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Phytoplankton, Macrophytes and Macroinvertebrates in Reservoirs: Response to Eutrophication

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Abstract. Eutrophication impact on key aquatic communities (phytoplankton, macrophytes and macrozoobenthos) was studied in three standing water bodies in Bulgaria (Kardzhali, Koprinka and Batak). The reservoirs had a high summer nutrient level and were between eu- and hypertrophic category. In the meso-eutrophic condition the highest number of phytoplankton species and functional diversity (Kardzhali Reservoir) and domination of functional groups (FGs) B, F, X3 and T were registered. The most intensive cyanobacterial growth was recorded in eu- to hypertrophic conditions (Koprinka Reservoir), as well as lower functional and species diversity, and domination of FGs M and J. Hydrological regime in hydroelectric reservoirs was a serious pressure for aquatic macrophytes and macrozoobenthos. Along the nutrient gradient, the Batak Reservoir had highest phytoplankton biovolume and macroinvertebrate taxa richness, well developed macrophyte community, dominated by eutrophication-tolerant species. The results suggested that measures for mitigating ecological impacts from pressures should be implemented without significant adverse effect on water use.

Key words: eutrophication, trophic state, biotic metrics, reservoirs.

Introduction

Eutrophication is one of the major environmental problems in reservoirs leading to water quality deterioration and restriction of their use (Kelly et al., 2016; Padedda et al., 2017). Among the symptoms of *eutrophication* is often the substantial loss of submerged plants and their replacement by dense *phytoplankton* communities (algal blooms). As key factors that reflect macroinvertebrate communities were found chlorophyll *a* and biomass of submerged macrophytes (Pan et al., 2015). The same research reported a loss of macroinvertebrate taxa richness and alteration of species composition along the eutrophication gradient. As far as eutrophication has a widespread impact, often its effects on invertebrate fauna and the remaining water biota is the result of combinations of other pollutants, hydromorphological changes and alien species (Donohue et al., 2009).

The trophic state of the Koprinka Reservoir for the period 2009-2011 was determined as mesotrophic, with good trophic integrity and with benthic community composed mostly of deposit feeders (Kenderov et al., 2014). The algal communities of the same reservoir were reported to be dominated by chlorophytes, cyanoprokaryotes and diatoms (Dochin et al., 2017a). The trophic state of the Kurdzhali Reservoir was assessed as meso- to eutrophic (Traykov, 2005). The last published data on phytoplankton of the Kurdzhali Reservoir reported 137 taxa from 6 divisions (Dochin & Stoyneva, 2014). Transparency, conductivity, pH, dissolved oxygen, total nitrogen were among the variables with the highest impact on the phytoplankton in the reservoir (Dochin et al., 2017b).

The phytoplankton community in Batak Reservoir included a total of 106 phytoplankton taxa and was dominated by diatoms, green algae and blue-green algae (Dochin et al., 2018).

The literature data on phytoplankton communities of Koprinka, Kurdzhali and Batak reservoirs focused mainly on phytoplankton taxonomic structure (species composition, dominant complexes, number of species), spatial and seasonal dynamics of the abundance and provided no data on macrophyte communities. Current research made an attempt to test new phytoplankton-based metrics sensitive to eutrophication and additionally to study how dominant functional groups, growth of cyanobacteria, species and functional diversity react along eutrophication gradient. The aim of this paper was to answer the question how phytoplankton, macrophytes and macroinvertebrates were affected by eutrophication in three large reservoirs.

Material and Methods

Study on three reservoirs Batak, Kardzhali and Koprinka (Table 1) was conducted in three sampling periods during 2011-2015. Water temperature (T, °C), pH, electrical conductivity (C, µS cm^{-1}) and dissolved oxygen (DO, mg L^{-1}) were measured *in-situ* using WTW pH/Conductivity/Oxygen meters. Water sampling followed EN ISO 5667-6. Total nitrogen (TN) and phosphorous (TP) and chemical oxygen demand (COD) were analyzed following the standards EN ISO 11905-1, EN ISO 11885, ISO 15705: 2002.

The nomenclature followed Lee (2008) for phytoplankton, Delipavlov et al. (2003) for vascular plants. Macroinvertebrate taxonomy followed Fauna Europaea (2013).

