FEATURES OF THE DISTRIBUTION OF THREE SPECIES OF FISH TREMATODES IN PAVLODAR REGION OF KAZAKHSTAN

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Abstract. The aim of the study is to determine the distribution of three species of fish trematodes in the Pavlodar region, as well as features of infection of definitive hosts and their parasitological analysis. The prospect of studying the fish trematode fauna of the North-East Kazakhstan is relevant because the life cycle of this group of parasitic worms depends on several groups of organisms that are intermediate, accessory and final hosts. 3 species of trematodes were identified: *Azygia lucii, Bunodera luciopercae, Sphaerostomum bramae*. The indicators of the invasion, prevalence and abundance index of parasites within the examined fish species were determined. The results demonstrated a good state of the intermediate hosts living in the Irtysh River and its reservoirs, infected by the three species of trematodes.

Key words: fish parasites; parasitological analysis; Trematoda.

INTRODUCTION

Azygia lucii (Müller, 1776) is a widespread species of fish trematodes found in Europe, North America, the European and Asian countries of the Commonwealth of Independent States. The main host of *A. lucii* is the pike *(Esox lucius)* but it could be also found in many other fishes (Akimova, 2016) such as perch, pikeperch and other predatory species (Bychkova *et al.,* 2017; Djikanovic *et al.,* 2012; Zhokhov, 2000) although less commonly. *E. lucius* presents the largest percentage of infection and intensity of invasion with *A. lucii*.

Sphaerostomum bramae (Müller, 1776) is a widespread parasite of carp fish, and less common occurs in fishes of other families. These trematodes exhibit high selectivity in relation to the final hosts: the main role in the life cycle of *S. bramae* is played by bream and silver bream (Lebedeva, 2005a). Distribution: Volga delta, Caspian Sea (Agrahan Bay), basins of the Black, Azov, Aral and Baltic Seas, water bodies of Kazakhstan (Djikanovic *et al.,* 2012; Kalmykov *et al.,* 2013; Mastitsky, 2007).

Bunodera luciopercae (Müller, 1776) infects freshwater fish from the families of perch and stickleback, rarely salmon. They are notable for the alternation of generations with a change of hosts: two intermediate and a final one. The role of the final hosts has representatives of many families of ray-finned fish. However, most parasites of freshwater fishes may infect the marine species as well. These trematodes are distributed in Eurasia, Africa, North and South America (Avdeeva *et al.,* 2017; Djikanovic *et al.,* 2012; Pulkkinen *et al.,* 2013).

Dzika *et al.* (from 2001 to 2004) carried out parasitological studies on fishes from Lake Kortowskie (Poland). 381 fish representing ten species, including perch *Perca fluviatilis* and pike *Esox lucius* were examined. *A. lucii* were noted in the alimentary tract of the ruffe *Gymnocephalus cernuus* (L.) (55 specimens examined - 5.5% infected). In turn, all examined pikes *E. lucius* (6) were infected with parasites. Two adult specimens of *A. lucii* were found in their branchial chamber (Dzika *et al.,* 2008). Maltseva and Avdeeva (2018) found marita of *A. lucii* and *B. luciopercae* in the gastrointestinal tract of pike perch *(Sander lucioperca)* in Curonian lagoon. *A. lucii* had the highest intensity of invasion (60%). Pulkkinen *et al.* (2013) studied the parasite species found in perch from the lakes Pa¨ija¨nne and Saimaa and their life cycles, namely the first and the second intermediate hosts and the

