



Can zooplankton species be used as indicators of trophic status and ecological potential of reservoirs?

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Received: 6 December 2020 / Accepted: 19 August 2021 / Published online: 9 September 2021
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Abstract The European Water Framework Directive implements the policies to achieve a good ecological status of all European waterbodies. To determine the ecological potential in freshwater environments, abiotic (morphology, physical and chemical variables) and biotics (algae, fishes, etc.) metrics are used. Despite their importance in trophic web, zooplankton was not included as one of the Biological Quality Elements (BQE) to determine the water quality. In the present research, we studied the zooplankton species that can be considered as indicators of trophic status and ecological potential for more than 60 water reservoirs. The data were obtained from more of 300 samples collected during 10 years from reservoirs at Ebro River watershed, which is the

largest basin in Spain. According to their physico-chemical and biological elements, the trophic status and ecological potential of these reservoirs were established. More than 150 zooplankton species were identified during the study. The results from this research indicate that species that are related with low water quality are: *Acanthocyclops americanus*, *Ceriodaphnia* spp., *Daphnia cucullata*, *Daphnia parvula*, *Diaphanosoma brachyurum*, *Brachionus angularis*, *Keratella cochlearis* and *Phompolyx sulcata*. An indicator of moderate quality was *Bosmina longirostris*, while *Daphnia longispina*, *Ascomorpha ovalis* and *Ascomorpha saltans* were considered as indicators of good water quality. The data obtained suggest that zooplankton species can be used as a valuable tool to determine the water quality status and should be considered, in a near future, as one more of the BQE within the WFD metrics.

Handling Editor: Nandini Sarma.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10452-021-09897-8>.

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Keywords Bioindicators · European Water Framework Directive · Dams · Ebro watershed · Water quality

Introduction

There is an ever-increasing pressure on water resources and freshwater cultural eutrophication (Schindler 2012). This cultural eutrophication is due

to the increase in nutrient input (mainly nitrogen and phosphorus) directly into lakes, reservoirs, rivers or inside the catchment basin area. The nutrient increase is principally due to industrial activity and high human population growth and, increasing use of fertilizers in agriculture together with the effects of climate change, and can result in the degradation of inland waters (Moss 2011).

The European Water Framework Directive (Directive 2000) was introduced to present the requirements and assessments to control the water quality and classify the waterbodies into different “Ecological Status” in the European Union. The aim of the Water Framework Directive (WFD) is to achieve a “good ecological status” in all waterbodies. The classification of waterbodies is obtained through the unique hydro-morphologic, physical and chemical characteristics and a Biological Quality Element (BQE). The last parameter comprises benthic invertebrates, fish fauna, macrophytes, phytobenthos and phytoplankton. The BQE algae is one of the most used and accepted indicators to evaluate the ecological potential using plankton data. However, zooplankton, despite their fundamental position in food webs (Haberman and Haldna 2014) in freshwater ecosystems, was surprisingly not included (Moss 2007) and without a scientifically sound explanation for their omission (Caroni and Irvine 2010; Jeppesen et al. 2011; Moustaka-Gouni et al. 2014).

Zooplankton play an important role in energy transfer in trophic webs between primary producers and higher consumers and thus contribute significantly to nutrient recycling (Lampert and Sommer 1997). Due to their pivotal position in aquatic environments, the zooplankton community is strongly related with higher and lower levels of the trophic web. They can be affected by phytoplankton blooms during bottom-up processes and respond quickly (Jeppesen et al. 2011; Stamou et al. 2019) or apply pressure in the top-down control and determine the phytoplankton composition and abundance (Naselli-Flores and Rossetti 2010). Also, physical and chemical parameters such as temperature, dissolved oxygen, pH, conductivity and turbidity can determine species assemblages in the water column (Lampert 1997; Devetter 1998; Špoljar et al. 2018). Zooplankton thus have characteristics to be indicators of environmental conditions and trophic status (Anas et al. 2013; Kuczyńska-Kippen et al. 2020).

Several studies in the past pointed to zooplankton as useful indicators (Gulati 1983; Sládeček 1983; Berziš and Pejler 1989). These days many authors have presented the utility of zooplankton as indicators of water quality and trophic state in water bodies using only one group of zooplankton such as rotifers (Duggan et al. 2001; May and O’Hare 2005; Ejsmont-Karabin 1995, 2012; Galir et al. 2018) or microcrustaceans (Boix et al. 2005; Pinto-Coelho et al. 2005; Haberman et al. 2007; Cheng et al. 2010; Ejsmont-Karabin and Karabin 2013; Jensen et al. 2013). Some other studies have considered both groups of zooplankton in general (Caroni and Irvine 2010; Jeppesen et al. 2011; Brito et al. 2011; Obertegger and Manca 2011; Haberman and Haldna 2014; Kehayias and Doulka 2014; Ochocka and Pasztaleniec 2016; Tasevska et al. 2017; Pocięcha et al. 2018; Stamou et al. 2019).

