

# Ecological Status Assessment of the Strumica River Watershed Based on Macroinvertebrates – A Step Towards the Implementation of the EU Water Framework Directive in the Republic of North Macedonia

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## Abstract

The aim of this study was to provide ecological status/potential assessment of the water bodies in the Strumica River Watershed based on macroinvertebrates, as a step towards the implementation of the WFD in the Republic of North Macedonia. The material collected during June 2015 from 13 river water bodies, as well as from 2 heavily modified water bodies - reservoirs Turija and Vodocha was examined. The following indices were used for ecological status assessment: EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa richness, Biological Monitoring Working Party Score (BMWP) and Average Score Per Taxon (ASPT). Canonical Analyses (CA) confirmed that macroinvertebrate communities significantly differentiate between water bodies, indicating different ecological conditions. The biological assessment based on applied indices BMWP, ASPT and EPT taxa richness indicated a “healthy” river sector on the Koleshinska, Lomnica and Bezgashtevska Rivers and could be selected as indicators for reference conditions. Dominance of Chironomidae and Oligochaeta, as well as metrics values indicated high levels of ecosystem stress or bad ecological status for the Radovishka River 4, Stara River 2 and Vodochnica River. The findings of this study contribute to the process of implementing macroinvertebrates as a mandatory component in future monitoring studies in the Republic of North Macedonia.

**Keywords:** Ecological status assessment, macroinvertebrates, Strumica River Watershed, Republic of North Macedonia.

## Introduction

The Water Framework Directive (WFD) (Directive 2000/60/EC) is the most comprehensive and overarching instrument of the European Union (EU) water policy. It applies to fresh, coastal and transitional waters and ensures an integrated approach to water management respecting the integrity of whole ecosystems. The environmental objectives of the WFD are to prevent deterioration of the status of water bodies and to protect, enhance and restore all water bodies (WBs), aiming to achieve

good ecological status or ecological potential and good chemical status for surface waters by 2027 at the latest (Arle et al., 2016).

The ecological status assessment of the WFD combines information on several hydromorphological, chemical and biological parameters to acquire a comprehensive picture of the overall status on the functioning and structure of the ecosystem (Noges et al., 2009). For surface water bodies, ecological status or ecological potential is to be assessed using different assessment methods in accordance

with the biological quality elements (**BQEs**): algae, macrophytes, macroinvertebrates and fish fauna (Arle et al., 2016).

Among the BQEs, macroinvertebrates are one of the most commonly used groups in the assessment of the quality of the structure and functioning of surface water ecosystems (Giorgio et al., 2016; Poikane et al., 2016). These organisms present a diverse and generally abundant group with a wide range of environmental tolerances and preferences which can act as long-term indicators of environmental quality (Rosenberg et al., 1993). Macroinvertebrates are found in all aquatic habitats, they are less mobile than most other groups of aquatic organisms, they are easily collected, and most have relatively long periods of development in the aquatic environment. As they are very sensitive to localized pollution loadings (Gresens et al., 2009) they should reflect deleterious events that have occurred in the aquatic environment during any stage of their development (Cairns and Pratt, 1993; Slavevska-Stamenković et al., 2011), fulfilling many of the criteria characterizing the ideal biomonitoring tool (Bonada et al., 2006; Deborde et al., 2016).

In the Republic of North Macedonia, ecological status/potential assessment based on macroinvertebrates started with research on the Mantovo Reservoir by Slavevska-Stamenković et al. (2008). Considerable progress has been made in recent years with the WFD based monitoring on the Pčinja River (Slavevska-Stamenković et al., 2011), Prespa Lake Watershed (Krstić et al., 2012), Ohrid Lake (Schneider et al., 2014) and its tributaries (Trajanovski et al., 2016) and on the Bregalnica River (Slavevska-Stamenković, 2013; Krstić et al., 2016). In regards to the Strumica River Watershed (**SRW**), to date, only the Strumica River Watershed Management Plan (2015) roughly discussed the ecological status of river water bodies (**RWBs**), as well as the ecological potential of a few heavily modified water bodies (**HMWBs**). Furthermore, there is no specific data on ecological status assessment based on different BQEs, including macroinvertebrates.

As a result of poor environmental legacy and an extended period of inadequate resource management, the SRW is experiencing continuous stress, hindering the overall ecological integrity of the ecosystem and the services it provides to the society. The ecosystem of the SRW plays an essential role in sustaining the livelihoods and wellbeing of some 124,500 people in the region. It provides a vital source of water for drinking and for agriculture, which is the chief source of income for the majority of the population. Covering almost seven per cent of the country's territory (with a total area of 1,649 km<sup>2</sup>), this valuable but fragile ecosystem also provides a vital habitat for a large

variety of animal and plant species. The health of the SRW ecosystem has been under threat in recent decades from pollution and rising demand for water from farming, industry and growing urban centers. Unsustainable farming practices, including excessive use of fertilizers and pesticides to grow vegetables and fruits and inefficient irrigation methods, have undermined water quality. Industrial and municipal water demands, coupled with current reservoir operating regimes, have exacerbated fluctuations in water levels, increasing the risk of droughts and floods. These accumulated pressures have made the ecosystem especially vulnerable to climate change, which is causing higher temperatures and extreme weather events which could lead to extreme water scarcity and jeopardize the livelihoods of the region's farming communities (Strumica River Basin District, River Basin Management Plan, 2015).