final host. According to their research, all parasite species belonged to Trematoda, which transmitted via snails or mussels, the free swimming cercariae produced as result of asexual multiplication, to the second intermediate host by penetration its skin or gills. Exceptions are *A. lucii* and *B. lucioperca*, of which cercariae are large and are eaten by the next host in the life cycle. All other species are transferred in the food chain. During parasitological examinations (Lake Lukomskoe (Belarus)) conducted by Mastitsky in May and August 2006, the snails were found to harbor cercariae of three trematode species: *Palaeorchis sp.*, *Rossicotrema donicum* and *S. bramae* (Mastitsky, 2007). Zhokhov (2000) studied fish parasites of three species: *B. luciopercae, S. bramae,* and *Phyllodistomum elongatum* and described the life cycle of all three trematodes. According to the author, maritae of *S. bramae* parasitize in the intestine of bream, *Ph. elongatum* in the urinary bladder of bream, and *B. luciopercae* in the intestine of perch, which are the main hosts of trematodes mentioned. Whereas parthenitae parasitize in molluscs: *Ph. elongatum* and *B. luciopercae* in the bivalve *Pisidium amnicum*, and *S. bramae* in the gastropod *Codiella inflata*. Djikanovic *et al.* (2012) presented data on freshwater fish parasitofauna investigations over the past 75 years in Serbian open waters. In total 170 parasitic species have been reported, including *A. lucii, B. lucioperca,* and *S. bramae*. Freshwater fish species were shown as definitive and intermediate hosts of parasites with larvae and mature stages infecting a variety of vertebrates, including humans. Thus, *A. lucii* was found in *E. lucius* (Linnaeus, 1758), *Acipenser ruthenus* (Linnaeus, 1758; Szidat, 1932), *Silurus glanis* (Linnaeus, 1758), *Perca fluviatilis* (Linnaeus, 1758), *Umbra krameri* (Walbaum, 1792), whereas *B. lucioperca* was registered in *Acipenser ruthenus* (Linnaeus, 1758), *Sander lucioperca* (Linnaeus, 1758), *E. lucius* (Linnaeus, 1758), *P. fluviatilis* (Linnaeus, 1758) and *S. bramae* found in *Abramis brama* (Linnaeus, 1758), *Bllica bjoerkna* (Linnaeus, 1758), *Leuciscus cephalus* (Linnaeus, 1758), *Rutilus rutilus* (Linnaeus, 1758), *Alburnus alburnus* (Linnaeus, 1758).

According to the literature review, the parasites of the fishes of the European continental water bodies have been well studied and described; in the Asian part, the studies focused on the waters of the Russian Federation. In Kazakhstan, a systematic study of the fauna, ecology, and parasitic features of trematodes is associated with the south and southeast Caspian Sea and rivers. In this regard, the Central, Northern and North-East of Kazakhstan remain less studied. In the last 30 years, systematic studies have not been conducted in the above region. In this context, the study of the fauna of fish trematodes of the largest rivers of Kazakhstan - the Irtysh River and its tributaries is very relevant.

MATERIAL AND METHODS

Fishes were caught from June to August within the 2013 to 2019 period in the North-Eastern part of the Republic of Kazakhstan within the Pavlodar region, namely in the Irtysh river, at various points along its course (in the territory of the Akkuly, Pavlodar regions) (Figure 1).

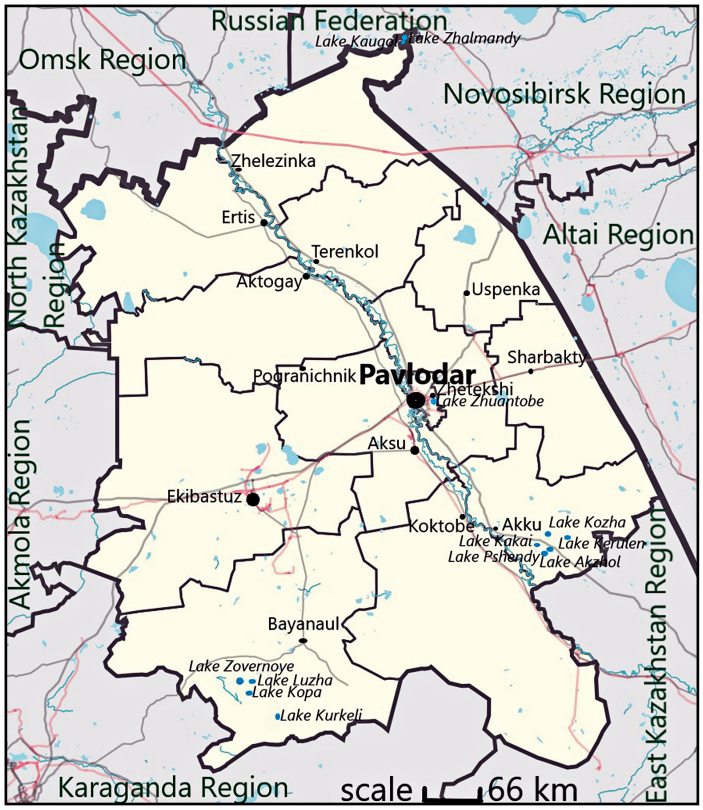

Fig. 1. Map showing the study location (Akkuly, Pavlodar region)

