FEATURES OF THE DISTRIBUTION OF THREE SPECIES OF FISH TREMATODES IN THE 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 this group of parasitic worms in their biology is connected by 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, abundance index, and the invasion intensity of fish of the examined species were analyzed. Analysis of the fauna of trematodes of the studied species shows a good state of intermediate hosts living in the Irtysh River and reservoirs associated with its water area.

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)*. In addition, it is 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. Apparently, *E. lucius* is the main (typical) host for *A. lucii*, as the largest percentage of infection and the intensity of invasion and the parasites themselves, as well, are large in pike. *Sphaerostomum bramae* (Müller, 1776) is a widespread parasite of carp fish, occuring less commonly 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) is a representative of the genus *Bunodera* that infect 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 final. The role of the final hosts have representatives of many families of ray-finned fish. However, most parasites of freshwater fishes may infect 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).

Thus, Dzika *et al.* (from 2001 to 2004) carried out parasitological studies of fish from Lake Kortowskie (Poland). 381 fish representing ten species, including perch *Perca fluviatilis*, pike *Esox lucius* were examined. *A. lucii* (5.5%) were noted in the alimentary tract of the ruffe *Gymnocephalus cernuus* (L.) (55 specimens examined). Also, all examined pikes *E. lucius* (6) were infected with parasites. Adult 2 specimens of *A. lucii* were found in branchial chamber (Dzika *et al.,* 2008). Maltseva and Avdeeva (2018) found marita of *A. lucii* and *B. luciopercae* in 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 from the perch at lakes Pa¨ija¨nne and Saimaa and their life cycles including the first and second intermediate hosts and final host. According to their research all species transmitted via snails or mussels belong to Trematoda producing free swimming cercariae via asexual multiplication, which then infect the second intermediate host by penetration through skin or gills. Exceptions are *A. lucii* and *B. lucioperca*, cercariae of which are large and 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 snail was 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 throughout 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* located 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 fairly 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 Caspian Sea and rivers in the south and southeast. In this regard, the Central, Northern and North-East of Kazakhstan remain less studied. Over 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 water artery of Kazakhstan - the Irtysh River and water bodies entering its water area is very relevant.

MATERIAL AND METHODS

Fishes were caught between 2013-2019 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). Fishes were harvested from June to August (annually). Helminths were collected using the method of complete helminthological dissection of fish according to the method of K.I. Scryabin. Appropriate identification keys were used for determination of parasite fauna representatives to the lowest taxonomic level. 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 indicators 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 infested 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 per 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).

Table 1. The indicators of infection level of some fishes 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 next 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.

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 individual species of trematodes is presented in Table 1.

A. lucii was found in 34 specimens of the pickerel family. The intensity of infection of this species of hosts is greatest in comparison with others. Thus, the indices of fish infection rate with *A. lucii* marites were much higher than in other species of detected trematodes. In terms of the prevalence of infection and the abundance index, *A. lucii* marita indices differed by far; as well as the invasion intensity with indicators, at least, twice as much. The maximum prevalence of invasion reaches 94.4% in pike, and the abundance index is 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 *S. bramae* indices, but significantly lower than *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 noticed in the ide. The abundance index was the lowest compared with the other two types of trematodes detected. The prevalence of invasion also differs significantly (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*).
Localization: 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)*.
Localization: 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)*.
Localization: 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 this species of hosts is greatest in comparison with others. Thus, the number of parasites in the stomach of pike reached 19 specimens. Localization of parasites - the esophagus and stomach. The diet of pike is quite diverse, but the intensity of nutrition is clearly seasonal in nature and is due to the climatic conditions of the reservoir and the characteristics of the biology of food objects (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 found in different research areas and 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 become infected with *A. lucii*, swallowing non-predatory fish and predators of a lower trophic level containing 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).

Nonpredatory 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) (Fig. 1).

The tail portion 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) (Fig. 1).