The aim of this study was to provide ecological status/potential assessment of the water bodies in the SRW based on macroinvertebrates, as a step towards the implementation of the European WFD in the Republic of North Macedonia.

## Methodology

### Study Area

The SRW is one of the four river basin districts in Macedonia (Figure 1). The watershed area is part of a larger trans-boundary river basin comprising parts of Bulgaria and Greece that gravitates toward the Aegean Sea. The SRW covers the furthestmost southeast part of North Macedonia and stretches in a northwest-southeast direction (Figure 1). The SRW contains a multitude of watercourses that are formed in the highest peaks of Mount Plachkovitsa and flow downwards. However, the actual source of the Strumica River is considered to be the spring of Radovishka River which is on an altitude of 1,540 meters. In the field of Radovish, the Radovishka River merges with the Oraovichka River and from that point on in the field bears the name of Stara River. Thence, where the Stara River crosses the short gorge between the Radovish and Strumica basins and enters the Strumica Valley it changes its name to the Strumica River. The Strumica River is a tributary of the Struma River, which flows into the territory of the Republic of Bulgaria (Popovska and Geshovska, 2014). The length of the Strumica River from the spring to the border is 68 km and the total area of the basin is 1,520 km<sup>2</sup> (Stojmilov, 2001).

The Strumica River has four major confluent rivers: Oraovichka River, Plavaja, Turija and Vodochnica. Major reservoirs in the SRW are Turija and Vodocha. The Turija Reservoir was built in 1972 on the Nivicanska River, 16 km northeast from Strumica. It is used for irrigation of about 10,000 ha

of arable land in the Strumica Valley as well as for municipal water supply and production of electricity. The Vodocha Reservoir was created in 1966 on the River Vodochica, 7 km west of Strumica. It is used as the municipal water supply for the City of Strumica and irrigation of roughly 3,100 ha of farmland in the Strumica Valley (Popovska and Geshovska, 2014).

## Materials and Methods

The macroinvertebrates were collected during June 2015 from 13 RWB and 2 HMWB - reservoirs Turija and Vodocha. More detailed information about water bodies is given in Table 1. A map showing the water bodies (sampling localities) in the SRW is also provided (Figure 1). All water bodies belong to the 7th ecoregion (Eastern Balkan) according to Illies (1978).

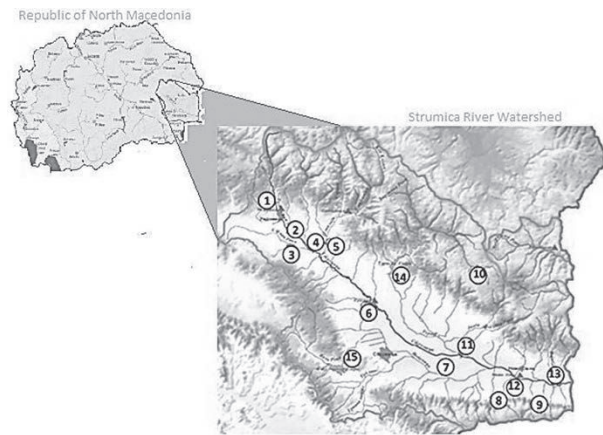


Figure 1: Map of the water bodies in the Strumica River Watershed (R. North Macedonia)

Table 1: Characteristics of the water bodies - WB in the Strumica River Watershed (Republic of North Macedonia)

No.	Water body	Altitude (m)	Latitude	Longitude	Code
1	Radovishka River 1	399	41°38'49.9"	22027°28.60"	WB_1
2	Radovishka River 2	363	41°37'45.02"	22028°9.88"	WB_2
3	Injevska River	370	41°36'57.17"	22025°45.41"	WB_3
4	Stara River 2	272	41°32'39.22"	22035°6.34"	WB_4
5	Plavaja River 2	342	41°36'42.15"	22032°41.19"	WB_5
6	Stara River 3	205	41°33'20.70"	22056°45.84"	WB_6
7	Vodochnica River	215	41°25'24.12"	22042°25.31"	WB_7
8	Koleshinska River	410	41°22'16"	22048°28"	WB_8
9	Smolare Waterfall (Lomnica)	440	41°22'13"	22054°00"	WB_9
10	Bezgashtevska River	964	41°33'52"	22047°47"	WB_10
11	Strumica 1	220	41°25'49"	22047°29"	WB_11
12	Strumica 2	200	41°24'12"	22051°56"	WB_12
13	Strumica 3	200	41°23'21"	22056°45"	WB_13
14	Turija Reservoir	396	41°33'28.07"	22039°19.45"	WB_14
15	Vodocha Reservoir	401	41°39'26.13"	22027°21.09"	WB_15

The macroinvertebrate specimens from different substrates were collected with a Surber sampler and Kick net with a mesh size of 500  $\mu$ m and in some cases (coarse sand and silt) with an Ekman grab, following standard methodology for collection of bottom fauna (EN ISO 10870: 2012). For preservation of macroinvertebrates in the field, 4% formaldehyde was used.

