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Associations of the Zooplankton Communities with the Trophic State and Ecological Potential of Reservoirs

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Abstract: The assessment of the current state of lakes and reservoirs is a prerequisite for the conservation and management of these ecosystems as well as for their restoration and mitigation of the damages triggered by various pressures. The aim of the present study was to explore associations of the zooplankton communities with the trophic state and (or) ecological potential (EP) of reservoirs, incorporating also standard physical and chemical factors and primary production. We recorded 48 zooplankton taxa: 32 rotifer, nine cladoceran and seven copepod taxa. Our results suggested a significant relationship of both zooplankton community and univariate metrics with trophic state index (TSI) and conductivity. Reservoirs with the highest correlation with TSI and chlorophyll a were with the highest trophic state. Most of the rotifers identified as indicator taxa evidenced eu-mesotrophic conditions or moderate-poor EP. The indicator cladocerans were significantly associated with moderate EP. The indicator copepods were associated with higher EP and oligo-mesotrophic conditions. Policy-driven approaches identified littoral macrozoobenthos as the key invertebrate group for the assessment of lentic water bodies, while science-driven studies focus more on the pelagial zooplankton communities in assessments of ecosystem health. The pelagial and the littoral of a lake (or a reservoir) are very different in the conditions, which they provide; they could be affected by distinct stressors, thus triggering a specific response of their communities. Studying lentic ecosystems in a more holistic way, across various water body types and for longer periods, would result in rigorous assessment schemes and improved management of the ecosystem health.

Key words: Bulgaria, ecological potential, environmental conditions, freshwater zooplankton, trophic state

Introduction

Biological communities, including zooplankton, are shaped by contrasting differences regarding origin and trophic state of reservoirs as well as by various anthropogenic stressors (Davidson et al. 2011, Goździejewska et al. 2016, García-Chicote et al. 2019). Studies on the impacts of these stressors on natural, artificial or highly modified aquatic ecosystems have triggered an increase in research on the development of metrics for assessment and biomonitoring of aquatic ecosystem health (e.g. Friberg

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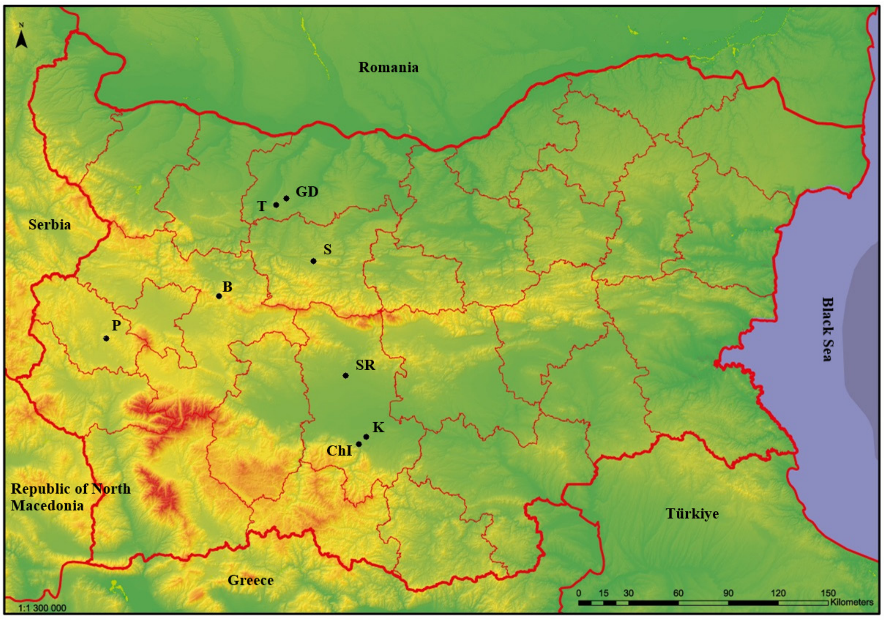


Fig. 1. Map of Bulgaria with location of the eight studied reservoirs (marked with black dots). Abbreviations: GD – Gorni Dabnik Reservoir; T – Telish Reservoir; S – Sopot Reservoir; B – Bebresh Reservoir; P – Pchelina Reservoir; SR – Sinyata Reka Reservoir; K – Konush Reservoir; ChI – Chetiridesette Izvora Reservoir.

et al. 2011, Azevêdo et al. 2015, Jurca et al. 2021). Scientists have acknowledged the suitability of zooplankton communities for the successful assessment of ecological conditions of various water bodies for a long time, with some studies dating back to the 1970s (Gannon & Stemberger 1978). Currently, for over a decade, there is an increase in research confirming its bioindicator potential (e.g. Caroni & Irvine 2010, Jeppesen et al. 2011, Ejsmont-Karabin 2012, Kozuharov et al. 2013, Krupa et al. 2020).

The number of reservoirs has been increasing since the 1950s (Lehner et al. 2011) and it is likely to increase even further, owing to the growing freshwater demand (Musie & Gonfa 2023). Zooplankton plays an essential role in the functioning and stability of lentic ecosystems, being caught in the middle of top predators and primary producers (Wetzel 2001). Zooplankton communities have an important share in secondary productivity (Sommer et al. 2012) and this has been long recognised. For example, it was estimated that zooplankton in Bulgarian reservoirs represented between 40 and 98% of the secondary productivity (Naidenov 1987).