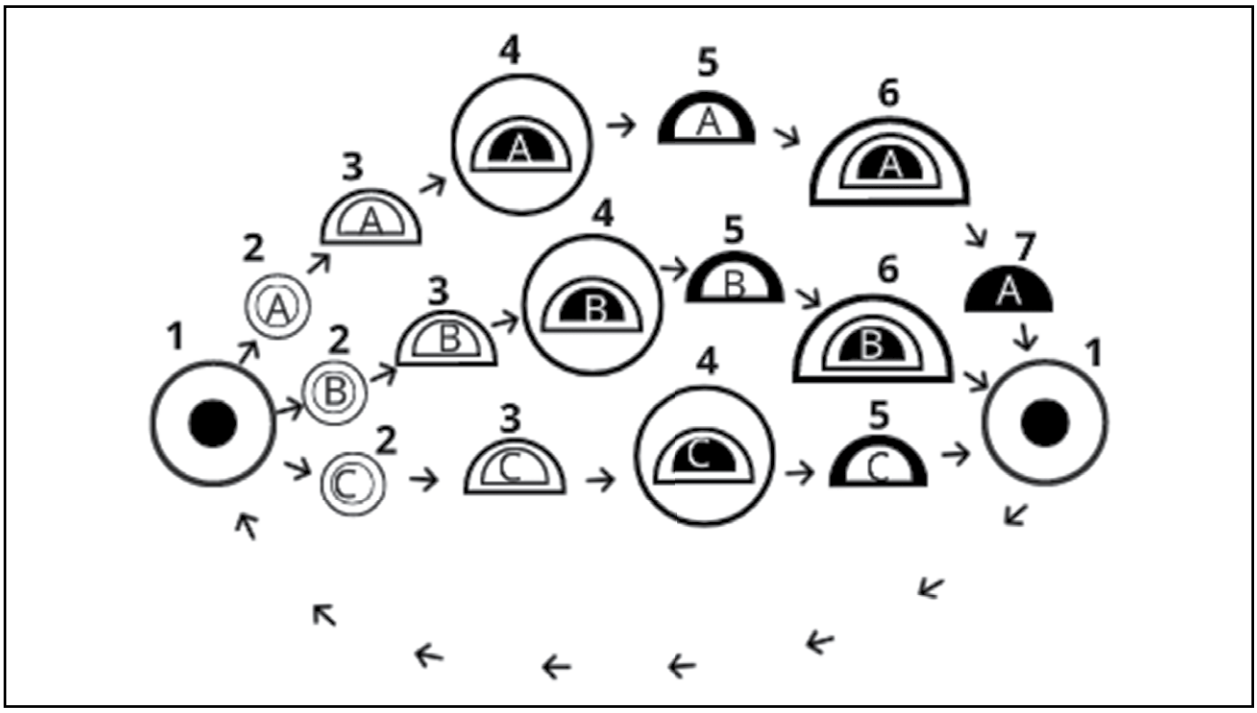

Fig. 1. Scheme of development cycles of helminths: **A** – *S. bramae;* **B** - *B. Luciopercae;* **C** – *A. lucii;* **1** – the final host (fish); **2** – eggs in water; **3** – 1st free-swimming larva; **4** – the first intermediate host - mollusk; **5** – 2nd free-swimming larva; **6** – the second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

According to the study, the next most widespread species of trematodes in the region is *B. luciopercae*, a widespread parasite of 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 reached 18 specimens in one individual. The localization of parasites is 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 is everything that moves along the bottom or in the waters of a reservoir, fry, small crustaceans, mollusks, insect larvae and eggs laid by other fish. Small perches emerging from caviar settle to the bottom, where they eat small crustaceans and insects. 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 belong 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 localized 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, and in summer plant food acquires great importance in nutrition (Fig. 1).

The dependence of the fish trematode fauna on the host feeding pattern is clearly expressed. Among the hosts studied, predators (pike, perch) and benthophages (ide, roach, bream) can be distinguished. However, among the latter there are representatives with mixed nutrition, eating benthos and vegetation and insects, mollusks, crustaceans, etc. (Lebedeva, 2005b; Lebedeva and Ieshko, 2008). It turns out that predators are infected with trematodes more strongly than other fishes, in the diet of which benthos occupies the main place. It should also be noted that in predatory fish, *i.e.* in perch two species of the three detected trematodes were found.

A. lucii is 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. It is worth noting that the *A. lucii* trematode was detected every year of the study, while *S. bramae* was found in all years of the study 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), indicators of 2019 were the lowest (11 spec.). The largest number of *B. luciopercae* specimens were found in 2017 (19), 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.

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

Thus, the fish trematode fauna in the Pavlodar region of the Republic of Kazakhstan is represented by 3 species: *A. lucii*, *S. bramae*, *B. luciopercae*. *A. lucii* is the most commonly found species of trematodes in the region. Trematodes have been found in the stomach and esophagus of pike. The maximum intensity and prevalence of infection was noted in pike (7.9 / 94.4%, respectively). The infection rate of *B. luciopercae* maritas in fish 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

eating intermediate hosts with larval stages of trematode become infected and, in it, 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 important food sources for human consumption that may become infected this way.

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