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Ecological status assessment of mountainous rivers (Prespa Lake watershed) via various biotic indices based on aquatic macroinvertebrates

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Abstract
The aim of this study was to provide indicative ecological status assessment of mountainous streams of Prespa Lake watershed, based on benthic macroinvertebrates indices according to the requirements of the EU Water Framework Directive (WFD). The sampling was conducted in spring period of 2021 and 2022 at 5 sites on three rivers. The benthic macroinvertebrates were collected by hydrobiological hand net applying kick & sweep technique and multihabitat procedure. For indicative ecological status assessment, the following indices were used: BMWP, ASPT and EPT-taxa. The water quality of the examined sites was evaluated from high to moderate (I - III class). In summary, the results assisted the selection of appropriate metrics for ecological quality assessment based on macroinvertebrates as BQE and are important in application of management protocols especially when such activities involve poorly investigated river ecosystems.

Key words: Macrozoobenthos, water quality, BMWP, ASPT, EPT, North Macedonia.

Introduction

Freshwater macroinvertebrates are important indicators of river health (Menezes et al. 2010), where its benthic assemblages occupy central role in the environmental processes in the aquatic ecosystems (Rosenberg and Resh 1993). They also present essential key element for their establishment in many biotic indices (eg. Wallace et al. 1996; Gabriels et al. 2010; Andem et al. 2015). Biotic indices, as numeric expressions linking complex ecological relations and taxa richness, enable results more accessible to decision makers that use this data before making crucial decision within the management of water bodies (Armitage et al. 1983).

The Prespa Lake watershed present transboundary basin shared between three countries. Studied watershed is situated in south-western part of the Republic of North Macedonia were four protected areas are established: the National Parks (NP) Pelister and Galicica (IUCN II), the Lake Prespa Monument of Nature (IUCN III) and the NP Erezani (IUCN IV category). According to Ilies (1978) this watershed belongs to the Ecoregion 6, Hellenic Western Balkans. The whole area belongs to the mountain region (higher than 800 m.a.s.l.), characterized as small water basin area (less than 100 km^2) and categorized as one type (type 1) (Petrovska et al., 2014). Although past data about the rivers in the Lake Prespa watershed are very scarce, the reference conditions were easily determined, as all rivers belong to one river type having very short and rapid flows prior to their inflow into Prespa Lake, where only the upstream sections of Kranska Reka and Brajchinska Reka Rivers are well protected from any significant human activities belonging to the NP Pelister (Petrovska et al., 2014). Thus, within the first thematic study conducted on Prespa Lake watersheds, the pressures from natural and anthropogenic origin, i.e. the input of diffuse and point sources pollutants, nutrients and hazardous substances (nutrient leaching from farmland, wastewater discharges from households and industry, emissions from industry and agriculture and leaching from disused landfills) and agriculture activities (drainage, watercourse maintenance and abstraction) were noted (Petrovska et al., 2014).

The ecological monitoring especially in the aquatic ecosystems, requires the development and

Table 1. List of the sampling sites with main data.

Site №	Code	River	Locality	Altitude (m)	Latitude	Longitude
1	**GR_S**	Golema Reka River	Upstream v. Izbishta	920	41°07'56.94"	21°00'23.36"
2	**KR_S**	Kranjska Reka River	Upstream v. Arvati	1022	40°56'42.57"	21°07'01.80"
3	**KR_M**	Kranjska Reka River	Downstream v. Krani	908	40°55'59.33"	21°05'59.66"
4	**BR_S**	Brajchinska Reka River	Upstream v. Brajchino	1050	40°54'33.34"	21°10'00.48"
5	**BR_M**	Brajchinska Reka River	Downstream v. Nakolec	860	40°53'50.41"	21°06'46.14"

implementation of the various biotic indices (eg. Rimcheska and Vidinova 2019, 2020). Within the river management, the point sources of pollution and diffuse loads of agricultural runoff affect macroinvertebrate communities, especially from the small watersheds of semi-mountainous and mountainous rivers (Benetti et al. 2012; Kali et al. 2012; Cooper et al. 2013).

