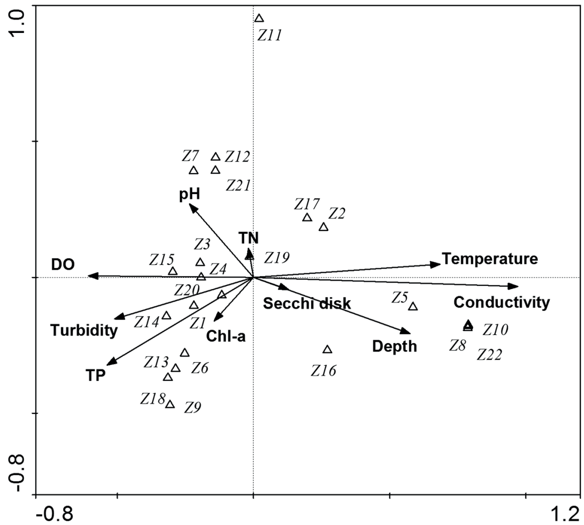

Figure 5. Canonical Correspondences Analysis of the 22 zooplankton main species. DO = Dissolved oxygen, TP = Total phosphorus, TN = Total nitrogen, Chl-*a* = chlorophyll *a*. *Análisis de Correspondencias Canónicas de las 22 especies principales del zooplancton, DO = Oxígeno disuelto, TP = Fosforo total, TN = Nitrogeno total, Chl-*a *= clorofila.* Z1 *Acanthocyclops americanus*, Z2 *Asplanchna priodonta*, Z3 *Bosmina longirostris*, Z4 *Ceriodaphnia pulchella*, Z5 *Ceriodapnia dubia*, Z6 *Chydorus sphaericus*, Z7 *Conochilus unicornis*, Z8 *Copidodiaptomus numidicus*, Z9 *Cyclops abyssorum*, Z10 *Cyclops* sp., Z11 *Cyclops vicinus*, Z12 *Daphnia cucullata*, Z13 *Daphnia galeata*, Z14 *Daphnia longispina*, Z15 *Daphnia pulicaria*, Z16 *Diaphanosoma mongolianum*, Z17 *Dreissena polymorpha*, Z18 *Eudiaptomus vulgaris*, Z19 *Neolovenula alluaudi*, Z20 *Polyarthra dolichoptera*, Z21 *Synchaeta pectinata*, Z22 *Thermocyclops dybowskii*.

was in the middle of the ordination plot, this rotifer was present in all reservoirs during both seasons, their highest abundances were during the summer at La Sotonera (279 ind/L) and Oliana (125 ind/L). The *Daphnia* group was related to DO, TP and turbidity. The *Daphnia* species were present in five reservoirs and their seasonality was split into those which had higher abundances in summer (*D. cucullata*, *D. galeata* and *D. pulicaria*) and in autumn (*Daphnia longispina*). Finally, the copepod *C. vicinus* is not related to any variable and the zebra mussels are slightly connected with pH and alkalinity (Fig. 5).

In the second CCA, we analyzed the environmental variables related with the zooplankton density and biomass, the first two axes represent the most explanatory value (93.2 %)

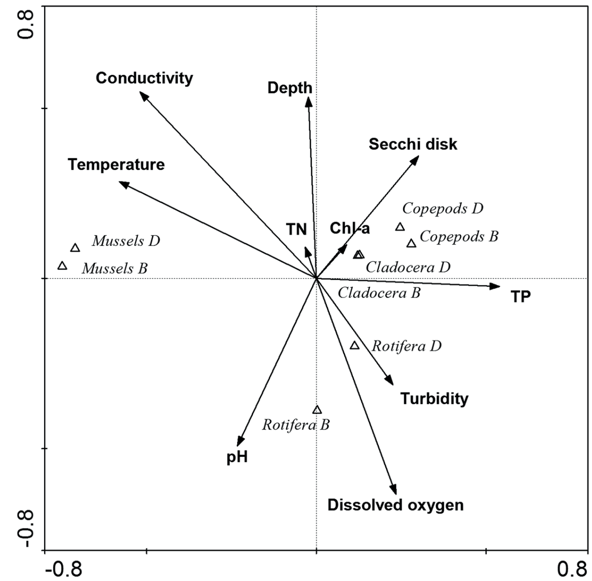

Figure 6. Canonical Correspondences Analysis of the zooplankton groups, D = density, B = biomass, TP = Total phosphorus, TN = Total nitrogen, Chl-*a* = chlorophyll *a*. *Análisis de Correspondencias Canónicas de los grupos del zooplancton, D = densidad, B = biomasa, TP = Fosforo total, TN = Nitrogeno total, Chl-*a *= clorofila.*

