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Softmouth trout (*Salmo obtusirostris*) in the Upper Neretva-River, Bosnia- Hercegovina



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Title page: Female softmouth trout at a spawning site near Glavatičevo, March 2024. (Photo: A. de Bruin & D. Harrewijn)

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1 Introduction

The vast mountain ranges of the Balkan Peninsula constitute the origin of numerous rivers and streams which include a rich diversity in freshwater species and can be considered biodiversity hotspots for fish and further aquatic organisms, many of which are endangered throughout Europe (Freyhof J. 2012). Up to now the Balkans represent an important European biodiversity hotspot where aquatic species can thrive in waterbodies that are often still unaffected by hydropower and river-regulation related structures (Belletti et al. 2020). Nevertheless due to increasing interest for renewable energies this particularity is at risk as an additional ~3,400 hydropower plants are planned to add to the energy production of ~1,400 plants already existing in the region (Carolli et al. 2023). This development is predicted to put 49 fish species at the risk of extinction or loss of 50-100 % of their Balkan distribution (Weiss et al. 2018). One example of this construction enthusiasm can be found at the Neretva River in southwestern Bosnia-Herzegovina. While the lower reaches of this stream have already been used for large-scale hydropower production since the Yugoslav-era, the untouched upper catchment is planned to be the scene for multiple dam projects one of which is already close to completion. This dam project near the towns of Ulog and Nedavici (Figure 1) will permanently degrade a previously undisturbed habitat that is known to host a wide diversity of terrestrial and aquatic species, many of which are endangered or protected, as well as numerous endemic species (Knokk et al. 2022). One affected species is the softmouth trout (*Salmo obtusirostris*), which occurs in the upper Neretva River from the Konjic regions up to the confluence with the Ljuta River which is located just downstream of the Ulog dam (Glamuzina et al. 2018, Neuburg et al. 2023). This species belonging to the family of salmonids is endemic to the Western Balkans and has so far only been recorded in the Neretva River, as well as in two river systems in Croatia (Krka River & Jadro River) and one in Montenegro (Zeta River) (Freyhof et al. 2020). Outside the Neretva system, it is very rare, and even within the Neretva-River, it has already lost large portions of its habitat due to river development, introduction of non-native species and is under angling and poaching pressure (Glamuzina et al. 2018). New damming operation puts additional stress on its already highly fragmented distribution area in the Neretva River.

The present document depicts findings from field research conducted during the reproductive period of the softmouth trout in the upper Neretva River, within an area that will be impacted by the Ulog hydropower project. Specifically, the focus was on mapping spawning sites and genetically monitoring the presence of the species using eDNA analysis in the upper Neretva River and the Ljuta River, a major tributary. Additionally, snorkel surveys and videography were employed to search for the softmouth trout in a river section that will be affected by residual flow after the completion of the Ulog hydropower-plant.

In 2011 and 2018, the Faculty of Agriculture and Food Sciences and the Faculty of Mathematics at the University of Sarajevo conducted extensive electrofishing surveys throughout the entire upper Neretva River basin (Ostojić & Brka 2018). Selected sections of the Neretva River, depicted in Figure 1, and its tributaries were sampled using backpack electrofishing equipment. The results obtained from these studies are mentioned in this work. During these electrofishing surveys, the softmouth trout was only detected at the lower end of the study area, near Glavatičevo, as shown in Table 1. Further electrofishing efforts and eDNA sampling conducted by BOKU University Vienna in late June 2022 (Neuburg et al. 2023) also confirmed the presence of the softmouth trout at Glavatičevo, but not further upstream. However, according to local fishermen, it is also found upstream near Rajac, an area located 8 km downstream of the confluence with the Ljuta-River, as well as in the Ljuta-River itself. Therefore, the presence of the softmouth trout in areas even further upstream and thus even closer to the dam construction site, as well as in larger tributaries, cannot be excluded and will be investigated in this study.

*Table 1; Results of electrofishing surveys conducted by the University of Sarajevo in 2011 and 2018 at sites that fall within the study area of this research. Values represent the number of individuals caught, standardized per 100 meters of river length. Values marked with * indicate absolute catch numbers, as the fished river length was not included in the data. (x = not caught, nd = no data as no fishing was conducted)*

Location & Year	<i>Salmo obtusirostris</i>		<i>Salmo marmoratus</i>		<i>Salmo trutta</i>		<i>Thymallus thymallus</i>		<i>Onchorynchus mykiss</i>		<i>Squalius cephalus</i>		<i>Phoxinus sp.</i>		<i>Cottus sp.</i>	
	2011	2018	2011	2018	2011	2018	2011	2018	2011	2018	2011	2018	2011	2018	2011	2018
L1-4 "Glavatičevo"	2.2	1.1	0.4	0.1	20.8	1.4	15.4	1.5	x	x	2.6	0.2	10.0	2.8	2.0	0.4
L1-3 "Ladanica"	x	x	x	x	7.8	*23	1.0	nd	x	*35	x	nd	10.4	nd	1.4	*20
L1-2 "Rajac"	x	x	x	x	14.0	9.0	x	1.5	x	x	x	x	x	x	5.5	x
L1-1 "Ocrkavlje"	x	nd	x	nd	7.0	nd	0.7	nd	x	nd	x	nd	x	nd	4.7	nd
L2-1 "Ljuta 1"	x	x	x	x	32.7	22.0	x	x	x	x	x	x	x	x	x	x
L2-2 "Ljuta 2"	x	nd	x	nd	10.7	nd	x	nd	x	*14	x	nd	x	nd	x	nd

