ASSESSMENT STUDY OF THE PRESPA TROUT

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Project title

MONITORING AND CONSERVATION OF THE ENDEMIC AND ENDANGERED TROUT (*SALMO PERISTERICUS*) IN NATIONAL PARK PELISTER AND PRESPA REGION

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CONTENT

ASSESSMENT STUDY
OF THE SPATIAL DISTRIBUTION, POPULATION SIZE, CONSERVATION STATUS, DETECTED THREATS AND RISKS, INCLUDING THE INFLUENCE OF HYDROPOWER PLANTS TO THE PRESPA TROUT

I. INTRODUCTION

1.1 Background

The Prespa region, comprising a part of National Park Pelister, is an area with high natural value, unique functional and living system with different habitats important for conservation of numerous rare and endemic species, including freshwater fish species. Regarding fish fauna, 9 fish species of the 23 taxa are endemic to the region. According to the "Red List of Freshwater Fish in the Mediterranean", Prespa region features as one of the 10 most important wetlands in the Mediterranean, both for the endemism of its fish species, as well as for its concentration of threatened species. In accordance with the IUCN Red List of Threatened Species, eight of the nine endemic fish species have been characterized as vulnerable (VU) or endangered (EN). The Prespa trout *Salmo peristericus* Karaman, 1938 is considered as an endangered species.

The Prespa trout lives in four rivers flowing into Lake Macro Prespa, from the north and east: three in the Republic of North Macedonia - Golema (Leva), Kranska and Brajchinska River systems and one in Greece (Agios Germanos River). Essentially, in North Macedonia, parts of Brajchinska River and their tributaries belong to protected area National Park Pelister. Initially, the Prespa trout was reported as endangered due to habitat destruction, illegal fishing, water abstraction and competition with the introduced rainbow trout and other salmonids (Economidis, 1995), while in 2006, the Prespa trout was classified as endangered by the IUCN criteria (Smith & Darwall, 2006).

Vulnerability and endemism of the Prespa trout have motivated scientists, managers and decision-makers at transboundary level to take the initiative in order to protect the population of the Prespa trout. The first Species Action Plan with proposed conservation measurements was prepared in order to ensure a long-term conservation of the Prespa trout (Crivelli et al., 2008; Koutseri et al., 2010). Beside this Action Plan as a useful conservation tool and apart from the positive result of embedding transboundary cooperation between scientists concerning conservation issues [expert studies: "Development of a Transboundary Monitoring System for the Prespa Park Area", (2009); "Transboundary Fish and Fisheries Management Plan for Prespa Lakes Basin", (2012)], none of them have been implemented by the management bodies to enable greater protection of the species. Additionally, all these actions and the documentation of the importance of the trout as an endangered endemic species of the Prespa basin was not sufficient to stop decision-makers at central level to approve the construction of small hydropower plants on the Brajchinska River and Kranska River in 2014. National Park Pelister as a protected area covers some parts of the trout habitats (on Brajchinska River and their tributaries), but conservation of the Prespa trout cannot be achieved only through measures and activities in the park.

Among other problems that slow down the process of species protection in the country is the insufficient information of the population distribution, abundance and trend of the abundance of many species, which are important parameters for proper species management and ensuring long-term recovery of the population. The abovementioned parameters are essential key aspects to be considered in the preparation of the National Red List. Currently, the evaluation of North Macedonian freshwater fish species for the National Red List has been delayed for a while, as data on population size and distribution range for most fish species are limited, while information on trends is available only indirectly for some species through fisheries data.

Based on all the mentioned facts and issues, the implementation of the project activities and expert tasks concerning the support in development of a Conservation Action Plan (CAP) for the endemic and endangered Prespa trout (*Salmo peristericus*) financed by PONT (Prespa Ohrid Nature Trust) and CEPF (Critical Ecosystem Partnership Fund), is of great importance, as it provides a new data on population distribution, size and structure of the Prespa trout in the borders of North Macedonia. The obtained data from project's field work activities combined with existing literature data provides a good basis for successful evaluation of the National conservation status of the Prespa trout, according to IUCN criteria. The assessment study is a basis for development of a Conservation Action Plan (CAP) for the Prespa trout, as the main goal of the Project. The obtained data are important parameters for proper species management and ensuring long-term recovery of the Prespa trout population. At the same time, these rivers provide essential services for the well-being of people. It is necessary to protect critical ecosystems that provide important services and therefore conservation measures ought to be implemented.

I.2. What do we know about the Prespa trout?

Answering the question "what do we know about *Salmo peristericus*" we emphasize that the Prespa trout is a well-recognized endemic species that gives special value to the Prespa Region. Prespa Lake and its watershed have been the main concern in many studies, followed up in this study, few of which contain data about *Salmo peristericus*. It seems evident that the question "what is there yet to be known about the Prespa trout" requires a profuse answer. Namely, there is still a lack of knowledge about Prespa trout's general biology and ecology, particularly on habitat preference, temperature range, feeding habits, phenology, fecundity, age structure and reproductive strategy.

Following the best scientific practice, before starting with project activities, desktop work was performed to consolidate data, as much as possible concerning the Prespa trout. More than 100 documents were analyzed (scientific papers, biodiversity studies, monitoring programs, national strategies for biodiversity conservation in the Prespa region, reports, etc.), and about 30 were selected in the development of the research field design. Some of them contain data that directly refer to the Prespa trout (distribution, population status), but most of them refer to other aspects of the Prespa Lake basin. However, the second mentioned studies are of great value for the project, serving as an information resource related to the trout's habitats.

We would like to emphasize once again that the following sections concerning taxonomy, population status, conservation status and trout's habitats are a summary of desktop analyses.

I.2.1. Taxonomic status

The Prespa trout was identified and described by Karaman (1938) as a subspecies (verbatim name *Salmo macedonicus peristericus*). The taxonomic status of the Prespa trout has been clearly identified at a level of species *Salmo peristericus* under the Phylogenetic Species Concept (PSC) (Kottelat, 1997; Kottelat and Freyhof, 2007). Identifying populations as *S. peristericus* based on morphological and molecular characters has been largely discussed (Crivelli et al., 1998; Koutseri et al., 2010). The only stable taxonomic characters that distinct the Prespa trout from other trout's from *Salmo trutta* complex are low gill rakers number and slender body (Karakousis et al., 1991; Delling, 2003). Mitochondrial DNA analyses have been aligning the Prespa trout to a certain Adriatic origin (Karakousis and Triantaphyllidis, 1990; Apostolidis, 1996; Snoj et al., 2009), while microsatellite analysis (Berrebi et al., 2013) confirm the distinctiveness of the species indicating presence of metapopulations along the individual rivers of Prespa Lake watershed.

1.2.2. Distribution

Karaman (1938) reports that there was evidence in the past that Lake Macro Prespa supported Prespa trout populations, but according to Crivelli, et al. (2008) today it is extremely rare to find this species in the lake. According to Kottelat & Freyhof (2007) the occasional lacustrine phenotype may also stem from stocking. Berrebi et al. (2013) in their study use some samples from the Prespa trout originating from the lake, not stating whether they originate from the Greek or Macedonian side of the lake. According to Shumka and Apostolou (2018), the Prespa trout is found very rarely in the fishermen catch in Macro Prespa. This statement is based on 3 years (2013-2015) ichthyological investigation in Prespa Lake (Ilik-Boeva et al., 2017). However, based on detailed analyses of results from this study we couldn't find specific data about the exact location where the Prespa trout was caught from the lake during the research period.

Based on the abovementioned literature data, the Prespa trout nowadays persist in small tributary systems of the lake; Leva Reka River (tributary of the Golema River), Kranska River and Brajchinska River in Macedonia, and the Agios Germanos River in Greece. The stream habitats where the Prespa trout lives are between 9 and 16 km long (Koutseri et al. 2010).

The lower parts of these four rivers do not sustain trout populations on a permanent basis due to water abstraction in summer for irrigation purposes (which causes low flow and absence of large pools, while it may render the riverbed totally dry for some weeks/months), habitat fragmentation, poaching and/angling, and pollution by sewage waste (Koutseri et al. 2010).

1.2.3. Population status and size

More details about the population size of the Prespa trout could be found in the study of Koutseri et al. (2010) as summarized results from a 10 years ichthyological investigation in Germanos River, as well as a 3-year survey (2006-2008) on Leva, Brajchinska and Kranska rivers. This study provided a trend status for the Prespa trout from the Germanos River which indicated that population size was decreasing. As a result of limited quantitative studies of the Prespa trout population from the Macedonian part in the past, the evaluation for population decrease was mainly based on information from local people (inhabitants).

According to the results from the same study, in some streams the population of trout is small and then potentially in danger of extinction such as the population from Upper Kranska River, Baltanska River and Sredna River. According to Kousteri et al. (2010) for some streams (e.g. Baltanska River, Sredna River) the low flow in summer and the absence of large pools could be the reason for low population densities. For other streams poaching and/or angling could be major factor. However, the authors point out that years of research are needed in order to draw definite conclusions, considering that trout populations fluctuate widely from one to another year. The obtained data on Aghios Germanos River show the necessity of long-term sampling before drawing any conclusion on the conservation status of the species.

Spirkovski et al. (2012), conducted research in 2011 on distribution and abundance of the Prespa trout in the same rivers of our country. During the research period (autumn/spring) trout was detected in Brajchinska River (between villages Brajchino and Ljubojno), in the part of the river after the confluence of Stanishar, in Rzhanska River and in the lower parts of Kriva Kobila River. Highest number of fish (per 100 m) were caught on the Kranska River after the Plitna River inflow, Srbina River (tributary of the Kranska River), main course of the Brajchinska River and Rzhanska River (tributary of the Brajchinska River). Low population density was recorded in the Leva River (Spirkovski et al., 2012). This study proved significant information for length classes of the Prespa trout. The smallest captured specimen's length is 31-50 mm, while the largest specimen's length is 271-290 mm. During this research, samples above 300 mm were not captured. According to Crivelli (2008) the maximum TL of *Salmo peristericus* is 35 cm.

It should be emphasized that during those researches mentioned above, species distribution and population size was not evaluated from the aspects of abiotic and biotic factors. The interaction of these biotic and abiotic factors determine the distribution of species arranged along longitudinal or altitudinal gradients in streams.

Desktop research raised (encouraged) several questions such as: What is the optimal temperature for the Prespa trout survival or optimal temperature for egg survival? What type of food do they prefer and what is the preferable habitat quality? What is the reason for their small size? What is the length and age of the first maturation? What are the effects of population isolation? These are still open and unanswered questions.

But, it is most important to emphasize the fact that population size of the Prespa trout is discussed only from the aspect of its abundance, not concerning the sexual maturity of the specimens. Namely, in ecology "population size is the number of individuals in a population", but for evaluation of conservation status according to the IUCN Red criteria, population size is "number of mature individuals".
The results within this project for the first time offer data concerning gender (sex) distribution (male and female) within the sampling sites of each river separately (see chapter 5). This parameter is extremely important for obtaining an answer of some hypotheses that refer to the reproductive strategy of the Prespa trout.

1.2.4. Conservation status and protection

As mentioned before, in 2006, according to IUCN criteria *Salmo peristericus* Karaman, 1938 was classified as endangered (EN) (Smith & Darwall, 2006). This evaluation was done mainly based on the data of population status of the Prespa trout in Agios Germanos River.

Crivelli et al. (2008), based on data from ichthyological surveys in all rivers that *Salmo peristericus* occupies, once again classified the Prespa trout as endangered (EN). Fragmentation of its habitat, limited extent and an observed continuing decline were the main criteria in assessing this status.

On the Greece National Red List (Crivelli and Nikolaou, 2008), the Prespa trout has the same endangered (EN) status.

The results from the assessment of the National conservation status are the first step in order to develop Conservation Action Plan (CAP) for the Prespa trout, planned as a result from the project activities.

Regarding the protection status at the National level, the Prespa trout as endemic and endangered species is on the lists of nationally protected or strictly protected species ("Official Gazette of the Republic of Macedonia" no. 139/2011). It is also included in Appendix III of the Bern Convention for the conservation of European Wildlife and Natural Habitats (Decision 82/72/EEC of the European Committee) and included in Annex II of the Directive 92/43/EEC, but it is referred to with another name (*Salmo macrostigma*) in the specific Annex (Freyhof and Brooks, 2011).

The following National laws and regulations offer protection of the river basins of the Prespa area and at same time offer trout protection:

- The Law on Nature Protection ("Official Gazette" 67/2004), according to which one of the four protected areas within the watershed of Prespa is the National Park Pelister (NPP);
- Law on Proclamation of part of Pelister Mountain as a National Park ("Official Gazette of the Republic of Macedonia" No. 150/07). With this law the borders of the park are extended including a portion of the upper part of the Brajchino River Valley. According to this law the waters and water habitats, including springs, streams and rivers are a natural wealth and are preserved in their natural state;
- Law on the Proclamation of Prespa Lake as a Natural Monument ("Official Gazette of the Republic of Macedonia" No. 51/11, 79/13);
- Law on the Proclamation of the site of Ezerani at Prespa Lake as a Nature Park ("Official Gazette of the Republic of Macedonia" No. 24/12);
- National Park Pelister is also a part of Emerald network (under the code *MK0000004*); IUCN (IUCN III); Green Belt (under the code *MK002*);
- NP Pelister is part of the Ohrid-Prespa Transboundary Biosphere Reserve;
- The area has already been identified together with the Ezerani Nature Park as a potential Natura 2000 site within the Natura 2000 project "Strengthening the implementation capacities of Natura 2000 - EUROPEAID / 136609 / IH / SER / MK".

1.2.5. Prespa trout habitats

Typology of rivers

Data for hydromorphological and morphological elements, as well as, data about chemical and physicochemical elements of Leva, Brajchinska and Kranska River could be found in the study "Development of Prespa Lake Watershed Management Plan; RFQ 50/2009, a technical report – part II. Identification of the major watershed management issues in Prespa Lake Watershed " (2010) and study "Prespa Lake Watershed Management Plan " (2014). According to the results from those studies Leva, Brajchinska and Kranska Rivers belong to Ecoregion 6, small rivers at the same river type (type 1), with very short and rapid flows prior to their inflow into Prespa Lake. Parts of Kranska and Brajchinska are with good hydraulic contact with their surroundings, rich riparian vegetation, clear water with very low conductivity (<100), slightly acidic, low in nutrients which are easily biodegradable, and with diverse natural flora and fauna in and around the watercourse. Some selected hydrology data and values of certain physicochemical parameters of water from Leva, Brajchinska and Kranska River are presented below (Table 1).

Table. 1. Selected hydrology data (period 1961-2009) and data for chemical and physicochemical elements for Leva, Kranska and Brajchinska River. Information from the study "Development of Prespa Lake Watershed Management Plan; Phase II - Identification of the major watershed management issues in Prespa Lake Watershed" (2010).

Parameters	Leva Reka	Kranska Reka River 1 (Kranska Reka upstream from Arvati village)	Kranska Reka River 2 (downstream of Arvati village)	Brajchinska Reka River 1 (upper section that is located into the territory of NP Pelister)	Brajchinska Reka River 2 (out of the territory of NP Pelister)
Altitude	871 m	1040 m	908 m	1043 m	866 m
Length of the water body	9,363 km	6,642 km	4,709 km	8,758 km	9,546 km
Area F (km²)	30.80	24.72	38.05	37.80	73.96
Flow (m³/s) Qmean	0.436	0.468	0.570	0.813	1.17
Runoff Module (l/s/km2)	14.1	19	15	21.5	15
River depth and width variation	H avg. =0.20 m-1.00 m, B avg. =4.00-8.00 m	H avg. =0.20 m-0.50 m, B avg. =1.00-4.00 m	H avg. =0.20 m-0.50 m, B avg. =4.00-8.00 m	H avg. =0.20 m-1.00 m, B avg. =3.00-8.00 m	H avg. =0.20 m-1.00 m, B avg. =3.00-8.00 m
Mean water width	4 m	2 m	5 m	3 m	6 m
Mean water depth	0.3 m	0.3 m	0.3 m	0.2 m	0.2 m
Structure and substrate of the river bed	Bedrock	Natural, embedded	Natural, coarse	Natural, embedded	Natural, coarse

Mean substratum composition	Large stones, pebbles, gravel	/	/	Large stones	/
Mean water slope	54.6 ‰	135.0 ‰	38.5 ‰	107.1 ‰	27.6 ‰
Mean air temperature	6.93	4.81	7.45	4.48	
Thermal conditions	Normal	Normal	Normal	Normal	Normal
Oxygenation conditions	High	Natural	Natural	Natural	High
Salinity	Low	Low	Natural	Low	Low
Acidification status	Slightly alkaline	Neutral to slightly acid	Variable alkaline	Slightly alkaline	Slightly alkaline
Nutrient conditions	Natural	Low	Variable slightly elevated	Slightly elevated	Slightly elevated

During the preparation of the Management Plan of the Prespa Lake basin, which was conducted during the period 2010-2014, a complex initial control monitoring of water quality was implemented in a way to identify ecological status of delineated water bodies. Based on hydromorphological and physicochemical quality elements, including biological (macroinvertebrates and algae) components, Leva Reka is classified as a river with “Good” ecological status; Upper Brajchinska (1) and Kranska Reka (1) with “High” status, Brajchinska 2 with “Poor”, while Kranska Reka (2) with “Moderate” ecological status.