The Water Framework Directive (WFD) (Directive 2000/60/EC) and European Environment Agensy – EEA (2018) requires the use of biotic indices BMWP and ASPT (based on benthic macroinvertebrates assemblages), which provide an integrated assessment of the ecological status of surface water ecosystems. Currently across Europe six versions of BMWP and three versions of ASPT have been adapted and used successfully (Rimcheska and Vidinova 2020). Within this paper was hypothesized that in the upper sections of the studied rivers the water quality would differ from the water quality of lower sections. The main reason behind that would be higher diversity and lower abundance of the benthic macroinvertebrates groups inhabiting less disturbed environment. In that order, the aim of this study was to provide the indicative ecological status assessment of mountainous streams of Prespa Lake Watershed (all of R3- river type), via benthic macroinvertebrates as biological quality element (BQE).

Materials and methods

Study area

The research was conducted in springs of 2021 and 2022 covering a total of 5 sites in the territory of Prespa Lake watershed (North Macedonia) (Figure 1; Table 1). The studied river stretches correspond to the characteristic mountainous (type 1) rivers in Hellenic Western Balkans (Ecoregion 6).

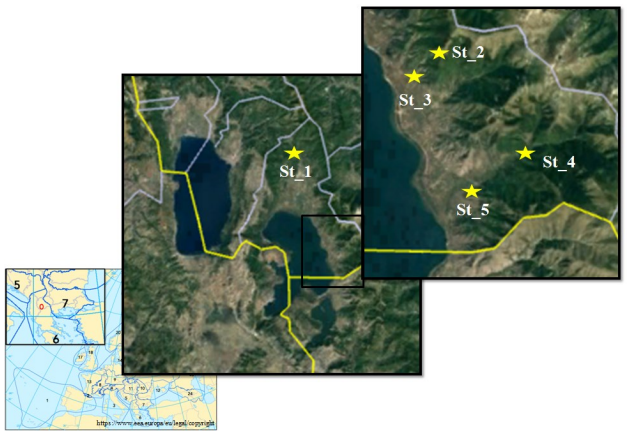

Figure 1: Map of the study area and location of the sampling sites.

The Prespa watershed area consists of permanent and seasonal streams that discharge into the two lakes: Macro and Micro Prespa. Within Macro Prespa Lake the major contributing rivers are Golema Reka, Brajchinska Reka and Kranska Reka in North Macedonia and Agios Germanos River in Greece. The Prespa Lakes form the deep points of an inner-mountainous basin that has no natural surface outflow where the drainage is only provided through karstic underground links by which water of the Macro Prespa Lake (approximately 845 m a.s.l.) drains westwards towards the Ohrid Lake lying approximately 150 metres below (Krstić et al., 2012). The major tributaries of Macro Prespa Lake (Golema Reka, Brajchinska Reka and Kranska Reka) have high variations of the discharge present in time and observed *in situ* at late summer period/s when the rivers have very low water discharges or even no water at all.

The sampling sites selection was done considering two main criteria, namely - natural or near to natural conditions, or the presence of a different

anthropogenic impact on the affected river stretches. Due to the high pollution observed *in situ* during the study period, the mouth part of Golema Reka River was not assessed based on the biological (benthic macroinvertebrates) component.

Macroinvertebrate sampling

Benthic macroinvertebrates were sampled twice per site: in spring period of 2021 and 2022. Exceptions was one site (KR_S), where the sampling was performed during both years, but due to the low water level observed *in situ* the collected material (spring 2021) was not applicable for this type of assessment. In total nine macroinvertebrate samples were collected with a standard pond net (mesh size 500 µm) applying a kick and sweep technique and multihabitat procedure (Cheshmedjiev et al. 2011) and implementing the methodology for collection of benthic fauna according to EN ISO 10870: 2012. At the laboratory macroinvertebrate specimens were elutriated from the inorganic substrates, passed through nested 1 mm and 0,5 sieves. Samples were sorted and labeled and the collected taxa identified to a different taxonomic level (Table 2). All collected and analyzed specimens were preserved in 70% ethanol.

Table 2: Level of identification of the systematic groups established during the study.