Phytoplankton was sampled three times during the vegetation season (June-October), close to the reservoir walls. Phytoplankton sampling and laboratory determination, including chlorophyll *a*, followed international standards: ISO 5667-3:2012, EN 15204:2006, ISO 10260:2002. Functional groups (FGs) of the phytoplankton species were determined by their codons following Reynolds et al. (2002) and Padisák et al. (2006, 2009). The descriptor species were selected based on their relative biovolume > 5% of the total biovolume. Percent cyanobacteria (% Cyano) was calculated as relative share of eutrophic species towards total biovolume. Trophic State Index (TSI) of Carlson (1977) was applied: TSI_{TP}, TSI_{SD} and TSI_{CHL} were calculated, and additionally TSI_{TN} (Kratzer & Brezonik, 1981). The classification of trophic categories was after Carlson & Simpson (1996): TSI < 40 - oligotrophy; 40 < TSI < 50 - mesotrophy; 50 < TSI < 70 - eutrophy; TSI > 70 -hypertrophy. Shannon index (Shannon, 1948) and functional diversity according to Borics et al. (2012) were calculated additionally for the phytoplankton communities.

The macrophyte surveys were carried out during the main vegetation period (end of June until September) in belt transects in correlation to the lake size. Species, their abundance and additional relevant parameters were recorded for the defined depth zones (0–1; 1–2; 2–4; and > 4 m). The abundance of each species was noted on a five-degree scale after Kohler (1978).

The macrozoobenthos sampling was made in compliance to the multi-habitat sampling method (Cheshmedjiev et al., 2011) and in accordance with the standards BDS EN ISO 5667-1:2007 and BDS EN ISO 5667-3:2012. After primary processing and taxonomic determination of the macrozoobenthos, a checklist was published (Vidinova et al., 2016). Feeding groups′ affiliation according Cheshmedjiev & Varadinova (2013) was made.

Ten metrics were applied: functional phytoplankton diversity (HFGsDiv) and species diversity (HSpD) of the phytoplankton, total phytoplankton biovolume, chlorophyll *a* (Chl *a*), % cyanobacteria (% Cyano), transparency according to Secchi (Transpar), depth zones of macrophyte colonization (DepthZ-MPH), number of macrophyte species (N-MPH), number of macrozoobenthos taxa (N-MZB) and PETI trophic index (Schweder, 1990).

We examined relationship between TN, TP and COD, and the ten metrics based on phytoplankton, macrophytes and macroinvertebrates with Principal component analysis (PCA). The data were transformed (x′ = log (x+1)), automatically centered and standardized with Canoco v.5 program (Smilauer & Budejovice, 2014).

Results

Trophic state

All three reservoirs had high summer nutrient levels at the border between eu- and hypertrophic category (Fig. 1, Table 2). Kardzhali Reservoir was between mesotrophic (TSI_{CHL}, TSI_{SD}) and eutrophic (TSI_{TP}, TSI_{TN}) category, while Koprinka Reservoir was in eutrophic condition with TSI_{TP} values in hypertrophy (Fig. 1). The strongest variation was observed for TSI in Batak Reservoir: from mesotrophic (TSI_{CHL}, TSI_{SD}), through eutrophic (TSI_{TN}) to hypertrophic (TSI_{TP}) state.

Phytoplankton

Phytoplankton species belonged to 7 taxonomic groups in the three reservoirs: Cyanoprokaryota, Chlorophyta, Chrysophyta, Bacillariophyta, Cryptophyta, Euglenophyta, Dinophyta (Table 2). Nine descriptor species were recorded in Batak Reservoir belonging to 8 FGs: **B**, **C**, **N**, **P**, **E**, **L_0**, **X_3** and **Y**. *Tabellaria fenestrata* var. *asterionelloides* **(N)** and *Fragilaria crotonensis* **(P)** had highest relative biovolume during the three sampling events (Table 2). The cryptophyte *Cryptomonas marssonii* from FG **Y** was recorded at the end of the vegetation season.

