REPORT

on biodiversity research for the section of the Mateševo - Andrijevica highway

Based on the contract signed with **WINsoft DOO** , a report on biodiversity research was prepared for the section of highway Matesevo - Andrijevica. The report consists of two sections: I - Baseline survey of the biodiversity of fish fauna in Drcka River and Lim River and II - Baseline survey of the biodiversity of benthic fauna in Drcka River and Lim River

The research team

Prof. Dr Drago Marić, ichthyology expert, team leader

M.Sc. Stevan Marić, ichthyology expert

Dr Danijela Šundić, expert for benthic fauna

Table of Contents

I - Baseline survey of the biodiversity of fish fauna in Drcka River and Lim River

Prepared by Prof. Dr Drago Marić and
M.Sc. Stevan Marić

Table of Contents

1. AIM AND OBJECTIVE OF THE RESEARCH

The aim of the research is to analyze and assess the condition of the fish fauna (the qualitative and quantitative status) in the Drcka River and Lim River) on the section of the Bar - Boljare highway, at sites where the influence of the highway is possible, in order to obtain the necessary data on the fish fauna in this area, for the purposes of preparation Environmental impact assessment study for the section of the Andijevica - Mateševo highway.

2. METHODS

2.1 Equipment and methodology of investigation

Uzorci se sakupljeni, odnosno ribe su lovljene, sa elektroagregatom (Sl. 1), a uzorci se obrađeni na mjestu ulov i ribe se nakon obrade vraćene u vodu.

The investigation was conducted using the methods that ichthyofauna researchers use in their specific field. These are methods that are widely known and based on which ichthyologists work and have been demonstrated in their numerous scientific and professional papers. For the realization of this work all necessary equipment for the field and laboratory research were used.

The assessment and analysis of the status of the fish fauna (qualitative-quantitative composition) was carried out at selected points in the field. Fishing was carried out in a continuous position-distance of about 150 m of river flow. The assessment of the ichthyofauna status was performed on the basis of the sample as an absolute indicator, as well as on the basis of relative indicators, and the abundance was estimated and expressed on one kilometer and on 1 ha of river surface area.

Samples of fish were collected, i.e. fished with an electric aggregate (Figure 1), and samples were treated at the site of catch, and after processing fish were returned to water.

Abundance of species is presented in the percentages, as well as on descriptive way: D – dominant species, C – common species, R – rare species, V – very rare species.

Figure 1. Fish catching with electroaggregate in Drcka River (foto: personal archive)

2.2 Investigated localities and time (date) of investigation

The research was conducted on the sampling sites in three rivers: Drcka River, Lim River and Zlorečica River. Basic parameters of investigated localities are presented in the figures 2, 3, 4, 5 and 6. Figures 2, 3, 4 and 6, 7, 8 show time of sampling, elevation, GPS coordinates.

Drcka River – First sampling site Mateševo was just above the stationary point, second sampling site was about 3 km upstream from Mateševo, and the third one was in the upper part of Kraljske Bare area.

Drcka River was investigated on 21 September 2019; position of 3 sampling sites are presented on the figures 1, 2, 3 and 4.

Lim River – The sampling was conducted on 2 sampling sites: first sampling site was nearby, bellow the Andrijevica, and the second sampling site was about 2 km downstream from the upstream (bridge on Lim River, for road direction to Seoce). Lim River was investigated at both localities on 22 September 2019. Sampling sites are shown on the figure 4.

Zlorečica River – the samples of fish were investigated only at one sampling site nearby the confluence of Lim River, below the bridge, nearby the veterinary station. Zlorečica River was investigated at both localities on 22 September 2019. Sampling sites are shown on the figure 4.

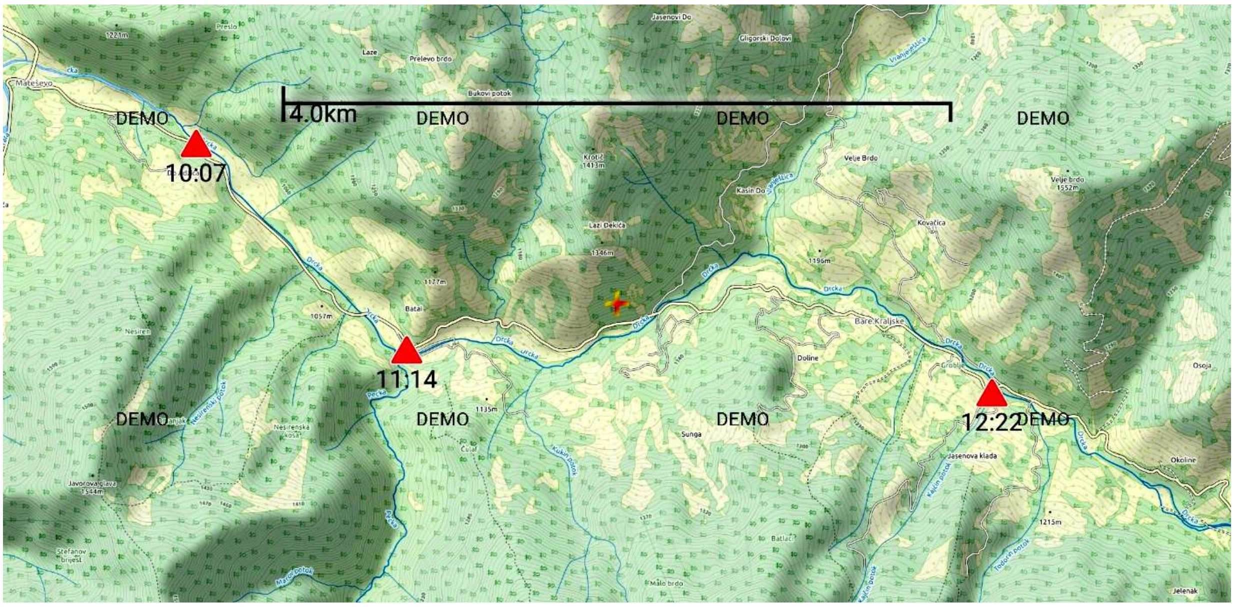


Figure 2. Position of investigated localities on Drcka River (presented position and time of sampling)

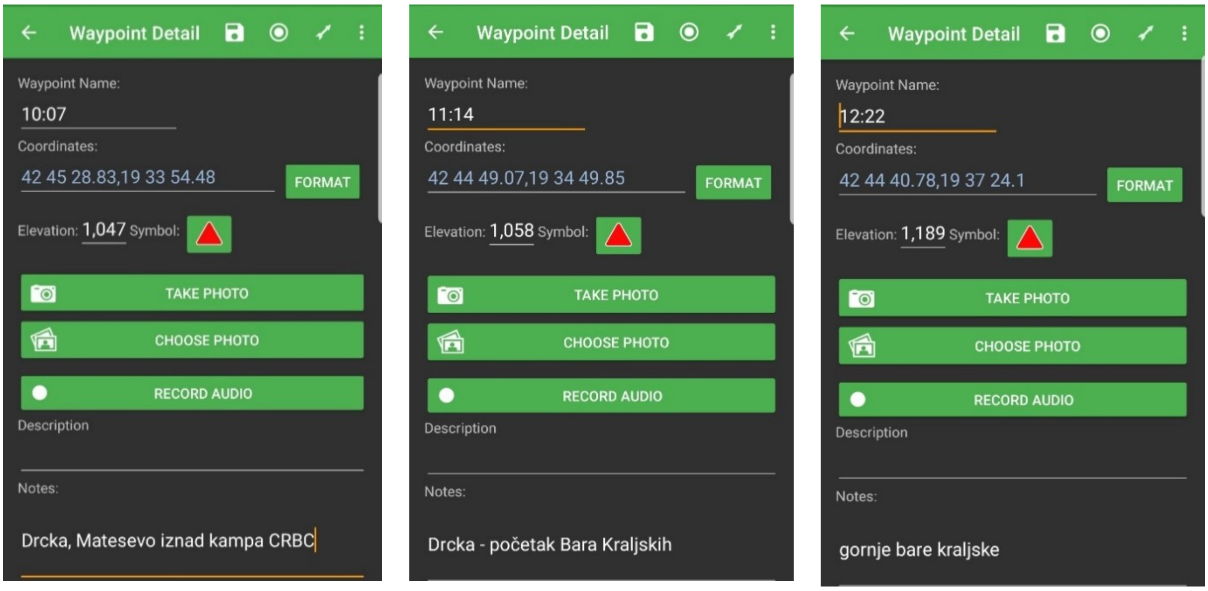


Figure 3. Basic parameters for investigated localities on Drcka River

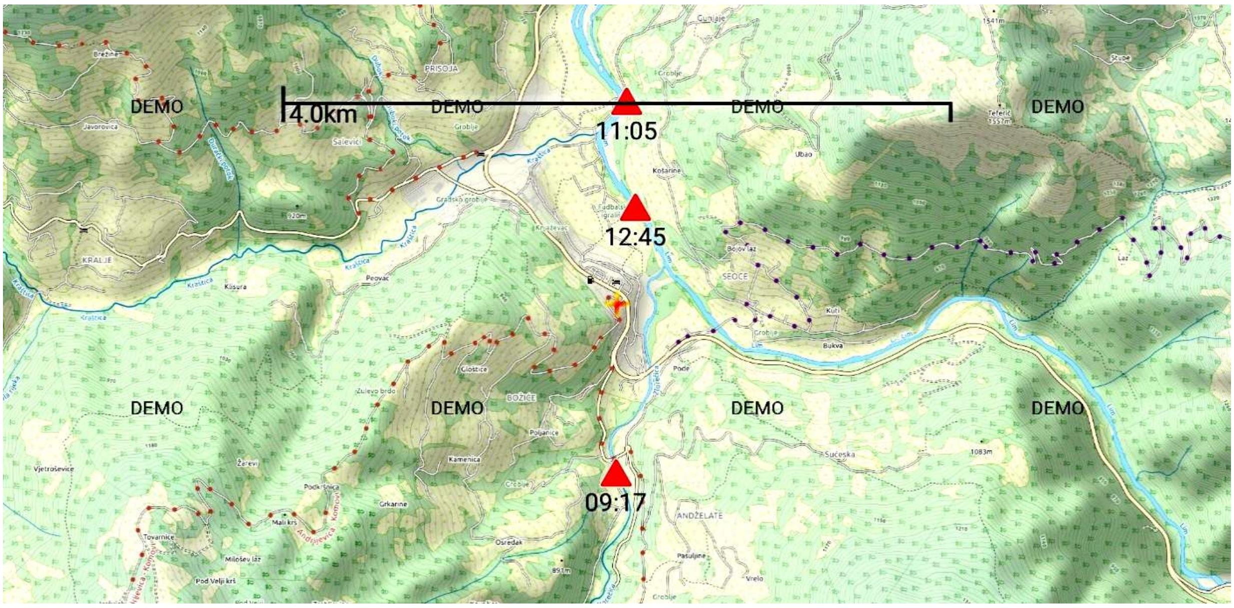


Figure 4. Position of investigated localities on Zlorečica River and Lim River (presented position and time of sampling)

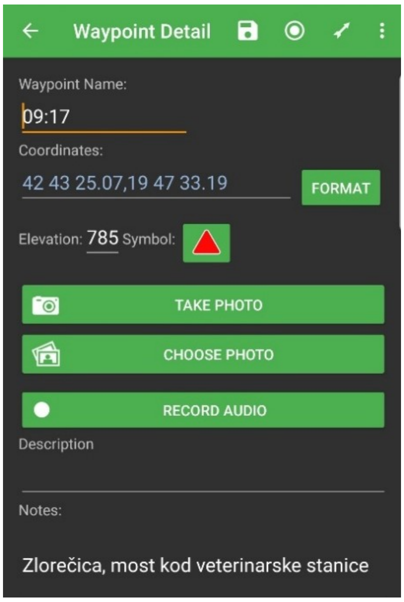


Figure 4. Zlorečica River

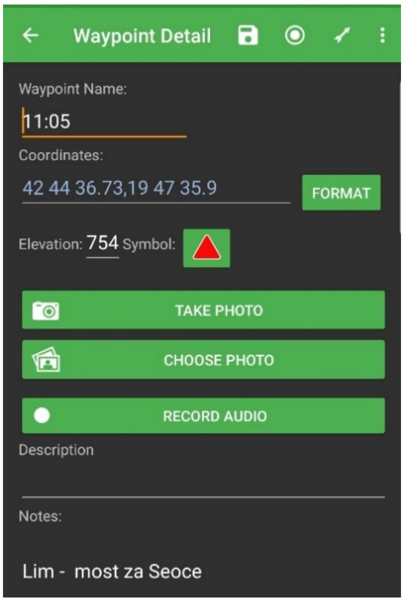


Figure 5. Lim River

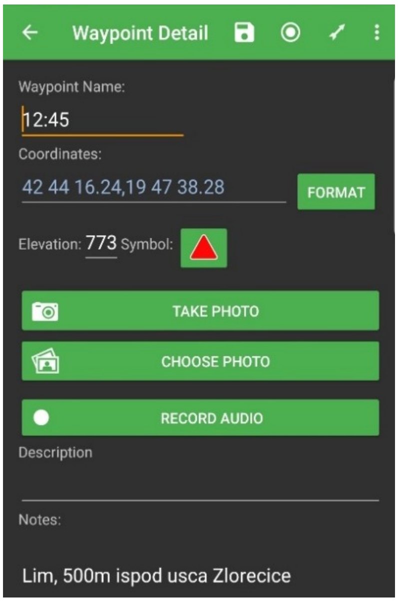


Figure 6. Lim River

Figure 4 – 6. Basic parameters for investigated localities

3. RESULTS OF THE INVESTIGATION

3.1 Ichthyofauna (structure-species)

a) Qualitative composition – diversity (number of species)

In the drainage basin of Tara River, nine (9) species of fish has been recorded up to now. Also, earlier investigation in the past confrmed the presence of five (5) species from 4 families in the Drcka River on the investigated sampling sites (Marić & Milošević, 2011; Marić, 2019) as it follows:

Familia: *Salmonidae*

Salmo labrax (Pallas.1814) – Black Sea trout

Familia: *Thymallidae*

Thymallus thymallus (Linn. 1758) - European Grayling

Familia: *Cyprinidae*

Barbus balcanicus (Kotlik, Tsigenopulos, Rab and Berrebi, 2002) - Large spot barbel

Phoxinus csikii (Hanko, 1922) - Danube Minnow

Familia: *Cottidae*

Cottus gobio (Linn. 1758) – European Bullhead

By this investigation, species *Phoxinus csikii* has not been recorded. According to former data, this species was represented in a small numbers, and was not recorded at all investigated sites within her areal of distribution. Table 1 shows list of fish species from Drcka River with their common name (English and Montenegrin).

Table 1. Findings of fish species from Drcka River

Latin name	**English common name**	**Montenegrin common name**	Literature data	This report
Salmo labrax	Black Sea trout	Crnomor. pot. pastrmka	+	+
Thymallus thymallus	European grayling	Lipljen	+	+
Barbus balcanicus	Large spot barbel	Balkanskaa mrena	+	+
Phoxinus csikii	Danube Minnow	Dunavska gaovica	+	-
Cottus gobio	European Bullhead	Peš	+	+

At the sampling site in the middle of investigated area (on the beggining of Bare Kraljske area) species *B. balcanicus* was not recorded, and at the third sampling site (the end of Bare Kraljske area), only species *S. labrax* and *C. gobio* were recorded (data presented in the table).

Other species from Tara River such as: *Hucho hucho* (Linnaeus, 1758) - huchen, *Chondrostoma nasus* (Linnaeus, 1758) - common nase and *Squalius cephalus* (Linnaeus, 1758) - chub, have not been registered in these localities in earlier (Maric & Milošević, 2011, Marić, 2019) or during this investigation. In this river, as well as in the whole Tara River Basin, there is no eel (*Anquilla anquilla*), as well as other species on the LISTS – APENDIX This qualitative composition of the fish community is expected, and all species found are indicators of clean water. Only common barbel can tolerate waters that are more heavily loaded with organic matter, regardless of their origin.

It should be emphasized here that such a qualitative composition of the community (biodiversity) is characteristic for the salmonid and salmonid-thymalid region in all rivers of this type in the Danube River Basin, as well as for all clean waters of the area.

