ACTA ZOOLOGICA BULGARICA

Acta Zool. Bulg., 76 (1): 129-134

Parasitology
Research Article

Published online 12 March 2024
DOI: https://doi.org/10.71424/azb76.1.002771

Infection of the Fish Species *Perca fluviatilis*, *Stizostedion lucioperca* and *Esox lucius* with *Eustrongylides excisus* Jägerskiöld, 1909 (Nematoda: Dioctophymatidae) in the Lower Section of the Dniprovske Reservoir: Site Preference and Pathogenicity

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Abstract: A helminthological survey of three fish species (*Perca fluviatilis, Stizostedion lucioperca* and *Esox lucius*) from the lower section of the Dniprovske Reservoir (also known as Zaporizhzhia) revealed the presence of the dioctophymatid nematode *Eustrongylides excisus* (third stage larvae). The study was conducted in 2020 and 2021. It was found that the total prevalence of infection was more than 40%. The nematode was found in the liver, abdomen and muscles. Significant tissue lesions were observed in some fish specimens. They were visually accompanied by mechanical damage, oedema and inflammation. The intensity of infection in *P. fluviatilis* and *S. lucioperca* was 14.18 ± 0.81 and 12.69 ± 0.73, respectively. In turn, the intensity of infection in *E. lucius* had the lowest value 2.40 ± 0.41. The abdomen and muscles of *P. fluviatilis* and *S. lucioperca* were affected more than the liver. No statistically significant differences were found between the intensity of infection of the abdomen and muscles in *P. fluviatilis*. Furthermore, the musculature of *S. lucioperca* was more heavily infected than the abdomen. In *E. lucius*, nematodes were found only in the abdominal cavity.

Key words: eustrongylidosis, abundance, infection intensity, predatory fish, prevalence, Ukraine.

Introduction

Fish is an important food product all over the world. In recent years, there has been a trend of increasing fish catch and consumption worldwide. For Ukraine and many countries of the world, the fish industry is an important commerce that contributes to the nutritional needs of the population (Burhaz & Soborova 2020, Korman, 2020). Unfortunately, the quality of fish products is affected by various diseases. The most common fish diseases worldwide are those caused by helminth parasites (Nguyen et al. 2021, Jyrwa et al. 2016). Such diseases are dangerous for many reasons. Helminths damage internal organs,

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lead to reduced immunity in fish and disrupt their metabolism (Souza et al. 2019). It is worth noting that a large number of fish helminth species can be pathogenic to humans (Adams et al. 1997).

Nematodes of the genus *Eustrongylides* Jägerskiöld, 1909 are typical parasites of a large number of fish species worldwide. Some species are predominantly associated with specific geographical regions (Honcharov et al. 2022). To date, there are known cases of *Eustrongylides* spp. infections of humans in the USA, Italy and South Sudan, which are a result of the consumption of raw or undercooked fish. They have inevitably been followed by medical intervention (Eberhard & Ruiz-Tiben 2014, Guardone et al. 2021).

Eustrongylides excisus Jägerskiöld, 1909 is a common parasite of fish, aquatic snakes and birds recorded in natural and artificial bodies of water in Europe, Asia and North and South America (Branciari et al. 2016, Lichtenfels & Stroup 1985, Melo et al. 2014, Novakov et al. 2013, Soylu 2013). *E. excisus* has a complex life cycle, comprising mainly aquatic and semiaquatic animals (Honcharov 2020). High infection rates of the nematode lead to severe diseases and increased mortality in the host animal populations (Oraić et al. 2023). Adult *Eustrongylides* are parasites in various wading birds, but the larvae are found in fish. Tissue lesions can occur when the parasite is ingested by humans. Therefore, high infection rates of this helminth species may affect the quality of fish products (Castiglione et al. 2023). Four cases of human infection have been reported among fishermen who consumed live bait minnows. In three of these cases, infection was associated with the consumption of sushi (Wittner et al. 1989, Narr et al. 1996). *Eustrongylides* are considered sporadic in humans but the infestation is possible. In this case, the human paratenic hosts required surgical intervention because worms penetrated the abdominal cavity (John & Petri 2006).