Systematic group	Level of identification
Turbellaria	genera
Oligochaeta	families, genera, species
Hirudinea	genera, species
Gastropoda	genera, species
Bivalvia	genera
Crustacea	genera, species
Ephemeroptera	genera, species
Odonata	genera, species
Plecoptera	genera, species
Coleoptera	genera, species
Megaloptera	genera, species
Trichoptera	genera, species
Diptera	families, genera, species
Nematoda	presence
Hydracarina	presence

Data analysis

For the indicative ecological status assessment the following indices were used: Biological Monitoring Working Party Score - BMWP, Average Score per Taxon - ASPT (Armitage et al. 1983) and EPT- index (Ephemeroptera, Plecoptera and Trichoptera taxa richness) (Bode et al., 1997). BMWP, ASPT and EPT indices were calculated using the ASTERICS software package (AQEM Consortium 2002).

Results

A total of 88 taxa belonging to 15 systematic groups of macroinvertebrates were recorded (Table 3, Figure 2). The most diverse groups were the insect orders – Ephemeroptera and Diptera. The upstream site of the Brajchinska Reka River (BR_S, spring 2021) was found to be the richest in total number of taxa (43), while the lowest richness (21 taxa) was observed at downstream site of Kranjska Reka River (KR_M, spring 2022) (Table 3).

Total abundances varied from 1858 ind/m^2 (KR_M) to 492 ind/m^2 (GR_S) in spring 2022 (Figure 2). It was evident that one site (KR_M) differs with the highest abundances during the sampled periods (Figure 2). As a whole, higher abundances occurred in autumn 2021 at GR_S, BR_S and BR_M with numerical dominance of Ephemeroptera and Trichoptera (Table 2). Comparing the study periods the negative trend of taxa decreasing at spring 2022 was recorded where the highest disturbances were evident for BR_S site (Figure 2).

The values of the selected biotic indices (BMWP, ASPT and EPT) suggest the water quality of the studied sites ranges from high (I class) to moderate (III class) (Figure 3). During spring 2022, GR_S and KR_M sites had higher BMWP and ASPT values compared to the previous year. At the same time, GR_S had decreasing EPT-Taxa index value, while KR_M had stable EPT-Taxa index values (Figure 3). More pronounced seasonal dynamics of the values was observed for BMWP and ASPT (spring 2021) at KR_M site that stood out as the most polluted one (III class). The indices values only for the GR_S site indicated excellent (I class) water quality (Figure 3).

Table 3. Taxa richness (number of taxa established per site) in the sampled area during the study.

SYSTEMATIC GROUPS/ SITES	NUMBER OF TAXA								
	GR_S		BR_S		BR_M		KR_M		KR_S
	2021	2022	2021	2022	2021	2022	2021	2022	2022
Turbellaria			1		1				
Nematoda						1			
Oligochaeta		1	1	1	1	2	4	1	1
Hirudinea	1	1					1		
Gastropoda		2	1	2	1	2	2	2	3
Bivalvia	1	1		1	1	1	1	1	1
Crustacea		1		1	2	1	1	1	2
Hydracarina	1		1	1	1	1			
Ephemeroptera	11	7	8	6	11	6	5	5	6
Odonata	1	2							
Plecoptera		3	6	2				1	2
Megaloptera	1		1						
Coleoptera	1		4	3	3	2	2	2	3
Trichoptera	7	7	8	6	8	3	3	3	6
Diptera	8	5	12	9	5	7	7	5	11
In total:	32	30	43	32	34	26	26	21	35