Helminths were collected using the method of complete helminthological dissection of fish according to the method of K.I. Scryabin. Statistical processing of the obtained materials was carried out in accordance with the recommendations of G.F. Lakin. Parasites found were bleached, stained, prepared and fixed for determination and collection. Isolation, sorting and identification of parasite fauna have been done within laboratory. Appropriate identification keys were used for determination of parasite fauna representatives to the lowest taxonomic level (Bauer, 1987a, 1987b; Ryzhikov, 1967). Based on the results of the autopsy of the hosts, the invasion prevalence, the abundance index, and the invasion intensity were calculated (Anikanova *et al.,* 2007; Lakin, 1990). In total, 81 fishes belonging to 5 species from 3 orders were studied over the entire period of research: Esociformes — common pike *Esox lucius* (n = 36); Perciformes - river perch, or common perch *Perca fluviatilis* (n = 15); Cypriniformes - ide *Leuciscus idus* (n = 23), common roach *Rutilus rutilus* (n = 2), common bream *Abramis brama* (n = 5).

RESULTS

Three basic indicators that are currently widely used in parasitology were calculated to identify the level of host infection: the invasion prevalence, invasion intensity, and abundance index (Table 1) (Anikanova *et al.,* 2007).

47 specimens (58%) out of the 81 fishes studied were infected with trematodes. They were found in three of the five studied fish species belonging to three families: pickerel *(Esocidae)*, perch *(Percidae)* and carp *(Cyprinidae)*, *i.e.* in pike, perch and ide. The prevalence of invasion of fish of the examined species varied from 6.7 to 94.4% (Table 1). The maximum abundance index was found in pike (7.5), while the minimum abundance index was found in perch (0.07). The intensity of invasion of the examined fish varied from 1 to 7.9 specimens. Trematodes were not found in two representatives of the carp family (roach and bream).

On average, 4.8 trematode specimens accounted for each infected fish. The highest invasion intensity was recorded in pike (7.9), which is significantly higher than the indices of other fish species (Table 1).

The trematode fauna of the examined fish species in the North-East of Kazakhstan was represented by 3 species: *Azygia lucii* (Müller, 1776), *Sphaerostomum bramae* (Müller, 1776) and *Bunodera luciopercae* (Müller, 1776). 89% of the detected 303 marites were identified as *A. lucii*, 8.4% were *B. luciopercae* and 2.6% were *S. bramae*. The combination of infection with trematodes of 2 species of different genera was recorded in perch. The prevalence of invasion of fish by each species of trematodes is presented in Table 1.

A. lucii was found in 34 specimens of the pickerel family. The intensity of hosts infection by this species is greater in comparison with others. Thus, the indices of fish infection rate with *A. lucii* marites were much higher than recorded by

Table 1. The indexes of infection of some fish species in the Pavlodar region of Kazakhstan

Host	Pike (*Esox Lucius*)			Perch (*Perca fluviatilis*)			Ide (*Leuciscus idus*)			Roach (*Rutilus rutilus*)			Bream (*Abramis brama*)		
Parasitic species	P. %	I, spec.	A, spec.	P, %	I, spec.	A, spec.	P, %	I, spec.	A, spec.	P, %	I, spec.	A, spec.	P, %	I, spec.	A, spec.
Azygia lucii	94.4	7.9	7.5	–	–	–	–	–	–	–	–	–	–	–	–
Sphaerostomum bramae	–	–	–	6.7	1	0.07	26.09	1.2	0.3	–	–	–	–	–	–
Bunodera luciopercae	–	–	–	40	4.2	1.7	–	–	–	–	–	–	–	–	–
Total species of trematodes	1			2			1			–			–		
Total number of examined fishes	36			15			23			2			5		

Note: P – prevalence, %; I – intensity, spec.; A – abundance, spec.; "–" – no parasite detected.

The indexes were calculated according to the following formulas (Anikanova *et al.*):

Prevalence: $P = \frac{Np}{n} \times 100\%$ where *Np* – number of infected hosts; *n* – total number of hosts.

Intensity: $I = \frac{Par}{Np}$ where *Par* is the number of detected parasites; *Np* number of infected hosts with this parasite.

Abundance: $A = \frac{Par}{n}$ where *Par* is the number of detected parasites in *n* examined animals.

other species of trematodes. The prevalence of infection, the abundance index and the invasion intensity of *A. lucii* marita were approximately twice higher. The maximum prevalence of invasion was reached in pike with 94.4%, whereas the abundance index was 7.5 specimens. On average, each infected fish accounted for 7.9 specimens (Table 1).

