Research Article

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# Summer Rotifer Assemblages in Three Reservoirs in the Republic of Macedonia

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**Abstract:** Rotifer and zooplankton assemblages in three reservoirs differing in their size and trophic state, i.e. eutrophic Konče 1 and Konče 3 and oligotrophic Špilje, were examined. Considerable differences were recorded among reservoirs in their environmental parameters, zooplankton composition, species diversity and abundance. High abundance and dominance of rotifers as well as higher species diversity in the zooplankton were observed in more productive, eutrophic reservoirs Konče 1 and Konče 3 compared to the oligotrophic Špilje Reservoir characterised by prevalence of planktonic crustaceans. Dominant rotifer species in eutrophic reservoirs were *Brachionus falcatus, Brachionus angularis* and *Hexarthra mira*; Špilje Reservoir was dominanted by *Kellicottia longispina*, a species characteristic for less productive waters. The significant correlation between trophic state index and rotifer abundance indicates that rotifers are useful biological indicators of water ecological status.

**Key words:** Rotifera, eutrophic, oligotrophic reservoir, abundance, diversity

## Introduction

Zooplankton plays important role in the energy transfer and nutrients cycling within communities of standing freshwaters; it is an important link between primary producers and higher trophic level (Chalkia & Kehayias 2013, Eskinazi-Sant'Anna *et al*. 2013, Špoljar *et al*. 2016). Zooplankters are known as sentinel organisms and indicators of pollution, and therefore are important for biomonitoring of water quality (Ejsmont-Karabin 2012, Gazonato Neto *et al*. 2014, Haberman & Haldna 2014).

Rotifers, beside cladocerans and copepods, are an important component of zooplankton community and are highly susceptible to physical and chemical changes in their environment due to their small size and permeable integument (Wallace *et al*. 2006). Their quick responses to environmental changes reflect short life cycle and numerous populations (Wallace *et al*. 2006). Variables related to the trophic state (nutrients, phytoplankton biomass) of the aquatic ecosystem determine the presence of particular species and seasonal succession of rotifer assemblage (Arora & Mehra 2003, Špoljar *et al*. 2011a, Špoljar 2013). Biology of rotifers in reservoirs has been studied in relation to environmental factors (Pociecha & Wilk-Wozniak 2007, Kozuharov & Stanachkova 2015) and temporal oscilations (Geraldes & Boavida 2006) across different geographical regions. Accordingly, the main aim of the present study was to ascertain rotifer assemblage as well as those of cladocerans and copepods, in three reservoirs differing in morphometric features and trophic state. We presumed that those properties will lead to imparity in rotifer and general zooplankton assemblage among reservoirs. We aimed to analyse differences and similarities of rotifer assemblage among three Macedonian reservoirs related to various environmental variables and their relationship with planktonic crustaceans. Results of this study will contribute to consideration of rotifer assemblages in biomonitoring and water quality assessment.

# Material and Methods

### Study area

This study was conducted in three reservoirs in the Republic of Macedonia: Konče 1, Konče 3 and Špilje (Fig. 1). Konče 1 (K1) and Konče 3 (K3) are located in the Southeast Macedonia, built in 1961, by collecting water from nearby sources in order to supply irrigation infrastructure for 90 ha of agricultural land. Špilje Reservoir (S, Debar Lake) is situated in Western Macedonia and constructed by damming the Crn Drim River in 1969; the main purpose of this reservoir is supplying of hydro-electric power (70 MW), irrigation of agricultural lands in the Debar Field, fishing, tourism and recreation.

### Zooplankton collection and processing

Sampling was carried out in three occasions at three sampling points in each reservoir during the summer of 2008. At each sampling point, zooplankton was collected in triplicates using 5-litre Ruttner sampler, filtered *in situ* through a sieve (45 µm mesh-sizes) and preserved in 4% formalin. Samples were quantitatively analyzed on Utermöhl chamber under an inverted Hydro–Bios microscope (100× to 600×). Identification of species was conducted at the lowest possible level according to identification guides (Kutikova 1970, Koste 1978, Amoros 1984, Einsle 1993, Segers 1995). Bdelloid rotifers were only counted (not identified).

