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TREMATODES OF *LYMNAEA STAGNALIS* (LINNAEUS, 1758) IN THE WATER BODIES OF LVIV AND ITS SURROUNDINGS (UKRAINE)

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Background. This publication highlights the results of studies on the trematode fauna of *Lymnaea stagnalis* (Linnaeus, 1758) in the water bodies of Lviv and its surroundings. It was established that *L. stagnalis* is an important component of local biodiversity and can serve as an indicator of environmental conditions. From a practical perspective, the research enables the detection of trematode species hazardous to humans and animals within the study area, the identification of local foci of trematodiasis, and provides data for ecological monitoring of aquatic ecosystems.

Materials and Methods. The study of the trematode fauna of *L. stagnalis* was conducted from June to August 2022 in five water bodies of Lviv and its surroundings, including Sand Lakes (Lviv), Black Lake (Sknyliv, Lviv District), the lake on Sadova Street (Sokilnyky, Pustomyty District), the pond on Krymska Street (Lviv), and the lake in the “Znesinnia” Park (Lviv). Molluscs were collected manually or using a dip net. During the study, 250 specimens of *L. stagnalis* were examined, and 750 temporary preparations of parthenitae were prepared for species identification. The intensity of invasion was determined during the helminthological dissection of molluscs. Statistical data analysis was performed using Statistica 8.0 and Excel software.

Results. The trematode fauna of *L. stagnalis* in water bodies within the city and suburbs of Lviv, characterized by partial anthropogenic impact, abundant macrophytes, and favorable conditions for mollusc development, was studied. In the Sand Lakes, larvae of 10 trematode species were identified, 10 species in Black Lake, 8 in the lake on Sadova Street, 10 in the pond on Krymska Street, and 6 species in the lake of “Znesinnia” Park. The identified digeneans belonged to 8 families: Echinostomatidae, Notocotylidae, Plagiorchiidae, Strigeidae, Diplostomidae, Haematoloechidae, Schistosomatidae, and Telorchiidae.

Conclusions. The species composition of the trematode fauna of *L. stagnalis* in the water bodies of Lviv and its surroundings comprises 14 species. The family Echinostomatidae exhibited the highest species richness. The most frequently encountered species were *Notocotylus attenuatus* (Rudolphi, 1809) and *Trichobilharzia ocellata* (La Valette, 1855). The prevalence of *L. stagnalis* infestation in the studied water bodies ranged from 42 % to 36 %, with the highest rates observed in the lake on Sadova Street (Sokilnyky) and the lowest in the Sand Lakes of Lviv. The presence of molluscs, waterfowl, fish, and macrophytes in the water bodies creates conditions for trematode circulation.

Keywords: trematode fauna, *Lymnaea stagnalis*, infestation extent, infestation intensity, cercaria, parthenitae

INTRODUCTION

Molluscs are an important group of animals inhabiting various aquatic environments. Freshwater molluscs play a significant role in biocenotic relationships, contributing to water purification as biotic filters, and serving as indicator species for assessing the degree of anthropogenic impact on biotopes. They are also typical obligate hosts for the larval stages of trematodes.

Parasitic worms are another critical component of the biosphere. Despite their small size, they constitute a significant portion of the biomass in ecosystems, thereby contributing substantially to energy flows within these systems. Contrary to their often-predicted negative effects, parasites can perform essential functions in ecosystems, such as concentrating and removing pollutants from the environment (Selbach *et al*., 2020). They can also serve as bioindicators for evaluating environmental conditions and changes due to their complex life cycles and interactions within ecosystems (Stadnychenko, 1998).

In Ukraine, a significant body of research has been dedicated to adult stages (maritae) of trematodes, but studies on the larval stages of these helminths parasitizing molluscs are far less common (Zhytova & Zhytov, 2016). Identifying the species composition of parthenitae and larval trematodes in *Lymnaea stagnalis* (Linnaeus, 1758) reliably reflects the parasite diversity in the studied region. This is a practical method for assessing sanitary-epidemiological situations, as the presence of schistosomatid cercariae indicates the potential risk of human cercarial dermatitis. Human infections by schistosomatid cercariae have been reported in many European countries, including Ukraine (Stadnychenko, 2002). Such cases have been observed particularly in Lake Svityaz and Lake Pisochne of the Shatsk National Nature Park, which are extensively used for recreational purposes (Koltun *et al*., 2020).