Table 2. Conservation status of fish in Drcka river

Latin name	**Bern**	**Habitats Directive Annexes**	**IUCN - Red List status** Evropa	-Montenegro	Natura 2000
Salmo labrax	-	-	LC	VU	-
			LC	VU	
Thymallus thymallus	II	V	LC	LC	-
			-	LC	

Barbus balcanicus	-	V	LC	LC	+
Phoxinus csikii	-	-			-
Cottus gobio	-	II			+

Conservation status in Montenegro according to Marić, 2019.

In the waters from Danube drainage basin, the migrator species such as European eel (*Anguilla anguilla*) and sturgeon (*Acipenser* spp.) are not presented.

3.2 *Abundance of species (quantitative composition)*

Drcka River

At the site in the middle course of the river, the abundance of grayling was higher than the abundance of Black Sea trout, both in number and weight (Table 3). However, considering the abundance, regardless of weight part, it follows that European Bullhead (Cottus gobio) is the dominant species throughout this river. The high abundance of this species in such waters is a normal occurrence, but it should be noted that this species also tolerates habitats that have been altered by anthropogenic activities. The most abundant species was C. gobo, which is generally similar in all rivers that belong to the Danube drainage basin in Montenegro. The table below shows the representation of species during field research in the Drcka River.

Table 3. Presence and abundance of species from locality Drcka River

Species	L-1. Iznad Mateševa br/grami	L-2. srednji dio toka br/grami	L-2. gornji dio toka br/grami
Salmo labrax	21 / 1.264	17 / 918	27 / 1.303
Thymallus thymallus	7 / 1.212	20/ 3.425	
Barbus balcanicus	9 / 678		
Cottus gobio	72 / 217	86 / 446	118 / 497
Total	109/3.371	114/4.789	145/1.800
kg/km (kg/ha)	23 (27)	32 (32)	12 (20)

In this area are visible different marks of human activities that have occurred earlier, but also more recently including river partitioning. The estimated total quantity of fish present in the investigated area ranges from 12 to 32 per on kilometer and from 20 to 32 per acre. These are relatively small quantities for these types of rivers, which are without significant anthropogenic influence, but these values show that anthropogenic influence is present. Why this is the case is not the subject of this study and will not be elaborated further. It is important to highlight here only the current facts and data that reflect the situation in this locality.

Table 4. Abundance (qualitative-quantitative composition) of ichthyofauna at investigated sampling site in Drcka River

Species	Sampling sites		
	L-1. above Mateševo, relative abundance %/descriptive	L-2. middle part of the river, relative abundance %/descriptive	L-2. upper part of the river, relative abundance %/descriptive
Salmo labrax (potoč. pastrmka)	19,3 / common	14,9 / common	18,2 / common
Thymallus thymallus (lipljen)	6,4 / rare	17,5 / common	-
Barbus balcanicus (pot. mrena)	8.3 / rare	-	-
Cottus gobio (peš)	66.1 / dominant	75,5 / dominant	81,8 / dominant

This analysis indicates that Black Sea trout is common or frequent species throughout the Drcka River, and that European Bullhead is numerously dominant throughout the stream. The barb was a rare species, found only in the lower course of the Drcka River, and in earlier studies, this species was more common in the downstream of this watercourse (Krivokapić & Marić, 1993). It is possible that the fish fauna has already been already influenced by the construction that took place near the first sampling site.

Lim River and Zlorečica River

Two localities on the Lim River and one on its tributaries Zlorečica River were selected for a more reliable view of the possible impacts of the construction highway on aquatic organisms: fish and aquatic macroinvertebrates. The Zlorečica River is in the immediate vicinity of the works, and its confluence with the Lim River is just below Andrijevica. Obtained data on the ichthyofauna of the Zlorečica River will, in subsequent studies (monitoring), show if there was an inpact and what was its extent during the construction and during the exploitation of the highway on the fish in its immediate surrounding.

Based on the available literature data (Drecun, 1962; Maric and Milosevic, 2011; Maric, 2019), in the Lim River and its whole drainage basin there are 24 fish species (+ 1 river lamprey) from 8 fish families and one lamprey family (Petromizonidae) (list below):

Check list of species in Lim River

Class Monorhina (Agnatha) - lamprey

Fam. Petromyzonidae

Eudontomizon sp. (cf. *E. vladikovi* Oliva & Zanandrea, 1959)

Class Osteichthyes

Oncorhynchus mykiss (Walbaum, 1792) – rainbow trout
Salmo labrax Pallas, 1814 – Black Sea trout
Hucho hucho (Linnaeus, 1758) – Huchen
Thymallus thymalus (Linnaeus, 1758) - European grayling
Alburnus alburnus (Linnaeus, 1758) – bleak
Alburnoides bipunctatus (Bloch, 1782) – spirlin,
Barbus balcanicus (Kotlik, Tsigenopulos, Rab and Berrebi, 2002) – large spot barbel

Barbus barbus (Linnaeus, 1758) – barbel
Chondrostoma nasus (Linnaeus, 1758) - nase
Gobio obtusirostris (Valenciennes, 1842) - Danube gudgeon
Leuciscus leuciscus Linnaeus,1758 – dace
Squalius cephalus (Linnaeus, 1758) – chub
Rutilus virgo (Linnaeus, 1758) - cactus roach
Telestes rysela (Heckel, 1852) - Danube riffle dace
Scardinius erythrophthalmus (Linnaeus, 1758) - rudd
Phoxinus csikii (Hanko, 1922, 1758) - Danube minnow
Cobitis elongata Heckel et Kner, 1858 – Balkan spined loach
Misgurnus fossilis (Linnaeus, 1758) - Weather loach [#]
Sabanajewia balcanica (Karaman, 1922) – Balkan golden loach
Barbatula barbatula (Linnaeus, 1758) – stone loach
Lepomis gibbosus Linnaeus, 1758 – pumpkinseed
Esox lucius Linnaeus, 1758 – pike
Lota lota (Linnaeus, 1758) – burbot
Cottus gobio (Linnaeus, 1758) – European bullhead

The fish list from the Lim River basin comprises 8 (eight) species that were not recorded during this survey. These species are cited in the literature (cited above), and two of them are present in the waters of Lim River, but downstream in the area of Bijelo Polje. The presence of three species: *S. erythrophthalmus*, *L. leuciscus*, and *M. fossilis* remain questionable and these species are only listed by Drecun (1962). The presence of the species *Esox lucius* is likely to be characteristic for the upper course of Lim River because the river flow of this river is not suitable for this species. *Oncorhynchus mykiss* and *Thymallus thymalus* are rare in this part of the river, although *O. mykiss* is cought only sporadically and originates from fish farm (escaped specimens). Data on the species presence in the two localities studied were grouped into one table (Table 5) because the data on them were similar or identical. The data presented in this way in all the tables that follow illustrate well the condition of the ichthyofauna in the part of the river below Andrijevica, in the river section about 3km, and near the foreseen works.

During this investigation 16 species of fish from 6 families were recorded (Table 5). Investigations at two localities on the Lim River have shown that these sites are statistically indistinguishable due to composition of ichthyofauna. Some differences in fish diversity (qualitative composition) were found, but since these differences are consequence of catch of one specimen of two species (Rutilus virgo, Hucho hucho), both of these specimens can be considered as one unit. Thus, on the basis of biodiversity and abundance, as well as the structure of populations of certain species, the situation in this part of the Lim River can be considered and the possible impact of the highway construction on this part of the river course, i.e. its ichthyofauna.

Table 5. Check list of the species from Lim River and its tributary Zlorečica River

Latin name	**English common**	**Montenegrin common**	Literature data	This report

	name	name		
Oncorhynchus mykiss	Rainbow trout	Kalifornijska pastrmka	+	–
Salmo labrax	Black Sea trout	Crnomorska pot. pastrmka	+	+
Hucho hucho	Huchen	Mladica	+	+
Thymallus thymallus	European grayling	Lipljen	+	–
Alburnus alburnus	Bleak	Zela, dunavska ukljeva	+	+
Alburnoides bipunctatus	Spirlin	Ukljevica	+	+
Barbus balcanicus	Large spot barbel	Balkanskaa mrena	+	+
Barbus barbus	Barbel	Velika mrena	+	+
Chondrostoma nasus	Nase	Skobalj	+	+
Gobio obtusirostris	Danube gudgeon	Dunavska mrenica	+	+
Leuciscus leuciscus	Dace	Klenić	+	–
Rutilus virgo	Cactus roach	Plotica	+	+
Telestes rysela (*souffia*)	Danube riffle dace	Jelšovka	+	+
Phoxinus csikii	Danube Minnow	Dunavska gaovica	+	+
Scardinious erythrophthalmus	Rudd	Crvenperka	+	–
Squalius cephalus	Chub	Klen	+	+
Cobitis elongata	Balkan spined loach	Velikli vijun	+	+
Misgurnus fossilis	Weather loach	Čikov	+	–
Sabanajewia balcanica	Balkan golden loach	Balkanski vijun	+	–
Barbatula barbatula	Stone loach	Brkica	+	+
Lepomis gibbosus	Pumpkinseed	Sunčanica	+	–
Esox lucius	Pike	Štuka	+	–
Lota lota	Burbot	Manic, derać	+	+
Cottus gobio	European Bullhead	Peš	+	+
Eudontomizon vladikovi	Danubian brook lamprey	Zmijuljica	+	–

Latin and Monteneegrin common names, from Marić, 2019

For this reason and for practical reasons, table 6 provides species and their status, because showing the results in several table, the same results would be repeated and could be confusing. Tables 7 and 8 show some specificities and differences between the ichthyofauna of the Lim River and its tributary Zlorečica River. A lower number of species have been identified in the gills, but this is due to the hydrologic-hydrographic characteristics of the river and, but in the monitoring, this could be a good basis for further research and comparisons.

Table 6. Conservation status of fish fauna in Lim River (Zlorečica River also included)

Latin name	**Bern Convention Annexes**	**Habitats Directive Annexes**	**Natura 2000**	**IUCN Red List status** Europa Crna Gora
Oncorhynchus mykiss				Introd.
Salmo labrax				LC – VU
Hucho hucho	III	II/V	+	EN – EN
Thymallus thymallus	II	V		LC – VU

Alburnus alburnus				LC – EN
Alburnoides bipunctatus	III			LC – LC
Barbus balcanicus		V	+	LC – LC
Barbus barbus		V		LC – VU
Chondrostoma nasus	III			LC – LC
Gobio obtusirostris				LC – LC
Rutilus virgo	III	II/V		LC – VU
Telestes rysela	III	II	+	– LC
Phoxinus csikii				LC – LC
Squalius cephalus				LC – LC
Cobitis elongata	III	II	+	LC – VU
Sabanajewia balcanica	III	II	+	LC – VU
Barbatula barbatula				LC – LC
Lota lota				LC – LC
Cottus gobio		II	+	LC – LC
E. vladikovi	III	II	+	LC – ?

Latin names and conservation status of fish species in Montenegro, from Marić, 2019

The fish fauna structure aforementioned in the tables is primarily conditioned by the structure of the river bed (bottom), then to a certain extent by the season of investigation, although seasonal differences are mainly shown by differences in spawning season and other seasons. During the spawning season, the higher presence of *C. nasus* is expected at the localities in the Lim River, or presence of *H. huho* when large specimens arrive at the spawning site. Since fishing was conducted in river rapids (lotic part), this structure reflects the state of the river only in such terrains, while the structure in whirlpools (more than 2m deep and over 100m long) is much different because large specimens of nase, chub, barbel, huchen and some others species, because such specimens can only be found in this type of habitat. Notwithstanding these differences, the established diversity of ichthyofauna faithfully reflects the situation in the entire study area or in the area covered by this study (about 3km).

The structure of ichthyofauna in the Zlorečica River faithfully represents the status in the lower course of this river, that is, the part of this river in which the possible impact of highway works could be indirectly manifested. It can be expected that part of the fish population from Lim River, if disturbed during construction in the immediate vicinity, will migrate upstream and part of the ichthyofauna will find shelter in the Zlorečica River.

Table 7. Presence and abundance of species at the studied sites in the Lim River and Zlorečica River

Species	Zlorečica River **no.**/grams	Lim- nearby confluence **no.**/grams	Lim – bridge to Seoce **no.**/grams
Salmo labrax	**19** / 2.264	**7** / 1.329	**3** / 111
Thymallus thymallus	–	–	–
Hucho hucho	–	–	**1**/10
Alburnus alburnus	**7**/61	**5**/47	**13**/119
Alburnoides bipunctatus	**14**/108	**16**/125	
Barbus barbus	–	–	**3**/74
Barbus balcanicus	**9** / 678	**5**/382	**7**/136
Chondrostoma nasus	–	–	**4**/430
Gobio obtusirostris	**2**/10	**3**/21	**9**/45
Rutilus virgo	–	–	**1**/7

Telestes rysela	**5**/39	**12**/53	**24**/127
Phoxinus csikii	**3**/14	**2**/9	**6**/19
Squalius cephalus	**11**/413	**14**/1.070	**23**/1.892
Cobitis elongata	–	–	**2**/18
Sabanajewia balcanica	–	–	–
Barbatula barbatula	–	–	**3**/23
Lota lota	–	**3**/43	**5**/72
Cottus gobio	**67**/302	**79**/360	**82**/368
E. vladikovi	–		
Total	**137**/5.065	**146**/3.799	**186**/3.450
kg/km river flow (kg/ha)	34 (42)	25 (32)	23 (23)

Table 8. Presence and abundance of species at the studied sites in the Lim River and Zlorečica River

Species	Zlorečica River relative abundance %/descriptive	Lim- nearby confluence relative abundance %/descriptive	Lim –bridge to Seoce relative abundance %/descriptive
Salmo labrax	13,9 / common	4,3 / very rare	1,6/ very rare
Thymallus thymallus	–	–	–
Hucho hucho	–	–	0,5/ very rare
Alburnus alburnus	5,1/ rare	3,0/ very rare	7,0 / rare
Alburnoides bipunctatus	10,2/ common	9,8/ common	–
Barbus barbus	–	–	1,6/ very rare
Barbus balcanicus	6,6 / rare	3,0/ vrlo rijetka	3,8/ very rare
Chondrostoma nasus	–	–	2,2/ very rare
Gobio obtusirostris	1,5/ very rare	1,8/ vrlo rijetka	4,8/ very rare
Rutilus virgo	–	–	0,5/ very rare
Telestes rysela	3,6/ rare	7,3/ common	12,9/ common
Phoxinus csikii	2,2/ very rare	1,2/ very rare	3,2/ very rare
Squalius cephalus	8,0/ common	8,4/ common	12,4/ common
Cobitis elongata	–	–	1,1/ very rare
Sabanajewia balcanica	–	–	–
Barbatula barbatula	–	–	1,6/ vrlo rijetka
Lota lota	–	1,8/ vrlo rijetka	2.3/ vrlo rijetka
Cottus gobio	48,9/ dominant	48,2/ dominant	44,1/ dominant

4. ANALYSIS OF THE IMPACT OF THE HIGHWAYS CONSTRUCTION ON THE AQUATIC ORGANISMS

The highway construction can impact aquatic organisms in rivers in two ways and with very different intensity. In general, it can be stated that all living organisms, regardless of habitat type are directly or indirectly affected by roads, in this case the highway. The direct impacts are easier to determine, i.e. to identify, and therefore it is easier to propose and implement possible protection measures. Indirect impacts are dificult to detect, especially in poorly investigated areas or ecosystems. All these impacts can be manifested during the construction phase of the highway, as well as its operational phase.

4.1 Main risks for ichthyofauna during construction works

The following impacts are possible during the preparatory work and construction of the highway:

1. The greatest impact on the fauna of fishes is at the **sites of bridge construction** over the rivers, and since this section of the highway does not cross the river bed, this impact will not occure.

2. A great impact on ichthyofauna also occures in cases where the **highway is built in the immediate vicinity of the river bed**, especially when direct construction works are carried out on the shores, for example, in order to prevent river banks collapse or at least their reinforcement for the purposes of stability of the highway. Due to the construction work and excavation, the natural habitat and appearance of the river banks are altered and even the river bed morphology is changed. These actions have a direct impact on habitat degradation and even habitat loss for aquatic organisms. This impact on habitats in the water or on the bank (ecotone) affects the entire living world of rivers, and for fish, it results in a decrease in biomass and production. These construction works affect several other variable characteristics of water, such as turbidity, pollution, or vibration during construction. All recorded species from the salmonid group (*S. labrax, H. huho, T. thymallus*) are sensitive to turbidity, and turbidity that lasts more than three days causing suffocation and death of species, especially young individuals and embryos. This is common knowledge, so fish farms are not built near rivers that have longer turbidity periods. In addition to trout, other species from the salmonid region are sensitive to high and long-lasting turbidity.