Table 1. Morphometric and typology characteristics of the selected reservoirs. Legend: L3 - Mountain lakes in the Eastern Balkans; L11 - Large deep reservoirs (Cheshmedjiev et al., 2010); HMWB - Heavily Modified Water Body.

Features	Batak	Kardzhali	Koprinka
Latitude/longitude	41.95786; 24.15654	41.63333; 25.31666	42.62183; 25.27348
Altitude, m a.s.l.	1106	330	380
Surface area, km^2	22.08	16.7	11.2
Maximal depth, m	30	93	43
Mictic type	dimictic	dimictic	dimictic
Volume, hm^3	310	533	140
Lake Type	L3	L11	L11
Water Body Category	HMWB	HMWB	HMWB
Use	Hydroelectric Power Plant, Water supply, Fishfarming	Hydroelectric Power Plant, Irrigation, Fishfarming	*Hydroelectric Power* Plant, Fishfarming

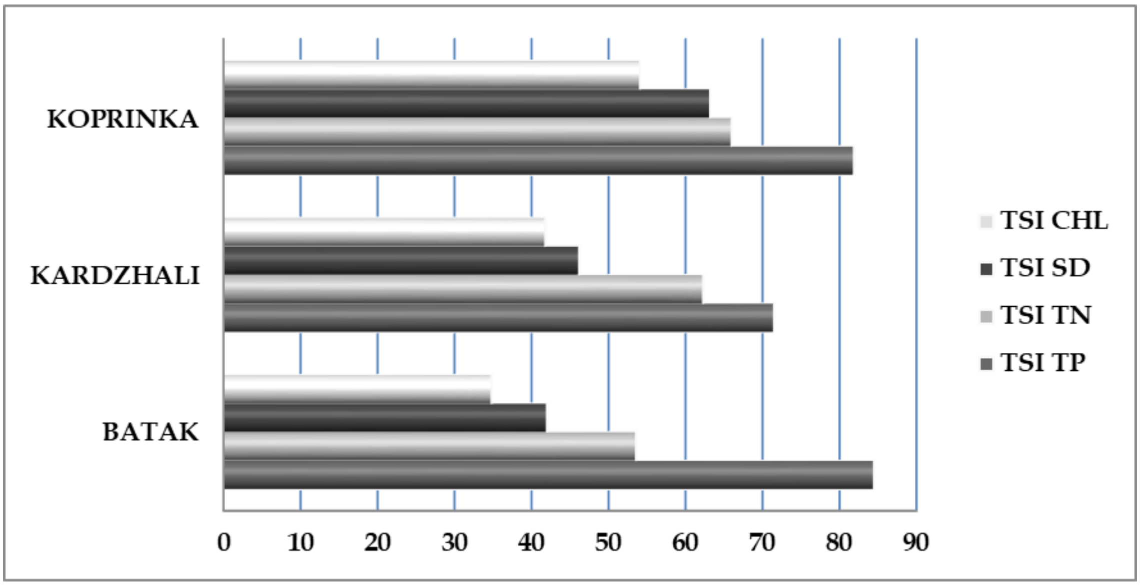

Fig. 1. Trophic State Index (TSI) for Chlorophyll *a* (TSI_{CHL}), Transparency (TSI_{SD}), total phosphorus (TP) and total nitrogen (TN). Classification of trophic categories: TSI < 40 - oligotrophy; 40 < TSI < 50 - mesotrophy; 50 < TSI < 70 - eutrophy; TSI > 70 -hypertrophy.

Table 2. Selected physico-chemical parameters and biota taxa at studied reservoirs. Legend: *average seasonal values for the main physicochemical and biological parameters (surface measurements) in the reservoirs; **descriptor species (> 5% of the total biomass), established in at least one sample and their FGs in parenthesis; ***relative taxa share (%) in the total biovolume during July/September/October.

