

Research Article

Challenges to preserving native brown trout diversity in the Western BalkansPredrag Simonović^{1,2}, Vojislav Sokolović¹, Milica Ristovska³, Ana Marić¹, Katarina Rebok³, Vera Nikolić^{1,2}, Tena Radočaj⁴, Ivan Špelić⁴, Tamara Kanjuh¹, Zoran Vidović⁵, Lorenzo Vilizzi^{6,7} and Marina Piria^{4,6}

¹University of Belgrade, Faculty of Biology, Institute of Zoology, 11000 Belgrade, Serbia; ²University of Banja Luka, Faculty of Natural Sciences and Mathematics, Department of Biology and Ecology, 78000 Banja Luka, Bosnia and Herzegovina; ³University “Ss Cyril and Methodius” of Skopje, Faculty of Sciences, Department of Biology, 1000 Skopje, North Macedonia; ⁴University of Zagreb Faculty of Agriculture, Department of Fisheries, Apiculture, Wildlife Management and Special Zoology, 10000 Zagreb, Croatia; ⁵University of Belgrade, Teaching Education Faculty, 11000 Belgrade, Serbia; ⁶University of Lodz, Faculty of Biology and Environmental Protection, Department of Ecology and Vertebrate Zoology, 90-237 Lodz, Poland; ⁷Department of Biological Sciences, College of Science, Research Center for the Natural and Applied Sciences, The Graduate School, University of Santo Tomas, Manila, 1008 Metro Manila, Philippines

Corresponding author: Predrag Simonović (pedja@bio.bg.ac.rs)

Co-Editors' Note: This paper was contributed as part of the Special Issue “Advances in the study of the management of biological invasions in inland waters and the legacy of Gordon Howard Copp (1956–2023)”. This collaborative effort has attracted some of the most prominent invasion biologists who have contributed a diverse collection of high-profile papers addressing current knowledge gaps, research advances, and future opportunities in the management of biological invasions.

Citation: Simonović P, Sokolović V, Ristovska M, Marić A, Rebok K, Nikolić V, Radočaj T, Špelić I, Kanjuh T, Vidović Z, Vilizzi L, Piria M (2025) Challenges to preserving native brown trout diversity in the Western Balkans. *Management of Biological Invasions* 16(1): 105–116, <https://doi.org/10.3391/mbi.2025.16.1.07>

Received: 23 February 2024**Accepted:** 16 June 2024**Published:** 15 January 2025**Handling editor:** Calum MacNeil**Copyright:** © Simonović et al.

This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International - CC BY 4.0).

OPEN ACCESS

Abstract

In the Western Balkans, fishery management and legislation do not recognize the high molecular diversity of native brown trout. For this reason, stocking of streams relies on brown trout of non-native origin. In recent years, there have been new findings about the life-history features, dispersal and molecular characterisation of nominal taxa of brown trout in the Western Balkans. This has prompted to a re-evaluation of their risk of invasiveness in the region. In this study, based on a screening of six non-native lineages of brown trout, four lineages were ranked as high risk and two as medium risk of invasiveness under current climate conditions, whereas one lineage was ranked as high risk and the other five as medium risk under predicted global warming scenarios. Variation in scores and risk outcomes were related to life-history traits differing among lineages, with most pronounced differences in dispersal mechanisms. The results of this study point to the need for improvement of relevant legislation in several sectors of fishery management. Management strategies should aim to mitigate the conflicts arising among stakeholders, which contribute to the deterioration of the native traits of brown trout stocks throughout the Western Balkans.

Key words: indigenous diversity, *Salmo trutta*, invasiveness risk, Aquatic Species Invasiveness Screening Kit (AS-ISK), administrative and fishery management

Introduction

Brown trout *Salmo trutta* L., 1758 is a highly valued resource for sport fishing. The first reliable record about the appreciation for brown trout fishing dates back to 1496 (Berners 1880), followed by the reports of Walton in 1653 (Walton 2019) and many others until the recent comprehensive review about trout fishing, particularly fly fishing, by Herd (2003). Almost two millennia have now elapsed since the first report on fly fishing for brown trout in Aegean Macedonia by Ælianus – an ancient Roman traveller.

Nowadays, in totally unrelated parts of the world, almost the same techniques for fly fishing (e.g. the Japanese “tenkara” and the Balkans’ hazelnut-pole-and-horsehair-line) have developed independently over time. As a result of its popularity, brown trout has spread out of its native range of Europe, Northwestern Africa and Asia Minor to 42 countries worldwide (Welcomme 1992), including streams in Kilimanjaro, Kashmir, Australia, New Zealand and Sri Lanka (Pawson 1987). This makes brown trout the 13th most widely introduced species in the world (Fausch 2007). Domestication occurred very early in 1748 in Westphalia, Germany (Leitritz and Lewis 1980), and then developed throughout the XIX century at Alsace and Baden-Württemberg (Müller 1956). Main exports out of the species’ native dispersal range were accomplished as fertilized roe loads from hatchery stocks of the United Kingdom and Germany (Pawson 1987; Tiano et al. 2007; Baisch 2012). However, the introduction of brown trout into distant streams on other continents has strongly affected the local native fish fauna including cutthroat trout *Oncorhynchus clarkii* (Richardson, 1836) in North America (Behnke 2007) and common river galaxias *Galaxias vulgaris* Stokell, 1949 in New Zealand (McIntosh et al. 1994; McIntosh and Townsend 1995). This makes brown trout one of the world’s most invasive fish species (Lowe et al. 2004).

