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### Article

## Features of the formation of the fish helminth fauna in the lower reaches of the Hrazdan River (Armenia) under anthropogenic pressure

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**Abstract.** Data from studies of the species composition of the fish helminth fauna and the degree of fish infection in the lower reaches of the Hrazdan River are presented. The studies were conducted in 2019–2020; 454 individuals of 15 fish species were examined. The detected helminth fauna was characterized by low species diversity, as a result, 5 species of helminths were identified: 1 species of Monogenea, 1 species of Trematoda, 2 species of Cestoda and 1 species of Nematoda. The total infection of fish by helminths was 34.1%. The detected helminths were localized mainly in the body cavity, intestine, eye lenses and on the gills of fish. The largest number of helminth species was noted in khramicarps and crucian carps. In almost all the studied areas of the lower reaches of the river, the most common of the detected species of helminths were plerocercoids of the cestode *Ligula intestinalis* (L., 1758) and metacercariae of the trematode *Diplostomum* sp. Nordmann, 1832.

**Keywords:** species composition of helminths, fish infection by helminths, contagious helminthiasis of fish, intermediate hosts of helminths

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*Научная статья*

# Особенности формирования гельминтофауны рыб нижнего течения реки Раздан (Армения) в условиях антропогенного пресса

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**Аннотация.** Приведены данные исследований видового состава гельминтофауны и степени инвазированности рыб нижнего течения реки Раздан. Исследования проводились в 2019–2020 гг., были обследованы 454 особи 15 видов рыб. Обнаруженная гельминтофауна характеризовалась невысоким видовым разнообразием, в результате выявлено 5 видов гельминтов: 1 вид Monogenea, 1 вид Trematoda, 2 вида Cestoda и 1 вид Nematoda. Общая инвазированность рыб гельминтами составила 34.1%. Обнаруженные гельминты были локализованы в основном в полости тела, кишечнике, хрусталиках глаз и на жабрах рыб. Наибольшее количество видов гельминтов отмечалось у храмуль и карасей. Практически на всех исследуемых участках нижнего течения реки наиболее распространенными из обнаруженных видов гельминтов были плероцеркоиды цестоды *Ligula intestinalis* (L., 1758) и метацеркарии трематод *Diplostomum* sp. Nordmann, 1832.

**Ключевые слова:** видовой состав гельминтов, инвазированность рыб гельминтами, контагиозные гельминтозы рыб, промежуточные хозяева гельминтов

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## Introduction

The Hrazdan River is the only river flowing from Lake Sevan, the largest freshwater body in the Caucasus. Lake Sevan is an oligotrophic lake located on the Armenian Highland at an altitude of 1900.30 m above sea level. The high mountain location and large volume of water of Lake Sevan determined its crucial importance for the development of the economy and energy of Armenia (Gabrielyan et al., 2022). The main energy and irrigation facilities of the Sevan-Hrazdan complex were built on it. The Hrazdan River is the second largest river in Armenia, flowing through the territory of Gegharkunik, Kotayk, Ararat regions and the city of Yerevan. It has an important economic, agricultural and recreational value. In the upper reaches, it flows south along a mountain valley, in the middle reaches it makes several sharp bends, in the lower reaches it flows along the Ararat plain and flows into the Araks River.

Over the past decades, the ecosystem of the Hrazdan River has undergone significant changes associated with various anthropogenic factors: the ongoing impact of hydroelectric power plants, the discharge of industrial and domestic wastewater, increased water intake for agricultural needs, etc. (Khosrovyan et al., 2022). This impact is especially noticeable in sections of the river located in the territories of large settlements and industrial enterprises, which leave a negative impact on almost all links in the trophic chain of its ecosystem (Kobelyan et al., 2021).

It is known that hydrobionts are capable of accumulating toxic substances contained in industrial wastewater (Stroganov, 1983). Studies have shown that their concentration in the organisms of hydrobionts is hundreds and thousands of times higher than the concentration in the aquatic environment (Stroganov, 1983). The circulation of toxic components of industrial wastewater through trophic chains creates a direct threat to human health. A loss of the economic value of commercial fish species is observed (Shakhmatova, 1983).