European perch *Perca fluviatilis* Linnaeus, 1758, pikeperch *Stizostedion lucioperca* (Linnaeus, 1758) and northern pike *Esox lucius* Linnaeus, 1758 are predatory fish species, serving as paratenic hosts for *E. excisus* larvae (Moravec 2013, Bjelić-Čabrilo et al. 2013, Dezfuli et al. 2015, Goncharov et al. 2018). It is worth noting that these fish species are common prey in recreational fishing and industrial catches at the Dniprovske Reservoir (Fedonenko et al. 2014, Kobyakov & Novitskyi 2022).

This study aimed to investigate the features of infection of *P. fluviatilis, S. lucioperca* and *E. lucius* with *E. excisus* (larvae) in the conditions of the lower section of the Dniprovske Reservoir (also known as Zaporizhzhia Reservoir) and to determine the difference of the site of infection in the different host species.

Materials and Methods

Fish sampling was conducted in the summer-autumn period of 2020 and 2021 during scheduled controlled fisheries. Fish were provided by local fishermen who legally caught fish in the water area of the lower section of the Dniprovske Reservoir, Dnipro River (Fig. 1). The boundaries of the lower section of the Dniprovske Reservoir are indicated according to Baranovsky (2000). A total of 146 adult specimens of three predatory fish species (*P. fluviatilis* – 81 specimens, *S. lucioperca* – 53 specimens and *E. lucius* – 12 specimens) were examined for the presence of nematodes.

The fish autopsy was conducted in a common way – the body cavity was dissected from the anus to the head. The organs removed from the body cavity were placed in Petri dishes with 0.9% saline. Some of the parasites were fixed in 70% ethyl alcohol. The detected nematode larvae were examined using a SIGETA MB-115 SE microscope. The identification of helminths was done according to Bauer (1987). All studies were performed in compliance with the requirements of the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes." (Strasbourg 1986) and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (Revision of 8 August 2021).

The infection characteristics, i.e. prevalence (%), the intensity of infection (range) and mean intensity of infection ± standard error (MI±SE) (see Table 1), were presented as suggested by Bush et al. (1997). The non-parametric Kruskal-Wallis (K-W) test was used to determine statistically significant differences, followed by Dunn's criterion. Statistically significant differences were accepted at $p < 0.05$. Statistical processing was performed using Origin Software, version 9.8 (Origin Lab Corp 2021).

Results

During the autopsy of fish, *E. excisus* larvae were found in the abdominal cavity of fish and on the organs' surfaces. We recorded both free and encapsulated nematodes (Fig. 1). Connective tissue capsules containing *E. excisus* larvae were found in the liver and muscles. The capsules were 5.0–5.5 mm in di-

Fig.1. Lower section of the Dniprovske Reservoir.