Although decision support tools that evaluate the risk of invasiveness of non-native organisms rely on nominal, valid taxonomic entities, brown trout is in a different position owing to discrepancies and incongruences in its taxonomy (Kottelat 1997). Polymorphism at the genetic level has provided an opportunity for in-depth research about brown trout phylogeography and phylogenetics and for introducing a new level of taxonomy relevant to risk assessments. Simonović et al. (2015) accomplished the first risk evaluation for the Balkans Region using the Fish Invasiveness Screening Kit (FISK: Copp et al. 2005). That study reported that rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and brown trout *S. trutta* (*sensu stricto*) of the Atlantic haplogroup posed the greatest risk of invasiveness, which was ranked as high. These species were followed by Macedonian brown trout *Salmo macedonicus* (Karaman, 1924) and Lake Ohrid brown trout *Salmo letnica* (Karaman, 1924) of the AD haplogroup, by Danubian brown trout *Salmo labrax* Pallas, 1814 of the Da1 (and Da2) haplotypes and by brook trout *Salvelinus fontinalis* (Mitchill, 1814), which were ranked as medium risk. Most brown trout haplotypes, albeit considered native, were domesticated and translocated. The only non-native brown trout of the Da2 haplotype was domesticated and historically introduced for aquaculture to be later translocated throughout the risk assessment area, despite different views recently presented in this regard (Veličković et al. 2023).

The Balkans Region is a remarkable biodiversity hotspot (Blondel and Aronson 1999; Médail and Quézel 1999; Hewitt 2011) as reflected by the original diversity of native trout *Salmo* spp. In addition to the soft-muzzled trout *Salmo obtusirostris* (Heckel, 1851), Lake Ohrid’s belvica trout *Salmo ohridanus* Steindachner, 1892 and marble trout *Salmo marmoratus* Cuvier,

1829, several nominal, valid or tentative taxa of brown trout have been described from the Western Balkans. Molecular markers, especially the non-coding sequence of the D-loop in the mitochondrial DNA control region (Bernatchez et al. 1992; Suarez et al. 2001; Cortey et al. 2004; Bardakçj et al. 2006), have been introduced and extensively researched in an attempt to resolve the brown trout polymorphism, though this has not helped resolve incongruencies in nomenclature. The use of molecular markers has enabled the discovery of three and four new haplotypes of the Danubian (DA) and Adriatic (AD) haplogroups in Serbia, respectively. (Marić et al. 2006). Recently, two more DA haplotypes were discovered (Tošić et al. 2014; Kanjuh et al. 2021) and another two reported from streams in northern Croatia (Kanjuh et al. 2020).

Apart from the native haplotypes, several non-native haplotypes have been reported, suggesting a long history of stocking streams with domesticated, hatchery-reared brown trout. Marić et al. (2006) reported for the first time the occurrence of *S. trutta* of the Atlantic (AT) haplogroup and *S. labrax* of the non-native haplotype Da2 from the River Crni Timok drainage in sympatry with the new, region-specific native haplotype described subsequently as Da23c (Tošić et al. 2014). The Da2 haplotype was reported later from some streams of the Western Balkans (Jadan et al. 2007; Mrdak et al. 2012; Škraba et al. 2017; Kanjuh et al. 2020), occurring always in sympatry with other haplotypes, both native (e.g. Da1) and non-native (e.g. Atcs1). An exception is the single occurrence in the River Vrijeka at the Dabar Karst Field in Eastern Herzegovina – a stream that was naturally void of brown trout (Simonović et al. 2017a). Meanwhile, three haplotypes of the AD haplogroup of *Salmo farioides* Karaman, 1938 native to the Adriatic Sea slopes in Montenegro (Škraba Jurlina et al. 2020) have revealed a migratory life history occurring together with the resident life history of fish in the same (sub)population. The recent occurrence of the Da2a haplotype of the tentative *S. labrax* has been reported in both brood stocks and streams of Serbia and North Macedonia (Marić et al. 2017; Veličković et al. 2023). This is similar to the haplotypes Adcs11 and Ad-Prz of native *S. farioides* of the AD lineage from the River Radika in the western part of North Macedonia, which were allegedly domesticated. The molecular identity of the North Macedonia's tentative *S. farioides* is different from that in Montenegro and Herzegovina (Škraba Jurlina et al. 2020). Currently, they are still horizon species for the majority of the catchments in the Western Balkans, except for streams in the River Vardar (Axios) drainage area belonging to the Aegean Sea basin in North Macedonia (Marić et al. 2017), where they have already been translocated owing to common practice of fishery managers and fish breeders to produce stocking material (Simonović et al. 2017b).

Marić et al. (2022) recently evaluated with the Aquatic Species Invasiveness Screening Kit (AS-ISK: Copp et al. 2016b, 2021) the risk of invasiveness in the Balkans Region of 13 extant and translocated non-native trout and four horizon species that are present in nearby regions but not yet found in the

risk assessment area. In that study, six of the screened species were found to pose a very high risk of invasiveness under current climate conditions, but only three under global warming scenarios. Among the species posing the highest risk of invasiveness was brown trout of the AT haplogroup. However, the study of Marić et al. (2022) did not focus on non-native brown trout lineages found in the broodstocks of local hatcheries, whose stocking may pose a threat of hybridization with native genetic material and detrimentally affect the local salmonid fish stock.

The aim of this study was to (1) re-evaluate the risk of invasiveness of domesticated brown trout lineages following the study by Simonović et al. (2015), and (2) perform a new screening of two recently identified non-native lineages of brown trout from local hatcheries in the Western Balkans. The present results are expected to contribute to the improvement of fishery management, particularly in terms of decisions regarding the selection of genetic material for stocking activities and the preservation of native salmonid fish stocks, not only in the risk assessment area but also on a broader scale.

Materials and methods

Risk screening was undertaken with AS-ISK v2.3.3 (available at www.cefas.co.uk/nns/tools/), which is a second-generation decision support tool for screening non-native aquatic organisms (Vilizzi et al. 2022b). This toolkit consists of 55 questions, with the first 49 focusing on the Basic Risk Assessment (BRA) and dealing with the biogeography, invasion history and biology/ecology of the screened species. The last six questions pertain to the Climate Change Assessment (CCA) and ask the assessor about the potential effects of future climate conditions on the risks associated with the species' introduction, establishment, dispersal and impact (as evaluated in the BRA). To achieve a valid screening, the standard protocol described in Vilizzi et al. (2022a, 2022b) and Vilizzi and Piria (2022) was followed. Screenings were conducted using a consensus approach that involved a minimum of two assessors who are familiar with each of the assigned species (Supplementary material, Appendix 1). To distinguish between medium- and high-risk species, the threshold value of 19.25 (after Marić et al. 2022) was used.