All these data are based on values that are measured only at one or two measuring points along the mean course of Leva, Brajchinska and Kriva rivers. It is obvious that for each small tributary that gravitates to these three main watercourses no data concerning these parameters existed. Variation among streams will result in variation in the trout population structure and health.

During the development of the Assessment Study and present field project activities the characteristics of the riverbed and values of certain physicochemical parameters of water from all sampling sites (27) were recorded.

Riparian forest study

According to the data present in the study “Annex for the Management Plan for Nature Monument Prespa Lake” (2019), Leva, Brajchinska and Kranska River runs along some of the habitats included in the Directive (Annex I):

- 9270 Hellenic beech forests with Abies borisii-regis - Beech forests (*Fagus sylvatica*), characterized by the presence of *Abies borisiiregis, Doronicum caucasicum, Galium laconicum, Lathyrus venetus, hellenicum*. In National Park Pelister, the habitat is associated with the spread of the community Fago-Abietetum meridionale. There are two well-developed communities in the area of the village Brajchino. The larger one is located at the Kalojzana site, on the left side of the Brajchinska River, and the smaller one is below the Baltan peak, on the right side of the Rzhanska River. The dominant species is *Abies borisii regis*, while the beech occurs as an accompanying species. Characteristic and differential species are *Galium rotundifolium, Pyrola minor, Orthilia secunda, Luzula luzulina, Veronica officinalis, Geranium robertianum* and others;

- 9280 Quercus frainetto woods Forest with *Fagus sylvatica/Fagus moesiaca*, occur in the transition zone between the supra-Mediterranean and mountain heights, which are characterized by the presence of a number of species from the Quercion frainetto union. The vegetation is represented by the communities *Quercetum frainetto-cerris, Carpino orientalis-Quercetum frainetto* and *Carici cuspidatae - Quercetum frainetto* Rizovski (1974). The dominant woody species are *Quercus frainetto* and *Quercus cerris*, and others include *Acer tataricum, Fraxinus ornus, Acer obtusatum*. Scrubs common species are *Rubus canescens, Cytisus nigricans, Colutea arborescens*, and on the grass level are *Helleborus odorus, Lathyrus laxiflorus, Trifolium pignantii, Festuca heterophylla, Silene italica, Clinopodula trachelium, Campopodium vulgare* and others. This habitat is found at the village of Brajchino;
- 91E0 * Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Albion incanae, Salicion albae). Tree species composition include flood tolerant species like *Alnus glutinosa, Salix alba, Salix fragilis, Populus nigra, Populus alba* and bush forming *Salix* spp. Their forests are located at lower areas and pools typical wetland vegetation, sometimes also floating hydrophyte communities.

This riparian forest ensures a good habitat quality as appropriate water temperature (shading), stability of bank, limited erosion or reduction of evaporation during the summer period. As rivers where trout lives are identified as small rivers, the availability of hiding places, based on wood debris is of great importance for population structure and health.

Based on QBR index (Kazoglou et al., 2010), total riparian cover values were found significantly higher in the upper parts of Brajchinska and Kranska River compared to upper parts of Agios Germanos River and Leva River, suggesting that the former provide better conditions for the population of the Prespa trout (more shade to the channel and better regulation of water temperatures). This study confirmed that the lower parts of the four rivers, below the altitude of 900 m are quite degraded. Beside the already known problems, such as reduced flow during summer, due to extraction for irrigation, it appears that there has been pronounced (evident) human intervention in these riparian habitats, leading to their fragmentation.

Stream habitat types

River system in the National Park Pelister, according to EUNIS habitat classification are identified as C2 rivers - epirhithral and metarhithral streams and temporary running waters. In correlation with the classification of HD 92/43/EEC they correspond to habitat type 3260: watercourses with vegetation of Ranunculion fluitantis and Callitricho - Batrachion. According to study "Annex for the Management Plan for Nature Monument Prespa Lake (2019)", the habitat covers the grass vegetation along the watercourses, from lowland to mountainous altitude. It refers to slow-flowing or medium-flowing rivers or streams. This includes a large number of floating or submerged (submersible) aquatic plant communities from the Ranunculion fluitantis and Callitricho-Batrachion vegetation. Plant species are characterized by *Ranunculus trichophyllus, Ranunculus fluitans, Ranunculus peltatus, Ranunculus aquatilis, Myriophyllum spp., Callitriche spp., Berula erecta, Mentha aquatica, Potamogeton spp., Fontinalis antipyretica* and others. Vegetation that is more related to mesotrophic and eutrophic streams and rivers are found in the middle and especially in the lower watercourses that flow into Prespa Lake.

Food resources for the Prespa trout

To fish, habitat represents a place that contains the biotic and abiotic components necessary for reproduction, growth and survival. The population structure depends on availability of hiding places, number and size of pools, presence/absence of wood debris, including carrying capacity of streams. Usually, criteria used to characterize trout's habitat are often based on observed correlations between physical habitat characteristics and trout abundances. Knowing the fact that habitat also contains the food resources necessary to support the growth and survival of salmonids it comes as a surprise that invertebrate food abundances are rarely evaluated as part of trout habitat monitoring programs (Fausch et al. 1988).

According to Koutseri et al., (2010) one of the threats to the Prespa trout is low carrying capacity of streams - food availability. From the abovementioned reasons, variation in invertebrate abundances across spatial and temporal scales are of great importance for evaluation of spatial and temporal variation of the Prespa trout. At this moment, the only data concerning the distribution of macroinvertebrates in Leva, Kranska and Brajchinska River could be found in the study "Development of Prespa Lake Watershed Management plan; RFQ 50/2009, a technical report – part II. Identification of the major watershed management issues in Prespa Lake Watershed" (2010) and Master Thesis of Shoreva (2015).

According to these studies, bottom fauna from the Leva River shows presence of 15 taxa, mostly belonging to insect groups. Among them aquatic insects, (Trichoptera, Diptera and Plecoptera) present dominant groups. Such a type of benthic community is characteristic for relatively healthy water courses. The average annual density of upstream macroinvertebrates is 169.83 $ind \cdot m^{-2}$. The fauna recorded in samples from Kranska 1 (upper part) was composed of 25 taxa. From the quantitative point of view, aquatic insects (Ephemeroptera, Trichoptera and Diptera: Chironomidae) were the most abundant groups in macrozoobenthos. The indicator of high water quality was *Baetis alpinus*, which was a dominant species in benthic fauna. The average annual density of macroinvertebrates upstream of Kranska River was 483.61 $ind \cdot m^{-2}$.

A total of 21 taxa were identified in bottom samples from Kranska 2. Diptera, followed by Oligochaeta, Trichoptera and Ephemeroptera were the most diverse groups. Moderate EPT taxa richness was detected. Presence of stoneflies species, which indicate increasing pollution were recorded at this sampling points. The most significant groups in terms of quantity were the non-insect group Oligochaeta and aquatic insects Diptera and Ephemeroptera. Quantitatively α-mesosaprobic indicators *Lumbriculus variegates* and *Cricotopus bicinctus* dominated, as well as, polysaprobic indicator *Tubifex tubifex*. The average annual density of macroinvertebrates downstream of Kranska River during the investigation period was 732.99 ind·m^{-2}.

Bottom fauna analyses from Brajchinska 1 (upper part) showed diverse benthic communities presented with dominated species from Ephemeroptera, followed by Trichoptera and Plecoptera, mostly characteristic for mountain, clean and fast flowing streams. The occurrence of sensitive to pollution flatworms *Crenobia alpina* and crayfish *Austropotamobius torrentium* clearly indicates a healthy river ecosystem. The average annual density of macroinvertebrates upstream of Brajchinska River was 400.39 ind·m^{-2}.

Bottom fauna analyses from Brajchinska 2 (lower part) showed low species richness. Existence of 8 taxa was evident. The greatest species diversity was recorded in Trichoptera. Low EPT taxa richness indicated an increasing level of ecosystem stress. The dominant species was *Cricotopus bicinctus*, which accounted for 59.68% of the community. The average annual density of macroinvertebrates downstream of Brajchinska River during the investigation period was 403.98 ind·m^{-2}.

The evaluation of distribution and density of the trout population in the watercourses on the Macedonian side of Lake Prespa were assessed according to the tasks set within the project. Additional activities undertaken by our team, such as determining the percentage of reproductively mature individuals in the population, age structure of the population and the fitness factor of the captured individuals, were done in order to obtain more knowledge about the biology of the Prespa trout (biotic factors). This information can provide an answer about the *potential* threats that could drift to further reduction of population size of this endemic and endangered fish species, at the same time providing a solution for further human activities in order to revitalize Prespa trout population. Are food availability or reproductive failure and low recruitment, as Koutseri et al. (2010) assumes, the reason for decline of population during the past; or maybe temporal and spatial variability of their environment causes a reproductive strategy that will lead to weakness of trout population are issues and topics that will be discussed in the frame of Final Project Results section of this Report.

I.3. Objectives of the Project (Assessment Study)

The Main objective of the project is to improve the conservation status of the Prespa trout population in North Macedonia through protection, monitoring and conservation actions in the National Park Pelister and the Macedonian part of the Prespa region.

The project has the following specific tasks and objectives:

- Assessment of the conservation status of the Prespa trout;
- Support of the development of a Conservation Action Plan (CAP) for the Prespa trout;
- Design of a long-term monitoring program for the Prespa trout;
- Design and implementation of a training program for the staff of the PIPNP and other stakeholders.

The key activities of the project (Assessment study) includes:

- assessment of the population distribution,
- assessment of population size and structure of the Prespa trout in North Macedonia,
- assessment of the risks and threats for the Prespa trout population,
- development of a conservation action plan for the species, including monitoring, and capacity building of the personnel of NP Pelister and other Stakeholders to implement the CAP.

II. FIELD PROJECT ACTIVITIES

The field activities within the project were performed in the period from 05.11 to 09.12.2020. At the beginning of the project, the sampling was planned to be performed at approximately 20 sampling points (SP), but the number of sampling points was expanded and the sampling was performed at a total of 27 sampling points: sixteen (16) on Brajchinska River, seven (7) on Kranska River and four (4) on Leva River. At each sampling point upon arrival, GPS coordinates were determined at the beginning of the sampling. The selection of the sampling points was based on the survey by Crivelli et al. (2008) conducted under field guidance by the participating field staff in the same survey.

Table 2. Assessment and monitoring sites (sampling points, description and relevant information: time, location, altitude, GPS coordinates, etc.)

Date of inv.	River	Description of Sampling Point	GPS coordinates	Altitude	Previous marking of SP	SP mark	Length of SP	Average width of SP	Fished area m^2
	Brajchinska River and tributaries								
04.12.2020	Rzhanska River	highest sampling point	40.8951556, 21.2169734	1571	T1	***B1***	180	1.2	216
04.12.2020	Brajchinska River	highest, Rupa	40.911569, 21.2366257	1518	T3	***B2***	130	1.3	169
04.12.2020	Rzhanska River	before and after the concrete bridge	40.9056922, 21.2046308	1381	T2	***B3***	144	1.5	216
05.12.2020	Drmishar	Before junction with Marushica	40.9287137, 21.1870883	1300	T6	***B4***	130	2.5	325
05.12.2020	Marushica Brajchinska	500 meters before junction with Drmishar	40.9260916, 21.1835457	1286	T7	***B5***	120	2	240
05.12.2020	Brajchinska River	Golem Dol Between SHPP and water intake	40.919247, 21.2062476	1228	T5	***B6***	120	3	360
06.11.2020	Baltanska River	Tributary of Brajchinska River	40.8981639, 21.1754088	1223	T10	***B7***	142	2	284
04.12.2020	Brajchinska River	after the water intake for SHPP PCC	40.9181438, 21.1943514	1202	T4	***B8***	100	3	300
05.12.2020	Stanishar	before (above) the water intake for SHPP	40.9199579, 21.1824587	1194	T25	***B9***	60	1	60
05.12.2020	Stanishar	after (under) the water intake for SHPP	40.9180173, 21.181943	1170	T8	***B10***	80	1	80
06.11.2020	Brajchinska River	Saint Ilija	40.9120561, 21.1789965	1111	T9	***B11***	160	3	480
06.11.2020	Brajchinska River	300 m under PCC SHPP, Above Brajchino village	40.909132, 21.1668358,	1040	T22	***B12***	142	2.5	355
09.11.2020	Brajchinska River	Between Brajchino and Ljubojno village	40.8992765, 21.150944	945	T11	***B13***	110	3	330
06.12.2020	Brajchinska River	Into the Brajchino village, under	40.8958916, 21.1371943	908	T26	***B14***	130	3	390

		the wooden bridge							
06.12.2020	Brajchinska River	Above the cascade	40.8968549, 21.1215941	871	T27	***B15***	160	3	480
06.12.2020	Brajchinska River	Below the cascade	40.8969053, 21.120313	859	T28	***B16***	180	3	540
							2088		**4825**
	Kranska River and tributaries								
07.12.2020	Srbina River	Before merging with Marushica Kranska	40.9640508, 21.1592977	1420	T13	***K1***	184	1	184
07.12.2020	Marushica Kranska	Before merging with Srbina River	40.9630681, 21.1616222	1410	T14	***K2***	160	1.5	240
07.11.2020	Kranska River	First SP after the forming	40.961847, 21.1555918	1341	T15	***K3***	140	1.5	210
08.11.2020	Kranska River	Before water intake for SHPP "A"	40.9576009, 21.146132,	1243	T16	***K4***	120	3	360
08.11.2020	Rechishte	Before inflow in Kranska River	40.9571672, 21.1420453	1225	T17	***K5***	130	1	130
08.11.2020	Kranska River	Between water intake and SHPP	40.9547416, 21.1355744	1171	T18	***K6***	159	3	477
08.11.2020	Kranska River	in to the Arvati village	40.9461383, 21.1198977	1034	T23	***K7***	120	3	360
							1013		**1961**
	Leva River and tributary								
09.11.2020	Biglichka River	200 m before (above) inflow in Leva River	41.1666794, 21.0368637	1058	T20	***L1***	200	0.8	160
09.11.2020	Leva River	highest sampling point	41.1736788, 21.0388894	1054	T12	***L2***	150	1.2	180
09.11.2020	Leva River	Middle sampling point	41.167068, 21.0368365	1035	T19	***L3***	120	1.2	144
09.11.2020	Leva River	before Leva Reka village	41.1661969, 21.0294434	1014	T21	***L4***	100	3	300
							570		**784**

Figure 1. Sampling points at Brajchinska River and tributaries

Figure 2. Sampling points at Kranska River and tributaries

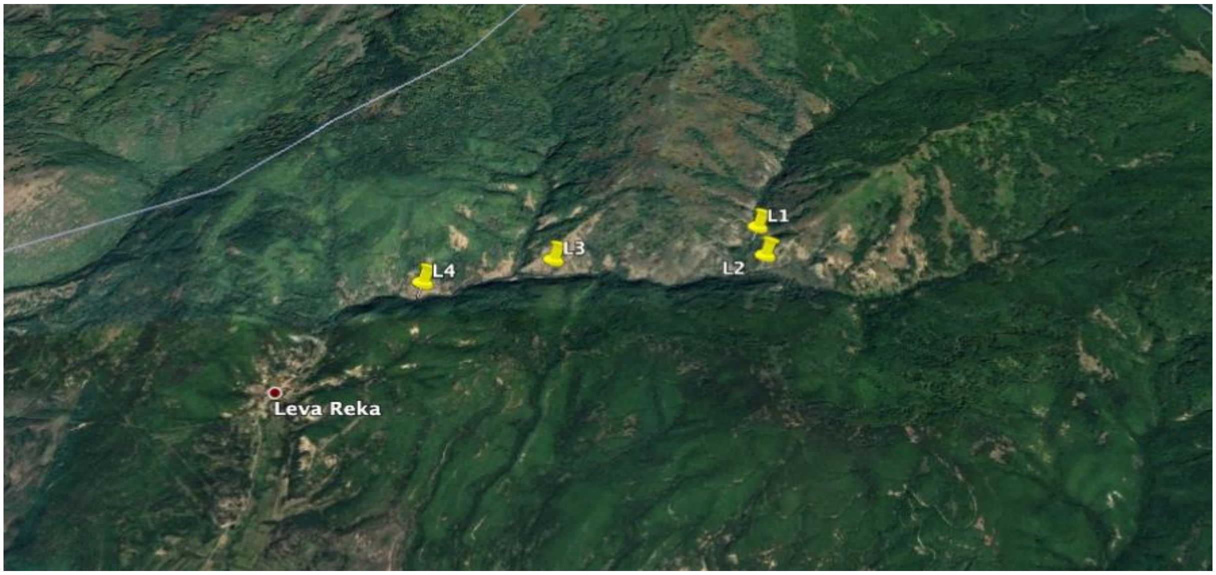

Figure 3. Sampling points at Leva River and tributaries

Electrofishing by electric generators Samus 1000 and Samus 725G was conducted according to a defined standard methodology of electrofishing (CEN Directive, 2003). The caught fish were kept alive and the basic measures were taken in the field. The mass of each fish was measured individually (to nearest 0.1g), the lengths were measured (total length, fork length, body length, to the nearest mm), the sex was determined (according to the fish sex dimorphism) and the scales were taken from each fish for determination of age structure. After the performance of all the field measurements, the fish were returned alive to the water on the same place from where they were caught.