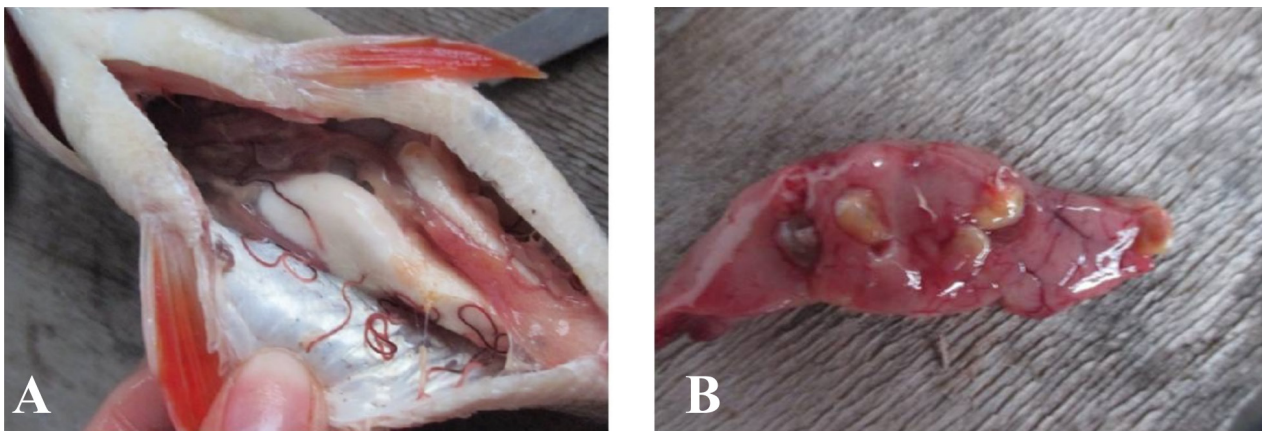

Fig. 2. Larvae of *Eustrongylides excisus* in the abdominal cavity (A) and on the liver (B) of *Perca fluviatilis.*

ameter and contained one larva each. The nematodes were twisted in a flat spiral forming a wide ring with a diameter of about 5–10 mm. The body length of the nematodes ranged from 20 to 35 mm. Ruptures, oedema and inflammation accompanied by significant tissue lesions were observed. Substantial liver lesions resulted in deformation of the liver surface. The affected livers were also characterised by a pale colour (Fig. 2).

The prevalence of *E. excisus* infection was more than 40%. Up to 22 larvae were observed in fish. The parasite studied was most frequent in *S. lucioperca* and least frequent in *E. lucius* (Table 1).

When comparing the intensity of infection, significant differences were found between *E. lucius* and the other two fish species (Dunn's test; $p < 0,05$). *P. fluviatilis* and *S. lucioperca* have similar intensity of infection values (Dunn's test; $p > 0,05$) (Table 1).

The present study showed that the parasite has preferable sites of infection in both *P. fluviatilis* and *S. lucioperca*. In *P. fluviatilis* these were the musculature and abdomen. No statistically significant differences in the intensities of infection between the two sites have been observed (Dunn's test; $p > 0,05$) (Fig. 3). The most preferable site of infection in *S. lucioperca* was the musculature. In both fishes, the least preferable site of infection was the liver (Fig. 3). In *E. lucius*, the nematode was found only in the abdomen (Fig. 3).

Table 1. Infection characteristics of *Eustrongylides excisus* in the fish species studied from the Dniprovske Reservoir. Legend: P — prevalence, %; MI — mean intensity of infestation; SE — standard error

Species	N	P %	Intensity	
			MI ± SE	Range
P. fluviatilis	81	45.60	14.18 ± 0.81[a]	2-22
S. lucioperca	53	49.06	12.69 ± 0.73[a]	3-19
E. lucius	12	41.70	2.40 ± 0.41[b]	1-3

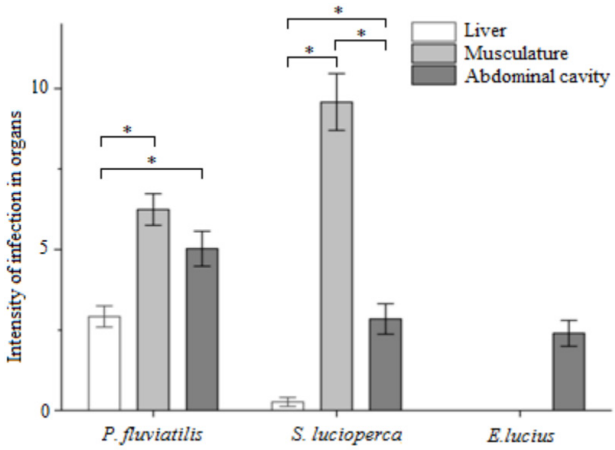

Fig. 3. Comparative analysis of the site preference of *Eustrongylides excisus* among the fish species examined.