Data obtained from studying *L. stagnalis* for trematode infections provide insight into the level of biological hazards associated with aquatic bodies of various uses and allow for the identification of existing foci of trematodiasis. This information can guide timely measures to reduce their activity.

To understand the life cycles of trematodes, it is essential to study their distribution and the environmental factors affecting them, including their impact on the host organisms. Therefore, identifying the species composition of trematodes in freshwater molluscs across different biotopes is crucial for effectively regulating their abundance and distribution.

The great pond snail (*L. stagnalis*) is one of the most common representatives of freshwater molluscs in Europe and is a good model object for malacological, parasitological, hydrobiological, and ecological studies (Kuroda & Abe, 2020).

The aim of this work was to study the trematode fauna of *L. stagnalis* in some water bodies of the city and suburbs of Lviv.

MATERIALS AND METHODS

The study of the trematode fauna of the great pond snail was conducted in five water bodies in and around the city of Lviv from June to August 2022 (**Fig. 1**):

The Sand Lakes (49°49′23.9″N 23°59′55.4″E) – are two water bodies located near Hordynskykh Street in the Frankivskyi district of Lviv. The total surface area of the lakes is 1.8 hectares. The shoreline is overgrown with macrophytes.

Black Lake or Aviation Lake (49°48′29.2″N 23°57′15.0″E) is located in the village of Sknyliv, Lviv District, of Lviv Region near Lviv Danylo Halytskyi International Airport. The lake is fed by the Vodianyi stream. Parts of the lake are covered with aquatic plants. Grazing of cattle and domestic waterfowl occurs in the vicinity of the lake.

The Lake on Sadova Street (49°46′46.6″N 23°57′49.7″E) is located south of the ring road in the village of Sokilnyky, Pustomyty District, Lviv Region. Parts of the shoreline are covered with plants. Domestic waterfowl and cattle are grazed near the lake.

The Pond on Krymska Street (49°49′18.7″N 24°02′56.0″E) is a water body in the Stryiskyi Park area of the Halytskyi district of Lviv, in the locality of Snopkiv. Together with two other water bodies, it forms part of the Snopkiv stream hydrosystem, which flows into the Zalizna Voda stream. The pond is small and shallow, surrounded by deciduous trees providing shade. Many macrophytes were observed along the shoreline.

The Lake in the Regional Landscape Park "Znesinnia" (49°50′54.0″N 24°03′07.8″E) is located in the Lychakivskyi district of Lviv, on a wooded hill of the Lviv Plateau in an area known as Kaiserwald, in the western part of the park. The shoreline is overgrown with aquatic plants.

A total of 250 specimens of *L. stagnalis* were studied from June to August 2022.

Molluscs were collected manually or with a dip net (15 cm in diameter) in shallow waters (0.5–1 m deep) from the surfaces of aquatic plants, and from objects such as stones and branches where they were found. The collected molluscs were placed in polyethylene bags or plastic containers with lids and transported carefully to avoid overheating. Mollusc density in the biotopes was determined using the transect method with a unit area of 1 m².

The species identification of molluscs was conducted according to standard conchological methods (Glöer & Meier-Brook, 1998; Gural & Gural-Sverlova, 2024; Jackiewicz, 1998).

To examine molluscs for trematode infections, two methods were applied: the method of live diagnosis and the dissection method.

Trematode larvae are localized in the hepatopancreas of freshwater molluscs (Nevidomska *et al.*, 2007). Infected organs, primarily the hepatopancreas, show enlargement (Zhytova *et al.*, 2021). The color of the hepatopancreas changes from the natural dark brown to white with red-brown hues, depending on the intensity of infection and the developmental stage of the larvae. At advanced stages of parasitism, the hepatopancreas becomes soft and granular. At low infection intensities or early developmental stages, the hepatopancreas of infected molluscs does not differ in color or consistency from that of healthy specimens.