For the re-screening, four brown trout molecular variants for which new knowledge has been acquired (Simonović et al. 2015) were selected due to their invasiveness risk potential previously identified with FISK (Copp et al. 2016a) (Table 1): *S. farioides* (haplotypes Adcs11 and Ad-Prz), *S. labrax* (haplotype Da2) and *S. macedonicus* (haplotype Adcs1). A new screening was performed on two lineages recently identified in the broodstock of the Western Balkans of *S. letnica* (haplotypes Haplo12 and Haplo14), which is native to Lake Ohrid and the River Crni Drim drainage area including the River Radika (Table 1). The risk assessment area encompassed the countries of the Western Balkans, namely Albania, Bosnia and Herzegovina, Croatia, Kosovo, Montenegro, North Macedonia and Serbia (Figure 1, Appendix 2).

Table 1. Non-native salmonids evaluated for their potential risk of invasiveness in the Western Balkans with details about native distribution and known introductions.

Species name	Common brown trout name	Haplotype	Distribution area		
			Native	Introduced	
<i>Salmo farioides</i>	Western Balkans	Adcs11	Adriatic Sea Basin in Montenegro and Northern Macedonia	Danube River Basin	Simonović et al. 2015
<i>Salmo farioides</i>	Western Balkans	Ad-Prz	Adriatic Sea Basin in Montenegro and Northern Macedonia	Danube River Basin	Simonović et al. 2015
<i>Salmo labrax</i>	Danubian	Da2	Northern slopes of Alps in Austria	Rivers Gacka, Gradac, Crni Timok, Vratna and many other streams in Serbia	Simonović et al. 2015; Veličković et al. 2023
<i>Salmo letnica</i>	Lake Ohrid	Haplo12	Lake Ohrid in Northern Macedonia	Vlasina Reservoir	Simonović et al. 2015
<i>Salmo letnica</i>	Lake Ohrid	Haplo14	Lake Ohrid in Northern Macedonia	Vlasina Reservoir	Simonović et al. 2015
<i>Salmo macedonicus</i>	Macedonian	Adcs1	Aegean Sea basin in SE Serbia and Northern Macedonia	River Jerma	Simonović et al. 2015

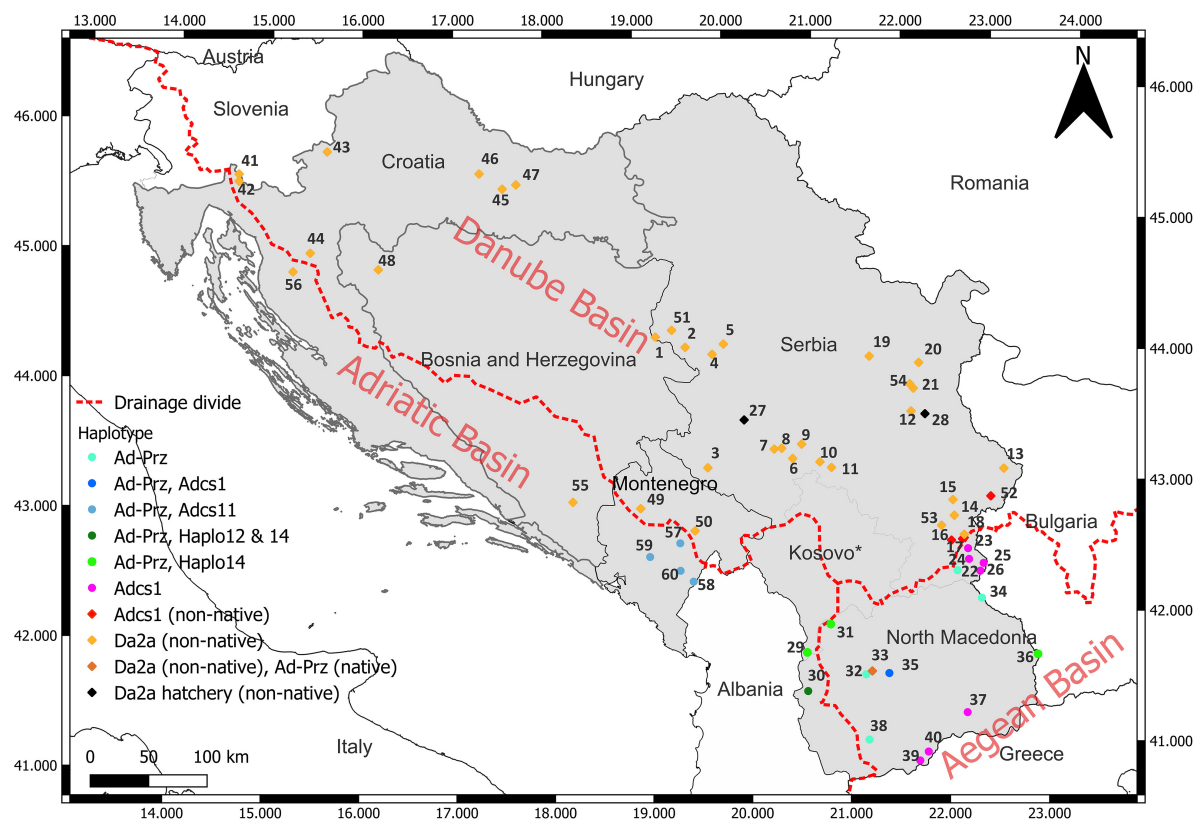


Figure 1. Localities where brown trout were sampled in the risk assessment area (numbers for each of sampling locations, rivers' and sampling locations' names, latitude and longitude coordinates, year of record and references for the source data are given in Appendix 2.