Beside the letal effect, water turbidity has additional negative effects such as:

-reduced light transmission, which directly reduces photosynthesis and decreased production of periphyton, which reduces the food base for many animals, including fish

-with decreasing light transmission, visibility is reduced, which is especially important for predators whose have sense of sight is the main organ in orientation, which is a large number of fish (most of them mentioned in this study), some birds (Cinclus cinclus - feed mainly on aquatic invertebrates) and mammals (Lutra lutra - mainly feeds on fish and crustaceans) (both species were recorded in the Lim River area under investigation, and C. cinclus and along the entire watercourse of Drcka River)

-it is known that suspended particles have impact on reduction of dissolved oxygen (see Maric and Rakocevic, 2009)

-suspended particles increase the temperature of the water, which disrupts natural conditions and adversely affects cold-water organisms;

-suspended particles directly or indirectly affect biological processes, for example the speed of embryonic development (may be lethal), the growth of fish (the growth is slow down), and even the taste of meat (make it worse);

3. Construction works can significantly affect **changes in the water regime** that in many indirect ways affect the living world, such as habitat loss, the problem of finding shelter, changes in physicochemical characteristics, such as temperature increase, changes in gas regime, pH, etc. For example, increased acidity slows growth, development of fertilized eggs and embryos (also causes mortality), then increases the toxicity of heavy metals (cadmium, mercury, aluminum, iron, copper, etc.), that affect the morphological changes in gills that have a lot of significant functions in fishes (breathing, release of decomposition products, osmoregulation, etc.)

Disturbances in the water regime can result in significant disturbances in the fish reproduction cycle, from site selection, spawning time, etc. and may lead to mismatches with other natural processes in the aquatic environment (development of other communities). In conditions of increased calcium content in water, the effect of heavy metals is mitigated (see Marić, 2019).

4. During construction works, especially during the operation of large machines, a great **noise and vibration** is created and this directly affects fish populations. This impact is only manifested if the works are performed directly in a water body or in the immediate vicinity of water bodies. It is known that fish avoid or remove away from the sources of these factors, thereby altering their behavior (due to stress) and under such conditions some forms of fishing cannot be practiced (sport fishing - fishing). Stress in fish changes the physiological functions that can significantly change the their condition state over time (fish are frightened and eat much less).

5. Construction works that are carried out near water bodies can cause **accidents** such as the outflow of harmful chemicals into the environment, e.g. petroleum products, etc. Such substances in large quantities cause the fish die-off and die-off of all organisms that are in the affected area.

6. One of the possible negative impacts of construction sites (recorded in Tara River) is the introduction of larger quantities of **particles originating from concrete** into the river system. The presence of fine particles (suspended in water) originating from concrete and concreting leads to their deposition in deeper layers of sand and gravel, their binding into solid conglomerates that prevent the development of stigoritron (living organisms in sand and gravel). It is known that for the hyporheic interstitial (stygorhithron), the composition of the sand particles is much more important than the chemical composition of the water, so disturbances in the substrate structure significantly alter the structure of biocenoses. These particles can lead to the complete closure of the lower layers (concreting) and / or inhibiting the circulation of oxygen. Both processes disable the development of living organisms.

7. In addition to the above potential impacts in the aquatic environment, i.e. in the living organisms and fish, the fish and their environment can also be affected by **changes in the surrounding land** in a narrow and wider area. By cutting larger forest areas the absorption of the terrain reduces, causing surface runoff, and it influences, in rainy periods, an increase of the speed of water flow, causing destabilization of the banks and erosion processes. This leads to changes in the characteristics of the watercourse in terms of its hydrology, the shape of the river channel, the amount of suspended matter, the chemistry and the biological properties of the water. All this can directly and indirectly affect fish population, and cause habitats loss for some species of fish and other organisms. Increased runoff from the surrounding terrain and more significant runoff from the pavement creates torrents that result in similar effects to the occurrence of high level of suspended matter due to works on highway construction. By this effect, the increased water speed acts erosively on the substrate and on living organisms.

It should be noted that these effects are manifested during both, the construction and operation phase of the highway.

4.2 Possible risks for ichthyophauna during operational phase of highway

After completion of the works and commissioning of the highway, some of the above risks may also occur at this stage (operational phase). During operational phase of the highway, a new threat to the aquatic living world arises, which is the **flashing of atmospheric waters from the road**, which introduces suspended matter and various pollutants (mainly petroleum products) into the aquatic recipient. Of course, this impact is possible if highway is built near river flows. Based on the enclosed documents, maps, these risks can be expected in several places because the envisaged route of the road from Mateševo to Andrijevica intersects a number of small streams, several bays, and in some positions passes in the vicinity of larger water bodies (Drcka River, for example).

The drainage of water from the highway in a larger amount can cause the **change in the water quality** of the recipient, its physical and chemical characteristics, which will directly affect the living organisms. The fish population will be affected directly and indirectly (through other communities). Changes in water quality will be caused by inorganic particles that are flushed out causing turbidity. In the long term, inorganic particles in larger quantities and for a longer period can also alter the physical characteristics of the river bed at the deposition point in the water.

During operational phase the highway, fish population can be mostly affected by decrease of water quality due to the flushing of atmospheric water from the pavement lane. This aspect of influence has been elaborated earlier in the text.

Increased turbidity, as a physical factor of water adversely affects several environmental factors such as: light level, oxygen level, water temperature, change of pH value, may cause embryon die-off. These negative impacts are also earlier elaborated.

The significance of these impacts in the final effect and in specific conditions can be characterized by low when it comes to ecosystems and common species, and moderate when it comes to species of importance.

4.3 Summary and impact assessment

As it is known in environmental protection there are 3 basic procedures:

1. Prevention of degradation and pollution of ecosystems or communities
2. Elimination or reduction of degradation and pollution,
3. Improvement of degraded and polluted environment.

There are works that cannot be completely prevented from environmental pollution, such as the construction of a highway. Therefore, in such situations, beside the preventing of the impact to the extent possible, remedial and improvement methods are applied.

All the above methods are foreseen and applied for the highway project. For the specific area of the Lim River and Drcka River, in addition to the applyingof the above methods, remediation should also be carried out through the stabilization of watercourses, green plantations and repopulation of river with fish.

All of this needs to be monitored over a longer period of time, i.e. the monitoring should be carried out from the beginning of the works to identifying visible signs that devastated habitats and wildlife are almost completely recovered.

5. DESCRIPTION OF MEASURES TO PREVENT, REDUCE OR REMOVE OF NEGATIVE IMPACTS

It is known that impacts on biodiversity are the most sensitive issue, given the permanent threat to the habitat of the living organisms. It has already been pointed out that the greatest risks are changes in the morphology of river banks and river beds, changes in the water regime and changes in the physico-chemical characteristics of water in rivers. To mitigate the negative effects on ichthyofauna and other aquatic organisms, preventative measures (not listed by priority or importance) should be taken:

1. In terrestrial ecosystems and communities, minimize degradation and arrange newly created biotopes (devastated areas) to be as close as possible to their previous state. This means creating the conditions for the return and functioning of the living organisms in them. This is important in order to prevent torrential waters and to flush the surrounding soil into the water recipient (explained above).

2. Dispose of **excavated material** (soil) at a predetermined landfill

3. In coastal communities (**ecotone**), also, minimize degradation, and arrange newly created biotopes (devastated areas) to be as close as possible to their previous state. It is desirable, for example, to leave old trees because they have strong roots that guard the river banks. This is important in order to prevent the surrounding land from spilling into the water recipient (explained above), and less devastated areas recover much faster.

4. During excavation, it is necessary to **prevent suspended matter** from being washed away by water, but to provide a temporary drainage channel, which will remove water from the work site and take it to landfills, sedimentation tanks etc. In this way, works can also be carried out under adverse conditions even in the immediate vicinity of rivers.

5. Waters that **flush the surrounding soil** (temporary and occasional turbidity), as well as wastewater, are treated through decanter, sediment separator, and system for additional treatment.

6. These adverse impacts can be reduced or avoided by good organization of work on construction sites, which includes more intensive work in the watercourse and its surroundings during the period, i.e. **months with low level of precipitation** (summer).

7. Work in the aquatic environment should be conducted at regular intervals, **2-3 days, to avoid permanent turbidity** (explained above).

8. Design **more frequent drainage channels** on or along the highway to avoid the collection and drainage of water from the surrounding area and pavement lanes. As the normal distribution and migration of small terrestrial organisms require special highway tunnels, water canals can be built in addition to the above.

9. Not to completely dam the riverbed, but if necessary, to do it partially.

10. In order to protect the ichthyofauna, it is recommended that during December and January, **work should be kept to a minimum** on the Drcka River, but of course only at sites adjacent to the river bed. For the Lim River this would be the period during the month of May, because the intense **fish spawning** occurs on these rivers in this period.

11. **Wastewaters generated by flushing of concrete handling equipment shall not be allowed to enter the aquatic environment** and must be adequately regulated and treated.

12. **Monitor** the living world for at least two seasons (late spring or early summer - June and fall in late September or early October). In the aquatic environment, the state of the watercourse or bottom is usually monitored via the periphyton and benthos. The condition of ichthyofauna is especially monitored because it has a broader significance (economically, etc.).

5.1 Specific protection measures for the endangered species of ichthyiofauna

In the rivers of Montenegro that belong to the waters of the Danube drainage basin, in this case the Lim River and the Tara River with tributaries that may be exposed to higher or lower impact of construction of the highway, several endemic species for the Danube Basin and several globally threatened species have been registered.

Endemic species to the Danube drainage basin are: *Huho huho*, *Cobitis elongata*, *Phoxinus csikii*, *Gobio obtusirostris*.

According to Marić (2019) in Montenegrin waters there are **no critically endangered (CR) species**, but only species from the **endangered category - EN**, and this is only *Huch hucho*, due to small areas of distribution and relatively low population density. The threat of the latest species, then the species *Sabanajewia balcanica*, *Cobitis elongata* and some other species is the highest in the whole area, so the greater level of protection has been granted (see Table 6). Of the endemic and endangered species, the smallest areal has *Phoxinus csikii*, which together with *Hucho hucho* should be especially emphasized, indicating the threatening factors.

Huho huho is a big species that is under great anthropogenic pressure through habitat change, river pollution, and intensive sport fishing. In addition, global climate change through disturbed water regimes affects big and long-lived species. Low water level disable normal spawning, and migration during spawning, etc. The huchen spawns during May, the incubation of the fertilized eggs lasts for a relatively long time as with all salmonids, and feeds mainly with fish. These basic characteristics indicate that all of the above threats to the aquatic ecosystem are factors that may, as described, threaten the survival of this species. In rivers that are devastated as Tara River or may be (for example, the Lim River) endangered by the construction of a highway, for the recovery of the population of this species, it is recommended to apply standard protection of the rivers, with a stocking with fry and to construct of a reprocenter in which fry of all salmonids will be produced.

Alburnus alburnus is a small species of short life that inhabits waters of different quality. This species prefers lentic regions in rivers and spawns in spring. Threatening factors are all mentioned above, and as the abundance is low, it is one of the rare species and its population in Montenegro is vulnerable. Therefore, additional protection or care is required in order to preserve its abundance, as well as for the huchen. The species is not of economic interest so other measures than those related to habitat protection are not being implemented.

6. EFFECTS OF THE PROPOSED MEASURES

1. Deteriorated water quality will be the first to be recovered and reach almost complete natural values within a few years after the finishing of construction activities. In order to restore the water quality (to its original state), it is necessary to put in order the river banks, as well as the wider area from which suspended matter (sludge, soil, etc.) may originated in the future.

2. The fastest recovery of fish populations can be achieved by stocking with quality fry, but stocking will be useless until normal biological processes have been established in degraded habitat, and under these prevailing conditions, normal biological processes will be established 5 to 10 years after the completion of construction activities. It should have in mind that the improvement of the situation will occur continuously from year to year, so by this dynamic the living organisms will be back to its original state.

3. After the construction works are completed on the entire section of the highway in the part of the area belonging to the Black Sea drainage basin, one of the measures of fish fauna rehabilitation is the construction of a hatchery in which the fish fry would be produced to recover the waters that have been exposed to devastation, all in accordance with the Law on Protection of the environment.

4. It is proposed to build a single reprocenter for the production of all salmonid fish species that live in the area or in the rivers belonging to the Danube drainage basin in Montenegro. They are: Black Sea trout, huchen and European grayling that inhabit naturally investigated rivers. Such a reprocenter can be organized in already existing fish ponds or new reprocenter could be constructed for this purpose only.

5. In accordance with previous mentioned, it is proposed to train one technologist (masters or doctoral studies) in specialized institutions and practice in fish ponds (immediately) that are in the same field (Slovenia), in order to produce a specialist to work in the reprocenter when the construction works are completed.

6. All of the above mentioned means that in parallel with construction and design, i.e. with expected and anticipated impact on the complete natural environment and the living organisms in targeted rivers and the surrounding area, immediate action must be taken to immediately mitigate and subsequently remedy the negative impacts.

7. Comprehensive monitoring will monitor the situation, and in case of problem detection, the intervention will be taken immediately.

II - Baseline survey on biodiversity of benthic fauna in Drcka River and Lim River

for the purposes of WB17-MNE-TRA-02
Technical assistance for the preparation of Preliminary Design and Environmental and Social Impact Assessment (ESIA) for section Mateševo - Andrijevica of Bar - Boljare highway (SEETO Road Route 4)

Prepared by Dr Danijela Šundić

November 2019

Table of Contents

1. INTRODUCTION

For the purposes of the ESIA study preparation for section Mateševo - Andrijevica of Bar - Boljare highway, biodiversity investigation was conducted in the areas of the rivers Drcka and Lim. The main objective of this one-time survey was to assess the current status of macroinvertebrate biodiversity, in order to enhance the biodiversity baseline. The survey comprised sampling in the field, work in the laboratory, as well as data processing and analysis.

As the main outcomes of the survey, in accordance with Terms of reference, detailed qualitative and quantitative analysis of benthic community which comprise species lists with number of specimens, exact locations of recording, list of registered species (Latin and English name) in each river and their national and international protection status, were provided. In addition, this report gives detailed description and assessment of community structure, population density of the macroinvertebrate species in each investigated watercourse, as well as water quality assessment in sampling sites of each water courses.

Also, this report analyzed the possible and expected impacts from the construction and operation of the highway, i.e. whether constructing the highway represent a threat to the benthic community, a treat to the status, survival or conservation of specific protected/endangered species as well.

The survey took place in September 2019, and the samples were collected one-time from five sampling sites in investigated water bodies.

2. METHODOLOGY

The samples of macroinvertebrates, were taken from 5 sampling sites from two water bodies: Drcka River and Lim River, once on 21 September 2019. The position of sampling stations and its characteristics (geographical coordinates, type of the substrate, etc.) are shown in Figure 1 and Table 1. The geographical coordinates were determined by GPSMAP 60CSx.

2.1. Investigated Area

Both water bodies belong to the Black Sea Drainage basin which surface in Montenegro is about 7,545 km2.

The area of the Tara drainage basin has 2,040 km2 in Montenegro and the length of the water stream is 147 km. The average yearly precipitation in this area is 1,628 mm, while the average flow is 77.5 m3/sec.

The hydrographic network of the Tara River drainage basin is well developed. The Tara River originates under the tops of the Maglić and Koriman, at the elevation of 1,100 MASL, where the Veruša meets Opasanica, while the Tara River forms the Drina River together with the Piva River, in Šćepan Polje.
The Tara River is a fast mountain river flowing through the canyon which is 1,000 m deep, even 1,300 at some parts, which makes it the deepest canyon in Europe. As the Tara River is the longest river in Montenegro, it receives a great number of constant or periodical tributaries. The most significant right tributaries are: the Opasanica, Drcka, Svinjača, Jezerštica, Rudnica, Selačka River and others, while the left tributaries are: the Pčinja, Plašnica, Štitarica, Bistrica and Sušica River.
Hydrographically, the Lim River represents the most developed Montenegrin river. The drainage basin area of this river has, in the territory of Montenegro 2,805 km2. The watercourse is 123 km long, the average water flow is 71 m3/sec while the average yearly precipitation is 1,235 mm.
The Lim River originates from the Lake Plavsko, at the elevation of about 908.9 MASL and flows to the north and northwest by places Andrijevica, Berane, Bijelo Polje, Brodarevo, Prijepolje, Priboj and Rudo all up to the mouth in the Drina River, downstream from Međeđa. In the area of Andrijevica Lim River receives its left tributary Zlorečica, and in the section from Berane to Bijelo Polje, the Lim River receives its left tributaries the Brzav River and the Ljuboviđa River and right tributaries the Dapskićka River, Lješnica River, Crnča River and the Goduška River.