The Water Act of Bulgaria (State Gazette 2000) regulates environmental protection and ensures good status (based on both biotic and abiotic metrics) of surface waters. In Europe, the Water Framework Directive (WFD: Directive 2000/60/EC) is a policy-driven legal act, which defines ecological potential of heavily-modified water bodies (HMWB) and artificial water bodies (AWB) as "an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters" (WFD CIS Guidance Document No 4 2003). Three sets of quality elements are used for the estimation of ecological status (potential) of surface water bodies: physical and chemical, hydromorphological and biological, the latter including phytoplankton, phytobenthos, macrophytes, littoral macrozoobenthos and fish (Directive 2000/60/EC). Despite their importance in lake and reservoir food webs, zooplankton communities have not been included as a biological quality element used in the assessment of the water quality in European surface waters as defined in the WFD.

The conservation of lake and reservoir ecosystems as well as their restoration and mitigation of the damages because of various pressures necessitate the assessment of the current state of these systems. As outlined above, policy-driven approaches (Directive 2000/60/EC) identified littoral macrozoobenthic invertebrates as one of the main biological elements for ecological assessment of lakes and reservoirs. However, science-driven studies focus more on the pelagial invertebrates (zooplankton communities) to be used in assessments of ecosystem health (Caroni & Irvine 2010, Jeppesen et al. 2011, Almeida et al. 2020).

The aim of the present study was to explore associations of the zooplankton communities with

Table 1. Coordinates and main characteristics of the studied reservoirs. R_N denotes reservoir number; sp – spring, su – summer and au – autumn. Code denotes the unique ID for each of the samples, collected during the respective season. Abbreviations: L2 – mountain lakes; L12 and L13 – medium and small semi-mountain reservoirs; L14 – large lowland reservoirs with medium depth; L16 and L17 – small- and medium-sized lowland reservoirs.

R_N	Reservoir name	Season	Sample code	Latitude N	Longitude E	Altitude (m)	Lake area (ha)	Max depth (m)	Lake type	Year sampled
1	Gorni Dabnik	sp	GD_sp	43.36828	24.32742	171	1180	23	L14	2016
		su	GD_su							
		au	GD_au							
2	Telish	sp	T_sp	43.3163	24.24247	230	232	28	L16	2016
		su	T_su							
		au	T_au							
3	Sopot	sp	S_sp	43.00786	24.42786	374	535	28	L12	2016, 2017
		su	S_su							
		au	S_au							
4	Bebresh	sp	B_sp	42.84597	23.77806	454	73.6	20	L2	2016
		su	B_su							
		au	B_au							
5	Pchelina	sp	P_sp	42.51723	22.84385	664	538	19	L13	2016
		su	P_su							
		au	P_au							
6	Sinyata Reka	sp	SR_sp	42.46888	24.70333	309	52.8	6	L17	2016, 2017
		su	SR_su							
		au	SR_au							
7	Konush	sp	K_sp	42.08147	25.03406	237	37.7	5	L17	2016, 2017
		su	K_su							
		au	K_au							
8	Chetiridesette Izvora	sp	ChI_sp	42.0053	24.93836	240	48.9	30	L17	2016, 2017
		su	ChI_su							
		au	ChI_au							

the trophic state and the ecological potential of reservoirs, incorporating also standard physical and chemical factors and primary production. We hypothesised that, on the one hand, the zooplankton assemblages could be associated with trophic state or ecological potential as defined using descriptors of the environmental conditions in the pelagial. On the other hand, we aimed to identify zooplankton taxa indicating various trophic states or specific ecological potentials. Lastly, we discuss and compare the conditions in the littoral and in the pelagial of the studied reservoirs as defined by our results.

Materials and Methods

Study area

We studied eight reservoirs located in Bulgaria: four in the Danube River Basin, three in the East Aegean River Basin and one in the West Aegean River Basin (Fig. 1). All of the selected reservoirs are included in the national monitoring system and represent a broad range of environmental conditions. Gorni Dabnik, Telish, Konush and Sinyata Reka Reservoirs are classified HMWB, while Bebresh, Pchelina, Sopot and Chetiridesette Izvora Reservoirs are classified as AWB (Marinov et al. 2016, RBMP of West Aegean Basin District 2016, RBMP of Danube Basin District 2016).

Environmental variables

Water temperature (T°C), concentration of dissolved oxygen (DO; mg.dm^{-3}) and electrical conductivity (Cond; µS.dm^{-3}) were measured *in situ* using WTW portable meters (series 330i). The concentrations of total phosphorus (P tot; mg.dm^{-3}) and pH were determined according EN ISO 6878:2005 and EN ISO 10523:2012, using portable photometer WTW, pHotoFlex Turb. Water transparency was measured using a Secchi disk with a diameter of 0.25 m and from here onwards is referred to as Secchi disk depth (Secchi; m). The concentration of chlorophyll a (Chl a; $\mu g.dm^{-3}$) was determined in the laboratory, following ISO 10260:2002.

Zooplankton: sampling and processing

Zooplankton was collected from the pelagial zone in spring, summer and autumn of 2016 or 2017, using an Apstein plankton net with mesh size of 40 µm, following EN 15110:2006. Zooplankters were identified following Kutikova (1970) and Bledzki & Rybak (2016). Absolute zooplankton abundance was calculated as $ind.dm^{-3}$. The semi-quantitative composite macroinvertebrate samples were collected from different microhabitats in the littoral of the reservoirs (EN ISO 10870:2012). Data on benthic invertebrates from the studied reservoirs were published in a previous paper (Subeva et al. 2019).