Further processing of the material was conducted in the Laboratory of Invertebrates at the Faculty of Natural Sciences and Mathematics, which included sorting of macroinvertebrates into groups for further identification, preserving, preparation of numerous permanent slides, as well as, adequate handling, labeling, and documentation of the sorted material. Macroinvertebrates were identified using an Olympus SZX9 binocular microscope and the appropriate taxonomic keys (Aubert, 1959; Hynes, 1977; Edington and Hidrew, 1981; Elliott et al., 1988; Waringer and Graf, 1997; Wallace et al., 2003; Zwick 2004; Waringer and Graf, 2013; Glöer,

2015), preserved in 80% ethanol and deposited in the Macedonian National Collection of Invertebrates (MNCI).

Total abundance was expressed as a relative contribution (%) of the species in a benthic community. Canonical Analyses (CA) was applied in order to display the variation in all the samples and species most efficiently. Ordination was performed on macroinvertebrates from both river and reservoir water bodies, using the computer program STATISTICA 8.0.

The most represented biotic indices or metrics such as: EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa richness (Plafkin et al., 1989; Bode et al., 1997), Biological Monitoring Working Party (BMWP) Score and Average Score Per Taxons (ASPT) (Armitage et al., 1983; Friedrich et al., 1996) were used in assessment of ecological status of the RWBs, while for the ecological potential of HMWBs, ASPT for littoral (Carvalho et al., 2002) was used. For calculation of the biotic indices the ASTERICs

software, version 3.0; [www.aqem.de](http://www.aqem.de) (AQEM 2002) was used. The lowest status determined by biotic indices dictated the ecological condition of the water body.

## Results and Discussion

During the investigation of the water bodies in the SRW the presence of 80 macroinvertebrates, mainly cosmopolitan taxa, was confirmed (Annex 1). Based on the macroinvertebrate communities found, the investigated water bodies were grouped in order to better visualize similarities in the community structure and composition. From graphical results of CA, it is obvious that the communities significantly differentiate between water bodies (Figure 4), indicating different ecological conditions.

Concerning RWBs, CA clustered together **WB\_8** - Koleshinska River, **WB\_9** - Lomnica and **WB\_10** - Bezashtevska River by a relatively large presence of macroinvertebrates, mostly characteristic for cold, fast flowing and well oxygenated streams (Figure 4). This group of water bodies, situated in a mountainous area is characterized by the greatest species diversity (21, 25 and 28 taxa) and high EPT taxa values (9-13) (Figure 2). Many authors (e.g. Moskova et al., 2008; Slavevska-Stamenković et al., 2011) associate high EPT taxa values with natural, or near natural conditions of river water bodies. From a quantitative point of view, Plecoptera, Amphipoda, Trichoptera, Ephemeroptera, and Coleoptera present dominant faunistic groups in the communities found in the Koleshinska Reka, Lomnica and Bezashtevska Reka (Figure 3). The most numerous were xeno- and oligosaprobic aquatic insects such as: *Ecdyonurus helveticus* Eaton, 1885 (Ephemeroptera), *Perla marginata* (Panzer, 1799), *Protonemura montana* Kimmins, 1941, *Leuctra hippopus* Kempny, 1899 (Plecoptera), *Hydropsyche fulvipes* (Curtis, 1834) (Trichoptera) and *Hydraena gracilis* Germar, 1824, *Elmis aenea* (Müller, P.W.J., 1806) (Coleoptera) (Annex 1) which according to list of Moog (2002) is indicative for high water quality. Additionally, the research showed occurrence of good populations of sensitive to pollution stone crayfish *Austropotamobius torrentium* (Schrank, 1803) in the Bezashtevska Reka. Obviously, well preserved habitat provides quality conditions for obtaining good populations of stone crayfish whose conservation requires a designation of Special Areas of Conservation (SACs) within the Natura 2000 network. Species is listed on Annex II of Habitats Directive and the stone crayfish is a priority species, which provides an even higher protection status. Further, *A. torrentium* represents a protected wild species in the Republic of North Macedonia (Official Gazette of the Republic of Macedonia no. 139/2011, 2011).

This species is intolerant to environmental change, so threats such as domestic and industrial pollution, agriculture, and household pollution, sedimentation, eutrophication, damming, water abstraction, and channelization have an extremely negative impact on it (Slavevska-Stamenkovic et al., 2016). In summary, macroinvertebrate assemblage, as well as metrics values (BMWP, ASPT, EPT taxa richness; Table 2) indicated a "healthy" river sector on **WB\_8**, **WB\_9** and **WB\_10** (high ecological status) and could be selected as an indicator for the **reference conditions**.

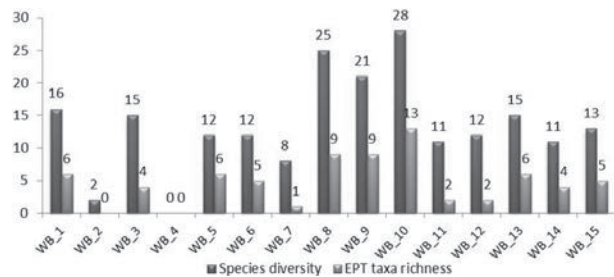


Figure 2: Species diversity and EPT taxa richness for the water bodies in the Strumica River Watershed for June, 2015.

