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Non-native Brook Trout Salvelinus fontinalis in Bulgaria: an Established Population in the Palakariya River (Balkan Peninsula, Iskar River Basin)

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Abstract. In the present study, we provide data on the first established, self-sustaining population of non-native brook trout (*Salvelinus fontinalis* Mitchill, 1814), family Salmonidae, in Bulgaria. The brook trout was detected in upland section of the Palakariya River (Iskar basin) at an altitude between 1350 and 1500 m a.s.l. Distribution, abundance and size structure of *S. fontinalis* were studied in the period 2019-2021. The coexistence of individuals of different sizes (from 4.1 cm to 24.6 cm); no restocking activities in the last 10 years and the suitable environmental habitat features support the contention of a self-reproducing population of *S. fontinalis* in the Palakariya River.

Key words: non-indigenous species, salmonid fish, self-reproducing, Bulgaria.

Introduction

The brook trout *Salvelinus fontinalis* (Mitchill, 1814) is a native salmonid species for North America (Page & Burr, 1991; Karas, 1997; Scott & Crossman, 1973). This fish inhabits oligotrophic streams, rivers and lakes and prefer cool, clear, well-oxygenated waters, pools with large woody debris and temperatures up to 20 °C (FishBase, 2021; Page & Burr, 1991; Karas, 1997).

Brook trout is a highly desirable fish for both sport fishing and aquaculture (Jansson, 2013). Since brook trout is relatively tolerant of acidic waters withstanding a pH as low as 5.0 (Dunson & Martin, 1973), it has been used as a replacement when populations of native salmonid species become locally extinct due to acidification of some Scandinavian lakes and rivers (Hesthagen et al., 1999). *S. fontinalis* were introduced in more than 50 countries all over the world (FishBase, 2021; Jansson, 2013; Welcomme, 1988). Reports for established population of *S. fontinalis* outside the species native range exist for 20 countries (Jansson, 2013). In Norway alone, 202 established populations of the species have been identified (Hesthagen et al., 2018).

In many countries *S. fontinalis* is considered as a medium-risk invasive species among non-native freshwater fish (Britton et al., 2010; Copp et al., 2009; Simonović et al., 2013). Many introductions of *S. fontinalis* were to previously fishless lakes which resulted in a dramatic reduction of amphibian populations, zooplankton and other invertebrates (Benjamin et al., 2013; Dunham, 2004; FishBase, 2021; Fuller &

Neilson, 2014; Jansson, 2013; Levin et al., 2002; Nakano et al., 1998; Spens et al., 2007). Brook trout have been found to compete with, displace, or replace many fish species throughout the world including golden trout (*Oncorhynchus aguabonita*), brown trout (*Salmo trutta*), cutthroat trout (*O. clarki*), bull trout (*Salvelinus confluentus*), and chinook trout (*O. tshawytscha*) (Levin et al., 2002; Korsu & Huusko, 2009; Korsu et al., 2009; Öhlund et al., 2008; Reiman et al., 2006). A study of 193 boreal lakes in Sweden found that introduced *S. fontinalis* pose a detrimental impact to native brown trout. Twenty percent of brown trout populations exposed to brook trout went extinct versus an only 2% extinction rate in unexposed lakes (Spens et al., 2007). Natural hybridization and other reproductive interactions between brook trout and brown trout that might detrimentally affect on native species have been recorded (Cucherousset et al., 2008). Hesthagen et al. (2018) supposed that invasiveness of the brook trout depends on site and conditions.

