PARASITES FOUND IN FISH OF THE PERCIDAE FAMILY FROM THE LAKES ON THE COURSE OF THE PREAJBA VALLEY RIVER, DOLJ, ROMANIA

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Abstract. The research carried out on the ichthyoparasitofauna of fish from the Percidae family (perch - *Perca fluviatilis*, walleye - *Sander lucioperca*, bream - *Gymnocephalus cernuus*), taken from the ten dam lakes on the course of the Preajba Valley river, lakes that are part of the *Preajba-Făcăi Lake Complex* site revealed the following aspects: 6 species of parasites were identified: 1 trematode (*Clinostomum complanatum*), 2 cestodes (*Diphyllobothrium latum, Triaenophorus nodulosus*), 1 nematode (*Eusstrongylides excisus*) and 2 crustaceans (*Lernaea cyprinacea, Argulus foliaceus*), in two of the three host species studied. Most species of parasites (6) were identified in *Perca fluviatilis* (perch): *Clinostomum complanatum, Diphyllobothrium latum, Triaenophorus nodulosus, Eustrongylides excisus, Lernaea cyprinacea, Argulus foliaceus* in *Sander lucioperca* (saddle), 1 parasite (*Eustrongylides excisus*) and *Gymnocephalus cernuus* (ghibort) in which no parasites were identified. The clinical signs of the disease were highlighted in the case of lerneosis and argulosis in perch by the presence of hemorhagic wounds and obvious ulcerations, as well as parasites.

Keywords: ichthyoparasitofauna, Percidae, Preajba Valley.

Rezumat. Paraziți întâlniți la pești din familia Percidae din lacurile de pe cursul râului Valea Preajba, Dolj, România. Cercetările efectuate asupra ihtioparazitofaunei la pești din familia Percidae (biban - *Perca fluviatilis*, șalău - *Sander lucioperca*, ghiborț - *Gymnocephalus cernuus*), prelevaţi din cele zece lacuri de baraj de pe cursul râului Valea Preajba, lacuri care fac parte din situl *Complex Lacustru Preajba-Făcăi* au relevat următoarele aspecte: au fost identificate 6 specii de paraziți: 1 trematod (*Clinostomum complanatum*), 2 cestode (*Diphyllobothrium latum, Triaenophorus nodulosus*), 1 nematod (*Eustrongylides excisus*) și 2 crustacee (*Lernaea cyprinacea, Argulus foliaceus*), la două din cele trei specii gazdă luate în studiu. Cele mai multe specii de paraziţi (6) au fost identificate la *Perca fluviatilis* (biban): *Clinostomum complanatum, Diphyllobothrium latum, Triaenophorus nodulosus, Eustrongylides excisus, Lernaea cyprinacea, Argulus foliaceus* la *Sander lucioperca* (șalău) s-a constatat prezența unui singur parazit (*Eustrongylides excisus*) iar la *Gymnocephalus cernuus* (ghiborț) nu au fost identificaţi paraziţi. Semnele clinice de boală au fost puse în evidenţă în cazul lerneozei și argulozei la biban prin prezența paraziților a rănilor hemoragice şi ulceraţiilor evidente.

Cuvinte cheie: ihtioparazitofaună, Percidae, Valea Preajba.

INTRODUCTION

In Romania, the parasitic fauna of fish was often limited to the level of biological studies, with a signaling character, both in artificial growth systems and in natural conditions. These studies are more frequent in the Danube Delta (ROMAN, 1956; OŢEL & CONSTANTIN, 1989) and much rarer in other regions (MIHALCĂ et al., 2003). The parasitism encountered in fish from the Preajba Valley lacustrine ecosystem is like an alarm signal due to the fish mortality that makes seasonal sport fishing difficult. The purpose of this work is to inventory the parasitic fauna of (3) species of fish from the Percidae family, species present in small lakes resulting from damming the course of the Preajba Valley river, with the revelation of some aspects of pathogenicity.

MATERIAL AND METHODS

832 fish from the ten lakes were examined (Fig. 1), of which 421 belong to the Percidae family with three species: perch (*Perca fluviatilis*), saddle (*Sander lucioperca*), walleye (*Gymnocephalus cernuus*).

The collection of ichthyological material started in 2008, seasonally for each individual lake, except for periods with unfavourable weather (late autumn, winter). In the absence of electronarcosis equipment, the sampling was done with various monofilament nets but also by fishing with fishing rods by amateur fishermen in the area. The harvesting stations of the fish material along the river were established starting from the upstream towards the downstream area, the three species of perch being taken from different basins (Figs. 2, 3, 4).