The diversity of the species composition of hydrobionts depends on the degree and nature of the impact of certain environmental factors (natural and anthropogenic). In connection with anthropogenic factors (reconstruction of natural reservoirs, increasing human economic activity), changes occur not only in the reproduction, development of hydrobionts and in the adaptive changes of populations, but also in communities as a whole – their species composition changes and eurybiontic organisms begin to predominate (Koshelev, 1984).

In the study of aquatic ecosystems, the state of the parasite fauna is used as one of the most sensitive bioindicators since the presence and abundance of parasitic organisms in fish can reflect the well-being of the aquatic community as a whole (MacKenzi et al., 1995).

Invasive fish diseases cause significant damage to the fisheries industry of the Republic of Armenia and significantly affect the viability of fish. The most pathogenic are those helminths for which fish are second intermediate or additional hosts: when fish die from the larvae of these helminths, they are transmitted to the definitive hosts. The assessment of the helminthological situation not only in artificial, but also in natural reservoirs is becoming very important (Vasilkov, 1999). The continuously deteriorating ecological conditions in natural water bodies lead to a decline in fish immunity against invasive pathogens, creating favorable conditions for the spread of various diseases (Vasilkov, 1999).

The literature contains some data on the study of the helminth fauna of fish in the Hrazdan River during the 1980s. For instance, an examination of 22 crucian carp from the upper reaches of the river revealed high levels of infection (up to 100%) by metacercariae of the trematode *Diplostomum* sp. in their eye lenses, as well as plerocercoids of the cestode *Ligula intestinalis* in their body cavities. Additionally, the trematode *Allocreadium isoporum* was found in the Kura khramicarp (Vardanyan, 1993). In 2016 and 2018, the species composition of the helminth fauna in fish from the upper (Hovhannisyan and Rukhkyan, 2018) and middle (Hovhannisyan and Rukhkyan, 2019) reaches of the Hrazdan River was studied (Fig. 1, sampling sites 1, 2, 3); the quantitative indicators of fish infection by various helminth

species, their localization, and hosts were identified. However, the helminth fauna of fish in the lower reaches of the Hrazdan River, where anthropogenic pressure significantly increases due to the presence of more populated areas, remains largely unstudied to this day.

The purpose of the research was to study the species composition and formation features of the helminth fauna of fish in the lower reaches of the Hrazdan River.

## Material and methods

The collections of helminths sampled from fish in the lower reaches of the Hrazdan River during 2019–2020 served as the research material.

Fish were caught near the settlements of Zorak, Ovtashen and Araksavan in the Ararat region. Sampling stations are shown in Fig. 1 (sampling sites 4, 5, 6).

Methods of parasitological examination were used to examine 454 individuals of 15 fish species. The species names and the number of examined fish by species are given in Table 1. Mature individuals from 14 to 27 cm in length were dissected.

The collection and cameral processing of fish helminths were carried out according to generally accepted methods (Bykhovskaya-Pavlovskaya, 1985; Gusev, 1983; Movsesyan, 1977; Shigin, 1986). The species identification of the detected helminths was carried out using various identification keys (Bauer, 1985, 1987; Moravec, 1975; Shigin, 1986).

To assess a fish infection rate by specific helminth species, standard helminthological indicators were used: Invasion Extensity (IE) and Invasion Intensity (II). IE is the ratio of the number of infested individuals of a particular species to the total number of individuals of that species examined, expressed as a percentage. II – is the number of parasites of a specific species present in an individual infested host.

Statistical analysis of the results was conducted using the software program “BioStat 2009.” The significance of the difference in EI of the same helminth species in different fish species was assessed using Fisher's criterion. The difference in the arithmetic mean II of the same helminth species in different fish species was assessed using Student's reliability criterion.

## Results

As a result of helminthological studies of fish from the lower reaches of the Hrazdan River, five species of helminths belonging to four taxonomic groups were recorded: Monogenea – *Dactylogyrus vastator* Nybelin, 1924; Trematoda – metacercariae *Diplostomum* sp. Nordmann, 1832; Cestoda – *Ligula intestinalis* (L., 1758), *Schyzocotyle acheilognathi* (Yamaguti, 1934); Nematoda – *Rhabdochona macrostoma* Moravec et Mikailov, 1970.

The overall helminth infection rate in fish was 34.1%. Of the 454 examined fish individuals, 155 were infected (Table 1).