Results

Based on the BRA scores, four species were ranked as high risk and two as medium risk. Based on the BRA+CCA scores, one species was ranked as high risk and the other five as medium risk (Table 2). Only *S. letnica* Haplo14 was ranked as high risk for both the BRA and BRA+CCA. Climate change predictions led to a decrease in the BRA score for all six species. The lowest score for the Biogeography/Historical section was assigned to *S. farioides* haplotype Adcs11, as the score in the Invasive elsewhere section was reduced by two points (Table 3). *Salmo letnica* haplo12 received five points in this part

Table 2. Risk ranks for the non-native salmonids screened with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for the Western Balkans. For each species, the following information is provided: Basic Risk Assessment (BRA) and BRA + Climate Change Assessment (BRA+CCA) scores with corresponding risk ranks (M = Medium; H = High) based on a threshold value of 19.25 (after Marić et al. 2022); CCA value; Confidence factor (CF) for all 55 questions (BRA and CCA) and separately for the 49 BRA questions and 6 CCA questions.

Species name	Haplotype	BRA		BRA+CCA		CCA	CF		
		Score	Rank	Score	Rank		Total	BRA	CCA
<i>Salmo farioides</i>	Adcs11	13.0	M	5.0	M	-8	0.85	0.86	0.75
<i>Salmo farioides</i>	Ad-Prz	28.0	H	16.0	M	-12	0.87	0.88	0.83
<i>Salmo labrax</i>	Da2	24.0	H	20.0	M	-4	0.83	0.83	0.83
<i>Salmo letnica</i>	Haplo12	15.0	M	3.0	M	-12	0.82	0.82	0.79
<i>Salmo letnica</i>	Haplo14	26.0	H	22.0	H	-4	0.83	0.83	0.83
<i>Salmo macedonicus</i>	Adcs1	25.0	H	13.0	M	-12	0.91	0.90	1.00

Table 3. Breakdown of the AS-ISK BRA scores for the non-native salmonids screened with the AS-ISK for the Western Balkans.

	<i>Salmo farioides</i> (Adcs11)	<i>Salmo farioides</i> (Ad-Prz)	<i>Salmo labrax</i> (Da2)	<i>Salmo letnica</i> (Haplo12)	<i>Salmo letnica</i> (Haplo14)	<i>Salmo macedonicus</i> (Adcs1)
Biogeography/Historical	2.0	16.0	16.0	5.0	16.0	16.0
Domestication/Cultivation	0.0	2.0	2.0	0.0	2.0	2.0
Climate, distribution, and introduction risk	4.0	4.0	4.0	3.0	4.0	4.0
Invasive elsewhere	-2.0	10.0	10.0	2.0	10.0	10.0
Biology/Ecology	11.0	12.0	8.0	10.0	10.0	9.0
Undesirable (or persistence) traits	4.0	5.0	3.0	5.0	5.0	5.0
Resource exploitation	7.0	7.0	7.0	7.0	7.0	7.0
Reproduction	4.0	4.0	4.0	4.0	4.0	4.0
Dispersal mechanisms	0.0	2.0	0.0	0.0	0.0	-1.0
Tolerance attributes	-4.0	-6.0	-6.0	-6.0	-6.0	-6.0

of the questionnaire, while the other four species received a score of 16. In the Biology/Ecology section, the highest score belonged to *S. farioides* haplotype Ad-Prz and the lowest to *S. labrax* Da2. In this section, the questions on Dispersal mechanisms resulted in the largest score differences.

Discussion

Despite previous screenings on brown trout and other trout species (Simonović et al. 2013, 2015; Marić et al. 2022), monitoring of the impacts and risks posed by non-native, translocated and horizon trout species should be updated with new information (Simonović 2016). In the context of the Western Balkans, there are concerns regarding the capacity of decision-makers to utilize decision support tools such as the AS-ISK in an effective way and to act according to the outcomes of the screenings. Furthermore, risk screening studies are closely intertwined with fishery and conservation issues both of which require management attention. The monitoring of non-native, translocated and horizon species (or of taxonomic units of high conservation value below the species level) falls within the remit of the administrative sector involved, with each sector playing a distinct role in preserving the original biological diversity of fish populations.

Based on the results of this study, there is a considerable threat posed by different haplotypes of non-native trout lineages to the native ones, ranging from medium to high risk. In the current state of brown trout conservation

practice in the risk assessment area, *S. fariooides* of the Adcs11 haplotype native from streams of the Adriatic Sea's slopes in Montenegro features a lake-dwelling subpopulation consisting predominantly of females that migrate to spawn with the predominantly resident males (Škraba Jurlina et al. 2020). This life-history feature superimposes them to the predominantly resident, stream-dwelling male counter-mates. However, this migratory life history also favours introduced (i.e. non-native) lake- or reservoir-dwelling brown trout stocks over the native resident, stream-dwelling stocks in the recipient headwater streams. As for *S. fariooides* of the AD haplotype Adcs11 in Montenegro and eastern Herzegovina, its domestication has been attempted several times in the last ten years without success. The legal obligation by fisheries managers for stocking only native species without quoting precisely the exact native haplotype raises attention towards the non-native haplotypes of this and other tentative brown trout species (e.g. *S. letnica*) that are already domesticated in North Macedonia. This justifies the need for a re-assessment of the risk of their introduction out of the native area.

Regardless of haplotype or species, all salmonids are cold-water fish of similar environmental demands (Simonović et al. 2020) and as such global warming could have a major impact on their habitat and populations. The tentative brown trout species analyzed in this study all showed a decline in score after accounting for climate change, hence confirming previous screenings (Marić et al. 2022). However, some screened species haplotypes (e.g. *S. letnica* haplo12) showed different CCA values resulting from adaptation to climate change. The detailed effects of temperature and precipitation on the distribution of particular species haplotypes is difficult to predict, though the BRA distinguished four haplotypes as more resilient (i.e. *S. fariooides* Ad-Prz, *S. labrax* Da2, *S. letnica* Haplo14 and *S. macedonicus* Adcs1), with *S. fariooides* Ad-Prz having the highest BRA score. Based on the BRA scores, *S. fariooides* Adcs11 and *S. letnica* Haplo12 were ranked as medium risk, possibly due to their low dispersal mechanisms and developmental plasticity, which could prevent them from adapting to changing environmental conditions (Marić et al. 2022). The BRA+CCA scores for *S. fariooides* Adcs11 and *S. letnica* Haplo12 were quite low, contrary to their Biology/Ecology scores, likely due to their higher tolerance and persistence compared to the other haplotypes screened.