In the Iberian Peninsula, recent studies have shown that zooplankton abundance (Garcia-Chicote et al. 2018) and community structure could be good indicators of trophic state in reservoirs in different basins, such as Jucar (Garcia-Chicote et al. 2019), Cavado (Almeida et al. 2020) and Ebro (Montagud et al. 2019). This last study presented the Zooplankton Reservoir Trophic Index (ZRTI) and can be considered as a preliminary approach to the present research. The reservoirs have a high importance in the socio-economic development of the Mediterranean region due to seasonal water scarcity. The main uses of these water resources are for human population requirements, large-scale agricultural irrigation and industrial use (Ibañez and Prat 2003; Cudennec et al. 2007).

The aim of this study was to determine the species of the three main zooplankton groups (rotifers, cladocerans and copepods) that are good indicators or are related to different trophic states in the reservoirs located in the Ebro watershed, using a robust data set collected during the last ten years in 66 reservoirs involving more than 300 sampling occasions. Also, following the guidelines of the WFD we assessed the water quality of the reservoirs and determined the species of zooplankton related to their ecological potential using the metrics specified in WFD, physicochemical and BQE algae metrics. The present research contributes to achieving a better zooplankton knowledge as water quality indicators by detecting key species related to trophic status and ecological potential.

Methods

Study site

The Ebro River is the largest river in Spain with a watershed area of 86,000 km², covering a fifth of the Spanish territory and one of larger basins in the Mediterranean region. The data presented in the current study were obtained from 66 reservoirs across the Ebro River watershed (Fig. 1) during summers of 2010 to 2019. To collect the corresponding samples in each reservoir, a sampling point was established in the deepest part of the reservoir at 300–500 m from the dam.

Environmental parameters, trophic state and ecological potential

At every sampling point, the following variables were measured in situ along a vertical profile: dissolved oxygen, temperature, conductivity, turbidity, pH and

chlorophyll-*a* using a multisensory device Sea-Bird 19 plus V2 (Seabird®, USA). The photic zone depth was calculated measuring the light penetration using a Li-Cor quanta-meter. The water transparency was determined measuring the Secchi disk depth (SD). For ex situ analysis, an integrative water sample was collected from the photic zone of each reservoir using a 25-mm-inner-diameter ballasted PET tube, and when photic zone was lower than 6 m deep, an integrative water sample was collected from the water surface until this depth or to the bottom (Vicente et al. 2005). We used standard methodology for estimating the following variables: suspended solids, turbidity, total phosphorus (TP) and chlorophyll *a* (Shoaf and Lium 1976; APHA 1998).

To determine the trophic state of each reservoir, we used the Trophic State Index (TSI) (Carlson 1977). To obtain a final trophic state, we used the average of the three variables of TSI (total phosphorus, chlorophyll-*a* and Secchi disk depth).