Figure 4-8. Electrofishing

Figure 9,10. Electrofishing – team work of experts and project team

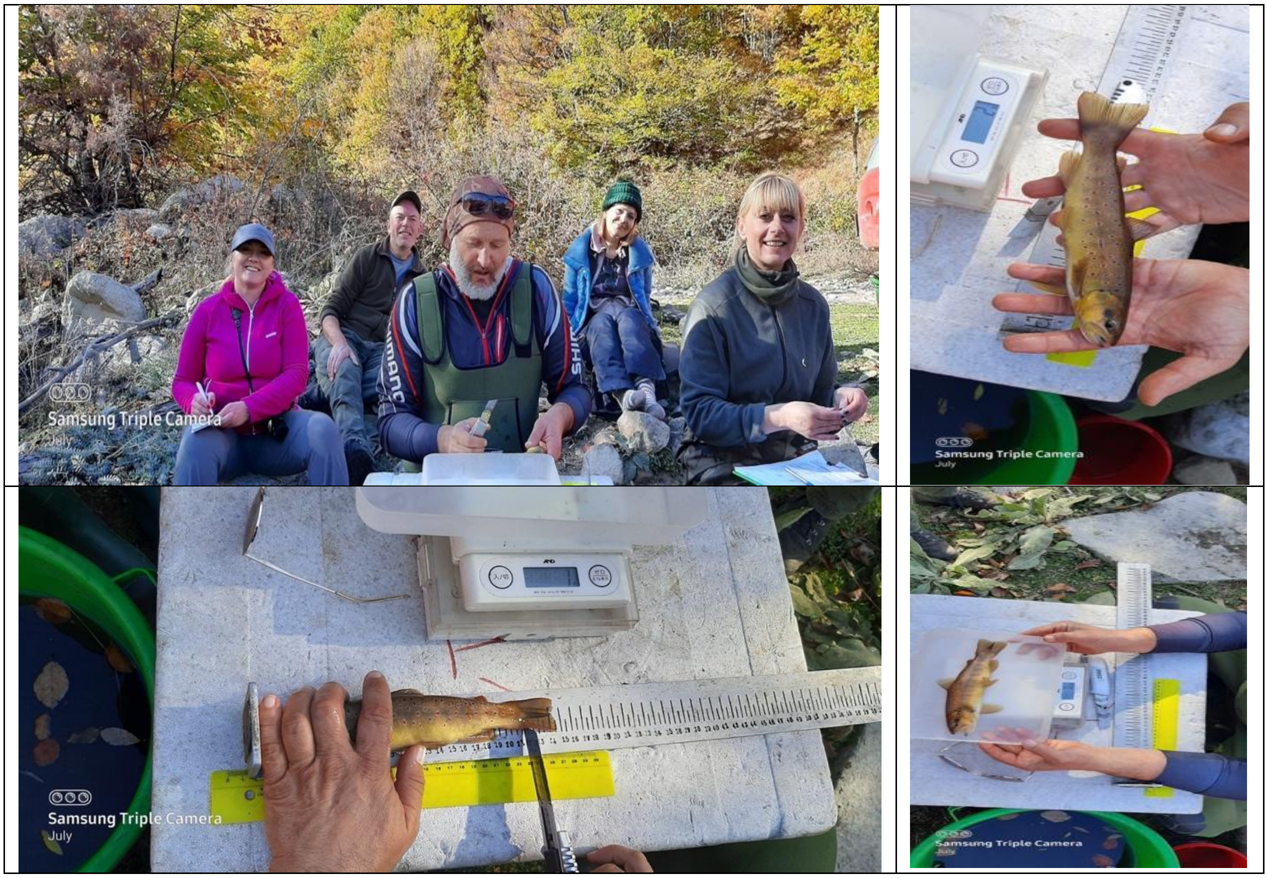

Figure 11-14. Taking basic measures in the field

The basic physicochemical parameters were taken at each sampling point, as follows: dissolved oxygen concentration, water saturation with oxygen, water pH, water temperature, air temperature, conductivity and salinity.

Sampling Protocols were filled in on each sampling point in which all relevant and necessary data were noted (Protocol attachment) such as: name and marking of sampling point, date, hydrographic basin, course, location description, reference site, GPS coordinates, time at the beginning of the sampling, time of ending of the sampling, altitude, sampling strategy, fished length, fished area, flow regime, site dimensions, width, depth, substrate, shadiness, weather, physicochemical measurements (conductivity, dissolved oxygen, pH, salinity, temperature of air, temperature of water), important pressures, bottom vegetation, habitat type, fish habitat details, other notes / interviews with locals, etc.

III. RESULTS OF THE FIELD RESEARCH

3.1.Spatial distribution, population size, abundance, and density of *S. peristericus*

Population size, density, spatial distribution and dispersal of fish provide basic information on population ecology, population dynamics, population genetics and evolutionary biology. Dispersal is important for the colonization of new habitats, it affects the genetic structure of a population (immigration and emigration) and influences demographic processes within the population. Limited dispersal favors, for example, local adaption, can lead to restricted gene flow between the population, and in turn may enhance reproductive isolation. The speciation processes could be consequential (e.g. Mayr, 1963; Futuyma, 1990).

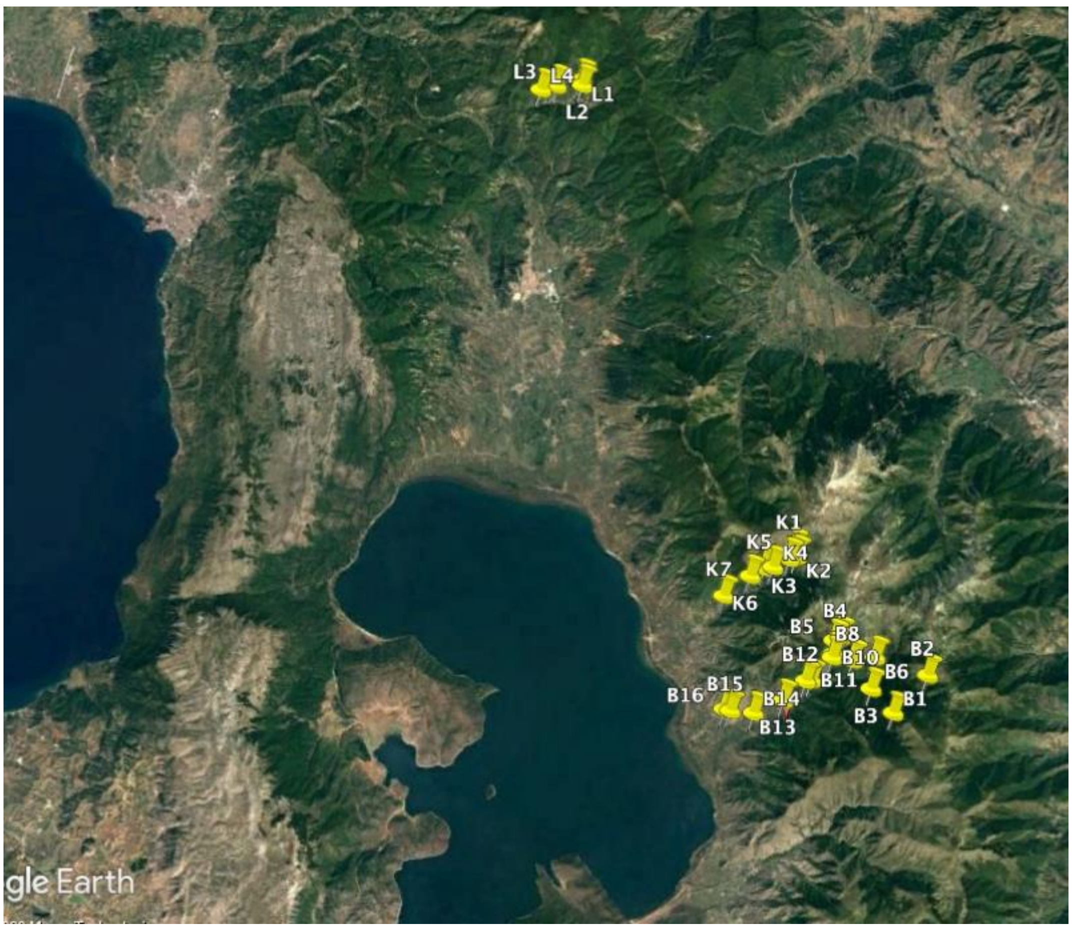

Figure 15. Spatial distribution of *Salmo peristericus* on the territory of Republic of North Macedonia.

The presence of *Salmo peristericus* is ascertained at almost all defined sampling points, except at the sampling point in the village Arvati (SP-K7) on the Kranska River and at the SP-M1 on the Biglichka River.

Table 3. Fish distribution, density and abundance of *S. peristericus* at Brajchinska River and tributaries

Date	Sampling Point (SP)	SP No	No of caught fish	Length of SP	Average width of SP	Wadded area m^2	fish/ $100m^2$	fish/ ha
04.12.2020	Rzhanska River	*B1*	4	180	1.2	216	2	**185**
04.12.2020	Brajchinska River	*B2*	5	130	1.3	169	3	**296**
04.12.2020	Rzhanska River	*B3*	17	144	1.5	216	8	**787**
05.12.2020	Drmishar River	*B4*	21	130	2.5	325	6	**646**
05.12.2020	Marushica Brajchinska	*B5*	6	120	2	240	3	**250**
05.12.2020	Brajchinska River	*B6*	21	120	3	360	6	**583**
06.11.2020	Baltanska River	*B7*	7	142	2	284	2	**246**
04.12.2020	Brajchinska River	*B8*	7	100	3	300	2	**233**
05.12.2020	Stanishar	*B9*	1	60	1	60	2	**167**
05.12.2020	Stanishar	*B10*	5	80	1	80	6	**625**
06.11.2020	Brajchinska River	*B11*	6	160	3	480	1	**125**
06.11.2020	Brajchinska River	*B12*	12	142	2.5	355	3	**338**
09.11.2020	Brajchinska River	*B13*	48	110	3	330	15	**1455**
06.12.2020	Brajchinska River	*B14*	16	130	3	390	4	**410**
06.12.2020	Brajchinska River	*B15*	19	160	3	480	4	**396**
06.12.2020	Brajchinska River	*B16*	32	180	3	540	6	**593**
	Total		**227**	**2088**	**36**	**4825**	**5**	**458**

Electrofishing on Brajcinska River and its tributaries was organized on a total of 16 sampling points and 227 specimens (units) of *Salmo pelistericus* were registered, 177 of them have been measured and processed. During the research, on Brajcinska River, 2088 meters of the river, with a total area of 4825 m^2 was wadded. The relative density of the population is determined on each profile and the total relative density of the population of *Salmo peristericus* over the whole course is calculated.

The calculated relative density of the population of *Salmo peristericus* in Brajcinska River is 458 fish/ha. The lowest relative density was found on the profile B11 (125 fish/ha), and the highest on the profile B13 (1455 fish/ha). On three profiles (B1, B9 and B11) the population density is below 200. However, observed densities lower than 200 fish/ha are quite low, and viability of those populations remains an issue.

Table 4. Fish distribution, density and abundance of *Salmo peristericus* at Kranska River and tributaries

Date	Sampling Point (SP)	SP No	No of caught fish	Length of SP	Average width of SP	Wadded area m^2	fish/100 m^2	fish/ha
07.12.2020	Srbina River	*K1*	13	184	1	184	7	707
07.12.2020	Marushica Kranska	*K2*	43	160	1.5	240	18	1792
07.11.2020	Kranska River	*K3*	24	140	1.5	210	11	1143
08.11.2020	Kranska River	*K4*	44	120	3	360	12	1222
08.11.2020	Recishte	*K5*	20	130	1	130	15	1538
08.11.2020	Kranska River	*K6*	34	159	3	477	7	713
08.11.2020	Kranska River	*K7*	0	120	3	360	0	0
	Total		**178**	**1013**	**14**	**1961**	**10**	**1016**

Electrofishing on Kranska River and its tributaries, was done at 7 sample points or (assessment and monitoring sites or assessment sites), a total of 178 fish were recorded, 144 were caught and processed (measured or analyzed). During the investigations of Kranska River, a total length of 1013 meters of the riverbed was wadded, with a total area of 1961m^2.

The relative population density of each sampling site was determined and the total relative density of the population of *Salmo peristericus* for the whole water course was calculated. The calculated relative density of the trout population in Kranska River is 1016 fish/ha. The lowest relative density was found on the profile K1 (707 fish/ha), and the highest on the profile K2 (1792 fish/ha). Not a single sample (fish) of *Salmo peristericus* was caught on profile K7 (in the village of Arvati).

A total of 8 fish were caught on the Leva River, on four profiles. The total relative density of the trout population in the Leva River is extremely small and it is 101 fish/ha. The densities of all examined profiles are below 200 fish/ha.

Summarizing the results of the field research, it can be concluded that the most numerous population and with highest density is the population of the Prespa trout in Kranska River. The lowest and generally below the minimum survival limits is the Prespa trout population in the Leva River.

Table 5. Fish distribution, density and abundance of *Salmo peristericus* at Leva River and tributaries

Date	Sampling Point (SP)	SP No	No of caught fish	Length of SP	Average width of SP	Wadded area m^2	fish/100 m^2	fish/ha
09.11.2020	Biglichka River	*L1*	0	200	0.8	160	0	0
09.11.2020	Leva River	*L2*	3	150	1.2	180	2	167
09.11.2020	Leva River	*L3*	2	120	1.2	144	1	139
09.11.2020	Leva River	*L4*	3	100	3	300	1	100
	Total		**8**	**570**	**6.2**	**784**	**1**	**101**

3.2. Population Size-structure – (Length classes distribution)

3.2.1. Length classes

Population size-structure refers to the density of fish within different size classes of a population. For the purpose of estimating production, breaking down a population into size classes is essential for applying methods used in estimating growth and the loss of fish over time due to mortality, as well as, providing a convenient way for estimating biomass. The use of length classes is both effective and convenient, however, length can be measured very precisely. The latter approach allows the rapid sorting of individuals into length classes that are suitable for most methods used to estimate production.

The length of the trout ranges from 4.1 cm to 28 cm. All caught trout are divided into 24 length classes (Figure 16). The largest distribution is in the length class 16.1 - 17.0 cm (11.1% of the caught fish). Total of 66.2% of the trout population in the river belongs to length classes from "11.1 – 12" to "19.1 – 20" cm.