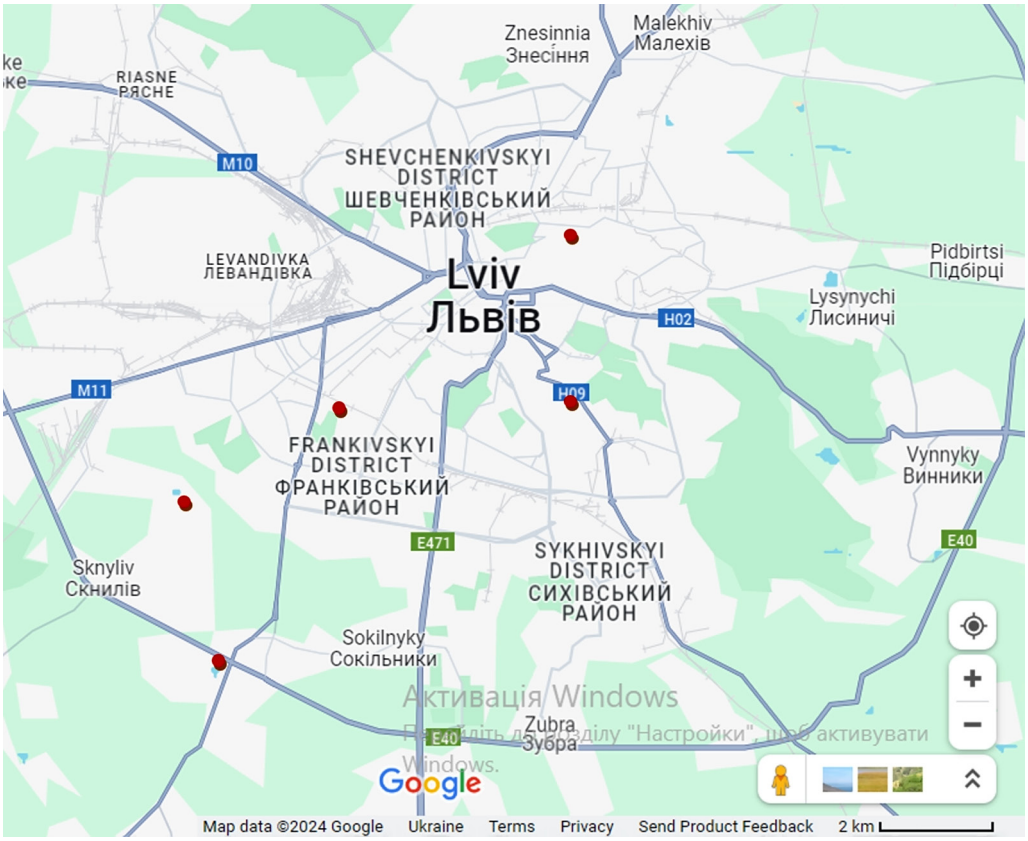

Fig. 1. Water bodies studied in the city of Lviv and its surroundings

Note: * – water bodies where *L. stagnalis* were collected for parasitological analysis

For parasitological studies, microscopes (PB-3320, Gemmy Industrial Corp. associated with Cannic, Inc., U.S.A., Taiwan) were used. Vital stains, such as methylene blue and neutral red, were applied.

Cercariae were identified based on morphological characteristics following established methodologies (Faltynková *et al*., 2007; Zhytova & Zhytov, 2016; Pantoja *et al.*, 2021; Zhytova, 2023).

Intensity and Prevalence of Infection. The intensity of infection is defined as the total number of parthenitae per infected mollusc (Bush *et al*., 1997).

The prevalence of infection (PI) is a measure that reflects the proportion of infected individuals in the sample, expressed as a percentage.

$$\mathrm{PI} = \frac{n}{N} \cdot 100\ \%,$$

where: PI – prevalence of infection; n – number of hosts infected with parasites; N – total number of hosts examined (sample size).

Statistical Analysis. Data analysis was conducted using STATISTICA 8.0 and Excel software. Significant differences between absolute infection frequencies (binary traits) were determined using the Chi-square (χ^2) test. In cases where the absolute

frequency values among comparison groups were less than 10, the Yates-corrected Chi-square test was applied. Results were considered statistically significant if the probability value (p) was less than 5 % (p <0.05).