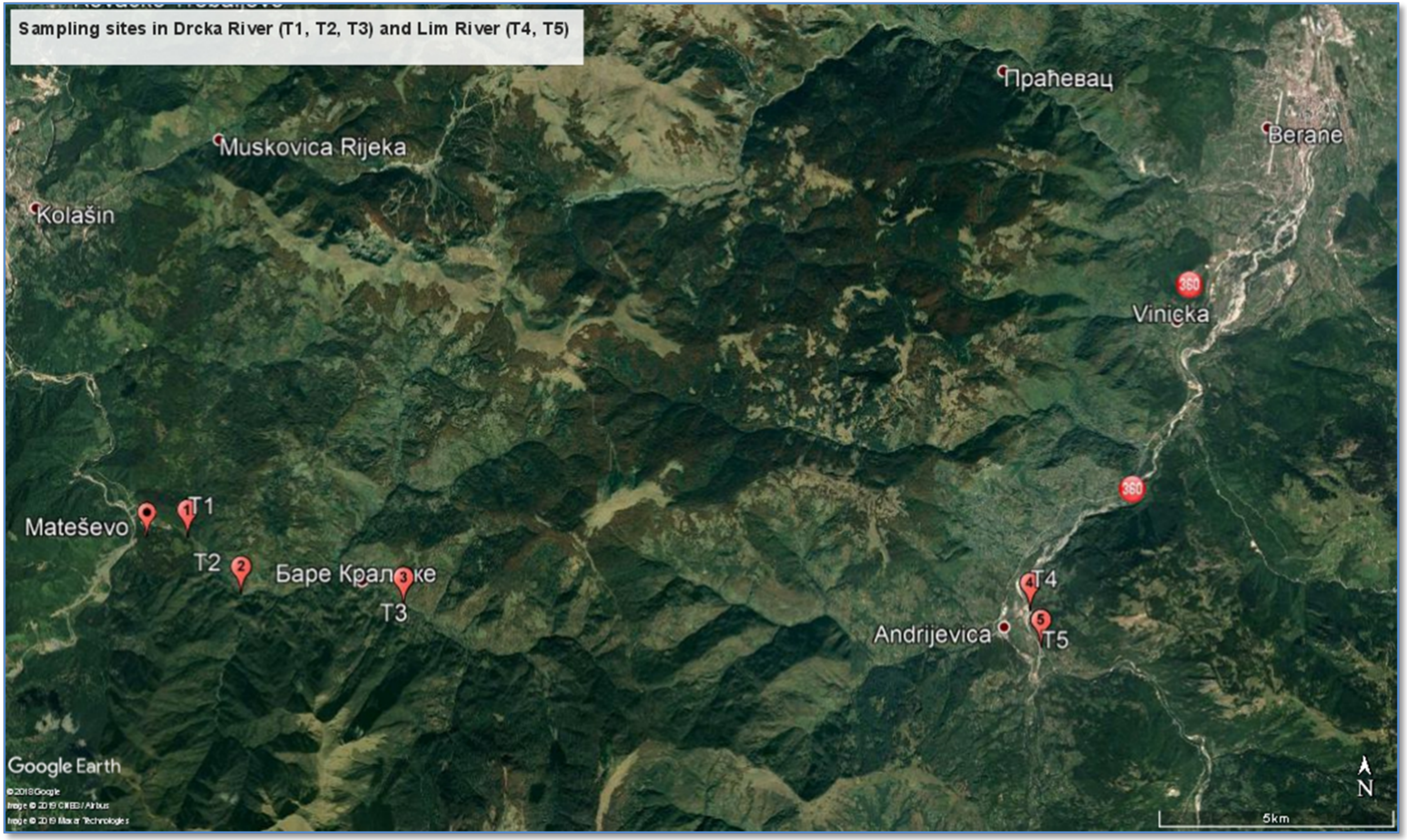


Figure 1. Investigated sampling sites in Drcka River (T1, T2, T3) and Lim River (T4, T5)

Table 1. Characteristics of investigated sampling sites (rst—rocks and stones; st—stones, gp—gravel, pebble; s—sand; m—mud; d—detritus).

Sampling site	*Coordinates*	*Substrate type (sand, stone, etc)*	*Water flow speed (medium, high, low)*	*Socio – economic activity*
T1- Drcka River	*42°45'29''* *19°33'56''*	*rst–5%, st – 25%, gp – 20%, s – 50%*	*lotic ecosystem, medium to high*	*Rural area*
T2 - Drcka River	*42°44'59''* *19°35'30''*	*rst – 10%, st – 20%, gp – 30%, s–30%, m–10%*	*lotic ecosystem, high*	*Rural area*
T3 - Drcka River	*42°44'33''* *19°37'58''*	*rst–15%, st – 35%, gp – 15%, s – 20%, m – 10%, d–5%*	*lotic ecosystem, medium*	*Rural area*
T4 – Lim River	*42°44'36''* *19°47'36''*	*rst–5, st – 70%, gp – 10%, s – 10%, m – 5%*	*lotic ecosystem, high*	*Urban area*
T5 – Lim River nearby the confluence of Zlorečica River	*42°44'40''* *19°47'40''*	*st – 40%, gp – 30%, s – 20%, m – 10%*	*lotic ecosystem, low*	*Urban area*

2.2 Benthic Invertebrate Sampling

The samples of sediment were taken by Surber's net with the surface area of 1225 cm^2 (35*35cm). The samples were stored in plastic jars, labelled and fixed in 6% formaldehyde and transported to the laboratory for further analysis. The following data were recorded in the field: habitat characteristics/type of supstrat, type of sampling method, type of macroinvertebrates collected.

2.3 Benthic Invertebrate Sorting and Identification

In the laboratory, the samples were washed through sieve of 0.5 mm in diameter. Using forceps, separate organisms from detritus and place one to several organisms representing each different taxon observed into a vial with alcohol. Each vial was clearly labelled. Labels contained data on sampling date, monitoring station number and name, names of macroinvertebrates taxa present in sample.

Afterwards, the separation and counting of macroinvertebrates were performed on binocular magnifier Stereomicroscope Stemi 2000. The material was fixed in 70% ethanol. The macroinvertebrates speciemen were identified mostly to the species level using bionocular Stereomicroscope Stemi 2000 and microscope on Carl Zeiss type AXIO Imager.

The relevant keys were used to determine macroinvertebrate taxa: Sperber, 1950; Chekanovskaya, 1962; Brinkhurst & Jamieson, 1971; Hrabe, 1981; Timm, 2009; Di Sabatino et al. 2003; Thorp and Covich, 1991; Pescador et al. 1995; Merritt and Cummins, 1996; 2008; Jessup et al. 1999; Epler, 2001; Pešić, 2002-2004; Nagel et al. 1989.

2.4 Data Analysis Methods

The following Biological Indices were used for qualitative and quantitative analysis of the investigated benthic community:

1) Numerical abundance NA (%) was calculated by formula:

$$NA = n_a/n \times 100$$

n_a – number of individuals of species in **a** sample
n – total number of individuals in sample
NA – Numerical abundance of species **a** in sample (%)

2) Diversity index (H) is calculated as per Shannon (Krebs, 2001) formula:

$$H = \sum_{i=1}^{n} pi \times \log pi$$

i = 1, n; ***pi*** = n/N
n – Number of individuals of ***i*** - species
N – total number of individuals in sample

3) Evenness (E) shows the extent to which the species, present in the sample, are similar regarding their number and the extent to which the abundance of species are uniform in the sample. (Shannon & Weaver, 1948; Pielou, 1977):

$$E = (H/Hmax) \times 100 = (H/lnS) \times 100$$

H – Shannon-Weaver index
S – total number of species in community

The index of evenness (E) has value from 0 to 100 where the maximal value (100) represents the complete evenness.

4) Taxa Richness (TR)

Taxa Richness (TR) indicates the health of the community through its' diversity (Plafkin *et al.*, 1989). TR equals the total number of taxa represented within the sample. The greater number of taxa found within community indicate the healthier benthic community.

In order to estimate water quality at investigated sampling sites, the following indices were calculated:

5) EPT Index (Plafkin et al., 1989) - The Ephemeroptera, Plecoptera, and Trichoptera index (EPT) displays the taxa richness within the insect groups which are considered to be sensitive to pollution, and therefore should increase with increasing water quality. The EPT index is calculated as the % of the individuals in the sample which belong to the aquatic insect orders Ephemeroptera, Plecoptera and Trichoptera.

% EPT Abundance =Total No. of EPT individuals/Total No. of individuals in the entire sample

Table 2. Reference value for EPT Index

EPT index	
Index value	**Water Quality**
> 50%	good
25 – 50%	moderate
< 25%	poor

6) Ratio of Ephemeroptera, Plecoptera, and Trichoptera and Chironomidae (EPT/C) The abundance of EPT and Chironomidae is indicator of balance of the benthic community, since EPT are considered to be more sensitive and Chironomidae less sensitive to environmental stress (Plafkin *et al.*, 1989). An even distribution among these four groups indicate community which is in good biotic condition, while high numbers of Chironomidae in community may indicate environmental stress (Plafkin *et al.*, 1989). The EPT/C index is calculated by dividing the sum of the total number of Ephemeroptera, Plecoptera, and Trichoptera individuals by the total number of Chironomidae individuals.

7) Family Biotic Index (FBI) (Hilsenhoff, 1982)

$$\mathbf{FBI = \Sigma (x_i \times t_i) / n}$$

x_i – number of individuals within a taxon
t_i – tolerance value of a taxon
n – total number of organisms in the sample

FBI is usually used for estimation of organic pollutants in water, but may be applicable for toxic pollutants also.

Table 3. Reference value for Family Biotic Index

Family Biotic Index		
Index value	**Water Quality**	**Degree of Organic Pollution**
0.00–3.50	Excellent	No apparent organic pollution
3.51–4.50	Very good	Possible slight organic pollution

4.51–5.50	Good	Some organic pollution probable
5.51–6.50	Fair	Fairly substantial pollution likely
6.51–7.50	Fairly poor	Substantial pollution likely
7.51–8.50	Poor	Very substantial pollution likely
8.51–10.0	Very poor	Severe organic pollution likely

8) Biological Monitoring Working Party (BMWP) (Friedrich et al., 1996)

The Biological Monitoring Working Party score (BMWP) provides single values, at the family level, representative of the organisms' tolerance to pollution; the greater their tolerance towards pollution, the lower the BMWP scores. BMWP was calculated by adding the individual scores of all families, and subclass Oligochaeta represented within the community. The BMWP index is calculated by summation of the tolerant values of each taxa presented in the sample. The tolerance values were for each taxa were taken from SNIFFER WFD72A: Revision and testing of BMWP scores.

Table 4. Reference value for BMWP index

BMWP index		
Index value	**Water quality**	
> 151	very clean	
100–150	clean	
51–99	moderate	
16–50	polluted	
0–15	very polluted	

9) Average Score per Taxon (ASPT) (Armitage et al., 1983), (Friedrich et al., 1996).

The Average Score per Taxon (ASPT) represents the average tolerance score of all taxa within the community, and was calculated by dividing the BMWP by the number of families represented in the sample.

ASPT=BMWP score/number of families

Table 5. Reference value for ASPT index

ASPT index	
Index value	**Biological water quality**
> 5.41	excellent
4.81–5.40	very good
4.21–4.80	good

3.61–4.20	medium
3.01–3.60	poor
< 3	very poor

3. RESULTS

3.1 Qualitative and quantitative analysis of benthic community

A total of 70 taxa from 5 phyla, 5 classes/subclasses, 10 orders and 41 families, have been recorded in the investigated rivers Drcka and Lim during one sampling survey performed in September 2019. Generally, community composition among sites in Drcka River and Lim River and between sites was very similar. In the Drcka River 21 species were recorded, in the Lim River 14, while 35 species were common for both water bodies (Annex 1, Annex 2).

The detailed analysis of benthic community as well as the estimation of water quality in the investigated watercourses is provided below, per each sampling site.

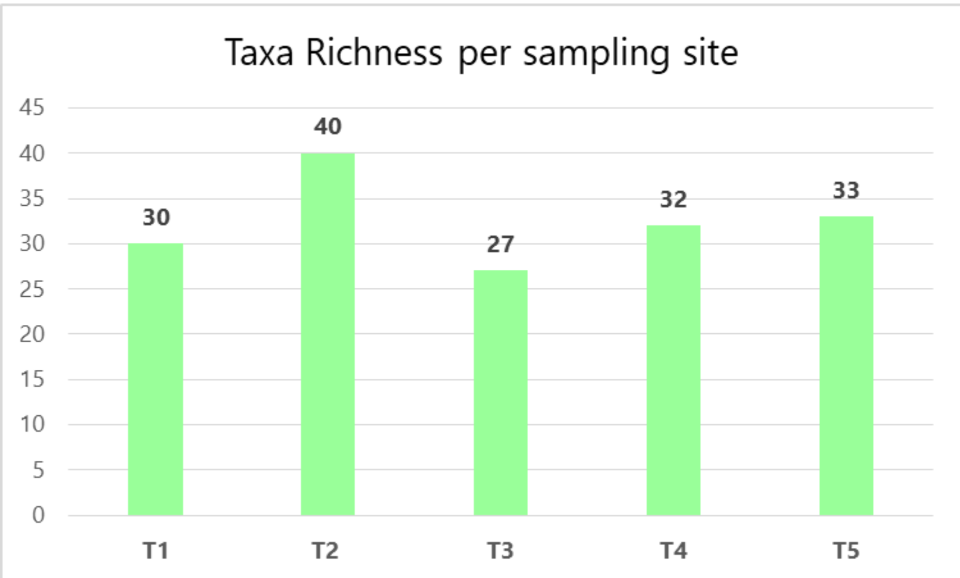


Figure 2. Taxa richness at investigated sampling sites, September 2019

3.1.1 Sampling Site T1 – Drcka River

At this station 8 macroinvertebrates groups were recorded (Annex 2), diversity index was relatively high (H=3.11), and evenness was high (E=0.91) (Figure 5). Order Trichoptera with abundance of 1477,55 ind/m2 or 57,64% was the most dominant group in the sample. The most abundant family within caddisflies was Lepidostomatidae with 1134,69 ind/m2. The total population density recorded in this site was 2563,27 ind/m2 (Table 6, Figure 3), and taxa richness was 30 (Figure 2). The second abundant group in this sampling site were oligochaetes with 457,14 ind/m2. The EPT index with value of 63,32% indicated very good condition of benthic community at this sampling site. The low number of Chironomidae in sample (10,03%), as well as EPT/C ratio 6,31 indicates benthic community which is in good biotic condition. Also, at this sampling sites Trichoptera, Coleoptera and Ephemeroptera dominated in the benthic community (Figure 4), indicating a good status of the water quality.

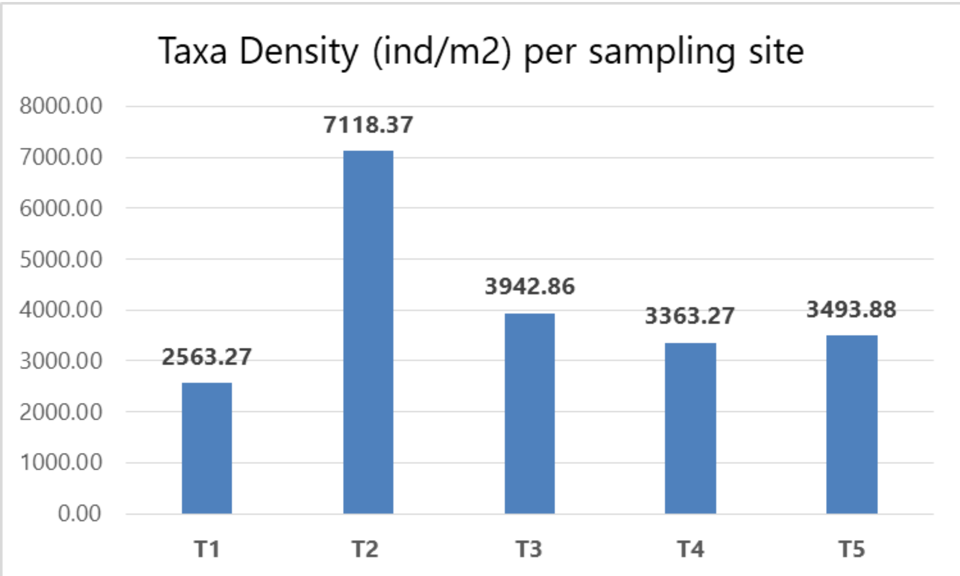


Figure 3. Taxa density (ind/m2) at investigated sampling sites, September 2019

The FBI value (3.55) indicates very good water quality with possible slight organic pollution. This water quality status was confirmed by ASPT and BMWP indices, whose values of 5,19 and 109 indicate very good biological water quality and clean water at this sampling site (Figure 7, Table 8).

