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The ichthyofauna of the upper Neretva River

Jakob NEUBURG[1], Thomas FRIEDRICH[1], Elisabeth HARING[2,3], Sarah HÖFLER[4], Ana MARIC[5], Saša MARIĆ[5], Stephanie POPP[1], Predrag SIMONOVIĆ[5,6], Aleš SNOJ[7], Simona SUŠNIK BAJEC[7], Simon VITECEK[1,8], Alexandra WANKA[2], Steven WEISS[9], Kurt PINTER[1]*

[1]University of Natural Resources and Life Sciences, Institute of Hydrobiology and Aquatic Ecosystem Management, Gregor-Mendel-Straße 33, 1180 Vienna, Austria
[2]Natural History Museum Vienna, Maria-Theresien-Platz 1010 Vienna, Austria
[3]University of Vienna, Department of Evolutionary Biology, Djerassiplatz 1, 1030 Vienna
[4]Blattfisch e.U. – Consultants in Aquatic Ecology and Engineering, Leopold-Spitzer-Straße 26, 4600 Wels, Austria
[5]University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia
[6]University of Belgrade, Institute for Biological Research »Siniša Stanković« - National institute of the Republic of Serbia, Blvd. Despot Stefan 142, 11000 Belgrade, Serbia
[7]University of Ljubljana, Department of Animal Science, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[8]WasserCluster Lunz – Biologische Station GmbH, Dr. Carl Kupelwieser Promenade 5, 3293 Lunz am See, Austria
[9]University of Graz, Faculty of Natural Sciences Universitätsplatz 2, 8010 Graz, Austria
*Corresponding author. E-mai: kurt.pinter@boku.ac.at

Abstract. Fish were sampled at nine locations of the upper Neretva River, upstream of Glavatičevo. Evidence of the endangered softmouth trout (*Salmo obtusirostris*) was obtained as well as the presence of other native and non-native salmonids. Genetic analysis suggests that the native brown trout (*Salmo trutta*) in the upper Neretva is homogeneous with no significant introgression by introduced Atlantic-lineage brown trout and quantitative catch data show that their populations are healthy with densities above 1,000 ind/ha. However, the relatively low biomass estimates may be the result of overharvesting. A new haplotype of the mitochondrial control region of the bullhead (*Cottus gobio*) was detected and confirmation of native *S. trutta*, *Barbatula barbatula*, *Phoxinus* sp., and *C. gobio* in the upper reaches, underlining the importance of this river stretch. The planned expansion of hydropower plants in the upper Neretva and its tributaries will have a significant negative impact on the largely untouched ichthyofauna, as is clearly demonstrated by the conditions found in the middle course of the river, which has been dedicated to hydropower production.

Key words: fish stock assessment, electrofishing, snorkelling, eDNA, genetics, hydropower, *Salmo obtusirostris*

Izvleček. Ihtiofavna gornje Neretve – Ribe so bile vzorčene na devetih lokacijah zgornjega toka reke Neretve, nad Glavatičevom. Ugotovljena je bila prisotnost ogrožene mehkoustne postrvi (*Salmo obtusirostris*) kot tudi drugih avtohtonih in neavtohtonih salmonidov. Genetska analiza kaže, da je avtohtona potočna postrv (*Salmo trutta*) v zgornjem toku Neretve homogena, brez pomembne introgresije uvedenih atlantskih linij potočne postrvi, kvantitativni podatki o ulovu pa kažejo, da so njihove populacije zdrave z gostotami nad 1000 osebkov/ha. Vendar pa relativno nizke ocene biomase lahko izhajajo iz prekomernega izlova. Odkrit je bil nov haplotip mitohondrijske kontrolne regije glavoča (*Cottus gobio*); potrditev avtohtonih vrst *S. trutta*, *Barbatula barbatula*, *Phoxinus* sp. in *C. gobio* v zgornjih delih poudarja pomen tega odseka reke. Načrtovana širitev hidroelektrarn v zgornjem toku Neretve in njenih pritokov bo imela negativen vpliv na večinoma nedotaknjeno ihtiofavno, kar kažejo razmere v srednjem toku reke, ki je že namenjena proizvodnji hidroenergije.

Ključne besede: ocenjevanje staleža rib, elektroribolov, potapljanje, eDNA, genetika, hidroenergija, *Salmo obtusirostris*

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Apstrakt. Ihtiofauna gornje Neretve – Ribe su uzorkovane na devet lokacija gornjeg toka rijeke Neretve, uzvodno od Glavatičeva. Dokazano je postojanje ugrožene mekousne pastrmke (*Salmo obtusirostris*) kao i prisutnost drugih autohtonih i alohtonih salmonida. Genetska analiza sugeriše da je autohtona smeđa pastrmka (*Salmo trutta*) u gornjem toku Neretve homogena bez značajne introgresije uvedenih atlantskih linija smeđe pastrmke, a kvantitativni podaci o ulovu pokazuju da su njihove populacije zdrave s gustoćama iznad 1.000 jedinki/ha. Međutim, relativno niske procjene biomase mogu biti rezultat prekomjernog izlova. Otkriven je novi haplotip mitohondrijske kontrolne regije peša (*Cottus gobio*). Potvrda autohtonih *S. trutta, Barbatula barbatula, Phoxinus sp.*, i *C. gobio* u gornjim tokovima naglašava važnost ovog dijela rijeke. Planirano širenje hidroelektrana na gornjoj Neretvi i njenim pritokama imaće značajan negativan utjecaj na uglavnom netaknutu ihtiofaunu, što jasno pokazuju uslovi u srednjem toku rijeke, koji je predviđen za proizvodnju hidroenergije.

Ključne riječi: procjena ribljeg fonda, elektroribolov, ronjenje, eDNA, genetika, hidroenergija, *Salmo obtusirostris*

Introduction

Rivers draining the slopes of the Western Balkans hold significant importance as biodiversity hotspots, with a high number of endemic and rare species (Freyhof & Brooks 2011; Oikonoumou et al. 2014; Schöffmann et al. 2019). Freshwater fish species diversity in this region is considered the highest in Europe (Skoulikidis et al. 2009). Unlike in many other European regions, the upper reaches of many Balkan rivers are free-flowing and relatively unaffected by major anthropogenic alterations such as channelisation or dams (Skoulikidis et al. 2009). In order to maintain the ecological integrity of the region and to foster ecosystem resilience in the face of climate change (cf. Keppel et al. 2012), management actions in these systems need to proceed with great care.

The Neretva River is one of the significant watercourses of the Western Balkan Peninsula. It originates in the Dinaric Alps and descends through rugged landscapes and a sequence of bedrock canyons and plains before entering the Adriatic Sea (Skoulikidis et al. 2009). The hydrological regime, as well as the sediment regime, are in a pristine state in the upper reaches where the river has a natural character with a pronounced diversity of habitats.

Thirty-four fish species are listed as native to the Neretva River basin, 17 of which are classified as endemic (Tutman et al. 2012; Vukić et al. 2019). Vukić et al. (2019) reported the presence of 32 introduced species. The salmonids native to the upper Neretva are the endangered softmouth trout (*Salmo obtusirostris*) (Crivelli 2006), marble trout (*Salmo marmoratus*), and native brown trout (*Salmo trutta*). Although we are aware that the taxonomic status of native Neretva trout (i.e., other than softmouth and marble trout) is complicated and uncertain, these considerations are beyond the scope of the present paper, so we will simply refer to them as (Neretva) brown trout (*Salmo trutta*). Brown and softmouth trout frequently interbreed and produce fertile hybrids. Adult purebreds are phenotypically clearly recognized and discerned from hybrids, while young specimens are more difficult to identify.

Predominantly in the impoundments of the middle reaches of the Neretva, numerous non-native species have been introduced through recreational fishing activities such as the European grayling (*Thymallus thymallus*), rainbow trout (*Oncorhynchus mykiss*), Alpine charr (*Salvelinus umbla*), brook trout (*Salvelinus fontinalis*) (Pavličević et al. 2016; Glamuzina et al. 2018; Vukić et al. 2019; Tutman et al. 2021), and non-native lineages of the brown trout (e.g., the Atlantic lineage; Razpet et al. 2007; Snoj et al. 2010). Genetic traces of the Danube lineage of brown trout have also been observed in the upper Neretva tributaries (Bernatchez et al. 1992; Razpet et al. 2007; Kalamujić et al. 2015), which raises the question of whether they naturally occur there or are the result of human-mediated translocations. Also, the fish fauna of the lower reaches of the river is currently undergoing a major transition, as seawater intrusion increases accompanied by the invasion of marine organisms (Glamuzina & Dobroslavić 2020; Tutman et al. 2021).

Studies related to fish biology and ecology on the Neretva have recently focused on the lower reaches and the estuary into the Adriatic Sea (Bartulović et al. 2004; Ivanković et al. 2010; Dulčić et al. 2017; Glamuzina et al. 2017; Glamuzina & Dobroslavić 2020). Even though studies describing the fish population in the upper reaches of the Neretva have been conducted (Razpet et al. 2007; Glamuzina et al. 2018), detailed population parameters like abundance and biomass or age class distributions are largely missing or not easily accessible to the international scientific community due to language barriers (Vegara et al. 2009; Muhamedagić et al. 2019). Molecular genetic studies on fishes of the Neretva were mainly focused on resolving genetic variation and taxonomic uncertainties within the genera *Salmo* (Bernatchez et al. 1992; Snoj et al. 2002; Razpet et al. 2007; Snoj et al. 2010; Kalamujić et al. 2015), *Cottus* (Bravničar et al. 2015), and *Phoxinus* (Palandačić et al. 2020). The majority of species in the Neretva remain understudied (ex. Tutman et al. 2017).

Here we present results of a fish ecological assessment that covers parts of the upper Neretva relying on both stock assessment and molecular genetic methods. The aims of this study were to provide a detailed stock assessment of the fish community in the upper Neretva, to provide additional insights into the systematics and the distribution of individual species as well as to potentially address some uncertainties on their native status. In addition to standard electrofishing and genetic analyses, a snorkelling survey and environmental DNA (eDNA) analysis were performed. Genetic analyses included a population genetic approach for brown trout using microsatellite markers, as well as the sequencing of mitochondrial DNA sequences for brown trout, minnow (*Phoxinus sp.*), and bullhead (*Cottus gobio*). Additionally, eDNA was extracted from water samples and analysed to try to detect softmouth trout in river sections that were either not sampled directly by other means, or where the species may have been so rare that they could have been missed with other approaches. A water sample from Glavatičevo, where softmouth trout are known to occur (Glamuzina et al. 2018), was included in the analysis as a positive control for the eDNA analysis.