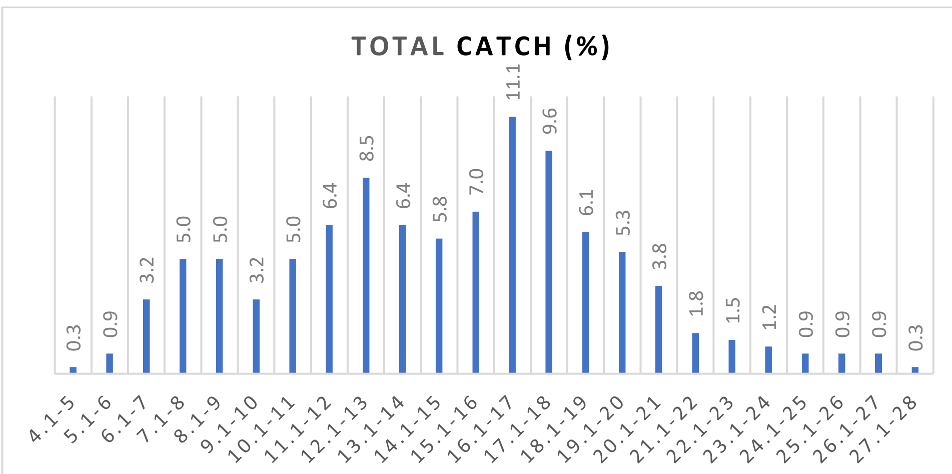

Figure 16. Distribution at length classes of total caught fish (%).

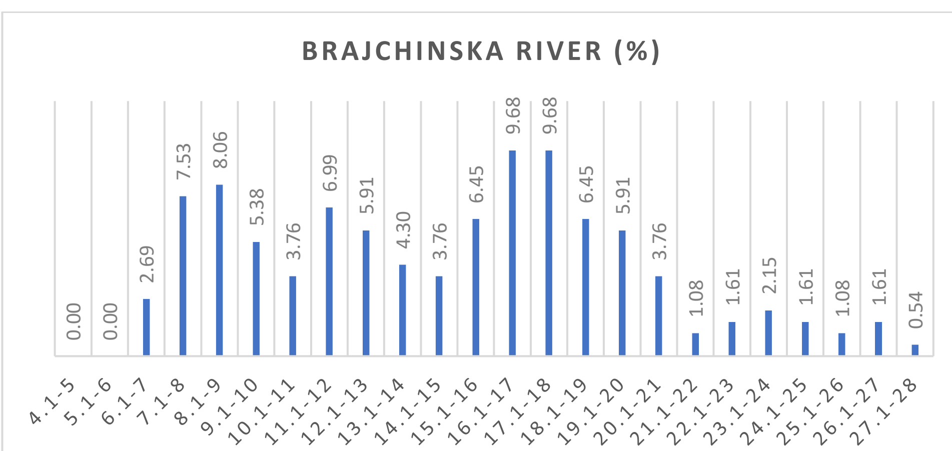

Figure 17. Population size (length) structure of *Salmo peristericus* from Brajchinska River (%).

The population structure of *Salmo peristericus* in Brajchinska River does not differ much from the defined structure of the entire population. The highest number of caught fish are with lengths between 16 and 18 centimeters (approximately 20% of the population. Relatively large presence in the catch (with over 5% in the catch) are the fish in the length classes from 7.1 to 9 cm (approximately 16% of the population).

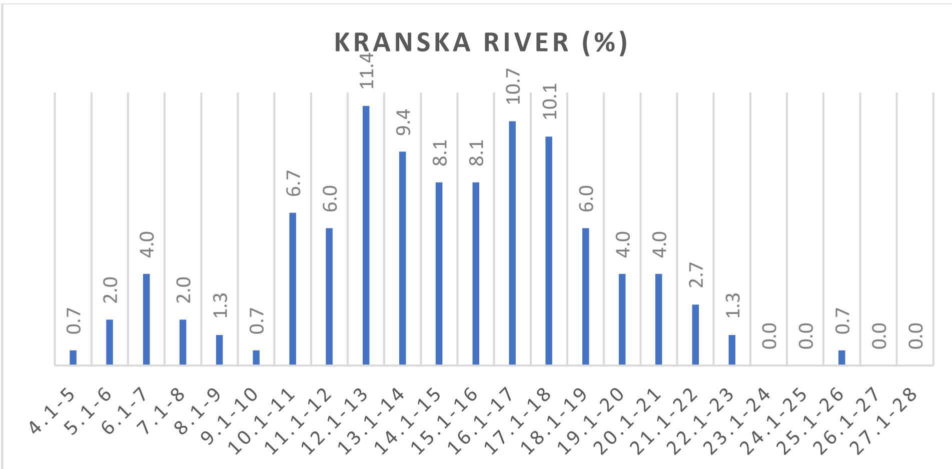

Figure 18. Population size (length) structure of *Salmo peristericus* from Kranska River (%).

In Kranska River, the largest part of the trout population is between 10.1 and 18 cm in size, and the fish from the length group 12.1-13 cm are the most represented.

In the Leva River, as already shown, a small number of fish were caught (6 in total) and they were: two males in length classes 12.1-13 and 14.1-15 and four females all in length class 16.1-17 cm.

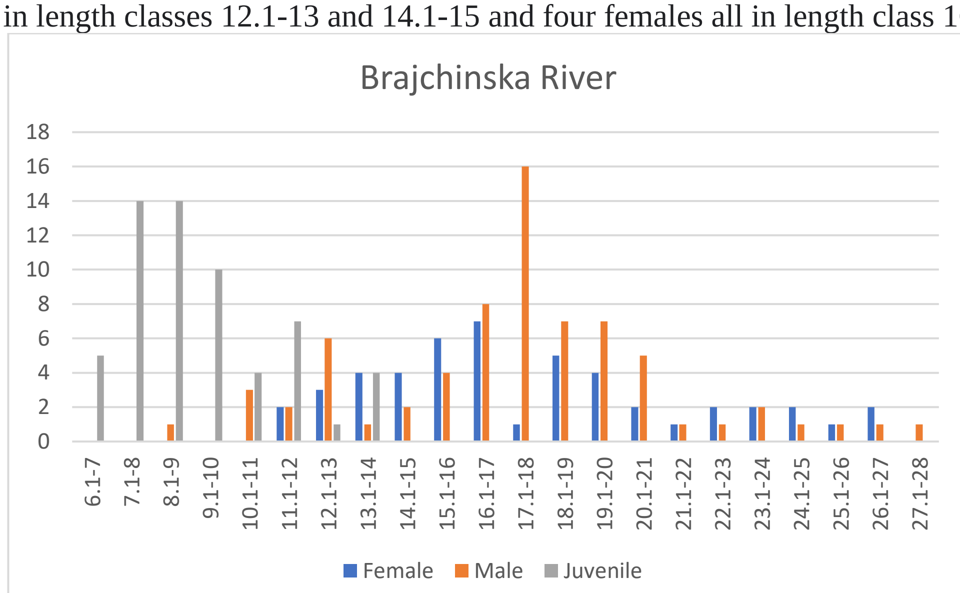

Figure 19. Population structure (length/sex) of caught *Salmo peristericus* from Brajchinska River (Number of caught fish in length classes).

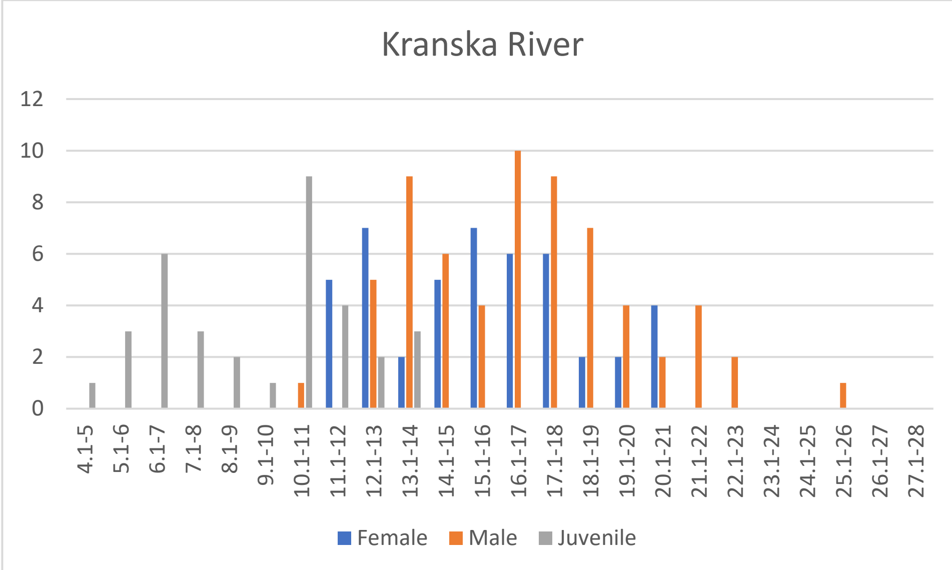

Figure 20. Population structure (length/sex) of *Salmo peristericus* from Kranska River, divided by sex and length classes. Number of caught fish in length classes.

From the Figure 20 above it can be seen that a large number of length classes are dominated by males, especially in those classes that are most numerous, starting from the length class 16.1-17 until the class 19.1 - 21 cm.

It can be concluded that in the Kranska River mostly length classes are dominated by male specimens. Their number in the length classes increases to the length class 16.1 - 17 cm, and then it decreases. The largest caught fish in the Kranska River is a male, with a length of over 25 cm.

3.3. Population Sex structure and length size maturity

In Brajchinska River, the first sexually mature males are registered in the length class 8.1 - 9 cm, which is an extremely short length for the occurrence of sexual maturity. The first female sexually mature fish appeared in the length class 11.1-12 cm. Juvenile individuals are present up to the length class 13.1 - 14 cm. All caught fish individuals from the trout population in Brajchinska River over 14 cm long are sexually mature and capable of reproduction.

Table 6. Population structure (length/sex) of caught *Salmo peristericus* from Brajchinska River, divided by sex and length classes.

Length class	Female	Male	Juvenile	Total
6.1-7	0	0	5	5
7.1-8	0	0	14	14
8.1-9	0	1	14	15
9.1-10	0	0	10	10
10.1-11	0	3	4	7
11.1-12	2	2	7	11
12.1-13	3	6	1	10

13.1-14	4	1	4	9
14.1-15	4	2	0	6
15.1-16	6	4	0	10
16.1-17	7	8	0	15
17.1-18	1	16	0	17
18.1-19	5	7	0	12
19.1-20	4	7	0	11
20.1-21	2	5	0	7
21.1-22	1	1	0	2
22.1-23	2	1	0	3
23.1-24	2	2	0	4
24.1-25	2	1	0	3
25.1-26	1	1	0	2
26.1-27	2	1	0	3
27.1-28	0	1	0	1
Total	**48**	**70**	**59**	**177**

Figure. 21-23. Male and female of *Salmo peristericus* at the moment of spawning, sperm and hatched eggs.

In Kranska River, the first sexually mature male trout appears in the longitudinal class 10.1 - 11 cm, and the first sexually mature female appears in the length class 11.1 - 12 cm. Juvenile fish in Kranska River trout population are found up to the length class 13.1 - 14 cm. All of the trout from Kranska River with a length over 14 cm are sexually mature and capable for reproduction, matching the results from Brajchinska River.

Table 7. Population structure (length/sex) of *Salmo peristericus* from Kranska River, divided by sex and length classes.

Kranska River				
Length class	**Female**	**Male**	**Juvenile**	**Total**
4.1-5	0	0	1	1
5.1-6	0	0	3	3
6.1-7	0	0	6	6
7.1-8	0	0	3	3
8.1-9	0	0	2	2
9.1-10	0	0	1	1
10.1-11	0	1	9	10
11.1-12	5	0	4	9
12.1-13	7	5	2	14
13.1-14	2	9	3	14
14.1-15	5	6	0	11
15.1-16	7	4	0	11
16.1-17	6	10	0	16
17.1-18	6	9	0	15
18.1-19	2	7	0	9
19.1-20	2	4	0	6
20.1-21	4	2	0	6
21.1-22	0	4	0	4
22.1-23	0	2	0	2
23.1-24	0	0	0	0
24.1-25	0	0	0	0
25.1-26	0	1	0	1
26.1-27	0	0	0	0
27.1-28	0	0	0	0
Total	**46**	**64**	**34**	**144**

Trout individuals caught in the Leva River were sexually mature and ready for spawning. The smallest female had a total length of 12.6 cm. All males were in the length class 16.1 - 17 cm.

Table 8. Population structure (length/sex) of *Salmo peristericus* from Leva River.

Leva River				
Length class	**Female**	**Male**	**Juvenile**	**Total**
12.1-13	1	0	0	1
13.1-14	0	0	0	0
14.1-15	1	0	0	1
15.1-16	0	0	0	0
16.1-17	0	4	0	4
Total	**2**	**4**	**0**	**6**

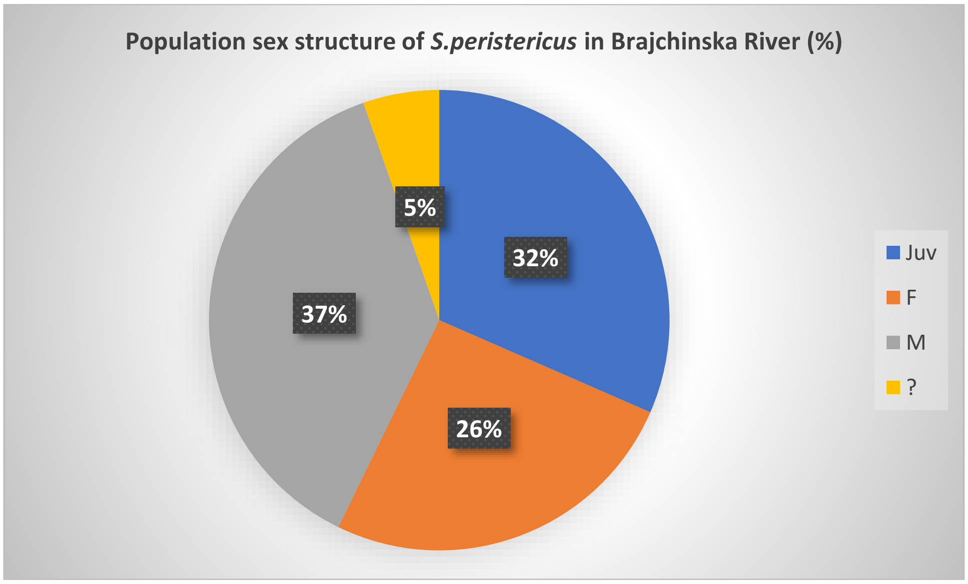

Figure 24. Sex structure of *Salmo peristericus* population in Brajchinska River.

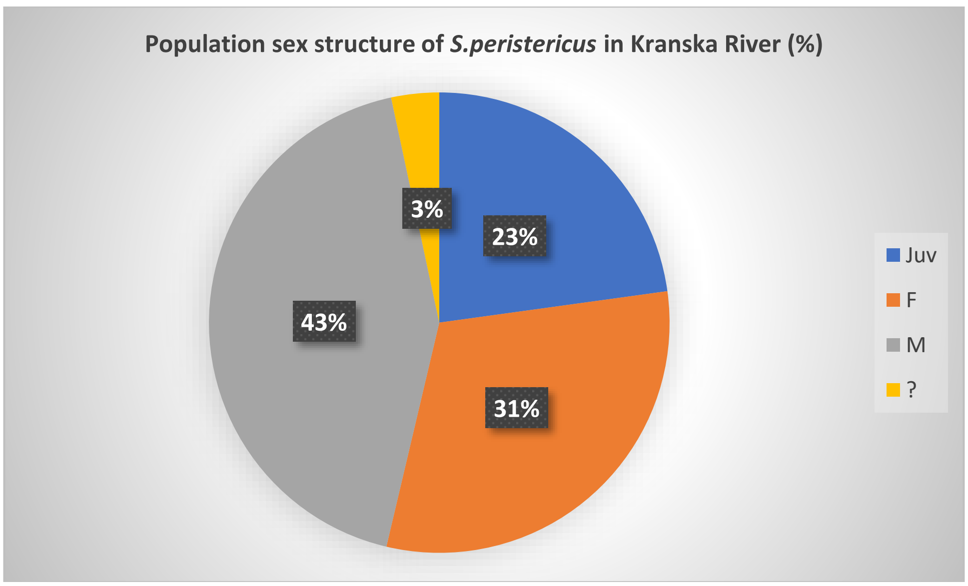

Figure 25. Sex structure of *Salmo peristericus* population in Kranska River.