### Measuring of environmental conditions

Temperature and pH were measured on the field using WTW probe (ProfiLine pH/mV-Meter pH 197). The following limnological parameters were also analyzed in the laboratory: dissolved oxygen (Winkler method, Apha-Awwa-Wpcf1998), dissolved organic matter (DOM) expressed as $KMnO_4$ consumption (Apha-Awwa-Wpcf 1998), total ammonia $N\text{-}NH_4$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ (Solorzano 1969) and total phosphorus (Strickland & Parsons 1972). Chlorophyll *a,* as indicator of phytoplankton biomass, was analysed spectrophotometrically after ethanol extraction according to ISO 10260 (1992).

### Data analysis

The Sørensen index (Sørensen 1948) and a cluster analysis based on determined taxa were used to compare the zooplankton species compositions between different reservoirs. The trophic state of the reservoirs was obtained by using Carlson Trophic Index (TSI), based according to the total phosphorous concentration (Carlson 1977). Normality for logarithmically [log (x+1)], transformed data, checked by

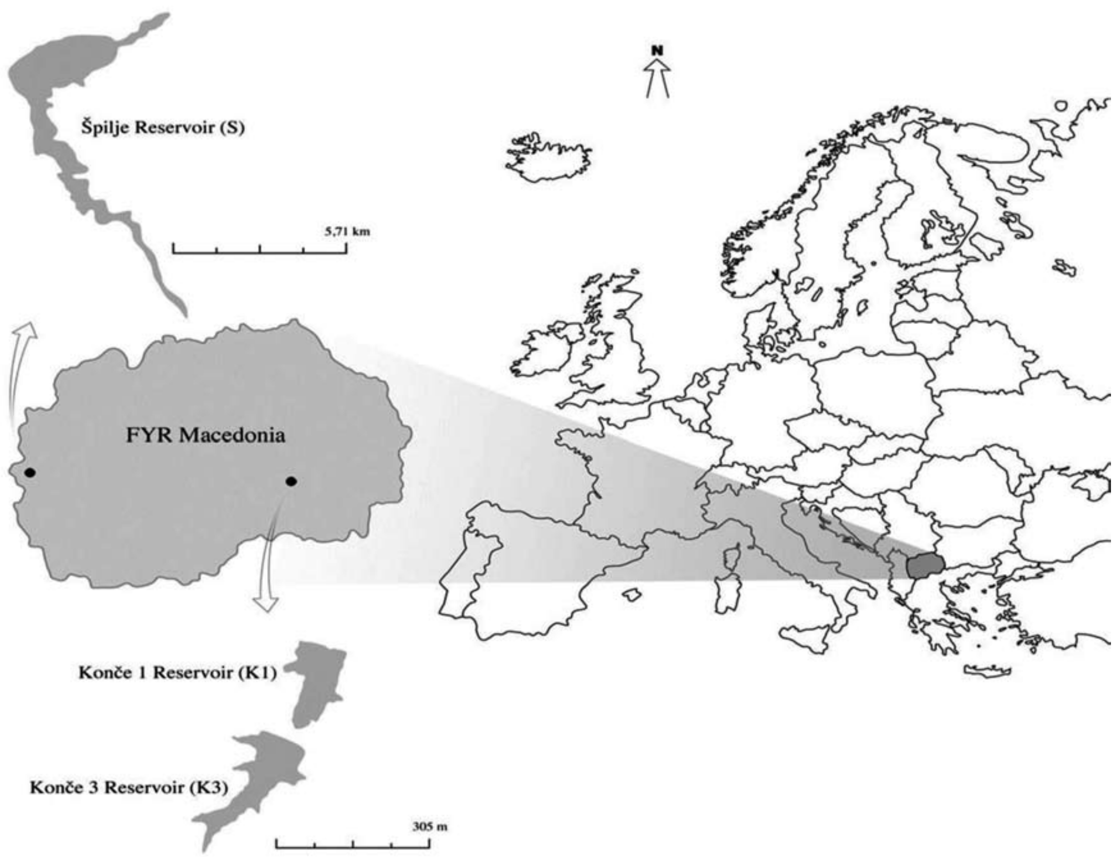


**Fig. 1.** Map of the investigated reservoirs in the Republic of Macedonia

Shapiro–Wilk's test and did not follow normal distribution, thus non-parametric analyses were applied. In each reservoir among studied sampling points significant differences were not observed (coefficient of variation < 10%), thus we considered their averages as value for each occasion in further analyses. Using Kruskal-Wallis H test, we analysed differences in environmental parameters and zooplankton assemblage among three reservoirs. Spearman's correlation was used to test for relationships between environmental variables and rotifer abundance. The statistical analyses were performed with the STATISTICA 8.0 (Statsoft Inc. 2007) analyses package. The relationships between rotifer abundance and environmental variables were explored by canonical correspondence analysis (CCA) using the program Canoco 4.2 (ter Braak & Šmilauer 1999).

# Results

### Environmental condition

Morphometric features of small and shallow reservoirs K1 and K3 opposite to large and deep reservoir S reflected in differences of environmental parameters (Table 1). Temperature, pH, nutrients (except $NO_3^-$ showed opposite pattern in S *vs*. K1 and K3), DOM, and phytoplankton biomass were significantly lower in S related to K1 and K3 (Kruskal-Wallis test $H_{(2, N=9)}$ =8 – 8.2, p = 0.02). These conditions classified K1 and K3 as a eutrophic and S as oligotrophic water body.