The most abundant species in sample were caddisflies *Crunoecia irrorata* with 1134,69 ind/m2 and *Glossosoma boltoni* with 179,59 ind/m2. The abundance of each species from this sampling site is presented in Annex 2.

Regarding protection status, only one gastropod species - *Ancylus fluviatilis* was designated with LC (Least Concern) IUCN status. None of the species collected is listed as nationally protected. One of the reason for low level of protected species is could be lack of investigation. Three mayfly species recorded at this site: *Ephemera danica*, *Centroptilum luteolum* and *Caenis horaria* are marked with LC status in Ireland Red List (Mary Kelly-Quinn and Regan, 2012) (Annex 1).

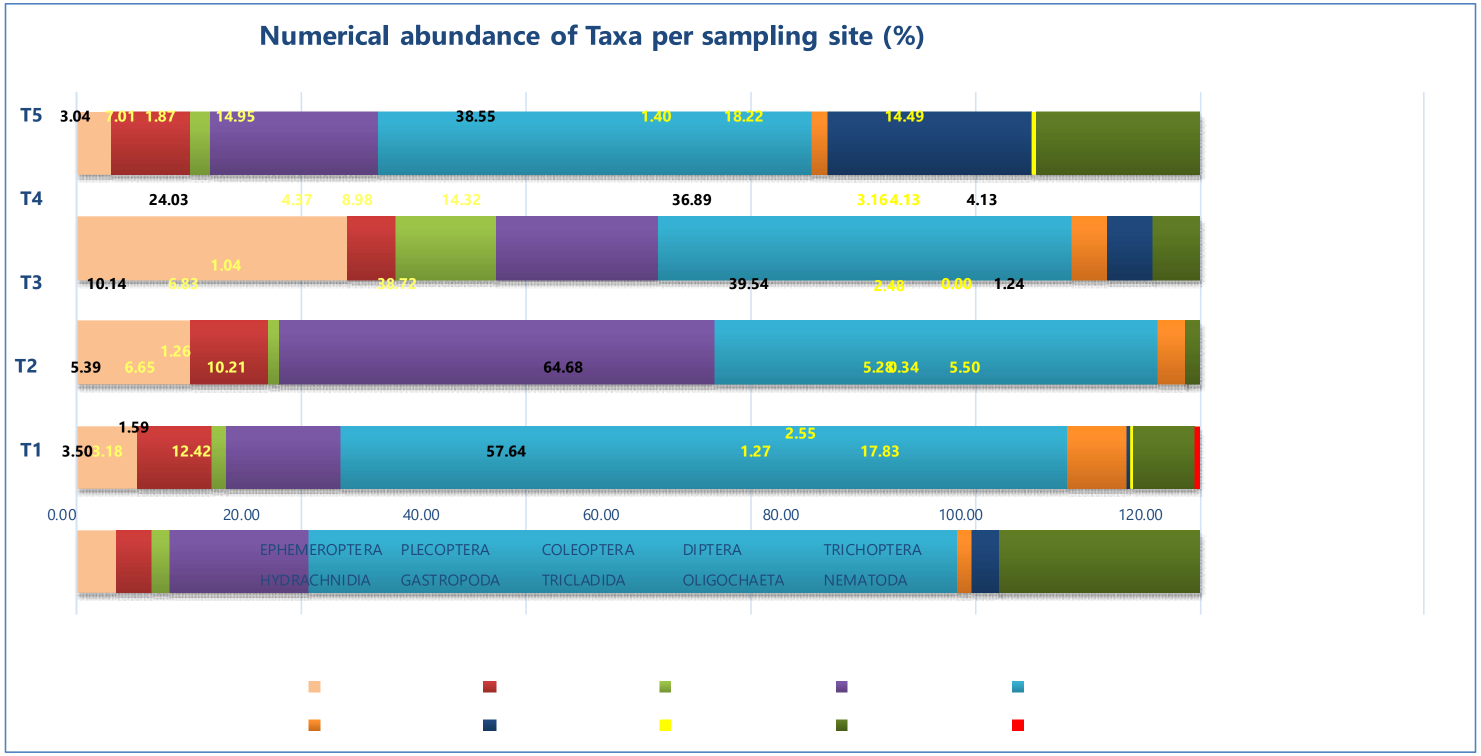


Figure 4. Comparative numerical abundance of investigated taxa per sampling site (%), September 2019

3.1.2 Sampling Site T2 – Drcka River

At this sampling site 10 macroinvertebrates groups were recorded (Annex 2), diversity index was the highest among investigated sites (H=3.47), and evenness was also high (E=0.93) (Figure 5). These values indicated undisturbed benthic community and very low level of pollution in the water. The taxa richness with 40 recorded taxa (Figure 2) and total taxa density of 7118,37 ind/m2 (Figure 3, Table 6) in this sampling site were the highest among the investigated sampling sites.

Order Trichoptera was the most dominant group in the sample with participation in the community of 64,68% (Figure 4) and population density of 4604,08 ind/m2 (Table 6), followed by Diptera with 10,21% and Plecoptera (6,65%). The value of EPT index was 76.72% at this monitoring station (Table 7). This value indicated a very good, unpolluted site. The ratio EPT/C of 11,15, as well as the low numbers of Chironomidae – 6,88% indicates community which is in good biotic condition, without environmental stress. In addition to this, the dominance of organisms that are sensitive to pollution such as Trichoptera, Ephemeroptera and Plecoptera shows good quality of the water at this sampling site.

Table 6. Population density of investigated Taxa per sampling site (ind/m2), September 2019

	T1	T2	T3	T4	T5
EPHEMEROPTERA	89,80	383,67	400,00	808,16	106,12
PLECOPTERA	81,63	473,47	269,39	146,94	244,90
COLEOPTERA	40,82	89,80	40,82	302,04	65,31
DIPTERA	318,37	726,53	1526,53	481,63	522,45
TRICHOPTERA	1477,55	4604,08	1559,18	1240,82	1346,94
HYDRACHNIDIA	32,65	375,51	97,96	106,12	48,98
GASTROPODA	65,31	24,49	0,00	138,78	636,73
TRICLADIDA	0,00	16,33	0,00	0,00	16,33
OLIGOCHAETA	457,14	391,84	48,98	138,78	506,12
NEMATODA	0,00	32,65	0,00	0,00	0,00
TOTAL	**2563,27**	**7118,37**	**3942,86**	**3363,27**	**3493,88**

On the basis of FBI index (3.96) it was stated very good water quality with possible slight organic pollution, at this sampling site. The values of ASPT index (5.52) indicated excellent water quality, and BMWP (160) indicated very clean water (Table 8, Figure 8).

The most abundant species in sample were caddisflies *Adicella filicornis* with 2530.61 ind/m2 and *Sericostoma personatum* with 1493.88 ind/m2. The abundance of the remaining taxa is given in Annex 2.

Only one gastropod species - *Lithoglyphus naticoides* was designated with LC (Least Concern) IUCN status. None of the species collected is listed as nationally protected. One of the reason for this low level of protected species is probably lack of investigation. In addition, one mayfly species recorded at this site: *Centroptilum luteolum* is designated with LC status in Ireland Red List (Mary Kelly-Quinn and Regan, 2012) (Annex 2).

3.1.3 Sampling Site T3 – Drcka River

At this sampling site 7 macroinvertebrates groups were recorded (Annex 2), diversity index (H=3.07), and evenness were relatively high (E=0.93) (Figure 5). These values indicated good biotic condition, without environmental stress. The taxa richness recorded in this site was 27, and total taxa density was 7118,37 ind/m2 which is the highest density among investigated sampling sites (Table 6).

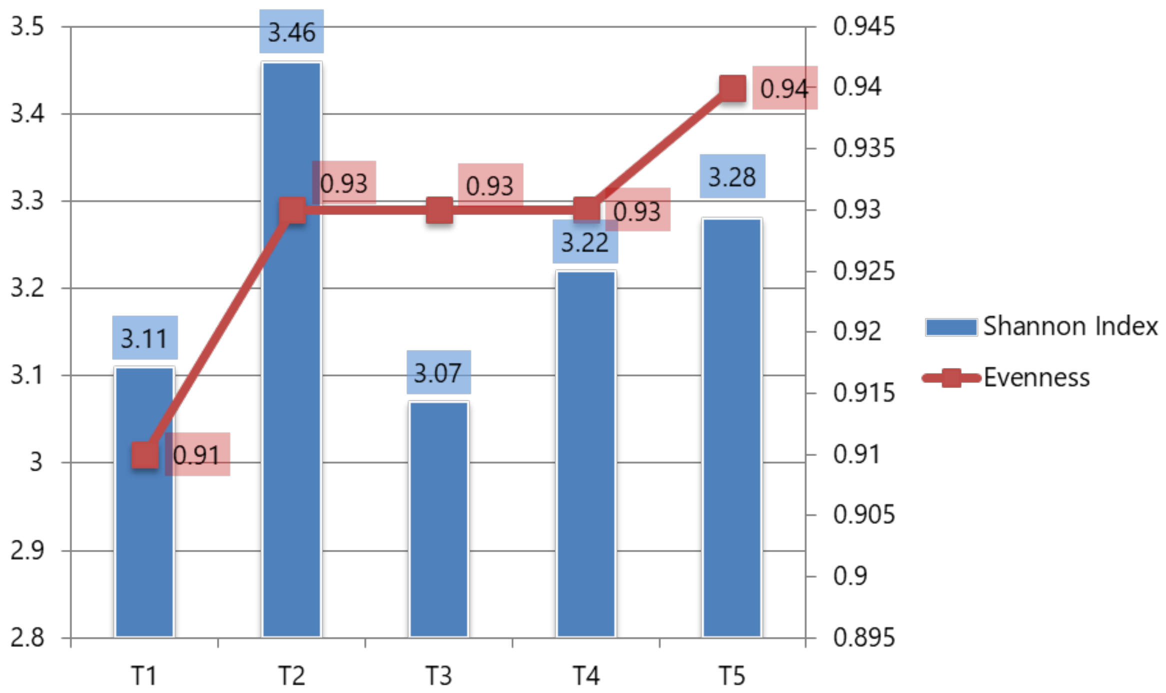


Figure 5. Values of Diversity Index and Evenness at investigated sampling sites, September 2019

Order Trichoptera was the most dominant group in the sample with participation in the community of 39,54% (Figure 4) and population density of 1559,18 ind/m2 (Table 6), followed by Diptera with 38,72% and Ephemeroptera (10,14%). The value of EPT index was 56,52% at this monitoring station. The ratio EPT/C was 1,58, and participation of Chironomidae – 35,82% indicates community which is in fairly good biotic condition (Table 7).

Table 7. Proportions of Ephemeroptera, Plecoptera and Trichoptera (%EPT), Chironomidae (%C), and ratio of Ephemeroptera, Plecoptera and Trichoptera and Chironomidae (EPT/C) at the sampling sites, September 2019

	T1	T2	T3	T4	T5
% EPT	63,32	76,72	56,52	65,29	48,60
% Chironomidae	10,03	6,88	35,82	9,95	14,02
Ratio EPT/Chironomidae	6,31	11,15	1,58	6,56	3,47

Values of FBI index (4,49) indicate very good water quality with possible slight organic pollution, at this sampling site. The values of ASPT index (5,27) indicated excellent water quality, what is confirmed by BMWP (116) whose value indicates very clean water (Table 8, Figure 9).

The most abundant taxa in sample was *Chironomus* sp. with 1004,08 ind/m2, then follows by caddisflies *Adicella filicornis* with 897,96 ind/m2 and *Glossosoma boltoni* with 489.80 ind/m2. The abundance of the remaining taxa is given in Annex 2.

Analysis of species protection status shows that none of the species collected is listed as nationally protected. Also, at this sampling site none of the species is on IUCN red list. Two mayfly species recorded at this site: *Centroptilum luteolum* and *Procloeon bifidum* are marked as least concern and vulnerable, in Ireland Red List (Mary Kelly-Quinn and Regan, 2012) (Annex 1).

3.1.4 Sampling Site T4 – Lim River

At this sampling site 8 macroinvertebrates groups were recorded (Annex 2). The value of diversity index was high (H=3.22), as well as the evenness (E=0.93) (Figure 5). These values indicated good biological status of benthic community and very low level of pollution in the water. The number of taxa recorded was 32 (Figure 2), and total taxa density of 3363,27 ind/m2 was the lowest in comparison to other investigated sites (Figure 3, Table 6).

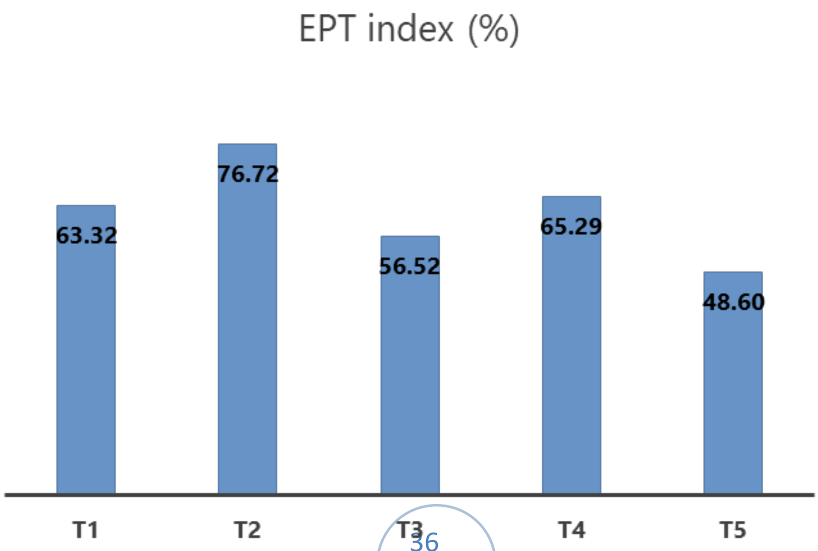


Figure 6. Values of EPT index (%) at investigated sampling sites

Orders Trichoptera and Ephemeroptera were the most dominant groups in the sample with participation in the community of 36,89% and 24,09% respectively (Figure 4). The population density Trichoptera was 1240,82 ind/m2, then followed by Ephemeroptera - 808,16 ind/m2. Group Hydrachnidia had the lowest population density in this sample, only 3,16% (Figure 4) or 106,12 ind/m2 (Table 6). The value of EPT index was 65,29%, which indicates a very good, unpolluted site with benthic community of good biological status. The ratio EPT/C of 6,56, as well as the low numbers of Chironomidae – 9,95% (Table 7) indicates community which is in good biotic condition, and shows that on this site there is no environmental stress. In addition to this, the dominance of organisms that are sensitive to pollution such as Trichoptera, Ephemeroptera and Plecoptera shows good quality of the water at this sampling site.

The FBI index showed very good water quality with possible slight organic pollution, at this sampling site (4,42). The values of ASPT index (5,27) and BMWP (137) indicates very good water quality and very clean water (Table 8, Figure 10).