Materials and methods

Study area

The upper Neretva flows roughly 100 km from its source to Jablaničko Reservoir downstream of Konjic (Fig. 1). Due to the high gradient throughout, the river's channel morphology can largely be described as constrained, with long sections running through gorges and occasional branching areas where the valley opens up. The annual mean discharge at Ulog amounts to 9 m^3/s and increases to 39 m^3/s at Glavatičevo (DIKTAS B&H 2012). Sampling was conducted along the main course of the upper Neretva and in two smaller tributaries, namely the Krupac, located in the upper part of the investigation area, and the lower part of the Jezernica downstream of Ulog (Fig. 1). Surveys were carried out at nine sites, eight of which are located close to the town of Ulog and one 30 km downstream at Glavatičevo, covering a river length of roughly 60 km (see also Tab. 1). The slopes at the sites Mjedenik, Confluence, Cerova, Swimming Beach, Nedavić, and Glavatičevo were 4.6%, 1.1%, 1.3%, 0.3%, 4.3%, and 0.2%, respectively.

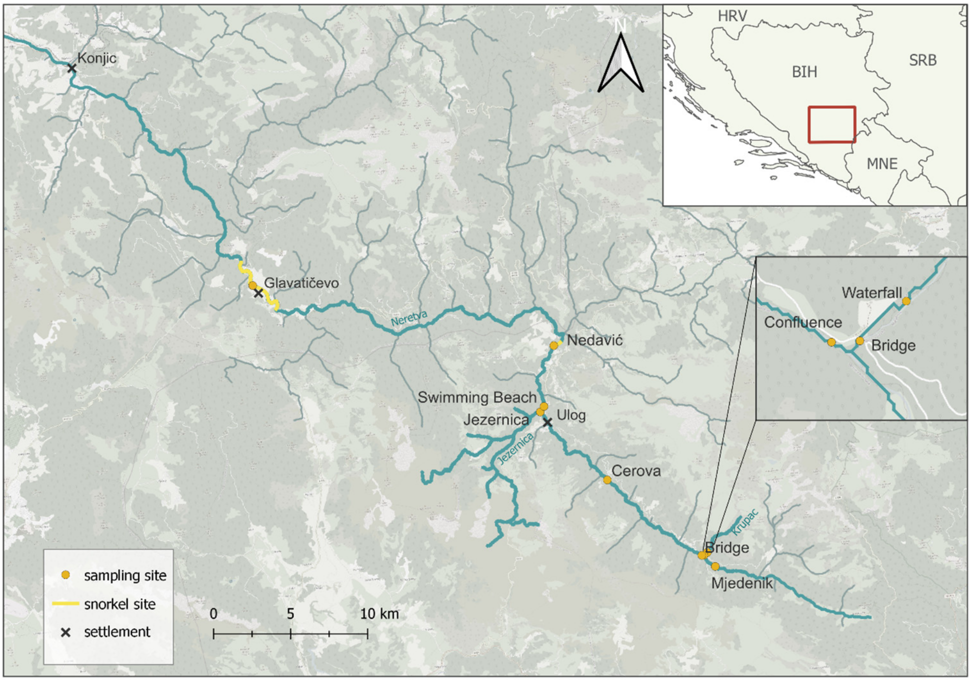

Figure 1. Study area with all respective sampling sites (orange dots) and the snorkelling site (yellow line).
Slika 1. Raziskovano območje z označenimi vzorčnimi mesti (oranžne pike) in lokacijo potapljanja (rumena črta).
Slika 1. Područje istraživanja sa svim odgovarajućim lokacijama uzorkovanja (narandžaste tačke) i mjestom ronjenja (žuta linija).

Field methods

Electrofishing

To assess the fish fauna, multiple-run depletion surveys, a quantitative method for the assessment of fish populations in wadeable streams, were conducted at three sites (Tab. 1) following Haunschmid et al. (2010). Each site was chosen to represent the typical instream habitats of the respective area. A block net with a mesh size of 20 mm was installed at the upper end of the reach to prevent fish from escaping and the length of the sampled stretch was measured using a handheld GPS. Beginning at the downstream end, three anode handlers waded upstream in parallel, with a maximum distance of 4 m between two anodes, covering the entire cross section. According to the requirements of the European Standard for the sampling of fish with electricity (EN 14011:2003) at least 50 m were covered at each site. Gasoline-powered backpack electrofishing units were used with unpulsed DC (300–600 V; 1.5–2.5 kW). All units were equipped with a 30-cm hoop anode and a cable cathode.

Table 1. Sampling sites and respective sampled length (L), width (W), area (A) and type of sampling (QN - quantitative, QL - qualitative, S - snorkelling, D - DNA analysis, eD - eDNA analysis). Quantitative and qualitative refer to electrofishing surveys.

Tabela 1. Vzorčna mesta in pripadajoče vzorčene dolžine (L), širine (W), površine (A) in tip vzorčenja (QN - kvantitativno, QL - kvalitativno, S - potapljanje, D-DNA analiza, eD - eDNA analiza). Kvantitativno in kvalitativno se nanaša na raziskave z elektro-ribolovom.

Tabela 1. Lokacije uzorkovanja i odgovarajuće uzorakovane dužine (L), širine (W), površine (A) i tip uzorkovanja (QN - kvantitativno, QL - kvalitativno, S - ronjenje, D-DNA analiza, eD - eDNA analiza).. Kvantitativno i kvalitativno se odnosi na istraživanja elektroribolovom.

Site name*	Latitude	Longitude	River	Date	L [m]	W [m]	A [m²]	QN	QL	S	D	eD
Mjedenik	43°19'09.79"	18°26'30.55"	Neretva	29.6.2022	190	2	380		●			
Waterfall	43°19'48.51"	18°25'39.83"	Krupac	29.6.2022	62	2	124		●		●	
Bridge	43°19'45.94"	18°25'32.63"	Krupac	29.6.2022	60	2	120		●			
Confluence	43°19'47.22"	18°25'25.45"	Neretva	29.6.2022	80	12	960	●			●	●
Cerova	43°22'42.43"	18°21'23.36"	Neretva	29.6.2022	66	2	132		●		●	
Swimming Beach	43°25'36.14"	18°18'39.91"	Neretva	28.6.2022	91	10	910	●	●			
Jezernica	43°25'32.90"	18°18'31.56"	Jezernica	30.6.2022	100	2	200		●			
Nedavić	43°27'28.64"	18°19'15.62"	Neretva	30.6.2022	118	11	1,298	●			●	●
				30.6.2022	464					●		
Glavatičevo	43°30'42.93"	18° 5'43.66"	Neretva	1.7.2022	4,950					●		●

* Shown in downstream direction.

Fish captured from each run were held separately in live wells at the stream margin. After the last run, all fish were identified to the lowest possible taxonomic level and measured to the nearest millimetre (Total Length (TL)). Fish biomass estimates were calculated based on existing length-weight relationships of the respective species. Population estimates were obtained using either the two-run (Seber & Cren 1967) or three-run depletion method (DeLury 1947). Estimated fish abundance and biomass were assessed for brown trout, standardised by area (hectare) and expressed as individuals/ha (ind/ha) and kg/ha. For other species, a reliable quantitative assessment was not possible due to sampling restrictions based on fish size and habitat use (e.g. cryptobenthic bullhead).

Using the same fishing gear described above, qualitative electrofishing surveys were carried out at six sites of the Neretva as well as in two tributaries (Tab. 1). At each site, selected microhabitats were sampled, aiming to supplement the species list and age class distribution. Fish were handled and measured as above. The fished area was estimated on the basis of length and width measurements.

Snorkelling survey

A snorkelling survey was conducted in two reaches of the Neretva (Tab. 1). One section was sampled downstream of Nedavić, where large boulders formed a cascade with deep pools. The second site was located at Glavatičevo, where the river had a width of >30 m and deep pools with >3 m depth. During both surveys, a high lateral visibility from shore to shore at Nedavić and of approximately 8 m at Glavatičevo provided suitable conditions. Two snorkelers drifted downstream side by side and identified, counted, and noted all visible fish following Thurow (1994). The mean number of counts per species from both snorkelers was taken as a qualitative sample. Since the primary aim was to count softmouth trout, the survey focused on habitats with a high probability of holding them. Therefore, smaller species and young age classes remain underrepresented.

DNA analyses

Fin-clips collected during electrofishing were used for genetic analyses (Tab. 3). DNA analyses were performed for three taxa (brown trout, minnow, and bullhead) in three different institutions. For clarity, despite some redundancy, laboratory procedures and data analyses are hereafter described separately for the various tasks and taxa.

eDNA analysis for the detection of softmouth trout

Analysis of eDNA samples was performed at the Central Research Laboratories of the Natural History Museum Vienna (NHM). eDNA samples were taken at three sites (Tab. 1) using a peristaltic pump and sterile disposable tubing to filter stream water through Spygen VigiDNA filters with a pore size of 0.45 µm. The mean filtration time was 27 minutes and the mean volume of water filtered was 37 L. Sampling was conducted at low flow conditions. Filters were stored in 80 mL of Spygen CL1 buffer (Pont et al. 2018) to prevent eDNA degradation. DNA extraction was carried out in the DNA clean room of the NHM following standard routines to avoid and detect contaminations. For DNA extraction, two protocols were used (1) DNeasy Blood &Tissue Kit (Qiagen) and (2) DNeasy Power Soil Pro Kit (Qiagen), and for each extraction method a negative control extraction was included to monitor contamination during DNA extraction. In addition, two fin-clip samples of softmouth trout were obtained from local fishermen and analysed to serve as a positive control. DNA extraction of those samples was performed (separated from the eDNA extractions) using the DNeasy Blood &Tissue Kit. Two marker sequences were used to test whether DNA of *Salmo* spp. could be detected in these samples: (1) partial regions of the mitochondrial cytochrome c oxidase subunit 1 gene (CO1), (2) partial regions of the mitochondrial control region (CR). Various Polymerase Chain Reaction (PCR) primers were designed to amplify fragments of these marker sequences (Tab. 2). The regions were selected according to two criteria: (1) short fragments, and (2) that they should contain positions where *S. obtusirostris* differs from other *Salmo* species. Furthermore, species specific primers were designed for both CO1 and CR that should (with 3´-mismatches) exclude *Salmo* species other than *S. obtusirostris*. Table 2 shows the primer sequences and the corresponding amplicon lengths ranging from 162 bp to 344 bp in *Salmo* spp.