The collected fish were transported with the help of plastic containers with water in a live state, and the dead ones were transported in a refrigerated state. The examination of the fish was carried out in the parasitology laboratory of the Dolj Veterinary Health and Food Safety Directorate.

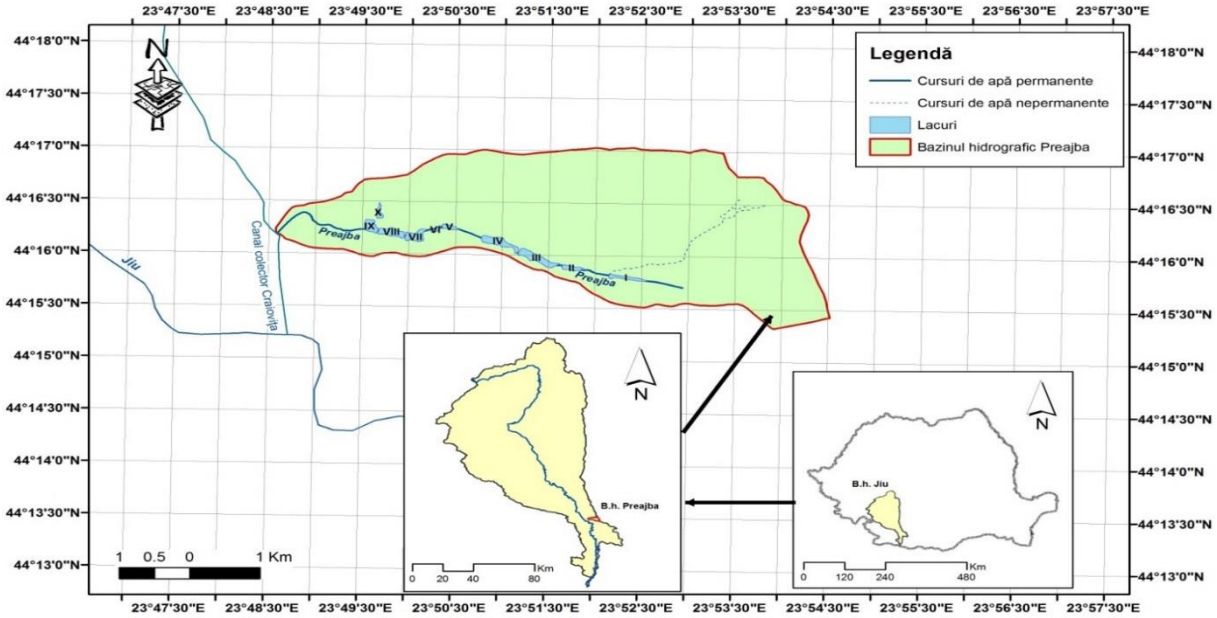


Figure 1. Framing of the Preajba hydrographic basin at the national and regional level (GIS processing after orthophotos, 2009).

Figure 2. Distribution of *Perca fluviatilis* species in the lake complex (original).

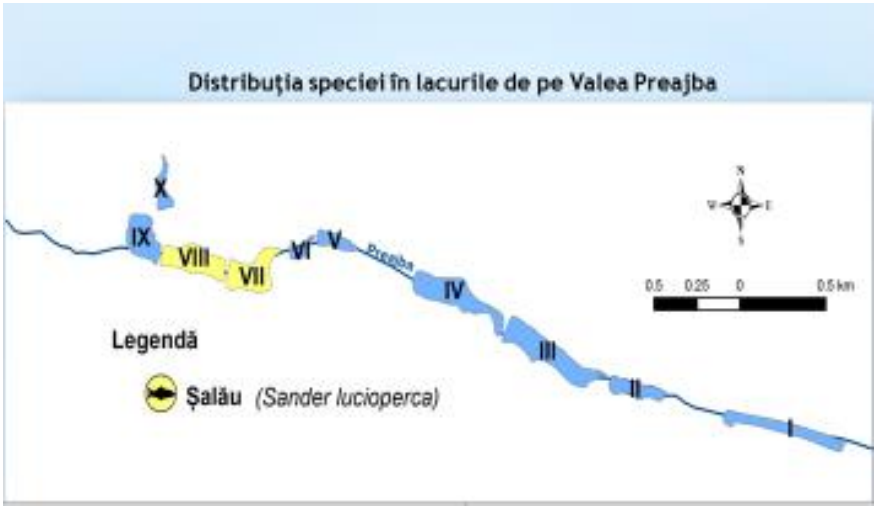


Figure 3. Distribution of *Sander lucioperca* species in the lake complex (original).