2 Study area

This study focuses on an area located in the upper reaches of the Neretva River, between the villages of Nedavic and Glavatičevo (Figure 1). The average yearly discharge of the Neretva at Ulog is 9 m³/s and, fed by numerous tributaries, increases to 39 m³/s at Glavatičevo. Downstream of Ulog, the river runs through a narrow valley, where its course is constrained by the rocky slopes of a forested gorge. This section features a diverse sequence of pool, run, and riffle habitats, with substantial input of rock material and deadwood from the surrounding slopes. Approaching Glavatičevo, the valley widens, and the river's width increases. Here, the Neretva-River flow is slower compared to the upstream gorge-section. Except for a few man-made structures in proximity to buildings, the river flows freely here and where the shores aren't lined by bedrock, naturally eroding riparian structures with flushed out bank areas of

varying depth are present. Additionally, the riverbanks are extensively equipped with deadwood of different size. The riverbed is characterized by significant sediment transport, and extensive gravel bars with side-branches of the mainstream can be found along the course of the river.

Adding to the Neretva-River, this investigation also includes one of its major right-shore tributaries in the upper part, the Ljuta-River which joins the Neretva-River about 10 km downstream of Ulog and is characterized by a steep flow gradient. Discharge data is not available for the Ljuta-River.

eDNA sampling focused on the Ljuta-River and a section of the future residual flow stretch of the Neretva-River. Snorkeling surveys were also conducted in this area. The mapping of spawning sites took place in the Glavatičevo area.

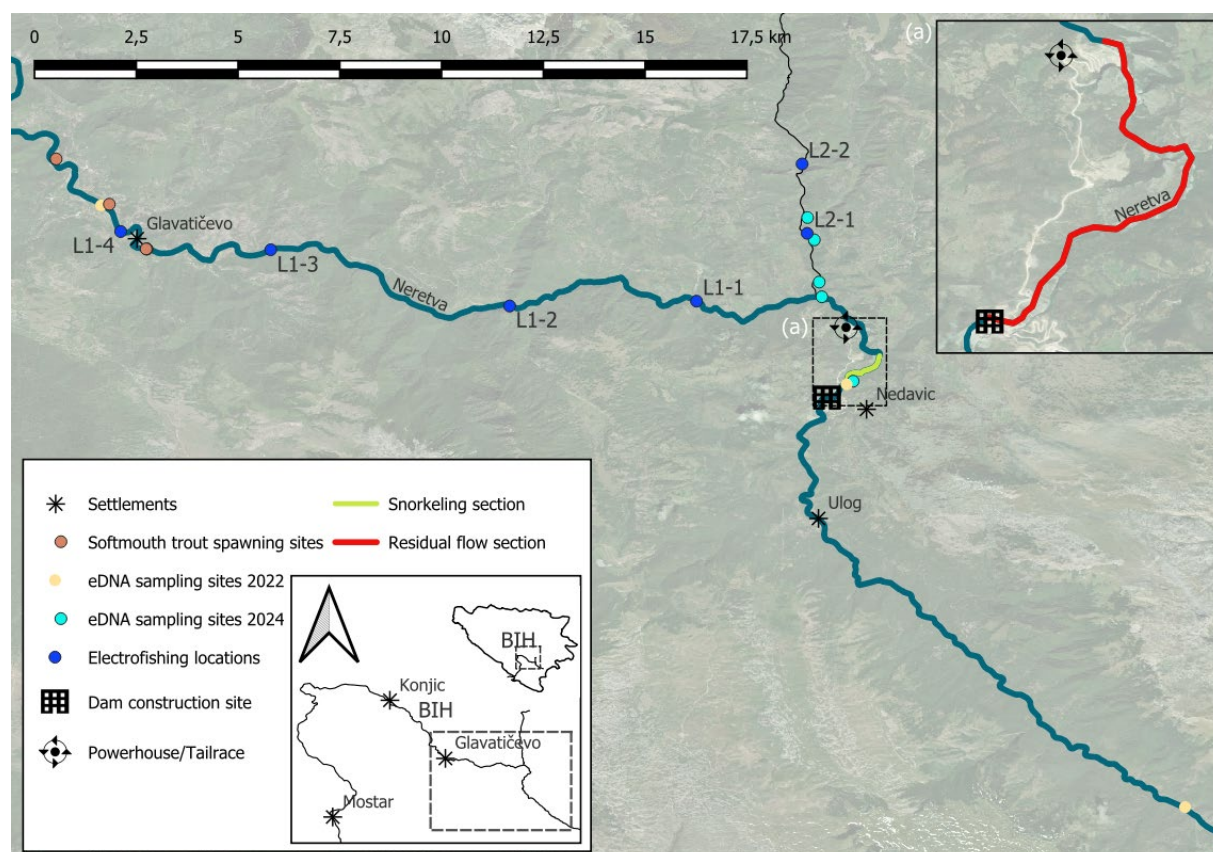


Figure 1: The location of the study area in southern Bosnia-Herzegovina, featuring the Neretva and Ljuta Rivers. Spawning sites and eDNA sampling locations are indicated. The detailed inset illustrates the Neretva-River's course within the residual flow stretch. In this section, eDNA sampling and snorkeling surveys were conducted. Additionally shown are the sites where electrofishing was conducted by the University of Sarajevo in 2011 and 2018 (Ostojić & Brka 2018).