PCR reactions were performed with the Multiplex PCR Kit (Qiagen, Hilden, Germany) in a total volume of 25 µl, containing: 12.5 µl Multiplex PCR Master Mix, 0.5 µM of each primer, and 1 µl template DNA. Cycling conditions were as follows: 94 °C for 5 min; 45 cycles of 94 °C for 30 s, annealing temperature for 30 s, and 72 °C for 30 s; final extension at 72 °C for 10 min. PCRs included control reactions without template DNA as negative controls. Thermocycling and post-PCR work was performed in a separate laboratory room. PCR products were extracted from agarose gels with the QIAquick Gel Extraction Kit and sequenced (bidirectionally) at Microsynth Austria (Vienna, Austria) using the PCR primers.

Table 2. PCR primers used for the detection of *Salmo* spp. in eDNA samples. Bold primers were specifically designed for *S. obtusirostris*.

Tabela 2. PCR primerji, uporabljeni za zaznavanje *Salmo spp*. v vzorcih eDNA. Primerji, označeni krepko, so bili posebej zasnovani za *S. obtusirostris*.

Tabela 2. PCR prajmeri korišteni za detekciju *Salmo* spp. u uzorcima eDNA. Boldirani prajmeri su posebno dizajnirani za *S. obtusirostris*.

Marker sequence	Primer name	Sequence (5´-3´)	Amplicon size (bp)
CO1	SalmCO1_1+	CGTAATTGTTACAGCCCATGCC	194
	SalmCO1_2-	CTTCAACTCCAGACGAGGCT	
	Salm Co1_3+	TTATGATCGGCGGCTTTGGG	210
	Salm Co1_4-	CGGAAGCTCCTGCGTGGGCG	
CR	SalmCR_1+	CATCAGCACTAACTCAAGGT	265
	SalmCR_4-	GATATAGGAACCAAATGCCAGG	
	SalmCR_2+	CACGTGATAATAACCAACTAAG	162
	SalmCR_3-	CAATAAGAGTATGCCTACTG	
	SalmCR_5+	GATAATAACCAACTAAGTTGTC	344
	SalmCR_6-	GGGAACCCTATGCATATAAG	

DNA analysis of brown trout

This part of the molecular genetic analyses was performed at the University of Ljubljana. DNA was isolated from fish tissue following the phenol-chloroform extraction procedure (Sambrook et al. 1989).

Microsatellite analysis

Thirty-two specimens were genotyped. Of these, 18 were caught at Swimming Beach, nine at Confluence and five in the Krupac tributary above the waterfall (Waterfall; Fig. 1). All specimens were genotyped using 12 microsatellite loci (Lerceteau-Köhler & Weiss, 2006; Marić et al. 2022), which have proved as efficient for characterising the genetic diversity in different species and lineages of brown trout in the Balkans and identifying interspecific hybrids (e.g., Snoj et al. 2010; Marić et al. 2022). The protocol for PCR amplification and genotyping procedure was described in Lerceteau-Köhler & Weiss (2006) and Marić et al. (2022).

For comparison, data from various brown trout from previous studies or data of the internal database of the University of Ljubljana was included: (i) Neretva sample (Ner-ref; N=15; Neretva near Glavatičevo; Razpet et al. 2007); (ii) Danubian lineage sample (DA-ref; N=20; Panjica River (Serbia); our unpublished data); (iii) Atlantic lineage sample (AT-ref; N=15; from Czech and Danish fish farms; Snoj et al. 2010; Marić et al. 2022); and (iv) Adriatic lineage sample (AD-ref; N=21; Dragovištica River (Aegean river basin); Marić et al. 2022).

For genetic differentiation and hybrid identification, Factorial Correspondence Analysis (FCA; Benzécri et al. 1973) implemented in Genetix v. 4.05 (Belkhir et al. 1996–2004) was applied. The genetic distances between compared groups were assessed by pairwise F_{ST} values calculated in FSTAT v. 2.9.3.2 software (Goudet 1995). For each group, genetic diversity was determined by calculating heterozygosity and allelic richness using the Genetix v. 4.05 software; Neretva samples from the present study and Ner-ref were considered as one group.

Sequence analysis of the mitochondrial control region

Sequencing of the CR of brown trout was performed only for those individuals sampled at the site Waterfall, which, due to the physical isolation from the downstream population, represented the most interesting material for a phylogenetic analysis. The complete CR (ca. 1100 bp) was PCR-amplified using LRBT-25 and LRBT-1195 primers (Uiblein et al. 2001) following the PCR conditions in Marić et al. (2022). They bind within the mitochondrial genes for tRNA Thr and tRNA Phe, respectively. DNA sequences were edited in Chromas Lite v.2.6.5 (Technelysium Pty Ltd, Australia; http://technelysium.com.au/wp/chromas/) and aligned by hand. BLAST was used to compare the sequences with those deposited in the NCBI GenBank.

DNA analysis of minnow

The DNA analysis of minnow samples was performed at the NHM. DNA was extracted from tissue of seven specimens caught at Swimming Beach (Fig. 1) using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. A partial section of the CO1 gene (652 bp) was amplified using primers FishF1 and FishR1 (Ward et al. 2005) and the protocol described in Palandačić et al. (2017). PCR products were purified with Qiagen PCR purification Kit according to the manufacturer's protocol and sent for sequencing in both directions with the PCR primers to Microsynth Austria.

DNA analysis of bullhead

The DNA analysis of six *C. gobio* samples (fin clips stored in 96% alcohol) collected at Swimming Beach (Fig. 1) was performed at the Center for Genotyping of Fishery Resources in Belgrade, Serbia. Total DNA was extracted using the Quick-gDNATM MiniPrep extraction kit according to the manufacturer's instructions (Zymo Research Corporation, Irvine, CA). The mitochondrial tRNA-Pro gene and the control region (CR) were amplified using the primers CotL1 (Šlechtová et al. 2004) and HN20 (Bernatchez & Danzmann 1993) following Šlechtová et al. (2004). They bind within the mitochondrial genes for tRNA Thr and tRNA Phe, respectively. Sequencing was done at the Center for Human Molecular Genetics, University of Belgrade, Faculty of Biology. Sequences were aligned and verified with MEGA 11 (Tamura et al. 2021) and compared with existing sequences using BLAST tool from NCBI.

To assess relationships of the Neretva haplotype and other bullhead populations, available sequence data was obtained from GenBank, and MAFFT (Katoh & Standley 2013) was used to construct a 949-bp alignment comprising 149 sequences. Phylogenetic relationships were inferred under Maximum Likelihood (ML) through the IQ-Tree webserver (Minh et al. 2020; Nguyen et al. 2015; Trifinopoulos et al. 2016) with standard settings using ultra-fast bootstrap to assess branch support (Hoang et al. 2017) and automatic model selection (Kalyaanamoorthy et al. 2017) on the unpartitioned alignment.

Results

Electrofishing

In total, an area of 3,168 m² was sampled quantitatively and another 1,156 m² qualitatively. With both methodological approaches, 381 individuals belonging to four species were caught (Tab. 3). Due to the limited catchability of small individuals of minnow (TL 10 – 30 mm) this species was underrepresented. Downstream of Cerova, the minnow occurred in schools of several hundred individuals (data not shown), marking the most abundant species in this part of the Neretva. The species distribution in the stretches downstream of Cerova is then followed by brown trout, bullhead, and stone loach (*Barbatula barbatula*). At Cerova and upstream of it, the species composition was dominated by brown trout followed by bullhead. The abundance and biomass of brown trout varied among sites, ranging between 113 and 1,180 ind/ha and 11 and 40 kg/ha, respectively.

The population structure of brown trout in the upper Neretva suggests an intact demography, although deficits also become visible (Fig. 3, Tab. 4). Due to the fishing date in late June, when Young-of-the-Year (YOY) individuals of brown trout often measured less than 4 cm, they could not be caught representatively. The low number of 0+ individuals (Fig. 3) was, therefore, mainly due to methodological limitations. The 1+ cohort was well represented and showed a clear decline from headwater to lower sections (Fig. 2). Similarly, older cohorts (2++) followed the same pattern as fish of the 1+ age class.

However, individuals of brown trout captured during quantitative sampling of all age classes were smallest at Confluence, the most upstream site, and size ranges increased in a downstream direction (Tab. 4).

Stone loach was only caught during the qualitative sampling at well-structured habitats of low flow and fine substrate. The highest number of stone loach could be found in the lower parts of the Jezernica tributary (Tab. 3). Length-frequency data indicate the presence of all age classes for stone loach, bullhead, and minnow (Fig. 3). As described for the brown trout, very small individuals of these other species were not caught representatively.

Table 3. Number of caught fish per sampling site and sampling method (M1 = quantitative sampling; M2 = qualitative sampling), estimated abundance and biomass of brown trout (SE in brackets), and number of spotted fish at snorkelling sites (M3 = snorkelling). The numbers in brackets next to the captured individuals indicate the number of individuals that were sampled for genetic analysis.

Tabela 3. Število ulovljenih rib na vzorčno mesto in metodo vzorčenja (M1 = kvantitativno vzorčenje; M2 = kvalitativno vzorčenje), ocena številčnosti in biomase potočne postrvi (SE v oklepajih) ter število opaženih rib na mestih potapljanja (M3 = potapljanje). Številke v oklepajih ob ulovljenih osebkih označujejo število osebkov, vzorčenih za genetsko analizo.

Tabela 3. Broj ulovljenih riba po lokaciji uzorkovanja i metodi uzorkovanja (M1 = kvantitativno uzorkovanje; M2 = kvalitativno uzorkovanje), procijenjena zastupljenost i biomasa smeđe pastrmke (SE u zagradama) i broj uočenih riba na ronilačkim mjestima (M3 = ronjenje). Brojevi u zagradama pored ulovljenih jedinki označavaju broj jedinki koje su bile uzorakovane za genetsku analizu.