	Batak	Kardzhali	Koprinka
T,°C*	16.0	23.8	21.7
Secchi depth, m*	3.5	2.6	0.8
pH*	7.45	7.97	8.56
C, μS cm^{-1*}	131.37	317	375.3
DO, mg L^{-1*}	7.32	6.32	8.05
TN, mg L^{-1*}	1.443	1.716	2.221
TP, mg L^{-1*}	0.263	0.107	0.22
COD, mg L^{-1*}	4.53	4.05	5.34
Phytoplankton-Descriptor species**	**Cyanoprokaryota (=Cyanobacteria)** *Woronichinia naegeliana*-***0/0/6.1 **(L_0)** **Chlorophyta** *Pseudosphaerocystis lacustris*-0/0/8.0 **(X_3)** **Chrysophyta** *Mallomonas caudata*-	**Chlorophyta** *Coelastrum microporum*-***9.6/0/0 **(J)** *Eutetramorus planctonicus*-0/4.7/0 **(F)** *Gloeotila monospora*-14.9/0/0 **(T)** *Oocystis lacustris*-7.8/0/0 **(F)**	**Cyanoprokaryota (=Cyanobacteria)** *Microcystis aeruginosa*-***0/18.5/0 **(M)** *Microcystis wesenbergii*-0/41.8/40.7 **(M)** **Chlorophyta** *Coelastrum reticulatum*-38.7/0/0 **(J)**

	0/0/9.1 **(E)** **Bacillariophyta** *Asterionella formosa*-0/0/5.1 **(C)** *Cyclotella meneghiniana*-0/7.4/0 **(C)** *Cyclotella ocellata*-0/12.9/0 (**B**) *Fragilaria crotonensis*-26.9/38.8/0 **(P)** *Tabellaria fenestrata* var. *asterionelloides*-70.1/36.0/45.3 **(N)** **Cryptophyta** *Cryptomonas marssonii*-0/0/19.0 **(Y)**	*Oocystis marssonii*-8.2/0/0 **(F)** *Closterium aciculare*-0/4.7/0 **(P)** *Cosmarium* sp.-0/12.1/0 **(N)** **Chrysophyta** *Chrysococcus minutus*-17.1/0/0 **(X_3)** *Chrysococcus rufescens*-18.6/0/0 **(X_3)** **Bacillariophyta** *Aulacoseira granulata*-0/14.6/0 **(P)** *Cyclotella radiosa*-0/0/15.3 **(B)** *Fragilaria crotonensis*-10.0/32.2/71.5 **(P)** *Fragilaria ulna* var. *angustissima*- 0/11.0/0 **(D)** **Dinophyta** *Ceratium furcoides*-0/15.1/0 (**L_0**)	*Eudorina elegans*-0/4.8/0 **(G)** *Oocystis marssonii*-0/8.5/0 **(F)** *Pediastrum simplex*-24.4/0/0 **(J)** *Staurastrum gracile*-11.4/0/0 **(P)** **Chrysophyta** *Chrysococcus rufescens*-12,7/0/0 **(X_3)** **Bacillariophyta** *Asterionella formosa*-0/0/6.1 **(C)** *Cyclostephanos invisitatus*-0/0/5.0 **(A)** *Cyclotella meneghiniana*-0/12.6/0 **(C)** *Cyclotella pseudostelligera*-0/0/7.5 **(B)** **Euglenophyta** *Trachelomonas volvocinopsis*-0/0/9.1 **(W_2)** **Dinophyta** *Ceratium furcoides*-0/0/15.3 **(L_0)**
Macrophytes	*Ceratophyllum demersum* *Elodea canadensis* *Elodea nuttallii* *Myriophyllum spicatum* *Potamogeton nodosus*	Macrophyte depopulation	*Myriophyllum spicatum*

Fourteen descriptor species were registered in Kardzhali Reservoir which belonged to 9 FGs: **B**, **F**, **N**, **X_3**, **P**, **D**, **J**, **T** and **L_0** (Table 2). The following FGs **F**, (*Oocystis lacustris*, *O. marssonii*), **X_3** (*Chrysococcus rufescens*, *Ch. minutus*) and **T** (*Gloeotila monostroma*) had highest relative abundance in midsummer. They were replaced by FGs **P** (*Fragilaria crotonensis, Aulacoseira granulata*) – 46.8% and **L_0** (*Ceratium furcoides*) – 15.1% during the seasonal succession. The same species from FGs **P** (71.5%) dominated at the end of the season.