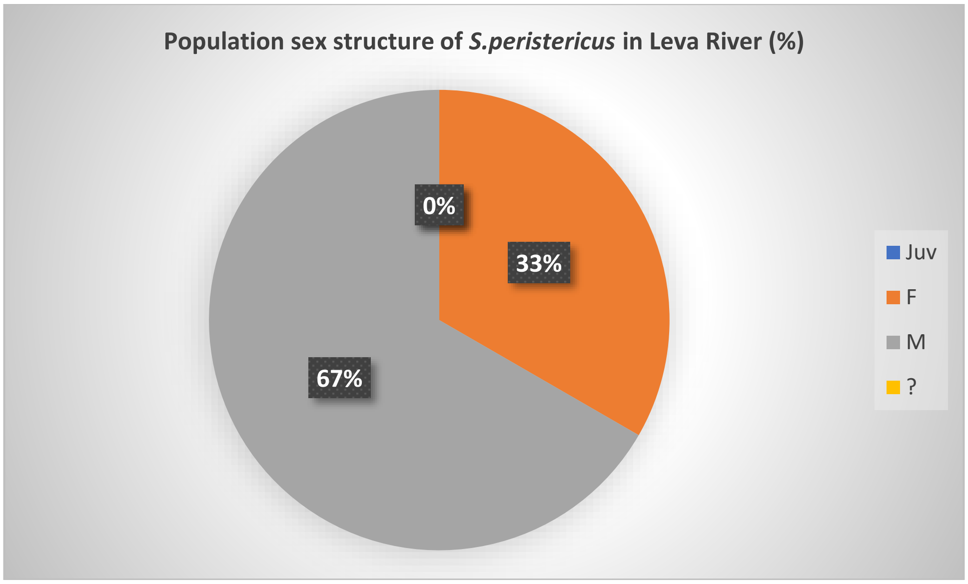

Figure 26. Sex structure of *Salmo peristericus* population in Leva River.

The percentage of sexes in the population of *Salmo peristericus* in Brajchinska River is presented in Fig.24. It can be concluded that males represent the largest part of the *Salmo peristericus* population in the Brajchinska River, total of 37% of the trout population, juveniles are 32% and females 26% of the trout population.

Similarly as in Brajchinska River, the obtained results of sex structure of trout population in Kranska River and Leva River, show domination by males (or male individuals), 43% males in Kranska River and 67% males in Leva River. Females in the trout population are 37% in Kranska and 33% in the Leva River. Juvenile fish in the Kranska River trout population account for 23%. Juvenile individuals in the Leva River have not been caught.

Sex distribution of *Salmo peristericus* population at all sampling points is presented at the figures 27, 28 and 29.

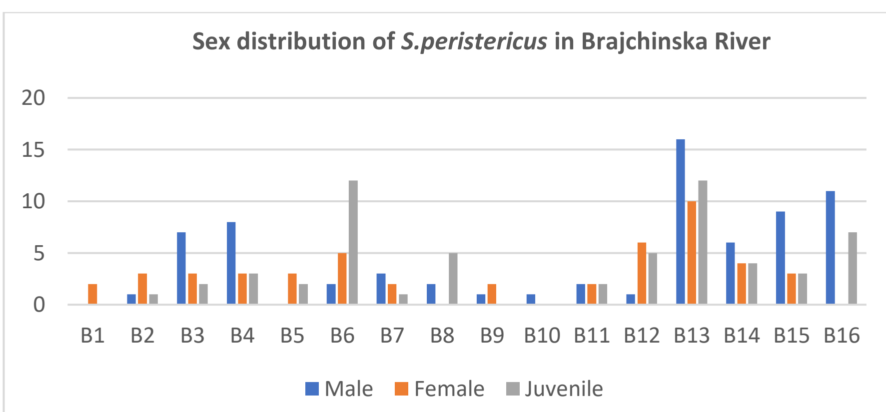

Figure 27. Sex distribution of *Salmo peristericus* population in Brajchinska River.

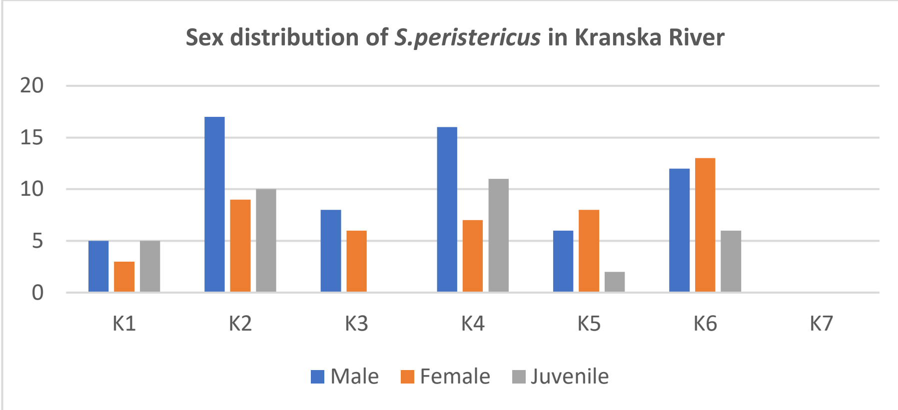

Figure 28. Sex distribution of *Salmo peristericus* population in Kranska River.

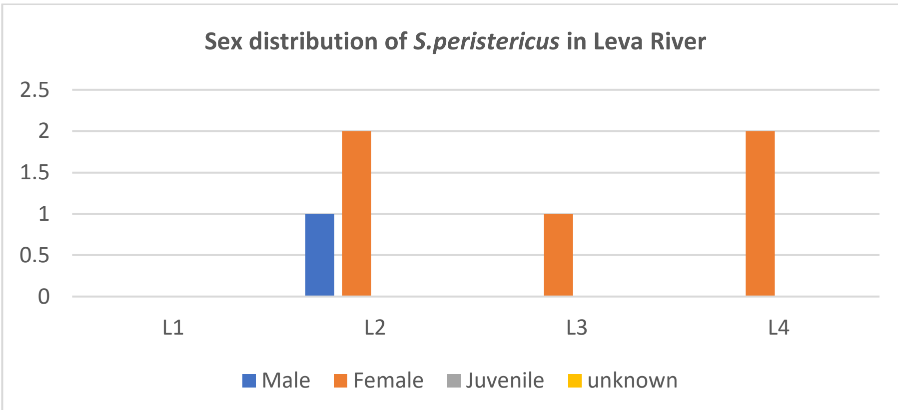

Figure 29. Sex distribution of *Salmo peristericus* population in Leva River.

3.4. Length-weight relationships

The length- weight relationship (LWR) and Fulton's condition factor (*K*) are the two main parameters used in fishery research, and have been closely related since they were first proposed. The LWR is the relationship between weight and length for a given species, and can be used to estimate the growth pattern.

Length-weight relationship was determined by fitting the data to a potential relationship based on the exponential equation (Le Cren 1951) in the form of:

$$W = aL^{b}$$

Where, **W** is the total weight (expressed in g), **L** is the total length (expressed in cm), **a** is a coefficient related to body form and **b** is an exponent indicating isometric growth when equal to 3, and indicating allometric growth when significantly different from 3 (Simon & Mazlan 2008; Simon et al 2009).

The parameters 'a' and 'b' of the exponential curve were estimated by linear regression analysis over log-transformed data expressed as:

$$\log W = \log a + b \log L.$$

For the parameters in the equation, the linear relationship between log *a* (logarithmic value for *a*) and *b* was used (Froese, 2006). The values of the constant 'a' and 'b' of the linear regression was determined by following Rounsefell and Everhart (1953) and Lagler (1966).

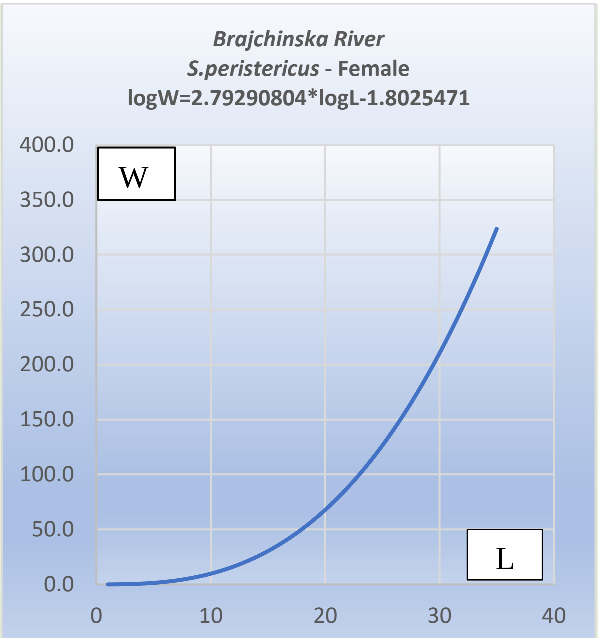

Figure 30. Length-weight relationship fema River

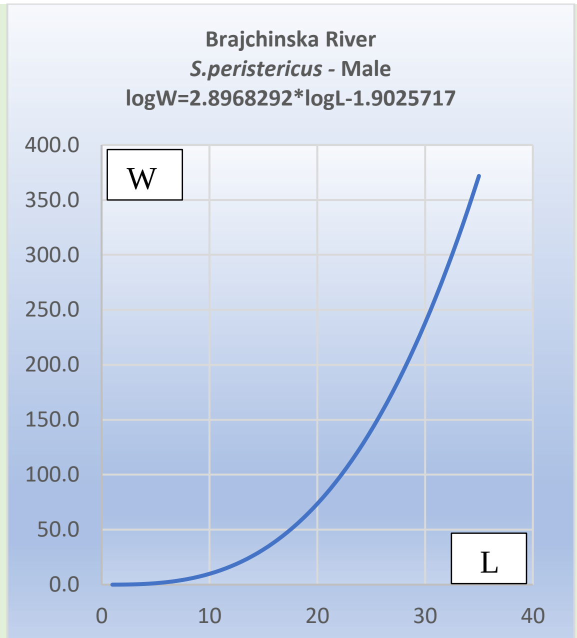

Figure 31. Length-weight relationship mal River

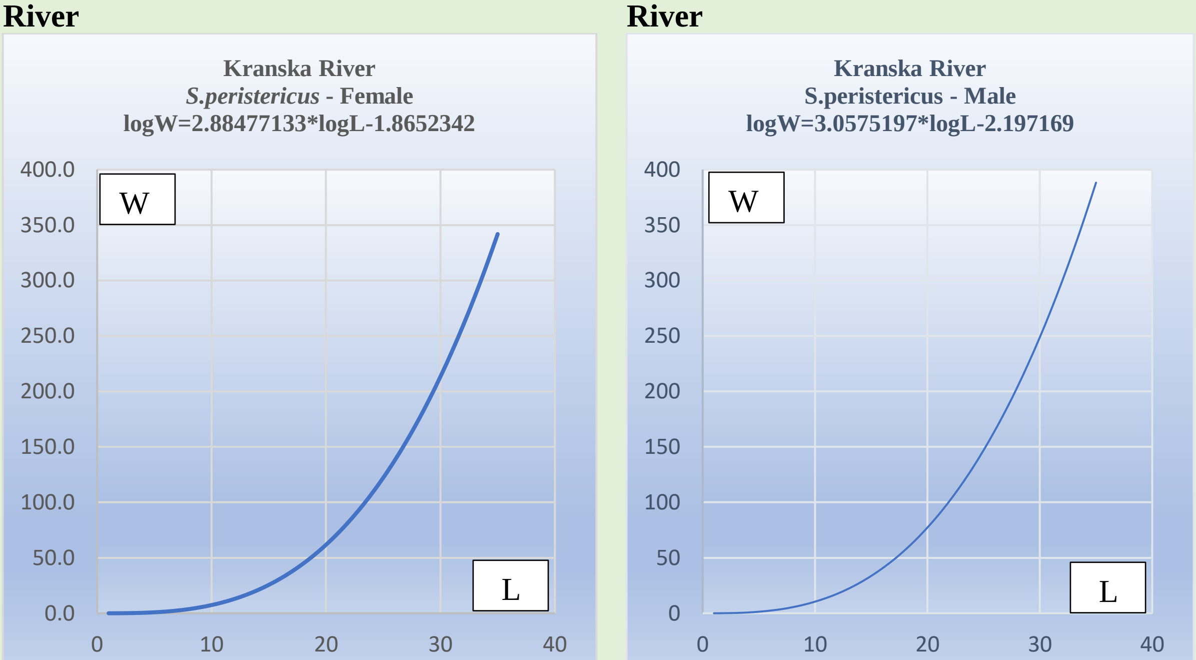

Figure 32, 33. Length-weight relationship in male and female population of *Salmo perister* River.

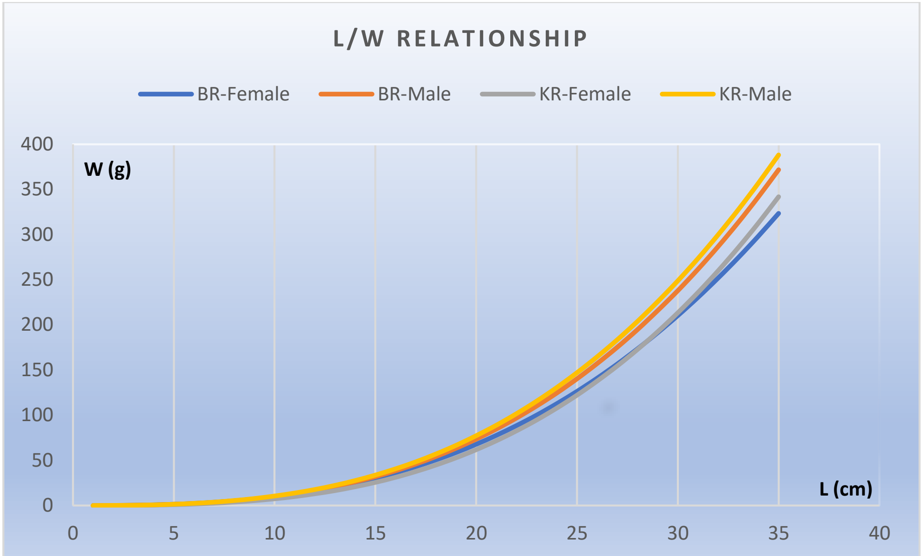

Figure 34. Length-weight relationship in male and female population of *Salmo peristericus* from Brajchinska and Kranska River.

The L-W relationship for the Prespa trout from Brajchinska, Kranska and Leva river basin is presented on Figure 30-34. Power function is describing the regression between the fish length and weight. The coefficient **b** from the equation for female from Kranska River and male and female from Brajchinska River are below 3. This indicates negative allometric population growth of *Salmo peristericus.* Negative allometric relationship means that the fish becomes thinner with increasing length (King, 1996). The male population from Kranska River has positive allometric relationship. This means that the fish become heavier with the increasing of length.

3.5. Condition factor (Fulton's condition factor K)

In order to answer questions referring to the Prespa trout nutritional status and within the frames of this Project, a condition factor (Fulton's condition factor K) was calculated of each single captured fish. The condition factor allows us to compare quantitatively the condition of individual fish within a population, individual fish from varied population, and two or more populations from different localities. K factor may also be used as an index of the productivity of a water. We are aware that K factor is influenced by the age of fish, sex, season, stage of maturation, fullness of gut, type of food consumed, amount of fat reserve and degree of muscular development (Rønshold, B., 1995; Nash et al., 2006) and for this reason interpretation of this value will be carefully discussed. However, the obtained results for the Fulton coefficient are of exceptional importance for obtaining initial information that will indicate the health condition of the trout. Knowing the fact that the K value is greatly influenced by the development of the reproductive organs, we followed recommendation from literature (Barnham and Baxter, 1998) that points out that the sampling of the fish ought to be done at the same time of the year, so that the individuals or populations are at the same stage of the reproductive cycle, as it was done in the frame of this project.

Fulton's condition factor, K was calculated by using the formula,

$$K = (W/L^3) \times 100$$

Where W = weight of fish in grams, L = total length in millimetres, 100 = factor to bring the value near to unity

For salmonids, K values usually fall in the range 0.8 to 2.0 (Barnham and Baxter, 1998).

As previously mentioned, the value of K is influenced by a range of physiological and ecological factors of fish. In some fish species, the gonads may weigh up to 15% or more of total body weight. With females, the K value will decrease rapidly when the eggs are shed.

On the basis of comparison of the K value with general appearance, fat content, etc, the following standards have been adopted for trout and salmon (Barnham and Baxter, 1998).