Confidence intervals for relative frequencies were calculated using the formula:

$$P \pm t \cdot \left(\sqrt{\frac{P(1-P)}{N}} + \frac{1}{2N} \right),$$

where: P – relative frequency of the event (e.g., infection or carrying a specific trematode species), expressed as a decimal fraction; N – total number objects in the sample; t – t-value (for calculating 95 % confidence intervals, approximated as 1.96); $\frac{1}{2N}$ – continuity correction to compensate for error when approximating the binomial distribution with the normal distribution (Altman *et al.*, 2000).

Results are presented as absolute frequencies or relative frequencies with ± 95 % confidence intervals.

RESULTS AND DISCUSSION

The study of *L. stagnalis* in the water bodies of Lviv and its surroundings revealed 14 species of trematodes belonging to 8 families.

All surveyed water bodies are located in Lviv and its suburbs, which experience varying degrees of anthropogenic impact. These water bodies are often overgrown with macrophytes and serve as favorable biotopes for the development of *L. stagnalis*. The presence of molluscs, along with definitive hosts of trematodes – waterfowl, fish, and amphibians – creates ideal conditions for the development and reproduction of these parasites.

The Sand Lakes. In the *L. stagnalis* population (50 specimens), 7 trematode species were identified: *Paryphostomum radiatum* (Dujardin, 1845), *Notocotylus attenuatus* (Rudolphi, 1809), *Opisthioglyphe ranae* (Fröhlich, 1791), *Haematoloechus similis* (Looss, 1899), *Trichobilharzia ocellata* (La Valette, 1855), *Plagiorchis elegans* (Rudolphi, 1802), and *Diplostomum spathaceum* (Rudolphi, 1819).

Black Lake (Aviation Lake). In the examined molluscs (50 specimens), larvae of 10 trematode species were found: *P. radiatum*, *Echinostoma revolutum* (Fröhlich, 1802), *N. attenuatus*, *O. ranae*, *H. similis*, *T. ocellata*, *Hypoderaeum conoideum* (Bloch, 1782), *Moliniella anceps* (Molin, 1859), *Echinoparyphium recurvatum* (von Linstow, 1873), and *P. elegans*.

The Lake on Sadova Street. Cercariae and metacercariae of 8 trematode species were detected in *L. stagnalis* (50 specimens): *E. revolutum*, *N. attenuatus*, *O. ranae*, *H. similis*, *H. conoideum*, *M. anceps*, *Echinoparyphium aconiatum* (Dietz, 1909), and *Echinoparyphium cinctum* (Rudolphi, 1802).

The Pond on Krymska Street. The parasitic fauna of *L. stagnalis* (50 specimens) included larvae of 10 trematode species: *P. radiatum*, *E. revolutum*, *N. attenuatus*, *O. ranae*, *T. ocellata*, *H. conoideum*, *M. anceps*, *P. elegans*, *Cotylurus cornutus* (Rudolphi, 1808), and *D. spathaceum*.

The Lake in the Regional Landscape Park "Znesinnia". The trematode species composition in *L. stagnalis* (50 specimens) consisted of 6 species: *P. radiatum*, *N. attenuatus*, *T. ocellata*, *E. aconiatum*, *E. recurvatum*, and *C. cornutus* (see **Table**).

