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ORIGINAL ARTICLE

PRELIMINARY PARASITOLOGICAL INVESTIGATION OF BROWN TROUT WITH NEW RECORDED SPECIES IN ARMENIA

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ABSTRACT

The brown trout *Salmo trutta* Linnaeus, 1758 is an important species for fisheries in Armenia, with a comparatively wide distribution in both wild habitats and aquaculture. Despite its significance and widespread presence, there is limited information regarding diseases and parasites affecting brown trout in Armenia. The aim of this study was to examine the parasite fauna of brown trout in the southern part of Armenia. The research was conducted in the Vayots Dzor Region, as well as at the Scientific Center of Zoology and Hydroecology, and Armenian National Agrarian University. Captured fish were examined using the method of complete parasitological research. Parasites were analyzed under microscope and identified using specialized manuals based on their morphological features. A total of 20 brown trout were captured between September and December 2023. Of these, 8 individuals (40%) were (40%) were infected with parasites. Six species of parasites were identified: *Dactylogyrus vastator* Nybelin, 1924 (Monogenea: Dactylogyridae), *Gyrodactylus* sp. (Monogenea: Gyrodactylidae), *Bunodera luciopercae* Müller, 1776 (Trematoda: Allocreadiidae), *Proteocephalus longicollis* Zeder, 1800 (Cestoda: Proteocephalidae), *Khawia armeniaca* Cholodkovsky, 1915 (Cestoda: Lytocestidae), and *Ichthyophthirius multifiliis* Fouquet, 1876 (Ciliata: Ichthyophthiriidae). Parasite infections were generally characterized by low intensity, except for *I. multifiliis*. *Gyrodactylus* sp. and *B. luciopercae* helminths were detected for the first time in Armenia. This pilot study provides baseline information for further large-scale investigations and offers a valuable insight for farmers to better understand the challenges associated with efficient production.

Keywords: Aquaculture, *Bunodera luciopercae*, Fishponds, *Gyrodactylus*, *Salmo trutta*.

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INTRODUCTION

The brown trout *Salmo trutta* Linnaeus, 1758 is an important species for fisheries, which has a comparatively wide distribution in Armenia, especially in small, fast-flowing rivers and high-mountain springs characterized by clean water, low water temperature, and high oxygen concentration (Pipoyan, 2012; Domingues *et al.*, 2022). In addition to its presence in the wild, this species is also cultivated in aquaculture, albeit in limited regions due to its specific ecological requirements (Krieg *et al.*, 2008; Domingues *et al.*, 2022). Additionally, *S. trutta* plays a crucial role as a reservoir and source of parasites for introduced fish species (Borgstrøm *et al.*, 2021).

Despite its wide distribution and a high significance, there is limited information available on diseases and parasites of brown trout in Armenia. To date, only two publications on *S. trutta* parasites in Armenia have been reported. Namely, *Ichthyophthirius multifiliis* ciliate parasites were found in the skin of fingerling brown trout in Hrazdan River, Central Armenia (Hovhannisyán and Rukhkyan, 2012), and *Diplostomum* sp. trematode metacercariae were detected in eye lenses of brown trout in river Kasakh, Central Armenia (Hovhannisyán and Rukhkyan, 2016).

It is important to highlight that research on parasites in brown trout is also relatively limited worldwide (Barskaya and Ieshko, 2004; Quilichini *et al.*, 2007; Mladineo *et al.*, 2009; Rolbiecki *et al.*, 2009; Dix, 2010; Gopko *et al.*, 2018; Laegran, 2022; Couso-Perez *et al.*, 2023).

In light of the above, the objective of this study is to investigate the parasite fauna of brown trout *Salmo trutta* in southern Armenia. It should be emphasized that the parasite fauna of fish in southern Armenia, especially in the Vayots Dzor and Syunik regions, has not been studied at all. Moreover, Data on the parasite fauna of brown trout in neighboring countries such as Iran, Azerbaijan, Georgia, and Turkey are also very limited (Sattari *et al.*, 2005).

MATERIALS AND METHODS

Study area: Brown trout *S. trutta* (For consistent tense) natural waters, and artificial fishponds of Vayots Dzor Region. This region was selected due to its rich biodiversity, as well as potential for introduction of parasite species and biological invasions from neighboring regions and countries. The main water body of the region is river Arpa (length: 128 km; drainage basin: 2630 km²) that begins from Vardenis Range (the natural border between the Sevan Lake and Arpa basins), and flows into river Araks, the longest river of Armenia (basin of Caspian Sea). Additionally, The Arpa River is connected to Lake Sevan Via the Arpa-Sevan Tunnel (length: 48 km). The river has several tributaries including the Yeghegis, Herher, and Martiros Rivers. Most of the rivers in Vayots Dzor are characterized by swift flow, deep fall, and inclination. There are also numerous small mountain lakes in the region.