The only available stocking material in the Balkans Region is that of domesticated brown trout of the AT lineage. The brood stocks of the native DA lineage originating from the majority of the streams in the Balkans are not present in local fish farms (Marić et al. 2022). However, stocking streams with non-native brown trout of the AT lineage complies with legal acts covering fishery management that do not recognize the high native diversity (as indicated by molecular variants or tentative taxa, as described here) of brown trout in the region. An occurrence of the allegedly native *S. labrax* of the Da2a

haplotype in brood stocks of several brown trout hatcheries (Veličković et al. 2023) was accompanied by its finding as the only one of the Da2 haplotypes occurring in several streams of Serbia. Regarding the character of the Da2a haplotype, both variants (corresponding to the DA and AT haplogroups) of brown trout stocking material from the River Soča drainage area (western Slovenia) at the beginning of the 20th century were obtained from the hatchery situated at the spring of the River Bosna at Ilidža near Sarajevo (Gridelli 1936 in Razpet et al. 2007). Records about stocking streams in Montenegro and Serbia with hatchery-reared brown trout are lacking, but it is certain that this took place in the second half of the 20th century when there was no native brown trout production at all. It is most likely that the source of the material for stocking was from hatcheries in Bosnia and Herzegovina, where salmonid aquaculture was a legacy of Austrian fish breeders (Aganović 1979).

Because of the risks that stocking poses to indigenous brown trout diversity (Simonović et al. 2017 b), it is likely that stocking has been the main vector of dispersal of *S. labrax* of the Da2 haplotype. The common driving force behind stocking is the fishermen's desire to catch trophy-size brown trout as fishery managers do not hesitate to pay for stocking material if it maintains or increases license sales. Fish farms produce readily available stocking material for this purpose. Thus, the income that serves as impetus for promoting stocking of brown trout fisheries, including the occurrence of Da2a in particular streams of Central Serbia and brown trout hatcheries currently holding brood fish of the Da2a haplotype (Veličković et al. 2023), are entirely in line with this practice. The occurrence of exclusive Da2a haplotype alone in streams of Serbia, as well as in streams throughout the Western Balkans, is incongruent with the native dispersal area of *S. labrax* of the Da2 haplotype group (i.e. streams in Western Austria), where three native sub-haplotypes (Da2a, b and c) occur in similar frequency (Baric et al. 2010). This monomorphism strongly indicates their introduction from aquaculture, hence their non-native origin.

The unresolved taxonomy of brown trout in the region strongly impinges on conservational issues as well as fishery management. Mitigating the current discrepancy between conservation and management, which involves contrasting interests, and implementing relevant legislative measures is not a straightforward task due to nomenclatural complexity (i.e. nominal species and molecular variants). Declaring the nominal *S. labrax* and *S. fariodes* species native to headwaters of the River Danube and Adriatic Sea slopes catchments, respectively would not adequately identify the nativeness of the stocking material. This is because some DA and AD haplotypes, which are already domesticated, may not be native to the recipient streams in the risk assessment area (e.g. the Da2a and the Ad-Prz in the River Danube and the River Neretva catchments, respectively). The actions to be taken depend on the strategy adopted for management. If the

aim for the improvement and maintenance of fisheries is stocking, then it will be necessary to upgrade relevant legislation by introducing the concept of either evolutionary or conservation significant units instead of nominal species and set the aim of domesticating and local rearing of such pure, indigenous brood stock lineages of brown trout. However, this is currently neither cost-effective nor feasible from the standpoint of administrative management and its capacity to survey it. A far more effective strategy would be to implement consistent control of fishing that ensures fishery sustainability, which is both the least costly and most effective way to avoid stocking, hence to reduce the risk from introduction of non-native brown trout haplotypes. This could be achieved as either conditional level of catch determined by minimal landing size and daily bag limit proportional both to natural stocks' yield and length at which yield is maximal (see Beverton 1992, as used in Simonović et al. 2018) or by unconditional catch-and-release, depending on fishing pressure. Furthermore, monitoring activities that would occasionally check the molecular markers' status of brown trout populations should be implemented, especially of those of greater conservation value holding local and isolated haplotypes. This is in accordance with recommendations for the conservation of native brown trout (Laikre et al. 1999).

Authors' contribution

PS conceptualized, designed research and coordinated contributors and prepared the final MS draft; VJ, MR and KR collected data and accomplished investigation by screening of brown trout haplotypes using the AS-ISK; ZV analysed the data and interpreted them; PS, MP, AM and TR drafted particular MS' sections; MP and LV checked all drafted versions and addressed statistical issues; IŠ created the map of localities; IŠ, TK and VN drafted the pre-final version of the MS; TK and VN prepared the citations and references and sorted out the literature. All authors inspected and approved the final version of the manuscript prior to submission.

Acknowledgments

PS, AM, VN, VS and TK were supported by Ministry of Science, Technological Development and Innovations of Serbia (Contracts No. 451-03-65/2024-03/ 200178 and No. 451-03-66/2024-03/ 200178), and MP, TR and IS by the EIFAAC/FAO Project "Management/Threat of Aquatic Invasive Species in Europe". Authors express a gratitude to anonymous reviewers' suggestions that improved the original manuscript.

Funding declaration

Publication of this study was supported by the European Union's Horizon Europe HORIZON-CL6-2024-BIODIV-01 project "GuardIAS - Guarding European Waters from IAS", under grant agreement no. 101181413 (Katsanevakis et al. 2024).

Ethics and permits

Sampling of brown trout in the Republic of Serbia was accomplished under the coverage with the License No. 324-04-007/2023-07 for Fishing for Scientific Purposes and Electrofishing issued on 02 March 2023 by the Ministry of Environment Protection of the Republic of Serbia, and the License No. 353-01-81/2023-04 for Research of Strictly Protected and Protected Fish Species for Scientific Purposes issued on 22 February 2023 by the Ministry of Environment Protection of the Republic of Serbia.