2.1 Characterization of the spawning habitat

The aim of the spawning site mapping was to assess the spatial extent of the spawning sites and to document the water depth, flow velocity, and grain size fractions of the substrate observed at the spawning pits as well as documenting the presence of woody debris and overhanging vegetation (cover) in proximity to spawning redds. Using measuring tapes and a laser distance meter, the distance from the outermost edge of each spawning site to the shore was measured. Subsequently, the length of each spawning site along the flow direction and the width perpendicular to the flow direction were recorded. For spawning sites with irregular shapes, the width was measured at multiple points. The individual spawning pits were measured for water depth and flow velocity. The water depth was recorded at the pit-front, pit bottom, and tail spill crest as shown in Figure 2. Flow velocity was determined at the same locations using a portable device (Flo-Mate) and measuring at the surface (V_{surface}) as well as at 60% of the water depth ($V_{0,60}$). The flow velocity (V_m) was then calculated using the following equation:

$$V_m = [0,31 \cdot v_{\text{surface}}] + [0,634 \cdot v_{0,60}]$$

The assessment of the quantitative composition of different substrate classes at spawning redds was conducted through a visual estimation across the spawning site. The classification was conducted according to abiotic choriotypes (Jungwirth et al. 2003). Hereby a discrimination between the grain sizes psammal (0.063-2 mm), akal (2-20 mm), microlithal (20-63 mm) and mesolithal (63-200 mm) as well as larger and smaller grain sizes is used. Moreover, the presence/absence of overhead cover and deadwood structures was also recorded.

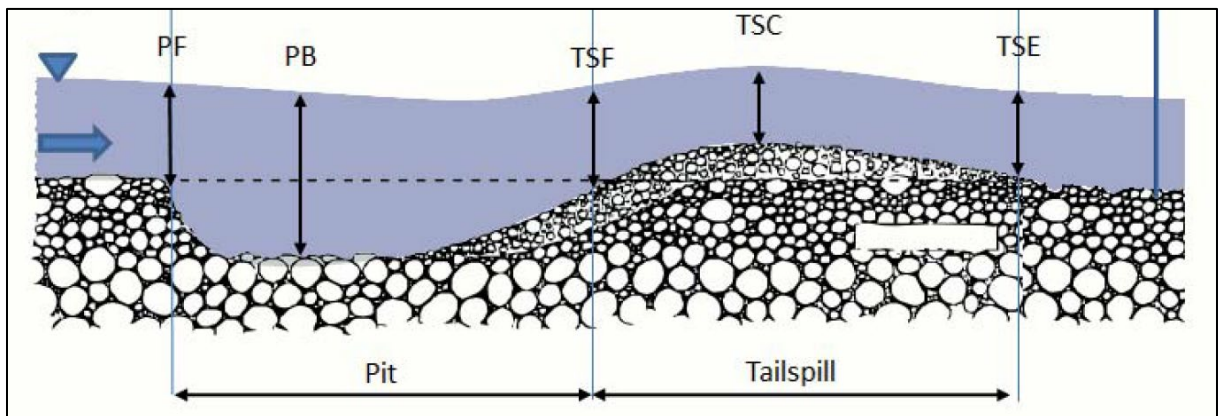


Figure 2: Illustration of the recording method for a salmonid redd (spawning pit) according to Johnson et al. (2007). (PF = Pit Front, PB = Pit Bottom, TSF = Tail Spill Front, TSC = Tail Spill Crest, TSE = Tail Spill End)

2.2 eDNA-sampling

The samples for eDNA analysis were collected at five locations depicted in Figure 1. Two of these locations were in the Neretva-River, specifically within the future residual flow section

below the newly constructed power plant and at the confluence with the Ljuta tributary. The other three samples were taken in the Ljuta River. For standardized sampling, 30 l of river-water were collected at each location using a peristaltic pump driven by a commercially available cordless drill and filtered through an eDNA filter (SpyGen-VigiDNA) with a pore size of 0.45 µm. The pumping and filtration process took ~15 minutes at each location. The filtrate was then stabilized with a buffer solution to prevent degradation of the DNA sample. The analysis of the samples was conducted by the Central Research Laboratories of the Natural History Museum of Vienna. In this study, we relied on previously conducted DNA extraction and analysis performed in 2022 (Neuburg et al. 2023). The methods included the use of DNeasy Blood & Tissue Kit (Qiagen) and DNeasy Power Soil Pro Kit (Qiagen) for DNA extraction, with appropriate negative and positive controls to monitor contamination and verify species identification. Marker sequences targeting partial regions of the mitochondrial cytochrome c-oxidase subunit 1 gene (CO1) and the mitochondrial control region (CR) were used to detect *Salmo* spp., following established protocols. Species-specific primers for *Salmo obtusirostris* were designed to exclude other *Salmo* species, ensuring precise detection. These DNA extraction and PCR protocols form the basis of the species identification results discussed in this report, allowing us to build upon the foundation established in the 2022 study.