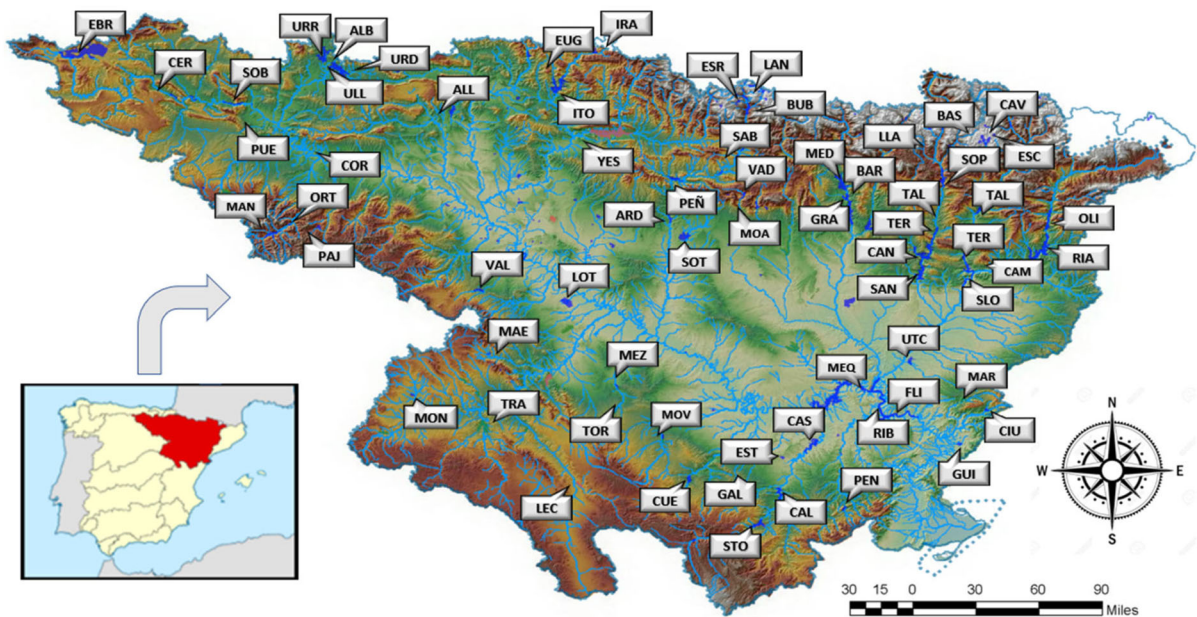


Fig. 1 Map of Ebro watershed with the approximate location of studied reservoirs. ALB Albiña, ALL Alloz, ARD Ardisa, BAL Balaguer, BAR Barasona, BAS Baserca, BUB Búbal, CAL Calanda, CAM Camarasa, CAN Canelles, CAS Caspe, CAV Cavallers, CER Cereceda, CIU Çiurana, COR El Cortijo, CUE Foradada, EBR Ebro, ESC Escales, ESR Escarra, EST Alcañiz, EUG Eugui, FLI Flix, GAL Gallipuéñ, GRA El Grado, GUI Guiamets, IRA Irabia, ITO Itoiz LAN Lanuza, LEC Lechago, LLA Llauset, LOT La Loteta, MAE Maidevera, MAN Mansilla,

MAR Margalef, MED Mediano, MEQ Mequinzena, MEZ Mezalocha, MOA Montearagon, MON Vicarías, MOV Moneva, OLI Oliana, ORT Ortigosa, PAJ Pajares, PEÑ La Peña, PEN Pena, PUE Puentelarra, RIA Rialb, RIB Ribarroja, SAB Sabiñanigo, SAN Santa Ana, SLO San Lorenzo, SOB Sobrón, SOP Soperia, SOT Sotenera, STO Santolea, TAL Talrn, TER Terradets, TOR Las Torcas, TRA Tranquera, ULL Ullivari, URD Urdalur, URR Urrunaga, UTC Utxa seca, VAD Vadiello, VAL Val, YES Yesa

The ecological potential (EP) was calculated following the methodology in “Spanish Legislation RD 817/2015” and Directive (2000). To obtain the EP, both biological and physicochemical indicators were assessed. The biological indices were obtained using the metrics obtained from four algal variables (chlorophyll *a*, biovolume, percentage of cyanobacteria and the IGA Index (Catalan and Ventura 2003)). Based on these, the classification scheme was Good or Superior, Moderate, Poor and Bad. The physicochemical indicator was obtained from the Secchi disk depth, hypolimnetic oxygen concentration and total phosphorus as variables. Their respective classifications were Very Good, Good, Moderate, Poor and Bad. To establish the representative classification of each biological and physicochemical indicator, we selected the average value of the algae and physicochemical variables. Following the WFD procedure using the “one-out, all-out” rule, the worst value between both indicators was selected as the ecological potential. A detailed methodology to obtain the ecological potential can be found in CHE 2016. In addition to determining the ecological potential using the standard procedure, we used the two previous indicators individually as the ecological potential to verify if there is a difference in the composition of zooplankton species classified as indicators.

Zooplankton samples

The zooplankton samples were collected using a vertical Ruttner bottle with capacity of 2.7 L. In each waterbody, we took two Ruttner bottles to obtain 5.4 L of sample; afterwards, it was filtered through a 30- μm mesh size Nylal. Also, a zooplankton vertical tow net of 45- μm mesh size Nylal was towed from 30 m depth or from the reservoir bottom until the surface; these vertical tow net samples were collected mainly for taxonomic purposes. Both vertical and Ruttner samples were fixed with formalin at 4% final concentration and stored in a hermetic glass vial. The depth at which the zooplankton samples were collected was established for each reservoir at the beginning of oxycline, which has been reported as the richest zone of zooplankton fauna (Miracle and Vicente 1983).

The zooplankton species were identified using Ruttner-Kolisko (1974), Koste (1978), Nogrady et al. (1995) and Nogrady and Segers (2002) for rotifers, Alonso (1996) and Błedzki and Rybak (2016) for

microcrustaceans. Since we detected the presence in several reservoirs of the veliger larvae of invader bivalve zebra mussel (*Dreissena polymorpha*), we counted them for further studies. The samples obtained from Ruttner samples were counted using a Sedgewick Rafter-type chamber (1 mL) under inverted microscope (Nikon Eclipse Ti-U, objective lens 4x-60 \times DIC) to obtain the corresponding specific richness, species abundance and biomass.