It should be underline that low level of disparity between water bodies in this group (Figure 2) is due to natural dissimilarities in structure of benthic fauna between water courses on higher (WB\_10) and lower altitude (WB\_8 and WB\_9) (Table 1). These results are in accordance with suggestions by Slavevska-Stamenković (2013) and Krstić et al., (2016) for Bregalnica River Watershed, that for different type of water bodies, type-specific biological reference conditions should be established.

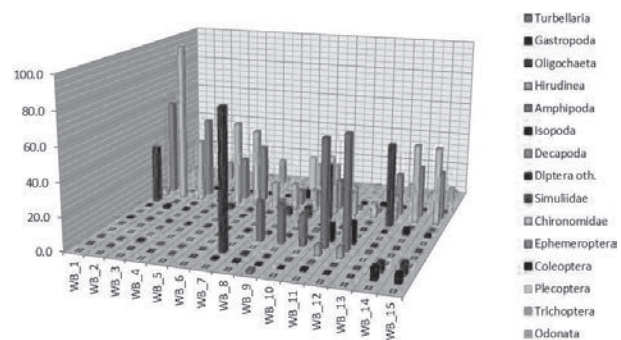


Figure 3: Relative contribution (%) of the macroinvertebrate groups from the water bodies in the Strumica River Watershed for June, 2015.

From Figure 4 it is evident that CA split **WB\_1** (Radovishka River 1) from the group of water bodies 8, 9 and 10. Certain differences in the composition and structure of the communities were probably caused by naturally unfavorable hydrological conditions throughout the year. The Radovishka River 1 is characterised by temporally low water or no water in separate parts of the river

bed, conditions under which a moderate decrease of EPT taxa richness (6) is expected (Figure 2). The Ephemeroptera and Diptera groups present a principal component in benthocenosis, while density of the stoneflies group (Plecoptera) slightly decreased. The effects of flow regime alterations on macroinvertebrates have been recently addressed by Kakouei et al. (2017) and Menció et al. (2018). According to the authors, some differences in faunal composition between perennial and temporary rivers occurred. Namely, some EPT taxa are more vulnerable to flow reductions and disappear, since they lack good dispersal abilities or flexible life cycles (Garcia et al., 2017). Among macroinvertebrates,  $\beta$ -mesosaprobic mayfly (Ephemeroptera) *Baetis vernus* Curtis 1834 was the most abundant species with a relative contribution of 57.2 % in benthic communities (Annex 1). As it was pointed out by Garcia et al. (2017) species from the genus *Baetis*, are more adapted to intermittent sites because they have good dispersal abilities and are less sensitive to flow reductions. Based on community structure and metrics values (Table 2) ecological status on the **WB\_1** is assessed as **good**.

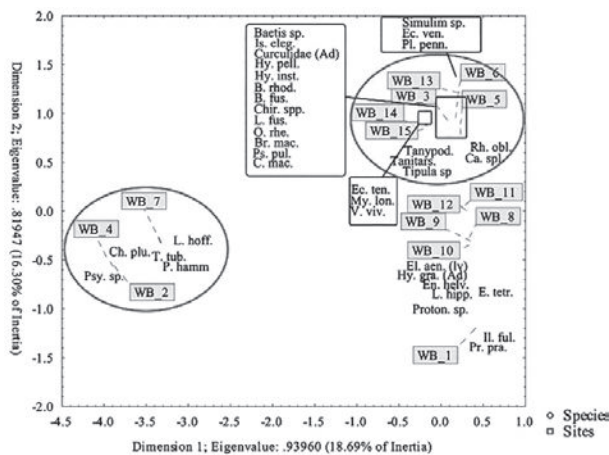


Figure 4: Graphical results of Canonical Analysis (CA). Abbreviations (code) for macroinvertebrate taxa are given in Annex 1.

Further, macroinvertebrate communities changed more and less on the other investigated RWBs in the SRW during the sampling periods (June, 2015). So, the water bodies **WB\_5** (Plavaja River 2), **WB\_6** (Stara River 3) and **WB\_13** (Strumica 3) with moderate impairment of the water quality (**moderate** ecological status; Table 2) were grouped together. It should be stressed that this group of WBs was impacted mainly by agricultural wastewaters, which caused dominance of quite different taxa (Annex 1). It is possible that higher concentrations of nitrate and phosphate from fertilizers contribute to moderate species diversity (12-15) and EPT taxa richness (5-6) (Figure 2). Water quality alterations caused by extended use of fertilizers in agricultural areas and its negative effects on macroinvertebrate

assemblages was discussed in detail by Menció et al. (2018). Additionally, riparian vegetation has been strongly degraded on the Plavaja River 2 and Stara River 3 and both WBs are under moderate impact by the erosion. Jovanovska et al. (2019) discussed the buffering effect of riparian vegetation in watersheds dominated by intense agriculture and point out that EPT taxa richness tends to decline with decline of a natural cover. Strumica 3, however, exhibits slightly better ecological characteristics than the Stara River 1 and 2, since it receives several rivers from the Belasica Mountain that brings in freshwater with low nutrient concentrations.