Fourteen descriptor species from 11FGs: **A**, **B**, **C**, **F**, **J**, **P**, **M**, **G**, **X_3**, **W_2** and **L_0** (Table 2) were recorded in Koprinka Reservoir. The seasonal succession began with domination of FGs **J** (*Coelastrum reticulatum*, *Pediastrum simplex*), **X_3** (*Chrysococcus rufescens*) and **P** (*Staurastrum gracile*), replaced by FGs **M** (*Microcystis aeruginosa, M. wesenbergii*) and **L_0** (*Ceratium furcoides*) at the end of the season.

Seasonal succession in the Batak Reservoir was characterized by domination of *Tabellaria fenestrata* var. *asterionelloides* from FG **N** during the whole season: **N→N,P→N,Y**.

In the meso-eutrophic Kardzhali Reservoir the seasonal succession of the dominant FGs had the following order: **X_3,F→P,L_0→P,** while for eutrophic state of the same lake type (Koprinka Reservoir) the order was different: **J→M→M,L_0**. Specific FGs for meso-eutrophic state of lake type L11 were **B**, **F**, **X_3** and **T,** for eutrophic were **M** (*Microcystis* species) and **J** (*Coelastrum*, *Pediastrum*) (Table 3).

Table 3. Dominant phytoplankton FGs (with average season abundance > 5%) in lake type L11. Legend: grey: common FGs for both reservoirs; black: specific FGs.

Reservoir	B	F	P	X3	M	J	Lo	T
Kardzhali	5.11	8.22	49.36	11.93			5.02	4.96
Koprinka			5.53		33.89	21.84	6.79	

The highest biovolume was registered in Batak Reservoir (Fig. 2). The biovolumes at Kardzhali and Koprinka Reservoirs were similar, but the percent of eutrophic cyanobacteria was very high in Koprinka Reservoir (36.6%). The parameters for species and functional diversity (H SpD, H FGs Div) had higher values in Kardzhali Reservoir (Fig. 2), while in eutrophic Koprinka Reservoir, species and functional diversity were lower.

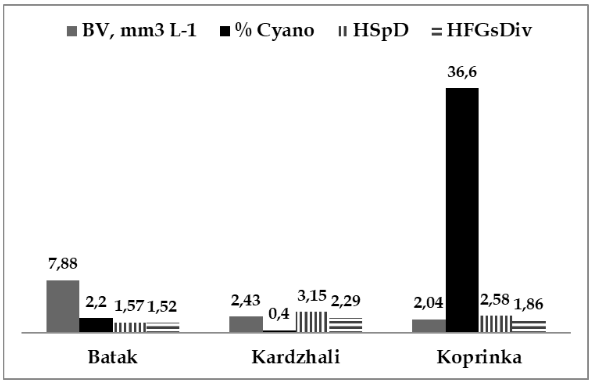

Fig. 2. Midseason values of phytoplankton metrics. Legend: BV – Total phytoplankton biovolume; % Cyano - % cyanobacteria; HSpD - Species diversity; HFGsDiv - Functional phytoplankton diversity.

Macrophytes

Five macrophyte species were registered in Batak Reservoir at 3 depth zones (Table 2). *Elodea nuttallii* was the species recorded with highest abundance and at maximum depth (2-4 m). Two of the registered species (*Ceratophyllum demersum* and *Elodea nuttallii*) were reported as tolerant to eutrophication (Penning et al., 2008).

Only one macrophyte species with low abundance was registered in Koprinka Reservoir, while Kardzhali was in macrophyte depopulation. Water abstraction in such large deep reservoirs results in water level fluctuations and specific conditions suppressing macrophyte communities' development.

Macroinvertebrates

Benthic invertebrates were under the negative influence of fluctuations of the water level caused by permanent water use in the result of negative for aquatic ecosystem anthropogenic activities such as water supply, hydroelectric power plant, fishfarming (Table 1). In the three studied reservoirs taxonomic composition of the macroinvertebrates was dominated by tolerant chironomids and aquatic oligochaetes (Fig.3).