Identifying ecological potential

Physical and chemical parameters, as well as the concentration of Chl a (all measured in the pelagial) were used to define ecological potential (EP) of reservoirs during the specific season and for the specific water body type according to the Ministry of Environment and Water (MoEW) Ordinance No H-4/2012 (with amendments in 2023). Some of the results have already been discussed in Subeva et al. (2019), before the final amendments in the national legislation from 2023. The ecological potential of the studied reservoirs was assessed also based on the new Bulgarian multi-metric index (BMMI) and the normalised ecological quality ratio (nEQR: Wolfram et al. 2022), included in the last amended version of MoEW Ordinance No H-4/2012. Colour coding of different ranges of ecological potential, as determined for each of the quality elements, was according WFD CIS Guidance Document No 4 (2003):

- maximum MEP and high HEP: blue and dark/ light grey stripes;
- good GEP: green and dark/light grey stripes;
- moderate (MoEP): yellow and dark/ light grey stripes;
- poor (PEP): orange and dark/ light grey stripes;
- bad (BEP): red and dark/ light grey stripes for HMWB/ AWB, respectively.

Trophic and saprobiological analysis

Trophic state indices (TSI%) were calculated following Carlson (1977) and Carlson & Simpson (1996), using the values for Secchi disk depth (m), the concentration of Chl a and of total phosphorus. Values of TSI below 40% corresponded to oligotrophic conditions, between 40 and 60 – mesotrophic, from 60 to 80 – eutrophic and above 80 – hypereutrophic conditions (Bajkiewicz-Grabowska 2007).

We calculated the ratio between the sum of the absolute abundance of all taxa of Cladocera and Copepoda in one sample and the total abundance of all taxa of Rotifera, Cladocera and Copepoda in the same sample or the RCC% index proposed by Kozuharov et al. (2013). It has been introduced for the assessment of the trophic state of lentic water bodies and it ranges from 0 (= hypertrophy) to 100 (= oligotrophy: Kozuharov et al. 2013).

Some classical metrics were also calculated using abundance for both zooplankton and macrozoobenthos communities: the saprobic index (S_{PB}; ranges from 0.50 to 4.50) according to Pantle & Buck (1955) and classification of saprobity levels (Sr%; ranges from 10-90) following Rothschein (1962). The highest values of S_{PB} indicated hypertrophy, while those for Sr% showed oligosaprobic conditions. The colour coding was based on their values and was done separately for S_{PB} and for Sr%; however, for each of the metrics, the values for macrozoobenthos and for zooplankton were merged into the same scale: from blue corresponding to oligotrophic conditions, through red for samples with the highest trophic state within our sample dataset.

Associations among environmental variables, aquatic invertebrates and trophic state or ecological potential

Canonical correspondence analysis (CCA) with forward variable selection was used to identify the environmental factors with the highest association with the zooplankton communities (ter Braak & Šmilauer 2002). The analysis was performed using zooplankton abundance data for taxa that are over 1% of the total abundance of zooplankton individuals. The taxa recorded in only one reservoir were not included in the analysis. Intraset correlation coefficients (ICC) are a quantification of relationships between the environmental variables and the canonical correspondence analysis axes for the zooplankton taxa; they are given in brackets after each of the discussed taxa, followed by the relevant axis (A1 for Axis 1 and A2 for Axis 2).

We used redundancy analysis (RDA) where the response data (RCC% and S_{PB}) are modelled as a function of one or more ordinate axes that are constrained to be linear combinations of the environmental variables. Monte Carlo unrestricted permutation tests (499 permutations) were used to test for statistical significance ($p > 0.05$) for both CCA and RDA. These analyses were done using the software statistical package CANOCO 4.5 for Windows.

Table 2. List of recorded zooplankton taxa with their taxon codes.

Taxon	Code	Taxon	Code
Rotifera			
Brachionus diversicornis (Daday, 1883)	R1	*Asplanchna priodonta* Gosse, 1850	R17
Brachionus angularis Gosse, 1851	R2	*Asplanchna herrickii* De Guerne, 1888	R18
Brachionus calyciflorus Pallas, 1766	R3	*Asplanchna* sp.	R19
Keratella tecta (Gosse, 1851)	R4	*Polyarthra euryptera* Wierzejski, 1891	R20
Keratella cochlearis (Gosse, 1851)	R5	*Polyarthra dolichoptera* Idelson, 1925	R21
Keratella irregularis (Lauterborn, 1898)	R6	*Polyarthra major* Burckhardt, 1900	R22
Keratella irregularis f. *wartmanni* (Asper & Heuscher, 1889)	R7	*Polyarthra vulgaris* Carlin, 1943	R23
Keratella quadrata (Müller, 1786)	R8	*Polyarthra remata* Skorikov, 1896	R24
Kellicottia longispina (Kellicott, 1879)	R9	*Synchaeta* sp.	R25
Trichocerca pusilla (Jennings, 1903)	R10	*Testudinella reflexa* (Gosse, 1887)	R26
Trichocerca cylindrica (Imhof, 1891)	R11	*Filinia longiseta* (Ehrenberg, 1834)	R27
Trichocerca similis (Wierzejski, 1893)	R12	*Filinia opoliensis* (Zacharias, 1898)	R28
Ascomorpha minima von Hofsten, 1909	R13	*Anuraeopsis fissa* Gosse, 1851	R29
Ascomorpha sp.	R14	*Ascomorphella volvocicola* (Plate, 1886)	R30
Ascomorphella sp.	R15	*Pompholyx sulcata* Hudson, 1885	R31
Asplanchna henrietta Langhans, 1906	R16	*Harringia eupoda* Gosse, 1887	R32
Cladocera			
Daphnia cucullata G.O. Sars, 1862	CL1	*Chydorus sphaericus* (O.F. Müller, 1776)	CL6
Daphnia longispina (O.F. Müller, 1776)	CL2	*Bosmina* (*Bosmina*) *longirostris* (O.F.Müller, 1785)	CL7
Daphnia galeata G.O. Sars, 1863	CL3	*Bosmina (Eubosmina) coregoni* Baird, 1857	CL8
Diaphanosoma cf. *mongolianum* Ueno, 1938	CL4	*Podon* sp.	CL9
Moina micrura Kurz, 1875	CL5		
Copepoda			
Copepodites	C1	*Megacyclops gigas gigas* (Claus, 1857)	C6
Cyclops vicinus vicinus Uljanin, 1875	C2	*Acanthocyclops robustus robustus* (Sars G.O., 1863)	C7
Thermocyclops crassus crassus (Fischer, 1853)	C3	*Eudiaptomus gracilis gracilis* (Sars G.O., 1863)	C8
Thermocyclops dybowskii dybowskii (Landé, 1890)	C4	Nauplii	C9
Megacyclops viridis viridis (Jurine, 1820)	C5		