Specimens` collection and processing: Twenty brown trout specimens, including both males and females aging one to four years, were captured from the wild (Arpa River, 39°45'04.6"N

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45°36'32.8"E, altitude 1500 m ASL), and from fish farms (Village Gndevaz, Vayots Dzor Region, 39°45'51.1"N 45°36'36.9"E, altitude 1523 m ASL), between September and December 2023. Ten wild fish, caught by hook, were brought to the Research Center of Veterinary and Sanitary Expertise at the Armenian National Agrarian University for parasitological analysis. The remaining ten fish, raised in aquaculture and caught using nets, were examined at the Laboratory of Molecular Parasitology, Scientific Center of Zoology and Hydroecology, NAS RA. Details of the samples are described in Table (1).

The age of the fish was determined by counting the number of paired opaque and translucent rings on their scales, as well as estimating the development stage of their gonads.

The specimens underwent a complete parasitological survey following the method of (Bykhovskaya-Pavlovskaya, 1985). All organs and tissues of the fish were examined. Skin, fins and gill scrapes were mounted between two large slide glasses and examined under a microscope (10X objectives, 16X eyepieces). Gastrointestinal contents were washed multiple times, and the sediments were examined under a binocular microscope (40X) and, for more detailed morphological examination, under a B-100 microscope (4x objective, 16x eyepiece).

Table (1): Number of Brown Trout *Salmo trutta* Samples Captured in Vayots Dzor Region.

Fish sex	Age of the captured fish, years								Total		
	1		2		3		4		W	Aq	Total
	W	Aq	W	Aq	W	Aq	W	Aq			
Males	-	1	1	1	1	1	1	1	3	4	7
Females	1	1	1	1	3	3	2	1	7	6	13
Total	1	2	2	2	4	4	3	2	10	10	20

Notes: W – wild fish; Aq – fish grown in aquaculture

Fish parasites were identified using identification manuals based on the morphological features (Bauer, 1984; Bauer, 1985; Bauer, 1987; Sitja-Bobadilla *et al.*, 2021).

Indices of rate of infection (prevalence) (A), intensity (B), and abundance index (C), were calculated:

A. Rate of infection =
$$\frac{\text{Number of the fish infected with the certain parasite}}{\text{Number of the examined fish}}$$

B. Intensity of infection is a quantity of parasite of the certain species detected in one fish/specimen.

Statistical analysis: To compare the prevalence of the same parasite in each group of fish, e.g. wild and artificially grown, Fisher exact probability test criterion was used.

Abbreviations: Aq: fish grown in aquaculture, ASL: above sea level, N/A: not applicable, NAS RA – National Academy of Sciences of the Republic of Armenia, sp: species, W: wild fish.

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RESULTS

The pattern of parasites registered in *Salmo trutta* in Armenia is shown in Table (2).

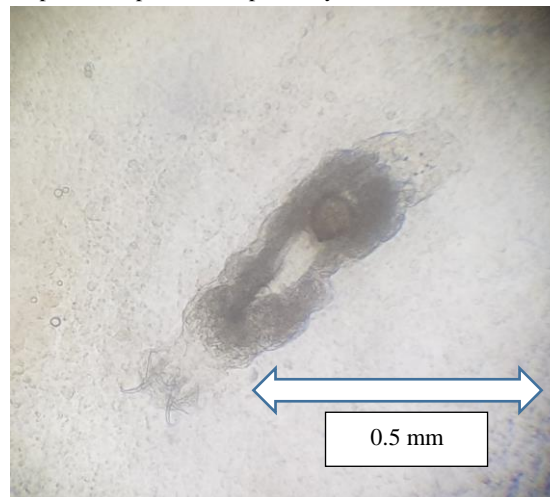
Table (2): Fauna of Parasites of Brown Trout *Salmo trutta* in Armenia.
N = 20 (10 wild and 10 grown in aquaculture)