Data analysis

A total of 304 samples were collected during 10 years of sampling. We considered each sample obtained as a datum, corresponding to the reservoir and date that was sampled. Using the total of zooplankton species presented in all reservoirs, we ran a similarity percentage analysis (SIMPER) to identify the species that most contributed to changes inside the communities. The SIMPER analysis was performed with the Bray–Curtis index with zooplankton abundances using PAST software (Hammer et al. 2001). To determine the relationship between environmental variables and zooplankton species, we ran a Canonical Correspondence Analysis (CCA). This analysis was performed using abundance data of zooplankton dominant species that are those species that were > 0.1% of the total zooplankton individuals (Table 1); also the species that were only present in only one reservoir were not included in the analysis. For this analysis, the selected environmental variables were: chlorophyll *a*, total phosphorus, turbidity, suspended solids, temperature, conductivity, dissolved oxygen, pH, Secchi disk depth and water residence time. All the data, except pH, were normalized transformed logarithmically $\text{Log}(x + 1)$. The model was tested using a Monte Carlo permutation ($n = 999$). The CCA was performed using the CANOCO 4.5 program for Windows system (ter Braak and Šmilauer 2002).

A second evaluation of indicator species related with trophic state and ecological potential was carried out using the Indicator Value (IndVal). This method uses and combines the species relative abundance (specificity) with the relative frequency of occurrence (fidelity) of the species in different habitats. The IndVal arranges the species in groups and gives values between 0 and 1; those species with values ≥ 0.50 and significance ($p < 0.05$) can be used as indicators (Dufrene and Legendre 1997; Cáceres and Legendre

Table 1 Dominant zooplankton species > 0.1% of total density

Code	Zooplankton Species	Code	Zooplankton Species
P1	<i>Acanthocyclops americanus</i>	R9	<i>Conochilus natans</i>
P2	<i>Copidodiaptomus numidicus</i>	R10	<i>Conochilus</i> sp.
P3	<i>Cyclops</i> sp.	R11	<i>Conochilus unicornis</i>
P4	<i>Cyclops vicinus</i>	R12	<i>Gastropus stylifer</i>
P5	<i>Eudiaptomus vulgaris</i>	R13	<i>Hexarthra fennica</i>
P6	<i>Neolovenula alluaudi</i>	R14	<i>Hexarthra intermedia</i>
P7	<i>Thermocyclops dybowskii</i>	R15	<i>Hexarthra mira</i>
P8	<i>Tropocyclops prasinus</i>	R16	<i>Hexarthra oxyuris</i>
C1	<i>Bosmina longirostris</i>	R17	<i>Kellicotia longispina</i>
C2	<i>Ceriodaphnia dubia</i>	R18	<i>Keratella cochlearis</i>
C3	<i>Ceriodaphnia pulchella</i>	R19	<i>Keratella cochlearis</i> f. <i>tecta</i>
C4	<i>Daphnia cucullata</i>	R20	<i>Keratella quadrata</i>
C5	<i>Daphnia galeata</i>	R21	<i>Polyarthra dolichoptera</i>
C6	<i>Daphnia longispina</i>	R22	<i>Polyarthra euryptera</i>
C7	<i>Daphnia parvula</i>	R23	<i>Polyarthra luminosa</i>
C8	<i>Daphnia pulicaria</i>	R24	<i>Polyarthra major</i>
C9	<i>Diaphanosoma brachyurum</i>	R25	<i>Polyarthra vulgaris</i>
C10	<i>Diaphanosoma mongolianum</i>	R26	<i>Pompholyx sulcata</i>
C11	<i>Diaphanosoma</i> sp.	R27	<i>Synchaeta kitina</i>
R1	<i>Anuraeopsis fissa</i>	R28	<i>Synchaeta longipes</i>
R2	<i>Ascomorpha ovalis</i>	R29	<i>Synchaeta oblonga</i>
R3	<i>Ascomorpha saltans</i>	R30	<i>Synchaeta pectinata</i>
R4	<i>Asplanchna girodi</i>	R31	<i>Synchaeta stylata</i>
R5	<i>Asplanchna priodonta</i>	R32	<i>Trichocerca pusilla</i>
R6	<i>Collotheca pelagica</i>	R33	<i>Trichocerca similis</i>
R7	<i>Collotheca</i> sp.	Dp	<i>Dreissena polymorpha</i>
R8	<i>Conochilus dossuarius</i>		

P = Copepoda species, C = Cladocera species, R = Rotifera species

2009). The analysis was performed with the *indic-species* package using R 4.0.0 “Arbor day” version (R Core Team 2020).

Results

Studied reservoirs, trophic state and ecological potential

Sampled reservoirs were classified by their trophic state according to the TSI' (Carlson 1977); then, samples were classified as: 123 oligotrophic, 123 mesotrophic, 55 eutrophic and only 3 as

hypereutrophic. Following WFD protocols, sampled reservoirs were assessed using both physicochemical and biological metrics to obtain their final ecological potential; samples were classified as: 99 Good or Superior, 202 Moderate and only 3 as Poor. Considering only the physicochemical metrics as final ecological potential, the results were the same as above. On the other hand, using only the algae metrics, the ecological potential of sampled reservoirs was better: 273 were Good or Superior, 28 Moderate and only 3 as Poor. The complete information related with the reservoirs trophic state and ecological potential can be found in Supplementary Table 1.