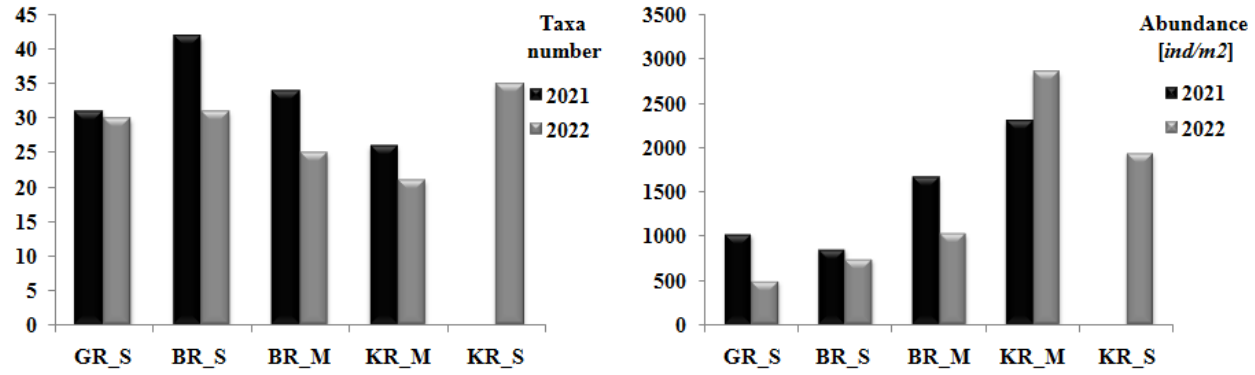

Figure 2. Number of taxa and the total abundance (ind/m2) of the macroinvertebrate communities at sampling sites for the study period.

Discussion

The macroinvertebrate communities from R3- river type (mountainous) rivers from Aegean watershed are characterized by specific conditions (Chesmedjiev and Marinov 2008). Within this study, the scores of selected biotic indices (BMWP, ASPT and EPT) assessed the water quality of the studied sites from high (I class) to moderate (III class) (Figure 3). Obtained results reflect adequately anthropogenic impact, mainly as a result of wastewater pollution, small hydropower plants and agriculture effluents (Krstić et al. 2012; Petrovska et al. 2014). All these kinds of disturbances cause domination of more tolerant macroinvertebrate taxa, which was especially evident at the mouths of the investigated rivers.

In general, the most dominant groups were the insect orders of Ephemeroptera and Diptera. From the results presented in Table 3, it is obvious that the highest taxa richness was detected at the sampling site BR_S (spring 2021). As a result of the high anthropogenic pressure (wastewater inflow and agricultural activities) evident on Kranjska Reka River from its uppermost sampling site observed *in situ,* the lowest taxa richness was observed on the site KR_M during spring 2022.