Table 8. Values of FBI, ASPT and BMWP Indices at investigated sampling sites, September 2019

	T1	T2	T3	T4	T5
FBI index	3,55	3,96	4,49	4,42	4,82
BMWP	109,00	160,00	116,00	137,00	117,00
ASPT	5,19	5,52	5,27	5,27	5,32

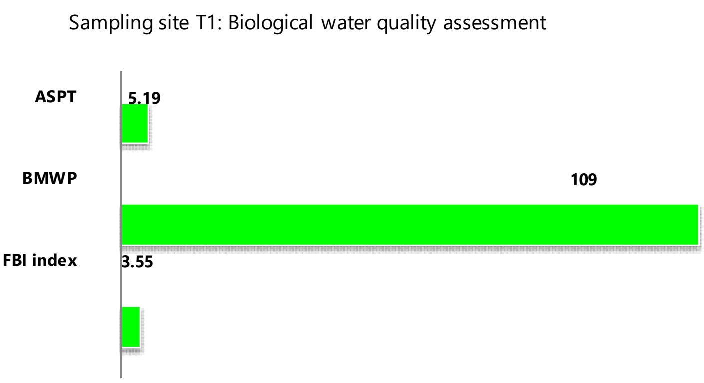


Figure 7. Values of FBI, BMWP and ASPT indices at sampling sites T1

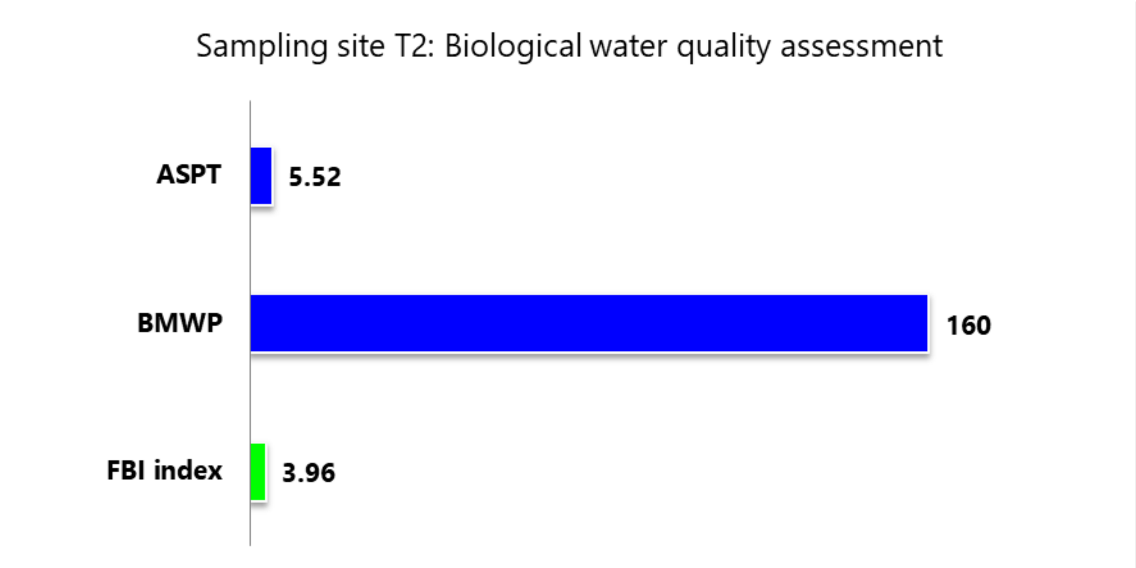


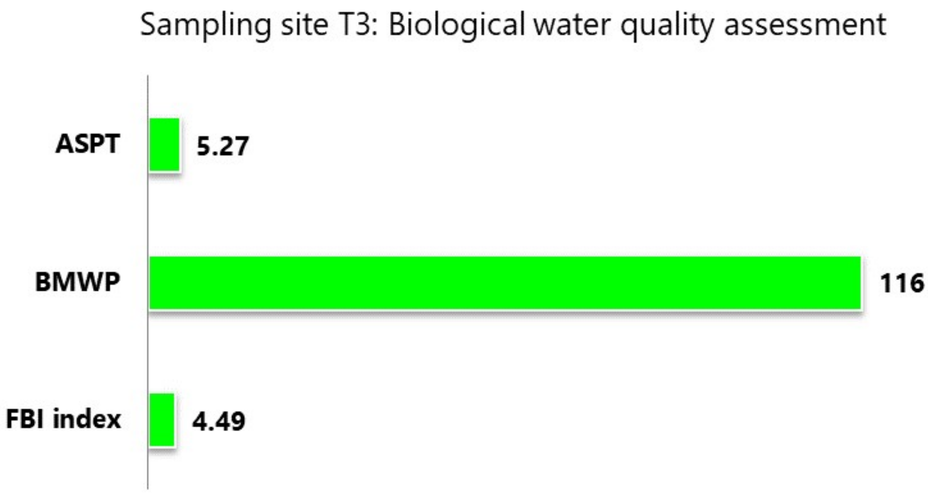
Figure 8. Values of FBI, BMWP and ASPT indices at sampling sites T2

The most abundant species in sample was caddisflies *Hydropsyche instabilis* with 710,20 ind/m2. The abundance of the remaining taxa is given in Annex 2.

Regarding protection status, three gastropod species - *Amphimelania holandrii*, *Ancylus fluviatilis*, and *Bithynia tentaculata* were designated with LC (Least Concern) IUCN status. None of the species collected is listed as nationally protected. The reason for low level of protected species is probably lack of investigation. One mayfly species *Centroptilum luteolum* is considered to be LC by Ireland Red List (Mary Kelly-Quinn and Regan, 2012) (Annex 1).

3.1.5 Sampling Site T5 – Lim River

Nine macroinvertebrate groups were recorded in this sampling site (Annex 2). The diversity index was high (H=3,28), and evenness as well (E=0.94) (Figure 5). These values indicated good biological status of benthic community and very low level of pollution in the water. The taxa richness was 33 (Figure 2) and total taxa density of 3493,88 ind/m2 in this



sampling (Figure 3, Table 6).

Figure 9. Values of FBI, BMWP and ASPT indices at sampling sites T3

Order Trichoptera was the most dominant group in the sample with participation in the community of 38,55% (Figure 4) and population density of 1346,94 ind/m2 (Table 6). The least dominant group was Tricladida with 0,4% or 16,33 ind/m2 (Figure 4, Table 6). The value of EPT index was 48,60% at this monitoring station. This value indicated a very good, unpolluted site. The ratio EPT/C of 3,47 as well as the participation of Chironomidae in community – 14,02% (Table 7) indicates community which is in good biotic condition.

On the basis of FBI index (4,82) it was stated very good water quality with possible slight organic pollution, at this sampling site. The values of ASPT index (5,32) indicated very good water quality, as well as BMWP (117) indicated clean water at investigated site (Table 8, Figure 11).

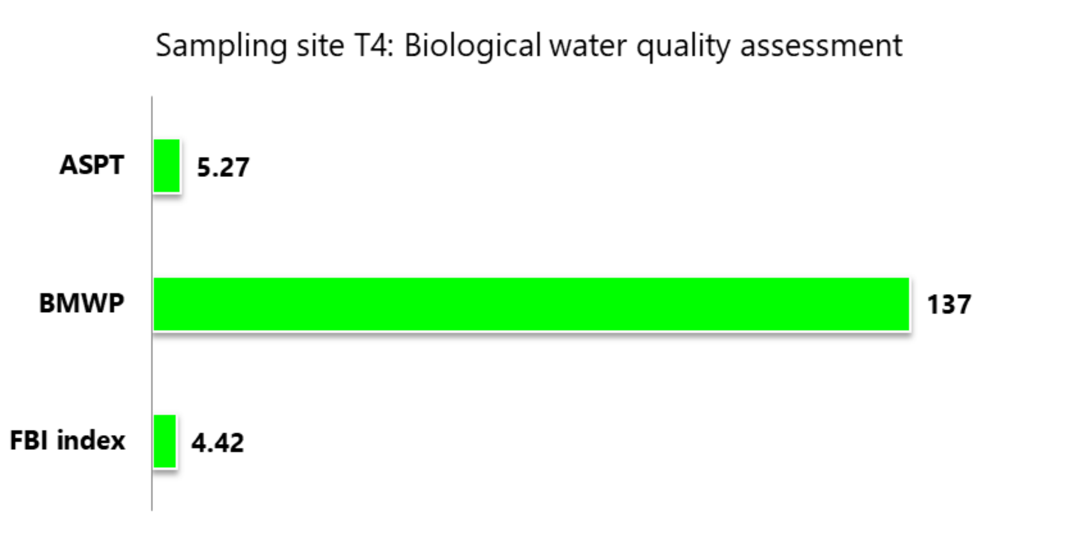


Figure 10. Values of FBI, BMWP and ASPT indices at sampling sites T1

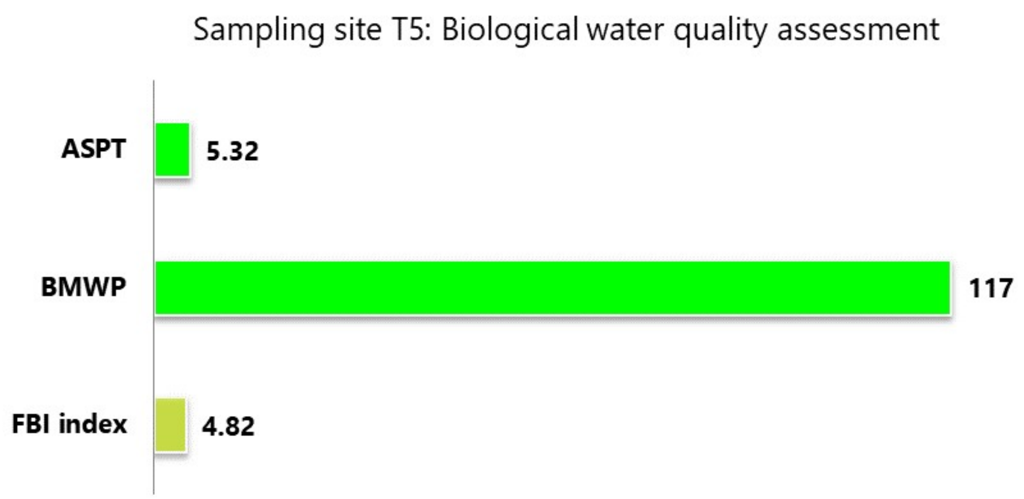


Figure 11. Values of FBI, BMWP and ASPT indices at sampling sites T5

The most abundant species in sample was *Sericostoma personatum* with 1020,41 ind/m2. The abundance of the remaining taxa is given in Annex 2.

Regarding protection status, two gastropod species - *Amphimelania holandrii*, and *Ancylus fluviatilis* were designated with LC (Least Concern) IUCN status. None of the species collected is listed as nationally protected. The reason for low level of protected species is probably lack of investigation. Two mayfly species *Centroptilum luteolum* and *Electrogena lateralis* are designated as LC, and pale evening mayfly, *Procloeon bifidum*, was designated as VU, by Ireland Red List (Mary Kelly-Quinn and Regan, 2012) (Annex 1).

3.2 Conservation status of investigated macroinvertebrate species

When it comes to conservation status of identified species, **only 4 species of gastropods are on the IUCN Red list and all of them have Least Concerned (LC) status. These species are: *Amphimelania holandrii*, *Ancylus fluviatilis*, *Bithynia tentaculata* and *Lithoglyphus naticoides*** (EU Red List - Gastropods: Cuttelod et al. 2011). None of 70 recorded species is not recognized as protected by EU Habitat directive, Bern convention or Bonn Convention (Annex 1). In addition to this, among investigated species there are no species with status of nationally protected species.

The reason for low level of protected species is probably lack of investigation and knowledge gaps on the autecological requirements and pollution sensitivity of these species. So, further assessment of conservation status could be expected. Several mayfly species recorded during this survey are currently protected in Ireland. These species are: *Ephemera danica*, *Centroptilum luteolum*, *Caenis horaria*, *Electrogena lateralis* with LC status and *Procloeon bifidum* with VU status (Mary Kelly-Quinn and Regan, 2012).

The complete List of registered species (Latin and English name) in each river and their national and international protection has been presented in the Annex 2.

3.3 Expected impacts on benthic fauna during construction and operation of the highway and proposal of mitigation measures

There are several impacts that could be expected on biota in the area where construction of the of the Highway section Mateševo – Andrijevica is planned, such as: physical and chemical effects, effects during construction phase, pollution effects on biota and ecosystems, effects of roads on biota and habitats, habitat fragmentation by roads.

The several expected impacts, recognized as most important for benthic fauna are provided below.

3.3.1 Impacts due to Construction phase

Expected Impact of the excavations

The excavations will probably result in short term increase in the suspended material in the water column of the rivers Drcka, Tara and Lim causing **increased turbidity**. If the suspended material (fine materials predominantly fine silty sands, silty clays and silt) remains for longer period at higher concentrations then the penetration of sunlight through the water column may be reduced. Furthermore, the turbidity can cause the clogging of gills and feeding structures of fish and benthic species including molluscs and worms. Hence, the increased level of turbidity will lead towards reduced productivity and may be fatal in some extreme cases, causing the inevitable removal of some benthic species at the construction site. Some of the species could be important in themselves or as a source of food for fishes. The re-suspended sediments can also be transported and re-deposited elsewhere in the surrounding causing negative impact on benthic fauna and therefore fish fauna.

Since excavation is a temporary activity, the impact is not expected to be very significant, because the severity of impact is dependent on the amount of sediment in suspension, sediment size distribution and the current movement in the area. It is anticipated that the turbidity thus caused would not have significant impact.

In addition to this release of contaminants / wastes (heavy metal, hydrocarbons, or other chemicals), can cause the negative effects and loss of benthic fauna may happen. Then, the release of organic wastes could cause the oxygen depletion in the water column creating stressful conditions for benthic fauna.

Mitigation: This is a temporary phase, so, no mitigation measures are required, except careful use of heavy machinery and precautions including careful and regulated excavation, back filling and construction methods. Also, if it is planned to excavate material from the river bed, the quality tests of the sediment should be done before the start of the construction works. If these tests demonstrate that the sediments are not contaminated with toxic chemicals, the dredging/excavation is not expected to have an adverse impact on water quality and biota. So, the potential toxic impacts on benthic fauna due to the bio-availability of any contaminants are not expected.

Expected impact of siltation and increasing/decreasing nutrient

The most damaging agent in aquatic habitats has been said to be **siltation and increasing nutrient loads** rather than by the many chemicals (Dickson, 1986). The siltation may affects benthic community, and can alters it, since it is a recognized as a stress for the benthic community. Siltation may cause changes in the bottom habitat, i.e. substrate, as well as in the physical and chemical characteristics of water (Milša et al., 2010). In addition, increasing or decreasing of nutrients can threat sensitive species, even lead to their extinction.

Mitigation: This is a temporary phase, so, no mitigation measures are required, except careful use of heavy machinery and precautions including careful and regulated excavation, back filling and construction methods. To mitigate the negative impacts on sensitive species, detailed investigation should be performed in order to recognize indicator species in benthic fauna. These indicator species should be monitor in all stages during construction phase, in terms of estimation of the status of population, community structure, and if disturbance occur conduct all measures necessary for species protection.

Expected impact of waste water discharged

During the construction phase there will be generation of some **sewage material** due to personnel engaged in the construction work. The disposal of these waste waters loaded with organic substance into the rivers will also have minimum adverse impact on the water quality as well as freshwater macroinvertebrates.

Mitigation: Since it is a temporary activity, no mitigation measure is required.

3.3.2 Impacts due to Operation phase

Expected impact of pollutants/chemicals

In the operation phase is expected impact of chemicals arising from roads, vehicles, fuels and corrosion. The pollutants in watercourse from roads may affect aquatic biota. These pollutants alter hydrology, increase sediment load, increase nutrients and therefore impact the benthic fauna. Pollutants that may impacting on freshwater biota of investigated water bodies include sand, dust and other particulates, metals such as Pb, Cd, Ni and Zn.

Mitigation: The plan to combat and rehabilitate oil and oil derivate spills in case of emergency has to be developed.

4. SUMMARY OF FINDINGS

A total of 70 taxa from the ten groups of macroinvertebrates were identified during this investigation, as follows: Ephemeroptera, Plecoptera, Coleoptera, Diptera, Trichoptera, Hydrachnidia, Gastropoda, Tricladida, Oligochaeta and Nematoda (Annex 1, Annex 2). The taxa richness at investigated sampling sites ranged from 27 taxa in site T3 to 40 taxa at sampling site T2. Based on this benthic invertebrate survey, total density in the Lim River (3363,27 to 3493,88 individuals/m2) was lower than the total density in the Drcka River (2563,27 to 7118,37 individuals/m2) (Figure 3).