Zooplankton assemblage

During this study, 169 zooplankton species were identified. The rotifers species richness was the highest (115) followed by cladocerans (36) copepods (17) and the veliger larvae of zebra mussel (*D. polymorpha*). The complete zooplankton species list can be found in Supplementary Table 2. The reservoir with the highest zooplankton richness was Santolea in the year 2010 with 26 species, where 18 species belong to rotifers; also was the reservoir with rotifera higher richness. The cladocera major richness was present in six reservoirs; in each of these reservoirs were found six cladocera species. In the case of copepods, two reservoirs presented higher species richness with five species each.

The richness found in eutrophic reservoirs was similar to oligotrophic reservoirs: oligotrophic 10.6 ± 3.3 , mesotrophic 12.2 ± 4 , eutrophic 11.2 ± 3.7 and hypereutrophic 11 ± 1.7 . The same tendency can be seen when classifying the reservoirs using the WFD metrics: Good or Superior 10.8 ± 3.9 , Moderate 11.6 ± 3.5 , Poor 16.5 ± 0.5 and Bad 10.

Statistical interpretation

The results from SIMPER analysis were divided in two steps. First, we ran the analysis using all trophic state data (oligotrophic until hypereutrophic) from all reservoirs, and next, we used the data of only maximum and minimum trophic state reservoirs. The same procedure was performed for ecological potential, using first the complete data, and then only the higher and lower potential. The species *P. dolichoptera*, *K. cochlearis*, *P. major*, *A. americanus*, *D. cucullata* and *D. polymorpha* were responsible of major variance in trophic state and ecological potential among reservoirs (Table 2).

The relationship between the selected environmental variables and the dominant zooplankton species carried out with the CCA showed that the first two ordination axes explain the 58.2% of variance, with a p value of 0.001 in the Monte Carlo permutation test. The first axis (39.8%) is related with the trophic state variables. Its positive part is related to oligotrophic variables as Secchi disk depth, and the species related were the cladoceran *D. longispina* and rotifers *Trichocerca similis*, *Gastropus stylifer*, *Ascomorpha saltans* and *A. ovalis*. The eutrophic elements such as

chlorophyll and total phosphorus are negatively correlated with this axis. The species that show a strong correlation with these eutrophic variables were the copepods *A. americanus* and *Cyclops vicinus*, cladocerans *B. longirostris*, *D. cucullata*, *Daphnia parvula* and rotifers *Pompholyx sulcata*, *Hexarthra intermedia*, *K. cochlearis* f. *tecta*, *Polyarthra vulgaris*, *Polyarthra euryptera* and *Asplanchna girodi* (Fig. 2).

The second axis (18.4%) explains the relationship among environmental variables and zooplankton species; in the negative part several species are related with conductivity, principally *Tropocyclops prasinus*, *Copidodiaptomus numidicus*, *Hexarthra fennica*, *Ceriodaphnia dubia* and *Diaphanosoma mongolianum*. The dissolved oxygen was related to some species such as *Diaphanosoma brachyurum*, *Synchaeta stylata*, *Synchaeta longipes*, *Conochilus unicornis* and *Anuraeopsis fissa*.

The Indicator Value analysis (IndVal; Table 3) using the data from the reservoirs trophic state indicates the presence of 14 species with significant values ($p < 0.05$); most of these species (11) were indicators of hypereutrophic status, while the rest of species were related to transition states: one as Eu-hypereutrophic, one as meso-eutrophic and one as oligo-mesotrophic.

The results of the IndVal with the ecological potential data exhibit that indicator species decreased to six: one species as Good or Superior and five as Poor. Also, we ran another IndVal using only the data from the algae metrics. The results obtained with this new analysis provide nine indicator species, two as Poor, two as Poor-Moderate and five as Moderate (Table 4).

Discussion

Through the statistical treatment applied in the present research to the large dataset obtained along the largest basin in Spain, we have been able to define the zooplankton species that are capable of being good indicators of different environmental conditions and trophic status. Some of these species can be used also to determine the water quality and ecological potential within the WFD.