Species Composition of Trematode Larvae in *L. stagnalis*

Species	Sand Lakes	Black Lake	Lake on Sadova Street	Pond on Krymska Street	Lake in the park "Znesinnia"
Echinostoma revolutum (Fröhlich, 1802)	-	+	+	+	-
Echinoparyphium cinctum (Rudolphi, 1802)	-	-	+	-	-
Echinoparyphium recurvatum (von Linstow, 1873)	-	+	-	-	+
Echinoparyphium aconiatum (Dietz, 1909)	-	-	+	-	+
Moliniella anceps (Molin, 1859)	-	+	+	+	-
Hypoderaeum conoideum (Bloch, 1782)	-	+	+	+	-
Paryphostomum radiatum (Dujardin, 1845)	+	+	-	+	+
Notocotylus attenuatus (Rudolphi, 1809)	+	+	+	+	+
Plagiorchis elegans (Rudolphi, 1802)	+	+	-	+	-
Opisthioglyphe ranae (Fröhlich, 1791)	+	+	+	+	-
Haematoloechus similis (Looss, 1899)	+	+	+	-	-
Trichobilharzia ocellata (La Valette, 1855)	+	+	-	+	+
Cotylurus cornutus (Rudolphi, 1808)	-	-	-	+	+
Diplostomum spathaceum (Rudolphi, 1819)	+	-	-	+	-

The analysis revealed that the common trematode species present in all the surveyed water bodies was *N. attenuatus*. Most water bodies also contained *P. radiatum*, *O. ranae*, and *T. ocellata*. Larvae of *E. cinctum* were found exclusively in molluscs from the lake on Sadova Street. The richest trematode species composition was observed in Black Lake and the pond on Krymska Street, with 10 species each. The lake in the Znesinnia Park exhibited the lowest species richness (6 species), though it included several species unique to one or two water bodies, such as *E. recurvatum*, *E. aconiatum*, and *C. cornutus*.

Thus, in *L. stagnalis*, we identified parthenitae and cercariae of 14 trematode species belonging to 8 families (**Fig. 2**). The investigated specimens contained larvae of digenean trematodes, including 7 species from the family Echinostomatidae (50.00 %): *E. revolutum*, *M. anceps*, *H. conoideum*, *P. radiatum*, *E. recurvatum*, *E. aconiatum*, *E. cinctum*. One species from the family Notocotylidae (7.14 %) – *N. attenuatus*; Plagiorchiidae (7.14 %) – *P. elegans*; Telorchiidae (7.14 %) – *O. ranae*; Strigeidae (7.14 %) – *C. cornutus*; Diplostomidae (7.14 %) – *D. spathaceum*; as well as Haematoloechidae (7.14 %) and Schistosomatidae (7.14 %), represented by larvae of *H. similis* and *T. ocellata*, respectively.

The highest occurrence frequency was recorded for *N. attenuatus*, found in 18 specimens of *L. stagnalis* (17.8 %), and for *T. ocellata*, detected in 10 specimens (9.9 %). The lowest occurrence frequency was observed for cercariae of *E. cinctum*, which infected only one mollusc (1 %) (**Fig. 3**).

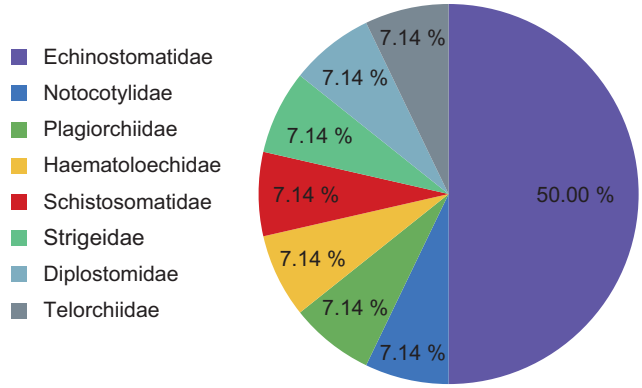

Fig. 2. Proportional representation of families based on the number of identified species