Site	**Method**	***Salmo trutta***	***C. gobio***	***Phoxinus sp.***	***Barbatula barbatula***	***S. obtusirostris***	***T. thymallus***	***O. mykiss***	***S. fontinalis***	**Total**	**ind/ha**	**kg/ha**
Confluence	M1	101 (9)	1							102	1,180 (±266)	35.2 (±10,3)
Swimming Beach	M1	39 (18)	17	23	1 (1)					80	429 (±56)	40.5 (±6,3)
Nedavić	M1	15	2 (1)	10						27	133 (±27)	11.3 (±3,4)
Total		**155**	**20**	**33**	**1**					**209**		
Mjedenik	M2	17								17		
Waterfall	M2	5 (5)								5		
Bridge	M2	15								15		
Cerova	M2	15	10 (7)	5						30		
Swimming Beach	M2	2 (2)	12 (2)	18 (2)	1 (1)					33		
Jezernica	M2	7	1	44 (3)	21 (5)					73		
Total		**61**	**23**	**67**	**22**					**173**		
Nedavić	M3	33	3	278						314		
Glavatičevo	M3	70				31	79	35	8	223		
Total		**103**	**3**	**278**		**31**	**79**	**35**	**8**	**537**		

Table 4. Delimitation of age classes of *Salmo* sp. based on TL (mm) measurements at each quantitative electrofishing site.

Tabela 4. Starostni razredi *Salmo* sp. na podlagi meritev TL (mm) na vzorčnih mestih kvantitativnega elektroribolova.

Tabela 4. Određivanje starosnih klasa *Salmo sp.* na osnovu mjerenja TL (mm) na svakom mjestu kvantitativnog elektrolovljenja.

Site name	**0+**	**1+**	**2++**
Confluence	0-79	80-149	150-229
Swimming Beach	0-99	100-189	190-269
Nedavić	0-139	140-229	230-309

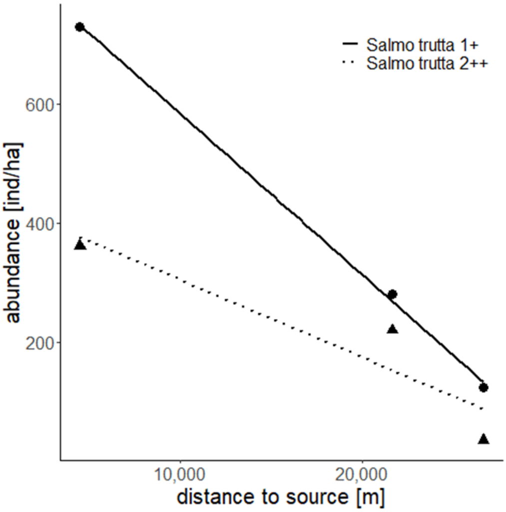

Figure 2. Abundance of 1+ and 2++ brown trout. at the respective distance from the source at the sites Confluence, Swimming Beach, and Nedavić. ● = abundance of brown trout 1+, ▲ = abundance of brown trout 2++.

Slika 2. Številčnost potočne postrvi 1+ in 2++ na posameznih razdaljah od izvira na vzorčnih mestih »Confluence«, »Swimming Beach« in »Nedavić«. ● = številčnost potočne postrvi 1+, ▲ = številčnost potočne postrvi 2++.

Slika 2. Zastupljenost smeđe pastrmke 1+ i 2++ na odgovarajućim udaljenostima od izvora na lokacijama Ušće, Plaža i Nedavić. ● = Zastupljenost smeđe pastrmke 1+, ▲ = Zastupljenost smeđe pastrmke 2++.

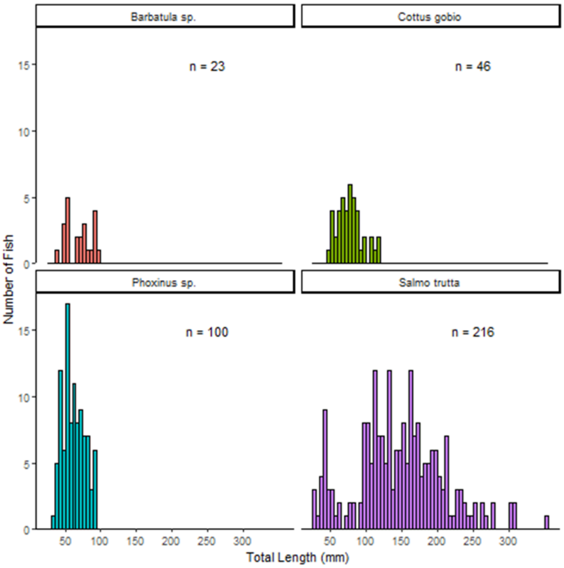

Figure 3. Population structure of species captured during quantitative and qualitative electrofishing of all sites (cumulated data).

Slika 3. Populacijska struktura vrst, ulovljenih z kvantitativnim in kvalitativnim elektroribolovom na vseh mestih (združeni podatki).

Slika 3. Struktura populacije vrsta uhvaćenih tokom kvantitativnog i kvalitativnog elektroroizlova na svim lokacijama (kumulativni podaci).

Snorkelling survey

Reaches of 464 m at Nedavić downstream of the electrofishing site and 4,950 m at Glavatičevo were surveyed through snorkelling. In total, 537 individuals from seven species were encountered. While individuals of the minnow were counted at Nedavić, they were not counted at Glavatičevo due to their high abundance and the subsequent risk of severe underestimation.

At Nedavić, a total of 33 brown trout of all age classes, three bullhead and 278 minnows with lengths between approximately five to ten cm were counted (Tab. 3). Several thousand minnow larvae and subadult fish could be identified. The most abundant species at Glavatičevo was the non-native grayling, followed by brown trout. Furthermore, 31 individuals of softmouth trout could be identified as well as rainbow trout and brook trout (Tab. 3). Around 10% of encountered brown trout could be clearly identified as stocked fish through either missing pectoral fins or gill covers. Young age classes were only observed for the brown trout. Fish from all other species ranged between 20-50 cm.

Analysis of environmental DNA (eDNA)

All primer combinations resulted in successful amplification in the positive controls. Using the primer pairs SalmCO1_1+/ SalmCO1_2-, SalmCR_1+/ SalmCR_4- , as well as SalmCR_2+/ SalmCR_3-, PCR products could be obtained from both marker sequences from all eDNA samples (both extraction methods). Sequencing revealed the presence of *Salmo* sp. haplotypes, but no haplotype specific for softmouth trout was obtained. Using the specific primers SalmCO1_3+/ SalmCO1_2- and SalmCR_4+/ SalmCR_6-, haplotypes characteristic for softmouth trout were obtained from the Glavatičevo samples for CO1, as well as CR.

DNA analysis of brown trout

Microsatellite analysis of brown trout

DNA was successfully extracted from all specimens except for one from the Confluence site. Genotyping was successful in all samples except for one from the Krupac above the waterfall, which later proved to be stone loach (see below).

FCA arranged the entire sample set into four genetically homogeneous groups: first, a Neretva brown trout group represented by most of the specimens collected in the present study including those sampled above the waterfall in the Krupac (Fig. 4; yellow), and Ner-ref (blue). The remaining three groups coincided with specimens from Adriatic (white), Danube (pink) and Atlantic (black) river basins. Six specimens from Swimming Beach deviate from the Neretva brown trout group (Fig. 4) with one located in the AT-ref group, and the others gravitating towards this group.

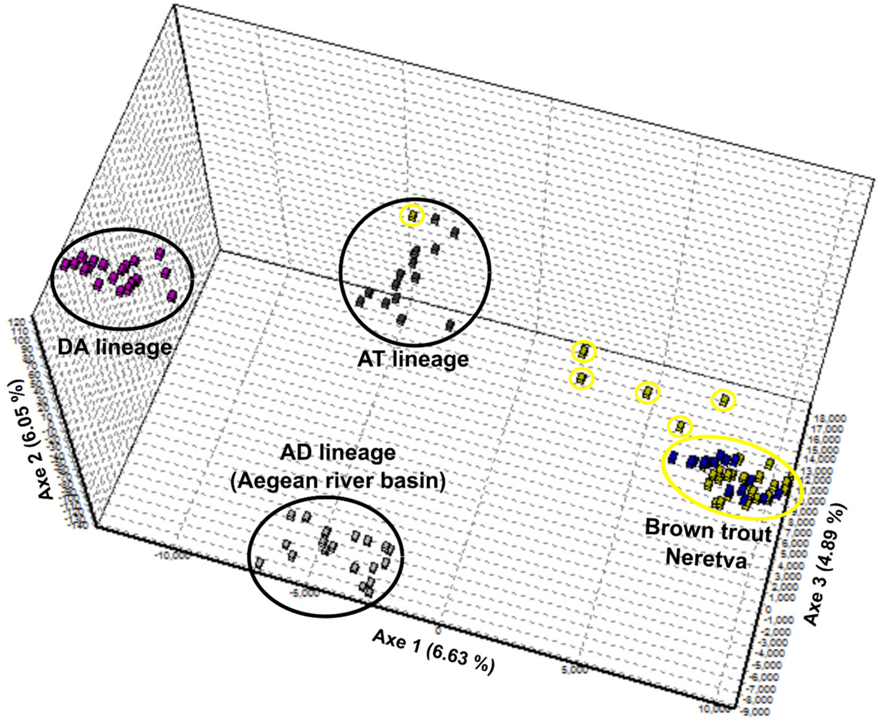

Figure 4. Factorial correspondence analysis (FCA) plot of the brown trout samples studied. AD, DA, AT stand for brown trout mitochondrial DNA lineages from the Adriatic, Danube and Atlantic river basins, respectively; for details on brown trout phylogeography, see Bernatchez et al. 1992. Genetic groups recognised by FCA are encircled. Yellow – specimens from the present study; blue –Ner-ref specimens. Individual specimens encircled in yellow are apparently introgressed with genes from Atlantic populations; one specimen (within AT-ref group) appears to be a 'pure' AT representative.

Slika 4. Diagram faktorske korespondenčne analize (FCA) vzorcev potočne postrvi. AD, DA, AT označujejo linije mitohondrijske DNK potočne postrvi iz Jadranskega, Donavskega in Atlantskega porečja; za podrobnosti o filogeografiji potočne postrvi glej Bernatchez et al. 1992. Genetske skupine, prepoznane s FCA, so obkrožene. Rumeno - primerki iz te študije; modro - primerki Ner-ref. Posamezni primerki, obrobljeni rumeno, imajo verjetno introgresirane gene atlantskih populacij; eden izmed primerkov (v skupini AT-ref) je najverjetneje 'čisti' predstavnik AT.