Even, CA includes **WB\_3** (Injevaska River) in this group of water bodies (Figure 4), which is under multiple pressures in comparison with the previous water bodies. It is influenced by wastewaters from agriculture where the riparian belt has not been preserved and its hydrological regime is disturbed since this river is used for irrigation purposes. This water body is characterized by shallow water and occasionally dries up which contributed to the increased abundance of lentic mayflies *Caenis macrura* Stephens 1835 (Annex 1). As a result of multiple pressures, the composition and structure of the benthic community changed, resulting in **poor** ecological status of **WB\_3** (Table 2). A completely distinctive type of benthic community was registered in **WB\_11** (Strumica 1) and **WB\_12** (Strumica 2) (Figures 4) which are under intensive human impact from municipal wastewaters. Compared to the previous group of water bodies (Figure 2), a significantly limited number of EPT taxa were registered (only 2), indicating an increased level of ecosystem stress. Amphipoda and Chironomidae groups quantitatively dominated in benthocenosis, accompanied by tolerant groups of Isopoda and Hirudinea (Figure 3). Macroinvertebrate assemblages are mainly presented with taxa indicative of higher nutrient concentrations in the water and more intensive decomposition processes, such as Chironomini larvae and  $\alpha$ -mesosaprobic *Asellus aquaticus* (Linnaeus, 1758) (Isopoda), *Erpobdella octoculata* Linnaeus 1758 and *Helobdella stagnalis* Linnaeus 1758 (Hirudinea) (Annex 1). The structure of the benthic community, as well as the metrics values given in Table 2, clearly shows **poor** ecological status on **WB\_11** and **WB\_12**. A group of three water bodies (**2**, **4** and **7**) positioned in the lower left of the ordination diagram in Figure 4, indicated that the benthic community significantly differentiated from other groups. Actually, two of the selected river water bodies, the Radovishka Reka 4 (**WB\_2**) and the Stara Reka 2 (**WB\_4**) were observed to be wastewaters whose sampling and analyses belong to special protocols and safety precautions. The Radovishka Reka 4 receives the communal wastewaters from Radovish, while the Stara Reka 2 receives industrial wastewaters and

waters that are leaching from the communal solid waste landfills. The highly deteriorated water quality conditions of the Radovichka Reka 4 (**WB\_2**), almost just *Chironomus plumosus* (Chironomidae) survived, which according to list of Moog (2002) is a highly tolerant species. Concerning to Stara Reka 2 (**WB\_4**) only a few specimens of *C. plumosus* were recorded near the river banks, exhibiting a trend which is characteristically associated with hypo- or anoxic conditions, which indicates a severe degradation of the benthic community by toxic compounds. With respect to **WB\_7** (Vodochnica Reka), a significantly low number of species (8), as well as EPT taxa (1) were registered (Figure 1). Furthermore, Oligochaeta (82.4%) and Chironomidae (15.6%) were the most significant groups in terms of quantity (Figure 3), with dominance of *Limnodrilus hoffmeisteri* Claparede 1862, *Tubifex tubifex* Muller 1774 (Oligochaeta) and *C. plumosus* (Annex 1). As this WB is under the strong influence of the wastewaters from city of Strumica and agricultural land, it is reasonable to assume why these polysaprobic species (Milbrink, 1994; Margaritora et al., 2003) significantly contributed to the benthic community. It is evident that the community structure indicated high levels of ecosystem stress or **bad** ecological status in **WB\_2**, **WB\_4** and **WB\_7**. Biological metrics confirmed this statement (Table 2).

Bearing in mind that many anthropogenic activities negatively affect lakes and reservoirs, and would be expected to drive changes in the littoral benthocenoses (Boon, 1992), in the frame of the current study special attention was given to the littoral region of two HMWBs - the Turija

(**WB\_14**) and Vodocha (**WB\_15**) reservoirs. Littoral invertebrates play an important role in the food web of lakes and in the sequestration and recycling of materials (Schindler and Scheuerell, 2002; Donhue et al., 2009). The same authors stated that this, in combination with their relatively long life cycles and large number of principally sedentary species, supports their potential for classifying the ecological status/potential of the lakes and reservoirs. Graphical results of CA confirmed similar benthic communities in the littoral region of the both reservoirs (Figure 4). Among macroinvertebrates, Tanitarsinii spp. and Chironominae spp. larvae (Chironomidae), as well as  $\beta$ -mesosaprobic *Caenis macrura* and  $\alpha$ -mesosaprobic *Cloeon dipterum* (Ephemeroptera) were the most abundant taxa in the benthocenosis (Annex 1; Figure 2). During the sampling campaign, the presence of highly eutrophic *Chironomus plumosus* was not identified in the sandy littoral region. The Caddisflies (Trichoptera) with  $\beta$ -mesosaprobic *Mystacides longicornis* (Linnaeus 1758) and  $\alpha$ -mesosaprobic *Ecnomus tenellus* (Rambur, 1842) as well as aquatic warms (Oligochaeta), such as polysaprobic *L. hoffmeisteri* moderately contributed in benthic community (Annex 1). Nascimento et al. (2009) suggest that *L. hoffmeisteri* is widely recognized as an effective indicator of organically polluted aquatic environments, so its presence in the littoral region indicated possible, further changes in the ecological potential of both reservoirs. In summary, the composition and structure of a benthic community, as well as the metric values (Table 2) indicated moderate ecological potential of **WB\_14** and **WB\_15**.

