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Alien Fishes in Some Tributaries of the Maritsa River in Bulgaria

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Abstract. The paper presents a study of alien fishes in the fish fauna of the Maritsa River tributaries' upper and middle zones. The study uses a large ichthyological sample, taken from four tributaries of the Maritsa River: the rivers Topolnitsa, Luda Yana, Stryama and Chepinska. The sample was collected in the period 2005-2011. Analysis identifies that out of 18 fishes, established in the studied rivers, three are invasive: the Prussian carp (*Carassius gibelio*), the Topmoouth gudgeon (*Pseudorasbora parva*), the Pumpkinseed sunfish (*Lepomis gibbosus*), and one is introduced: the Rainbow trout (*Oncorhynchus mykiss*). Alien species do not exceed 3% of the ichthyofauna composition of the studied rivers. It is identified that this pattern is partially caused by the fast water flow and absence of suitable habitats for these species. *O. mykiss* is only present in proximity to fish firms, wherefrom they have been accidentally released. This species comprises less than 0.2% of the total sample. *C. gibelio* and *L. gibbosus* are distributed in all studied water courses and are the most numerous (1.2% and respectively 0.7% of the sample*). P. parva* is found only in the rivers Topolnitsa and Luda Yana and represents 0.5% of the sample. In general, the number of non-native fish in the studied rivers is relatively small and this is why they are expected the impact on local fish fauna was small. However human activities can cause an increase in the population density of alien fishes in the Maritsa River tributaries.

Key words: Non-indigenous fish, Middle and Upper river zone, Topolnitsa, Luda Yana, Stryama, Chepinska river.

Introduction

Continental waters are surrounded by land and represent isolated basins. The colonization of such waters by new fish requires that the latter overcome large land and sea barriers (Haury & Patee, 1997). Often the distribution of aquatic organisms, in particular fish, has taken place via dispersal by birds (e.g. fish eggs may stick to water birds' feathers or feet), tornadoes, or trapping of one river by another in past geological times, such as the Danube and Rhine Rivers, Rhine and Rona Rivers. (Keith & Allardi, 1997). Fish species dispersal continues today, and European fauna continues to be enriched with species from Asia such as *Carassius gibelio* (Bloch, 1782) (Arcadievitch, 2006) and *Perccottus glenii* (Dybowski, 1877) (Jurajda et al., 2006). Human activities are also an important pathway for alien fishes' dispersal. For example, fish's introduction has been traced back to antiquity.

Consequently, the number of alien fishes in many European countries has gradually increased. For example, the inland waters of France are inhabited by 27 nonindigenous fishes (Keith & Allardi 1997).

In Italy there are 30 species of alien fishes, which is almost half of the Italian fresh water fish fauna (Marcanto et al., 2000). In the inland waters of Greece, alien fishes amount to 25 species (Koutsikos et al. 2018).

The impact of alien species has not only been positive, but it has also posed a strong threat to local ichthyofauna. In many cases, they have proven to be food competitors to the native fish species. In this regard Marcanto et al. (2000) discovered that the acclimatization of species of the family Coregonidae in the Italian mountain lakes was the main reason for the decrease of *Alburnus arborella* (Bonapart, 1841) catches. Others fish, such as *Ameliurus melas* (Rafinesque, 1821), which was imported to France in the 19th century, became not only food competitors, but also dangerous predators, destroying the spawning and larvae of native fish (Bruslé & Quinquard, 2013). In some cases, food competition and predation by alien fish species have even led to the extinction of local fish fauna. The acclimatization of fresh water fishes to Morocco was considered as the main reason for the disappearance of the local trout species *Salmo pallaryi* (Pellegrin, 1824) (Azeroual, 2000). The introduction of alien fish species in Europe has also contributed to the transmission of dangerous new diseases to the native fish. Such was the introduction pathway of the pathogen parasite *Bothriocephalus acheilognathi* (Yamaguti 1934), which spread to European fish farms, cultivating grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) (Ahmad et al., 2018).