Slika 4. Analiza faktorske korespondencije (FCA) proučavanih uzoraka potočne pastrmke. AD, DA, AT označavaju mitohondrijalne DNK linije potočne pastrmke iz jadranskog, dunavskog i atlantskog riječnog sliva; za detalje o filogeografiji potočne pastrmke, vidi Bernatchez et al. 1992. Genetske grupe koje priznaje FCA su zaokružene. Žuta – primjerci iz ove studije; plavi –Ner-ref uzorci. Pojedinačni uzorci zaokruženi žutom bojom su očigledno introgresirani genima iz atlantskih populacija; čini se da je jedan primjerak (unutar AT-ref grupe) 'čisti' AT predstavnik.

All pairwise F_{ST} values were statistically significant (Tab. 5). The minimum value was observed between the Neretva sample from the present study and Ner-ref sample. The remaining values ranged from ca. 0.2 to 0.4; the highest values were observed in pairs with the DA-ref sample.

Table 5. Pairwise FST values between the brown trout specimens from the present study and the reference samples, i.e. Neretva reference (Ner-ref), Danubian reference (DA-ref), Atlantic reference (AT-ref) and Adriatic reference (AD-ref). Asterisks denote statistical significance (p>0.095).

Tabela 5. Parne vrednosti FST med primerki potočne postrvi iz te študije in referenčnimi vzorci, tj. referenca Neretva (Ner-ref), donavska referenca (DA-ref), atlantska referenca (AT-ref) in jadranska referenca (AD-ref). Zvezdice označujejo statistično značilnost (p>0,095).

Tabela 5. Parne FST vrijednosti između uzoraka smeđe pastrmke iz ove studije i referentnih uzoraka, tj. neretva referenca (Ner-ref), dunavska referenca (DA-ref), atlantska referenca (AT-ref) i jadranska referenca (AD-ref). Zvjezdice označavaju statističku značajnost (p>0.095).

	Neretva, present study	**Ner-ref**	**AD-ref**	**AT-ref**	**DA-ref**
Neretva, present study		**	**	**	**
Ner-ref	0.0275		**	**	**
AD-ref (Aegean river basin)	0.3081	0.2874		**	**
AT-ref	0.2516	0.2363	0.2035		**
DA-ref	0.4019	0.3957	0.4087	0.3363	

The observed parameters show a high genetic diversity for all genetic groups except for DA-ref, which reflects a medium genetic diversity (Tab. 6; see Berrebi et al. 2021).

Table 6. Genetic diversity assessment inferred from heterozygosity (He = heterozygosity expected; Ho = heterozygosity observed), and allelic richness (Ar). AD-ref = reference Adriatic lineage; AT-ref = reference Atlantic lineage; DA-ref = reference Danubian lineage.

Tabela 6. Ocena genetske raznolikosti, izpeljana iz heterozigotnosti (He = pričakovana heterozigotnost; Ho = opažena heterozigotnost) in alelna bogatost (Ar). AD-ref = referenčni jadranski izvor; AT-ref = referenčni atlantski izvor; DA-ref = referenčni donavski izvor.

Tabela 6. Procjena genetske raznovrsnosti izvedena iz heterozigotnosti (He = očekivana heterozigotnost; Ho = uočena heterozigotnost) i alelnog bogatstva (Ar). AD-ref = referentna jadranska linija; AT-ref = referentna atlantska linija; DA-ref = referentna dunavska linija.

	He	***Ho***	***Ar***
Neretva (present study +Ner-ref)	0.609	0.501	7.28
AD-ref (Aegean river basin)	0.630	0.629	5.59
AT-ref	0.744	0.743	7.74
DA-ref	0.389	0.408	3.53

Mitochondrial control region of brown trout from locality Waterfall

Sequences for the complete CR were obtained for four specimens from the Krupac tributary above the waterfall; three had a haplotype previously found in the Neretva brown trout (Delling et al. 2020; ADcr4; NCBI GenBank accession number MK184929). Another published, much shorter (309-bp) sequence (Bernatchez et al. 1993; Ad-s3; NCBI GenBank accession number: M97967) was identical in the overlapping section. The fourth specimen proved to be another species, as it exhibited a not yet described haplotype, which showed 98.14% similarity to the species *B. barbatula* from Croatia (NCBI GenBank: GU583680; Jakovlić et al. 2013).

DNA analysis of minnow

All minnow specimens exhibited the same haplotype, previously detected in the Bunica River, confluence to Neretva (GenBank accession numbers MF407681) and clustering to Clade 2 (*Phoxinus* sensu lato; clade named according to Palandačić et al. (2015)). This haplotype was also detected in Karanovac, Lepenica, Ugar and Fojnica Rivers.

DNA analysis of bullhead

A novel, not yet detected CR haplotype, Cot73 (NCBI GenBank accession number QQ379171), was obtained from all bullhead samples. In our analyses this haplotype was nested within a clade comprising haplotypes detected in the Lower Danube drainage (unpublished sequences from GenBank without more detailed geographic information), the Kolpa/Kupa River and the Tounjčica River (Šlechtová et al. 2004), which was the sister group of a well-supported clade comprising haplotypes from the Pčinja River (Fig. 5).

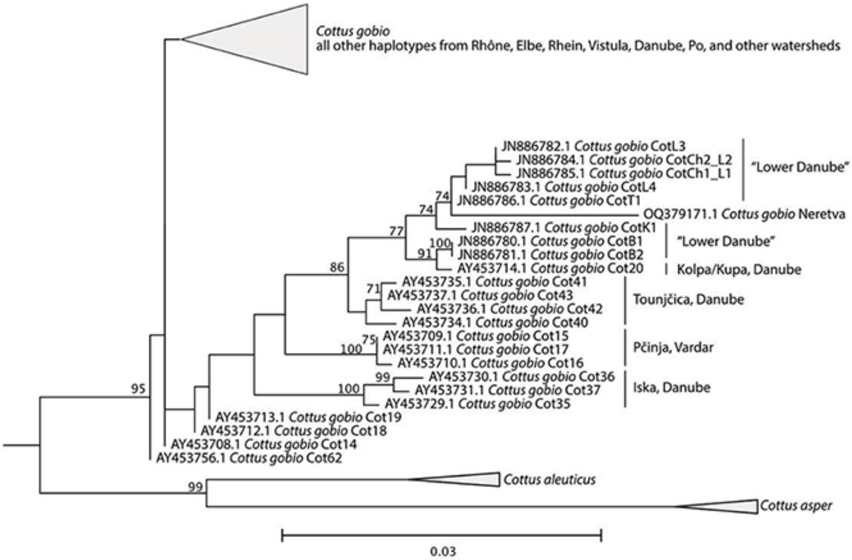

Figure 5. Relationships amongst *Cottus* spp. CR haplotypes. The haplotype representing the Neretva samples is nested within a clade comprising haplotypes from the Lower Danube drainage and haplotypes from Sava tributaries Kolpa/Kupa and Tounjčica. Besides this clade, the remaining *Cottus gobio* haplotypes are collapsed. The same is true for the outgroup species: *C. aleuticus*, and *C. asper*. For a detailed description of haplotypes, see Šlechtová et al. (2004). Alphanumeric codes before species names correspond to GenBank accession numbers, codes after species names to previously identified haplotypes (Šlechtová et al. 2004).

Slika 5. Razmerja med CR haplotipi *Cottus* spp. Haplotip, ki predstavlja vzorce iz Neretve, je vgnezden v kladu, ki zajema haplotipe iz spodnjega toka Donave in haplotipe pritokov Save Kolpe/Kupe in Tounjčice. Preostali haplotipi *C. gobio* so združeni. Enako velja za zunanje skupine vrst: *C. aleuticus* in *C. asper*. Za podroben opis haplotipov glej Šlechtová et al. (2004). Alfanumerične kode pred imeni vrst ustrezajo kodam GenBank, kode po imenih vrst pa že identificiranim haplotipom (Šlechtová et al. 2004).

Slika 5. Odnosi među CR haplotipovima *Cottus* spp. Haplotip koji predstavlja uzorke iz Neretve ugniježđen je u kladus koji obuhvata haplotipove iz donjeg toka Dunava i haplotipove iz pritoka Save Kolpa/Kupa i Tounjčica. Pored ovog kladusa, ostali haplotipovi C. gobio su kolabirali. Isto vrijedi i za vanjske vrste: *C. aleuticus* i *C. asper*. Za detaljan opis haplotipova pogledajte Šlechtová et al. (2004). Alfanumerički kodovi prije naziva vrsta odgovaraju brojevima pristupa GenBank, kodovi nakon naziva vrsta prethodno identificiranim haplotipovima (Šlechtová et al., 2004).

Discussion

This study provides a quantitative and qualitative description of the ichthyofauna of the upper Neretva, including the lower parts of two tributaries. The length-frequency diagrams showed the presence of all age classes of brown trout, bullhead, minnow and stone loach underlining the integrity of this river stretch. The number of species is low in the upper reaches and increases downstream, following the classical pattern of cold, oxygen-rich rivers with strong currents of the montane and submontane zone, with the trout region in the upper reaches (Huet 1949). With increasing distance from the source, structural composition and the species spectrum diversifies. The Neretva around Glavatičevo, which corresponds to the so-called hyporhithral of alpine rivers, was naturally colonised by the softmouth trout, marble trout, and brown trout (Glamuzina et al. 2018). As the Neretva River drainage offers the largest remaining natural habitats for the endangered softmouth trout, this species was in part a focus of our investigation.

Softmouth trout

Evidence of the softmouth trout was found in the course of the survey through both a snorkelling survey and eDNA analysis. During the snorkelling survey, the majority of softmouth trout was encountered in deep runs and pools spread out over the whole reach sampled at Glavatičevo. The DNA analysis of two softmouth trout samples showed that they carried specific haplotypes reported earlier for CR and CO1, respectively (Snoj et al. 2008; Tougard et al. 2018). Moreover, it could be shown that these haplotypes can be detected via eDNA analyses, albeit evidence was obtained only from the water sample at Glavatičevo. Thus, for future analyses, systematic sampling should be performed with a sufficient number of replicates at different localities. Yet, further optimisation of primers is necessary to increase specificity and, thus, the sensitivity of the analyses considering the potential rarity of the species in some localities. It should also be noted that hybridisation between the softmouth trout and brown trout has been reported and is relatively extensive in the Neretva basin, posing a major threat to the long-term survival of the species (Razpet et al. 2007; Snoj et al. 2007). As the typical eDNA protocol targets mitochondrial DNA only, this approach is unable to distinguish between hybridised, pure-bred, or introgressed individuals.