Parasite species	Rate of infection/prevalence, %			Infection intensity, parasites per fish	
	W	Aq	Total	W	Aq
<i>Dactylogyrus vastator</i>	20	0	10	2 in one fish, and 3 in another	0
<i>Gyrodactylus</i> sp.	0	10	5	0	1
<i>Bunodera luciopercae</i>	10	0	5	37	0
<i>Proteocephalus longicollis</i>	10	0	5	2	0
<i>Khawia armeniaca</i>	10	0	5	1	0
<i>Ichthyophthirius multifiliis</i>	0	40*	20	N/A	N/A

* -P < 0.05 (by Fisher exact test)

A mixed parasitic infection was recorded in 40% of cases (8 out of 20 examined fish specimens). The rate of parasitic infection was similar in both wild and farmed, with an infection rate of 40% in each group. Between one and three parasite species were detected in each infected fish. The following parasite species were identified in the current study:

- 1. *Dactylogyrus vastator*** Nybelin, 1924 (Monogenea, Dactylogyridae). A comparatively large monogenean (Pl. 1) (1.25 x 0.25 mm) was detected in the gills of two females trout (10%) aged 2 and 3 years, captured from the wild in the Arpa River. The intensity of infection was low, with 2 and 3 specimens per fish, respectively.



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Plate (1): *Dactylogyrus vastator* in *Salmo trutta* gills.

2. *Gyrodactylus* sp. (Monogenea, Gyrodactylidae). A small monogenean (mean length 0.8 mm) (Pl. 2) was found on the skin of a single male trout aged 3 years, grown in aquaculture. The intensity of infection was very low, with only one helminth detected. It should be noted that it was impossible to identify the exact species of *Gyrodactylus*: *G. salaris*, *G. truttae* or *G. derjavini* based solely on morphological features. This finding represents the first recorded case of *Gyrodactylus* in Armenia, as no prior data on its presence in the country are available.

3. *Bunodera luciopercae* Müller, 1776 (Trematoda, Allocreadiidae). A small trematode (0.5-2.3 x 0.2-0.7 mm) with a highly mobile anterior body region (Pl. 3) was found in the intestine of a single female trout (5%) aged 3 years, captured in the wild. A total of 37 specimens were collected. This trematode is detected for the first time in Armenia.

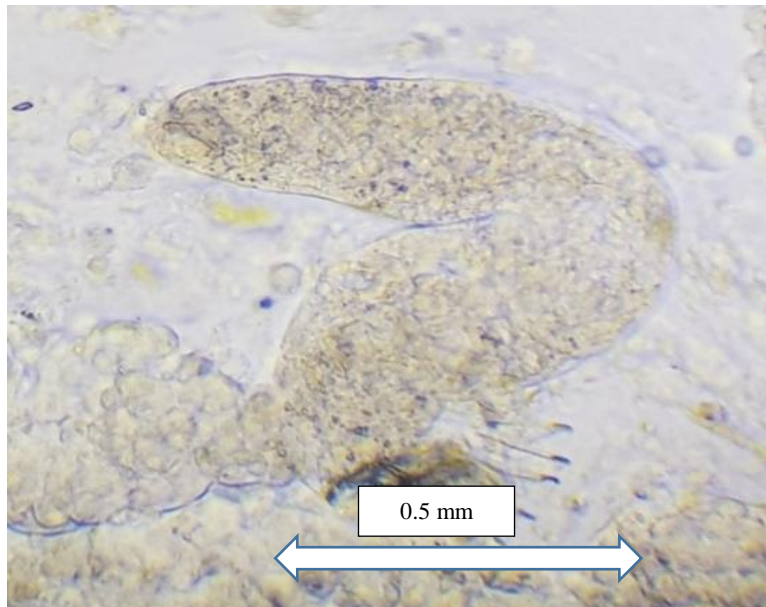


Plate (2): *Gyrodactylus* sp. monogenean in skin of *S. trutta*.

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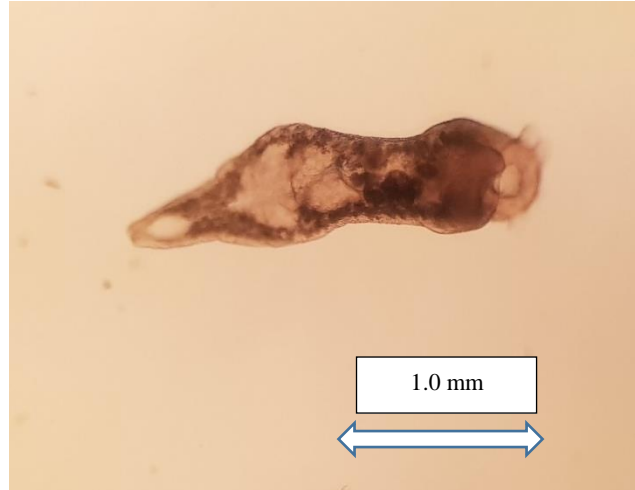


Plate (3): *Bunodera luciopercae* in the intestine of *S. trutta*.