In Bulgaria, *S. fontinalis* has been introduced in the 1930 from Czechoslovakia (Wellcomme, 1988). To middle of 2010s, *S. fontinalis* was produced mainly on a state hatchery in the town of Samokov. Annual production varies between 5 and 12 tones. Since the middle of the 20th century, brook trout has been introduced into more than 20 alpine lakes and rivers throughout the country (T. Trichkova, G. Raikova, unpublished data). Some of these water bodies, such as glacial lakes were naturally fishless. There are observations that in the areas where stocking with rainbow trout and brook trout is carried out, the native brown trout is not found or has a low density. However, the reasons for this have not been studied. Despite suspicions of a negative impact of these nonnative salmonid species on local fauna, no research has been conducted in this regard (Uzunova, 2006). At the beginning of this century, the official policy in Bulgaria regarding the distribution of alien species was changed and brook trout stocking ceased. However, probably due to the lack of effective control over stocking conducted by individual and private organizations, brook trout continue to be released in wild.

River Palakariya is a part of Iskar River basin. The valley of the Palakariya River is included in the European ecological network Natura 2000 (BG0000617) (38.1 rkm). The uppermost part of the river (6.6 rkm) is on the territory of Vitosha Nature Park. At the beginning and middle of the last century the ichthyofauna of the Iskar River and its tributaries was studied by Drenski (1921) and Paspalev & Peshev (1955). More recent studies on ichthyofauna of the Palakariya River show totally 15 fish species and subspecies from three families (Dikov et al., 1988; Kenderov et al., 2017; Pavlova & Pehlivanov, 2009). The following fish species listed in Annex 2 of the Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora are found in the river: *Sabanejewia aurata*, *Rhodeus amarus*, *Barbus petenyi*, *Cobitis taenia*, *Gobio uranoscopus* and *Gobio kessleri*. In 2015, the reintroduction of bullhead (*Cottus gobio*) was carried out with the aim to restore its population in the river (Uzunova et al., 2017). Faunistic research has not documented the presence of brook trout so far, but the surveys did not cover the uppermost sections of the river. However, a few angler records of this species posted in different internet platforms suggested that a small population of brook trout exist. The first confirmed record of brook trout in the Palakariya River was in 2016 when ten adult individuals were caught near to the village of Yarlovo (lat. 42°27' 55, long. 23°16' 55, 1120 m a.s.l.) (EU pers. obs.). The following year, five sexually mature brook trout were caught at the same place (EU pers. obs.). Despite the long period of acclimatization of *S. fontinalis* in Bulgaria, the existence of a self-sustaining population has not been established so far.

The aim of the present study is to find evidences that the non-native brook trout has formed a self-sustaining population in the Palakaria River.

Material and methods

Study area. The study was conducted on the Palakariya River. With its 44, 7 km of length it is the one of largest left tributaries of the Upper Iskar River (Danube River Basin). The watershed area is 400 km^2 (Hristova, 2012). The Palakaryia River rises at the foot of the Kupena peak in the south part of Vitosha Mountain at the altitude of about 2113 m a.s.l. The study covered the area from weir located above the village of Yarlovo to the spring zone of the river or a total of 6.1 km. The entire study area is natural without any significant modifications except two fragmentations caused by weirs higher than two meters (Fig. 1). Regarding habitat characteristics, fast flowing, shallow riffles with some interspersed pools (0.9 m max depth) were dominated. Bottom substrate was coarse, composed by gravel, boulders and rocks. River wetted width was between 1 and 3.5 m. Above site 4 the river passes into its spring zone, characterized by significant steepness and width ~1 m (in low water period). Water level varied from 0.1 m to 0.5 m depending on the hydrological conditions. Water temperature during winter months varied between 1.5 and 4 °C, in summer max temperature reached 12 °C. Dissolved oxygen range from 9.2 to 11.5 mg/l; conductivity was ranged from 20 to 40 µS cm^{-2}; pH - from 7.7 to 8.4. Just above weir 2 (site 1) a pool ~ 15 m^2 and 1.2 m max depth was formed.

Data collection. Samples were collected between 2019 and 2021 in five expeditions (May, July, October, November, and December). The fish sampling was performed during the day time (9.00:17.00). Two different collection types were conducted. Point Abundance Sampling by Electrofishing approach (PASE) SAMUS-725G device (12 cm diameter ring anode, average voltage of 200 - 350 V, operating at an average of 3 - 8 A) was used in area from weir 1 located upstream of Yarlovo village to weir 2 (site 1, where the presence of brook trout has been found). 261 sampling points or 1 point every 20 m were randomly selected in an upstream direction. Anode was activated for a 10-s period at each point sample (Copp, 1989).