References

- Aganović M (1979) Salmonidne vrste riba i njihov uzgoj. IGKRO Svjetlost-OOUR Zavod za udžbenike, Sarajevo, Yugoslavia, 320 pp
- Baisch D (2012) Origin of Great Lakes brown trout, *Salmo trutta*: a phylogeographic analysis using mtDNA sequence variation. Master Theses 9–2012, Grand Valley State University, Michigan, United States, 40 pp
- Bardakçi F, Degerli N, Ozdemir O, Basibuyuk HH (2006) Phylogeography of the Turkish brown trout *Salmo trutta* L.: mitochondrial DNA PCR-RFLP variation. *Journal of Fish Biology* 68: 36–55, <https://doi.org/10.1111/j.0022-1112.2006.00948.x>
- Baric S, Riedl A, Meraner A, Medgyesy N, Lackner R, Pelster B, Dalla Via J (2010) Alpine headwater streams as reservoirs of remnant populations of the Danubian clade of brown trout. *Freshwater Biology* 55, 866–880, <https://doi.org/10.1111/j.1365-2427.2009.02318.x>
- Behnke RJ (2007) About trout: The best of Robert Behnke from Trout Magazine. Lyons Press, Guilford, Connecticut, USA, 259 pp
- 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, <https://doi.org/10.1111/j.1365-294X.1992.tb00172.x>
- Berners DJ (1880) A Treatise of Fysshynge wyth an Angle. Wynkyn de Worde, Westminster. A facsimile reproduction of 1496 edition. Elliot Stock, London, England, 23 pp.
- Beverton RJH (1992) Patterns of reproductive strategy parameters in some marine teleost fishes. *Journal of Fish Biology* 41 (Suppl. B): 137–160, <https://doi.org/10.1111/j.1095-8649.1992.tb03875.x>
- Blondel J, Aronson J (1999) Biology and wildlife of the Mediterranean region. *Journal of Natural History* 38:1723–1724, <https://doi.org/10.1080/002229303100015621>
- Copp GH, Garthwaite R, Gozlan RE (2005) Risk identification and assessment of non-native freshwater fishes: Concepts and perspectives on protocols for the UK. Science Series Technical Reports No. 129. Cefas, Lowestoft, Great Britain, <https://doi.org/10.1111/j.1439-0426.2005.00692.x>
- Copp GH, Russell IC, Peeler EJ, Gherardi F, Tricarico E, MacLeod A, Cowx IG, Nunn AN, Occhipinti-Ambrogi A, Savini D, Mumford J, Britton JR (2016a) European Non-native Species in Aquaculture Risk Analysis Scheme – a summary of assessment protocols and decision making tools for use of alien species in aquaculture. *Fisheries Management and Ecology* 23: 1–11, <https://doi.org/10.1111/fme.12074>
- Copp GH, Vilizzi L, Tidbury H, Stebbing PD, Tarkan AS, Moisse L, Gouletquer P (2016b) Development of a generic decision-support tool for identifying potentially invasive aquatic taxa: AS-ISK. *Management of Biological Invasions* 7: 343–350, <https://doi.org/10.3391/mbi.2016.7.4.04>
- Copp GH, Vilizzi L, Wei H, Li S, Piria M, Al-Faisal AJ, Almeida D, Atique U, Al-Wazzan Z, Bakiu R, Bašić T, Bui TD, Canning-Clode J, Castro N, Chaichana R, Çoker T, Dashinov D, Ekmekçi FG, Erős T, Ferincz Á, Ferreira T, Giannetto D, Gilles Jr AS, Glowacki Ł, Gouletquer P, Interesova E, Iqbal S, Jakubčinová K, Kanongdate K, Kim JE, Kopecký O, Kostov V, Koutsikos N, Kozic S, Kristan P, Kurita Y, Lee HG, Leuven RSEW, Lipinskaya T, Lukas J, Marchini A, González-Martínez AI, Masson L, Memedemin D, Moghaddas SD, Monteiro J, Mumladze L, Naddafi R, Năvodaru I, Olsson KH, Onikura N, Paganelli D, Pavia Jr RT, Perdikaris C, Pickholtz R, Pietraszewski D, Povž M, Preda C, Ristovska M, Rosiková K, Santos JM, Semenchenko V, Senanan W, Simonović P, Smeti E, Števove B, Švolíková K, Ta KAT, Tarkan AS, Top N, Tricarico E, Uzunova E, Vardakas L, Verreycken H, Zięba G, Mendoza R (2021) Speaking their language – Development of a multilingual decision-support tool for communicating invasive species risks to decision makers and stakeholders. *Environmental Modelling & Software* 135, e104900, <https://doi.org/10.1016/j.envsoft.2020.104900>
- Cortey M, Pla C, Garcia-Marin JL (2004) Historical biogeography of Mediterranean trout. *Molecular Phylogenetics and Evolution* 33: 831–844, <https://doi.org/10.1016/j.ympev.2004.08.012>
- Fausch KD (2007) Introduction, establishment and effects of non-native salmonids: considering the risk of rainbow trout invasion in the United Kingdom. *Journal of Fish Biology* 71 (Suppl. D): 1–32, <https://doi.org/10.1111/j.1095-8649.2007.01682.x>
- Gridelli E (1936) I pesci d'acqua dolce della Venezia Giulia. *Bollettino della Societa Adriatica di Scienze Naturali in Trieste* 35: 7–140.
- Herd A (2003) The fly: Two thousand years of fly fishing. 2nd edition. Medlar Press, Ellesmere, Shropshire, Great Britain, 381 pp.
- Hewitt GM (2011) Mediterranean peninsulas: the evolution of hotspots. In: Zachos F, Habel J (eds) Biodiversity hotspots. Springer, Berlin, Heidelberg, Germany, pp, 123–147, https://doi.org/10.1007/978-3-642-20992-5_7
- Jadan M, Čož-Rakovac R, Topić Popović N, Strunjak-Perović I (2007) Presence of unexpected phylogenetic lineages of brown trout *Salmo trutta* L. in Gacka River, Croatia. *Aquaculture Research* 38: 1682–1685, <https://doi.org/10.1111/j.1365-2109.2007.01832.x>
- Kanjuh T, Marić A, Piria M, Špelić I, Maguire I, Simonović P (2020) Diversity of brown trout, *Salmo trutta* (Actinopterygii: Salmoniformes: Salmonidae), in the Danube River basin of