(*p* value > 0.05 in the Monte Carlo permutation test). This CCA indicates that in the first axis the trophic indicators are related (SD, Chl-*a* and TP). These principal indicators are related with both cladocera and copepoda density and biomass, while rotifer density is related to combination of TP, turbidity and DO. The biomass of rotifera group presents a similar relation with pH instead of turbidity. Finally, both density and biomass of zebra mussels are related to temperature and conductivity (Fig. 6).

DISCUSSION

Sommer *et al.* (1986) together with the PEG (Plankton Ecology Group) proposed a model where sequential statements describe the changes in zooplankton and phytoplankton communities in lakes. In these statements, they described that in summer the smaller groups with short generational life cycle dominate and during autumn large species appear. In our study, we found that most of the changes in reservoirs' communities followed these statements, for example, the general tendency at Oliana was that rotifers had high abundances during summer, then, during autumn this group tended to decrease and microcrustaceans increased in number becoming the dominant group. Ullibarri-Gamboa presented a similar tendency but with higher abundances of rotifers and mussels during the summer, followed by the increase of copepods and cladocerans during autumn. At the Ebro and La Sotonera reservoirs, the number of all groups increase in autumn but with the rotifers being the dominant group. However, at Mequinenza the zebra mussels and copepods were dominants during summer reaching up 350 ind/L, but with a decrease during autumn. Sobrón shows a similar tendency, the summer was dominated by the zebra mussel (540 ind/L) and in the next period densities of all groups decreased dramatically. For these two last reservoirs several factors could explain these changes, such as an extreme fish predation (Amundsen *et al.*, 2009; Ginter *et al.*, 2019), the establishment of the sessile stage of mussels in any surface decreasing the number of the planktonic larvae (Claudi & Mackie, 1994) or even some criteria that were not taken in count in the previous model, such as the food quality and the trophic level of each reservoir (Sommer *et al.*, 2012).

Biodiversity is strongly related with environmental factors (Jeppesen *et al.*, 2000), while some physiochemical parameters such as temperature, dissolved oxygen, pH, etc., can have positive or negative effects on zooplankton (Wetzel, 2001). One of the more efficient analyses to correlate the zooplankton communities with the physical and chemical variables is the CCA (Attayde & Bozelli, 1998). Data from our CCA analysis shows the rotifer *P. dolichoptera*, which was positioned in the middle of the ordination plot, due to their high tolerance to different environments conditions (Bērziņš & Pejler, 1989), nowadays it has a wide distribution in many water bodies around the world (Segers, 2007). The copepod *Neolovenula alluaudi*, that is typically from the Mediterranean area (Miracle, 1982), also was positioned near the center of the CCA. We can infer that they possess high tolerance, however, compared to the previously mentioned rotifer, it was only present at La Sotonera reservoir. The populations of this copepod are moving from the south and are now found in several water bodies along the Iberian Peninsula (Alfonso & Belmonte, 2013; Miracle, 1982). Thus, this copepod was reported at Mequinenza 30 years ago (De Manuel & Jaume, 1993), but not found during the present study. Furthermore, at the Mequinenza reservoir the presence of silurids is well documented and the early stages of this fish can consume copepods and large cladocerans individuals as the *Daphnia* species, they can promote the small-size species such as *C. dubia* and *D. mongolium*, (Miranda *et al.*, 2010). Also, in this reservoir no *Daphnia* species were recorded, probably due the combination of predation and lower levels of oxygen compared to other reservoirs (Hanazato, 1996).

The copepods *C. numidicus* and *T. dybowskii* were correlated with conductivity, temperature and depth. It is well known that big-sized zooplankton species perform a daily vertical migration to avoid depredation (Hays, 2003; Lampert, 1989). The study of Caramujo & Boavida (2000) found that these two copepod species can be consumed in large numbers by fishes, for this reason, their populations are settled in deepest water bodies. In this study, we found both species only at Mequinenza, which has an average 50 m of depth in both seasons. The biggest copepod found in this study was *C. numidicus* and it provides a high percentage of total biomass and density of all copepods, thus, Pearson correlation was significative in terms of depth for this group.