Considerable differences were found in the rotifer and zooplankton assemblage regarding to abundance, diversity and the occurrence pattern of dominant species between eutrophic reservoirs K1 and K3 and oligotrophic reservoir S (Fig. 2).

Summer zooplankton composition in three reservoirs showed small oscillations in diversity (15 to 19 taxa). A total of 17 taxa of Rotifera, 6 species of Cladocera, and 3 species of Copepoda have been found in the studied reservoirs (Fig. 2, Table 2). The Sørensen index indicates considerable similarity between K1 and K3 (76%), moderate between K3 and S (57%) and low between K1 and S (38%). Similar results were confirmed by cluster analysis (Fig. 3). Zooplankton abundances achieved high values, $1152 \pm 85 \times 10^3$ ind $m^{-3}$ in K1 and $906 \pm 61 \times 10^3$ ind $m^{-3}$ in K3 (Fig. 2). Rotifers dominated (Kruskal-Wallis test: $H_{(2, N=9)}$ =8.1 p = 0.02) in eutrophic reservoirs K1 and K3, 63% and 74%, respectively (Fig.

**Table 1.** Morphometrical features and physical and chemical parameters (mean ± SE) of the investigated reservoirs

| | Konče 1 (K1) | Konče 3 (K3) | Špilje (S) |
|---|---|---|---|
| Coordinates | 41°30′51″N, 22°23′33″E | 41°30′41″N 22°23′28″E | 41°49′52″N 20°51′32″E |
| $Length_{max}$ (km) | 0.274 | 0.364 | 22 |
| $Width_{mean}$ (km) | 0.145 | 0.137 | 0.6 |
| Surface area (ha) | 3.9 | 4.9 | 1320 |
| Max. depth (m) | 5 | 6 | 94 |
| Total volume ($10^6$ $m^3$) | 19 | 20 | 520 |
| Transparency (m) | 0.3 | 0.3 | 4.75 |
| Macrophyte coverage (%) | 20–30% | 20–30% | / |
| Type of macrophytes | Submerged | Submerged and emerged | |
| Dominant macrophytes | *Potamogeton gramineus* L, *Ceratophylllum demersum* L. | *Potamogeton gramineus* L. *Ceratophylllum demersum* L. *Phragmites australis* (Cav.) Trin ex Steud. *Schoenoplectus lacustris* (L.) Palla | |
| Temperature (°C) | 29.2±0.25 | 27.9±0.2 | 14±0.36 |
| Dissolved oxygen (mg $l^{-1}$) | 12.05±0.13 | 11.71±0.15 | 7.383±0.25 |
| pH | 9.48±0.01 | 9.37±0.06 | 7.764±0.02 |
| Total Phosphorus (µg TP $l^{-1}$) | 147.81±1.55 | 132.73±1.4 | 16.175±1.36 |
| Ammonia (µg N-$NH_4$ $l^{-1}$) | 17.81±0.53 | 55.3±0.4 | 10.812±3.44 |
| $NO_2$ (µg N-$NO_2$ $l^{-1}$) | 2.85±0.11 | 4.88±0.2 | 0.491±0.019 |
| $NO_3$ (µg N-$NO_3$ $l^{-1}$) | 2.1±0.15 | 9.39±0.22 | 208.698±17.16 |
| DOM (mg $O_{2Mn}$ $l^{-1}$) | 16.01±0.07 | 23.39±0.9 | 4.555±0.38 |
| Chl *a* (µg $l^{-1}$) | 13.87±0.16 | 18.16±0.18 | 1.548±0.68 |
| TSI (TP) | 55.32±0.42 | 54.78±0.52 | 37.77±0.52 |