Of the 15 fish species, 10 were infected; 5 species (Kura and Angora char, eastern chub, mosquitofish and sand goby) were not infected. The data on the detected helminths are summarized in Table 2.

The higher number of helminth species was found in khramicarps – 4 species. Three species of helminths were identified in crucian carps. In the Kura barbel, riffle minnow, Kura bleak, sunbleak, common carp, as well as the white bream, roach and gudgeon (from a small sample), 1 species was noted (Table 2).

In our studies, among the helminths, the most common are the plerocercoids of the tapeworm *Ligula intestinalis*, which parasitize the fish body cavity, and the metacercariae of trematode *Diplostomum* sp., which parasitize the eye lenses. Both species of helminths are among the most invasive ones and cause significant harm to fish (Vasilkov, 1999).

In the helminth fauna of fish, 4 species of biohelminths were noted (multi-host parasites with a complex life cycle requiring two or more hosts) and 1 species with a direct life cycle (*Dactylogyrus vastator*). Most helminths enter the fish body through the trophic route, not excluding the percutaneous method of fish invasion by diplostomes. Two helminth species (*Ligula intestinalis* and *Diplostomum* sp.) are classified as generalist species (biological species capable of surviving in various habitat conditions and utilizing different types of resources). Three species are classified as specialist species (biological species that exist only in a narrow range of habitats and/or have a limited diet): *D. vastator*, *S. acheilognathi* and *R. macrostoma*.