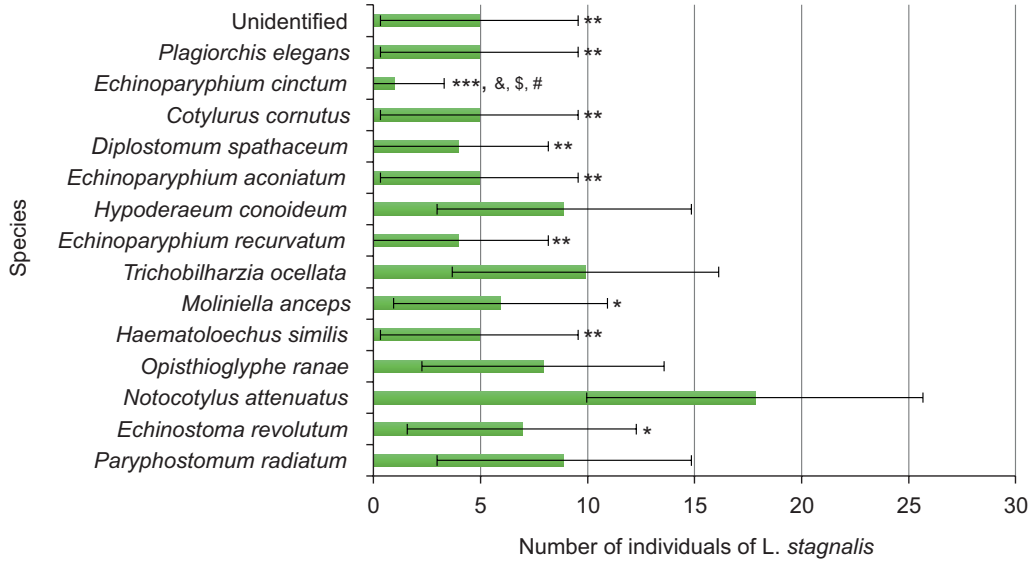

Fig. 3. Frequencies (and confidence interval ranges) of trematode parthenitae occurrence in the examined *L. stagnalis*

Notes: * – p <0.05; ** – p <0.01; *** – p <0.001 – significant compared to *N. attenuatus*; & – p <0.05 – significant compared to *T. ocellata*; $ – p <0.05 – significant compared to *P. radiatum* and *H. conoideum*; # – p <0.05 – significant compared to *O. ranae*

It is worth noting that the presence of *T. ocellata* in the studied water bodies may pose a risk to humans, as it causes cercarial dermatitis. Additionally, *N. attenuatus* is a pathogen responsible for a severe disease in waterfowl, notocotylidosis (**Fig. 4**).

Prevalence and Intensity of Infection. A comparison of the prevalence of infection (PI) in molluscs across the studied water bodies revealed that the maximum PI was 42 % ± 13.6 % (Lake on Sadova Street), while the minimum was 36 % ± 14.0 % (the Sand Lakes). However, these differences were not statistically significant (**Fig. 5**).

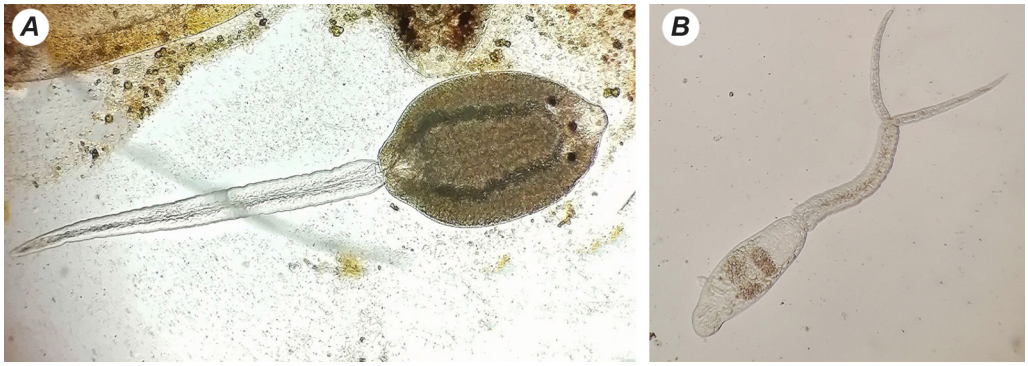

Fig. 4. ***A*** – *Notocotylus attenuatus;* ***B*** – *Trichobilharzia ocellata* (magnification: ×280; photo by: A. Serbina)