In the CCA for groups (Fig. 6) the rotifer biomass was also correlated to pH, other studies have shown that this parameter can affect the rotifer occurrence (Bērziņš, 1987) and their assemblage in reservoirs (Devetter, 1998).

The complex of abundances and biomasses of microcrustaceans (copepods and cladocerans), were related with the components that conform the trophic state since they are influenced by the Secchi disk, TP and Chl-*a*. Some authors have indicated that large species of these groups can be used as an indicator of oligotrophic state (Pejler, 1983; Moss *et al.*, 2003; Kane *et al.*, 2009; Haberman & Maldna, 2014). Usually, at higher trophic level, large species are replaced by small species (Lampert & Sommer, 1997). The Ebro, Oliana and La Sotonera reservoirs, during the autumn, were mesotrophic to eutrophic, and densities of larger species as *Daphnia* spp. decay while smaller cladocerans such as *B. longirostris* and *Ceriodaphnia* spp. increase.

Despite the limitations of this work (and taking in count the low number of reservoirs sampled compared with the watershed size), with the data obtained from the CCA we could hypoth-

Table 3. Density (ind/L), Biomass (mg/L) and their percentage (%) of zooplankton groups present on the six reservoirs. *Densidad (ind/L), Biomasa (mg/L) y el porcentaje (%) de los grupos del zooplancton presentes en los seis embalses.*

	Summer				Autumn			
	ind/L	mg/L	ind/L %	mg/L %	ind/L	mg/L	ind/L %	mg/L %
Ullibarri-Gamboa								
Cladocerans	23.46	31.15	14.4	47.14	47.12	95.06	49.8	73.95
Copepods	2.31	0.34	1.42	0.52	38.85	32.87	41.06	25.57
Rotifers	107.69	22.23	66.12	33.64	8.27	0.47	8.74	0.36
Mussels larvae	29.42	12.35	18.06	18.7	0.38	0.16	0.41	0.13
Total	162.88	66.07	100	100	94.62	128.55	100	100
Mequinenza								
Cladocerans	24.62	47	7.31	11.3	15	29.54	22.61	36.75
Copepods	118.08	287.74	35.05	69.19	29.81	43.88	44.93	54.59
Rotifers	13.08	5.03	3.88	1.21	5.77	0.33	8.7	0.41
Mussels larvae	181.15	76.08	53.77	18.3	15.77	6.62	23.77	8.24
Total	336.92	415.86	100	100	66.35	80.37	100	100
Ebro								
Cladocerans	3.85	14.62	5.12	23.46	16.73	29.96	9.25	23.29
Copepods	15.96	42.38	21.23	68.04	55.19	91.83	30.5	71.39
Rotifers	55.38	5.29	73.66	8.5	109.04	6.83	60.26	5.31
Total	75.19	62.29	100	100	180.96	128.62	100	100
Oliana								
Cladocerans	34.8	75.9	14.22	44.69	24.81	36.71	20.31	48.05
Copepods	76.92	86.86	31.42	51.14	89.42	39.29	73.23	51.43
Rotifers	133.08	7.09	54.36	4.17	7.88	0.4	6.46	0.52
Total	244.8	169.85	100	100	122.11	76.4	100	100
Sobrón								
Cladocerans	54.23	104.17	8.24	29.75	0.77	1.62	19.05	64.52
Copepods	3.08	3	0.47	0.86	1.73	0.35	42.87	13.83
Rotifers	60.58	16.21	9.21	4.63	0.38	0.06	9.53	2.32
Mussels larvae	540	226.8	82.08	64.77	1.15	0.48	28.55	19.34
Total	657.88	350.19	100	100	4.04	2.5	100	100
Sotonera								
Cladocerans	16.92	24.19	9.19	39.5	15.77	24.15	3.74	23.44
Copepods	16.73	20.68	9.08	33.77	15.58	25.92	3.7	25.16
Rotifers	134.62	9.67	73.07	15.78	302.5	16.29	71.83	15.81
Mussels larvae	15.96	6.7	8.66	10.95	87.3	36.67	20.73	35.59
Total	184.23	61.24	100	100	421.15	103.03	100	100

esize the zooplankton groups, such as, copepods and cladocerans could be affected firstly and their structure modified if the variables that are more related or affect these groups change for several factors, such as, climate change, new invasive species and or anthropogenic impacts.