The trophic status in reservoirs normally exhibits similar tendencies over the years; for example, Mequinenza was oligotrophic or mesotrophic for most

Table 2 SIMPER analysis results

Trophic State		Oligotrophic versus hypereutrophic			Ecological potential			Good or superior versus bad			
Species	Contrib. %	Cumulative %	Species	Contrib. %	Cumulative %	Species	Contrib. %	Cumulative %	Species	Contrib. %	Cumulative %
<i>P. dolichoptera</i>	18.85	18.85	<i>A. americanus</i>	14.46	14.16	<i>P. dolichoptera</i>	18.85	18.85	<i>K. cochlearis</i>	34	34
<i>K. cochlearis</i>	9.65	28.5	<i>D. cucullata</i>	13.6	27.76	<i>K. cochlearis</i>	10.22	29.07	<i>P. dolichoptera</i>	21	55
<i>D. polymorpha</i>	8.13	36.63	<i>P. major</i>	12.2	39.96	<i>P. major</i>	7.68	36.75	<i>A. ovalis</i>	7.3	62.3
<i>P. major</i>	7.97	44.6	<i>P. dolichoptera</i>	8	47.96	<i>D. polymorpha</i>	6.7	43.45	<i>P. luminosa</i>	4.6	66.9

Contrib % = variance contribution percentage of each species

of time. The only three reservoirs classified as hypereutrophic were Mezalocha 2012, Utxesa 2016 and Moneva 2017, due to an increase in the values of chlorophyll *a*, total phosphorus and low Secchi Disk transparency compared with previous or later years. In the case of the first two reservoirs, the rest of the years were classified as eutrophic; however, Moneva ranged between oligotrophic and hypereutrophic throughout the monitoring period. Despite the increase in their trophic state, the ecological potential of these three reservoirs was cataloged as Moderate, regardless of the use of physicochemical or algae metrics. The low sensitivity of these variables leads to the opportunity to test other biological strategies with higher sensibility, such as zooplankton, to obtain more precise results.

Worldwide, algae are one of the most accepted groups to obtain metrics to assess trophic conditions and water quality due to the dynamics of their species assemblage, functional groups, density and response to environmental conditions (Reynolds et al. 2002; Padišák et al. 2006). Also, several algae metrics were established to be reassessed within the WFD, such as biovolume, composition and chlorophyll *a* (Ptacnik et al. 2008; Poikane et al. 2009; Phillips et al. 2013). The zooplankton can also provide valuable information with various types of metrics to determine the trophic conditions, i.e., functional groups (Obertegger and Manca 2011; Sun et al. 2019; Kuczynska-Kippen et al. 2020), density (May and O’Hare 2005; Ejsmont-Karabin 2012; Ejsmont-Karabin and Karabin 2013) and species composition (Attayde and Bozelli 1998; Pinto-Coelho et al. 2005; Montagud et al. 2019; Muñoz-Colmenares et al. 2021).

Species composition and their relationship with the environmental variables through the CCA analysis indicate that the set of variables related to eutrophic conditions as chlorophyll *a* and total phosphorus together with suspended solids and turbidity were decisive for the presence of a significant number of species such as *A. americanus*, *D. parvula*, *P. sulcata*, *K. cochlearis* f. *tecta* and *A. girodi*; these species were reported also as eutrophic species in the ZRTI index (Montagud et al. 2019). While species such as *B. longirostris* and *D. cucullata* have been related to meso-eutrophic environments (Haberman et al. 2007; Jensen et al. 2013), the species from our analysis related to Secchi disk and oligotrophic state including *D. longispina*, *T. similis*, *G. stylifer*, *A. saltans* and *A*

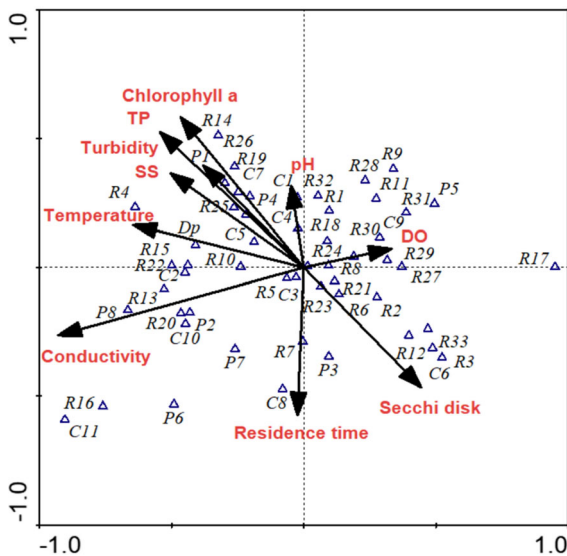


Fig. 2 Canonical Correspondence Analysis of dominant zooplankton species and environmental variables. TP = total phosphorus, SS = suspended solids, DO = dissolved oxygen. Zooplankton species codes names are as denoted in Table 1

ovalis were also reported in the ZRTI as oligo-mesotrophic species. Besides, temperature exhibits a strong relation with the species *A. girodi*, *P. euryptera*, *H. mira*, *C. dubia* and *D. polymorpha*; this variable was located near variables of high trophic states. Recently, it has been suggested that temperature can be responsible of community composition and size structure of cladocerans (Haberman et al. 2007), rotifers (Chalkia and Kehayias 2013) and zooplankton metrics as abundance and biomass, especially in a global warming scenario (Cremona et al. 2020; Dziuba et al. 2020). The use of CCA analysis together with other biological indicators methods such as IndVal and SIMPER analysis can be useful to determine properly the species associated with certain environments, habitats and highlight the differences between the fauna present in different trophic state and ecological potential levels.