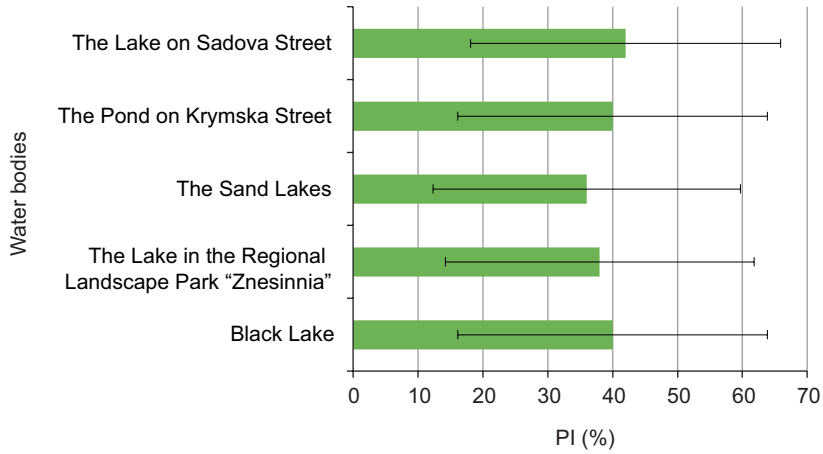

Fig. 5. Comparison of infection prevalence of *L. stagnalis* by trematode parthenitae and larvae, along with their confidence intervals

Analyzing the average values of trematode sporocyst infection intensity in *L. stagnalis* across the studied water bodies, the highest infection level was observed in the pond on Krymska Street. This can be attributed to the high density of mollusc populations, favorable environmental conditions, and the presence of definitive hosts that facilitate parasite circulation. The lowest infection level was recorded in Black Lake, which may indicate the presence of certain ecological or biological barriers limiting the development of trematode hosts. In Znesinnia Lake and the Sand Lakes, the infection intensity by parthenitae was relatively similar, suggesting comparable environmental conditions in these water bodies.

A comparison of these findings with studies conducted by other Ukrainian researchers (Zdun, 1962; Astakhova, 2002; Hural & Yavorski, 2004; Zhytova, 2011; Stadnychenko, 2014; Astakhova & Muzh, 2016; Zhytova, 2023) demonstrates similar patterns in trematode distribution. The widespread occurrence of species from the family Echinostomatidae in various Ukrainian water bodies, especially those with significant diversity of waterfowl, aligns with our results. When compared to studies of trematodes in the Polissia region, we find overlapping species, such as *H. conoideum*, *O. ranae*, and *C. cornutus*.

This suggests that both regions share a similar composition of definitive hosts for these trematodes. However, certain species, such as *P. radiatum*, *N. attenuatus*, and *T. ocellata*, are more prevalent in the water bodies of Lviv and are less common in Polissia.

CONCLUSIONS

The species composition of trematodes in *Lymnaea stagnalis* from the studied water bodies in Lviv and its surroundings comprises 14 species: *Moliniella anceps*, *Trichobilharzia ocellata*, *Haematoloechus similis*, *Opisthioglyphe ranae*, *Notocotylus attenuatus*, *Paryphostomum radiatum*, *Hypoderaeum conoideum*, *Echinostoma revolutum*, *Echinoparyphium recurvatum*, *Echinoparyphium aconiatum*, *Plagiorchis elegans*, *Cotylurus cornutus*, *Diplostomum spathaceum*, and *Echinoparyphium cinctum*. The family Echinostomatidae exhibited the highest species richness, with 7 species.

In the surveyed water bodies, *L. stagnalis* was most frequently infected by parthenitae and cercariae of trematodes whose maritae parasitize birds. These include *M. anceps*, *T. ocellata*, *N. attenuatus*, *P. radiatum*, *H. conoideum*, *E. revolutum*, *E. recurvatum*, *E. aconiatum*, *C. cornutus*, *D. spathaceum*, and *E. cinctum*. Larvae of trematodes whose definitive hosts are amphibians, fish, or mammals (*H. similis*, *O. ranae*, *P. elegans*) were recorded much less frequently. The highest occurrence frequencies were recorded for *N. attenuatus* (17.8 %) and *T. ocellata* (9.9 %). *E. cinctum* was found in a single instance (1 %).

The prevalence of infection in the studied water bodies ranged from 42 % to 36 %. Among all the water bodies examined, *L. stagnalis* from the lake on Sadova Street in Sokilnyky village showed the highest infection prevalence, while those from the Sand Lakes in Lviv exhibited the lowest prevalence.