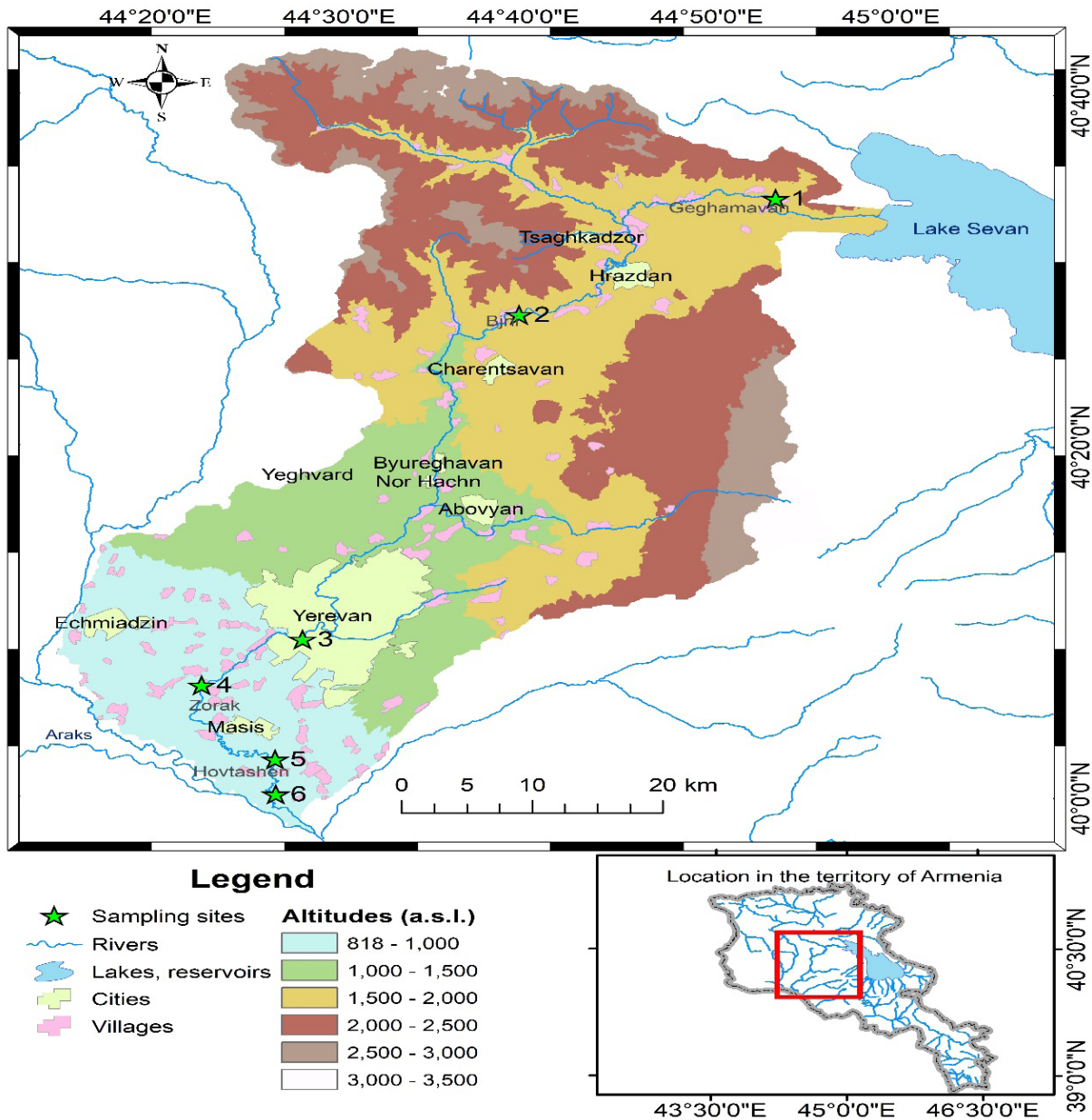


Fig. 1. Sampling stations in various sections of the Hrazdan River.

## Discussion

As a result of helminthological studies of fish from the lower reaches of the Hrazdan River, monogenea, trematodes, cestodes, and nematodes were detected.

Metacercariae of *Diplostomum* sp. localized in the eye lenses of fish (Fig. 2, Application: Metacercaria *Diplostomum* sp. in crucian carp (video)). In case of high IE level, they can lead to blindness and, consequently, to fish death. Dead fish float to the surface, becoming easy prey and at the same time a source of infection for fish-eating birds (Shigin, 1986).

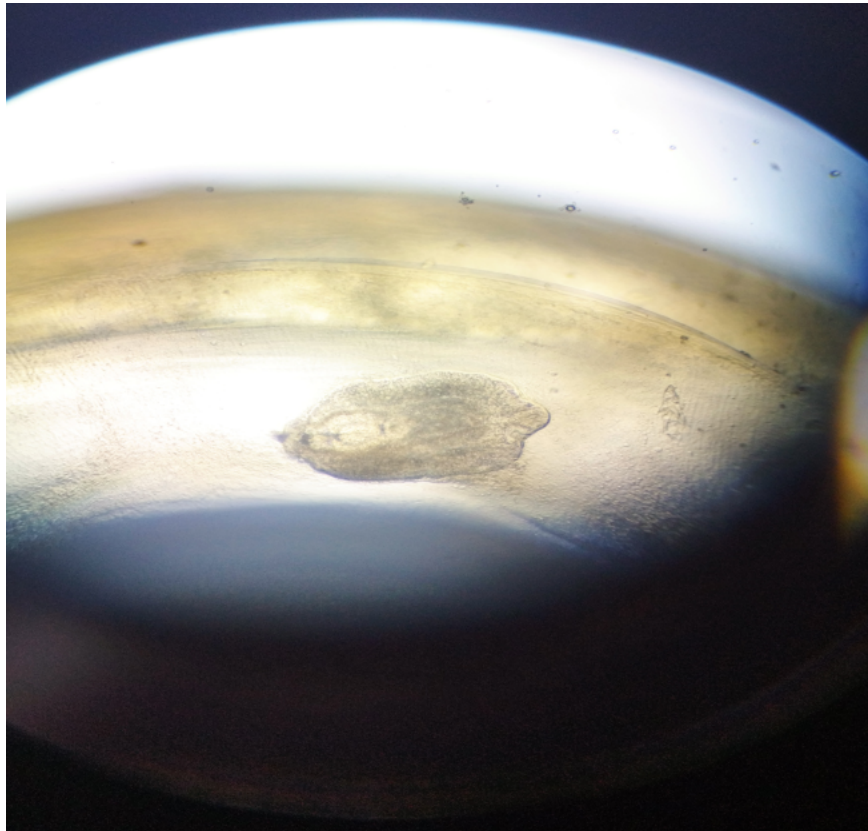
Infection of fish by metacercariae of diplostomes was observed from early summer to mid-autumn. However, the highest infection rates were recorded from July to mid-September, with maximum IE values of 37.5% in khramicarps and 32.2% in crucian carps. This can be attributed to the peak abundance of pond snails – gastropod mollusks of the family *Lymnaeidae* (the first intermediate hosts of diplostomes) – and fish-eating birds (definitive or final hosts), which introduce the infectious stage into reservoirs (Shigin, 1986).