4. *Proteocephalus longicollis* Zeder, 1800 (Cestoda, Proteocephalidae). A comparatively long tapeworm (up to 25 cm in length and 2 mm in width) (Pl. 4) was isolated from the pyloric caeca of a single female trout (5%) aged 3 years, captured in the wild. This is the first record of this species outside Lake Sevan basin.



Plate (4): Fragment of *Proteocephalus longicollis* strobila from the pyloric caeca of *S. trutta*

5. *Khawia armeniaca* Cholodkovsky, 1915 (Cestoda, Lytocestidae). A medium - sized tapeworm (65 x 5 mm) (Pl. 5) was detected in the intestine of a single female trout (5%) aged 3 years, captured in the wild. One specimen was found. This represents the second record of this tapeworm outside the Lake Sevan basin, as well as the first record of its presence in brown trout in Armenia.

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Plate (5): Rear end of *Khawia sinensis* from the intestine of *S. trutta*.

6. *Ichthyophthirius multifiliis* Fouquet, 1876 (Ciliata, Ichthyophthiriidae). A large cosmopolitan ciliate (Up to 1mm in length) (Pl. 6) was identified for the first time parasite in southern part of Armenia. Only fish grown in aquaculture were infected with *I. multifiliis*. The difference in infection prevalence between wild and farmed fish was statistically significant ($P < 0.05$).

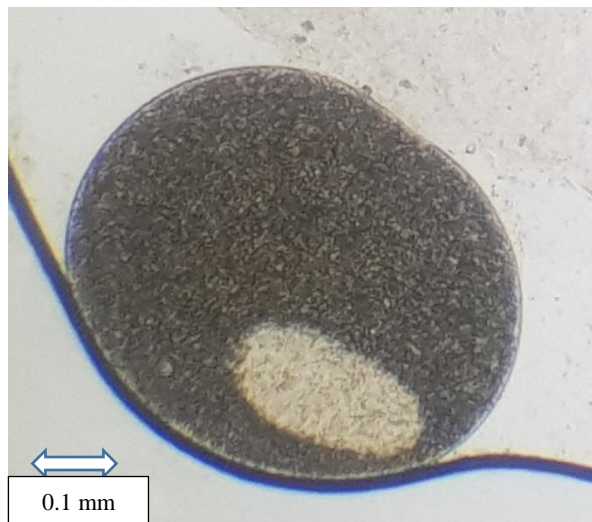


Plate (6): *Ichthyophthirius multifiliis* in the skin of *S. trutta*.

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I. multifiliis infection was characterized by pronounced clinical manifestation, including severe skin and fin lesions (Pl. 7). The infection was further complicated by a secondary infestation with *Saprolegnia* spp., a group of oomycetes (Pl. 8).



Plate (7): Skin and fin lesions in *S. trutta*.

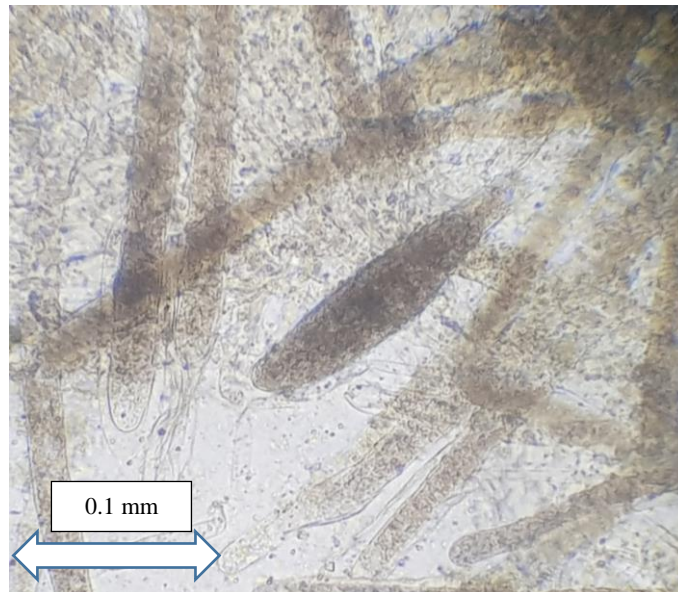


Plate (8): Growth of *Saprolegnia* sp. oomycetes in *S. trutta* skin.