Indicator taxa

As the focus of this paper was the zooplankton communities, we tested for associations among specific taxa and trophic state or ecological potential as defined based on environmental variables or TSI %. The explanatory variables for this analysis were identified through CCA and RDA. We used the Indicator Value (IndVal) test in PAST 4.15 statistical software in order to identify potential indicator zooplankton taxa. This method uses and combines the relative abundance of species with the relative frequency of occurrence of the species in different habitats. Those taxa with significance (p <0.05) and an indicator value > 50% can be used as indicators (Dufrene & Legendre 1997).

Results

Aquatic invertebrates

We recorded 48 zooplankton taxa, of which 32 rotifers, nine cladocerans and seven copepods in the pelagial of the eight reservoirs (Table 2). The total taxon number (TTN) in each zooplankton sample ranged from seven to 17. Details on the composition of benthic macroinvertebrates in the littoral of the eight reservoirs can be found in Subeva et al. (2019).

Ecological potential and saprobic indices

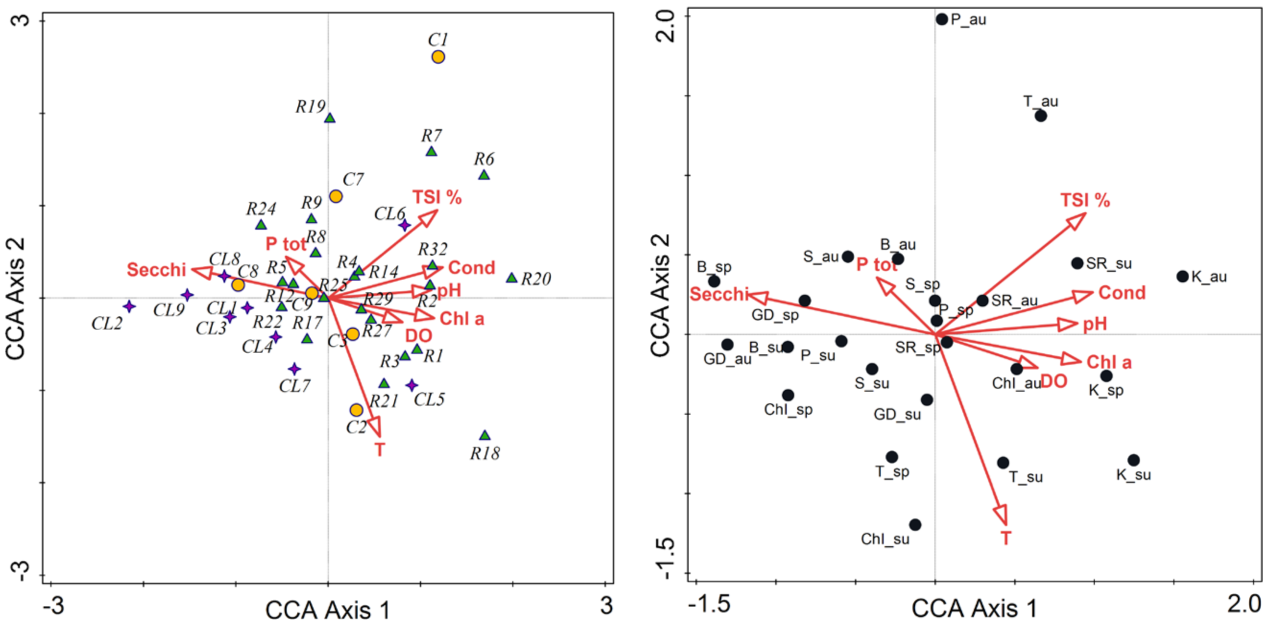
Physical and chemical parameters, as well as the concentration of Chl a (all measured in the pelagial) suggested the ecological potential (EP) of the studied reservoirs during our study varied from good to poor. Different quality elements identified a different potential, with conductivity suggesting the highest and concentrations of Chl a – the lowest EP (Environment: Table 3). Overall, the EP defined based on the macroinvertebrates metric was worse but the metrics did not provide a consistent result with the EP defined based on the environmental parameters. Similar are the results for S_{PB} and Sr%, suggesting more oligotrophic conditions in the pelagial than in the littoral of the studied reservoirs (Table 3).

Associations among abiotic factors and primary production vs. zooplankton communities

Canonical correspondence analysis identified the main factors affecting zooplankton communities, with the first two axes accounting for 44.94% of the cumulative percentage variance in zooplankton (Figs. 2a, b). Monte Carlo unrestricted permutation tests indicated statistical significance of the first axis (pseudo-F = 2; $p = 0.02$) and of all axes (pseudo-F = 1.4; $p = 0.004$). The zooplankton taxon – environmental correlations for the two axes (with pseudo-canonical correlations 0.91 and 0.90, respectively) suggested a clear separation of the taxa in the ordination space. The forward variable selection testing showed that the most important factors were TSI% (pseudo-F = 1.9; $p = 0.008$), conductivity (pseudo-F

Table 3. Ecological potential (EP) of the studied reservoirs based on physical and chemical parameters, primary production and macrozoobenthos. For abbreviations of environmental variables and metrics and details on colour coding, see Materials and Methods. For reservoir codes (Code), see Table 1. TTN denotes the total number of zooplankton taxa.