Neretva brown trout

As inferred from the present study, brown trout in the upper Neretva do not differ from the downstream population at Glavatičevo; together they form a genetically homogeneous unit that is clearly distinct from geographically separated genetic entities such as the brown trout lineages from the Danube and Aegean watersheds and Atlantic lineage (anthropogenically introduced into the Neretva system) brown trout. The genetic data suggest that Atlantic lineage brown trout have already invaded the upper Neretva population but have apparently not yet extended to the highest reaches as all introgressed specimens were restricted to the downstream sampling site near the village of Ulog (Swimming Beach). Stocked brown trout were only observed around Glavatičevo corresponding to previous genetic tests from the same location (Razpet et al. 2007).

The presence of stocked fish at Glavatičevo was also corroborated in real time via the snorkel survey at that locality, as typical hatchery phenotypes (missing or damaged fins, deformations and missing gill covers) of three salmonid species (brown trout, rainbow trout and brook trout) were easily observed. The stocking is likely also related to angling pressure, which is supported by the overall low total biomass (Tab. 3) and low numbers of trout exceeding 25 cm TL at all sites (Glamuzina et al. 2018). In total, low biomass, low numbers of large fish, and direct observations of remnant fishing gear, as well as hooking injuries, all potentially suggest that overfishing and harvest may be taking place.

The introgression of brown trout with non-indigenous genes was also reported to be undeniably a result of stocking (Kalamujić et al. 2015). The same is assumed for introgression from Danube populations that were observed mainly in the river Rakitnica (the right tributary of the Neretva below Glavatičevo) and in the Neretva near the confluence with Rakitnica (Razpet et al. 2007; Kalamujić et al. 2015). In this study, however, with our limited sampling campaign, no trace of a Danubian-lineage genes was detected. The question as to whether or not Danubian-lineage genes in the Neretva detected by Razpet et al. (2007) and Kalamujić et al. (2015) arrived by natural or human-mediated means remains unanswered.

Despite observations of stocked salmonids and the documented evidence of introgression in the lower courses of the surveyed reaches, the uppermost reaches of the Neretva were characterised by pristine habitat and natural population structure of all occurring species. The 1+ cohort of brown trout appears well represented and shows a typical pattern for trout populations (Unfer & Pinter 2018) in that the abundance decreases with increasing distance from the river's source. At the site Confluence, a high number of brown trout was caught, but the biomass of 35 kg/ha is low for the calculated abundance, highlighting a lack of large individuals. The results underline the importance of the upper reaches as nursery areas that support high densities of juveniles, while the lower reaches serve as feeding areas for older stages. Large fish (>25 cm TL) were mainly observed during snorkelling at Glavatičevo. In the upper reach, large individuals were predominantly captured at the lower sites Swimming Beach and Nedavić.

Non-salmonid native species

Comparing the species list from Muhamedagić et al. (2010) for the upper reach of the Neretva (source to Jablanica lake), three presumably native species, namely the European chub (*Squalius cephalus*), the gudgeon (*Gobio gobio*), and the marble trout, could not be identified during this survey. Moreover, those authors reported a relatively low abundance of minnow of only 4% (15 individuals) with respect to the whole species composition of the upper Neretva. In contrast, during this present study, several thousand minnows were observed.

Tutman et al. (2017) indicated that the upper Neretva might be inhabited by several species of stone loaches. During this survey, one specimen was identified as *B. barbatula.* However, for a more precise identification of the genetic lineage, the mitochondrial cytochrome b gene should be sequenced as it is a much more frequently used and more informative marker gene in *Barbatula* studies compared to the CR. The stone loach was only found at Swimming Beach presumably because the nearby gently flowing Jezernica tributary offered the typical habitats of stone loaches, characterised by shallow, and stagnant conditions.

Both, bullhead and minnow are considered native to the upper Neretva (Palandačić et al. 2015; Pilić et al. 2021). In the Western Balkans, highly divergent mitochondrial clades of *Phoxinus phoxinus sensu lato* have been found (Palandačić et al. 2015). The present results confirmed that the minnows sampled belong to Clade 2 of Palandačić et al. (2015), comprising populations from the lower Sava catchment and rivers flowing directly into the Adriatic Sea (Palandačić et al. 2015, 2017, 2020). Similarly, several divergent genetic clades had been identified in bullhead (Šlechtová et al. 2004; Bravničar 2012; Bravničar et al. 2015). The specimens sampled in the Neretva appeared to share an evolutionary origin with populations found in northern Balkan Danube tributaries, despite the fact that the Neretva drains towards the Adriatic basin (Bravničar et al. 2015). Phylogeographically, this would suggest colonisation of the Neretva and other North-Western Balkan rivers across major mountain systems, as previously proposed (Bravničar et al. 2021; Šlechtová et al. 2004). Detecting this unique and apparently derived haplotype in the Neretva demonstrates the significance of the stream as a refuge and source of genetic diversity in bullhead.

Non-native species

The presence of several alien species, especially the grayling, suggests potential competition with native species (Glamuzina et al. 2018). At Glavatičevo, obviously stocked individuals of brown trout, rainbow trout and brook trout were present. These specimens could be easily identified by typical symptoms of rearing environments such as missing or damaged fins, deformations, and missing gill covers. The relative abundance of grayling and rainbow trout among salmonids were higher during the present study compared to reports of Muhamedagić et al. (2010).

Summary conclusions

Through the assessment of the ichthyofauna, healthy population structures of brown trout, bullhead, minnow, and stone loach were identified as expected in a well-structured river that provides functional habitats for all age classes. The occurrence of the endangered softmouth trout and the discovery of a new mitochondrial CR haplotype of *Cottus gobio* highlight the value of the upper Neretva as a biodiversity hotspot. The construction of dams changed the natural state of the middle Neretva from a salmonid river to a chain of impoundments that is dominated by cyprinids. The subsequent restriction of longitudinal connectivity further contributed to a vast decline of salmonids in the upper reaches (Muhamedagić et al. 2010). Preserving the upper reaches of the Neretva from ecological deterioration and destruction through planned HPP expansion is paramount to preserve this river’s function as a biodiversity refuge in the present human-made ecological crisis.

Povzetek

Reke zahodnega Balkana so izjemnega pomena kot vroče točke biotske raznovrstnosti, z velikim številom endemičnih in redkih vrst. Raznolikost vrst sladkovodnih rib v regiji velja za najvišjo v Evropi, tudi zaradi ohranjenosti zgornjih tokov rek, ki so pogosto prosto tekoči in brez večjih antropogenih posegov, kot so kanaliziranja in jezovi. Kljub temu so te dragocene naravne danosti močno ogrožene zaradi širitev hidroelektrarn (HE).

V Neretvi, eni najpomembnejših rek na zahodnem Balkanu, živi 34 domorodnih vrst rib, od tega 17 endemičnih. Zabeleženih je bilo tudi 32 tujerodnih vrst, ki so bile pretežno vnesene v zajezitve srednjega toka za namene rekreacijskega ribolova. Med njimi so lipan (*Thymallus thymallus*), šarenka (*Oncorhynchus mykiss*), potočna zlatovčica (*Salvelinus fontinalis*) in tujerodne vrste potočne postrvi (*Salmo trutta*), ki tekmujejo z avtohtonimi salmonidi, kot so ogrožena mehkousta prostrv (*Salmo obtusirostris*), soška postrv (*Salmo marmoratus*) in avtohtona potočna postrv. Pretekle študije rib so bile osredotočene na srednji ali spodnji tok. Študije, ki so se osredotočale na zgornji tok, niso dostopne ali nimajo podrobnih populacijskih parametrov. Namen pričujoče analize je bil preučiti stalež ribje združbe v zgornji Neretvi, zagotoviti dodatne vpoglede v sistematiko in razširjenost posameznih vrst ter odpraviti nekatere negotovosti glede statusa avtohtonosti vrst.

Uporabili smo standardni elektroribolov in napravili genetske analize, ki so zajemale populacijsko genetski pristop za potočno postrv, pisanca (*Phoxinus* spp.) in kaplja (*Cottus* spp.). Poleg tega smo se potapljali na dah in odvzeli vzorce okoljske DNK (eDNK), da bi odkrili globočka v rečnih odsekih, ki bodisi niso bili vzorčeni na druge načine bodisi bi ga zaradi njegove redkosti lahko zgrešili.

Prisotnost mehkouste postrvi je bil potrjen s potapljanjem na dah in eDNA. Vsi osebki so bili zaznani okoli Glavatičevega, njegovo morebitno pojavljanje gorvodno ostaja nepotrjeno. V prihodnosti bo potrebno sistematično vzorčenje vode za eDNA na različnih lokacijah. Poleg tega bi bilo, zaradi redkosti pojavljanja vrste, potrebno nadalje optimizirati primerje za povečanje specifičnosti in s tem občutljivosti analiz.

Potrdili smo vse starostne razrede potočne postrvi, kar kaže na ekološki pomen zgornjega toka Neretve. Številčnost in biomasa pa sta bili nizki, gibali sta se med 113 do 1180 osebkov/ha oziroma 11–40 kg/ha. Številčnost mladic potočne postrvi se je zmanjševala dolvodno, kar nakazuje pomen Zgornje Neretve za rast mladic. Genetske analize so pokazale, da so ribe iz zgornjega toka in nižje populacije okoli Glavatičevega genetsko homogena enota, ki se jasno razlikuje od geografsko ločenih genetskih entitet. Čeprav je bil atlantski izvor potočne postrvi vnesen v Neretvo, naši podatki kažejo, da se še ni razširila na območje gorvodno od Uloga. Nizka biomasa, majhno število velikih rib in neposredna opazovanja ostankov ribolovnega orodja ter poškodbe zaradi trnka potencialno kažejo na prekomerni izlov. Poleg tega so tujerodni salmonidi v okolici Glavatičevega številčno presegli avtohtone salmonide.

Sedanji rezultati so potrdili, da vzorčena pisanec in kapelj pripadata genetskim kladom, ki obsegajo populacije iz rek, ki tečejo neposredno v Jadransko morje. To nakazuje kolonizacijo Neretve in drugih rek severozahodnega Balkana prek večjih gorskih sistemov, kot so že predlagali drugi raziskovalci. Odkritje edinstvenih in izpeljanih haplotipov v Zgornji Neretvi dokazuje pomen reke kot zatočišča in vira genetske raznolikosti teh vrst. Poleg tega pojav vseh starostnih razredov nakazuje zdrave strukture populacije obeh vrst.