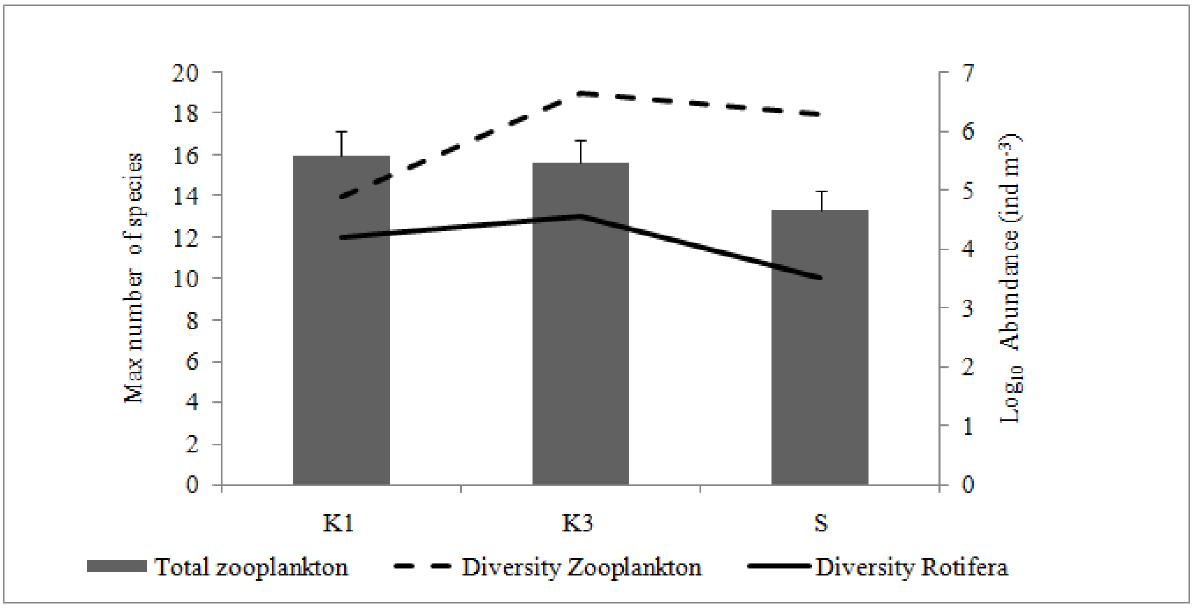


**Fig. 2.** Differences in total zooplankton diversity and abundance (mean ± SE) and diversity of rotifers among the three studied reservoirs (K1, K3 and S)