2.3 Snorkeling & Filming

A snorkeling survey was conducted in the residual flow section of the upper Neretva River, directly downstream of the Ulog dam building site at Nedavić (Figure 1). Using dry suits and masks, this section was inspected by allowing the current to carry the observers downstream. Fish of all possible species were targeted in the present habitats. During the snorkeling survey, the investigated structures were video documented using a GoPro camera. Efforts were made to minimize disturbance during the survey to avoid skewing results by scaring away potentially present fish. In addition, GoPro action cameras mounted on extension poles were used to search for fish in promising structures, such as deeper pools or calm waters behind large rocks in the current. This was carried out using wading equipment, rather than through snorkeling.

3 Results

3.1 Characterization of the spawning habitat

Five softmouth trout spawning sites could be localized near Glavatičevo between March 20 and 21, 2024. The water level of the Neretva River was 60 cm, measured at the gauge near Glavatičevo (43.5018436 N, 18.1064895 E). No water temperature measurements were conducted.

At all spawning sites the substrate had been dug up and cleared of fine sediment by spawning softmouth trout. Additionally, at one spawning site where seven individuals were encountered, two individuals were observed engaging in a spawning act, during which milt and eggs were released into the substrate and subsequently covered with gravel by the female.

Table 2: Depiction of measured softmouth trout spawning grounds (SG) near Glavatičevo. Vegetation cover refers to overhanging vegetation from the banks covering the spawning area. Deadwood describes woody debris encountered at the spawning sites near the observed redds. Distance to shore describes the maximum distance of the SG to the nearest shore.

Spawning site	Lat (WGS 84)	Lon (WGS 84)	Area [m ²]	Number of pits	Vegetation cover	Deadwood	Distance to shore [m]
SG1	43,498714	18,107547	0.8	1	yes	yes	4.0
SG2	43,498691	18,107482	3.3	1	yes	yes	3.4
SG3	43,49874	18,107311	0.8	1	yes	no	4.7
SG4	43,512258	18,096162	64.5	9	yes	yes	7.45
SG5	43,525964	18,08005	17.0	2	yes	yes	4.4

All five spawning sites around Glavatičevo were situated in close proximity to the riverbank, with their outer edges ranging between a minimum- of 3.4 m and a maximum-distance of 7.5 m from the shore. Overhanging riparian vegetation provided cover at all spawning sites, and deadwood structures in the proximity of the spawning sites were observed at all sites, except one. The spawning areas measured an average length of 6.8 m and width of 1.7 m. The largest spawning site was 22 m long and 5 m wide at its broadest section. The smallest spawning site measured 0.8 m², the largest 64.5 m². A total of 9 dug-out pits were counted at the largest site shown in Figure 5 whereas the two smallest spawning sites contained one single pit each. On average, the softmouth trout had dug 8 cm deep into the substrate at their spawning pits. We were able to observe two fish at a spawning site during the survey, but our guide informed us that he had seen 13 fish at the largest spawning site the day before, eight of which were males and five females. During an initial inspection of the Glavatičevo area on the previous day, we were able to observe two and seven softmouth trout at two spawning sites, respectively.

Table 3: Representation of the measured water depths and flow velocities at the spawning pits of the softmouth trout.

	Water-depth [cm]			Flow velocity [m/s]			distance shore [m]
	pit-front	pit-bottom	tail-spill-crest	pit-front	pit-bottom	tail-spill-crest	
Mean	0.63	0.61	0.46	0.57	0.59	0.68	4.0
Median	0.53	0.58	0.45	0.55	0.57	0.65	3.4
Max	1.12	1.10	0.60	0.90	0.84	0.95	4.7
Min	0.43	0.47	0.23	0.39	0.46	0.53	7.45
SD	0.26	0.32	0.24	0.23	0.30	0.35	4.4

The average water depth measured at the pit-fronts was 0.63 m (0.53 m median), with maximum depths reaching 1.12 m and minimum depths recorded at 0.43 m. In contrast, the average water depth at the pit-bottoms was 0.61 m (0.58 m median), with maximum depths of 1.10 m and minimum depths of 0.47 m. At the highest point of each spawning pit, the tail-spill crest, the average water depth measured was 0.46 m (0.45 m median), with a maximum depth of 0.6 m and a minimum depth of 0.23 m.