**Table 1.** The fish species, the number of examined and infected fish.

Fish species	Number of examined fish, individuals	Number of infected fish, individuals
Crucian carp <i>Carassius auratus gibelio</i> Bloch, 1782	118	61
Riffle minnow <i>Alburnoides bipunctatus eichwaldii</i> (De Filippi , 1863)	85	18
Khramicarp <i>Capoeta capoeta</i> (Guldenstadt, 1773)	72	49
Kura barbel <i>Barbus cyri</i> De Filippi, 1865	62	12
Common carp <i>Cyprinus carpio</i> Linnaeus, 1758	20	2
Kura bleak <i>Alburnus filippii</i> Kessler, 1877	14	4
Sunbleak <i>Leucaspis delineatus</i> (Heckel, 1843)	21	3
Kura loach <i>Oxynoemacheilus brandtii</i> Kessler, 1877	10	–
Chub <i>Squalius orientalis</i> Heckel, 1847	14	–
Roach <i>Rutilus rutilus</i> (Linnaeus, 1758)	6	2
White bream <i>Blicca bjoerkna</i> (Linnaeus, 1758 )	8	3
Eastern mosquitofish <i>Gambusia holbrooki</i> Girard, 1859	4	–
Monkey goby <i>Neogobius fluviatilis</i> (Pallas, 1814)	5	–
Angora loach <i>Oxynoemacheilus angorae</i> (Steindachner, 1897)	9	–
Gudgeon <i>Gobio gobio</i> (Linnaeus, 1758)	6	1
<b>Total</b>	<b>454</b>	<b>155</b>

**Table 2.** Helminth infection of the examined fish. Significance criteria: \* –  $p < 0.05$  (according to Fisher); \*\* –  $p < 0.05$  (according to Student).

Class, helminth species	Host	Helminth localization	Number of infected fish, individuals	IE, %	II, individuals (mean $\pm$ error of the mean)
<b>Monogenea</b>					
<i>Dactylogyrus vastator</i>	Crucian carp	gills	6	5	1.17 $\pm$ 0.17
<b>Trematoda</b>					
	Khramicarp	eye lens	27	37.5	1.96 $\pm$ 0.17
	Crucian carp	eye lens	38	32.2	1.92 $\pm$ 0.13
	Kura barbel	eye lens	12	19.4*	1.17 $\pm$ 0.11
<i>Diplostomum</i> sp.	Rifle minnow	eye lens	18	21.2*	1.22 $\pm$ 0.13
	Kura bleak	eye lens	4	29	1.25 $\pm$ 0.25
	Sunbleak	eye lens	3	14.3	1
	Common carp	eye lens	2	10*	1
<b>Cestoda</b>					
<i>Ligula intestinalis</i>	Crucian carp	body cavity	17	14.4	4.06 $\pm$ 0.29**
	Khramicarp	body cavity	12	17	2.17 $\pm$ 0.27**
<i>Schizocotyle acheilognathi</i>	Khramicarp	intestine	4	5.5	1.25 $\pm$ 0.25
<b>Nematoda</b>					
<i>Rhabdochona macrostoma</i>	Khramicarp	intestine	6	8	2.17 $\pm$ 0.31



**Fig. 2.** Metacercaria of *Diplostomum* sp. in the eye lens of a crucian carp.

The diversity of the species composition of the helminth fauna of fish depends on the number of different hosts of helminths in the reservoir, composition of their food base, dietary spectrum, and other factors (Dogel, 1958). According to the faunistic research of the Hrazdan River, a comparative analysis of the zoobenthos composition in the Hrazdan hydroecosystem conducted in the 1930s and in 2004–2007 revealed changes in the quantitative indicators of the benthic fauna. In particular, gastropod mollusks of the family *Lymnaeidae* were present both in those years and more recently; however, their quantitative indicators have significantly decreased (Dallakyan, 2011). This likely explains the trend of decreasing levels of fish infection by metacercariae of diplostomes compared to those recorded in 1980s when IE was rather high and often reached 100% (Vardanyan, 1993). It is noteworthy that some non-commercial fish species, such as gudgeon, sunbleak, riffle minnow and others, accumulating parasites, play a significant role in the spread of diplostomiasis among other fish species (Vasilkov, 1999).