Abundance of *S. fontinalis* in river section between site 1 and site 4 was assessed by using CEN standard (CEN, 2003) with the same equipment except ring anode, which was replaced with a 30 cm diameter. A single pass electrofishing without block nets was applied. Sampling transects were between 30 and 165 m long, depending on river width. The total fishing area per site varied between 380 and 420 m^2. The electrofishing was conducted from one bank to the other over the entire river stretch. The electrofishing crew was consisted of one operator and one netter. Electrofishing was performed in zigzag pattern, upstream covering all available habitats.

All the collected brook trout specimens were fixed in formaldehyde solution and stored in the collection of the Department of General and Applied Hydrobiology (GAH). All other fish species caught have returned to places of their capture after recovering from electronarcosis. Each brook trout was photographed with a Canon EOS Digital 350D camera and the photos were used for morphometric measurements (Digimizer Version 4.6.1 MedCalc Software). The collected fish were counted and measured: total length (TL), standard length (SL) in 0.1 cm accuracy and total weight (W) in 0.1 g accuracy. The measurement of the plastic and meristic characteristics was performing according to the scheme proposed by Pravdin (1966). The identification keys of Bacon (1954), Karas (1997), Kottelat & Freyhof (2007), Martinez (1984) and Stauffer & King (2014) were used.

At each site, the physical and chemical parameters, i.e. conductivity (µS/cm), pH,

dissolved oxygen content (D.O., mg/l), and water temperature (°C) were measured, using Portable Hanna Instr.

Data analysis. All sampled specimens were split in size groups (every 10 mm TL) and the number of individuals in each group was plotted as a histogram. The total length frequency formula is as follows:

$$TLi = Ni/ N \times 100\%$$
$$(i = 4{,}1 – 5{,}0\ cm, 5{,}1 – 6{,}0\ cm...24{,}1 – 25{,}0\ cm),$$

where TLi is the frequency for a certain interval, Ni is the number of specimens in one total length interval, and N is the total number of specimens.

The fish density was expressed as number of fishes caught per 100 m^2 and the biomass in kg per 100 m^2. Abundance and biomass of *S. fontinalis* were calculated separately for each sampling site.

Results

The following fish species were collected in the river section between weir 1 and weir 2 (Fig.1): *Salmo trutta* (n=34), *Phoxinus phoxinus* (n=14) and *Barbus petenyi* (n=7). The survey detected *S. fontinalis* (n=51) in the highest parts of the Palakariya River at altitude of 1352 m (Site 1 - N 42° 30' 44,43"; E 23°16' 2.56") to about 1500 m a.s.l. (Site 4 - N 42° 30' 48.78"; E 23°17' 4.515") (Fig. 1). The length of the river section inhabited by brook trout was about 900 m. No other fish species than *S. fontinalis* were found in the river section upstream weir 2. The zone above site 4 was fishless.

Collected fish specimens in sites 1 to 4 had a spindle-shaped body with an adipose fin close to the tail. The adult specimens had dark green to brown back, with a distinctive marbled pattern of lighter color, described as vermiculations. These vermiculations break up into light spots and relatively small red spots surrounded by blue halos along their sides. Pectoral, pelvic, and anal fins have white edges followed by a contrasting black stripe (Fig. 2). Juvenile fish have 8-10 parr marks.