In the early 20th century, an overview of the Bulgarian ichtyofauna by Kovachev (1923) and an examination of the fish fauna of the Aegean watershed by Shishkov (1939) presented no data about the presence of non-native fish species. Only during the 1970s did Michajlova discover two alien fishes in the above-mentioned Bulgarian watershed: *Gambusia holbrooki* (Baird & Girard, 1853) and *Oncorhynchus mykiss* (Walbaum, 1792). Inspecting the species composition of the Maritsa River's ichthyofauna, Velcheva & Mechterov (2005) found three non-native fish species: *Pseudorasbora parva* (Temminck & Schlegel, 1842), *C. guibelio* and *Lepomis gibbosus (*L., 1758). In the same year Apostolou (2005) reported that the same species have been found in the Mesta River. In an overview of the Bulgarian fish fauna composition Stephanof (2007) listed 24 alien fishes in the Aegean watershed in Bulgaria, including invasive species, introduced fishes for aquaculture and for sports fishing in the dams, in the fish farms and also in the rivers. In 2010, Apostolous et al.'s guide to the fish of the Mesta River referred to 7 non-native fishes on the territories of both Bulgaria and Greece. Four of these species were freshwater fish: *G. holbrooki, L. gibbosus, P. parva* and *C. gibelio*. In their studies of alien fishes in Bulgaria Uzunova & Zlatanova (2007), Yankova (2016) conclude that the main introductory pathway was aquaculture. The other authors (Koutsikos et al., 2018; Welcomme, 1988) confirm that the major entrance pathway for alien species in Europe has been aquaculture, with sports fishing coming in the second place; other factors have also been acknowledged as important. More recently Uzunova et al. (2019) reported the presence of *Micropterus salmoides* (Lacepede, 1802) in the Bulgarian stretch of the Struma River, and in some dams in the South-Western part of the country.

Bulgarian inland waters are connected to these of other European countries. Many alien fishes, which initially dispersed to other countries of Western or Eastern Europe, are now found in Bulgaria. Population pressures, such as food competition, predation and new diseases threatening native fish, are also be expected to take place. So, an assessment of the distribution of non-native species in the Bulgarian rivers is particularly important. In particular the invasion rate of alien species in the rivers Topolnitsa, Luda Yana, Stryama and Chepinska, has not yet been determined. Therefore, purpose of this study is to assess the presence, distribution and abundance of alien fishes in the local ichthyofauna of these four rivers.

Materials and Methods

Study area. The study area includes the four tributaries of the Maritsa River, the biggest

river of the Aegean watershed. These are: the Chepinska River flowing from the Western Rhodope Mountains, and the rivers Topolnitsa, Stryama and Luda Yana originating from the Sredna Gora Mountain (Fig. 1).

Fig. 1 shows the catchment area of the Maritsa River. This river springs from the Rila, the highest Bulgarian Mountain. The river basin includes the Thracian valley, which is enclosed by two major mountain ranges. On the north the Maritsa watershed is bound by the Sredna Gora Mountain and on the south by the Rodopes Mts.

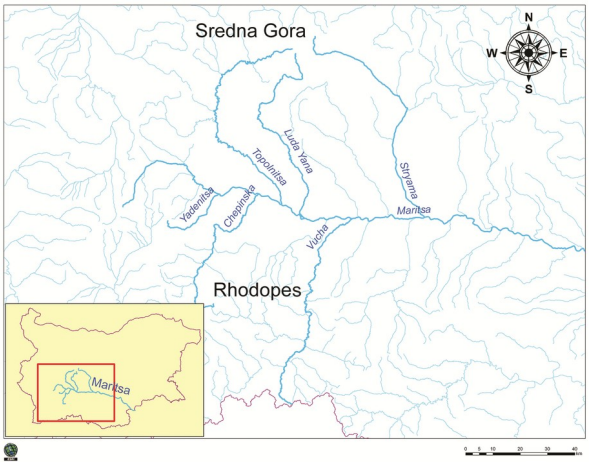

Fig. 1. Location of the study area.

The Chepinska River is a right tributary of the Maritsa River. The tributary's length is 81.7 km, with a catchment surface area of 899.6 km^2. The river originates from the Western Rhodopes Mountains (Hristova, 2012); it then flows into the Maritsa River near the village of Kovachevo (Pazardzhik).