The cestode *Schyzocotyle acheilognathi*, detected in khramicarp, parasitizes the intestine, exerting mechanical, toxic, and trophic effects on the infected fish. In case of high level of II, intestinal blockage and mortality in young fish can be registered (Protasova, 1977). The development of cestodes takes place through intermediate hosts – copepodes *Mesocyclops crassus* (Fischer, 1853), *Cyclops strenuus* Fischer, 1851, *C. vicinus* Uljanin, 1875 etc. (Protasova, 1977). The last two species are found in the zooplankton of the Hrazdan River (Ayrapetyan, 2012) and, accordingly, can participate in the life cycle of this helminth in the river.

The nematode *R. macrostoma* first identified in the fauna of Armenia in the khramicarps of Sevan Lake (Hovhannisyan and Rukhkyan, 2013). In our studies, this species was also found in the khramicarps of the lower reaches of the Hrazdan River that indicates a fairly rapid spread of the species. The intermediate hosts of this nematode are rheophilic larvae of amphibious insects, including mayfly larvae (Ephemeroptera) from the genera *Ephemerella*, *Heptagenia*, and others (Moravec, 1975). It is known that among benthic organisms, the khramicarp mainly eats mayfly larvae and chironomids (Gabrielyan, 2010). A large number of mayflies of the genus *Heptagenia* were found in the Hrazdan River (near the village of Atarbekyan) (Asatryan and Dallakyan, 2019), which participate in the life cycle of this nematode in the river.

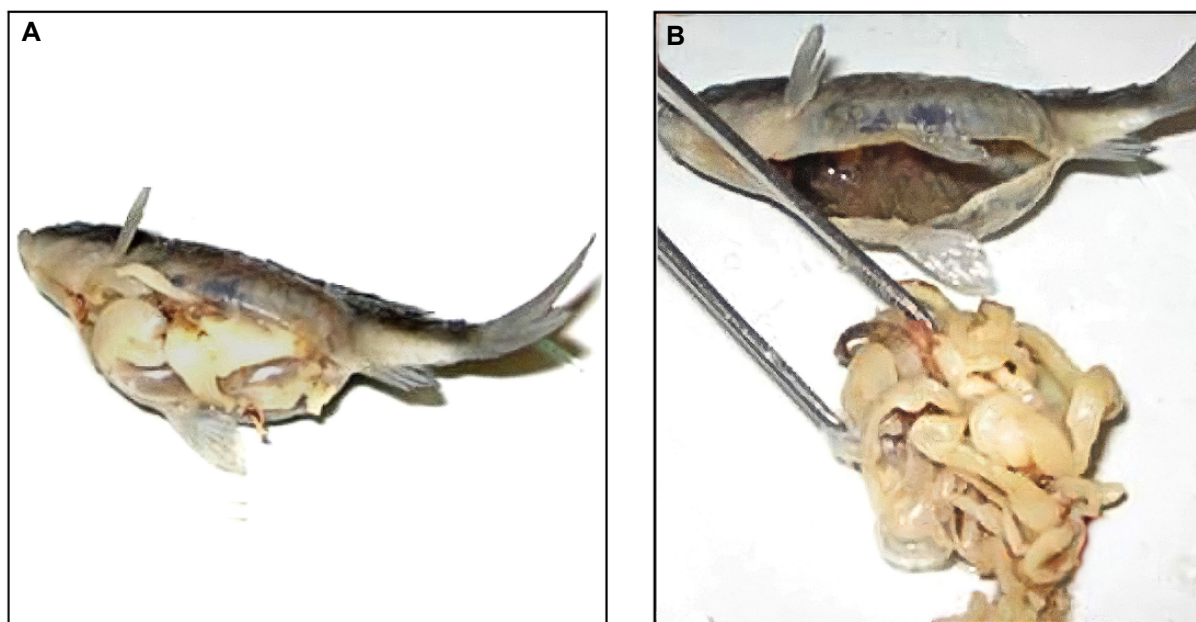
Monogenea *D. vastator* have been found on the gills of crucian carps. From the destruction of the gill apparatus and the disruption of the respiratory function due to the effects of this type of parasite, mainly carp and crucian carp fry die (Vasilkov, 1999).