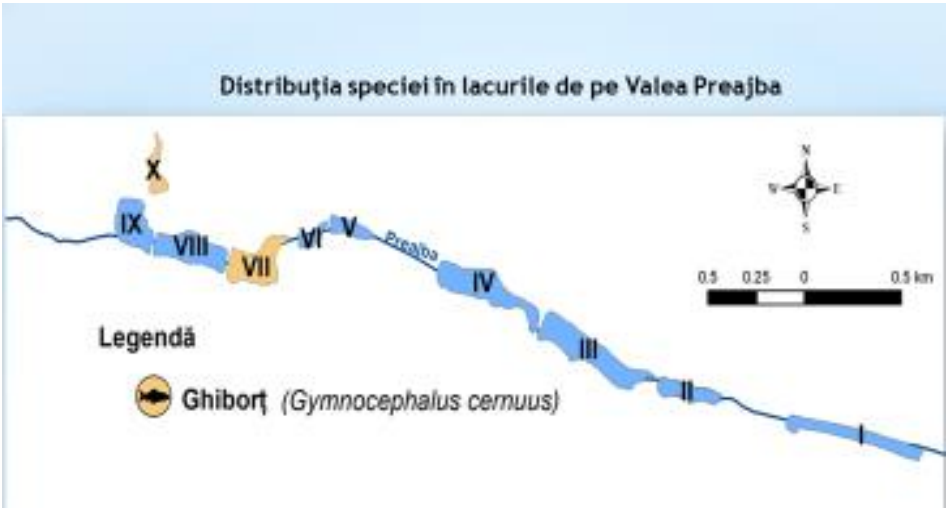


Figure 4. Distribution of *Gymnocephalus cernuus* species in the lake complex (original).

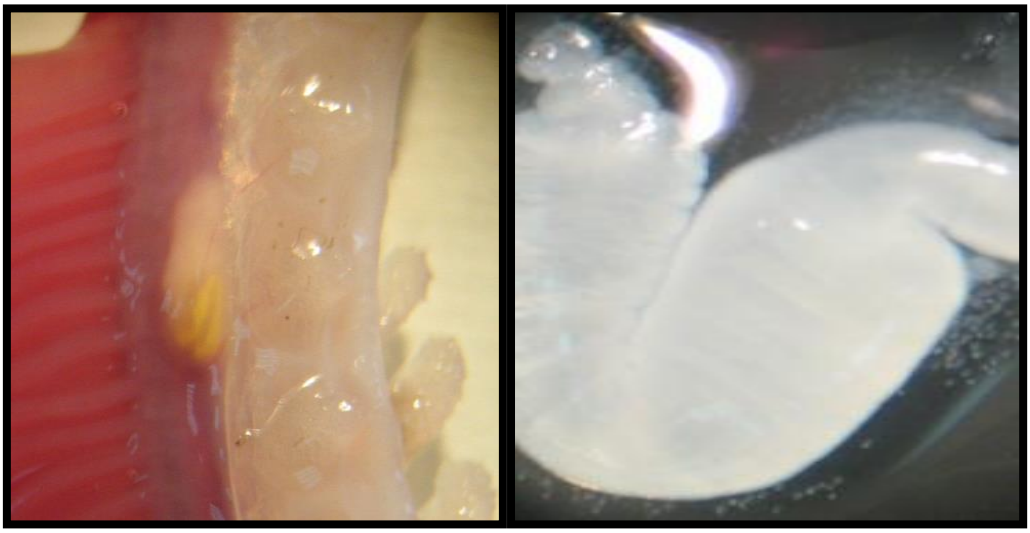
The macroscopic examination was done by observing the entire surface of the body, eyes and gills, to highlight external parasites, the integrity of the skin and skin lesions (cysts, ulcers, destroyed fins, lack of scales, etc.), and even the presence of parasites (*Lernaea cyprinacea*, *Argulus foliaceus*). With the help of scissors and a scalpel, the fish was opened on the abdomen, the intestine was sectioned along its entire length, examining the contents and appearance of the mucosa. Several cuts were also applied in the thickness of the muscles and internal organs, in order to detect areas with changes, such as: necrosis, cysts, hypertrophies or atrophies of the organs, colour changes or macroscopic parasites. By taking small amounts from the stomach and intestinal contents as well as from the gill scraping, native slide - slide preparations were made which were examined microscopically. Trematodes, nematodes and crustaceans were fixed in 70^0 alcohol.