The average intensity of trematode infection in *L. stagnalis* across all studied water bodies indicates moderate to high levels of infection, likely due to favorable conditions for trematode circulation.

In the analyzed molluscs, the human-pathogenic schistosome *T. ocellata*, which causes cercarial dermatitis, was detected in all water bodies except the lake on Sadova Street. Additionally, *N. attenuatus*, the causative agent of notocotylidosis in waterfowl, was present in all studied water bodies.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

Animal studies: all international, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [I.K.; I.Kh.]; methodology, [A.S.; I.K.]; validation, [I.Kh.]; formal analysis, [A.S.; I.K.]; investigation, [A.S.]; resources, [A.S.; I.K.; I.Kh.]; data curation, [I.K.]; writing – original draft preparation, [A.S.; I.K.]; writing – review and editing, [I.K.; I.Kh.]; visualization, [A.S.; I.K.]; supervision, [I.Kh.].

All authors have read and agreed to the published version of the manuscript.

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ТРЕМАТОДИ *LYMNAEA STAGNALIS* (LINNAEUS, 1758) У ВОДОЙМАХ ЛЬВОВА ТА ЙОГО ОКОЛИЦЬ (УКРАЇНА)

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Обґрунтування. У публікації висвітлено результати досліджень трематодофауни *Lymnaea stagnalis* (Linnaeus, 1758) у водоймах Львова та його передмістя. Встановлено, що *L. stagnalis* є важливим компонентом місцевого видового багатства та може слугуватити індикатором стану довкілля. З практичної точки зору дослідження дають змогу реєструвати на дослідженій території небезпечні для людини і тварин види трематод, виявляти локальні осередки трематодозів, а також здійснювати екологічний моніторинг водних екосистем.

Матеріали і методи. Дослідження трематодофауни *L. stagnalis* виконували протягом червня – серпня 2022 року у п'яти водоймах Львова та його околиць, зокрема, у Піскових озерах (м. Львів), озері Чорному (с. Скнилів Львівського району), озері на вул. Садовій (с. Сокільники Пустомитівського району), ставу на вул. Кримській (м. Львів) та озері парку "Знесіння" (м. Львів). Збирали молюски вручну або за допомогою сачка. За період дослідження обстежено 250 екземплярів *L. stagnalis*. Виготовлено 750 тимчасових препаратів партеніт для визначення їхньої видової приналежності. Визначення інтенсивності інвазії здійснювали під час гельмінтологічного розтину молюсків. Статистичний аналіз даних проводили за допомогою програм Statistica 8.0 та Excel.

Результати. У місті й передмісті Львова досліджували трематодофауну *L. stagnalis* у водоймах, що частково зазнають антропогенного впливу, багаті на макрофіти та є сприятливими для розвитку молюсків. У Піскових озерах виявили личинки 10 видів трематод, 10 видів у Чорному озері, 8 – у озері на вул. Садовій, 10 – у ставку на вул. Кримській та 6 видів у парку "Знесіння". Під час аналізу спостерігали дигеней із 8 родин: Echinostomatidae, Notocotylidae, Plagiorchiidae, Strigeidae, Diplostomidae, Haematoloechidae, Shistosomatidae, Telorchiidae.

Висновки. Видовий склад трематодофауни *L. stagnalis* у водоймах міста Львова і його околиць налічує 14 видів. Найбільшим видовим багатством характеризувалася родина Echinostomatidae. Найбільшу частоту трапляння виявили у *Notocotylus attenuatus* (Rudolphi, 1809) і *Trichobilharzia ocellata* (La Valette, 1855). Екстенсивність інвазії *L. stagnalis* у досліджених водоймах коливалася від 42 % до 36 %, з найвищими показниками в озері на вул. Садовій (с. Сокільники) та найнижчими у Піскових озерах м. Львова. Наявність молюсків, водоплавних птахів, риб і макрофітів у водоймах створює умови для циркуляції трематод.

Ключові слова: трематодофауна, *Lymnaea stagnalis*, екстенсивність ураження, інтенсивність ураження, церкарія, партеніти

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