The zooplankton community normally varied through months, seasons or years, and the species replacement can happen quickly or change gradually with time (Lampert & Sommer, 1997). Some of these species' substitutions can be observed in the current research compared with data of previous works, at Mequinenza, from species reported previously for summer season we found only two shared species. Larger filter species such as *N. alluaudi* and *D. galeata* together with the main predator *A. robustus*, were substituted for *C. numidicus* and *T. dybowskii*. A similar case occurred at Sobrón, where only three species were shared. From three cyclopids species to only *C. vicinus* and the presence of *D. galeata* and *C. pulchella*.

The reservoir with the most shared species was Oliana, with seven of the nine species reported for this study. The main change observed was *C. abyssorum* to *A. americanus*. The Sotonera reservoir was the only reservoir where the two previous copepod species did not suffer any variation, however, cladocerans from two *Daphnia* species changed to one species (*D. galeata*) and medium-size filters as *C. dubia* and *D. mongolianum*. The study of Higgins & Vander Zanden (2010) suggests that *D. polymorpha* can reduce the zooplankton biomass to 40-77 % in pelagic areas and replace them, this affect the species richness and diversity. The low replacement at Oliana could be related to the non-presence of them. In contrast, some changes can be appreciated at La Sotonera and Sobrón with low diversity (Fig. 3) for their increase in density and biomass (Table 3).

For the Ebro reservoir, there is a great difference in the number of zooplankton species between studies since previously 20 species were reported, where almost half of the species were microcrustaceans including several species of *Daphnia* and cyclopids, however, we registered only four shared species. Nowadays, only eight species are present, where five of them belong to rotifers and only *D. pulicaria* and *C. abyssorum* were reported before. Thus, all these data indicate that the communities have changed, increasing, or decreasing the number of species and being replaced for others. Several explanations such as competition, natural succession or even variations of environmental variables (Devetter, 1998; Dodson *et al.*, 2009) could explain these changes, however, since there is a lack of information for all non-reported years, the question of which exact events caused these changes remains unanswered.

Due to diverse factors, including management, most of water bodies cannot be sampled on a regular basis to confirm the species presents and like in this study, can take a long time until having new data. Nevertheless, having a monitoring program could help us to understand the community changes. But this is not the only benefit, thus, it can be a tool to have complete knowledge of species richness and to identify the already reported and the newly invasive species. For the invasive fauna, correct actions could prevent their introduction and dispersal along the watershed area, which could not only affect local diversity and become one of the major aquatic stressors, as is the case with zebra mussels (Strayer, 2010), but also create economic losses due to their impact on important infrastructures (Duran *et al.*, 2012).

The Zebra mussels were detected for first time at the Ebro watershed in 2001 (Duran & Anadón, 2008). Previously at La Sotonera reservoir the presence of *D. polymorpha* was not detected, however, now the veliger larvae can be found at both seasons and it's a dominant component of zooplankton. Thus, the mussel invasion has progressed throughout the years and among different reservoirs. The two reservoirs were mussels were not present are Oliana and Ebro, this last is under special protection (Duran & Anadón, 2008). Due to the lack of natural predators, efficient competition and non-intentional dispersion of invaders caused by the interaction between people among the reservoirs in the area, this invader could be detected in the Ebro reservoir in the upcoming years. Consequently, they would be present from the beginning until the end of watershed.

CONCLUSION

Our results show that abundances and biomass values were in general two times higher in summer than values in autumn. However, there is not an equal tendency for all reservoirs and each one works in a different way. The data suggest that the changes in the zooplankton community during both seasons are related mainly with physico-chemical variables as Chl-*a*, SD, TP, pH and reservoir depth, as well as with biotic interactions, like competition with alien species such as *D. polymorpha*. The relation between the zooplankton groups and the environmental variables could help us understand the main changes that could occur in a shifting world. All reservoirs presented new records in zooplankton species. La Sotonera had the highest number of new registers with ten species, followed by the Ebro with seven, while Oliana, Sobrón and Mequinenza have five. For Ullibarri-Gamboa reservoir we showed for the first time a record of zooplankton species. Also, we detected for first time the presence of zebra mussels at La Sotonera reservoir, indicating that this invader is dispersing throughout the watershed. Therefore, zooplankton composition knowledge, regular monitoring of species inhabiting in the reservoirs and the understanding of environmental variables that affect species and zooplankton structure (specific richness, density and biomass) can be a helpful tool for watershed management and early detection of invasive species.

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