Table 9. Adopted standards for trout and salmon for condition

K value	Comments
1.60	**Excellent** condition, trophy class fish
1.4	**Good**, well-proportioned fish.
1.2	**A fair fish**, acceptable to many anglers.
1	**A poor fish**, long and thin.
0.8	**Extremely poor fish**, resembling a barracouta; big head and narrow, thin body

Figure 35. *Salmo peristericus* from Brajchinska River basin with poor K

Table 10. Condition coefficient's results (Fulton Condition coefficient) for *Salmo pelistericus* from Brajchinska River, Kranska River and Leva River

Sampling point	Male	Female	Juvenile
Brajchinska River			
B1	-	0.9	-
B2	0.9	1	0.8
B3	0.9	0.8	0.9
B4	0.9	0.8	0.9
B5	-	1	1.1
B6	0.8	0.8	0.9
B7	0.8	0.7	1
B8	0.9	-	1
B9	0.8	0.8	-
B10	0.8	-	-
B11	0.8	0.8	0.8
B12	1	1	1
B13	0.9	0.9	1
B14	1	0.9	0.9
B15	1	0.9	1
B16	1	-	0.9
average	**0.89**	**0.87**	**0.94**
minimum	**0.8**	**0.7**	**0.8**
maximum	**1**	**1**	**1**
Kranska River			
K1	1	1	0.9
K2	0.9	0.9	0.9
K3	1	0.9	-
K4	1	0.8	0.9
K5	0.9	0.9	1
K6	1	1	1
average	**0.97**	**0.92**	**0.94**
minimum	**0.9**	**0.9**	**0.9**
maximum	**1**	**1**	**1**
Leva River			
L1	-	-	-
L2	0.9	0.8	-
L3	0.9	-	-
L4	1	-	-
average	**0.93**	**0.80**	**-**

The obtained results for the condition coefficient of *Salmo peristericus* from Brajcinska River, Kranska River and Leva River indicate that there are populations that are composed of fish that have poor to very poor condition. The condition factor of Brajcinska River fish for all males, females and juveniles in the population is on average below 1 (0.89 for males, 0.87 for females and 0.94 for juveniles). The condition factor for the fish from Kranska River is relatively higher compared to Brajchinska River fish population (0.97 for males, 0.92 for females and 0.94 for juveniles), then again it is as well below "1".

3.6. Basic physicochemical parameters of water

At the field, at each assessment site (sampling point), the temperature, oxygen concentration, pH, conductivity and salinity in the water were measured.

The average measured temperature of the water in Brajchinska River was 5.87°C, with variations from 3.4°C on B1 to 8.3 °C on B12. Kranska River has water with a relatively higher temperature. The average water temperature was 6.7°C. The lowest measured temperature was found at K2 (5.4°C), and the highest at K7 (8.1°C).

It can be concluded that these are relatively cold watercourses, with extremely high concentrations of dissolved oxygen in water, with low values for conductivity and salinity, and with a low alkaline value for pH (7.9 at Brajchinska, 7.6 at Kranska and 7.8 at Leva Rivers).

Table 11. Basic physicochemical parameters of water from Brajchinska, Kranska end Leva River.

Date of inv.	Sampling Point	fish/ha	Water temp.	pH	O^2	Conductivity	Salinity
Brajchinska River							
04.12.2020	*B1*	**185**	3.4	8.0	12.1	36.8	16.4
04.12.2020	*B2*	**296**	5.4	7.5	13.5	35.8	16.6
04.12.2020	*B3*	**787**	5.2	8.2	12.3	51.2	23.7
05.12.2020	*B4*	**646**	5.6	7.8	13.4	112.0	52.0
05.12.2020	*B5*	**250**	6.1	7.9	12.4	77.3	36.3
05.12.2020	*B6*	**683**	3.8	8.0	12.9	172.6	78.0
06.11.2020	*B7*	**246**	7.7	8.1	12.1	70.3	32.8
04.12.2020	*B8*	**233**	5.2	7.8	12.6	66.2	30.1
05.12.2020	*B9*	**167**	5.3	7.8	13.4	70.3	31.1
05.12.2020	*B10*	**625**	5.2	7.8	12.1	73.7	33.2
06.11.2020	*B11*	**125**					
06.11.2020	*B12*	**338**	8.3	7.6	10.3	66.8	57.5
09.11.2020	*B13*	**1455**	8.1	7.6	10.8	71.1	32.4
06.12.2020	*B14*	**410**	5.7	8.0	12.2	122.5	56.4

06.12.2020	*B15*	**396**	6.5	7.8	12.6	125.7	58.6
06.12.2020	*B16*	**593**	6.6	7.9	12.7	125.7	58.6
		458	**5.9**	**7.9**	**12.3**	**85.2**	**40.9**
Kranska River							
07.12.2020	*K1*	707	6.1	7.4	11.3	85.2	4.6
07.12.2020	*K2*	1792	5.4	7.5	11.1	27.6	12.5
07.11.2020	*K3*	1143	6.6	8.0	10.1	82.2	38.8
08.11.2020	*K4*	1222	5,7	7.8	12.5	37.8	17.6
08.11.2020	*K5*	1538	7.1	7.7	11.7	111.1	50.5
08.11.2020	*K6*	713	6.7	7.4	12.3	48.4	22.4
08.11.2020	*K7*	0	8.1		10.5		
		1016	**6.7**	**7.6**	**11.4**	**65.4**	**24.4**
Leva River							
09.11.2020	*L1*	0	5.4	7.6	10.5	88.7	40.6
09.11.2020	*L2*	167	6.1	7.6	10.3	17.3	50.7
09.11.2020	*L3*	139	6.7	7.9	10.9	120.3	55.6
09.11.2020	*L4*	100	5.3	8.0	10.6	109.8	49.8
		101	**5.9**	**7.8**	**10.6**	**84.0**	**49.2**

3.7. Prespa trout population (density) trend in Brajcinska, Kranska and Leva river basin

The density population of *Salmo peristericus* in Brajcinska, Kranska and Leva River and their tributaries is presented in Table 12. The summary of this table contains original data in detail presented and discussed in Crivelli et. al. (2008). Additional summary is presented from the study by Spirkovski et al. (2011) conducted on Leva, Kranska and Brajcinska rivers in spring and autumn, where during the late survey the construction of hydropower plants in the Prespa region was ongoing. Study reports by Kostov (2017) and Kostov et al. (2017) conducted in Brajchinska River show the presence of the Prespa trout at sampling points before and after the hydropower plant's intakes.

According to Crivelli et al. (2008) the general densities of Brown trout and Marble trout are determined as above 5000 fish/ha. Compared to these two trouts, the Prespa trout have much lower densities. However, densities below 200 fish/ha are considered too low and their viability remains doubtful (Crivelli et al., 2008). Some of the low densities can be explained with the general habitat of the Prespa trout, and the hydrology of the mountain streams. Low flow in the summer, and absence of large pools can explain the low densities of Baltanska and Sredna tributaries (Crivelli et al., 2008). Illegal and even legal fishing are considered as a human factor contributing to the low densities of Upper Kranska River (Crivelli et al., 2008).

Table 12. Densities of the Prespa trout population in Brajchinska, Kranska and Leva Rivers (according: Crivelli et al., 2008, Spirkovski et al. 2012, Kostov et al. 2017 and current study 2020)

Reference	Site and stations	Years (number of stations)	Surface sampled (m^2)	Length sampled (m)	Mean N trout >1+/ha	Mean N trout >1+/100m of stream
	Brajchinska River					
Crivelli et al. (2008)	Main river	2006 (2)	858	205	664	28
Crivelli et al. (2008)		2007 (4)	1468	405	660	24
Crivelli et al. (2008)		2008 (4)	1468	405	858	31
		2011 (2)		200		21
Kostov (2017)		2016 (2)		90		20
Kostov et al. (2017)		2017 (2)		160		12
Current study		**2020 (9)**	**3404**	**1232**	**492**	**13**
Crivelli et al. (2008)	Baltanska	2006 (1)	220	100	136	3
Crivelli et al. (2008)		2007 (2)	474	210	42	1
Crivelli et al. (2008)		2008 (2)	474	210	657	15
Spirkovski et al. (2011)		2011 (1)		100		17
Current study		**2020 (1)**	**284**	**142**	**246**	**5**
Crivelli et al. (2008)	Rzhanska	2007 (2)	455	200	1121	26
Crivelli et al. (2008)		2008 (2)	455	200	1297	30
		2011 (2)		200		33
Current study (2020)		**2020 (2)**	**432**	**324**	**486**	**7**
Crivelli et al. (2008)	Drmishar	2007 (2)	490	210	878	20
Crivelli et al. (2008)		2008 (2)	490	210	694	16
Spirkovski et al. (2011)		2011 (1)			0	0
Current study (2020)		**2020 (1)**	**325**	**130**	**646**	**16**
Crivelli et al. (2008)	Kriva Kobila	2007 (1)	263	105	1709	43
Crivelli et al. (2008)		2008 (2)	565	217	1007	26
Spirkovski et al (2011)		2011 (2)		200		16
Kostov et al. (2017)		2017 (2)		100		9
Kostov (2017)	Stanishar	2016 (2)		60		10
Current study (2020)		**2020 (2)**	**140**	**140**	**396**	**4**
Current study (2020)	Brajchinska Marushica	**2020 (1)**	**240**	**120**	**250**	**5**

	Kranska Basin					
Crivelli et al. (2008)	Main river	2006 (1)	289	98	519	15
Crivelli et al. (2008)		2007 (4)	1298	408	593	19
Crivelli et al. (2008)		2008 (4)	1298	408	778	25
Spirkovski et al. (2011)		2011 (2)		200		16
Current study (2020)		**2020 (4)**	**1407**	**539**	**770**	**19**
Crivelli et al. (2008)	Upper Kranska	2007 (1)	287	100	174	5
Crivelli et al. (2008)	Srbina	2007 (1)	268	113	485	12
Spirkovski et al. (2011)		2011 (2)		200		25
Current study (2020)		**2020 (1)**	**184**	**184**	**707**	**7**
Spirkovski et al (2011)	Rechishte	2011 (2)		200		16
Current study (2020)		**2020 (1)**	**130**	**130**	**1538**	**15**
Current study (2020)	Kranska Marushica	**2020 (1)**	**240**	**160**	**1792**	**27**
	Leva Reka Basin					
Crivelli et al. (2008)	Sredna	2007 (2)	431	200	186	4
Crivelli et al. (2008)		2008 (2)	431	200	162	3
Spirkovski et al. (2011)		2011 (2)		200		1
Spirkovski et al. (2011)	Main river	2011 (1)		200		1
Current study		**2020 (3)**	**624**	**370**	**135**	**2**
Spirkovski et al. (2011)	Biglichka	2011 (1)		100		1
Current study		**2020 (1)**	**160**	**200**	**0**	**0**

The results from this study show very low densities of the Prespa trout population from the Leva River basin. The density of *Salmo peristericus* in Kranska River ranges from 707 to 1792 fish per hectare. The density of the Prespa trout in Brajchinska River varies between 246 fish/ha in Baltanska River to 646 fish/ha in Drmishar tributary. The main Brajchinska River course has lower number of fish/ha than the previous studies.

The data from the following studies are not always presented with area of wading, so the number of caught fish/100m length of stream are taken into consideration in order to show the trend line of population density (Figures 36-38). The timeframe of published data begins with 2006 (published by Crivelli et al., 2008) and ends with results from 2020 presented as original first-time published data within the current study aligned with the aims of the project. It is evident that the population of the Prespa trout in Brajchinska River is the most studied within the watershed of Prespa Lake. There is a lack of data in certain longer gaps between 2011 and 2016, and smaller gaps from 2009 and period of 2018-2019. However, based on the presented studies it is possible to determine the decreasing direction of the Prespa trout population in the main Brajchinska River, Stanishar River, Rzhanska River, Kriva Kobila River and Brajchinska Marushica River. The density of trout population in the Stanishar River and Brajchinska Marushica River is to be taken with great reserve because of the serious lack of studies throughout the period of time. Prespa trout populations in Baltanska River and Drmishar River do not have a negative trend line, however, the lack of data and low densities in general are noted.

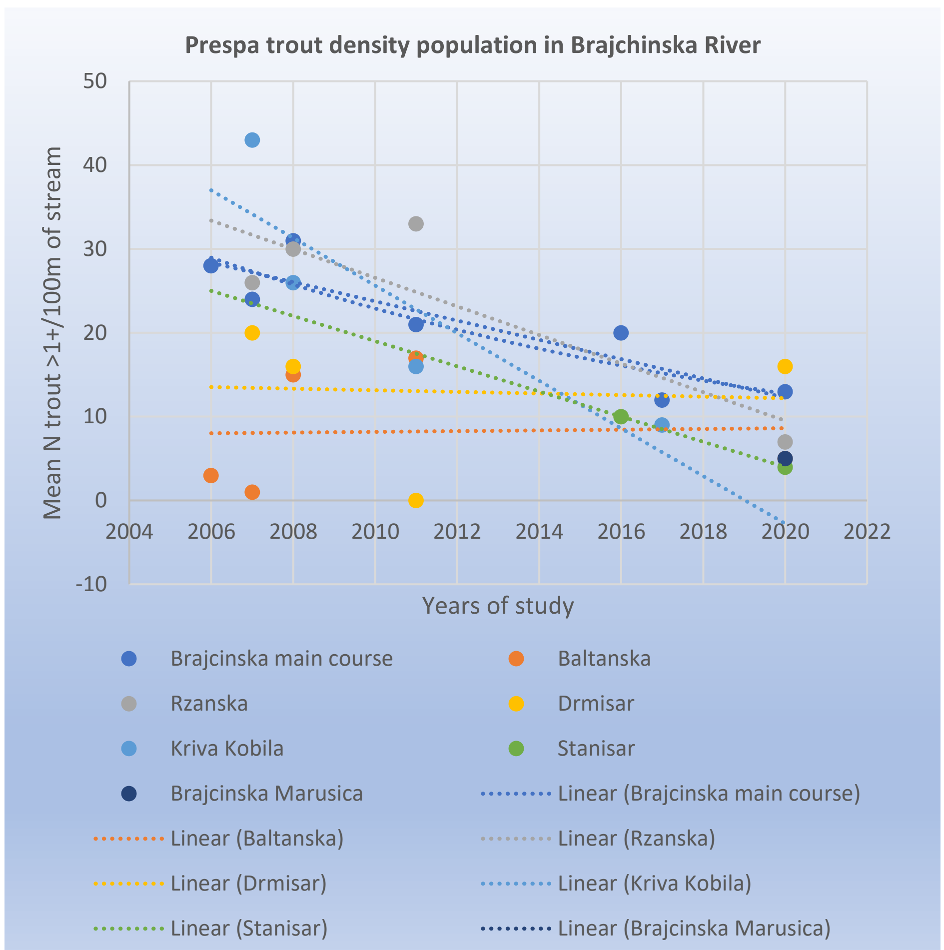

Fig 36. Linear trend line of number of caught fish of *Salmo peristericus* per 100m of stream in Brajchinska River based on collected and published data (see reference in Table 12).