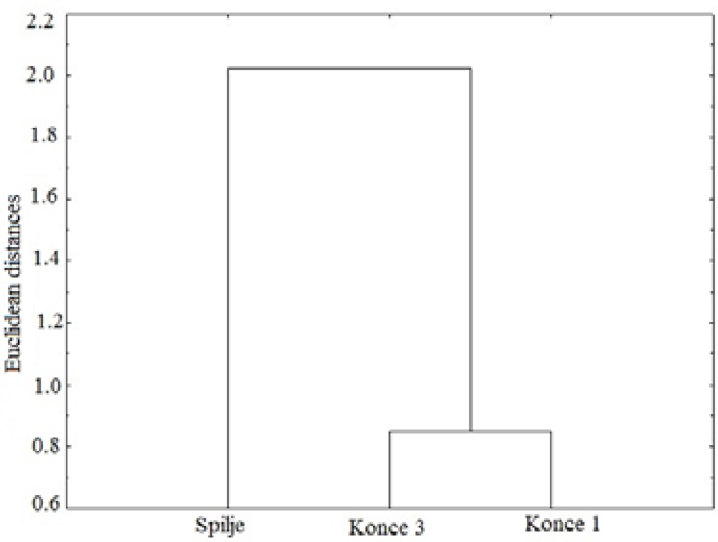


**Fig. 3.** Dendrogram based on differences in zooplankton diversity between the three studied reservoirs

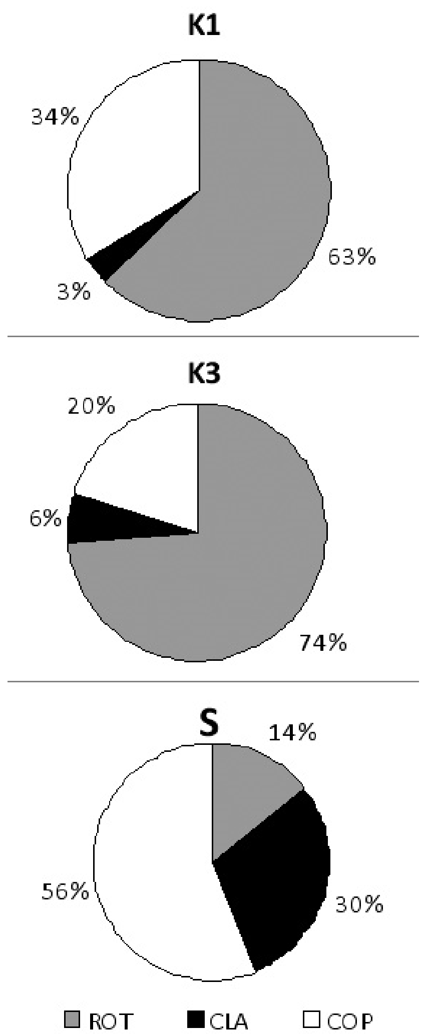


**Fig. 4.** Relative abundances of main zooplankton groups: Rotifera, Cladocera, Copepoda in three studied reservoirs: K1, K3, S

4). Otherwise, cyclopoid nauplii overall dominated among planktonic crustaceans in all three reservoirs. Planktonic crustaceans prevailed within zooplankton relative abundance in S with ratio of 86% opposite to significantly less share in K1 and K3, 37% and 26 % (Fig. 4). Otherwise their absolute abundance was the lowest in S (Kruskal-Wallis test: $H_{(2, N=9)}$ =7.9 p = 0.02).

Detritivorous microfilter-feeder rotifer species achieved higher abundance in eutrophic reservoirs, e.g. *Brachionus falcatus* (K1), *B. angularis* (K3) and in both reservoirs K1 and K3 *Hexarthra mira* and *Keratella cochlearis.* In S, *Kellicottia longispina* comprising 44% of the total rotifer abundance.

**Table 2.** Zooplankton species found in investigated sites

| Species | K1 | K3 | S |
|---|---|---|---|
| Rotifera | | | |
| *Asplanchna* sp. | | ** | * |
| Bdelloidea | * | ** | |
| *Brachionus angularis* Gosse, 1851 | ** | **** | |
| *Brachionus falcatus* Zacharias, 1898 | **** | | |
| *Filinia opoliensis* (Zacharias, 1898) | | ** | |
| *Gastropus stylifer* Imhof, 1891 | ** | * | * |
| *Hexarthra mira* (Hudson, 1871) | *** | *** | |
| *Kellicottia longispina* (Kellicott, 1879) | * | * | * |
| *Kerattella cochlearis* Gosse, 1851 | *** | *** | * |
| *Kerattella quadrata* (Müller, 1786) | | | * |
| *Lecane luna* (Müller, 1776) | * | | |
| *Lecane lunaris* (Ehrenberg, 1832) | ** | * | |
| *Ploeosoma* sp. | | | * |
| *Polyarthra* sp. | ** | ** | * |
| *Pompolyx* sp. | ** | * | |
| *Synchaeta* sp. | | | * |
| *Trichocerca* sp. | ** | * | |
| *Trichocerca similis* (Wierzejski, 1893) | | * | * |
| Cladocera | | | |
| *Bosmina longirostris* (O. F. Müller, 1776) | | * | * |
| *Ceriodaphnia quadrangula* (O. F. Müller, 1785) | ** | ** | |
| *Chydorus sphaericus* (O. F. Müller, 1785) | | * | |
| *Daphnia cucullata* Sars, 1862 | * | * | * |
| *Diaphanosoma brachyurum* (Liévin, 1848) | | * | ** |
| *Leptodora kindtii* (Focke, 1844) | | | * |
| Copepoda | | | |
| *Cyclops* sp. | ***** | *** | * |
| *Eudiaptomus gracillis* (Sars, 1862) | | | ** |
| *Mesocyclops leuckarti* (Claus, 1857) | | | ** |