Flow velocities were highest at the tail-spill crest, with an average of 0.68 m/s (0.65 m/s median). The maximum flow velocity recorded at this structure was 0.95 m/s, while the minimum was 0.53 m/s. At the pit-bottom, the average flow velocity was 0.59 m/s (0.57 m/s median), with a maximum velocity of 0.84 m/s and a minimum of 0.46 m/s. At the pit-front, the average velocity was 0.57 m/s (0.55 m/s median), with a maximum of 0.9 m/s and a minimum of 0.39 m/s. Figure 3 presents the water depths and flow velocities at the various sampled structures of the spawning pits.

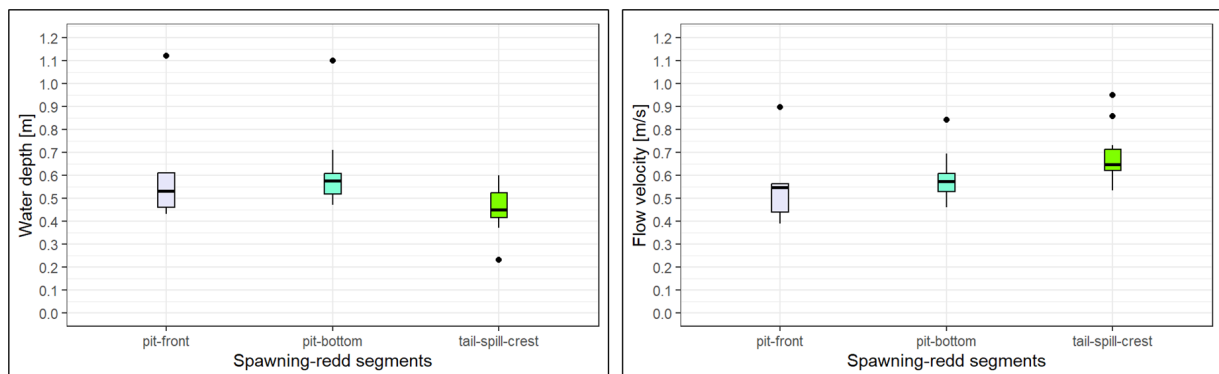


Figure 3: Boxplots illustrating the distribution of water-depth as well as flow-velocity measurements at selected spawning redd segments.

As can be seen in Figure 4, the dominant substrate class at all spawning sites is gravel (microlithal), consisting of particles with a grain size of 20-63 mm. This substrate class accounts for 55 % of the total substrate composition at the spawning sites as shown in Figure 4. The second most common substrate class is pebbles (akal), which have a grain size of 2-20 mm and make up an average of 28 % of the substrate. Stones (mesolithal) with a grain size of 63-200 mm are the least represented, comprising only 17 % of the overall substrate composition.

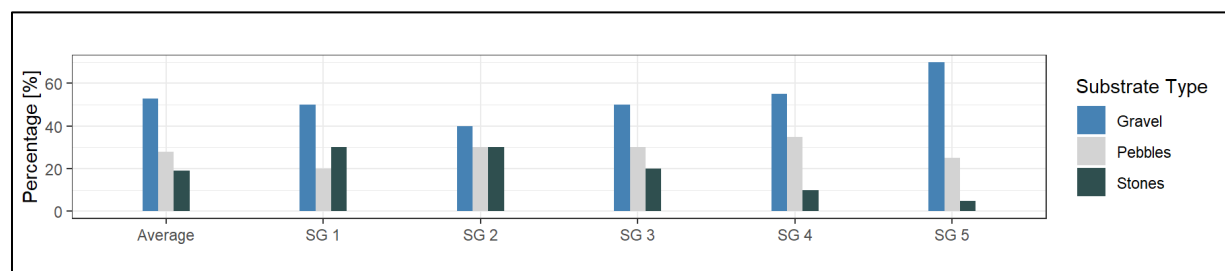


Figure 4: Substrate composition at spawning sites depicted by bar charts. Average percentages of substrate composition at all sites is shown at the left-hand site. Individual spawning sites are labelled "SG".



Figure 5: The picture shows the largest spawning site (SG4) found near the village of Glavatičevo. ©Kurt Pinter

3.2 eDNA

Summary taken from Pinter et al. (2025): *Our results revealed S. obtusirostris haplotypes in 8 out of 11 sampling sites, confirming its presence in Glavatičevo, at the Ljuta estuary, and within the Ljuta River. The findings suggest that the species may inhabit the entire river stretch between Glavatičevo and the Ljuta estuary, a distance of about 25 km, and that it is also capable of colonizing steep and turbulent habitats such as those of the Ljuta River. However, no S. obtusirostris haplotypes were detected upstream of the Ljuta confluence (sites Ner_2, Ner_3, Ner_6), supporting previous observations that the upper distribution limit of the species in the Neretva River has already been reached in this region. An important methodological insight is that the primers employed were not entirely species-specific. While the CR primer pair showed comparatively high specificity, mixed haplotypes (S. obtusirostris / S. trutta) occurred repeatedly in CO1-based amplifications.*

3.3 Snorkeling

During the snorkeling survey at the future residual flow section, a diverse range of run, pool, and riffle habitats was examined within an approximately one-kilometer-long river section. The visibility conditions were good, and except for very deep pools, all structures in the riverbed could be clearly observed. A significant layer of fine sediments was observed covering most of the streambed (Figure 6). The construction of the Nedavic power station caused this layer of sediment of unknown material. Overall, no fish except for a single bullhead (*Cottus sp.*) were observed in the entire snorkeled section.