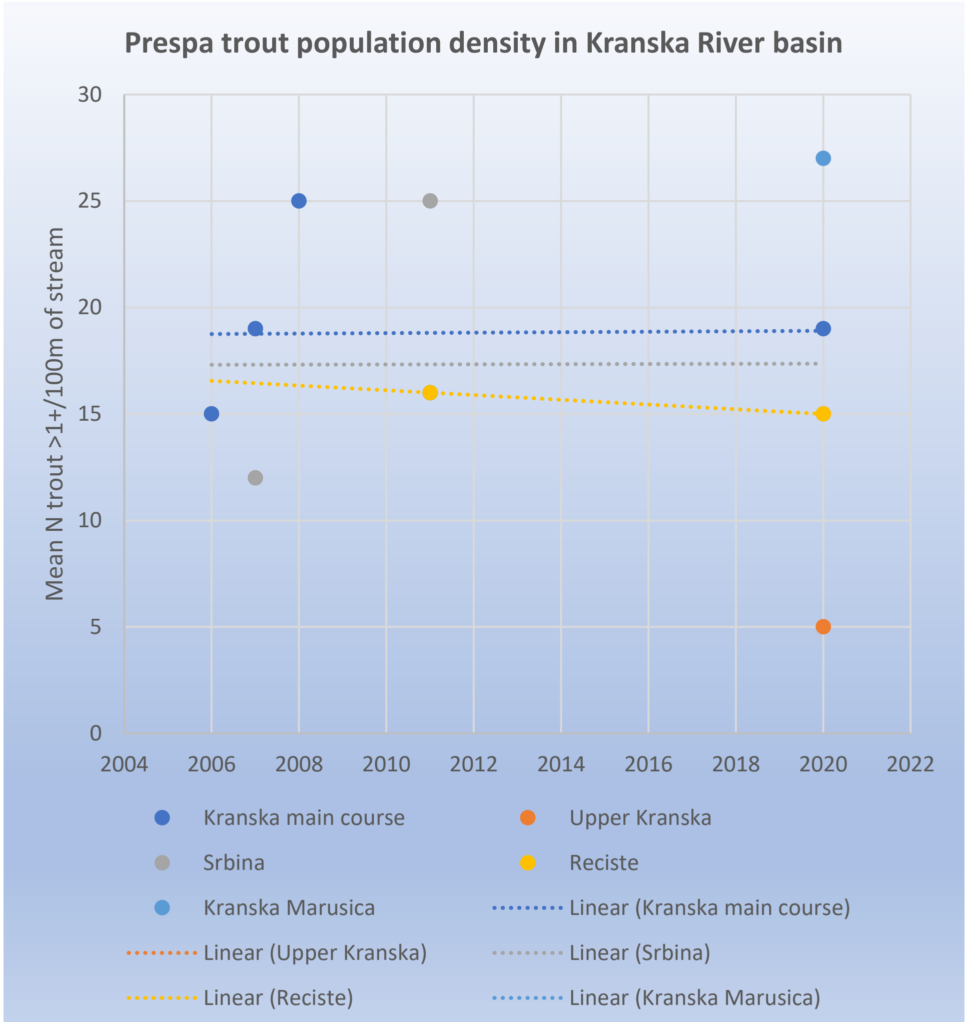

Figure 37. Linear trend line of number of caught fish of *Salmo peristericus* per 100m of stream in Kranska River based on collected and published data (see reference in Table 12).

The density of the Prespa trout population in Kranska River shows stability throughout the period from 2006 to 2020. However, there is a serious lack of knowledge (data) that expands throughout almost the entire last decade. It seems that the Prespa trout population in Kranska River does not decrease, but it is currently impossible to know if this population has fluctuations. Lastly, the density of *Salmo peristericus* in the Leva River shows all the possible trend lines, with severely low density numbers.

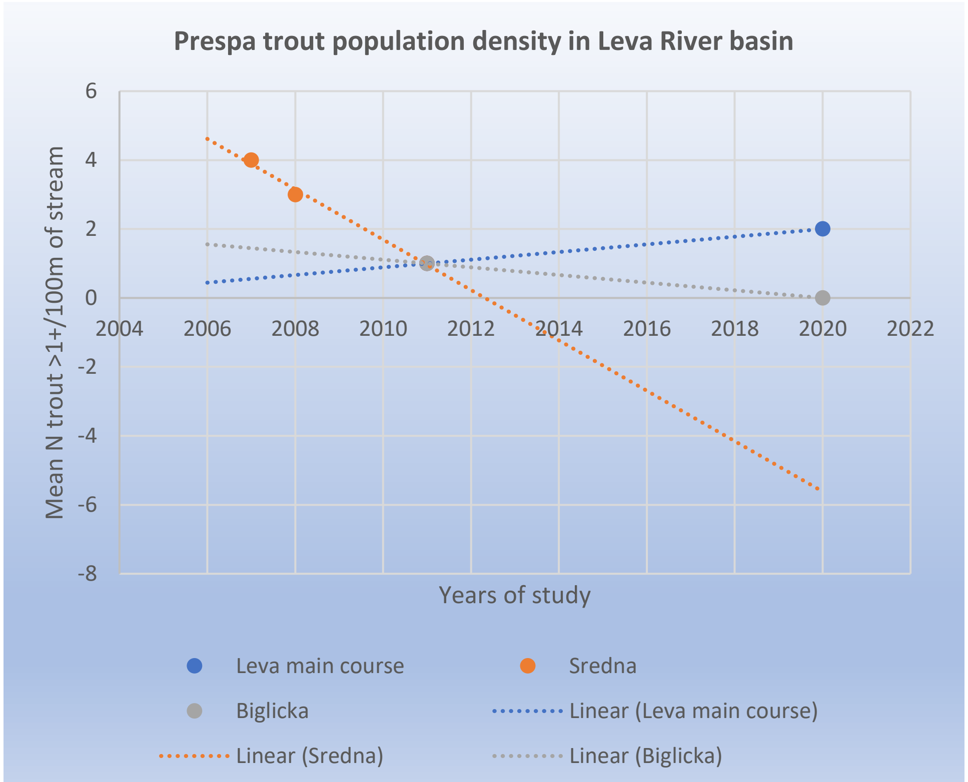

Figure 38. Linear trend line of number of caught fish of *Salmo peristericus* per 100m of stream in the Leva River basin based on collected and published data (see reference in Table 12).

IV. DETERMINED THREATS AND INFLUENCE OF SMALL HYDRO POWER PLANTS ON PRESPA TROUT POPULATION

In the process of assessing the conservation status of *Salmo peristericus* it is of great importance to detect all the potential threats to the Prespa trout population. In that context, the registration of all threats during the field research have been noted, and in addition, interviews with locals have been made, and they were all of great importance in order to find out more about this issue.

During our research in the frame of this project, a total of 27 sampling points (SP) at the Brajchinska River, Kranska River and Leva River and their tributaries were surveyed. At each investigated sampling profile among fish sampling, their measurements, habitats and environmental conditions in the riverbed, all the possible threats were reviewed and assessed. One of the goals of this project is to detect and determine possible negative impacts on the Prespa trout populations as a result of human factor activities and/or natural impacts.

The conservation status assessment of the Prespa trout, and future measures for developing the Conservation Action Plan will depend on the possible exposure to threats and pressures of its population density, size and structure.

The following text presents all the threats defined on each sampling point separately.

Brajchinska River

B1- Rzhanska River (highest SP)
During the research of this sampling point there are no direct threats detected considering the location in the frame of National park Pelister.

B2 - Brajchinska River (highest SP- Rupa)
This sampling point is within a strictly protected area of National park Pelister and there are no detected threats to the trout populations.

B3 - Rzhanska River (before and after the concrete bridge)
Along this sampling point which also belongs to the National park, no threats have been detected.

B4 – Drmishar (before junction with Marushica)
The electrofishing was performed at a location of 500-700m before the confluence with the river Marushica. There are no threats detected on this sampling point considering the area in the frame of the National park.

B5 – Marushica (500m before junction with Drmishar)
No threats detected on this sampling point.

Figure 39 - 44. Near the water intake and SHPP's.

B6 – Brajchinska River (Golem dol, between SHPP and water intake)

The location of this sampling point is between the water intake and the machine building at distance of 1.5km from the intake of the SHPP. Detected threats at this SP are the water capture, resulting in reduced amount of water flow and the fragmentation. It is also noticed that the riverbed bottom is covered with a large amount of leaf mass and the presence of sludge in the parts of the riverbed.

B7 – Baltanska river (tributary of Brajchinska river)

At this sampling point we recorded the presence of a natural barrier (1.5m height waterfall).

Figure 45 - 48 Part of the irrigation systems that take water direct from the rivers

Figure 49 – 50. Barriers and rock cascades made during the construction

B8 - Brajchinska River (after a water intake of SHPP PCC)

This SP is under the water intake for SHPP ``PCC” (Brajchino II) and SHPP “Brajchino 1” machine building. At this SP we recorded the presence of rock cascades in the riverbed dating from the construction period of SHPP. Some of the cascades are impassable barriers for the small fish. Habitat modification has been noticed, since the construction of the SHPP. We also found a reduction in the amount of water flowing into the riverbed.

B9 - Stanishar (before-above the water intake of SHPP)
A direct threat is the presence of the water intake of SHPP.

B10 - Stanishar (under the water intake of SHPP)
At this sampling point the considered detected threat is the presence of water intake resulting in a decreased water flow and fragmentation of the riverbed.

B11 - Brajchinska River (Saint Ilija)
Electrofishing sampling at this profile is performed at the location between the intake and the power plant. The water is captured for the purposes of SHPP. Irrigation water capture was detected as well.

B12 - Brajchinska River above Brajchino village (300m under PCC SHPP)
At this sampling point the following threats are detected:

- presence of irrigation water captures;
- occurrence of fishing;
- surrounding apple orchards as possibility of presence of herbicides and pesticides into the river;
- information obtained from locals about frequent water amount variation (fluctuations) depending on SHPP activities and
- the riverbed often remains dry due to the intake from SHPP "A".

B13 - Brajchinska River (between Brajchino and Ljubojno village)
Detected threats at this sampling point are the following:

- fishing and poaching;
- water intake for drinking water;
- apple seedlings near the river as possibility of herbicides spilling into the river and
- sewerage drainage pipes from the local households direct into the water.

B14 - Brajchinska River (into the Brajchino village under the wooden bridge)
Threats identified are the following:

- Irrigation water intakes;
- Possibility of water pollution from waste water (sewerage) pipes from the households into the river;
- Received information from the locals about the water flow variation and
- Fishing and poaching.

B15 - Brajchinska River (above the cascade)
Detected threats at this SP are:

- the poaching;
- water pollution

- presence of cascade disabling fish migration upstream

Figure 51 - 52 Brajchinska River below the cascade

B16 - Brajchinska River (below the cascade)
From the obtained interviews with the local residents while conducting the field work, it is found that during the summer the river is very often without water. The direct impact and threat is the existing cascade, poaching and fishing.

Kranska River

K1 - Srbina River (tributary of Kranska River)
From information gained from local residents it is stressed that illegal fishing is a threat for the trout.

K2 - Kranska Marushica (tributary of Kranska River)
From information gained from local residents at this sampling point the illegal fishing is a suspected threat for the Prespa trout population.

Figure 53 - 54. Dry riverbed of Kranska River before the inflow into Prespa Lake

K3 - Kranska River
The location of this sampling point is in the course of the river after the formation of Kranska River from Kranska Marushica and Srbina river. There are no threats detected on this SP.

K4 - Kranska River
The location of the sampling point is the part of the river before the intake of water for SHPP ''A'' Marushica, and there are no direct threats detected.

Figure 55 - 56. Sewerage drainage pipes spilling direct into Kranska River

K5 - Kranska River (Rechishte)
The SP is at the location before the inflow of Kranska River above the water intake of SHPP. There are no threats detected considering the Prespa trout populations.

K6 - Kranska River
The location of the sampling point is above the water intake of SHPP and the machine building. Frequent capturing of water due to activities of SHPP at the section form intake along the fish passage.

Figure 57 – 58. Cascades and irrigation water intake in Arvati village

K.7 - Kranska River (Arvati village)
The location of the sampling point is in Arvati village after water inflow from the machine building. Following threats have been detected:

- waste water (sewerage) pipes from households spilling direct into the river (Fig. 55-56)
- presence of cascades (Fig. 57-58) and
- water irrigation intake.

Leva River

L1 - Biglichka River)
This sampling point has no detected threats of Prespa trout populations.

L2 Leva River (Golema River)
Based on the interviews with the local residents the poaching of the Prespa trout at this part of the river is a threat.

L3 Leva River (Golema River)
At the sampling point the Prespa trout is threatened by poaching.

L4 Leva River (Golema River)
The poaching is a detected threat, according to the local residents at this part of the river.

For Leva River we can say that no direct threats have been identified. We received information from the local residents that poachers are regularly present in this part, during the summer months. It was also pointed out that the amounts of water flowing in the river are significantly lower compared to 20 to 30 years ago. It is emphasized that this is a natural process.

Table 13. Sampling profile marks, description of sampling spots and detected threats during the field survey of Brajchinska, Kranska and Leva rivers

SP mark	Description of sampling point	Detected threats
B1	Rzhanska River (highest SP)	none (National park protected area)
B2	Brajchinska River (highest Rupa)	none (National park protected area)
B3	Rzhanska River (before and after the concrete bridge)	none (National park protected area)
B4	Drmishar (before the junction with Marushica)	none (National park protected area)
B5	Marushica River (500m before the junction with Drmishar)	none (National park (protected area)
B6	Brajchinska river (Golem Dol between SHPP and water intake)	-SHPP intake; -reduced water inflow; -fragmented riverbed and -large amount of leaf mass of the river bottom and presence of sludge of the certain parts of the profile
B7	Baltanska River (tributary of Brajchinska river)	natural barrier (1,5m height waterfall)
B8	Brajchiska River (after the water intake of SHPP PCC	rock cascade presence in the riverbed dating from SHPP construction

B9	Stanishar River (before (above) the water intake for SHPP)	presence of the water intake of SHPP
B10	Stanishar River (after (under) the water intake of SHPP)	-reduced water inflow -fragmentation
B11	Brajchinska River (between water intake and SHPP)	presence of irrigation water capture (two pipes ϕ 110)
B12	Brajchinska River 300m under PCC SHPP (above Brajchino village)	-presence of irrigation water captures; -information obtained for fishing; -surrounding of apple orchards as a possibility of presence of herbicides and pesticides into the river; -information obtained from local residents about frequent water flow variation depending on SHPP activities and -the riverbed is often remained dry due to the intake from SHPP “A”.
B13	Brajchinska River (between villages Brajchino and Ljubojno)	-fishing and poaching; -water intake for drinking water; -apple seedlings near the river as possibility of herbicides spilling into the river and -sewerage drainage pipes from the local households direct into the water
B14	Brajchinska River (Ljubojno village) under the wooden bridge	-irrigation water intakes; -possibility of water pollution from sewerage pipes from the households into the river; -received information from the locals about the water flow variation and -fishing and poaching
B15	Brajchinska River (above the cascade)	-poaching and -water pollution.
B16	Brajchinska River (below the cascade)	-during the summer the river is very often left without water -the existing cascade -poaching and fishing
K1	Kranska River (Srbina river)	-illegal fishing
K2	Kranska Marushica River (before merging with Srbina River)	-illegal fishing
K3	Kranska River (first SP after the forming)	no detected threats
K4	Kranska River (before water intake for SHPP “A”	no detected threats
K5	Kranska River Rechishte	no detected threats

K6	Kranska River (between water intake and SHPP)	water is often captured due to the SHPP activities from the intake along the fish passage
K7	Kranska River (village Arvati) after water inflow from the machine building	-presence of cascades; -sewerage pipes from households spilling direct into the river and - irrigation water intake
L2	Leva River (Golema River)	poaching
L3	Leva River (Golema River)	poaching
L1	Biglichka River	no detected threats
L4	Leva River (Golema River)	poaching

There is an obvious contradiction with the construction of SHPP conducted on Brajchinska River, which is considered opposite of the prescribed Law for nature protection (Broken rivers, 2017). Among the fact that Brajchinska River belongs within the National Park Pelister, where fishing of all streams and tributaries is prohibited, it is not clear why the construction of the SHPP was even allowed at all (Broken rivers, 2017).

With the construction of SHPP Brajchinska 1 and Brajchinska 2, the existence of the Prespa trout and the cumulative impact of SHPPs, fish passage construction as referred to in environmental protection elaborates by SHPP, is considered as eventual mitigation.

The published data in Broken rivers (2017) report that Brajchinska River and its tributary Kriva Kobila River were detected as completely dried. In the period 2006-2007, the section between Brajchinska 1 SHPP intake and machine building were the most important Prespa trout habitats with the presence of 28 and 43 individuals per 100 m, respectively. As a result of increased water flow along the river in 2017 the situation was slightly improved related to the increased presence of trout on the profile between machine building and the water intakes of Brajchino 1 and 2 SHPPs. The fact that should not be ignored is the concerning situation related to the presence of Prespa trout populations due to the decreased number of captured individuals, compared to the section over the water intakes of Brajchino 1 (Crivelli et.al., 2008).

The biological minimum prescribed for SHPP Brajchinska 1 does not correspond to the field survey conducted in 2017. The fish passage was found inadequate due to the blockage of the upper exit and absence of organic substrate at the bottom of the riverbed (Broken rivers, 2017). Beside the direct impact on the water flow, the environment of the riverbed is endangered by construction materials (concrete, stones, rocks, blocks), disturbing and altering the river habitat.

Kostov (2017), indicates the necessity of future extended surveys and research in order to asset the efficacy and functionality of fish passages and presence of Prespa trout populations. It also points out the necessity of video monitoring of fish passages. The number of fish crossing the fish passage will be monitored, as well as the time and direction of fish migration. These data would be valuable for determining the size of the populations and proposing the mitigation measures for further protection of Prespa trout populations.