Relative abundance of each species, in terms of maximum density recorded, is marked as follows: * <$10\times10^3$; ** >10-$100\times10^3$; *** > 100-$200\times10^3$; **** >200-$300\times10^3$; ***** >$300\times10^3$ ind $m^{-3}$

We point out on findings of rotifer (*Asplanchna* and *Ploesoma*), and cladoceran (*Leptodora kindtii*) predators peculiarly in oligotrophic S reservoir (Tab. 2). Temperature, dissolved oxygen, pH, TP ($r = 0.87 - 0.89$, $p < 0.05$) mainly positively impacted rotifer abundance. Thus TSI positively correlated ($r = 0.89$, $p < 0.05$) with rotifer abundance an abundance of *B. falcatus*. Abundances of rotifers and species *B. falcatus* negatively correlated with presented planktonic invertebrate predators ($r = 0.83$, $p < 0.05$), *L. kindtii* and *Asplanchna*, respectively.

According to CCA analysis, the first two axes explained 73% of variation (Fig. 5). As is shown by CCA plotting, detritivorous microfilter-feeder species *Hexarthra mira*, *Brachionus angularis* and bdelloids achieved their maximum abundances in eutrophic K3 reservoir indicated by TSI. Their abundances were positively affected by temperature, dissolved oxygen, DOM, Chl *a*. Contrary, omnivorous *Asplanchna* and *Trichocerca* achieved their maximum abundances in oligotrophic S reservoir.

# Discussion

Our results showed considerable differences in the environmental parameters and zooplankton assemblage, i.e., species diversity and abundance. The analyses of zooplankton reflect the significant dominance of rotifers in more productive, eutrophic reservoirs Konče 1 and Konče 3 in comparison to the oligotrophic Špilje Reservoir.

Higher temperature, nutrients and food availability (algal biomass) were expected in shallow lake of higher trophy (Gazonato Neto *et al*. 2014, Haberman & Haldna 2014). We presumed that notable macrophyte coverage contributed by photosynthetic ac-