Rezultati ponovno dokazujejo ekološki pomen zgornje Neretve kot vroče točke biotske raznovrstnosti. V luči velikih sprememb ihtiofavne srednjega toka Neretve zaradi gradnje hidroelektrarn je nujno zaščititi gornji tok Neretve pred ekološkim osiromašenjem in uničevanjem habitatov, ki bi ga povzročila širitev HE. S tem bi ohranili funkcijo zatočišča biotske raznovrstnosti v sedanji antropogeno povzročeni ekološki krizi.

Sažetak

Neretva, jedna od najvažnijih rijeka na zapadnom Balkanu, dom je 34 autohtone vrste riba, od kojih je 17 endemskih. Zabilježene su i 32 alohtone vrste, koje su uglavnom uvedene u rezervoare srednjeg toka za potrebe rekreativnog ribolova. Među njima su: lipljen (*Thymallus thymallus*), kalifornijska pastrmka (*Oncorhynchus mykiss*), potočna pastrmka (*Salvelinus fontinalis*) i alohtone vrste potočne pastrmke (*Salmo trutta*), koje se takmiče sa autohtonim salmonidima kao što su: ugrožena mekousna pastrmka (*Salmo obtusirostris*), glavatica (*Salmo marmoratus*) i autohtona potočna pastrmka. Prethodne studije o ribama fokusirale su se na srednji ili donji tok. Studije koje se fokusiraju na uzvodno nisu dostupne ili im nedostaju detaljni parametri populacije. Cilj ove analize bio je ispitati fond riblje zajednice u gornjoj Neretvi, dati dodatni uvid u sistematiku i rasprostranjenost pojedinih vrsta, te otkloniti neke nejasnoće u pogledu statusa autohtonosti vrste.

Koristili smo standardni elektroribolov i izvršili genetičke analize koje su uključivale populacijski genetski pristup za potočnu pastrmku, pijor (*Phoxinus* spp.) i peš (*Cottus* spp.). Pored toga, ronjenjem smo prikupili i uzorke DNK iz životne sredine (eDNA) kako bismo detektovali potočnu pastrmku u dijelovima rijeke koji ili nisu uzorkovani na drugi način ili su, zbog rijetkosti vrste, mogli biti propušteni.

Prisustvo mekousne pastrmke potvrđeno je ronjenjem i eDNK. Svi primjerci su otkriveni oko Glavatičeva, moguće prisustvo vrste uzvodno ostaje nepotvrđeno. U budućnosti će biti neophodno sistematsko uzorkovanje vode za eDNK na različitim lokacijama. Osim toga, zbog rijetkosti vrste, bilo bi potrebno dodatno optimiyovati parametre kako bi se povećala specifičnost, a time i osjetljivost analiza.

Potvrđeno je prisustvo svih dobnih klasa potočne pastrmke, što odražava ekološki značaj gornjeg toka Neretve. Broj i biomasa su bili niski, u rasponu od 113 do 1180 jedinki/ha, odnosno 11–40 kg/ha. Broj mlađi potočne pastrmke se smanjio nizvodno, što ukazuje na značaj gornjeg toka Neretve za rast mlađi. Genetske analize su pokazale da su ribe gornjeg toka i niže populacije oko Glavatiča genetski homogena jedinica koja se jasno razlikuje od geografski odvojenih genetskih entiteta. Iako je atlantska linija potočne pastrve unesena u Neretvu, naši podaci pokazuju da se još nije proširila na područje uzvodno od Uloga. Niska biomasa, mali broj velikih riba i opažanja ostataka ribolovne opreme i oštećenja nastala od udica potencijalno ukazuju na prekomjeran ribolov. Osim toga, alohtonih salmonida u okolini Glavatičeva bilo je više od autohtonih salmonida.

Dosadašnji rezultati su potvrdili da uzorkovani pijor i peš pripadaju genetskim kladama koje čine populacije iz rijeka koje se ulijevaju direktno u Jadransko more. Ovo sugeriše kolonizaciju Neretve i drugih rijeka sjeverozapadnog Balkana putem većih planinskih riječnih sistema, kao što su ranije sugerirali drugi istraživači. Otkriće jedinstvenih i izvedenih haplotipova u gornjoj Neretvi pokazuje važnost rijeke kao utočišta i izvora genetske raznolikosti za ove vrste. Osim toga, pojava svih starosnih klasa ukazuje na zdravu strukturu populacije obje vrste.

Rezultati još jednom dokazuju ekološku važnost gornje Neretve kao žarišta biodiverziteta. U svjetlu velikih promjena u ihtiofauni srednjeg toka Neretve zbog izgradnje hidroelektrana, potrebno je zaštiti gornji tok Neretve od ekološkog osiromašenja i uništavanja staništa koje bi prouzrokovalo proširenje HE. Time bi se očuvala funkcija utočišta za biodiverzitet u trenutnoj ekološkoj krizi izazvanoj antropogenošću.

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References

Bartulović V, Glamuzina B, Conides A, Dulcic J, Lucic D, Njire, J, Kozul V. 2004. Age, growth, mortality and sex ratio of sand smelt, Atherina boyeri Risso, 1810 (Pisces: Atherinidae) in the estuary of the Mala Neretva River (middle-eastern Adriatic, Croatia). Journal of Applied Ichthyology. 20(5): 427–430. https://doi.org/10.1111/j.1439-0426.2004.00560.x

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 1996-2004. GENETIX v. 4.04, Logiciel sous WindowsTM pour la génétique des populations. Montpellier: Université Montpellier 2, Laboratoire Génome et Population. https://kimura.univ-montp2.fr/genetix/

Benzécri JP, Benzécri F, Birou A, Blumenthal S. 1973. L'analyse des données. Volume 2: L'analyse des correspondances. Paris: Dunod.

Bernatchez L, Danzmann RG.1993. Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of brook charr (Salvelinus fontinalis Mitchill). Molecular Biology and Evolution. 10: 1002–1014.

Bernatchez L, Guyomard R, Bonhomme F. 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout Salmo trutta populations. Molecular Ecology. 1: 161-173.

Berrebi P, Horvath Á, Splendiani A, Palm S, Bernaś R. 2021. Genetic diversity of domestic brown trout stocks in Europe. Aquaculture. 544: 737043. https://doi.org/10.1016/j.aquaculture.2021.737043

Bravničar J. 2012. Taxonomic analysis of bullhead (*Cottus* sp.) in Slovenia with application of molecular methods [bachelor's thesis]. [Ljubljana]: Univerza u Ljubljani, Biotehniška Fakulteta, Oddelek za Biologijo.

Bravničar J, Palandačić A, Jelić D, Podgornik S, Snoj A. 2021. Molecular data reveal distinct phylogenetic position of *Cottus metae*, establish its distribution range, and invalidate the species status of *C. scaturigo*. Journal of Zoological Systematics and Evolutionary Research. 59(2): 428–441. https://doi.org/10.1111/jzs.12434

Bravničar J, Palandačić A, Marić S, Šanda R, Snoj A. 2015. Phylogeny of European bullhead (*Cottus* sp.) in Western Balkan. In: Abstract book. XV European Congress of Ichthyology; 7-11 September; Porto. p. 35-36.

Crivelli AJ. 2006. *Salmo obtusirostris*. The IUCN Red List of Threatened Species 2006: e.T19862A9056634; [accessed on 21.8.2023]. https://dx.doi.org/10.2305/IUCN.UK.2006.RLTS.T19862A9056634.en.

DeLury DB. 1947. On the estimation of biological populations. Biometrics. 3(4): 145. https://doi.org/10.2307/3001390

DIKTAS B&H. 2012. DIKTAS - Protection and sustainable use of the Dinaric Karst transboundary aquifer system. National Report Bosnia and Herzegovina. http://diktas.iwlearn.org

Dulčić J, Dragičević B, Ugarković PTP. 2017. The largemouth black bass (*Micropterus salmoides*): first record in the Neretva River delta, Adriatic drainage system of Croatia. Cybium. 41(1): 77–78.

EN 14011:2003. Water quality-sampling of fish with electricity. https://standards.iteh.ai/catalog/standards/cen/cd873486-340e-4ee6-ad71-2fbdf24907cc/en-14011-2003

Freyhof J, Brooks E. 2011. European Red List of freshwater fishes. Luxembourg: Publications Office of the European Union.

Glamuzina B, Bartulović V, Glamuzina L, Dobroslavić T. 2017. Records of new fish species in the River Neretva estuary: potential threat to Coastal Adriatic nursery*.* Naše More. 64(3): 86–89. https://doi.org/10.17818/NM/2017/3.2

Glamuzina B, Stanić-Koštroman S, Matić-Skoko S, Glamuzina L, Muhamedagić S, Rozić I, Weiss S, Pavličević J. 2018. Recent status and life history traits of endangered soft-mouth trout, *Salmo obtusirostris* in the River Neretva catchment (Bosnia and Herzegovina) as a consequence of river alteration. Journal of Applied Ichthyology. 34(5): 1160–1168. https://doi.org/10.1111/jai.13780

Glamuzina L, Dobroslavić T. 2020. Summer fish migrations in the River Neretva (South-Eastern Adriatic Coast, Croatia) as a consequence of salinization. Naše More. 67(2): 103–116. https://doi.org/10.17818/NM/2020/2.3

Goudet J. 1995. FSTAT (Version 1.2): a computer program to calculate F-statistics. Journal of Heredity. 86: 485-486. https://doi.org/10.1093/oxfordjournals.jhered.a111627

Haunschmid R, Schotzko N, Petz-Glechner R, Honsig-Erlenburg W, Schmutz S, Spindler T, Unfer, G, Wolfram G, Bammer V, Hundritsch L, et al. 2010. Leitfaden zur Erhebung der biologischen Qualitätselemente Teil A1 - Fische. Wien: Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft.

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS, Rosenberg MS. 2017. UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution. 35(2): 518–522. https://doi.org/10.5281/zenodo.854445

Huet M. 1949. Aperçu des relations entre la pente et les populations piscicoles des eaux courantes. Zeitschrift Für Hydrologie. 11: 333–351.