Considering Brajchinska River 1 SHPP there is an urgent and immediate need for return to the natural course of the river flow. Brajchinska 2 is considered for further extended monitoring of the impacts of Prespa trout populations, such as fragmentation and isolation, low water flow and water variations, and efficacy of fish passages. According to Broken rivers (2017), there are specific recommendations regarding SHPP:

a) avoidance of future construction of new SHPPs in protected areas;
b) implementation of stricter criteria for prescribed biological minimum of water;
c) regular monitoring of fish passages and their functionality and efficacy;
d) implementation of a procedure for comprehensive environmental impact assessment in addition to elaborates of SHHPs.

It could be concluded that SHPPs are serious threats and pressures for the Prespa trout due to their water intakes, as eventual impassable barriers for fish migration considering condition, preventing access to food availability, fragmentation and isolation of habitats, disabling trout to migrate towards spawn localities, as well as further growth and development due to limited life area-water.

V. CONSERVATION STATUS OF PRESPA TROUT (*Salmo peristericus*)

One of the tasks of this Project is to assess the National conservation status of *Salmo peristericus*. It is well known that the Red List provides scientific information and analysis on the status, trend and extent of endangered species, in order to draw the attention of the public, especially decision makers (nationally and globally). The ultimate goal is to design appropriate strategies and programs and undertake biodiversity conservation activities. Based on the data collected from the project activities (distribution and population size), as well as, literature data from the past, assessment of the National conservation status will be performed according to IUCN Red List Categories and Criteria.

5.1. Previous classification

The early assessment of the population of *Salmo peristericus* was defined as endangered species (Economidis, 1992). Later, the Prespa trout was assessed against the risk of global extinction following the classification system of the IUCN. According to the IUCN Red List, based on the geographic range, *Salmo peristericus* was classified in the category of endangered species (Smith & Darwall, 2006). The IUCN evaluation of the Prespa trout was conducted only on the long-term data collection from Agios Germanos stream (Criveli et al., 2008) (Figure 29). Based on this study it has been estimated that the threatened Prespa trout is facing very high risk of extinction in the wild. The assessment of the geographical range of *Salmo peristericus* estimated that the extent of occurrence of the Prespa trout is less than 5000km^2, while the area of occupancy is less than 500 km^2, existing at no more than 5 locations. The estimates also indicate observed continuous decline for the Prespa trout population, severe fragmentation, as well as, limitation of the extent and quality of the habitat.

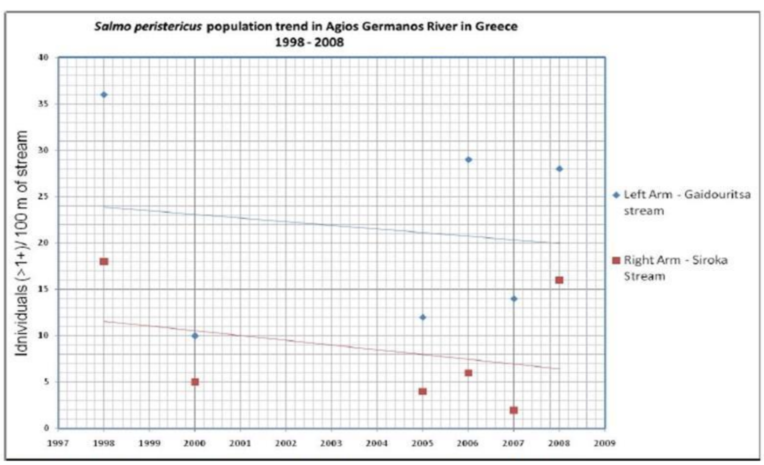

Figure 59. Population trend in Agios Germanos River (1997-2007) - published data in Crivelli et al. (2008) shows large fluctuations of population size.

5.2. Present (Current) classification

The International Union for Conservation of Nature (IUCN) is a global network aiming to create authority on nature conservation. The evaluation of the species' extinction risk, The IUCN Red List of threatened species uses a classification system of a set of Categories and Criteria (A-E). The process of global risk extinction assessment requires evaluation against all criteria (A-E).

Taxa are qualified for the Criterion A when they have undergone a significant reduction in the near past, or are projected to experience a significant reduction in the near future. The applying Criterion A falls under the objective that there is a greater chance of extinction when the decline rate is greater (Mace et al. 2008). Criterion A is based only on population reduction for the reason that even low population densities may lead to decline, exposing the population to even more and new threats. Thus, the decline of population, if not stopped, will lead to extinction. Criterion A is based only on the rate of decline – it is practical because even abundant species can become extinct as a result of decline in their population in answer to a certain threat (Stanton, 2014; (Lande et al. 2003). Criterion A contains four parts: A1, A2, A3 and A4, dealing with reductions in the past 10 years or three generations in which the threats can be reversible understood/not understood, ceased/not ceased. The population reduction can be estimated, projected, inferred or suspected for the determined period of time. The basis of the reduction is listed from (a) to (d): (a) direct observation, (b) an index of abundance appropriate to the taxon, (c) a decline in area of occupancy, extent of occurrence and/or quality of habitat, (d) actual or potential levels of exploitation, and/or (e) the effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites. In order to scale the decline rate threshold in this criterion the three years generation length is used. The data require calculation of mature individuals. Having in mind that there are certain gaps in the data collection for *Salmo peristericus*, it is unlikely that this species can meet criterion A. The noted decline in the Prespa trout population so far is considered as continuous; however, criterion A does not require continuing decline. Thus, the risk assessment of extinction of the Prespa trout, based on the results from this research does not qualify for criterion A, because of lack of knowledge on the length of generation in *Salmo peristericus* and continuous data collection for a period of 10 years.

Taxa can be qualified under Criterion B based on geographic ranges or certain patterns of habitat occupancy. If a taxon has a restricted distribution that is fragmented or exists in a few locations, it qualifies for Criterion B. The population also needs to undergo some form of continuing decline, and/or to have current or future extreme fluctuation. The extent of occurrence (EOO) or the area of occupancy (AOO) are ways to define the distribution range of the taxon. Further, at least two out of three sub criteria, listed from (a) to (c), must be met to qualify for this taxon. Namely, (a) the taxon has to be severely fragmented or to exist in no more than a certain number of locations; (b) the taxon has to have continuing decline; (c) the taxon also has to exhibit extreme fluctuation. The results from this study show that *Salmo peristericus* can be found at a small geographic range, that is both fragmented and exists in a certain number of locations. Thus, this species meets Criterion B. More specifically the habitat of population of the Prespa trout extends in less than 5000km^2 under EOO, and less that 500km^2 under AOO. Altogether, the geographic range is fragmented and the Prespa trout exists in more than one, but less than 5 locations, a sub criterion that along with continuing decline observed in extent of the habitat places this fish species in the endangered category.

To qualify for Criterion C, the taxa need to be presented with small populations that are currently declining or may decline in the near future. Yet, the leading factor is the small population which is based on the number of mature individuals within the population.

Considering the number of mature individuals, the Criterion D deals with very small or restricted populations. However, the calculations are based on data collection from a much longer period of time, that can be from two years of generation up to 100 years.

For the Criterion E, a quantitative population variability analysis (PVA) has to be conducted. The extinction risk has to be calculated for three time periods: 10 years or three generations, 20 years or 5 generations, and 100 years. The generation length of the taxon will determine how many assessments are needed.

The results presented in this study are based on the current data collection survey. The number of mature individuals were determined in each sub basin of Prespa Region, however, there is a lack of data in previous studies that are explicitly stating the numbers of mature individuals. This is understandable as the determination of mature individuals requires much more time in the field survey and it is usually based on some fundamental knowledge for the biology of the species. Having in mind that *Salmo peristericus* is largely understudied species, practically every aspect of IUCN Red List Categories and Criteria that deals with quantitative analysis based on certain generation periods will not be met.

The criteria require quantitative studies over a longer period of time and/or knowledge of the distribution of the species in assessment. The lack of consistent sampling throughout a longer period of time is limiting the criteria used for assessing the fish population within this study. Therefore, within this study, in the assessment of conservation status of *Salmo peristericus* only criterion B of IUCN classification system can be used.

Salmo peristericus is a rare species, native to the country and the region of Prespa. Within this research, the distribution of the species was studied at sampling sites that are considered to provide an appropriate habitat. Part of these sampling sites were previously defined as sampling points in the early attempts of monitoring the Prespa trout. The assessment of the distribution of the Prespa trout within the research is conducted as a one-time evaluation and confirms the restricted geographical range to Brajchinska River, Kranska River and Leva River, which is less than 5000 km^2.

All the three watersheds are not connected at the upper parts. The only possible connection is the Prespa Lake itself. However, the study shows that *Salmo peristericus* is facing certain threats (listed in details within this document) that indicate fragmentation of the area severely affecting the habitat occupancy.

The extinction risk of a species can be assessed at global, regional or national level. One species can have a different category in the Global Red List and a Regional Red List. For example, a species that is common worldwide and classed as Least Concern (LC) in the Global Red List could face a high level of threat and fit the Endangered category (EN) in a particular region (see Figure 1 for the explanation of the IUCN categories). In order to avoid an over- or underestimation of the regional extinction risk of a species, the Guidelines for the application of IUCN Red List Criteria at Regional Level should be applied (IUCN 2003). **Logically, an endemic species should have the same category at regional and global level, as it is not present in any other part of the world.** (IUCN 2003, 2019)

The threat to the risk of extinction requires the population of the Prespa trout to remain in the threatened **endangered** category.

5.3. Future classification

The dynamicys of a long-term study has to be re-established and maintained, since consistency in data collection is key in forming a fundament of more comprehensive and authoritative procedural follow-up of the assessment. A reevaluation of the population assessment of *Salmo peristericus* focused on population trends from all the Prespa subbasins based on long-term data collection with standardized methods is of urgent need within the next 5 years period of time.

VI. SUMMARY

Within this project, the following aspects of the population dynamics and biology of *Salmo peristercius* from Brajchinska, Kranska and Leva river basins have been researched: spatial distribution, population density, sex structure of the population, length structure of the population, time and length of first sexual maturation, length weight ratio and coefficient of condition of fish. The trends in the population, the threats and the impact of the small hydro power plants on the population of *Salmo peristericus* have been determined and the conservation status of the trout has been assessed in accordance with the criteria defined in the IUCN.

Population size, density, spatial distribution and dispersal of fish provide basic information on population ecology, population dynamics, population genetics and evolutionary biology. Dispersal (distribution) is important for the colonization of new habitats, affects the genetic structure of a population (immigration and emigration) and influences demographic processes within the population.

The sampling was performed at a total of 27 sampling points: sixteen (16) on Brajchinska River, seven (7) on Kranska River and four (4) on the Leva River.

The results obtained within the project showed that in all the sites where the presence of trout was registered, the community consisted of fish with different length classes and different sex structure. The length of the trout ranges from 4.1 cm to 28 cm. The largest fish caught were 27.7 cm. The largest distribution of fish is in the length class 16.1 - 17.0 cm (11.1% of the caught units). The large number of length classes are dominated by males, especially in those classes that are most numerous, starting from the length class 16.1-17

The calculated relative density of the population of *Salmo peristericus* in Brajchinska River was 458 fish/ha. The lowest relative density was found on the SP B11 (125 fish/ha), and the highest on the profile B13 (1455 fish/ha). On three profiles (B1, B9 and B11) the population density is below 200 fish/ha. The calculated relative density of the trout population in Kranska River was 1016 fish/ha. The lowest relative density was found on the SP K1 (707 fish/ha), and the highest on the SP K2 (1792 fish/ha). The total relative density of the trout population in the Leva River is extremely small and it is 101 fish/ha. The densities of all the examined sampling points are below 200 fish/ha. However, the observed densities lower than 200 fish/ha are quite low, and the viability of those populations remains an issue.

The results obtained for the coefficient of condition and length-weight relationship showed that *Salmo pelistericus* from the investigated watercourses is in very poor condition. These are fish that are poorly fed, elongated, with a long slender body and low body weight. The results of the condition factor analysis indicate values lower than 1 and 1.

The analysis showed that within the communities, fish with small length dimensions are sexually mature. The first sexually mature males are registered in the length class 8.1 - 9 cm, which is an extremely short length for the occurrence of sexual maturity. The first female sexually mature individuals appear in the length class 11.1-12 cm. All fish from the trout population in Brajchinska River and Kranska River over 14 cm long are sexually mature and capable of reproduction. Sexual maturity at small size is a serious problem because maturation retards the growth rate of the fish and often causes increased mortality (Nedval et al., 1981). It seems that many strategies have evolved to shape patterns in fecundity and early maturation. Life-history theory predicts that selection will favor a pattern of allocation of resources to reproduction over the lifetime of an individual that will maximize its contribution of offspring to the next generation (Roff, 1992, 2002); or to be more specific, the fish population under a pressure will develop a reproductive strategy in a way to maintain the existence of the population for the longest time possible.

Early sexual maturation prevents reaching larger dimensions, and thus in the population individuals will be present that will have difficulties to cope with the migration route (natural obstacles through the riverbeds due to the presence of larger rocks along the rivers, but also the presence of hydropower plants). This creates naturally isolated populations within the same river. In such isolated populations, in order to maintain the populations, greater recruitment of young individuals is necessary.

This recorded situation is in favor of the literary data which indicate that trout that live in small rivers, but also in isolated populations are characterized with smaller body size and early maturation (Nikolsky 1969; Nedval et al., 1981; Jonsson et al. 2001), but in some cases with low condition factor (Sandlund and Jonsson, 2016).

The population of the Prespa trout in Brajchinska River basin is the most studied within the watershed of Prespa Lake. There is a lack of data in certain longer gaps between 2011 and 2016, and smaller gaps from 2009 and the period of 2018-2019. However, based on the available studies it is possible to determine the decreasing trend direction of the Prespa trout population in the main Brajchinska River, Stanishar, Rzhanska, Kriva Kobila and Brajchinska Marushica. The density of trout population in Stanishar and Brajchinska Marushica is to be taken with great reserve because of the serious lack of studies throughout the period of time. Trout populations in Baltanska and Drmishar do not have a negative trend line, however, the lack of data and low densities in general are noted. The density of the Prespa trout population in Kranska River shows stability through the time period of 2006 – 2020. However, there is a serious lack of knowledge that expands through the almost entire last decade. Lastly, the density of *Salmo peristericus* in the Leva River Basin shows all the possible trend lines with severely low density numbers. At the same time, this watershed is the least studied. Future efforts must be headed towards determining the stress factors that influence the low numbers.

According to the results, the SHPP water intakes are an additional barrier for fish migration. In addition, the absence of longitudinal connectivity of watercourse (downstream and upstream), also negatively affects the health and density of trout populations, as well as, fragmentation and isolation of populations to the size and the condition of the trout. The existence of fish passages along with water intakes is not enough passable for trout migration to reach the upper parts of the river.

It could be concluded that SHPPs are serious threats and pressures for the Prespa trout due to their water intakes, as eventual impassable barriers for fish migration considering condition, preventing access to food availability, fragmentation and isolation of habitats, disabling trout to migrate towards spawning localities, as well as, further growth and development due to limited life area-water.

An additional fact that should be pointed out for salmonid fishes is that they exhibit season migrations, moving between spawning areas in lake or coastal areas. Such seasonal migrations are often regarded as an adaptive behavior to increase growth or survival and to maximize fitness, in seasonally fluctuating environments (Gross, 1987). But, this migration and good fitness is possible only if there is no limitation in their migration (no barriers).

The Prespa trout and its populations in the three rivers are separated from the lake. In the past, the migration from the rivers into the Prespa Lake and opposite was possible. The Lake offers a large source of food, and the migration itself and the source of food from the lake provides the trout with better fitness, and thus larger dimensions. There is data that in the past the dimensions of the trout were much larger. There is also data that indicates that in the past the trout was present along the Golema River, but as a result of the pollution of this river basin, the fish withdrew and today lives only in the Leva River. The Prespa trout in Kranska River and Brajchinska River are also prevented from migrating freely into the lake today. All this indicates that these are isolated populations within which close bonds have been established for a long time.

The assessment of the distribution of the Prespa trout within the research confirms the restricted geographical range to Brajchinska River, Kranska River and Leva River, which is less than 5000 km^2.

All the three watersheds are not connected at the upper parts. The only possible connection is the Prespa Lake itself. However, the study shows that *Salmo peristericus* is facing certain threats (listed in details within this document) that indicate fragmentation of the area severely affecting the habitat occupancy.

The threat of extinction, spatial distribution, population size and decreasing tendency are the main criteria according to which the population of the Prespa trout needs to remain within the threatened **endangered** category.

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