Implemented indices reviled sensitivity to organic

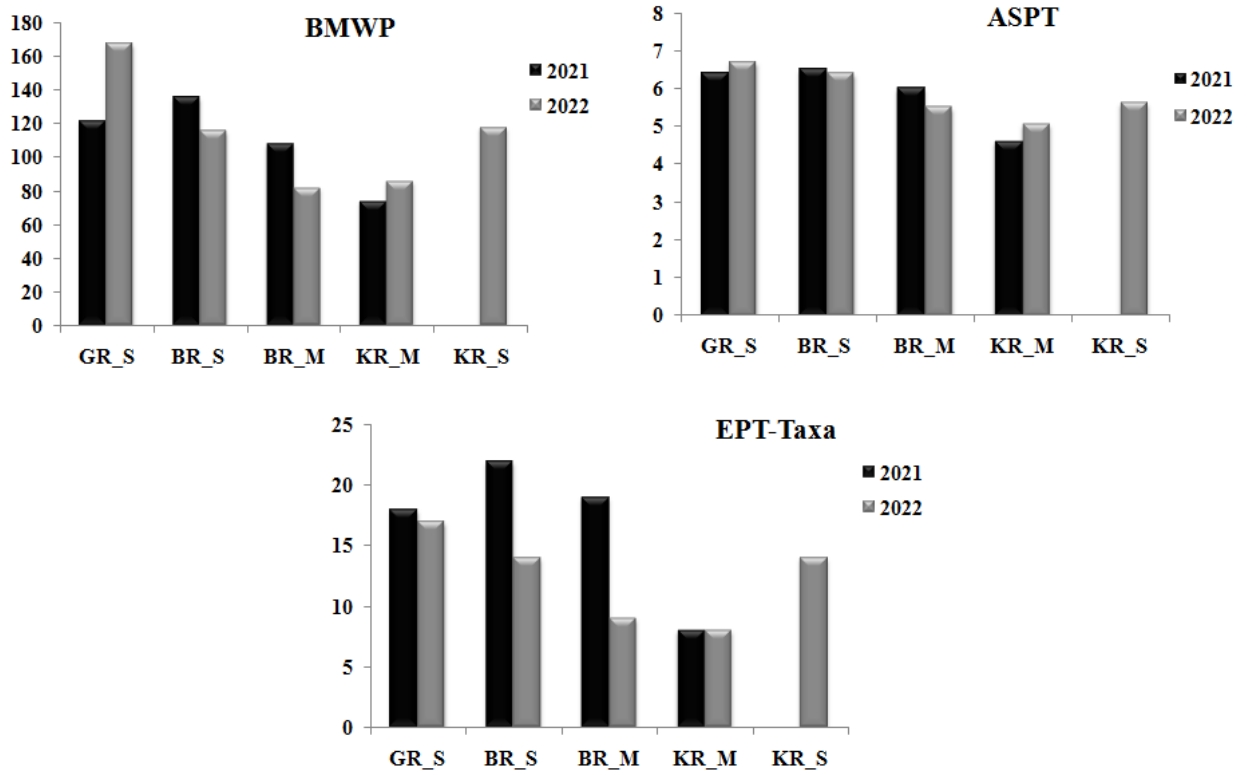

Figure 3. Values of the calculated indices (y-axis) per studied sites (x- axes).

pollution and habitat loss (degradation) at some of the sites. In case of GR_S and BR_S sites the values of EPT and ASPT referred an ecological quality higher than BMWP with one class, which is an indication on the higher sensitivity of BMWP index (Armitage et al. 1983; Rimcheska and Vidinova 2020). The BMWP index responds negatively to increase of organic pollution (Armitage et al. 1983; Ferreira et al. 2004), situation also observed at the most polluted site within this study (mouth part of Kranjska Reka River - KR_M). As given by Rimcheska and Vidinova (2020) not always the low indices scores can be a result of an organic pollution, pointing out that it can be a result of low habitat heterogeneity caused by physical/mechanical barriers (e.g. Hydropower plants), confirmed by lowest values of the BMWP, EPT-taxa and ASPT indices. Probably this situation occurs and at the upper stretch of the Brajchinska Reka River (above sampling site BR_S), where two small hydropower stations were built in the near past (Petrovska et al. 2014). Unlike BMWP, ASPT is more related to seasonal dynamics (Zamora-Muñoz et al. 1995), and the closest assumption within this study can be pointed out on the higher water level observed during 2021 sampling period.

Calculated indices revealed sensitivity to organic pollution and habitat loss at selected sites, as was established by Rimcheska and Vidinova (2020). It is known that BMWP scores are more sensitive than EPT and ASPT (Armitage et al. 1983; Bode et al., 1997; Rimcheska and Vidinova 2019, 2020). That is why comparing the values of BMWP with the those of EPT and ASPT they usually referred to neighboring classes of ecological quality (differs with one class) (e.g. KR_M site).

Bearing in mind the results of the conducted study and the ecological assessment based on the benthic macroinvertebrates, as the closest to "reference" conditions can be pointed out only for the site GR_S. Further more natural and reference sites should be searching in the most upstream sites of the studied rivers.

Conclusion

In summary, few recommendations for future studies are provided: i) the number of studied sites should be increased, including more upstream localities at the studied rivers within Prespa Lake watershed; ii) type- and stressor specific system should be developed;

iii) identification of reference and "near natural" sites within the studied watershed should be done; and iv) clearly defined type-specific macroinvertebrates communities for mountainous (type 1) rivers within studied Ecoregion 6 (Hellenic Western Balkans) should be listed. Implementing the proposed measures will enable to achieve the environmental conditions that will contribute to improvement of the ecological status of studied mountainous rivers and consequently will ensure and preservation of the mountainous benthic macroinvertebrates diversity within the Prespa Lake watershed.

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References

Andem, A.B., Esenowo, I.K., Bassey, D.O., 2015. Application of biotic indices and pollution tolerance index in assessing macro-invertebrate assemblage of Ediba River, cross river state, Nigeria. Journal of Environmental & Analytical Toxicology (S7): 1.

AQEM Consortium, 2002. Manual for application of the AQEM system. A comprehensive method to access European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive. Version 1.0, February 2002. Contract No: EVK1-CT1999-00027, 198 pp. http://www.aqem.de/mains/products.php (accessed September 2022).

Armitage, P., Moss, D., Wright, J., Furse, M., 1983. The Performance of a New Biological Water Quality Score System Based on Macroinvertebrates Over a Wide Range of Unpolluted Running - Water Sites. Water Research 17: 333-347.