Plerocercoids of ligula, which are the most invasive stage of this parasite in terms of the degree of pathogenic effect on the host organism, were found in khramicarp and crucian carps. They cause a dangerous helminthiasis in fish – ligulosis. Parasitizing in the body cavity and having large sizes, plerocercoids destroy internal organs, which leads to their atrophy and infertility of fish; at high level of IE, a rupture of the body cavity occurs, leading to the death of fish (Dubinina, 1966). We have repeatedly observed cases of perforation of the body cavity of fish by plerocercoids of ligula (Fig. 3A, B).

The fish infection by plerocercoids of ligules occurred from June to mid-October, with the maximum infection of fish observed in mid-summer – early autumn: IE in khramicarp made 17%, II avg. – 2 sp., in crucian carps – 14.4% and 4 sp., respectively. This is accounted by the presence of optimal conditions for the reproduction of the ligulosis pathogen: abundance of the first intermediate hosts (copepods) of the cestode *L. intestinalis* and definitive hosts – fish-eating birds, which serve as the main distributors of the pathogen (Dubinina, 1966).

The comparative analysis of the data on the IE of fish from the upper, middle, and lower reaches of the Hrazdan River by ligula plerocercoids showed significant differences (Table 3). In the upper reaches of the river, the IE of ligula in both crucian carps and khramicarp is lower than in other areas. This is probably due to the fact that in the upper reaches the river flows through a mountain valley at high speed; in addition, the water temperature in this area is quite low for the vital activity of intermediate hosts of ligula. The IE of fish reaches its maximum value in the middle reaches of the river.

It should also be noted that the water pollution is higher in the lower reaches of the river. As a result of the studies of 2016–2018, the ecological state of the Razdan River was assessed, in particular, the quality of water in various sections of the river was determined (Kobelyan et al., 2021). The river is periodically polluted and self-cleansed along the flow. However, the volumes of runoff coming into the river from the city of Yerevan, populated areas, and fish farms, are increasing in the lower reaches. As a result, the river ecosystem does not have time to implement the self-purification process and the level of pollution increases. The water quality up to the lower reaches of the river corresponds to the category “clean” (quality class 2), and in the lower reaches – “polluted” (quality class 4) (Kobelyan et al., 2021). The lower infection of fish in the lower reaches, compared to the middle reaches, presumably, is also associated with the impact of anthropogenic factors (pollution of the river by runoff, etc.), which negatively affect all links of the trophic chain of the river ecosystem.



**Fig. 3.** Perforation of the body cavity of a crucian carp by plerocercoids of *Ligula intestinalis* (A), plerocercoids of *L. intestinalis* from the body cavity (B).

**Table 3.** Invasion Extensity of fish of various sections of Hrazdan River by plerocercoids of the cestode *Ligula intestinalis*

Fish species	Upper reaches, Village Geghamavan (Hovhannisyanyan and Rukhkyan, 2018)	Middle reaches, Village Bjni (Hovhannisyanyan and Rukhkyan, 2019)	Lower reaches, Villages Zorak, Hovtashen, Araksavan (our data)
Crucian carp	7%	45.5%	17%
Khramicarp	6%	25%	12%

## Conclusion

Thus, during the helminthological examination of fish in the lower reaches of the Razdan River, 5 species of helminths were registered, including 1 species of monogeneans, 1 species of trematodes, 2 species of cestodes and 1 species of nematodes.

The total infection of fish by helminths was 34.1%. The most common species of the discovered helminths are the metacercariae of trematode *Diplostomum* sp. and the plerocercoids of the cestode *Ligula intestinalis*. The main periods of fish infection by helminths cover the period from early summer to mid-autumn that is associated with the relatively higher water temperatures during this period.

The depletion of the species composition of the fish helminth fauna in the lower reaches of the Razdan River and the changes of the fish infection by helminths depend on the degree and nature of the complex impact of certain environmental factors, both natural and anthropogenic.

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