RESULTS AND DISCUSSION

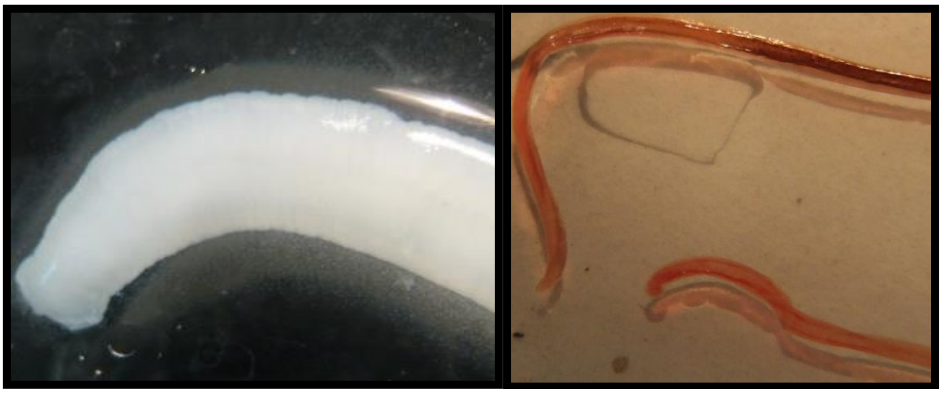
Observations and ichthyological items collected since 2008 have highlighted the fact that, due to the anthropogenic impact and the intense phenomenon of eutrophication, the forms of parasitism on fish populations are increasingly evident. In the collected percids, in the 20 catches, 6 species of parasites were highlighted (Figs. 5. A, B, C, D, E, F).

Infection with the trematode *Clinostomum complanatum* (Rudolphi, 1819) was identified in the summer of 2010 in ten specimens of perch (*Perca fluviatilis* LINNAEUS, 1758), (7♂ and 3♀), weighing between 91-422g, caught with the monofilament net in the lakes (lake VII and VIII). Each examined sample had at least six cysts. Macroscopic examination revealed the attachment sites of the parasite. The metacercaria rolled inside some ellipsoidal yellow-white cysts measured approx. 2 mm long were detected in the upper angle and the inner face of the operculum, gill arches and gill lamellae. This shows the parasite's preference for well-vascularized areas with intense activity. Fixation of the parasite as a fresh slide-slide preparation allowed examination under the optical microscope (Fig. 5A) (ob. 20x, eyepiece 10x), with the metacercaria being placed in a drop of water and visualized through transparency under a stereomicroscope. When determining the trematode, the place of parasitism in the host's body was considered, as well as the issues related to the size and shape of the suction cups, the position of the ventral suction cup compared to the oral one, as well as the morphology of the digestive tube (MUNTEANU & BOGATU, 2008). The ecosystem of the area offers optimal conditions for the development of the parasite through the presence of intermediate, complementary and final hosts. The absence of these parasites from fish species in the same habitat that do not have an ichthyophage trophic regime could be explained by a low receptivity to them. Trematode worms use predatory fish as a second intermediate host (complementary host). The cercariae that reach their body with the help of gastropods or bivalves (the first intermediate host), which are the trophic base for these fish, lose their tails, close up, turning into metacercariae. From the point of view of pathogenesis, peaceful fish infested with metacercariae can be ingested by predatory fish species, in this case the perch, and the trematode infection can take on the skin (gill, muscle) form, or affect the internal organs (ROMAN, 1955). The metacercar is very similar to the adult both in colour and shape. Ventral sucker very voluminous with long cecums, extending to the front of the oral sucker (Fig. 6). It should be mentioned that after surveying the locals who fish only for recreational purposes, fish with parasites were not reported until the moment of our observation. The subclinical evolution of this parasitosis is also due to the fact that metacercariae can survive two years in the body of fish (EIRAS et al., 1999), and the insufficient presence of ichthyophage birds to continue the biological cycle can lead to a process of self-cleaning. In the small lakes placed along the Preajba Valley, full of aquatic vegetation, with concrete downstream banks, the two hosts that continue the evolutionary cycle of this parasite are present, with hosts represented by gastropods belonging to the genera Planorbis, Physa, Viviparus, as well as a large number of ichthyophage birds: swans, cormorants, herons, pond hens. The lack of macrophyte vegetation on small portions of the banks Favors the excessive development of algae, which