The rivers Toponitsa, Luda Yana and Stryama are left tributaries of the Maritsa River. The Toponitsa is 154.8 km long and the surface of its catchment area is 1788.8 km^2. The river springs from the Sredna Gora Mountain (Hristova, 2012). Three dams have been built on the Topolnitsa: Dushanci, Jekov vir and Topolnitsa. The river flows into the Maritsa River, west of the town of Pazardzhik.

The length of the Luda Yana River is 74 km and the surface of its catchment area is 685.3 km^2. The river springs from the Sredna Gora Mountain (Hristova, 2012); it flows into the Maritsa River near the village of Sinitovo.

The Stryama River is 110.1 km long. The surface of its catchment area is 1394.5 km^2. The stream Kavardjikliiska is generally considered as its source stream. The stream springs from the Stara Planina Mountain in the Teteven area. (Hristova, 2012). The river flows into the Maritsa River, near the village of Manole.

Taking into account their length, the Topolnitsa and Stryama rivers rank among the longest Bulgarian rivers, and the Chepinska and Luda Yana are classified as medium size rivers (Hristova, 2012). All four studied rivers are classified as a mountainous rivers (Protected Waters Act. 2012).

Study materials

Study material has been collected over a period of five years: 2005 – 2007 and 2010 - 2011. During this period, a sample of more than 3500 fish were caught in the rivers Topolnitsa, Luda Yana, Stryama and Chepinska by electrofishing (Kolev, 2010, 1913). This material is supplemented by data obtained during a study of density and biomass of *Salmo sp.* in 2008, in the upper zones of the same four rivers (Kolev, 2010). A SAMUS 725G converter was used, providing up to 640V direct current (DC), with a frequency of 50 Hz and output power reaching up to 200W. The catch was performed according to the EN 14011: 2004 instruction (Water quality – Sampling of fish with electricity). In order to collect the material for the study, 15 sampling areas were used (Table 1).

Each fish was measured in order to establish the following parameters: standard lengths (*L*) of the fish with a precision of 1 mm and net weight (*NW*) with a precision of 1 g. Total weight (TW) also was measured with a precision of 1 g. Total weight was used to estimate the alien-native fishes mass ratio. Fish age was determined by measuring fish scales, by using a microscope with a 40×

magnification. The identification of fish species was made according to the classification of Kottelat & Freyhof (2007).

The study uses the classification of alien species by Richardson et al. (2000).

Results

Among the 18 identified fish species of the rivers Topolnitsa, Luda Yana, Stryama and Chepinska (Kolev, 2010, 2013), three fishes we found to be invasive: C. *gibelio*, *P.*, *L. gibbosus*. One species – *O. mykiss* was identified as an introduced species (Bruslé & Quinquard, 2013; Jurajda et al., 2006; Kateřina, 2013; Van Kleef et al., 2008, see Table 1 and 2).

No alien fish species have been found in the upper parts of the studied rivers – i.e. in river stretches, located higher than 800 meters above the sea level. Hydrologic particularities of the middle zone of the surveyed rivers determined the strong dominance of two local ichthyofauna: *Barbus cyclolepis* (Heckel, 1848) and *Squalius orpheus* (Kottelat & Economidis, 2007). These two fishes accounted for over 85% of the local fish population. Each of the other fish species did not exceed 4% of the fish fauna. Overall, the four non-native species comprised 2.6% of the local fish fauna (Kolev, 2010, 2013).

Measuring 0.2% of the total fish sample, the abundance of *O. mykiss* was the smallest. The fish has been farmed in the trout farms "Kleptuza" (Velingrad) and "Dabene" (Karlovo) and was accidentally released into the rivers Chepinska and then Stryama. Consequently, the species was found only in the middle zone of these rivers (Table 1). Overall the rainbow trout is less abundant (Fig. 2). The specimen were one-year-old (1+), shorter than 300 mm and weighing less than 250g.

C. gibelio was widely distributed in all surveyed rivers (Fig.2). It was the most numerous fish amongst the nonnative species, comprising 1.2% of the total sample. *C. gibelio* was frequently detected in proximity to the confluence of the surveyed rivers and the Maritsa River. The fish was also present in the inert river materials extraction reservoir by the village of Lozen (Table 1). The age of the fish varies between 1 (0+) and 4 years. The biggest specimen, a four years old fish caught in Luda Yana, was 147 mm long and weighted 91g.