B. luciopercae was recorded in 6 specimens of the perch family. The infection rate of *B. luciopercae* maritas reached 40% in representatives of the perch family. The abundance index is higher than of *S. bramae* indices, but significantly lower than of *A. Lucii* indices. (Table 1).

S. bramae was found in 7 representatives of the examined specimens of fish of the perch and carp family. On average, 1.1 specimens accounted for each infected fish. The maximum intensity of invasion (1.2) was observed in the ide. The abundance index was the lowest compared with the other two types of trematodes detected. The prevalence of invasion also differed significant (Table 1).

A summary of the occurrence of fish trematodes in the North-East of the Republic of Kazakhstan is given below.

Family Azygiidae Lühe, 1909
Subfamily Azygiinae Lühe, 1909
Genus Azygia Looss, 1899
Azygia lucii (Müller, 1776)
Host: common pike (*Esox lucius)*.
Tissue: esophagus, stomach.
Place of discovery: Karatal river (channel of the Irtysh river, Kenzhekol, Pavlodar region); Irtysh river (Podpusk, Akkuly region); Irtysh river (Pavlodar); Irtysh river (Beskaragai, Akkuly region); Irtysh river (Tlektes, Akkuly region).

Family Allocreadiidae Looss, 1902
Subfamily Bunoderinae Looss, 1902
Genus Bunodera Railliet, 1896
Bunodera luciopercae (Müller, 1776)
Host: river perch *(Perca fluviatilis)*.
Tissue: intestines.
Place of discovery: Irtysh river (Podpusk, Akkuly region); Irtysh river (Tlektes, Akkuly region).

Family Opecoelidae Ozaki, 1925
Subfamily Plagioporinae Manter, 1947
Genus Sphaerostoma Rudolphi, 1809
Sphaerostomum bramae (Müller, 1776)
Host: river perch *(Perca fluviatilis)*, ide *(Leuciscus idus)*.
Tissue: intestines.
Place of discovery: Irtysh river (Pavlodar); Irtysh river (Tlektes, Akkuly region); Irtysh river (Podpusk, Akkuly region).

DISCUSSION

As already noted, 89% of the detected 303 maritas of trematodes are identified as *A. lucii*. Most often, *A. lucii* affects pike, and the intensity of infection of hosts by this species is greatest in comparison with others. Thus, the number of parasites in the stomach of pike reached 19 specimens. The parasites can be found in the hosts esophagus and stomach. The diet of pike is quite diverse and the intensity of nutrition is clearly seasonal depending on the climatic conditions of the reservoir and the food items availability (Grunin, 2009).

Most of *A. lucii* trematodes were nobilous, which indicates the infection of fish in the spring-summer period during active feeding. *A. lucii* is an obligate pike parasite (Lebedeva, 2005a; Liberman and Kozlov, 2018; Savelyeva and Petrova, 2016). However, puberty of this parasite is possible in other fishes: perch *(Percidae)*, salmon *(Salmonidae)*, burbot *(Lotidae)* – its optional final hosts. Large predatory fishes may become infected with *A. lucii*, by swallowing other infected fishes with maritas of this parasite. The degree of puberty of *Azygia* during reinfestation does not matter, since both juvenile and nubilous individuals of this parasite live in predator and continue to develop (Odening, 1976; Szidat, 1932).

Non-predatory fish become infected with *A. lucii*, swallowing cercariae floating in the water column. In terms of size, body shape, and the nature of the movements, cercariae of this species have some similarities with the larvae of long-winged dipterans and are attractive to fish (Odening, 1976; Szidat, 1932). Predatory fish, as noted above, are infected with this trematode from eaten victims (Sokolov *et al.,* 2017) (Figure 1).

The tail of *A. lucii* cercariae increases significantly after they leave the mollusk and fall into the water, and the fishes swallow them, taking them for mosquito larvae. In the body of fishes, these cercariae do not turn into metacercariae, but in adults – maritas. They all use aquatic mollusks as their first intermediate host. The eggs enter the aquatic environment, where the miracidium emerges from them, which then actively penetrates the mollusk. In the body of the latter, sporocysts are formed, and then redia, cercariae come out of them, which leave the mollusk's body and enter the external aquatic environment. After this, *A. lucii* cercariae enter the body of the fish and develop there into an adult (Frolova and Shcherbina, 1899; Shakaralieva, 2017) (Figure 1).