Code	Environmental						Macrozoobenthos				Zooplankton			
	pH	Cond	DO	P tot	Secchi	Chl a	BMMI	nEQR	S_{PB}	Sr%	TTN	S_{PB}	Sr%	RCC%
GD_sp	8.7	250	9.9	0.8	3.7	2.4	0.33	0.2	2.09	48	10	1.55	62	44.60
GD_su	8.7	232	7.8	0.08	3.4	1.78	0.5	0.4	2.32	44.54	9	1.54	60	69.38
GD_au	8.8	273	10.4	0.025	1.7	0.79	0.3	0.2	2.33	40.6	12	1.4	63	72.00
T_sp	8.7	224	9.8	0.77	0.5	11.85	0.33	0.2	2.56	39.11	9	1.62	59	32.84
T_su	8.8	198	7.1	0.08	0.5	12.44	0.78	0.6	2.67	38.96	13	1.52	60	39.52
T_au	9	261	9.7	0.27	0.8	8.29	0.56	0.4	2.03	47.88	11	1.23	65	12.81
S_sp	8.6	304	8	0.08	3.4	0.74	0.31	0.2	2.25	43.45	10	1.63	59	15.80
S_su	8.9	263	7	0.9	2.6	4.15	0.55	0.6	1.97	50.48	7	1.33	64	67.70
S_au	9.7	270	7.4	0.33	1.6	1.78					14	1.4	62	27.08
B_sp	8.8	146.8	8.8	0.86	2.8	4.145	0.29	0.2	NA	NA	8	1.27	68	98.37
B_su	8.5	176.4	7.8	1.09	3.8	5.18	0.57	0.4	2.46	40.71	9	1.41	62	54.85
B_au	8.6	220	9.6	0.06	0.8	0.42					14	1.44	62	22.11
P_sp	8.7	652	20.8	1.41	1.2	36.72	0.48	0.4	2.36	44.47	11	1.68	59	23.32
P_su	8.4	637	10.3	2.24	1.5	4.81	0.57	0.6	1.98	51.46	7	1.52	61	21.83
P_au	8.1	728	4.5	1.95	2.9	0.59	0.55	0.6	2.11	47.83	10	1.45	63	15.87
SR_sp	9.4	370	7.81	0.87	0.8	0.59	0.25	0.2	2.18	46.81	17	1.76	55	23.44
SR_su	9.7	414	3.5	0.821	0.5	1.18					12	1.88	55	26.06
SR_au	12	267	24.9	0.85	0.2	57.26					13	1.82	53	11.42
K_sp	8.9	529	17.73	1.46	0.8	78.19	0.49	0.4	2.14	51.31	13	1.77	55	0.65
K_su	9.2	604	13.8	0.22	0.6	1.78	0.49	0.4	2.13	41.39	13	1.88	55	15.28
K_au	11	667	19.3	0.1	0.25	76.02					10	1.83	55	7.13
ChI_sp	8.4	347	13.16	1.71	1.4	25.45	0.44	0.4	2.5	39.91	14	1.6	58	58.56
ChI_su	8.6	315	6.5	0.024	1.5	2.37					16	1.64	57	42.32
ChI_au	11	338	20.9	0.3	0.6	78.98					18	1.38	63	10.27



a

b

Fig. 2. Biplot with spatial ordination resulting from canonical correspondence analysis of physical and chemical environmental factors and concentrations of chlorophyll a vs. zooplankton taxa (**a**) and studied sites (**b**). For site codes, see Table 1; for taxon codes – Table 3 and for abbreviations of environmental variables - Materials and Methods.

= 1.7; $p = 0.008$) and total phosphorus (pseudo-F = 1.5; $p = 0.058$), with contributions of 17.3%, 16.8% and 14.6%, respectively. They were followed by water temperature (T, bordering on significance), the concentration of Chl a and Secchi disk depth. Two main gradients were evident for Axis 1: negative (Secchi: −0.54) and positive (Cond: 0.45, TSI%: 0.43 and Chl a: 0.43). Furthermore, Secchi disk depth and Chl a were strongly negatively correlated. Axis 2 was related to water temperature (T: −0.61) and total phosphorus (P tot: 0.18) and separated the sites with high values of water temperature.

Our results suggested that TSI % and Chl a (represented by Axis 1) had the highest positive correlation with the rotifers *Polyarthra euryptera* (R20; ICC = 1.99), *Asplanchna herricki* (R18; ICC = 1.70), *Harringia eupoda* (R32; ICC = 1.13), *Brachionus diversicornis* (R1; ICC = 0.96), *B. calyciflorus* (R3; ICC = 0.83), *P. dolichoptera* (R21; ICC = 0.61, A1), *Keratella tecta* (R4; ICC = 0.30, A1) and *K. cochlearis* (R5: ICC = -0.40), as well as the cladocerans *Chydorus sphaericus*(CL6) and *Moina micrura* (CL5; ICC = 0.91: Fig. 2a). The rotifer *Asplanchna* sp. (R19) was also positively correlated with TSI %, though its ICC was higher (1.94) for Axis 2 and we recorded a high association with lower T. The second highest was the correlation with T and P tot (Axis 2) of the copepod *Cyclops vicinus vicinus* (C2: ICC = -1.21), followed by the rotifers *Kellicottia longispina* (R9; ICC = 0.85) and *P. remata* (R24; ICC = 0.79), both of which were correlated with total phosphorus, in particular; however, they were also associated with Secchi disk depth. Positively correlated with Secchi disk depth (along Axis 1) were the rotifer *Trichocerca similis* (R12; ICC = -0.49) and the cladoceran *Daphnia longispina* (CL2; ICC = -2.1539; Fig. 2a).