Table 1. Sampling areas. Presence of non-native fish species. *Legend:* "+" - the species was present; "-" - the species was not present; PrCp - prussian carp (*C. gibelio*); TmGd – topmoouth gudgeon (*P. parva*); Psun – pumpkinseed (*L. gibbosus*); sunfish; RbTr - rainbow trout (*O. mykiss*).

River	Location	PrCp	TmGd	PSun	RbTr
Chepinska	1. East of the village of Zlokuchene, near the road bridge of Belovo (Pazardzhik).	+	-	-	-
	2. A reservoir for inert materials extraction near the village of Lozen (Pazardzhik).	+	-	+	-
	3. Near the mineral baths of the Varvara village (Pazardzhik).	-	-	-	-
	4. Near the railway station M. Nikolov (Pazardzhik).	-	-	-	-
	5.Near the park "Kleptuza" (Velinfrad).	-	-	-	+
	6. On the road from Velingrad to Ablanitsa village (Pazardzhik).	-	-	-	-
Topolnitsa	7. Near the Boshulya village, next to the bridge under the Pazardjik-Septemvry road.	+	+	+	-
	8. On the road between the villages Lesichevo and Muhovo (Pazardzhik).	-	+	-	-
	9. Next to the dam wall of Topolnitsa reservoir, near	-	-	-	-

	the village of Muhovo (Pazardzhik).				
LudaYana	10. Next to the bridge under the Pazardzik-Plovdiv road.	+	+	+	-
	11. Next to the bridge under the Pazardzik-Chernogorovo village road.	-	-	+	-
	12. West of the the bridge under the Plovdiv – Rakovski village road.	-	-	-	-
	13. Near the Rajevo Konare village (Plovdiv).	-	-	+	-
Stryama	14. Next to the trout farm, near the Dabene village (Karlovo).	+	-	+	+
	15. West of the Rozino -Slatina villages road bridge (Plovdiv).	-	-	-	-