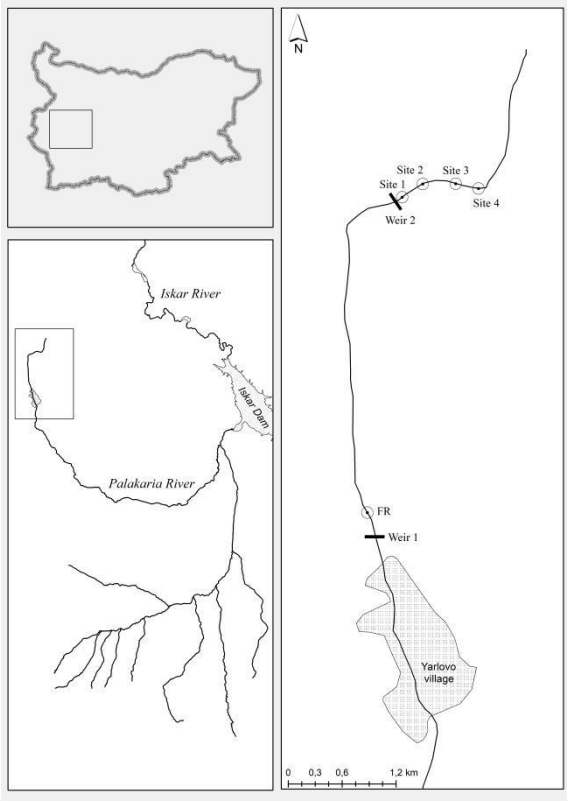

Fig. 1. Confirmed records of *S. fontinalis* in the Palakariya River: Site 1,2,3,4 - sites of established brook trout in period 2019-2021; FR – site of established brook trout in period 2016-2017; (—) – unpassable fragmentation (weir).

The total body mass of the collected *S. fontinalis* individuals ranged from 2.0 g to 155 g. The longest specimen was a female of 246 mm (TL), and the smallest was 4.1 cm (TL). The most common were fish with size from 4 to 10 cm (Fig. 3). No external malformations were found among collected individuals. The adult brook trout caught during the autumn months were sexually mature and released eggs and milt when their abdomens were gently pressed.

The density of *S. fontinalis* in sites 1-4 varied from 6 to 28 ind. 100 m^{-2}. Mean density (mean number of fish per 100 m^{-2} ± SE) was 13 ± 5.07. The highest density was observed at the site 1, while the lowest at site 4 (Fig. 4). Site 1 was dominated by fish of the smallest size classes, so the biomass measured here is the lowest, while in site 2 was found few, but relatively large fish (Fig.4).

Fig. 2. Brook trout specimens caught in the Palakariya River. A) Specimen Cat. N GAH 123194, 13.5 cm TL; B) specimen Cat. N GAH 12531912, 4.1 cm TL, (on the right). Photographs by Eliza Uzunova.

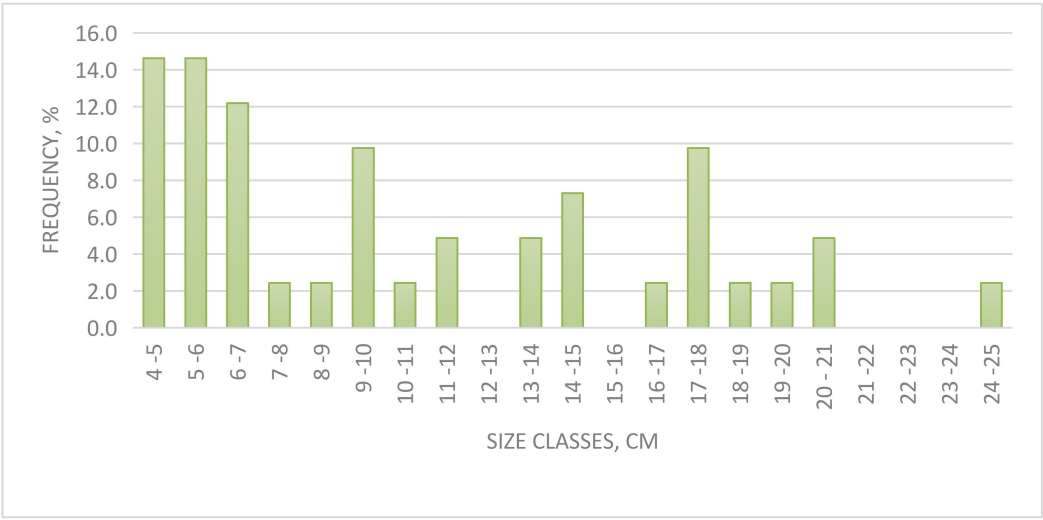

Fig. 3. Frequency distribution of the body size (TL, cm) of brook trout specimens caught in the Palakariya River in the period 2019-2021. (n = 51).