The trophic state index and the concentration of Chl a were mostly associated with zooplankton communities in spring samples of Telish, Konush and Sinyata Reka Reservoirs, as well as with the autumn samples of Bebresh, Pchelina and Sinyata Reka Reservoirs. The spring conditions in Gorni Dabnik and Bebresh Reservoirs had the highest (positive) relationship with Secchi disk depth (Fig. 2b).

Relationship among abiotic factors and primary production vs. zooplankton indices

According to the RDA, the first two axes explained 71.58% of the total variance in the saprobiological (S_{PB}) and trophic (RCC%) zooplankton indices (Fig. 3). Monte Carlo unrestricted permutation tests indicated statistical significance of the first axis (pseudo-F = 19.1; $p = 0.002$) and all axes (pseudo-F = 4.4; $p = 0.002$). The most significant were the associations between the studied zooplankton indices and the following variables: Cond (pseudo-F = 8.1; $p = 0.004$), pH (pseudo-F = 4.9; $p = 0.008$), T (pseudo-F = 4.9; $p = 0.012$), TSI% (pseudo-F = 4.2; $p = 0.022$) and P tot (pseudo-F = 3.4; $p = 0.044$).

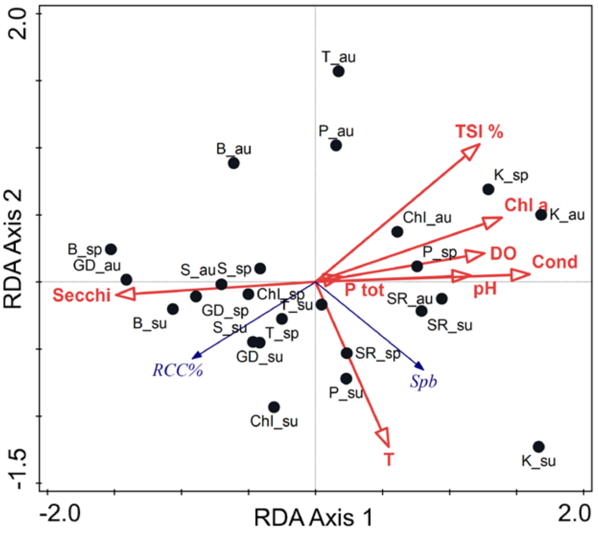


Fig. 3. Triplot of spatial representation of redundancy analysis of the physical and chemical environmental factors and concentrations of chlorophyll a vs. zooplankton indices S_{PB}, RCC% and studied sites. For site codes, see Table 1. The abbreviations of environmental variables are explained in Materials and Methods.

The plotting of the RCC% (ICC = -0.92, A1) in the bottom left part of the triplot indicated a strong negative relationship with the situated in the upper right quadrant TSI% and a positive one with Secchi disk depth. The saprobic index S_{PB} (ICC = 0.81, A1) was positively associated with TSI% and water temperature.

Results of CCA and RDA identified several parameters that appear to be important for structuring zooplankton communities, i.e. TSI%, Chl a, Secchi, P tot and Cond. For each of them, we tested for potential indicator species and selected only the ones with significant *p*-values (Table 4). Copepodites (C1) and nauplia (C9) were excluded from the discussion, as they should not be counted as separate taxa, being early stages of Copepoda.

A total of 21 zooplankton taxa were identified as significant indicators (Fig. 4), 13 of which had an indicator value > 50%. Of these, most of the rotifers indicated eu-mesotrophic conditions (with one exception) or MoEP/PEP (with two exceptions).

Table 4. Indicator zooplankton taxa per metric, with % contribution and *p*-values. Only taxa with significant contributions ($p < 0.05$) are included in the table. Highlighted taxa have a contribution > 50% (shaded), while bold font represents ≥ 50% (rounded values). EP denotes ecological potential. For other abbreviations, see Materials and Methods.

Taxon	**Code**	**TSI%**			**Chl a**			**Secchi/ P tot/ Cond**		
		Trophic state	**Ind-Val%**	***p***	**EP**	**Ind-Val%**	***p***	**EP**	**Ind-Val%**	***p***
Brachionus angularis	R2				Poor	**49.04**	0.0072			
Keratella tecta	R4				Poor	**66.74**	0.0204	Moderate$_{\text{Secchi}}$	**80.75**	0.0036
Keratella cochlearis	R5	Mesotrophic	**58.12**	0.0449				Moderate$_{\text{Ptot}}$	**79.30**	0.0404
Keratella irregularis	R6	Eutrophic	**98.07**	0.0009						
Trichocerca pusilla	R10				Poor	34.19	0.0483			
Trichocerca similis	R12	Oligotrophic	43.76	0.0325						
Asplanchna priodonta	R17	Mesotrophic	**57.03**	0.0036						
Asplanchna sp.	R19	Eutrophic	**60.49**	0.0272						
Polyarthra euryptera	R20				Poor	40	0.0353			
Polyarthra major	R22				Reference	**48.82**	0.0032			
Polyarthra remata	R24							Good$_{\text{Cond}}$	**86.18**	0.0174
Testudinella reflexa	R26				Moderate	**95.23**	0.0183			
Filinia longiseta	R27				Reference	**52.52**	0.0035			
Anuraeopsis fissa	R29							Moderate$_{\text{Secchi}}$	**46.54**	0.0287
Daphnia longispina	CL2				Moderate	**88.04**	0.0351			
Daphnia galeata	CL3				Moderate	**84.15**	0.0291			
Moina micrura	CL5				Reference	36.36	0.0297			
Cyclops vicinus vicinus	C2	Mesotrophic	39.56	0.0484	Moderate	**88.44**	0.0223			
Thermocyclops crassus crassus	C3				High	**72.33**	0.0034			
Acanthocyclops robustus robustus	C7				Reference	36.36	0.0317			
Eudiaptomus gracilis gracilis	C8	Oligotrophic	**47.24**	0.0354				Good$_{\text{Secchi}}$	**66.45**	0.009