According to the this study, the next widespread species of trematodes in the region was *B. luciopercae*, very common also in perch fish in most of the Palaearctic and Neoarctic, the main definitive host being *P. fluviatilis* (Kuperman *et al.,* 1997). The infection rate of *B. luciopercae* maritas reached 40%. The maximum intensity of infection was of 18 specimens in one individual. The location of parasites are the intestines. *B. luciopercae* is distinguished by alternating generations with a change of hosts: two intermediate and final. The first intermediate host of *B. luciopercae* are mollusks of the family

Pisidiidae, the second - planktonic crustaceans, the final hosts - fishes of the family *Percidae* (Kuperman *et al.,* 1997). Perch is considered one of the most voracious and indiscriminate predators in food: the food of perch includes everything that moves on the bottom or in the waters: fry, small crustaceans, mollusks, insect larvae and eggs laid by other fishes. By mid-summer, the grown individuals move closer to the coast, where small roach and verkhovka become their food (Maltseva and Avdeeva, 2018) (Figure 1).

The lowest indicators of the infection index in the study region belonged to *S. bramae* maritas (1-2 specimens per individual). However, this is the only species that was found in two species of fishes of different families: perch and ide. According to the literature, *S. bramae* marites are located in the intestines of many carp fish, less often in fish of other orders. Concerning the life cycles of *S. bramae* trematodes, it was found that the mollusk *Bitynia tentaculata* serves as the first intermediate host, and the leeches of *Herpobdella octoculata* play the role of the second intermediate hosts (Serbina, 2013; Sinitsin, 1905). Cercariae, leaving the body of the mollusk, climb onto its shell and concentrate on the tentacles of its intermediate host. When meeting with the *Herpobdella* leech, these cercariae attack it, penetrate the skin and encyst under the skin, turning into metacercariae (Kalmykov *et al.,* 2013). As already noted, *S. bramae* was discovered in perch and ide. The perch is a predator, and the ide is a benthophage. However, the ide's food is diverse - mollusks, crustaceans, earthworms, insect larvae, medium-sized fish, while in summer the plants are of great importance in nutrition (Figure 2).

The dependence of the fish trematode fauna on the host feeding pattern is clearly expressed. Among the host predators (pike, perch) and benthophages (ide, roach, bream) can be distinguished. However, among the latter there are representatives with mixed nutrition, consuming from benthos and vegetation to insects, mollusks, and crustaceans. (Lebedeva, 2005b; Lebedeva and Ieshko, 2008). It turns out that predators are infected with trematodes more strongly than other fishes, due to their diet constituted mainly of benthos. It should also be noted that in predatory fishes, such as the perch, two of the three detected trematodes species were found.

A. lucii was the most often found fish trematodes of the 3 species identified in the water bodies of the North-Eastern part of the Republic, which was registered with a single host – pike, and showed the highest rates of infection indices. *A. lucii* trematode was detected every year of the study, while *S. bramae* was found in all years except for 2013 and 2014, and *B. luciopercae* was found in 2013, 2016 and 2017. The majority of *A. lucii* specimens were found in 2013 and 2016 (81 and 72, respectively), while the indicators of 2019 were the lowest (11 specimens). The largest number of *B. luciopercae* specimens (19) were found in 2017, whereas in the other years the number of trematodes varied from 2 to 4 spec. The number of *S. bramae* found was approximately 3-5 specimens per year.