Ivanković P, Treer T, Piria M, Knezović Z. 2010. Diet and growth of the endemic Neretva chub, *Squalius svallize* from the Neretva river area, Bosnia and Herzegovina. Folia Zoologica. 59(1): 51–56. https://doi.org/10.25225/fozo.v59.i1.a8.2010

Jakovlić I, Qing Jiang W, Treer T, Šprem N, Jian Fang Gui JF. 2013. Introgression evidence and phylogenetic relationships among three (*Para*)*Misgurnus* species as revealed by mitochondrial and nuclear DNA markers. Archives of Biological Sciences. 65(4): 1463-1467. https://doi.org/10.2298/ABS1304463J

Kalamujić B, Škrijelj R, Pojskić N. 2015. Genetic diversity of contemporary populations of *Salmo trutta* L. in the basin of the river Neretva, Bosnia and Herzegovina. Frontiers in Marine Science. Conference Abstract: XV European Congress of Ichthyology. https://doi.org/10.3389/conf.fmars.2015.03.00049

Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods. 14(6): 587–589. https://doi.org/10.1038/nmeth.4285

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution. 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Keppel G, Van Niel KP, Wardell-Johnson GW, Yates CJ, Byrne M, Mucina L, Schut AGT, Hopper SD, Franklin SE. 2012. Refugia: Identifying and understanding safe havens for biodiversity under climate change. Global Ecology and Biogeography. 21(4): 393–404. https://doi.org/10.1111/j.1466-8238.2011.00686.x

Lerceteau-Köhler E, Weiss S. 2006. Development of multiplex PCR microsatellite assay in brown trout Salmo trutta, and its potential application for the genus. Aquaculture. 258: 641-645. https://doi.org/10.1016/j.aquaculture.2006.04.028

Marić S, Stanković D, Sušnik Bajec S, Vukić J, Šanda R, Stefanov T, Nikolić D, Snoj A. 2022. Perils of brown trout (*Salmo* spp.) mitigation driven translocations: a case study from the Vlasina Plateau, Southeast Serbia. Biological Invasions. 24: 999-1016. https://doi.org/10.1007/s10530-021-02688-0

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R, Teeling E. 2020. IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution. 37(5): 1530–1534. https://doi.org/10.1093/molbev/msaa015

Muhamedagić S, Gjoen HM, Vegra M. 2010. Salmonids of the Neretva river basin – present state and suggested sustainable selection programme to protect and strengthen salmonid populations. Proceedings of the EIFAC Symposium on Interactions between Social, Economic and Ecological Objectives of Inland Commercial and Recreational Fisheries and Aquaculture; 21-24 May 2008; Antalya, Turkey.

Muhamedagić S, Pavličević J, Savić N. 2019. Composition of ichthyopopulations in the upper stream of the Neretva River, half a century after the construction of the first dam. Works of the Faculty of Agriculture and Food Sciences, University of Sarajevo, Vol. LXIV, No. 69/1.

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution. 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Oikonoumou A, Leprieur F, Leonardos ID. 2014. Biogeography of freshwater fishes of the Balkan Peninsula. Hydrobiologia. 738: 205-220.

Palandačić A, Bravničar J, Zupančič P, Šanda R, Snoj A. 2015. Molecular data suggest a multispecies complex of *Phoxinus* (Cyprinidae) in the Western Balkan Peninsula. Molecular Phylogenetics and Evolution. 92: 118–123. https://doi.org/10.1016/j.ympev.2015.05.024

Palandačić A, Kruckenhauser L, Ahnelt H, Mikschi E. 2020. European minnows through time: museum collections aid genetic assessment of species introductions in freshwater fishes (Cyprinidae: *Phoxinus* species complex). Heredity. 124(3): 410–422. https://doi.org/10.1038/s41437-019-0292-1

Palandačić A, Naseka A, Ramler D, Ahnelt H. 2017. Contrasting morphology with molecular data: An approach to revision of species complexes based on the example of European *Phoxinus* (Cyprinidae). BMC Evolutionary Biology. 17(1). https://doi.org/10.1186/s12862-017-1032-x

Pavličević J, Glamuzina L, Conides A, Savić N, Rozić I, Klaoudatos D, Kazić A, Glamuzina B. 2016. Pikeperch, *Sander lucioperca* invasion in the Neretva River watershed (Bosnia and Herzegovina, Croatia) after alteration of river flow. River Research and Applications. 32(5): 967–974. https://doi.org/10.1002/rra.2923

Pilić S, Gajević M, Vesnić A, Šljuka S, Korjenić E, Đug S, Mušović A. 2021. A preliminary study of morphometric characteristics and reproductive potential of the fish species *Cottus gobio* Linnaeus, 1758 on selected sites from Bosnia and Herzegovina. Veterinaria. 70(3): 293–310. https://doi.org/10.51607/22331360.2021.70.3.293

Pont D, Rocle M, Valentini A, Civade R, Jean P, Maire A, Roset N, Schabuss M, Zornig H, Dejean T. 2018. Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. Scientific Reports. 8(10361). https://doi.org/10.1038/s41598-018-28424-8

Razpet A, Sušnik Bajec S, Rozman T, Snoj A. 2007. Genetic variation among trout in the river Neretva basin, Bosnia and Herzegovina. Journal of Fish Biology. 71(sa): 94-110. https://doi.org/10.1111/j.1095-8649.2007.01392.x

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning. A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press. New York (NY): Cold Spring Harbor.

Schöffmann J, Marić S, Snoj A. 2019. Trout of Southeast Europe, Western and Central Asia. In: Kershner LK, Williams JE, Greswell RE, Lobón-Cerviá J, editors. Trout and char of the world. Bethesda (MD): American Fisheries Society.

Seber GAF, Cren EDL. 1967. Estimating population parameters from catches large relative to the population. The Journal of Animal Ecology. 36(3): 631. https://doi.org/10.2307/2818

Skoulikidis NT, Zogaris S, Karaouzas I. 2009. Rivers of the Balkans. In: Rivers of Europe. [place unknown]: Elsevier. p. 595–655. https://doi.org/10.1016/B978-0-08-102612-0.00015-8

Šlechtová V, Bohlen J, Freyhof J, Persat H, Delmastro GB. 2004. The Alps as barrier to dispersal in cold-adapted freshwater fishes? Phylogeographic history and taxonomic status of the bullhead in the Adriatic freshwater drainage. Molecular Phylogenetics and Evolution. 33(1). 225–239. https://doi.org/10.1016/j.ympev.2004.05.005

Snoj A, Bogut I, Sušnik S. 2008. Evidence of a genetically distinct population of Vrljika softmouth trout *Salmo obtusirostris* Heckel evolved by vicariance. Journal of Fish Biology. 72(8). 1945–1959. https://doi.org/10.1111/j.1095-8649.2008.01816.x

Snoj A, Glamuzina B, Razpet A, Zablocki J, Bogut I, Lerceteau-Köhler E, Pojskuć N, Sušnik Bajec S. 2010. Resolving taxonomic uncertainties using molecular systematics: *Salmo dentex* and the Balkan trout community. Hydrobiologia. 651(1): 199-212. https://doi.org/10.1007/s10750-010-0297-5

Snoj A, Melkić E, Sušnik Bajec S, Muhamedagić S, Dovć P. 2002. DNA phylogeny supports revised classification of *Salmothymus obtusirostris*. Biological Journal of the Linnaean Society. 77(3): 399-411. https://doi.org/10.1046/j.1095-8312.2002.00130.x

Snoj A, Razpet A, Tomljanović T, Treer T, Sušnik S. 2007. Genetic composition of the Jadro softmouth trout following translocation into a new habitat. Conservation Genetics. 8(5): 1213–1217. https://doi.org/10.1007/s10592-006-9262-2

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution. 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

Thurow RF. 1994. Underwater methods for study of salmonids in the Intermountain West. General Technical Report - US Department of Agriculture, Forest Service. INT-GTR-307. https://doi.org/10.5962/bhl.title.100074

Tougard C, Justy F, Guinand B, Douzery EJP, Berrebi P. 2018. Salmo macrostigma (Teleostei, Salmonidae): Nothing more than a brown trout (*S. trutta*) lineage? Journal of Fish Biology. 93: 302–310. https://doi.org/10.1111/jfb.13751/

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research. 44(W1): W232–W235. https://doi.org/10.1093/NAR/GKW256

Tutman P, Buj I, Ćaleta M, Hamzić A, Korjenić E, Adrović A, Glamuzina B. 2017. Status and distribution of spined loaches (Cobitidae) and stone loaches (Nemacheilidae) in Bosnia and Herzegovina. Folia Zoologica. 66(4): 211–226. https://doi.org/10.25225/fozo.v66.i4.a2.2017

Tutman P, Dragičević B, Dulčić J, Bukvić V, Bekh V, Glamuzina B. 2021. Records of invasive European catfish *Silurus glanis* (Linnaeus, 1758) in the Neretva River delta (Croatia): Social Media as Information Source. Croatian Journal of Fisheries. 79(1), 47–52. https://doi.org/10.2478/cjf-2021-0005

Tutman P, Dragičević B, Dulčić J, Glamuzina L, Bukvić V, Vekić J. 2021. Unusual records of marine organisms in the Neretva River (Croatia and Bosnia and Herzegovina). Acta Adriatica. 62(2): 139–148. https://doi.org/10.32582/aa.62.2.2

Tutman P, Glamuzina B, Dulčić J, Zovko N. 2012. Ichthyofauna of the Hutovo Blato wetland (lower River Neretva, Bosnia and Herzegovina); status and vulnerability. Croatian Journal of Fisheries. 70(4): 169–185.

Uiblein F, Jagsch A, Honsig-Erlenburg W, Weiss S. 2001. Status, habitat use, and vulnerability of the European grayling in Austrian waters. Journal of Fish Biology. 59: 223-247. https://doi.org/10.1111/j.1095-8649.2001.tb01388.x

Unfer G, Pinter K. 2018. Recreational fisheries: The need for sustainability in fisheries management of Alpine rivers. In: Schmutz S, Sendzimir J, editors. Riverine Ecosystem Management. Cham: Springer. p. 253–270. https://doi.org/10.1007/978-3-319-73250-3_14

Vegara M, Muhamedagić S, Hamzić A. et al. 2009. Mid-term improvement program of fisheries for the fishing area of Kalinovik municipality. Norwegian University of Life Sciences (UMB), NORAGRIC, AAS, Norway, Norwegian SEE programme: Institutional collaboration between academic institutions in agriculture, forestry and veterinary medicine in Norway and Bosnia and Herzegovina, Croatia and Serbia and Montenegro, 2006-2009.

Vukić J, Eliášová K, Marić D, Šanda R. 2019. Occurrence of alien spirlin (*Alburnoides* sp.) in the Neretva River basin. Knowledge & Management of Aquatic Ecosystems. 420: 15. https://doi.org/10.1051/kmae/2019007

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences. 360(1462): 1847–1857. https://doi.org/10.1098/rstb.2005.1716