When examining taxonomic groups, Trichoptera (caddisflies) were observed as dominant taxa at all sampling sites. This group participated in benthic fauna with ratio of 38,55% to 64,68% (Figure 4) including their dominant families Lepidostomatidae, Leptoceridae and Sericostomatidae (Annex 2). The least dominant and least abundant group was Nematoda with participation at sampling ite T2 only with abundance of 32,65 ind/m2.

The diversity index was fairly high across all sites and ranged from 3,07 at sampling site T3 to 3,49 at sampling site T2 in Drcka River (Figure 5). The evenness was also high across investigated sites, with almost uniform values of 0,91 at T1 to 0,94 at T5 (Figure 5).

The proportion Chironomidae (%C) was low at all investigated sites and %EPT was fairly high (Table 7), which indicates good quality of the benthic community overall. These trends were also recorded when it comes to the EPT/C ratio.

The performed analysis during one-time survey in September 2019, and values of different community indices suggest that benthic fauna at all investigated sampling sites in Drcka River and Lim River is of good biotic condition. Therefore, these water bodies are assessed as those with good water quality. Also, the dominance of highly pollution-sensitive taxa belonging to Trichoptera, Ehemeroptera, Plecoptera (Henriques-de-Oliveira et al. 2007; Narangarvuu et al. 2014; Zaiha et al. 2015) indicates good water quality all sampling site.

Metrics used for rapid assessment of water quality (FBI, BMWP, ASPT), which are based on composition and abundance of benthic community indicated the high water quality at all investigated sites in Drcka River and Lim River.

Only four gastropod species: *Amphimelania holandrii*, *Ancylus fluviatilis*, *Bithynia tentaculata* and *Lithoglyphus naticoides* are on the IUCN Red list having (LC) Least Concerned status. In addition to this, among investigated species there are no species with status of nationally protected species

The excavations, siltation and increasing/decreasing nutrient, waste water discharged are recognized as most important expected impacts of construction of highway section Mateševo-Andrijevica on benthic fauna during construction phase. In operational phase, the impact of pollutants such as chemicals arising from roads, vehicles, fuels and corrosion is recognized as expected.

5. CONCLUSIONS

Taking into consideration the complexity of planned works on construction of the highway section Mateševo-Andrijevica, the impacts on benthic fauna that inhabits Drcka and Lim River water bodies from both, construction phase and operational phase of the roads are expected. Since water resources are classified in strategic resources it is necessary to monitor, permanently, their quality and secure their protection. The preservation of benthic community is important because this community represents the basis of fish feeding in the rivers. Defragmentation of habitats or degradation of benthic community can consequently influence the fish populations in these watercourses.

Considering the results of the performed one-time survey, we can conclude that all sampling sites from both investigated water courses Drcka River and Lim River have good ecological quality. Also, the findings show that benthic community is in a good biological condition.

In order to preserve this ecological status in the future, and mitigate alterations in benthic community, and therefore in fish community from these watercourses, it is strongly recommended proposal of the Environment Management Plan for implementation at the construction and operational stage of Project activity in order to closely monitor the performance.

6. REFERENCES

Dickson, K.L. 1986. Neglected and forgotten contaminants affecting aquatic life. Environmental Toxicology and Chemistry, 5: 939–940.

Henriques-de-Oliveira C, Baptista DF, Nessimian JL. 2007. Sewage input effects on the macroinvertebrate community associated to Typha domingensis Pers in a coastal lagoonin southeastern Brazil. Brazilian Journal of Biology, 67(1): 73-80.

Narangarvuu D, Hsu CB, Shieh SH, Wu FC, Yang PS. 2014. Macroinvertebrate assemblagepatterns as indicators of water quality in the Xindian watershed, Taiwan. Journal ofAsia-Pacific Entomology, 17(3): 505-513.

Zaiha A N, Ismid MM, Azri, M. S. 2015. Effects of logging activities on ecological waterquality indicators in the Berasau River, Johor, Malaysia. Environmental Monitoring andAssessment, 187(8): 1-9.

Miliša, M., Živković, V., Matoničkin Kepčija, R. and Habdija, I. 2010. Siltation disturbance in a mountain stream: aspect of functional composition of the benthic community. *Periodicum Biologorum*, 112 (2): 173–178.

Nagel, P., Bäthe, J., Günther, R.H., Kunz, M., Potel, S., Schönleber, Y., Steinhause, S. and Trischker, M. 1989. Bildbestimmungsschlüssel der Saprobien. Makrozoobenthos, 183 pp. Stuttgart, New York: Gustav Fischer Verlag 1989/ISBN 3-437-20438-6.

Kelly-Quinn, M. and Regan, E.C. 2012. Ireland Red List No. 7: Mayflies (Ephemeroptera). National Parks and Wildlife Service, Department of Arts, Heritage and the Gaeltacht, Dublin, Ireland.

Pešić, V. 2002. Two interesting species of the genus *Atractides* Koch 1837 (Acari, Actinedida) from Crna Gora (Balkan Peninsula). *Lauterbornia*, 44: 65-71.

Pešić, V. 2003. New records of water mites (Acari, Actinedida) from running waters from Yugoslavia and Macedonia (SE Eeurope). *Acta Entomologica Serbica,* 6 (1-2): 131-138.

Pešić, V. 2003a. New records of the water mite genera *Atractides* Koch and *Sperchon* Kramer from the Balkan, with the description of one new species. *Zootaxa*, 168: 1-12.

Pešić, V. 2004. Water mites (Acari: Hydrachnidia) of the Biogradska Gora National Park (Serbia and Crna Gora). *Biodiversity of the Biogradska Gora National Park*, No 1, 82-103, Centre for Biodiversity of Montenegro/ISBN 86-905195-0-5.

Di Sabatino, A., Gerecke, R., Smit, H., Pesic, V., Panesar, A. 2003. Water mites of the family Torrenticolidae (Acari, Actinedida) from the Eastern Mediterranean region. *Arch. Hydrobiol. Suppl.*, 139 (3): 393-431.

Gerecke, R. 1991. Taxonomische, faunistische und ökologische Untersuchungen an Wassermilben (Acari, Actinedida) aus Sizilien, unter Berücksichtigung anderer aquatischer Invertebraten. *Lauterbornia*, 7: 1-303.

Epler, J.H. 2001. Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. Special Publication SJ2001-SP13. North Carolina Department of Environment and Natural Resources, Raleigh, NC and St. Johns River Water Management District, Palatka FL. 526 p.

Brinkhurst, R.O. and Jamieson, B.G.M. 1971. (Eds.). Aquatic Oligochaeta of the World. Oliver – Boyd, Edinburg, 860 p.

Hrabě, S. 1981. Vodni máloštetinatci (Oligochaeta) Ceskoslovenska, Acta Universitatis Carolinae – Biológica, 1979: 1–167.

Merritt, R.W., K.W. Cummins, and M.B. Berg. 1996. An Introduction to the Aquatic Insects of North America. 3rd Edition. Kendall Hunt Pub. Co.

Merritt, R.W., K.W. Cummins, and M.B. Berg. 2008. An Introduction to the Aquatic Insects of North America. 4th Edition. Kendall Hunt Pub. Co.

Jessup, B.K., Markowitz, A. and Stribling, J.B. 1999. Family-Level Key to the Stream Invertebrates of Maryland and Surrounding Areas. Maryland Department of Natural Resources, 83 p.

Pescador, M.L., Rasmussen, A.K., Harris, S.C. and Hulbert, J.L. 1995. Identification manual for the caddisfly (Trichoptera) larvae of Florida. State of Florida, Department of Environmental Protection Division of Water Facilities Tallahassee; Entomology, Center for Water Quality Florida A&M Univerisity, 186 p.

Timm, T. 2009. A guide to the freshwater Oligochaeta and Polychaeta of northern and central Europe. Lauterbornia, International journal of faunistics and floristics of European Inland waters, 66: 1–235.

Sperber, C. 1950. A guide for determination European Naididae. Zoologiska Bidrag Fran Uppsala, (29): 45–81.

SNIFFER, 2007. WFD72A: Revision and testing of BMWP scores.

Leader of the scientific team

Prof. dr Drago Marić

7. ANNEXES

Annex 1. List of registered species with their protection status

	Common name	River	**IUCN status** LC – Least Concern NE – Not Evaluated	**Ireland Red List (Mary Kelly-Quinn and Regan, 2012)** LC – Least Concern VU – Vulnerable	**EU Red List (Gastropods:Cuttelod et al. 2011)** LC – Least Concern
EPHEMEROPTERA	**MAYFLIES**				
Ephemeridae	Burrowing mayfly				
Ephemera danica Müller, 1764	Green drake	Drcka River	NE	LC	
Baetidae	Small minnow mayflies				
Centroptilum luteolum Müller, 1776	Small spurwing	Drcka River, Lim River	NE	LC	
Procloeon bifidum (Bengtsson, 1912)	Pale evening mayfly	Drcka River, Lim River	NE	VU	
Caenidae	Small Squaregill Mayflies		NE		
Caenis horaria (Linnaeus, 1758)	Anglers' curse	Drcka River	NE	LC	
Heptageniidae	Stream mayfly				
Electrogena lateralis (Curtis, 1834)	Dusky yellowstreak	Lim River	NE	LC	
Epeorus yougoslavicus (Samal, 1935)	–	Drcka River, Lim River	NE		
Heptagenia sp.	–	Lim River	NE	LC	
PLECOPTERA	**STONEFLY**				
Nemouridae	Forestfly stonefly				
Nemoura cinerea (Retzius, 1783)	–	Drcka River	NE	LC	

Nemurella pictetii (Klapálek, 1900)	–	Drcka River	NE
Chloroperlidae	Sallflies		
Chloroperla tripunctata (Scopoli, 1763)	–	Drcka River	NE
Leuctridae	Rollwing stonefly		
Leuctra nigra (Olivier, 1811)		Drcka River, Lim River	NE
Perlidae	Summer stoneflies		
Dinocras cephalotes (Curtis, 1827)	–	Drcka River	NE
Perla bipunctata Pictet & F.J., 1833	–	Drcka River, Lim River	NE
Taeniopterygidae	Taeniopterygid winter stoneflies		
Brachyptera sp.	–	Lim River	NE
Capniidae	Capniid winter stoneflies		
Capnia vidua Klapálek, 1904	–	Lim River	NE
COLEOPTERA			
Elmidae	Riffle beetles		
Limnius volckmari (Panzer, 1793)	Volckmar's Water-beetle	Drcka River, Lim River	NE
Elmidae sp. 1 larvae	–	Drcka River, Lim River	NE
Elmidae sp. 2 larvae	–	Drcka River, Lim river	NE
Elmidae sp. 3 larvae	–	Drcka River	NE
Elmidae sp. 4 larvae	–	Drcka River	NE
Dytiscidae	Predaceous Diving Beetle		

Hydroporus discretus Fairmaire & Brisout de Barneville, 1859	–	Drcka River	NE
DIPTERA			
Athericidae	Snipe flies or Ibis flies		
Atherix sp.	–	Drcka River	
Chironomidae	Midges		
Chironomus riparius Meigen, 1804	–	Drcka River, Lim River	NE
Chironomus sp.	–	Drcka River, Lim River	NE
Tabanidae	Horse-flies		
Tabanus sp.	–	Drcka River, Lim River	NE
Chaoboridae	Phantom midges or glassworms		
Chaoborus sp.	–	Drcka River	NE
Tipulidae	Crane fly		
Tipula sp.	–	Drcka River, Lim River	NE
Simuliidae	Black fly		
Simulium sp.	–	Drcka River, Lim River	NE
TRICHOPTERA	**CADDISFLIES**		
Leptoceridae	Long-horn caddisflies		
Adicella filicornis (Pictet, 1834)	–	Drcka River, Lim River	NE
Glossosomatidae	Tortoise or Saddle-Case Makers		

Glossosoma boltoni Curtis, 1834	–	Drcka River, Lim River	NE
Lepidostomatidae	Little Brown Sedges		
Crunoecia irrorata (Curtis, 1834)	–	Drcka River, Lim River	NE
Rhyacophilidae	Primitive caddisflies		
Rhyacophila dorsalis (Curtis, 1834)	–	Drcka River, Lim River	NE
Rhyacophila fasciata Hagen, 1859	–	Drcka River	NE
Hydropsychidae	Net-spinning caddisflies		
Hydropsyche instabilis (Curtis, 1834)	–	Drcka River, Lim River	NE
Hydropsyche rhadamanthys Malicky, 2001		Drcka River, Lim River	NE
Sericostomatidae	Sericostomatid Case-Maker Caddisflies		
Sericostoma personatum (Kirby & Spence, 1826)	–	Drcka River, Lim River	NE
Polycentropodidae	tube-making caddisflies		
Polycentropus flavomaculatus (Pictet, 1834)	–	Lim River	NE
HYDRACHNIDIA	**WATER MITES**		
Lebertiidae	–		
Lebertia sparsicapillata Thor, 1905	–	Drcka River, Lim River	NE
Lebertia sp.	–	Drcka River	NE
Torrenticolidae	–		
Torrenticola sp.	–	Drcka River, Lim River	NE
Hydryphantidae	–		

Protzia sp.	–	Drcka River, Lim River	NE		
Hydrodromidae	–				
Hydrodroma cf. despiciens (Müller, 1776)	–	Drcka River, Lim River	NE		
Hydrodroma sp.	–	Drcka River, Lim River	NE		
Sperchonidae	–				
Sperchon sp. 1	–	Drcka River, Lim River	NE		
Sperchon sp. 2	–	Drcka River	NE		
Sperchon sp. 3	–	Drcka River	NE		
Limnesiidae	–				
Limnesia sp.	–	Drcka River	NE		
Lebertiidae	–				
Hygrobatidae	–				
Atractides sp.	–	Lim River	NE		
Hygrobates sp. 1	–	Lim River	NE		
Hygrobates sp. 2	–	Lim River	NE		
GASTROPODA					
Planorbidae	Ramshorn snails				
Ancylus fluviatilis Müller, 1774	Common river limpet	Drcka River, Lim River	**LC**		**LC**
Lithoglyphidae	–				
Lithoglyphus naticoides (C.Pfeiffer, 1828)	Gravel snail	Drcka River	**LC**		**LC**
Amphimelaniidae	–				

Amphimelania holandrii (C.Pfeiffer, 1828) –		Lim River	**LC**	**LC**
Bithyniidae	Faucet snails			
Bithynia tentaculata (Linnaeus, 1758)	Common bithynia	Lim RIver	**LC**	**LC**
TRICLADIDA	**Freshwater trclad**			
Geoplanoidea	–			
Dugesiidae	Dugesiid triclads			
Dugesia gonocephala (Duges, 1830)	–	Drcka River	NE	
Planarioidea	Freshwater planarian			
Dendrocoelidae	–			
Dendrocoelum lacteum subsp lacteum (Müller, 1774).	–	Lim River	NE	
OLIGOCHAETA	**AQUATIC WORMS**			
Naididae/Naidinae	Sludge-worms			
Nais barbata Müller, 1774	–	Lim River	NE	
Nais bretscheri Michaelsen, 1899	–	Drcka River, Lim River	NE	
Nais communis Piguet, 1906	–	Drcka River	NE	
Nais elinguis Müller, 1774	–	Drcka River, Lim River	NE	
Nais pardalis Piguet, 1906	–	Drcka River, Lim River	NE	
Nais pseudobtusa Piguet, 1906	–	Lim River	NE	
Nais variabilis Piguet, 1906	–	Drcka River, Lim River	NE	

Stylaria fossularis Leidy, 1852	–	Drcka River	NE
Enchytraeidae	Pot-worms		
Enchytraeus albidus Henle, 1837	–	Lim River	NE
Cernosvitoviella atrata (Bretscher, 1903)	–	Drcka River, Lim River	NE
Lumbriculidae	–		
Stylodrilus heringianus Claparède, 1862	–	Drcka River, Lim River	NE
Stylodrilus lemani (Grube, 1879)	–	Drcka River, Lim River	NE
Lumbricidae	Earthworms		
Eiseniella tetraedra (Savigny, 1826)	–	Drcka River, Lim River	NE
NEMATODA	**ROUNDWORMS**	Drcka River	NE