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Pattern of various age groups fish infection is presented in Table (3).

Table (3): Rate of Infection in Different Age Groups of Brown Trout (%).

Index	Age of the Fish, years				Total
	1	2	3	4	
Rate of infection/prevalence %	33.33	50.0	62.5	0	40.0

DISCUSSION

Based on the results of this research, we conclude that the parasite fauna of brown trout in Armenia is comparatively poor, comprising six species. These include two monogeneans, one trematode, two tapeworms, and one ciliate. The absence of roundworms (nematodes) and acanthocephals is a distinctive feature of the parasite fauna of *Salmo trutta* in Armenia.

Dactylogyrus vastator is a native Palearctic species that originally was infected cyprinids in both wild and aquaculture environments (Abdullah and Abdullah, 2013; Benovics, 2020). As an invasive species in Armenia, *D. vastator* was as an invasive species in Armenia, in cyprinids grown in aquaculture in 1983 (Grigoryan and Poghosyan, 1983). However, a recent trend of this parasite infecting salmonids in Armenia has been observed (Naghashyan *et al.*, 2015).

Gyrodactylus sp. is a native monogenean parasitizing the skin of salmonids in Europe (Rokicka *et al.*, 2007; Hansen *et al.*, 2016). However, it hasn't been previously detected in fish in Armenia.

Bunodera luciopercae is a typical parasite of perciform fish. However, this trematode is also able to infect salmonids, namely, *Salmo trutta* (Wootten, 1973).

Proteocephalus longicollis is an autochthonous species typical for salmonids (*Salmo ischchan*) and cyprinids (*Capoeta capoeta sevangi* and *Barbus goktschaicus*) in Lake Sevan. It was first recorded in the lake in 1933 (Dinnik, 1933) has been periodically detected later in cyprinids in wild salmonids and cyprinids of Sevan lake (Platonova, 1963; Voropaeva *et al.*, 2011), as well as for wild salmonids worldwide (Brabec *et al.*, 2023). This is the first record of this tapeworm outside the Lake Sevan basin. It is likely that *P. longicollis* entered the Arpa River via the "Arpa-Sevan" tunnel transported by infected copepods, which serves as intermediate hosts for the helminth.

Khawia armeniaca is an Armenian endemic species primarily associated with fish in Lake Sevan. It was first recorded in the lake in 1933 (Dinnik, 1933) and has since been periodically detected in wild salmonids and cyprinids of the lake, including *Capoeta capoeta sevangi*, *Salmo ischchan*, and *Coregonus lavaretus* (Rubenyan, 2008; Voropaeva and Tolstenkov, 2008). This has also been described in *Capoeta capoeta* in Iran (Williams *et al.*, 1980). In the current research, this tapeworm was recorded for the second time outside the Lake Sevan basin and represents the first record of *k. armeniaca* in brown trout in Armenia. Probably, this

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tapeworm entered the Arpa River Via the “Arpa-Sevan” tunnel, carried by infected *Tubifex* sp. oligochaetes, which are intermediate hosts for the helminth.

Ichthyophthirius multifiliis is a cosmopolitan ciliate that parasitizes numerous fish species, including brown trout (Grigoryan and Poghosyan, 1983; Sigh and Buchmann, 2002; Hovhannisyanyan and Rukhkyan, 2012). In the current research, this ciliate was detected on the skin and gills of 4 trout (20%), comprising two males and two females aged 1 to 3 years that were grown in aquaculture.

In total, three of the detected parasites- *Bunodera luciopercae*, *Proteocephalus longicollis*, and *Khawia armeniaca* - were biohelminthes; three were ectoparasites, and three were endoparasites. Our observations indicate that ectoparasites are generally more common in brown trout grown in aquaculture, while endoparasites, especially biohelminthes, are generally typical of wild trout.

The rate of parasitic infection was the same in both wild and farmed fish, at 40%. The infection rate in females (50%) was higher than males (30%), however, the difference was not significant ($P > 0.05$). According to the results of research, the highest infection rate was registered in trout with an of age 3 years (62.5%), followed by trout aged 2 years (50.0%), and the lowest level was observed in fish aged 1 year (33.33%). Trout aged 4 years were free of parasites. However, the difference was not statistically significant ($P > 0.05$). The results obtained, in general, correspond to the concept that the level of parasite infection in fish, specifically rainbow trout, increases with age (Couso-Perez, 2023).