Figure 6: A layer of fine material covering most of the streambed downstream of the Ulog Hydropower plant.

4 Discussion

As part of this study, during spring 2024 the distribution of the softmouth trout (*Salmo obtusirostris*) was investigated in the upper reaches of the Neretva River as well as within the Ljuta River, a major tributary of the upper catchment. This investigation was conducted through environmental DNA (eDNA) sampling and analysis. Furthermore, a description of the softmouth trout's spawning grounds including the prevailing habitat conditions was carried out.

4.1 Distribution of *S.obtusirostris*

The results of the eDNA analysis indicate the presence of the species in the Neretva-River right up to the confluence with the Ljuta-River (Figure 1). This signifies, that the softmouth trout occurs significantly further upstream than previously documented in scientific literature (Glamuzina et al. 2018; Neuburg et al. 2023; Škrijelj et al. 2011). There were anecdotal reports from local fishermen suggesting the presence of the softmouth trout in the Neretva-River up to the confluence with the Krupac tributary, a short and dwell fed tributary just upstream the Ljuta River. Based on the eDNA result, the occurrence of the species in these upper parts of the

Neretva basin can thus be proven (see also Pinter et al. 2025). Moreover, the species is also proven to be present in the Ljuta-River itself. Our findings, based on the localization of softmouth trout haplotypes, support the occurrence of the species in the areas indicated by local fishermen. Both the sample taken at the confluence of the Ljuta and Neretva rivers and the three samples from the Ljuta River tested positive for softmouth trout haplotypes. Pinter et al. (2025) could bring additional prove of the species occurrence just downstream the road-bridge crossing the Ljuta River some 3 km upstream the confluence with the Neretva River. However, the results indicate that eDNA-based detection in this system may be influenced both by primer specificity and by the relative abundance of haplotypes in the water. Furthermore, haplotype detection alone cannot determine whether sequences originated from pure *S. obtusirostris* individuals or from hybrids with the *S. trutta* complex. Previous reports of hybridization in the Neretva catchment (Razpet et al. 2007; Sušnik et al. 2007) and suspicions of fish farm introductions highlight the need for caution in interpretation. Despite these uncertainties, the findings are consistent with oral reports from local fisheries managers that point to the presence of *Salmo obtusirostris* along this river section and in the Ljuta tributary. The absence of detections in electrofishing surveys (Ostojić & Brka 2018) may reflect methodological limitations due to the species' low abundance, elusive behavior, and preference for hidden habitats such as deep pools.



Figure 7: The Ljuta River, a tributary in the upper reaches of the Neretva River, derives its name from the Bosnian word for "angry"—a designation that becomes evident when observing the exposed bedrock scoured by its turbulent flows during periods of high discharge. Despite its steep gradient and hydrologically dynamic character, this stream sustains ecologically significant habitats for the endangered softmouth trout (*Salmo obtusirostris*).

According to our findings, the distribution range of the species thus extends to the immediate vicinity of the Ulog hydropower plant. Nevertheless, the habitats present in the residual flow section correspond to the habitats in the Neretva River downstream of the confluence with the Ljuta River and the historical presence of softmouth trout cannot be excluded there. It remains unclear whether the high input with fine sediments (Figure 6) or other disturbances due to the construction activities at the Ulog dam caused the absence of softmouth trout (and fish in general) in the section below the dam or whether other reasons are responsible for the lack of this species.