Benetti, C.J., Pérez-Bilbao, A., Garrido, J., 2012. Macroinvertebrates as indicators of water quality in running waters: 10 years of research in rivers with different degrees of anthropogenic impacts. In: Voudouris K. (Ed.): Ecological Water Quality-Water Treatment and Reuse, InTech, pp. 95-122.

Bode, R.W., Novak, M.A., Abele, L.A., 1997. Biological stream testing. NYS Department of Environmental Protection. Division of Water. Bureau of Monitoring and Assessment. Stream monitoring unit. Albany. USA.

Cheshmedjiev, S., Marinov, M., 2008. Typology of water ecosystems in Bulgaria (implementation of Water Framework Directive 2000/60/EC). In: Velcheva I.G. & Tsekov A.G. (Eds.): Proceedings of the Anniversary Scientific Conference of Ecology, Plovdiv, November 1, 2008, pp. 371-383 (In Bulgarian).

Cheshmedjiev, S., Soufi, R., Vidinova, Y., Tyufekchieva, V., Yaneva, I., Uzunov, Y., Varadinova, E., 2011. Multihabitat sampling method for benthic macroinvertebrate communities in different river types in Bulgaria. Water Research and Management 1(3): 55-58.

Cooper, S.D., Lake, P.S., Sabater, S., Melack, J.M., Sabo, J.L., 2013. The effects of land use changes on streams and rivers in mediterranean climates. Hydrobiologia 719(1): 383-425.

EEA 2018. https://www.eea.europa.eu/themes/water/european-waters/water-quality-and-water-assessment/water-assessments/ecological-status-of-surface-water-bodies

EN ISO 10870:2012. Water quality - Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters. Finnish Standards Association, Helsinki. 26 pp.

EU Water Framework Directive - 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Communities: L 327, 22.12.2000.

Ferreira, V., Graça, M.A., Feio, M.J., Mieiro, C., 2004. Water quality in the Mondego river basin: pollution and habitat heterogeneity. Limnetica 23(3-4): 295-306.

Gabriels, W., Lock, K., De Pauw, N., Goethals, P.L., 2010. Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). Limnologica-

Ecology and Management of Inland Waters 40(3): 199-207.

Kail, J., Arle, J., Jähnig, S.C., 2012. Limiting factors and thresholds for macroinvertebrate assemblages in European rivers: empirical evidence from three datasets on water quality, catchment urbanization, and river restoration. Ecological Indicators 18: 63-72.

Krstić, S.S., Zdraveski, N., Blinkova, M., 2012 Implementing the WFD in River Basin Management Plans–A Case Study of Prespa Lake Watershed. https://balwois.com/wp-content/uploads/old_proc/530.pdf

Menezes, S., Baird, D.J., Soares, A.M., 2010. Beyond taxonomy: a review of macroinvertebrate trait based community descriptors as tools for freshwater biomonitoring. Journal of Applied Ecology 47(4): 711-719.

Petrovska, M., Conevski, T., Krstić, S., Blinkov, I., Stavric, V., Cukaliev, O., Longholt, J., Ristovski, I., Kočovski, Z., Minčev, I., Dinev, Ġ., Mileski, J., Dimov, M., Bojkovska, R., Talevski, T., Talevska, M., Slavevska Stamenković, V., Stafilov, T., Kaevski, I., Spirovska, S., Krango, N., Soreva, I., Blinkova, M., 2014. Prespa Lake Watershed Management Plan. Report, Ministry of Environment and Physical Planning, 127 pp.

Rimcheska, B., Vidinova, Y., 2019. Ecological status assessment of mountainous and semi-mountainous streams via different biotic indices based on benthic macroinvertebrates - the case study of Macedonian -Bulgarian cross-border territory.- 11th Seminar of Ecology - 2018, Sofia, Bulgaria, Proceedings, 26-27 April: 32-38pp.

Rimcheska, B., Vidinova, Y., 2020. Ecological status assessment of mountain and semi-mountain streams in the aegean watershed: applicability of biotic indices BMWP and ASPT based on macroinvertebrates. Acta Zoologica Bulgarica 72(1): 49–60.

Rosenberg, D.M., Resh, V.H., (Eds.), 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall: New York, USA.

Wallace, J.B., Grubaugh, J.W., Whiles, M.R., 1996. Biotic indices and stream ecosystem processes: results from an experimental study. Ecological applications 6(1): 140-151.