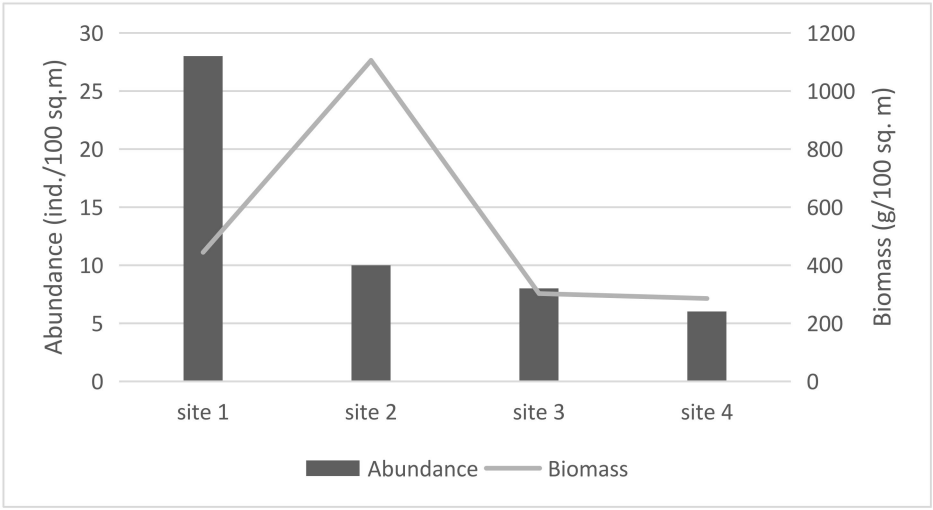

Fig. 4. Abundance (ind.100 m^{-2}) and biomass (g100 m^{-2}) of brook trout in the Palakariya River (2019-2021).

Fish density calculated from 261 sampling points in area between weir 1 and weir 2 were as follow: *S. trutta* - 141.1 ind. 100 m^{-2}; *P. phoxinus* - 84.8 ind. 100 m^{-2}; *B. petenyi* – 42.4 ind.100m^{-2}.

Discussion

According to internet posts, *S. fontinalis* in the Palakariya River originated from a single introduction of brook trout juveniles in the early-2010s. Anglers' posts are proving to be a very important source of information related to the distribution of invasive fish species (e.g. Banha et al., 2015; Kalous et al., 2018). That is how we found the person who carried out the single illegal stocking of brook trout in Palakaryia River in the 2010s. There is no evidence that this introduction has been repeated since then. We discovered that in the 10 years following that introduction, the brook trout has spread 1 km upstream from its point of initial release. This is probably due to the fact that the brook trout is a highly mobile invader that has spread extensively in stream ecosystems, occupying steep stretches of streams, even those with gradients up to 31% (Korsu et al., 2007). It was found that brook trout have a high capacity to disperse and a single introductory population may spread throughout an entire river system in search of territory and food resources (Hesthagen et al. 2018; Karas, 1997). The spread of the brook trout to the lower reaches of the Palakariya River is prevented by weir 2 (site 1, 1350 m a.s.l.), serving as a fish passage barrier. However, in periods with high water levels it could be possible for a few individuals to overcome this fragmentation in the downstream direction. This explains the discovery of single specimens in the lower reaches of the river.