Discussion

Nematodes of the genus *Eustrongylides* have a wide geographical distribution and complex life cycles (Anderson 2000, Honcharov et al. 2022). The definitive hosts are fish-eating birds, while aquatic oligochaetes and fish are the first and second intermediate hosts, respectively. Mortality due to infection has been reported in both fish and birds (Sutherland et al. 2018). *Eustrongylides* spp. are also of zoonotic importance (Eberhard & Ruiz-Tiben 2014), causing symptoms such as gastritis and intestinal perforation (Rusconi et al. 2022). The genus *Eustrongylides* belongs to the family Dioctophymatidae and has been reported in humans in Sudan as well as in the USA after consuming raw fish (Eiras et al. 2018). Current data indicate that the eustrongylidosis is common in populations of *P. fluviatilis*, *S. lucioperca* and *E. lucius* of the Ponto-Caspian and Mediterranean regions (Bjelić-Čabrilo et al. 2013, Moshu 2014, Ozmen et al. 2021, Pekmezci & Bolukbas 2021).

The species we studied are characterised by an average prevalence of infection with *E. excisus* larvae. Goncharov et al. (2018), when surveying predatory fish of the Dnipro-Bug Estuary, found that populations from different ecosystems had differences in terms of the prevalence and intensity of infection. It is worth noting that in all ecosystems, the percentage of affected fish was more than 40% and, in some sites, reached 100%. Therefore, in the Dniprovske Reservoir, the infection rates may have a different nature, which requires further research. According to Yesipova (2013), *E. excisus* has been recorded in fish in the Zaporizhzhia (Dniprovske) Reservoir since 2008. According to Yesipova's testimonies in recent years there has been a rapid increase in cases of infected commercial fish species in all parts of the reservoir. This is possibly due to the increase in the number of great cormorants, which are the definitive host of *E. excisus* (Bulakhov et al. 2008, Rusconi et al. 2022).

Perca fluviatilis and *Stizostedion lucioperca* had a significant number of nematodes in the muscles and abdomen, which caused mechanical damage and inflammatory processes. Such lesions in the fish tissues activate immune and repair processes (Dezfuli et al. 2015). *E. excisus* can also affect the gonads and walls of the gastrointestinal tract of the fish, which should be taken into account in further observations (Honcharov et al. 2022).

It should be noted that the dice snake *Natrix tessellata* (Laurenti, 1768) is a common aquatic reptile species in the Dniprovske Reservoir. This snake is also a reservoir host for the fourth-stage larvae of the nematode studied (Sharpilo 1976) that is due to its similar feeding habits to those of predatory fish. It is worth noting that dice snakes from the middle and lower sections of the Dniprovske River have a high infection rate (over 80 %) (Yermolenko et al. 2022). This may be due to the fact that in the ecosystems where *P. fluviatilis*, *S. lucioperca* and *E. lucius* were studied, they consume fewer benthic fish and more invertebrates (Didenko & Gurbyk 2011, 2012, 2016) than *N. tessellata* (Hutinec & Mebert 2011).

Conclusion

In the conditions of the lower section of the Dniprovske Reservoir, *P. fluviatilis*, *S. lucioperca* and *E. lucius* are typical reservoir hosts of the nema-

tode *E. excisus* larva. More than 40% of fish of each species were affected by this helminth. Biological abnormalities in the fish body occurred due to this infection. The parasite, being in the larval stage in the body of the fish, causes mechanical damage, as evidenced by tissue raptures, necrosis and inflammatory processes. It is worth noting that *E. excisus* also parasitizes humans. Thus, there is a need to monitor the infection of fish populations by this parasite. However, due to the current war in Ukraine, the necessary parasitological monitoring is quite difficult. In addition, the explosion of the Kakhovka HPP dam led to an ecological disaster, which could lead to significant changes in the structure of fish and parasite communities.

Acknowledgments: The research was a part of the project funded by the Ministry of Science and Education of Ukraine, state registration number 0124U000608.

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Received: 04.12.2023
Accepted: 07.03.2024