The use of indicator value of species, to assess water quality, community preferences and pollution levels, has been widely used in diverse aquatic environments. Some examples of applying this IndVal with diverse aquatic groups are its use with fishes in the Mediterranean Sea (Carlucci et al. 2018), macrophytes in urban reservoirs (Silva et al. 2014), plankton groups in alpine lakes (Catalan et al. 2009), diatoms in saline lakes (Stenger-Kovács et al. 2014), marine

zooplankton (Mazzocchi et al. 2011) and recently freshwater zooplankton to determine the trophic state in reservoirs in Spain (Garcia-Chicote et al. 2019).

The rotifers *A. girodi*, *P. sulcata* and *K. tropica* reported by Garcia-Chicote et al. (2019) at Jucar watershed as indicators of high trophic status are in accordance with our IndVal results (Table 3); however, their results do not show any species related to oligotrophic conditions; meanwhile, our data suggest that *D. longispina* indicate the oligo-mesotrophic status. The characterization of this low trophic indicator species probably is due to the difference in the trophic state of reservoirs, since in the present study the proportion of oligotrophic reservoirs was higher than in the Jucar study. This same cladoceran *D. longispina* was the only indicator of a Good or Superior status; meanwhile, some species related with low ecological levels were similar to those related with high trophic states such as *A. americanus* and *K. cochlearis*. Using only the algae metrics, there is no species with good potential; in contrast, some species catalogued previously as indicators of high trophic condition and low ecological potentials are located as indicators of Moderate state such as *C. dubia*, which has been reported as tolerant to eutrophication (Azevêdo et al. 2015). The total number of indicator species was higher using the trophic state than the elements of the WFD, so this suggests that zooplankton can be more sensitive to changes in trophic status than in ecological potential.

In oligotrophic waterbodies, the zooplankton density and biomass are lower compared with those reported with higher trophic status (Lampert and Sommer 1997; May and O'Hare 2005; Garcia-Chicote et al. 2018). The IndVal method is based principally on the detection of species density associated with certain variables or particular conditions (i.e., trophic state); therefore, applying this method to zooplankton can be reliable to determine the species related especially with higher trophic levels or associated with low ecological potential status.

Normally, as trophic state increases, zooplankton large filter species decrease considerably and are replaced by smaller-sized species (Jeppesen et al. 2000; Pinto-Coelho et al. 2005). A similar tendency was detected in the SIMPER results comparing the lowest and highest trophic level. The small sized species were responsible for community change among oligotrophic to eutrophic–hypereutrophic

Table 3 Indicator value (IndVal) from zooplankton species and trophic state, only those species with $p < 0.05$ are shown

Species	IndVal	p value	Trophic state
<i>Acanthocyclops americanus</i>	0.938	0.0002	Hypereutrophic
<i>Polyarthra major</i>	0.837	0.0077	Hypereutrophic
<i>Pompholyx sulcata</i>	0.808	0.0027	Hypereutrophic
<i>Daphnia cucullata</i>	0.805	0.0058	Hypereutrophic
<i>Cyclops abyssorum</i>	0.781	0.0024	Hypereutrophic
<i>Keratella cochlearis</i> f. <i>tecta</i>	0.702	0.0119	Hypereutrophic
<i>Lecane stichaea</i>	0.574	0.0062	Hypereutrophic
<i>Cyclops vicinus</i>	0.544	0.0325	Hypereutrophic
<i>Keratella tropica</i>	0.525	0.0353	Hypereutrophic
<i>Asplanchna girodi</i>	0.518	0.0257	Hypereutrophic
<i>Daphnia parvula</i>	0.499	0.0480	Hypereutrophic
<i>Bosmina longirostris</i>	0.829	0.0102	Eu-Hypereutrophic
<i>Keratella quadrata</i>	0.510	0.0485	Meso-Eutrophic
<i>Daphnia longispina</i>	0.616	0.0500	Oligo-Mesotrophic

Table 4 Indicator value (IndVal) of zooplankton species and ecological potential using WFD (water framework directive) and algae metrics. Only those species with $p < 0.05$ are shown