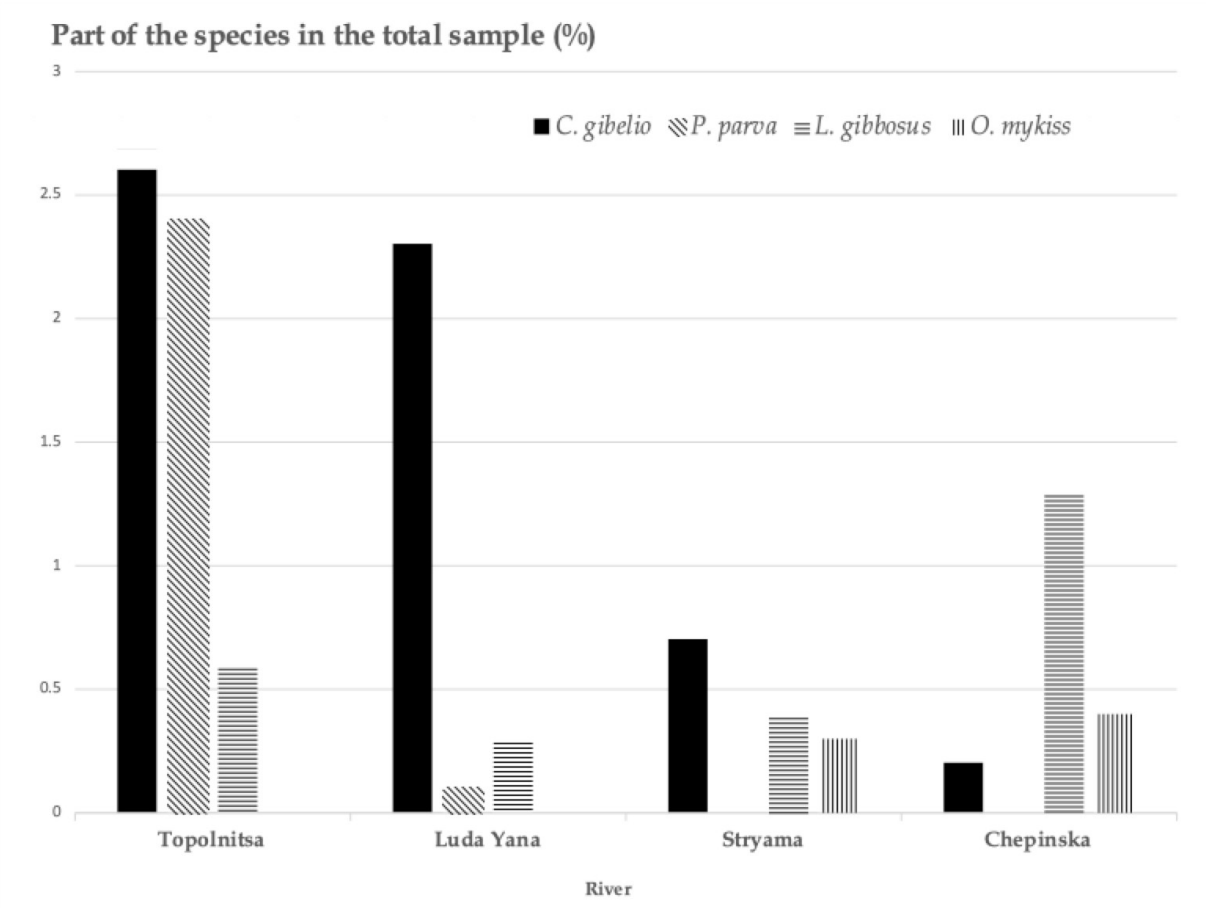

Fig. 2. Proportion of nonindigenous fishes of the total fish simple, caught in the rivers Topolnitsa, Luda Yana, Stryama and Chepinska.

L. gibbosus was the second most abundant alien fish in all four surveyed rivers; it accounted for 0.2% of the total fish population (Table 1). The sunfish was most numerous in the inert river materials extraction reservoir adjacent to the village of Lozen (Pazarzik). The biggest specimen caught in that water body, a five years' old (5+) sunfish, weighted 33g and reached up to 100 mm in length.

P. parva populations are established in the rivers Topolnitsa and Luda Yana, mainly in slow flow areas. The fish comprises 0.5% of the overall fish population. *P. parva* was most abundant in the Topolnitsa River (Fig. 2), wherein the catch of the biggest specimen took place: a four-year-old fish with a length of 80 mm and weighing 9.2 g.

Table 2 present data about the total weight of each fish, of each river. The table data was calculated by using the total weight of the alien and native fishes from all samples.

Table 2. Alien vs. native fishes mass ratio. Total fish weight (TW) (g) from samples 2005-2007, 2008, 2010-2011, collected in the upper and middle zone of the studied rivers. *Legend:* "*" – missing data, because of species higher conservation status.

Species / River	Topolnitsa	Stryama	Luda Yana	Chepinska	Total	%
Alien species weight (g)						
Carassius gibelio (Bloch, 1782)	315	105	331	171	*921*	
Oncorhynchus mykiss (Walbaum, 1792)	0	270	0	0	*270*	
Lepomis gibbosus (L., 1758)	41	67	15	119	*241*	
Pseudorasbora parva (Temminck & Schlegel, 1842)	74	0	5	0	*78*	
Total alien species weight (g)					1570	2
Native species weight (g)						
Squalius orpheus (Kottelat & Economidis, 2007)	10156	13768	3800	6350	*34074*	
Barbus cyclolepis (Heckel, 1848)	5651	6825	3570	6671	*22718*	
Salmo sp.	485	453	353	5150	*6441*	
Rutilus rutilus (L., 1758)	339	55	857	558	*1801*	
Chondrostoma vardarense (Karaman, 1928)	251	24	0	365	*639*	
Gobio bulgaricus (Drensky, 1926)	255	107	77	142	*581*	
Esox lucius (L., 1758)	0	300	0	0	*300*	
Perca fluviatilis (L., 1758)	0	31	101	132	*264*	
Alburnus alburnus (L., 1758)	30	25	1	160	*215*	
Vimba melanops (Heckel, 1837)	6	152	0	16	*174*	
Phoxinus phoxinus (L., 1758)	0	23	0	0	*23*	
Rodeus amarus (Bloch, 1782)	*	*	*	*	*	
Cobitis strumicae (Karaman, 1955)	*	*	*	*	*	
Sabanejewia balcanica (Karaman, 1922)	*	*	*	*	*	
Total native species weight (g)					67230	98
Total all species weight (g)					**68800**	100

Alien fishes' part of the weight of the total fish biomass in all catches remained negligible, less than 2 %. More than half of alien fishes' mass belonged to *C. gibelio*.