Table 2: Assessment of the ecological status / potential of the investigated water bodies in the Strumica River Watershed for June, 2015, based on BMWP, ASPT and EPT indices

Metrics / Water Bodies	WB_1	WB_2	WB_3	WB_4	WB_5	WB_6	WB_7	WB_8	WB_9	WB_10	WB_11	WB_12	WB_13	WB_14	WB_15
BMWP	G	B	P	B	M	P	B	H	H	H	P	P	M	/	/
ASPT	G	B	M	B	H	G	B	H	H	H	M	M	G	M	M
EPT taxa richness	G	B	M	B	G	M	B	H	H	H	P	P	G	/	/
Ecological status / potential	G	B	P	B	M	M	B	H	H	H	P	P	M	M	M

\* Legend: H - high status; G - good; M - moderate; P - poor; B - bad.

## Conclusions

The quality of surface waters in the SRW is a very sensitive issue because anthropogenic actions degrade surface waters and impair their use for drinking, industrial, agricultural, recreation or other purposes. A comprehensive water quality monitoring program (number of water bodies increased, reference conditions properly checked

and confirmed, biological communities clearly defined for the various types of water courses) is necessary in order to safeguard public health and to protect the valuable fresh water resources. In this context, the macroinvertebrate community in the SRW changes according to the water quality, thus, the use of benthic macroinvertebrate indicators greatly enhances the states' ability to identify and

subsequently improve impaired water (Machado et al., 2015). The biological assessment showed that water bodies in the SRW with high or good ecological status are associated with the presence of natural areas and a high number of EPT taxa, while dominance of Chironomidae and Oligochaeta species caused by agricultural activities and urbanisation at the lowest altitude are important predictors of ecological degradation and bad/poor ecological status.

Furthermore, the findings of this study contribute to the process of implementation of the macroinvertebrates as a mandatory component in monitoring studies in the Republic of North Macedonia. For further work on the ecological status assessment of the Strumica River Watershed, type and stressor specific system should be developed. This involves the work on typology of water bodies, identification of reference and "near natural" sites, selection of appropriate metrics in addition to development of type specific reference conditions.

Overall, ecological status/potential assessment of the water bodies in the Strumica Reka Watershed based on macroinvertebrates, presents a step towards the implementation of WFD in the Republic of North Macedonia. We believe that this information can be useful in bringing awareness to the local communities and politicians of the urgent need for measures aimed at minimizing the anthropic impacts observed in the Strumica River Watershed.

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## Annex 1. Relative contribution (%) of the macroinvertebrate taxa from the water bodies in the Strumica River Watershed for June, 2015 and code for macroinvertebrate taxa for Canonical Analyses (CA).

No.	Animal Groups and Taxa / Water Bodies	WB_1	WB_2	WB_3	WB_4	WB_5	WB_6	WB_7	WB_8	WB_9	WB_10	WB_11	WB_12	WB_13	WB_14	WB_15	Code for CA
<b>Turbellaria</b>																	
1	<i>Dugesia gonocephala</i> Duges 1830	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	<i>Du_gon</i>
<b>Gastropoda</b>																	
2	<i>Ancylus fluviatilis</i> Müller 1774	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.4	0.2	0.0	0.0	0.0	0.0	0.0	<i>An_flu</i>
3	<i>Radix (Lymnaea) auricularia</i> Linnaeus 1758	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.3	2.7	<i>Ra_aur</i>
4	<i>Galba (Galba) truncatula</i> Müller 1774	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.5	0.0	0.0	0.0	<i>Ga_tru</i>
5	<i>Physella acuta</i> Draparnaud 1805	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Ph_acu</i>
6	<i>Viviparus viviparus</i> (Linnaeus, 1758)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	<i>Vi_viv</i>
<b>Oligochaeta</b>																	
7	<i>Pristina rosea</i> Piguët 1906	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	<i>Pr_ros</i>
8	<i>Limnodrilus hoffmeisteri</i> Claparede 1862	0.0	0.0	0.7	0.0	0.0	0.0	56.9	0.0	0.0	0.0	0.0	0.0	0.0	5.5	6.8	<i>Li_hof</i>
9	<i>Potamothenis hammoniensis</i> Michaelsen 1902	0.0	0.0	0.0	0.0	0.0	0.0	8.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Po_ham</i>
10	<i>Tubifex tubifex</i> Müller 1774	0.0	0.0	0.2	0.0	0.0	0.0	17.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Tu_tub</i>
11	<i>Enchytraeus albidus</i> Henle 1837	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	<i>En_alb</i>
12	<i>Eiseniella tetraedra</i> Savigny 1826	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Ei_tet</i>
<b>Hirudinea</b>																	
13	<i>Erpobdella octoculata</i> Linnaeus 1758	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	4.7	0.0	0.0	0.0	<i>Er_oct</i>
14	<i>Helobdella stagnalis</i> Linnaeus 1758	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	1.9	0.0	0.0	0.0	<i>He_sta</i>
<b>Amphipoda</b>																	
15	<i>Gammarus balcanicus</i> Schaefer 1922	0.1	0.0	0.1	0.0	0.0	0.6	0.0	23.2	23.6	18.6	63.6	67.1	0.8	0.0	0.0	<i>Ga_bal</i>
<b>Isopoda</b>																	
16	<i>Asellus aquaticus</i> (Linnaeus, 1758)	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	10.2	12.7	0.0	0.0	0.0	<i>As_a_bal</i>
<b>Decapoda</b>																	
17	<i>Austropotamobius torrentium</i> Schrank 1803	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	<i>Au_tor</i>
<b>Trichoptera</b>																	
18	<i>Potamophylax latipennis</i> Curtis 1834	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.4	0.9	0.0	0.0	0.0	0.0	0.0	<i>Po_lat</i>
19	<i>Halesus digitatus</i> von Paula Schrank 1781	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	<i>Ha_dig</i>
20	<i>Hydropsyche instabilis</i> Curtis 1834	0.0	0.0	0.0	0.0	15.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Hy_ins</i>
21	<i>Hydropsyche pellucidula</i> Curtis 1834	0.0	0.0	11.3	0.0	0.0	15.8	0.0	0.0	0.0	0.0	0.0	0.0	8.0	0.0	0.0	<i>Hy_pel</i>
22	<i>Hydropsyche fulvipes</i> Curtis 1834	0.4	0.0	0.0	0.0	0.0	0.0	0.0	14.4	13.8	0.0	0.0	0.0	0.0	0.0	0.0	<i>Hy_ful</i>
23	<i>Silo pallipes</i> (Fabricius 1781)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	<i>Si_pal</i>
24	<i>Sericostoma flavicorne</i> Schneider 1845	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	<i>Se_fla</i>