The Sørensen coefficients demonstrated 21% similarity between the Batak and Kardzhali Reservoirs, 19% between Batak and Koprinka. The greatest resemblance (26%) was observed between the Kardzhali and Koprinka Reservoirs. It should be noted that benthic samples were collected close to the reservoir wall, as well both standing water bodies belong to national type L11 (large deep reservoirs) and had a similar hydromorphological and hydrogeological characteristics. Batak was distinguished by the richest taxonomic composition, the prerequisite of which was smallest depth, well-formed sampling littoral zone, where more diverse environmental conditions and changes in the trophic status were observed. During the studied period Batak Reservoir was determined in maximum ecological potential according metric H-MZB, Kardzhali and Koprinka were defined in good ecological potential. (Varadinova, 2013; Varadinova et al., 2019).

Incomplete trophic structure of the bottom communities was formed in the studied modified water bodies. Permanent presence of deposit feeders, tolerant taxa of scrapers and predators were observed. The group of shredders was significantly reduced or completely missing. The deposit feeders (fam. Tubificidae, subfam.

Chironominae) predominated in the trophic structure of the macrozoobenthos in the three reservoirs. This functional group coposed 70% of the benthic community in the Koprinka and Kardzhali and more than half of the invertebrate taxa in Batak (Fig.3).

Relationship between metrics and pressure

Among the tested 10 metrics, chlorophyll *a* levels and percent share of cyanobacteria positively correlated with COD and total nitrogen, while PETI was in negative correlation with COD (Fig. 4). The eigenvalues of the first two PCA axes were 0.662 and 0.338. The second axis revealed the importance of total phosphorous. Aquatic macrophyte community in Batak Reservoir was species richer and occupied deeper zones, as well as macrozoobenthos was represented by highest number of species.

Discussion

The studied communities were highly influenced by site-specific conditions formed by the hydrological regime and environmental variables. They often are correlated to each other, so it is difficult to separate the influence of single factors in determining the assemblage composition (Larocque et al., 2001).

Common result for the three reservoirs was that nutrients indicated higher trophic status then chlorophyll *a* and transparency. The obtained result $TSI_{TP} > TSI_{Chl} = TSI_{SD}$ could be linked to zooplankton grazing or nitrogen limitation (Brown & Simpson, 1998). The type-specific for L11 functional groups, linked with meso-eutrophic state were FGs **B**, **F**, $\mathbf{X_3}$ and **T** (Kardzhali Reservoir)**,** replaced by **M** and **J** in eutrophic state (Koprinka Reservoir). Habitat templates of codons **M** and **J** are highly enriched systems, eutrophic to hypertrophic (Padisák et al., 2009). It was confirmed that blooming cyanobacteria FG **M** (*Microcystis*) domination results in lower phytoplankton functional diversity (Borics et al., 2012). The intense cyanobacterial growth in Koprinka Reservoir could be linked to total nitrogen concentration (2.22 mg L^{-1}) due to the fact that in high phosphorus levels, cyanobacterial development has linear link with nitrogen (Dolman et al., 2012).