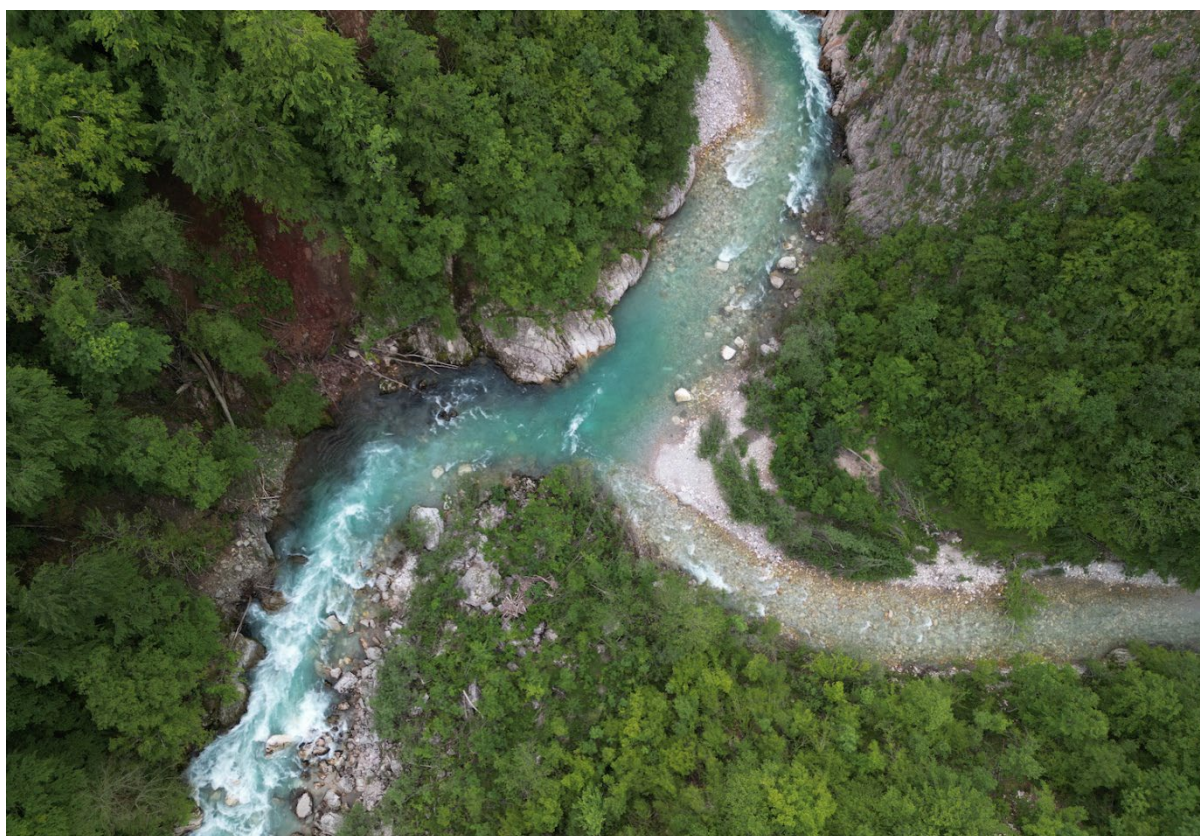


Figure 8: Confluence of the Ljuta River and the Neretva River. © Joshua Lim

4.2 Spawning habitat of *S. obtusirostris*

The habitat characteristics observed at five spawning sites show typical characteristics for spawning sites of salmonids. For instance, the substrate class of microlithal was predominant, followed by akal. Fine sand (psammal) constituted the smallest portion of the substrate composition. This composition is, for example, also characteristic of European grayling (*Thymallus thymallus*) spawning sites. However, there are differences in the water depth at which spawning occurred. While European grayling prefer areas with a water depth of 0.2-0.4 m, the spawning pits of the softmouth trout in the Neretva River were found at depths of 0.4-0.6 m, with one even located in water 1.1 m deep. The flow velocities are similar to those preferred by grayling, which spawn in areas with moderate flow velocities of 0.5-0.7 m/s

(Sempeski 1995; Nykänen and Huusko 2002; Gönczi 1989). Softmouth trout dug their spawning pits in areas with flow velocities of 0.5-0.6 m/s. Brown trout (*Salmo trutta*) typically create their spawning pits in shallower (headwater) sections than softmouth trout, typically between 0.1-0.5 m below the water surface; the flow velocity at brown trout spawning sites is also more moderate (0.2-0.5 m/s) compared to those of the softmouth trout (Louhi et al. 2008; Zimmer and Power 2006; Gauthey et al. 2017). For brown trout, microlithal also constitutes the main fraction of the spawning substrate (Louhi et al. 2008). It was noticeable that all of the spawning sites observed were located in the riparian zone, providing a degree of protection from the surrounding vegetation and also from accumulations of woody debris.

4.3 Hydropower impact

The upper reaches of the Neretva-River and the entire course of the Ljuta-River are free of constructions, which is why a multitude of diverse habitats can be found here that are characteristic of an unregulated wild river. An interdisciplinary ecological study from 2022 emphasizes the great importance of the upper Neretva-River catchment area for both terrestrial and aquatic biodiversity and addresses the ideal habitat quality for all developmental stages of the softmouth trout (Knokk et al. 2022). The construction and operation of a hydropower plant means a certain loss in such a valuable area in every respect. This is especially true for the softmouth trout.

The confluence area of the Ljuta River, representing the location of our most upstream positive eDNA sample is located approximately 1.4 km downstream from the tailrace of the newly constructed hydropower. Additionally, it is possible that the species is present further upstream in the residual flow section, despite a negative eDNA sample, since the habitat characteristics are similar to those areas of the Neretva where we have already detected the species. Due to this high proximity, hydromorphological anomalies in the Neretva River caused by the plant's operation will have a pronounced impact in this area but also further downstream sections like the Glavatičevo area will encounter pressures caused by the HPP.

The newly documented distribution area of the soft-mouth trout is likely to experience significant stress. Transverse structures associated with hydropower usage act as migration barriers for migratory fish species (Schmutz and Sendzimir 2018), and following the construction of the Ulog dam, the upstream migration of soft-mouth trout to areas further upstream will no longer be possible. Reduced migration capabilities can result in isolation and increased genetic homogeneity (Wofford et al. 2005). Furthermore, downstream-migrating individuals are likely to experience increased mortality due to turbine passage (Schmutz and Sendzimir 2018). Adding to this, comes the blockage of sediments behind the dam which

influences the availability of crucial habitats e.g. spawning and feeding grounds. This is particularly true for larger sediment fractions, while the transport of fine sediments remains possible. This imbalance in sediment transport can lead to the downstream clogging of the riverbed, as fine sediments increasingly accumulate over time. Sediments that accumulate behind the dam might periodically be flushed downstream to increase the reservoir's storage capacity (Schmutz and Sendzimir 2018). This process has severe impacts on the biocenosis downstream of the dam, as it releases large amounts of sediment in short periods of time. Early developmental stages of rheophilic fish species are particularly affected by this phenomenon.