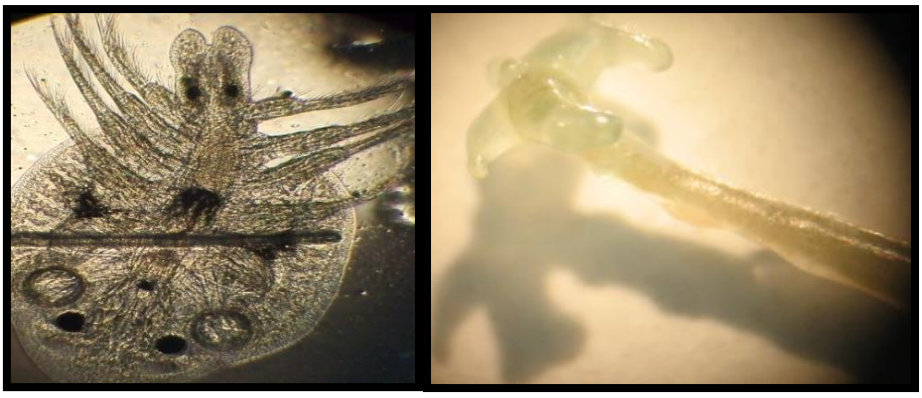
allow the development of gastropods and the perpetuation of the parasite. The prevention of this parasitosis in natural waters is impossible. In order to limit as much as possibly the infestation of fish populations in lakes, the relevant literature recommends prophylaxis, through periodic drying that can lead to a decrease in the number of molluscs, avoiding the formation of excess vegetation, but also preventing the penetration of fish species through repopulation in fish facilities potential wild animals carrying parasites.



A. *Clinostomum complanatum* (metacercar); **B.** *Triaenophorus nodulosus*;



C. *Diphyllobothrium latum* (larvae**)**; **D**. *Eustrongylides excisus* (larvae);



E. *Argulus foliaceus*; **F**. *Lernaea cyprinacea.*

Figure 5. Parasite species identified in dam lakes in the Preajba Valley hydrographic basin (original).

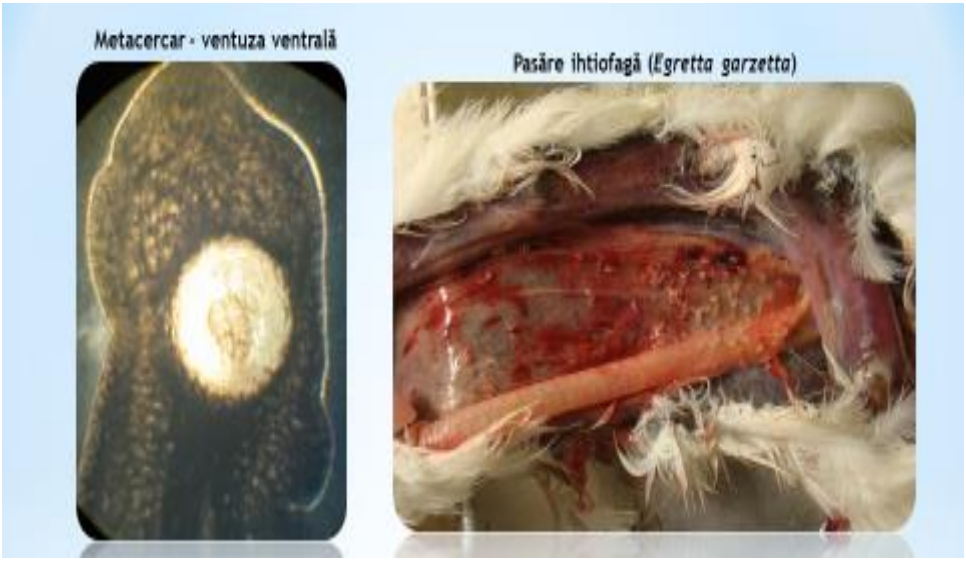


Figure 6. Metacercar and parasitized ichthyophaga bird (original).