No.	Animal Groups and Taxa / Water Bodies	WB_1	WB_2	WB_3	WB_4	WB_5	WB_6	WB_7	WB_8	WB_9	WB_10	WB_11	WB_12	WB_13	WB_14	WB_15	Code for CA
25	<i>Rhyacophila obliterata</i> Zetterstedt 1840	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	<i>Rh_obl</i>
26	<i>Tinodes rostocki</i> McLachlan 1878	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0	<i>Ti_ros</i>
27	<i>Psychomyia pusilla</i> Fabricius 1781	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Ps_pus</i>
28	<i>Philopotamus montanus</i> Donovan 1813	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	<i>Ph_mon</i>
29	<i>Athripsodes</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	<i>Ath_sp.</i>
30	<i>Brachycentrus maculatus</i> Fourcroy 1785	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	<i>Br_mac</i>
31	<i>Mystacides longicornis</i> (Linnaeus 1758)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	2.7	<i>My_lon</i>
32	<i>Ecnomus tenellus</i> (Rambur, 1842)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	4.1	<i>Ec_ten</i>
Ephemeroptera																	
33	<i>Ecdyonurus venosus</i> Fabricius 1775	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	<i>Ec_ven</i>
34	<i>Ecdyonurus helveticus</i> Eaton 1885	1.2	0.0	0.0	0.0	0.0	0.0	0.0	2.8	4.4	3.6	0.0	0.0	0.0	0.0	0.0	<i>Ec_hel</i>
35	<i>Serratella ignita</i> Poda 1761	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.2	0.0	0.0	<i>Se_ign</i>
36	<i>Baetis fuscatus</i> Linnaeus 1761	0.0	0.0	4.4	0.0	0.0	31.5	0.9	0.0	0.0	0.0	1.7	0.9	12.3	0.0	0.0	<i>Ba_fus</i>
37	<i>Baetis vernus</i> Curtis 1834	57.2	0.0	0.0	0.0	0.0	0.0	0.0	5.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Ba_ver</i>
38	<i>Baetis rhodani</i> Pictet 1843	0.0	0.0	4.7	0.0	23.3	0.0	0.0	0.0	0.0	6.1	0.0	0.0	12.4	0.0	0.0	<i>Ba_rho</i>
39	<i>Baetis alpinus</i> Pictet 1843	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.6	0.0	0.0	0.0	0.0	0.0	<i>Ba_alp</i>
40	<i>Ephemera danica</i> Müller, 1764	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	<i>Ep_dan</i>
41	<i>Cloeon dipterum</i> Linnaeus 1761	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.2	16.4	<i>Cl_dip</i>
42	<i>Habrophlebia fusca</i> Curtis 1834	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	<i>Ha_fus</i>
43	<i>Oligoneuriella rhenana</i> Imhoff 1852	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Ol_rhe</i>
44	<i>Caenis macrura</i> Stephens 1835	0.0	0.0	39.8	0.0	0.0	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.7	12.3	<i>Ca_mac</i>
Plecoptera																	
45	<i>Perla marginata</i> Panzer 1799	0.1	0.0	0.0	0.0	0.0	0.0	0.0	3.4	4.0	0.6	0.0	0.0	0.0	0.0	0.0	<i>Pe_mar</i>
46	<i>Perlodes microcephalus</i> Pictet 1833	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	<i>Pe_mic</i>
47	<i>Protonemura montana</i> Pictet 1841	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.4	21.8	0.3	0.0	0.0	0.0	0.0	0.0	<i>Pr_mon</i>
48	<i>Protonemura praecox</i> Morton 1894	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Pr_pra</i>
49	<i>Leuctra fusca</i> Linnaeus 1758	0.0	0.0	0.0	0.0	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Le_fus</i>
50	<i>Leuctra hippopus</i> Kempny 1899	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	<i>Le_hip</i>
Chironomidae																	
51	<i>Orthocladiinae</i> spp.	0.8	0.0	3.9	0.0	35.7	25.3	0.0	14.1	12.4	9.6	0.0	0.0	1.5	0.0	0.0	<i>Ort_spp.</i>
52	<i>Tanypodinae</i> spp.	0.8	0.0	14.7	0.0	0.0	4.0	2.7	2.2	0.9	3.7	2.8	0.9	0.0	1.8	4.1	<i>Tany_spp.</i>
53	<i>Tanitarsini</i> spp.	3.0	0.0	15.4	0.0	4.0	6.6	0.0	0.0	0.0	4.4	0.0	0.0	10.0	23.6	26.0	<i>Tani_spp.</i>
54	<i>Chironomini</i> spp.	0.0	0.0	3.7	0.0	12.0	11.9	0.0	0.0	0.0	0.0	11.9	8.5	3.8	21.8	16.4	<i>Chir_spp.</i>
55	<i>Chironomus plumosus</i> (Linnaeus 1758)	0.0	98.4	0.0	0.0	0.0	0.0	12.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Ch_plu</i>
Simuliidae																	
56	<i>Simulium</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.9	0.2	0.0	0.0	0.1	0.0	0.0	<i>Pr_tur</i>
57	<i>Prosimulium</i> sp.	0.1	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	<i>Dic_sp.</i>
Diptera oth.																	