Discussion

Non-indigenous species percentiles of the ichthyofaunal populations of the rivers Topolnitsa, Luda Yana, Stryama and Chepinska are relatively small. The middle zone of the Maritsa River tributaries is strongly dominated by two fishes: *B. cyclolepis* and *S. orpheus*, which together comprise more than 86% of the local fish populations (Kolev, 2010, 2013). This fact is also strongly supported by the alien vs. native fishes mass ratio (Table 2). However, *C. gibelio*, *L. gibbosus*, *P. parva and O. mykiss* comprise about one fifth (18%) of the total fish population, with the remaining 15 fish species comprising altogether the remaining 82% (Kolev, 2010, 2013). Alien fishes are most abundant in the rivers Topolnitsa and Luda Yana, amounting to 5.7% and 2.7%, respectively, of the fish

population. There are fewer alien fishes in the rivers Chepinska and Stryama: 1.9% in the former and 1.4% in the latter.

Strong water flow in the upper and middle zones of the surveyed rivers represents unfavorable habitat conditions for *L. gibbusus* and therefore it has a limiting impact on its distribution and abundance (Fedonenko & Marenkov, 2013). Consequently, the resultant small species density diminishes the negative impact of the *L. gibbusus* presence on local ichthyofauna. However, in areas with slow water speed, such as the inert river materials extraction reservoir, in proximity to the village of Lozen (Pazarzik), the numbers of *L. gibbusus* rapidly increase. High *L. gibbusus* density in the reservoir confirms the opinion of some authors (Uzunova et al., 2012; Van Kleef et al., 2008), that aggregates extraction reservoirs are one of the favorite habitats of this species. The study discovered that in the Lozen reservoir *L. gibbusus* has more significant influence on the rest of the fish community. This impact may be expressed mainly in competition for food, reduction of planktonic and benthic food and in the direct destruction of other fish's caviar (Uzunova et al., 2012). The study also found that many adult specimen inhabiting the Lozen gravel reservoir had length greater than 75 mm. These adult fish are fully capable of ensuring the reproduction of the *L. gibbusus* population at that location (Fedonenko & Marenkov, 2013).

The higher *C. gibelio* density in all the surveyed rivers is in full accordance with its widespread distribution in all Bulgarian water bodies (Apostolou, 2005; Apostolou et al., 2010; Boyadjiev, 1969; Karapetkova & Dikov, 1986; Stefanov & Trichkova, 2006; Stefanov, 2007). This is due to the great adaptability, reproductive potential and omnivorous nature of the species (Lorenzoni et al., 2007), even though its density in the middle zone of the Maritsa River's tributaries is lower than in standing water bodies (Boyadjiev, 1969). The present study found most of this species in zones, characterized by anthropogenic modifications, or so-called corrections, of the river bed, as well as the construction of dykes along the shores of the rivers Topolnitsa and Luda Yana, right before their confluence with the Maritsa River. *C. gibelio* competes for food with native fish species. Behavior, such as caviar predation and destruction, are very rare in this species. *C. gibelio* preferred habitats are different from these of the main fish species, inhabiting the area (Arkadievitch, 2006, Ulianovsk, Russia – pers. com.). *C. gibelio*'s, lack of suitable breeding sites, as well as sports fishing do not allow the fish to increase its abundance in the middle zone of the Maritsa River. In consequence, its impact on local fauna is not significant.