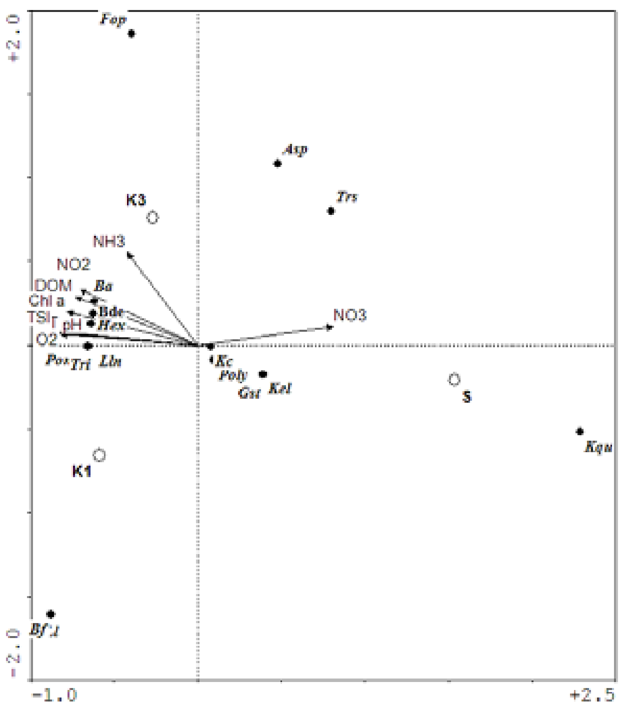

**Fig. 5.** CCA triplot illustrating the relationships between rotifer abundances and environmental parameters Asp *Asplanchna*, Bde Bdelloidea, Ba *Brachionus angularis*, Bf *B. falcatus*, Fop *Filinia opoliensis*, Gst *Gastropus stylifer*, Hex *Hexarthra mira*, Kel *Kellicottia longispina*, Kc *Keratella cochlearis*, Kqu. *K. quadrata*, Ll *Lecane luna*, Lln *L. lunaris*, Plo *Ploesoma*, Poly *Polyarthra*, Pom *Pompholyx,* Syn *Synchaeta*, Tri *Trichocerca*, Trs *Trichocerca similis* (for environmental parameters refer to Table 1)

tivity to higher dissolved oxygen concentrations in Konče 1 and 3, and thus increased nitrogen oxidation (higher nitrate concentration in K1 and K3) in those reservoirs. A positive correlation between temperature and species assemblage in freshwater enhance the rate of population growth at higher temperatures (Galkovskaya 1987) has been previously described for zooplankton in particular (Hessen *et al*. 2007, Kaya & Altindağ 2007, Kaya *et al*. 2010). A common factor causing pH levels to rise over 9 is intense photosynthetic activity resulting from algal growth in nutrient–enriched waters (Gracia *et al*. 2007). These conditions are optimal for the mass development of dominant rotifers, *Brachionus* species and *Hexarthra mira* (Špoljar *et al*. 2011b). Berziņš & Pejler (1987) concluded that rotifer species found in oligotrophic waters prefer pH ≤ 7, whereas for the species common in eutrophic waters favour pH ≥ 7. Total phosphorus, as the most important nutrient, indicates the trophic status and may partly determine the species presence and dynamics of rotifer assemblages. In addition, there are reports of significant correlations of rotifer populations with phosphorus by Stemberger (1995) and Arora & Mehra (2003), which is in concordance with the results in our study.

The higher zooplankton abundance in the eutrophic water bodies, influenced by abundance of opportunistic organisms, such as rotifers and juvenile crustaceans, is also confirmed by other authors (Almeida *et al.* 2009, Lodi *et al.* 2011, Tasevska *et al*. 2012, Špoljar 2013). Eutrophic water bodies contain higher amounts of suspended organic matter (detritis) and therefore easily support higher biomass and productivity of microzooplankton with short generation time (Sed'a & Devetter 2000, Nogueira 2001, Gazonato Neto *et al.* 2014, Haberman & Haldna 2014).

At the species level, many authors have reported a strong relationship between certain rotifer species and lake trophic state (Duggan *et al*. 2001, Baião & Boavida 2005; Špoljar *et al*. 2016). Rotifer assemblage dominated by detritivore species *B. falcatus, B. angularis H. mira*, *K. cochlearis* and their high abundance noted during our study suggested high trophic state as was evident in several studies (Nogueira 2001, Žiliukienė 2003, Ejsmont-Karabin 2012, Malekzadeh-Viayeh & Špoljar *et al*. 2012). May *et al*. (2014) pointed out the abundance as a more sensitive indicator of trophic state, compared with species composition and that even simple measures of rotifer abundance and relative species composition could provide sensitive indicators of water quality. This is in accordance with results of our study because, in terms of species diversity, Konče 1 and Konče 3 despite their eutrophic character, showed higher biodiversity than Špilje Reservoir. We supposed, that due to macrophyte coverage in Konče 1 and Konče 3 habitats heterogeneity promote many microhabits, higher food availability and refuge from predation and contributes to higher species diversity (Kuczyńska-Kippen 2005, Špoljar *et al.* 2012).

In the deep and oligotrophic Špilje Reservoir prevalence of planktonic crustaceans explain that aquatic ecosystems with limited sources of energy do not possess a suitable environment for smaller body sized microzooplankton because of their higher specific rates of metabolism compared with larger sized macrozooplankton (Sed'a & Devetter 2000).

In conclusion, our results showed that rotifers are useful biological indicators of the structure and function of freshwater ecosystems and their ecological status.

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