Species	IndVal	p value	Ecological potential
WFD			
<i>Keratella cochlearis</i>	0.836	0.032	Poor
<i>Collotheca pelagica</i>	0.654	0.009	Poor
<i>Acanthocyclops americanus</i>	0.571	0.011	Poor
<i>Brachionus angularis</i>	0.562	0.011	Poor
<i>Diaphanosoma brachyurum</i>	0.552	0.016	Poor
<i>Daphnia longispina</i>	0.683	0.045	Good or superior
Algae metrics			
<i>Collotheca pelagica</i>	0.703	0.009	Poor
<i>Diaphanosoma brachyurum</i>	0.541	0.039	Poor
<i>Bosmina longirostris</i>	0.848	0.017	Poor–moderate
<i>Keratella cochlearis</i>	0.828	0.039	Poor–moderate
<i>Acanthocyclops americanus</i>	0.788	0.015	Moderate
<i>Keratella cochlearis</i> f. <i>tecta</i>	0.734	0.015	Moderate
<i>Pompholyx sulcata</i>	0.668	0.013	Moderate
<i>Daphnia cucullata</i>	0.641	0.033	Moderate
<i>Ceriodaphnia dubia</i>	0.585	0.034	Moderate

reservoirs, and some of small species are also shared in the high eutrophic results of CCA and in the IndVal test. Some of these species were the rotifers *P. sulcata*, *K. cochlearis*, *A. girodi*, cladoceran *D. cucullata* and the cyclopoid copepod *A. americanus* that are consider

typical from eutrophic waters (Attayde and Bozelli 1998; Duggan et al. 2001; Smakulska and Górnica 2004; Haberman et al. 2007; Kehayias and Doulika 2014). The use of multiples tests, as CCA, SIMPER and Indval, together with species that are present and

shared among them, can give us a more precise acquaintance data about species that have high potential to be used as indicators.

Cyclopoid copepods are more abundant in high trophic environments in temperate and tropical regions (Pinto-Coelho et al. 2005). In concordance with our results, *A. americanus* has been reported in eutrophic waterbodies in Spain (Garcia-Chicote et al. 2019; Montagud et al. 2019) and Mexico (Nandini et al. 2016). Besides, other *Acanthocyclops* spp. and *Cyclops* genera can be found worldwide in meso- to eutrophic waters, such as Asia (Chengxue et al. 2019), Oceania (Duggan et al. 2020), Europe (Haberman et al. 2014) and South America (Perbiche-Neves et al. 2016).

In contrast, large filtering microcrustacean such as calanoid copepods and large *Daphnia* species are found worldwide in low production waters, as in Europe (Ejsmont-Karabin and Karabin 2013; Stamou et al. 2019; Montagud et al. 2019), North America (Pinto-Coelho et al. 2005; Muñoz-Colmenares et al. 2017) and South America (Brito et al. 2011; Picapedra et al. 2020) together with the rotifers *A. ovalis* and *A. saltans* (Montagud et al. 2019; Duggan et al. 2020) that are present in the current research.

Previously, to determine the ecological potential using phytoplankton the follow metrics are used under the WFD criteria: biovolume, chlorophyll *a*, Catalán index (IGA) and percentage of cyanobacteria. In the case of zooplankton, it should have their own methodology comparable to those in phytoplankton. We suggest that similar metrics for zooplankton could be the species that in our present research were found as indicators of different trophic state and ecological potential levels along with the species provided in the ZRTI index (Montagud et al. 2019). Besides, the use of abundance and biomass of zooplankton groups (Garcia-Chicote et al. 2019) could be a good complementation. For sample collection, a standard methodology would work good, quantitative samples using Ruttner bottles or any other technique that permits obtain accurate numerical estimations that lead the correct implementation of metrics and indexes.

The integrative capacity of zooplankton species of the environmental factors that determine the trophic state and the ecological potential gives us a broader picture over time compared to phytoplankton. Since this last group has a shorter life span and their communities can vary in less time compared to

zooplankton (Reynolds, 2006) and sometimes under specific environmental pressures, the phytoplankton could not give a so accurate picture of how the aquatic system is really in general. However, the use of both phytoplankton and zooplankton species present in the waterbodies can be complementary and would give us a more precise picture of the water quality, trophic state or ecological potential. Zooplankton sample collection is not complicated and generally can be taken at the same time with phytoplankton and can be included in monitoring programs easily. Thus, we recommend with great emphasis, as many other authors before us, that zooplankton should be included as one more of BQE for Water Framework Directive.

Acknowledgements The first author thanks CONACyT (447079) for doctoral scholarship financial support. We are grateful to the Confederación Hidrográfica del Ebro (Ebro Basin Authority—Ministry of Ecological Transition of Spain Government) for the support provided for the continuous sampling campaigns from 2010 to present day, without which it would have been impossible to obtain the data necessary for this study. We thank Universitat de Valencia for their laboratory support, and the fieldwork team, Xavi Soria and Maria Dolores Sendra, for their help during the sampling campaigns. Finally, we thank Olga Krammer for the chemical data and the laboratory work. **In memoriam Professor Ramesh Gulati and Professor Maria Rosa Miracle.**

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. The first author MCME received a doctoral scholarship from CONACyT (México, 447079).

Availability of data and material Data are available at public site <http://www.chebro.es>.

Code availability All used packages and software tools have been cited properly in the manuscript. There is not a specific code.

Declarations

Conflict of interest The authors have no conflicts of interest or competing interest.

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