P. parva is more abundant in the Topolnitsa River, wherein this fish was introduced by the water outflow of the Topolnitsa dam. *P. parva* is most commonly introduced with the larvae of *Hypophtalmichthys molitrix* (Valenciennes, 1844) and *Hypophtalmichthys nobilis* (Richrdson, 1845) (Boltachov et al., 2006; Yankova, 2016). Fish stocking with these species in the Topolnitsa Dam (unpublished data from local forestry officials) have introduced *P. parva* there. The fishes are absent in the section of the river, located directly below the dam wall, which is adjacent to the village of Muhovo, because of a strong water current found there. Adapted to calmer waters (Kotovska & Hristenko, 2013) *P. parva* migrates to calm areas of the water course. These are the lower parts of the rivers Topolnitsa and Luna Yana, right before their inflow into the Maritsa River. Since this fish has a very large nutritional spectrum (Kotovska & Hristenko, 2013), it competes for food with native fish species, especially with the smallest-size fish groups. In three of the four studied sites, wherein *P. parva* is established, this fish co-inhabits with the *C. gibelio* (Table1). According to Didenko (2013), *P. parva* prefers areas, inhabited by *C. gibelio*. The fish not only enters into food competition with *P. parva*, but also parasites

on it. Parasitic behaviour causing damage to the skin and gnawing on fins (Abramenko, 2012; Boltachev et al., 2006). It is also probable that caviar predation of *P. parva* also takes place in the surveyed rivers, as described in other water bodies by Kotovska & Hristenko (2013). The study found that the length of a high number of specimen from the rivers Topolnitsa and Luda Yana exceeds 30 mm, so the specimen represent fish, which has probably already matured (Boltachev et al., 2006). The high ecological plasticity, eurythermality, early sexual maturation and high reproductive potential of *P. parva* (Boltachev et al., 2006) make it a serious potential threat to native fish species in the middle zone of the Maritsa River. Koutsikos et al. (2018) assess the presence of *P. parva* and *L. gibbosus* in Greece, as particularly threatening to the local ichthyofauna.

O. mykiss has a limited spread and very small abundance in the Maritsa River tributaries. Its presence is due to accidental release from trout farms. The trout's negative impact is expressed in food competition with the *B. cyclolepis*, *S. orpheus*, *P. fluviatilis* and *E. lucius*. *O. mykiss* supplants other fish from their habitats and destroys their offspring. However, due to its low abundance, the negative impact of this fish is negligible. Domesticated in fish farms, the *O. mykiss* has now lost its instinct for self-preservation (Plasseraud, 1990) and the fish has become an easy catch for anglers. Sports fishing and absence of natural reproduction, observed only in the Rila Mountain by Konstantinov (1964), explain the low survival rate of this species in the Maritsa River tributaries. Thus, the presence and impact of *O. mykiss* in these rivers is fully controllable.

Currently, the small biomass of alien fish species still does not allow them to have a significant negative impact on the local ichthyofauna. Predation and food competition remain negligible. Transmission of new diseases to local fish has not yet been reported. At the same times, the most commonly represented species, both in abundance and in mass: *C. gibelio,* is also a desirable object for sports fishing, which suppresses its population increase..

Conclusions

Of the four non-native fishes, discovered in the middle zone of the Maritsa River tributaries, the most widespread are *C gibelio* (1.2% of the sample) and *L. gibbosus* (0.7% of the sample). Both species are observed in areas with slow water flow or standing water, such as river stretches with corrected riverbeds, as well as habitats with the river run gravel. However, the faster water flow speed and the dominant habitat of gravel riverbeds limit the penetration of these species to the majority of river beds and limit their impact on local fish fauna. The presence of *P. parva* is linked to fish stocking with *H. molitrix* and *H. nobilis*, and presents a significant potential threat to the local ichthyofauna. Overall, the habitat conditions in the upper zone of the Maritsa River tributaries are completely inappropriate for the four alien species and non-indigenous fish have not been able to penetrate there. The spread of alien species in the investigated rivers has been particularly facilitated by human activity.

Recommendations

Limiting the negative impact of *C. gibelio* and *L. gibbosus* in the Maritsa River tributaries requires a cessation of the anthropogenic river bed alterations and restoration of their primary appearance and water flow.

Strengthening the dominant habitat of control of the existing fish farms can prevent further increase in Rainbow trout abundance in the surveyed rivers.

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