- Croatia revealed by mitochondrial DNA. *Acta Ichthyologica et Piscatoria* 50: 291–300, <https://doi.org/10.3750/AIEP/02939>
- Kanjuh T, Tomić T, Marić A, Škraba Jurlina D, Nikolić V, Simonović P (2021) Trout *Salmo* spp. (Salmoniformes: Salmonidae) molecular diversity in streams on the southern slopes of the Stara Planina Mts. in Serbia. *Acta zoologica bulgarica* 73: 425–429, <http://acta-zoologica-bulgarica.eu/2021/002487.pdf>
- Katsanevakis S, Zaiko A, Olenin S, Costello MJ, Gallardo B, Tricarico E, Adriaens T, Jeschke JM, Sini M, Burke N, Ellinas K, Rutten S, Poursanidis D, Marchini A, Brys R, Raeymaekers JAM, Noé N, Hermoso V, Blaaliid R, Lucy FE, Verbrugge LNH, Staehr PAU, Vandepitte L, de Groot D, Elliott M, Reuver M, Maclaren J, Li M, Oldoni D, Mazaris A, Trygonis V, Hablützel PI, Everts T, Pistevos JCA, Dekeyzer S, Kimmig SE, Rickowski FS, Panov VE (2024) GuardIAS – Guarding European Waters from Invasive Alien Species. *Management of Biological Invasions* 15: 701–730, <https://doi.org/10.3391/mbi.2024.15.4.14>
- Kottelat M (1997) European freshwater fishes. An heuristic checklist of the freshwater fishes of Europe (exclusive of former USSR), with an introduction for non-systematics and comments on nomenclature and conservation. *Biologia* 52 (Suppl. 5): 1–271.
- Laikre L, Antunes A, Apostolidis A, Berrebi P, Duguid A, Ferguson A, Garcia-Marin HL, Guyomard R, Hansen MM, Hindar K, Koljonen ML, Largiader C, Martinez P, Nielsen EE, Palm S, Ruzzant D, Ryman N, Tryantaphyllidis C (1999) Conservation genetic management of brown trout (*Salmo trutta*) in Europe. Report by the Concerted action on identification, management and exploitation of genetic resources in the brown trout (*Salmo trutta*), “Troutconcert”, EU Fair CT97-3882.
- Leitritz E, Lewis RC (1980) Trout and salmon culture (hatchery methods). *California Fish Bulletin* 164. Publication 4100. University of California, Division of Agriculture and Natural Resources, Oakland, CA, USA.
- Lowe S, Browne M, Boudjelas S, Poorter MD (2004) 100 of the world’s worst invasive alien species: A selection from the Global Invasive Species Database. Invasive Species Specialist Group (ISSG), a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), World Conservation Union, Auckland, Australia, 12 pp.
- Marić S, Sušnik S, Simonović P, Snoj A (2006) Phylogeographic study of brown trout from Serbia, based on mitochondrial DNA control region analysis. *Genetic, Selection, Evolution* 38: 411–430, <https://doi.org/10.1051/gse:2006012>
- Marić S, Sušnik Bajec S, Šhöffman J, Kostov V, Snoj A (2017) Phylogeography of stream-dwelling trout in the Republic of Macedonia and a molecular genetic basis for revision of the taxonomy proposed by S. Karaman. *Hydrobiologia* 785: 249–260, <https://doi.org/10.1007/s10750-016-2930-4>
- Marić A, Špelić I, Radočaj T, Vidović Z, Kanjuh T, Vilizzi L, Piria M, Nikolić V, Simonović P (2022) Changing climate may mitigate the invasiveness risk of non-native salmonids in the Danube and Adriatic basins of the Balkan Peninsula (south-eastern Europe). *NeoBiota* 76: 135–161, <https://doi.org/10.3897/neobiota.76.82964>
- McIntosh R, Crowl TA, Townsend CR (1994) Size-related impacts of introduced brown trout on the distribution of native common river galaxias. *New Zealand Journal of Marine and Freshwater Research* 28: 135–144, <https://doi.org/10.1080/00288330.1994.9516602>
- McIntosh AR, Townsend CR (1995) Contrasting predation risks presented by introduced brown trout and native common river galaxias in New Zealand streams. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 1821–1833, <https://doi.org/10.1139/f95-175>
- Médail F, Quézel P (1999) Biodiversity hotspots in the Mediterranean Basin: Setting global conservation priorities. *Conservation Biology* 13: 1510–1513, <https://doi.org/10.1046/J.1523-1739.1999.98467.X>
- Mrdak D, Nikolić V, Tošić A, Simonović P (2012) Molecular and ecological features of the soft-muzzled trout *Salmo obtusirostris* (Heckel, 1852) in the Zeta River, Montenegro. *Biologia* 67: 222–233, <https://doi.org/10.2478/s11756-011-0150-y>
- Müller H (1956) Die Forellen. Die einheimischen Forellen und ihre wirtschaftliche Bedeutung. Neue Brehm-Bücherei A. Ziemsen Verlag, Wittenberg, Lutherstadt, Germany, 164pp.
- Pawson T (1987) Fly Fishing Around the World: The International Guide for the Gamefisher. Unwin Human Ltd., London & Sidney, United Kingdom, 235 pp
- Razpet A, Marić S, Parapot T, Nikolić V, Simonović P (2007) Re-evaluation of *Salmo* data by Gridelli (1936) – description of stocking, hybridization and repopulation in the River Soča basin. *Italian Journal of Zoology* 74: 63–70, <https://doi.org/10.1080/11250000601090081>
- Simonović P (2016) Brown Trout *Salmo* cf. *trutta* L. – a case species for fishery- and aquaculture-vectorized invasiveness. FINS II Conference, 11–14 July 2016, University of Zagreb, Faculty of Agriculture, Zagreb, Croatia, Book of Abstracts, pp. 14–15.
- Simonović P, Tošić A, Vassilev M, Apostolou A, Mrdak D, Ristovska M, Kostov V, Nikolić V, Škraba D, Vilizzi L, Copp GH (2013) Risk identification of non-native freshwater fishes in four countries of the Balkans Region using FISK. *Mediterranean Marine Science* 14: 369–376, <https://doi.org/10.12681/mms.337>