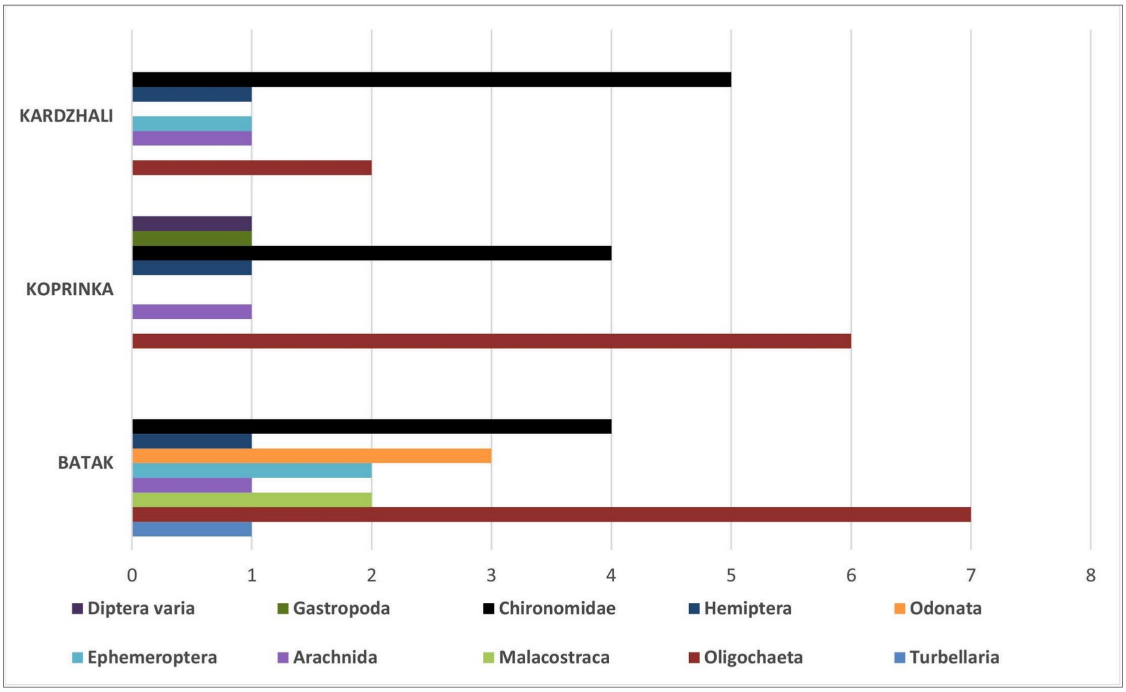

Fig. 3. Distribution of main taxonomic groups of the macrozoobenthos in the studied reservoirs.

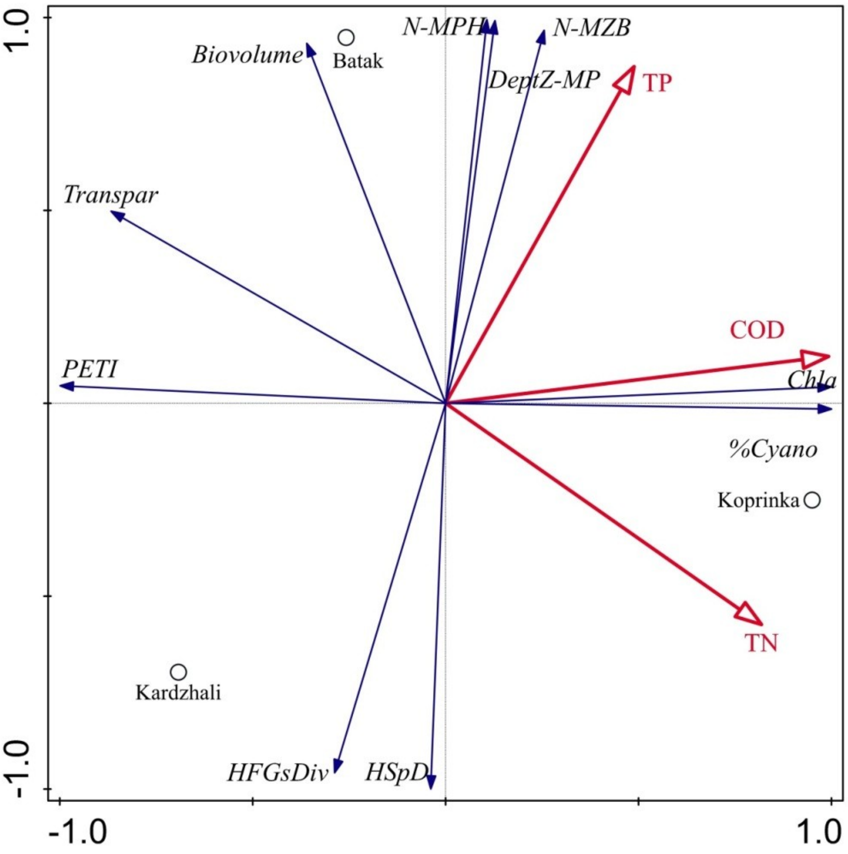

Fig. 4. PCA scatterplot of the studied metrics and selected physico-chemical variables (Chla - Chlorophyll *a*; COD - chemical oxygen demand; DepthZ-MPH – depth zones of macrophyte colonization; HFGsDiv - Functional phytoplankton diversity; HSpD - Species diversity; N-MPH - number of macrophyte species; N-MZB - number of macrozoobenthos taxa; PETI - trophic index PETI; TN - total nitrogen; TP - total phosphorous; Transpar - transparency; % Cyano - % of cyanobacteria).