Annex 2. List of identified macroinvertebrate species from the sampling sites

Phylum	Class/Subclass	Order	Family/Subfamily	Species	No. Individuals Per m2
SAMPLING SITE T1					
ARTHROPODA	INSECTA	EPHEMEROPTERA	Ephemeridae	*Ephemera danica*	24,49
ARTHROPODA	INSECTA	EPHEMEROPTERA	Baetidae	*Centroptilum luteolum*	16,33
ARTHROPODA	INSECTA	EPHEMEROPTERA	Baetidae	*Caenis horaria*	48,98
ARTHROPODA	INSECTA	PLECOPTERA	Perlidae	*Perla bipunctata*	16,33
ARTHROPODA	INSECTA	PLECOPTERA	Nemouridae	*Nemoura cinerea*	65,31

Phylum	Class/Subclass	Order	Family/Subfamily	Species	No. Individuals Per m2
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Elmidae sp. 1 larvae*	40,82
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Elmidae sp. 2 larvae*	8,16
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Elmidae sp. 3 larvae*	24,49
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Elmidae sp. 4 larvae*	8,16
ARTHROPODA	INSECTA	DIPTERA	Athericidae	Atherix sp.	40,82
ARTHROPODA	INSECTA	DIPTERA	Chironomidae	*Chironomus riparius*	97,96
ARTHROPODA	INSECTA	DIPTERA	Chironomidae	*Chironomus sp.*	163,27
ARTHROPODA	INSECTA	DIPTERA	Tabanidae	*Tabanus sp.*	16,33
ARTHROPODA	INSECTA	TRICHOPTERA	Leptoceridae	*Adicella filicornis*	138,78
ARTHROPODA	INSECTA	TRICHOPTERA	Glossosomatidae	*Glossosoma boltoni*	179,59
ARTHROPODA	INSECTA	TRICHOPTERA	Lepidostomatidae	*Crunoecia irrorata*	1134,69
ARTHROPODA	INSECTA	TRICHOPTERA	Rhyacophilidae	*Rhyacophila fasciata*	24,49
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Lebertiidae	*Lebertia sparsicapillata*	8,16
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hydrodromidae	*Hydrodroma cf. despiciens*	8,16
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Torrenticolidae	*Torrenticola sp.*	8,16
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hydryphantidae	*Protzia sp.*	8,16
MOLLUSCA	GASTROPODA		Planorbidae	*Ancylus fluviatilis*	65,31
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*Nais elinguis*	16,33
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. communis*	106,12
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. pardalis*	97,96
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. variabilis*	106,12
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*Stylaria fossularis*	8,16
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Enchytraeidae	*Cernosvitoviella atrata*	89,80
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Lumbriculidae	*Stylodrilus heringianus*	16,33
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Lumbriculidae	*S. lemani*	16,33
SAMPLING SITE T2					
ARTHROPODA	INSECTA	EPHEMEROPTERA	Baetidae	*Centroptilum luteolum*	106,12

Phylum	Class/Subclass	Order	Family/Subfamily	Species	No. Individuals Per m2
ARTHROPODA	INSECTA	EPHEMEROPTERA	Heptageniidae	*Epeorus yougoslavicus*	277,55
ARTHROPODA	INSECTA	PLECOPTERA	Chloroperlidae	*Chloroperla tripunctata*	48,98
ARTHROPODA	INSECTA	PLECOPTERA	Leuctridae	*Leuctra nigra*	187,76
ARTHROPODA	INSECTA	PLECOPTERA	Nemouridae	*Nemoura cinerea*	57,14
ARTHROPODA	INSECTA	PLECOPTERA	Perlidae	*Dinocras cephalotes*	57,14
ARTHROPODA	INSECTA	PLECOPTERA	Perlidae	*Perla bipunctata*	122,45
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Elmidae sp. 3 larvae*	89,80
ARTHROPODA	INSECTA	DIPTERA	Athericidae	Atherix sp.	57,14
ARTHROPODA	INSECTA	DIPTERA	Chaoboridae	*Chaoborus sp.*	16,33
ARTHROPODA	INSECTA	DIPTERA	Chironomidae	*Chironomus riparius*	285,71
ARTHROPODA	INSECTA	DIPTERA	Chironomidae	*Chironomus sp.*	204,08
ARTHROPODA	INSECTA	DIPTERA	Tabanidae	*Tabanus sp.*	81,63
ARTHROPODA	INSECTA	DIPTERA	Tipulidae	*Tipula sp.*	81,63
ARTHROPODA	INSECTA	TRICHOPTERA	Glossosomatidae	*Glossosoma boltoni*	367,35
ARTHROPODA	INSECTA	TRICHOPTERA	Hydropsychidae	*Hydropsyche instabilis*	8,16
ARTHROPODA	INSECTA	TRICHOPTERA	Hydropsychidae	*H. rhadamanthys*	32,65
ARTHROPODA	INSECTA	TRICHOPTERA	Lepidostomatidae	*Crunoecia irrorata*	8,16
ARTHROPODA	INSECTA	TRICHOPTERA	Leptoceridae	*Adicella filicornis*	2530,61
ARTHROPODA	INSECTA	TRICHOPTERA	Rhyacophilidae	*Rhyacophila dorsalis*	163,27
ARTHROPODA	INSECTA	TRICHOPTERA	Sericostomatidae	*Sericostoma personatum*	1493,88
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hydrodromidae	*Hydrodroma cf. despiciens*	40,82
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hydrodromidae	*Hydrodroma sp.*	130,61
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Torrenticolidae	*Torrenticola sp.*	73,47
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hydryphantidae	*Protzia sp.*	24,49
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Limnesiidae	*Limnesia sp.*	16,33
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Sperchonidae	*Sperchon sp. 1*	57,14
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Sperchonidae	*Sperchon sp. 2*	24,49
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Sperchonidae	*Sperchon sp. 3*	8,16

Phylum	**Class/Subclass**	**Order**	**Family/Subfamily**	**Species**	**No. Individuals Per m2**
PLATYHELMINTHES	RHABDITOPHORA	TRICLADIDA	Dugesiidae	*Dugesia gonocephala*	24,49
MOLLUSCA	GASTROPODA	LITTORINIMORPHA	Lithoglyphidae	*Lithoglyphus naticoides*	16,33
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*Nais bretscheri*	24,49
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. communis*	24,49
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*Nais elinguis*	122,45
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. pardalis*	73,47
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. variabilis*	73,47
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*Stylaria fossularis*	8,16
ANNELIDA	CLITELLATA/OLIGOCHAETA	HAPLOTAXIDA	Enchytraeidae	*Cernosvitoviella atrata*	48,98
ANNELIDA	CLITELLATA/OLIGOCHAETA	HAPLOTAXIDA	Lumbricidae	*Eiseniella tetraedra*	16,33
NEMATODA					32,65
SAMPLING SITE T3					
ARTHROPODA	INSECTA	EPHEMEROPTERA	Baetidae	*Centroptilum luteolum*	171,43
ARTHROPODA	INSECTA	EPHEMEROPTERA	Baetidae	*Procloeon bifidum*	89,80
ARTHROPODA	INSECTA	EPHEMEROPTERA	Heptageniidae	*Epeorus yougoslavicus*	138,78
ARTHROPODA	INSECTA	PLECOPTERA	Nemouridae	*Nemoura cinerea*	146,94
ARTHROPODA	INSECTA	PLECOPTERA	Nemouridae	*Nemurella picteti*	32,65
ARTHROPODA	INSECTA	PLECOPTERA	Perlidae	*Dinocras cephalotes*	89,80
ARTHROPODA	INSECTA	COLEOPTERA	Dytiscidae	*Hydroporus discretus*	8,16
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Limnius volckmari*	16,33
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Elmidae sp. 1 larvae*	16,33
ARTHROPODA	INSECTA	DIPTERA	Athericidae	Atherix sp.	65,31
ARTHROPODA	INSECTA	DIPTERA	Chaoboridae	*Chaoborus sp.*	16,33
ARTHROPODA	INSECTA	DIPTERA	Chironomidae	*Chironomus riparius*	408,16
ARTHROPODA	INSECTA	DIPTERA	Chironomidae	*Chironomus sp.*	1004,08
ARTHROPODA	INSECTA	DIPTERA	Tabanidae	*Tabanus sp.*	8,16
ARTHROPODA	INSECTA	DIPTERA	Tipulidae	*Tipula sp.*	8,16
ARTHROPODA	INSECTA	DIPTERA	Simuliidae	*Simulium sp.*	16,33

Phylum	Class/Subclass	Order	Family/Subfamily	Species	No. Individuals Per m2
ARTHROPODA	INSECTA	TRICHOPTERA	Glossosomatidae	*Glossosoma boltoni*	489,80
ARTHROPODA	INSECTA	TRICHOPTERA	Hydropsychidae	*Hydropsyche instabilis*	48,98
ARTHROPODA	INSECTA	TRICHOPTERA	Lepidostomatidae	*Crunoecia irrorata*	24,49
ARTHROPODA	INSECTA	TRICHOPTERA	Leptoceridae	*Adicella filicornis*	897,96
ARTHROPODA	INSECTA	TRICHOPTERA	Rhyacophilidae	*Rhyacophila dorsalis*	97,96
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hydrodromidae	*Hydrodroma sp.*	16,33
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hydryphantidae	*Protzia sp.*	40,82
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Lebertiidae	*Lebertia sp.*	40,82
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. communis*	8,16
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. pardalis*	24,49
ANNELIDA	CLITELLATA/OLIGOCHAETA	HAPLOTAXIDA	Enchytraeidae	*Cernosvitoviella atrata*	16,33
SAMPLING SITE T4					
ARTHROPODA	INSECTA	EPHEMEROPTERA	Baetidae	*Centroptilum luteolum*	416,33
ARTHROPODA	INSECTA	EPHEMEROPTERA	Heptageniidae	*Epeorus yougoslavicus*	391,84
ARTHROPODA	INSECTA	PLECOPTERA	Capniidae	*Capnia vidua*	89,80
ARTHROPODA	INSECTA	PLECOPTERA	Perlidae	*Perla bipunctata*	24,49
ARTHROPODA	INSECTA	PLECOPTERA	Taeniopterigidae	*Brachyptera sp.*	32,65
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Limnius volckmari*	171,43
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Elmidae sp. 1 larvae*	130,61
ARTHROPODA	INSECTA	DIPTERA	Chironomidae	*Chironomus riparius*	212,24
ARTHROPODA	INSECTA	DIPTERA	Chironomidae	*Chironomus sp.*	122,45
ARTHROPODA	INSECTA	DIPTERA	Tabanidae	*Tabanus sp.*	16,33
ARTHROPODA	INSECTA	DIPTERA	Tipulidae	*Tipula sp.*	8,16
ARTHROPODA	INSECTA	DIPTERA	Simuliidae	*Simulium sp.*	122,45
ARTHROPODA	INSECTA	TRICHOPTERA	Glossosomatidae	*Glossosoma boltoni*	114,29
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hydrodromidae	*Hydrodroma sp.*	8,16
ARTHROPODA	INSECTA	TRICHOPTERA	Hydropsychidae	*Hydropsyche instabilis*	710,20
ARTHROPODA	INSECTA	TRICHOPTERA	Hydropsychidae	*H. rhadamanthys*	171,43

Phylum	Class/Subclass	Order	Family/Subfamily	Species	No. Individuals Per m2
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hydryphantidae	*Protzia sp.*	16,33
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hygrobatidae	*Hygrobates sp. 1*	16,33
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hygrobatidae	*Hygrobates sp. 2*	8,16
ARTHROPODA	INSECTA	TRICHOPTERA	Lepidostomatidae	*Crunoecia irrorata*	114,29
ARTHROPODA	INSECTA	TRICHOPTERA	Leptoceridae	*Adicella filicornis*	81,63
ARTHROPODA	INSECTA	TRICHOPTERA	Rhyacophilidae	*Rhyacophila dorsalis*	48,98
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Torrenticolidae	*Torrenticola sp.*	57,14
MOLLUSCA	GASTROPODA	CAENOGASTROPODA	Amphimelaniidae	*Amphimelania holandrii*	24,49
MOLLUSCA	GASTROPODA	LITTORINIMORPHA	Bithyniidae	*Bithynia tentaculata*	57,14
MOLLUSCA	GASTROPODA		Planorbidae	*Ancylus fluviatilis*	57,14
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. pardalis*	8,16
ANNELIDA	CLITELLATA/OLIGOCHAETA	HAPLOTAXIDA	Enchytraeidae	*Cernosvitoviella atrata*	57,14
ANNELIDA	CLITELLATA/OLIGOCHAETA	HAPLOTAXIDA	Enchytraeidae	*Enchytraeus albidus*	16,33
ANNELIDA	CLITELLATA/OLIGOCHAETA	LUMBRICULIDA	Lumbriculidae	*Stylodrilus heringianus*	8,16
ANNELIDA	CLITELLATA/OLIGOCHAETA	LUMBRICULIDA	Lumbriculidae	*S. lemani*	24,49
ANNELIDA	CLITELLATA/OLIGOCHAETA	HAPLOTAXIDA	Lumbricidae	*Eiseniella tetaedra*	24,49
SAMPLING SITE T5					
ARTHROPODA	INSECTA	EPHEMEROPTERA	Baetidae	*Centroptilum luteolum*	40,82
ARTHROPODA	INSECTA	EPHEMEROPTERA	Baetidae	*Procloeon bifidum*	8,16
ARTHROPODA	INSECTA	EPHEMEROPTERA	Heptageniidae	*Electrogena lateralis*	8,16
ARTHROPODA	INSECTA	EPHEMEROPTERA	Heptageniidae	*Epeorus yougoslavicus*	40,82
ARTHROPODA	INSECTA	PLECOPTERA	Capniidae	*Capnia vidua*	16,33
ARTHROPODA	INSECTA	PLECOPTERA	Leuctridae	*Leuctra nigra*	228,57
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Elmidae sp. 1 larvae*	16,33
ARTHROPODA	INSECTA	COLEOPTERA	Elmidae	*Elmidae sp. 2 larvae*	48,98
ARTHROPODA	INSECTA	DIPTERA	Chironomidae	*Chironomus riparius*	416,33
ARTHROPODA	INSECTA	DIPTERA	Chironomidae	*Chironomus sp.*	73,47
ARTHROPODA	INSECTA	DIPTERA	Tabanidae	*Tabanus sp.*	32,65

Phylum	Class/Subclass	Order	Family/Subfamily	Species	No. Individuals Per m2
ARTHROPODA	INSECTA	TRICHOPTERA	Hydropsychidae	*Hydropsyche instabilis*	146,94
ARTHROPODA	INSECTA	TRICHOPTERA	Hydropsychidae	*H. rhadamanthys*	32,65
ARTHROPODA	INSECTA	TRICHOPTERA	Lepidostomatidae	*Crunoecia irrorata*	81,63
ARTHROPODA	INSECTA	TRICHOPTERA	Polycentropodidae	*Polycentropus flavomaculatus*	48,98
ARTHROPODA	INSECTA	TRICHOPTERA	Rhyacophilidae	*Rhyacophila dorsalis*	16,33
ARTHROPODA	INSECTA	TRICHOPTERA	Sericostomatidae	*Sericostoma personatum*	1020,41
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hydrodromidae	*Hydrodroma cf. despiciens*	8,16
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Hygrobatidae	*Atractides sp.*	8,16
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Lebertiidae	*Lebertia sparsicapillata*	16,33
ARTHROPODA	ARACHNIDA	TROMBIDIFORMES	Sperchonidae	*Sperchon sp. 1*	16,33
MOLLUSCA	GASTROPODA	CAENOGASTROPODA	Amphimelaniidae	*Amphimelania holandrii*	236,73
MOLLUSCA	GASTROPODA		Planorbidae	*Ancylus fluviatilis*	400,00
PLATYHELMINTHES	RHABDITOPHORA	TRICLADIDA	Dendrocoelidae	*Dendrocoelum lacteum*	16,33
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*Nais barbata*	8,16
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. bretscheri*	8,16
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. elinguis*	24,49
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. pseudobtusa*	8,16
ANNELIDA	CLITELLATA/OLIGOCHAETA	TUBIFICIDA	Naididae/Naidinae	*N. variabilis*	32,65
ANNELIDA	CLITELLATA/OLIGOCHAETA	HAPLOTAXIDA	Enchytraeidae	*Cernosvitoviella atrata*	81,63
ANNELIDA	CLITELLATA/OLIGOCHAETA	LUMBRICULIDA	Lumbriculidae	*Stylodrilus heringianus*	277,55
ANNELIDA	CLITELLATA/OLIGOCHAETA	LUMBRICULIDA	Lumbriculidae	*S. lemani*	65,31

Annex 3. Photos from investigated sampling sites