No.	Animal Groups and Taxa / Water Bodies	WB_1	WB_2	WB_3	WB_4	WB_5	WB_6	WB_7	WB_8	WB_9	WB_10	WB_11	WB_12	WB_13	WB_14	WB_15	Code for CA
58	<i>Atherix ibis</i> Fabricius 1798	0.0	0.0	0.0	0.0	2.4	0.4	0.0	0.0	0.0	0.0	0.0	0.0	49.6	0.0	0.0	<i>Sim_sp.</i>
59	<i>Clinocera</i> <i>stagnalis</i> Haliday 1833	35.0	0.0	0.2	0.0	0.0	0.0	0.0	4.4	6.7	33.8	0.0	0.0	0.0	0.0	0.0	<i>Pro_sp.</i>
60	<i>Tipula</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0	<i>At_ibi</i>
61	<i>Prionocera turcica</i> Fabricius 1787	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Cl_sta</i>
62	<i>Dicranota</i> sp.	0.1	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Tip_sp.</i>
63	<i>Tabanus</i> sp.	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Tab_sp.</i>
64	<i>Psychoda</i> sp.	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Psy_sp.</i>
65	<i>Bezzia</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6	1.4	<i>Be_sp.</i>
Coleoptera																	
66	<i>Cyphon</i> sp. (lar)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Cyp_sp.</i> (lar)
67	<i>Limnius</i> sp. (ad)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	<i>Lim_sp.</i> (ad)
68	<i>Gerris lacustris</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	<i>Ge_lac</i>
69	<i>Ilybius fuliginosus</i> (Fabricius, 1792) (ad)	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Il_ful(ad)</i>
70	<i>Elmis aenea</i> (lar)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.4	1.5	0.0	0.0	0.0	0.0	0.0	<i>EL</i> <i>aen(lar)</i>
71	<i>Elmis aenea</i> (ad)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	<i>EL</i> <i>aen(ad)</i>
72	<i>Curculionidae</i> (ad)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Curcu(ad)</i>
73	<i>Hydraena gracilis</i> Germar 1824 (ad)	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.9	1.3	1.2	0.0	0.0	0.0	0.0	0.0	<i>Hy</i> <i>gra(ad)</i>
74	<i>Hydrophilidae</i> sp. (ad)	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.6	0.4	0.0	0.6	0.9	0.0	0.0	0.0	<i>Hyd_sp.</i> (ad)
75	<i>Peltodytes caesus</i> (Duftschmid, 1805) (ad)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	<i>Pe</i> <i>cae(ad)</i>
Neuroptera																	
76	<i>Osmylus</i> <i>fulvicephalus</i> (Scopoli, 1763)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	<i>Os_ful</i>
Odonata																	
77	<i>Calopteryx</i> <i>splendens</i> Harris 1782	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.2	0.6	0.9	0.5	0.0	0.0	<i>Ca_spl</i>
78	<i>Platycnemis</i> <i>pennipes</i> Pallas 1771	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	<i>Pl_pen</i>
79	<i>Ischnura elegans</i> (Vander Linden, 1820)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	2.7	<i>Is_ele</i>
Heteroptera																	
80	<i>Corixa dentipes</i> Thomson 1869 (lar)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.5	0.0	0.0	0.0	<i>Co</i> <i>den(lar)</i>