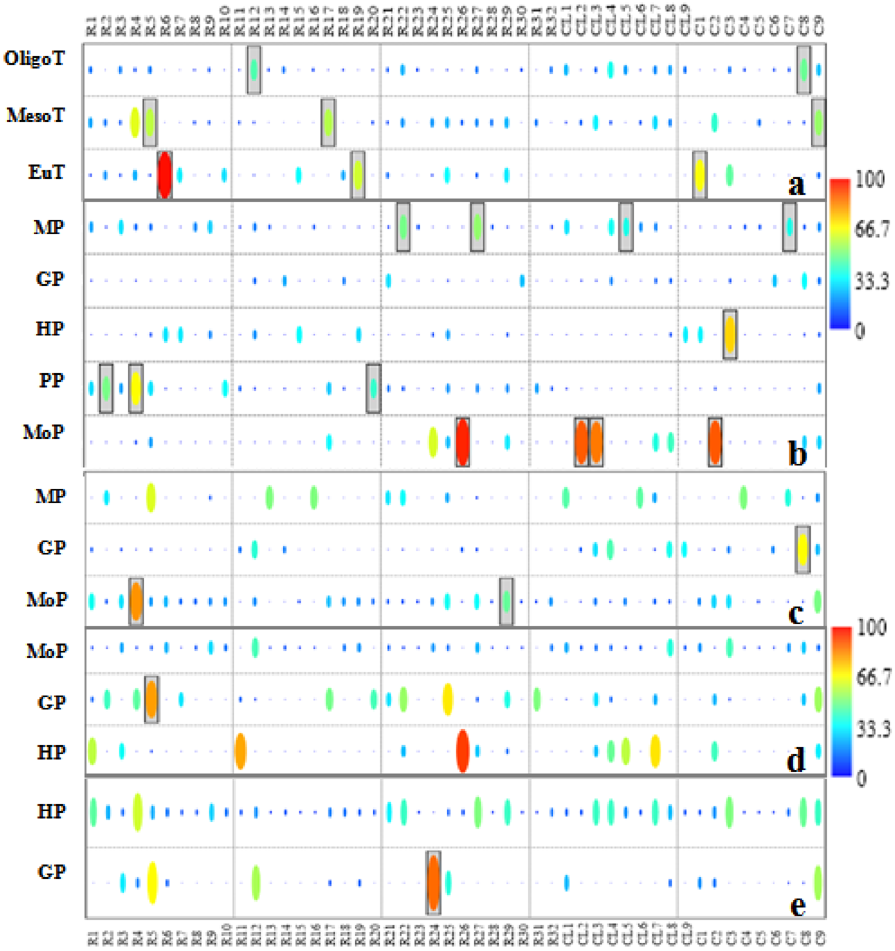


Fig. 4. Visual representation of the individual indicator value (from 0 to 100%) for each zooplankton taxa indicator of different trophic state: identified using TSI% (**a**) or ecological potential as based on Chl a (**b**), Secchi (**c**); P tot (**d**) and Cond (**e**). Rectangles enclose indicator values for taxa with significant contribution to a specific trophic state or ecological potential. Legend: OligoT – oligotrophic; MesoT – mesotrophic; EuT - eutrophic; MP – maximum ecological potential; GP – good ecological potential; HP – high ecological potential; PP – poor ecological potential; MoP – moderate ecological potential.

Three of the species of the genus *Keratella* had indicator values > 75%. Two of the indicator cladocerans were significantly associated with MoEP, both being daphnids with indicator values > 80%. Taxa of Copepoda were indicators of higher EP or oligo-mesotrophic conditions.

Discussion

Lentic ecosystems are under continuous stress for decades now, owing to numerous anthropogenic activities, climate change or natural long-term cyclic events. The never-ending need for freshwater is among the main reasons for the construction of reservoirs. Based on estimations by Lehner et al. (2011), about 16.7 million reservoirs larger than 0.01 ha may exist worldwide, while through sensitivity analyses the authors outline the importance and impacts of smaller reservoirs. The above entails enhanced efforts towards evaluation of ecosystem health of reservoirs, a key facet of current environmental protection and sustainable management.

We found 48 zooplankton taxa, of which 32 rotifers, nine cladocerans and seven copepods. Dominance of rotifers is sometimes associated with less favourable conditions or even pollution (Mukhopadhyay et al. 2007). Based on our analysis, we can conclude that the most significant and persistent relationship of both zooplankton community and univariate metrics were recorded with trophic state index and conductivity. On the other hand, the highest number of identified indicator species was for the trophic state of the reservoirs. Thus, we could speculate that TSI% is likely a good descriptor of changes in zooplankton communities, as demonstrated also by the CCA and RDA. Almeida et al. (2020) and Muñoz-Colmenares et al. (2021) also found that many of the species shifts in zooplankton communities were correlated with changes in trophic state.