Furthermore, the hydropeaking operation of hydropower plants pose a significant threat to the softmouth trout population. Hydropower plants do not always operate at full capacity or at a constant rate, leading to fluctuations in the water volume passing through the turbines. These fluctuations cause considerable variations in the discharge of the affected water bodies which is especially expressed in shallow areas such as riverbanks and gravel bars. Since juvenile fish and fish larvae don't yet have fully developed swimming capabilities, they tend to colonize these structures and often get stranded with suddenly decreasing water levels (Hayes et al. 2019). On the other hand, rapid increases in the water levels come with increased flow velocities which can result in down drifting of the mentioned early developmental stages of river-fish (Schmutz and Sendzimir 2018). Both effects boost each other e.g. fish that seek shelter during peakflow in shallow areas face an even higher stranding risk as soon as the water level drops. Both stranding and- drifting cause high mortality rates (Auer et al. 2017). For European grayling, a species with similar ecologic traits as the softmouth-trout, hydropeaking is the main cause of the drastic population decline in parts of its distribution range (Hayes et al. 2021). At the same time as this report is being finalised, the Ulog power station is starting trial operations and unnatural and pronounced water level fluctuations are already being observed at a downstream gauge (Figure 9). These water level fluctuations are likely to already have a negative impact on reproductive success in 2025. This underlines the urgent need for action to protect this rare species and the aquatic fauna in general.



Figure 9: Records from the water level station at Kašići. The fluctuations observed on 15 March 2024 are of anthropogenic origin and are due to the operation of the Ulog power plant (source: <https://www.fop.fhmzbih.gov.ba/>).

4.4 Poaching

In addition to habitat loss and competition from invasive species such as the European grayling, the illegal capture and killing of softmouth trout also poses a problem (Glamuzina et al. 2018). A fisheries inspector from a local fishing association mentioned to us several times that poaching is a widespread phenomenon in the area. Furthermore, the Neretva River is difficult to monitor in its narrow gorge sections, which complicates oversight by personnel. Illegal fishing was already an issue before the construction of the new power plant. However, habitat degradation due to hydropower utilization in the upper Neretva can significantly weaken the population, thereby exacerbating the impact of poaching.

5 Conclusion and recommendations

In this study, we explored a part of the Neretva River catchment that hasn't so far been impacted by hydropower exploitation. This situation is now changing with the operation of the Ulog power plant near Nedavic, which, according to our eDNA analyses, is in close proximity to or within the distribution range of the softmouth trout. Additionally, there are about a dozen other hydropower projects planned in this area (Schwarz 2022), affecting both tributaries and the Neretva River. Therefore, it is expected that the softmouth trout population will face significant pressure and that the stocks will decline. Continuous monitoring of its population status is essential to detect changes in biomass, abundance, and age structure early and to implement countermeasures.

Since the Ljuta-River has also been identified as a softmouth trout habitat, it is crucial, to protect it as well as other similar-sized tributaries from further hydropower-related interventions. Intact tributaries can serve as refuge habitats in the affected area. Currently, a total of eight small scale hydropower plants are planned in the Ljuta-River alone (Schwarz 2022), and other tributaries in the area are affected by future construction projects (Schwarz 2022). The best scenario for the survival of the softmouth trout involves the decommissioning of the power plant at Nedavic and the avoidance of further hydropower developments in this ecologically significant area.

The endangered softmouth trout has a very restricted distribution range and is very rare outside the Neretva-System (Freyhof J. 2012). Our findings underline that the upper Neretva still supports a reproductively viable softmouth trout population capable of sustaining itself through natural reproduction. The lower reaches of the Neretva-River have historically been heavily exploited for hydropower production, resulting in the species being partially absent or only present in larger tributaries there (Glamuzina et al. 2018). Numerous hydropower projects in

the upper reaches of the Neretva pose a risk of putting the species under the same pressure in its last remaining undisturbed habitat. It is anticipated that the population of the softmouth trout in the affected area will decline. Given that the species is already very rare or absent in the lower Neretva-River, and is potentially threatened by hydropower projects in rivers within its very limited distribution area outside the Neretva-System (Schwarz 2022), an integrated conservation strategy is urgently required. Stocking activities, particularly with hybrids, should be prohibited to protect the genetic integrity of the softmouth trout population. Non-native fish species such as European grayling should be reduced. Hydropower-induced discharge fluctuations must be prevented and further hydropower development in the Neretva and its tributaries abandoned. Habitat of softmouth trout requires strict protection, while poaching must be effectively controlled. Finally, long-term monitoring should further clarify the species' distribution, population status, habitat requirements and degree of hybridisation.

6 Literature

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