- Simonović P, Vidović Z, Tošić A, Škraba D, Čanak-Atlagić J, Nikolić V (2015) Risks to stocks of native trout of the genus *Salmo* (Actinopterygii: Salmoniformes: Salmonidae) of Serbia and management for their recovery. *Acta Ichthyologica et Piscatoria* 45: 161–173, <https://doi.org/10.3750/AIP2015.45.2.06>
- Simonović P, Tošić A, Škraba Jurlina D, Nikolić V, Piria M, Tomljanović T, Šprem N, Mrdak D, Milošević D, Bećiraj A, Dekić R, Povž M (2017a) Diversity of brown trout *Salmo* cf. *trutta* in the River Danube basin of western Balkans as assessed from the structure of their mitochondrial control region haplotypes. *Journal of Ichthyology* 57: 603–616, <https://doi.org/10.1134/S0032945217040154>
- Simonović P, Tošić A, Škraba Jurlina D, Čanak Atlagić J, Nikolić V (2017b) Stakeholders participation in conservation of brown trout stocks in Serbia. Wild Trout XII Symposium "Science, Politics, and Wild Trout Management: Who's Driving and Where are We Going?", September 26–29, 2017, West Yellowstone, MT, USA, pp. 55–62.
- Simonović P, Grubić G, Tošić A, Čanak Atlagić J, Škraba Jurlina D, Nikolić V (2018) Justification for retention of the Catch-and-Release in the wild brown trout *Salmo* cf. *trutta* fishery. *Fisheries Research* 200: 17–24, <https://doi.org/10.1016/j.fishres.2017.12.010>
- Simonović P, Marić A, Škraba Jurlina D, Kanjuh T, Nikolić V (2020) Determination of community structure, population, productivity and growth features of stream-dwelling brown trout *Salmo* cf. *trutta* in Serbia by their habitat characteristics. *Biologia*, Section Zoology 75: 103–114, <https://doi.org/10.2478/s11756-019-00284-1>
- Škraba D, Bećiraj A, Šarić I, Ićanović I, Džaferović A, Piria M, Dekić R, Tošić A, Nikolić V, Simonović P (2017) Genotypization of brown trout (*Salmo trutta* L.) populations from River Una drainage area in Bosnia and Herzegovina and implications for conservation and fishery management. *Acta Zoologica Bulgarica* 69: 25–30.
- Škraba Jurlina D, Marić A, Mrdak D, Kanjuh T, Špelić I, Nikolić V, Piria M, Simonović P (2020) Alternative life-history in native trout (*Salmo* spp.) suppresses invasive effect of alien trout strains introduced into streams at the western part of the Balkans. *Frontiers in Ecology and Evolution* 8: 188, <https://doi.org/10.3389/fevo.2020.00188>
- Suarez J, Bautista JM, Almodovar A, Machordom A (2001) Evolution of the mitochondrial control region in Palaearctic brown trout (*Salmo trutta*) populations: the bio-geographical role of the Iberian Peninsula. *Heredity* 87: 198–206, <https://doi.org/10.1046/j.1365-2540.2001.00905.x>
- Tiano TJ, Willis CM, Noble AA, Luttenton M, Nikitin A (2007) Genetic identification of the hatchery stocks of brown trout using mitochondrial DNA polymorphism. *North American Journal of Fisheries* 27: 965–970, <https://doi.org/10.1577/M05-208.1>
- Tošić A, Škraba D, Nikolić V, Mrdak D, Simonović P (2014) New mitochondrial DNA haplotype of brown trout *Salmo trutta* L. from Crni Timok drainage area in Serbia. *Turkish Journal of Fisheries and Aquatic Sciences* 14: 37–42, <https://doi.org/10.4194/1303-2712-v14i05>
- Veličković T, Snoj A, Simić V, Šanda R, Vukić J, Barčić D, Stanković D, Marić S (2023) A new perspective on the molecular dating of the brown trout complex with an extended phylogeographic information on the species in Serbia. *Contributions to Zoology* 92: 362–389, <https://doi.org/10.1163/18759866-bja10046>
- Vilizzi L, Piria M (2022) Providing scientifically defensible evidence and correct calibrated thresholds for risk screening non-native species with second-generation Weed Risk Assessment-type decision-support tools. *Journal of Vertebrate Biology* 71: 22047, <https://doi.org/10.25225/jvb.22047>
- Vilizzi L, Copp GH, Hill JE, Piria M (2022a) A protocol for screening potentially invasive non-native species using Weed Risk Assessment-type decision-support toolkits. *Science of the Total Environment* 832, 154966, <https://doi.org/10.1016/j.scitotenv.2022.154966>
- Vilizzi L, Piria M, Copp GH (2022b) Which calibrated threshold is appropriate for ranking non-native species using scores generated by WRA-type screening toolkits that assess risks under both current and future climate conditions? *Management of Biological Invasions* 13: 593–608. <https://doi.org/10.3391/mbi.2022.13.4.01>
- Walton I (2019) *The Compleat Angler: The Contemplative's Man Recreation*. Richard Marriot, London, England, 217 pp E-book edition in 2019. Digireads.com Publishing, London.
- Welcome RL (1992) A history of international introductions of inland aquatic species. *ICES Marine Science Symposium* 194, 3–14.

Supplementary material

The following supplementary material is available for this article:

Appendix 1. AS-ISK reports for the six non-native salmonids screened for the Western Balkans.

Appendix 2. Sampling locations in water bodies of the risk assessment area (Western Balkans), with latitude and longitude coordinates, year of data recording and reference study.

This material is available as part of online article from:

http://www.reabic.net/journals/mbi/2025/Supplements/MBI_2025_Simonovic_et_al_Appendix_1.xlsx

http://www.reabic.net/journals/mbi/2025/Supplements/MBI_2025_Simonovic_et_al_Appendix_2.xlsx