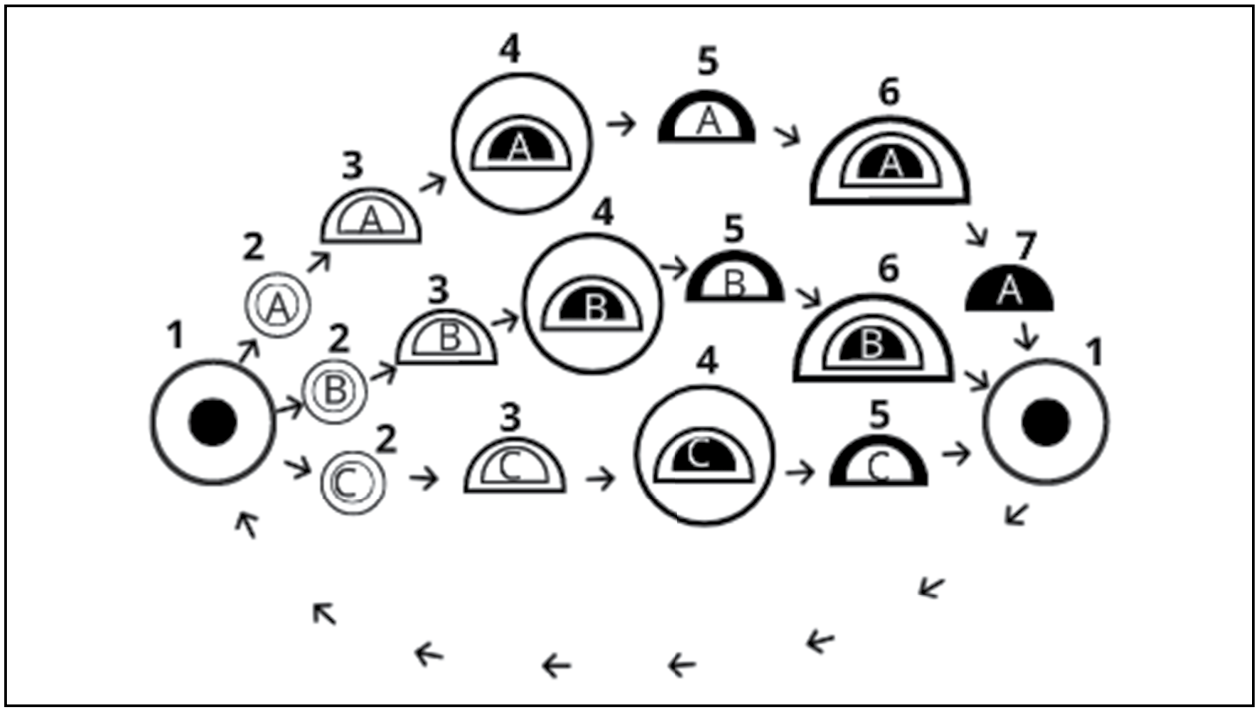

Fig. 2. Scheme of development cycles of helminths: **A** – *S. bramae;* **B** - *B. Luciopercae;* **C** – *A. lucii;* **1** – the final host (fish); **2** – eggs in water; **3** – 1st free-swimming larva; **4** – the first intermediate host - mollusk; **5** – 2nd free-swimming larva; **6** – the second intermediate host (a - the leech; b - planktonic crustaceans); **7** – 3rd free-swimming larva.

In our opinion, the reason for the high contamination of the representatives of the pickerel family is the abundance of intermediate hosts, *i.e.* aquatic mollusks that inhabit water bodies, as well as an indiscriminate and varied diet of pike.

CONCLUSION

The fish trematode fauna in the Pavlodar region of the Republic of Kazakhstan was represented by 3 species: *A. lucii*, *S. bramae*, *B. luciopercae*. *A. lucii* was most commonly found species of trematodes in the region. The maximum intensity and prevalence of infection was noted in pike's (7.9 / 94.4% respectively) stomach and esophagus The infection rate of *B. luciopercae* maritas in fishes of the region was 40%. The prevalence of invasion of *S. bramae* ranged from 6.7 to 26.09%. *S. bramae*, according to the study, is the only species found in two hosts of a different species. The abundance index of *S. bramae* was several times lower compared to the other two types of detected trematodes.

According to the results of the studies, it can be noted that the trematodes of predatory fish of the Irtysh River are more specialized for hosts, like the trematode *A. lucii* was registered only in pike. According to the research, fish of different ages turned out to be infected with this type of helminths, and infection with this trematode occurs regardless of whether this helminth is present in a particular specimen of pike or not. It can be seen from the results of the autopsy of captured fish – one pike has both mature maritas of trematode and specimens with immature sexual gonads.

According to our observations, the trematode *B. luciopercae* under the conditions of the Irtysh River is a specialized parasite of perch and does not occur in pike, although both fish species belong to predatory representatives of the ichthyofauna. In our opinion, such a situation is associated with the biology of the hosts of the trematode *B. luciopercae*, namely, perch. Possibly, the infection of perch with this trematode occurs in juveniles, and in a more mature state, the fish do not become infected, because the fish diet changes. Young perches willingly eat intermediate hosts with larval stages of trematode so becoming infected and thus, the marital stage of helminth begins to develop. In perches in which trematodes of *B. luciopercae* were found, all maritas of helminths were mature and had eggs in the uterus. This is one more evidence that the invasion of perch occurs in the juvenile stage.

The data we have established may be of practical importance for discussing the biology and nutrition of fish and especially for fish farm, naturally ponds/rivers management and not ultimately because those fish species are an important food sources for human consumption that may become infected in this way.

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