Water level fluctuations have great influence on aquatic plant communities (Coops et al., 2003). Aquatic macrophytes both in Kardzhali and Koprinka Reservoirs were not represented mainly due to the rapid water level change (> 10 m increase in spring/about 10 m decrease in summer). These results suggested that in such cases, some mitigation measures to reduce impact of maintenance could be recommended. Nevertheless, if such measures will impact the specified water uses significantly, then macrophytes can be applied only as supplementary indicators to eutrophication pressure.

On the contrary, in Batak Reservoir mass development of submerged disturbance indicators was recorded.

Benthic taxa composition in aquatic environments depends mainly on factors such as substratum type, water trophic status, and hydro-period (Sanseverino et al., 1998; Kownacki et al., 2000). Specific invertebrates' communities were formed in the littoral zone of the reservoirs not only as a result of the environmental factors, but also under the conditions of permanent fluctuation of the water level. Important factor for the macrozoobenthos species distribution was and inflow of nutrients and the trophic resources availability. However, when organic pollution is more intense, it is oxygen concentration rather than food that limits the species survival and determines the community composition (Larocque et al., 2001). Although the majority of benthic organisms do not have a strict differentiation with regard to the trophic status, the predominant part of the found species were pollution-tolerant, adapted to nutrient heavily loaded aquatic environment. Some oligochetes like a *Limnodrilus hoffmeistery* and chironomids are probably the most useful profundal indicator of trophic status (Solimini, 2006). They occur over the whole spectrum of nutrient conditions but individual species water worms and non-biting midges have specific ecological preferences. The predominant part of these species belongs to the group of deposit feeders. However, in this study no close connection was found between the trophic status and the percentage of the deposit feeders. Thus, Kardzhali (meso-eu-trophic) and Koprinka (eutrophic) are characterized by different trophic conditions, but deposit feeders in the both reservoirs formed the same share in the trophic structure of the macrozoobenthos. Batak Resevoir, which trophic status varied (meso-ey-hyper-trophic), had the lower percentage of the deposit feeders and maximum ecological potential.

Positive correlation between chlorophyll *a* and % cyanobacteria with COD and TN confirmed previous results of Phillips et al. (2008) and Borics et al. (2013) that the link Chl a = f (TP) is linear only in conditions of low phosphorous levels (TP < 5-100 μg L^{-1}) and that when nitrogen levels are high (TN ≤ 1700 μg L^{-1}) a linear connection was established with nitrogen (Chl a = f (TN)). The number of macrophyte and macroinvertebrate species, as well as the colonized depth zones were positively correlated with the total phosphorous gradient. This could be a result of interactions between *macrophytes* and *phytoplankton* that had led to indirect facilitation among plants and the maintenance of higher *macrophyte* diversity in *eutrophic* conditions.

Conclusion

Eutrophication changes the aquatic environment conditions and has a structurally significant impact on aquatic communities. Phytoplankton was characterized by (i) replacement of FGs: **B**, **F**, **X3** and **T** were replaced by **M** and **J**; (ii) growth of cyanobacteria from FG **M** (*Microcystis* species) and thus a loss of species and functional diversity along eutrophication gradient. Macrophytes are growing at the littoral zone and even small changes in water level affect their distribution and lead to macrophyte depopulation registered in Kardzhali Reservoir and similar limited development in Koprinka. Reduction in macrophytes had a negative impact on the macrozoobenthos because plants provide refuges and impede predation. Anthropogenically induced water level fluctuation and changes in the trophic status alter the food base, which reflects on the taxonomic composition and the trophic structure of the macrozoobenthos. Thus, biota communities′ reaction towards eutrophication in highly modified water bodies should be first differentiated from hydromorphological pressure. The determination of HMWBs′ types based on the use (e.g. for electric power, fish farming, etc.) is therefore crucial. Given the received preliminary results, phytoplankton-based metrics sensitive to eutrophication could be suggested as key metrics in assessing eutrophication in HMWBs.

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