The reservoirs with the highest correlation with TSI% and Chl a had the highest trophic level among all studied samples. Environmental variables related to meso-eutrophic conditions, such as the higher trophic state index and concentration of Chl a, correlated with the presence of potential indicator species. These were the rotifers *Polyarthra euryptera*, *Haringia eupoda*, *Branchionus diversicornis*, *P. dolichoptera*, *Keratella tecta* and *K. cochlearis* (the latter two confirmed also by the indicator value analysis) as well as the cladocerans *Chydorus sphaericus* and *Moina micrura*. Most of these species have been reported as eutrophic species (Ejsmont-Karabin 2012, Muñoz-Colmenares et al. 2021). Previous studies have found *Asplanchna herricki* in low trophic conditions (Gannon & Stemberger 1978, Ejsmont-Karabin 2012) and thus it is excluded from the above list with potential indicators of meso-eutrophic conditions.

Our results suggest that some of the other recorded rotifers, together with one daphnid and one cyclopoid, preferred less polluted environments. The rotifers *Kellicottia longispina* and *P. remata* (identified as an indicator of GEP by IndVal) were correlated with total phosphorus, in particular; however, together with the copepod *Cyclops vicinus vicinus* (IndVal: mesotrophic & MEP), these two species were associated also with more transparent waters. Positively correlated with Secchi disk depth were also the rotifer *Trichocerca similis* (IndVal: oligotrophic) and the cladoceran *Daphnia longispina* (IndVal: MEP), as recorded also by Muñoz-Colmenares et al. (2021).

The identified indicator taxa are based on an abundance data matrix, thus accounting for the share of taxa and not only their mere presence. Overall, most of the rotifers, identified as indicator taxa, discriminated eu-mesotrophic conditions or moderate-poor EP, while indicator cladocerans were significantly associated with moderate EP and indicator copepods were indicators of higher EP and oligo-mesotrophic conditions. One of the effects of eutrophication is an increased share of rotifers or crustaceans that prefer eutrophic waters (Karabin 1985, Ejsmont-Karabin 2012, Ejsmont-Karabin & Karabin 2013). Such species are *Keratella tecta, K. cochlearis, Trichocerca pusilla* and species of the genus *Brachionus* (Gannon & Stemberger 1978, Karabin 1985) and the cladoceran *Chydorus sphaericus* (Ejsmont-Karabin & Karabin 2013), as demonstrated also by the results of our indicator values analyses and partially by CCA. Earlier studies confirm that rotifers could be good indicators of trophic state (Gannon & Stemberger 1978, Kozuharov et al. 2013), especially when combined with monitoring physical and chemical water parameters (Jeppesen et al. 2011, Jurczak et al. 2019), as opposed to information provided by the environmental data alone (Caroni & Irvine 2010, Almeida et al. 2020). The above was confirmed also by this study. Our assessments of EP based on physical and chemical parameters resulted in different ecological potential (from MEP through PEP) or were outside the ranges. Furthermore, according to the Bulgarian multi-multimetric index and nEQR, the ecological potential varied from GEP to PEP and differed from assessment using environmental variables.

The zooplankton in most of the studied reservoirs has been poorly studied with the exception of Pchelina Reservoir (Kozuharov 1994, 1996, Kozuharov et al. 2007, Kozuharov et al. 2013). Moreover, to the best of our knowledge, this is the first study to test the applicability of zooplankton in the assessment of reservoirs in Bulgaria and to compare indices based on zooplankton abundance S_{PB}, Sr% and RCC%. Regarding the univariate analyses, the reservoirs with the highest RCC% values are assigned to oligotrophic state, for instance, B_sp and GD_au. According to the S_{PB} index, B_sp and GD_au are oligosaprobic. According to Naidenow (1981), the saprobity of a standing water body depends mostly on its eutrophication status and intensity of production of organic matter. Therefore, we could speculate that the higher values of S_{PB} and Sr% indicated an important inflow of organic matter, where oxidation processes predominate, accompanied by a large amount of free carbon acid, ammonia, hydrogen sulphide and fatty acids (Russev 1972). When comparing our results on saprobic indices, there is a mismatch between the zooplankton community (oligosaprobic to β-mesosaprobic conditions) and the macrozoobenthic communities (β-mesosaprobic to α-mesosaprobic conditions). A possible explanation could be that specific taxon composition is influenced by numerous factors, natural or anthropogenic, e.g. variability of hydrological regime and the higher degree of immediate impacts on the littoral zone. Arias et al. (2022) suggests that both zooplankton and benthic macroinvertebrates should be included in ecological quality management, assessments and even in restoration of water bodies. Further studies are needed to test trophic and saprobic indices for the assessment of ecological potential and to adapt them for the assessment of HMWB and AWB. Future successful assessments should consider re-assigning water body types with updated reference conditions in the frame of changing climate (Free et al. 2024).

Conclusion

Policy-driven approaches identified littoral macrozoobenthos as the key invertebrate group for the assessment of lentic water bodies, while science-driven studies focus more on the pelagial zooplankton communities in assessments of ecosystem health. The pelagial and the littoral of a lake or reservoir are very different in the conditions, which they provide. They could be affected by various stressors, thus triggering a specific response of their communities. Studying lentic ecosystems in a more holistic way, across various water body types and for longer periods, is challenging. However, it would result in rigorous assessment schemes and improved conservation and management of inland surface waters at the European level and, ultimately, in improved ecosystem health. Future challenges for successful assessments include not only larger scale and longer periods but also re-assigning water body types with updated reference conditions in the frame of changing climate.

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