The self-sustaining status of *S. fontinalis* in the Palakariya River is supported by information obtained from the body size structure of the collected specimens. The brook trout population had an overall balanced body size distribution consisting of individuals of all size classes between 4 and 25 cm. The dominating size for juvenile fish was between 4 and 10 cm.

The combination of morphological characteristics and specific body coloration allow for the definitive conclusion that the adult fish found in the uppermost section of the Palakaryia River are in fact brook trout and not any other synoptic species (Karas, 1997; Kottelat & Freyhof, 2007; Page & Burr, 1991; Scott & Crossman, 1973; Stauffer & King, 2014). The identification of the smallest brook trout (< 6-7 cm) was made on the basis of the length of the pectoral fins and the length and pigmentation of the adipose fin (Bacon, 1954; Martinez, 1984). Lastly, the distinction between wild and stocked salmonid fish, although remaining quite difficult, was done on the basis that among farm *S. fontinalis*, malformations are quite common. The absence of such malformations in the specimens caught in the Palakariya River suggests that the hypothesis for the presence of successful natural reproduction of the brook trout in the river still stands.

In the upper reaches of the Palakaryia River, the main native fish is the brown trout. The consequences of coexistence of brown trout and brook trout are contradictory. According to Spens et al. (2007) brook trout actively displace brown trout through competition, predation, disease spreading, or a combination of these. A long-term detrimental impact of brook trout on brown trout was documented in higher-altitude lakes (Eby et al., 2006; Spens et al., 2007). Cucherousset et al. (2008) observed mixed spawning groups composed of native brown trout and non-native brook trout, interspecific subordinate males, and presence of natural hybrids („tiger trout") and suggested that these reproductive interactions might detrimental effects on native species. In the majority of cases where the two species coexist, however, it has been documented that

brown trout dominate and gradually displace brook trout from river ecosystems (Fausch, 1988; Öhlund et al., 2008). Due to the lack of previous ichthyological studies in the headwater parts of the Palakariya River, we cannot conclude with certainty whether the absence of brown trout in this section of the river is a result of displacement by the brook trout, or the area was originally fishless. It seems possible that the brook trout have found an empty niche in the upper headwater reaches of the Palakaryia river system and that is why no brown trout were found. Moreover, environmental conditions in the upper section of the Palakaryia River are more favourable for brook trout than brown trout. *S. fontinalis* prefer colder waters and generally inhabit areas with higher altitudes than *S. trutta* (Rahel & Nibbelink, 1999). Water temperature is considered the single most important factor that limits its geographic range (MacCrimmon & Campbell, 1969). It has been suggested that brook trout have a competitive advantage against brown trout in such cold habitats because of their higher juvenile growth rates (Öhlund et al., 2008). Brook trout also favour smaller streams, as they mature at a smaller body size and can reproduce in shallow systems with limited spawning habitats (Rahel & Nibbelink, 1999). Hesthagen et al. (2018) conclude that in small streams, brook trout will sustain viable populations and are unlikely to be wiped out by brown trout through competition. Korsu et al. (2007) and Korsu & Huusko (2009) found that in Finland, brook trout are mostly established in tributary streams, where they may form dense populations that can be harmful to the local brown trout, while in larger streams brown trout are usually not affected. Therefore, the brown trout population in the lower parts of the Palakariya River (600-1350 m a.s.l.) is vulnerable to a potential increase in the number of brook trout. The influence of the non-native brook trout on native species such as *Barbus peteyi, Sabanejewia aurata, Gobio kessleri, G. uranoscopus* has not been studied at this stage and we can only assume that the presence of a predator such as *S. fontinalis* would have negative consequences for the listed species.

Despite the fact that studies consider the brook trout as a non-native species with moderate invasiveness in countries of the Balkans region, its potential harmful effects to native ecosystems should not be underestimated (Simonović et al., 2013). The control over the resettlement of brook trout must be increased and the release of fish in the oligotrophic lakes, as well as in the high-altitude mountain streams inhabited by brown trout, must be prevented.

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