Parasite infections were generally characterized by low intensity, except for *I. multifiliis* infections which were characterized by high intensity, and pronounced clinical manifestation. A similar pattern is generally typical in aquaculture, where various ectoparasites infect fish more intensively than in the wild. This process process is often complicated by infection with *Saprolegnia* sp. and other parasitic oomycetes (Cieplinski *et al.*, 2018; Kaminskas, 2021; Buchmann, 2022). The tight stocking density of fish in ponds, as well as numerous stress factors, promotes the rapid development of secondary infections, especially mycoses (Kayis *et al.*, 2018; Casa-Mulet *et al.*, 2021). None of the detected pathogens are dangerous to human health; however, they can seriously affect the commercial condition, and as well as the sanitary and hygienic status, of the fish (Aloo, 2000; Tessema, 2020).

CONCLUSIONS

According to current research, 40% of the examined fish individuals were infected with parasites. Six species of parasites were identified: *Dactylogyrus vastator* Nybelin, 1924, *Gyrodactylus* sp., *Bunodera luciopercae* Müller, 1776 (Trematoda: Allocreadiidae), *Proteocephalus longicollis* Zeder, 1800, *Khawia armeniaca* Cholodkovsky, 1915, and *Ichthyophthirius multifiliis* Fouquet, 1876. Parasite infections were generally characterized by low intensity, except for *Ichthyophthirius multifiliis*.

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Notably, *Gyrodactylus* sp. and *Bunodera luciopercae* helminthes were detected for the first time in Armenia. The results indicate that the parasite fauna of fish parasites in the southern regions of Armenia is distinctive. This pilot study provides baseline information for further large-scale investigations and offers valuable insights for farmers to better understand the challenges associated with efficient fish production.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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دراسة اولية لطفيليات سمكة السلمون المرقط البني مع الأنواع المسجلة حديثاً في أرمينيا

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الخلاصة

تُعدّ سمكة الترويت البنية *Salmo trutta* Linnaeus, 1758 واحدة من الأنواع ذات الأهمية الاقتصادية في مجال مصايد الأسماك، نظرًا لانتشارها الواسع في أرمينيا، حيث توجد في بيئتها الطبيعية وتُربى أيضًا في نظم الاستزراع المائي. على الرغم من هذه الأهمية الواسعة والانتشار الجغرافي الملحوظ، تظل المعلومات المتاحة حول الأمراض والطفيليات التي تصيب الترويت البني في أرمينيا محدودة. هدفت هذه الدراسة إلى تحليل الفونة الطفيلية لسمكة الترويت البنية في المناطق الجنوبية من أرمينيا. وقد أُجريت الدراسة في منطقة فايوتس دزور، بالإضافة إلى المركز العلمي لعلم الحيوان والهيدرولوجيا، والجامعة الزراعية الوطنية الأرمينية. إذ خضعت الأسماك التي تم اصطيادها لفحص شامل باستخدام منهجية البحث الطفيلي الكاملة، وتم تحليل

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الطفيليات باستخدام المجهر و تشخيصها استنادًا إلى الأدلة المعيارية و الصفات المظهرية. خلال الفترة من سبتمبر إلى ديسمبر 2023، تم اصطياد 20 سمكة ترويت بنية، حيث أظهرت النتائج إصابة 8 منها (40%) بالطفيليات. وتم تحديد ستة أنواع من الطفيليات هي:

Dactylogyrus vastator Nybelin, 1924 (Monogenea: Dactylogyridae), *Gyrodactylus* sp. (Monogenea: Gyrodactylidae), *Bunodera luciopercae* Müller, 1776 (Trematoda: Allocreadiidae), *Proteocephalus longicollis* Zeder, 1800 (Cestoda: Proteocephalidae), *Khawia armeniaca* Cholodkovsky, 1915 (Cestoda: Lytocestidae), and *Ichthyophthirius multifiliis* Fouquet, 1876 (Ciliata: Ichthyophthiriidae).

عمومًا، كانت الإصابات الطفيلية منخفضة الشدة باستثناء عدوى *Ichthyophthirius multifiliis* التي تميزت بشدة أعلى. تُمثل هذه الدراسة الاستكشافية قاعدة بيانات أولية لمزيد من الأبحاث الواسعة النطاق، كما تزود المزارعين بمعرفة أكثر عمقًا بالتحديات المرتبطة بعمليات الإنتاج الفعّالة.