Infection with the cestode *Triaenophorus nodulosus* was detected in May 2011 in a single specimen of perch (*Perca fluviatilis*). It presented a bloated abdomen due to the accumulation of a significant amount of exudate, a fact highlighted following the opening of the abdominal cavity. The adult parasite was extracted from the intestine with a forceps and examined under a stereomicroscope. It had the appearance of a ribbon, a length of 20 cm and a width of 4 mm, white-translucent, it had a slightly ovoid scolex, with two slightly devolved botrydia and two pairs of hooks with a trident appearance Fig. 5B and Fig. 7. The unsegmented strobilus on the outside showed a well-developed longitudinal musculature that simulated segmentation, but with an obvious metamery on the inside. Complementary species of zooplanktonophagous fish (*Pseudorasbora parva*, *Alburnus alburnus*), found partially digested in the stomach of perch, consuming crustaceans infected with procercoids, are interposed in the evolutionary cycle of the identified *T. nodulosus* parasite.

As preventive measures, the entry of parasite-carrying fish and eggs that circulate with the surplus water, which enters from one basin to another through the surface spillways, must be prevented.

Figure 7. Parasite extracted from the intestines and viewed under a stereomicroscope (original).

Infection with the cestod *Diphyllobothrium latum* was identified in April 2011 in perch (*Perca fluviatilis*). The identified plerocercoid larvae were extracted from the musculature and visualized under the optical and stereomicroscope, then preserved in a special plastic container in 2.5% formaldehyde. According to the morphological characteristics, it could be established that the larvae taken from the muscles belonged to the genus *Diphyllobothrium*, respectively *D. latum*, as a larval stage – plerocercoid (sparganum) (Fig. 5G and Fig. 8). Plerocercoid larva with an elongated wrinkled body (up to 45-60 mm long; 2-3 mm wide). It was possible to observe an elongated cephalic area that is not separated from the rest of the body and equipped with two more or less prominent botrydial grooves; and the cephalic end is not differentiated. The larva is off-white or cream in colour, slightly transparent with a transversely wrinkled surface. *D. latum* larvae taken from predatory fish species were reported in Romania on pike by Antipa, 1909 and Iamandi, 1936 in the Jijia pond after V. Babeş in 1853 had identified *Esox lucius* as an intermediate host for *D. latum*. These data were mentioned by ROMAN (1955), who discovered the parasite in a specimen of *Perca fluviatilis* in the Danube Delta (Mila 23). VULPE (2007) also reported the presence of *D. latum* in fish in ponds (Greece) formed by floods. The location we found is in accordance with the one mentioned in specialized literature (MUNTEANU & BOGATU, 2008).

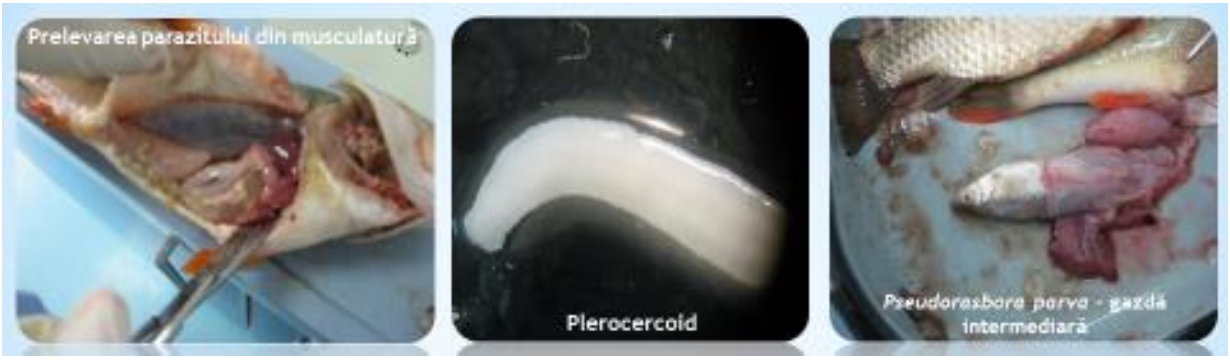


Figure 8. Plerocercoid larva extracted from abdominal musculature (original).

Infection with the nematode *Eustrongylides excisus* was detected in *Perca fluviatilis* (6 specimens) and *Sander lucioperca* (8 specimens), both as a closed larva surrounded by a thick capsule of connective tissue, on the mesentery and gonads, and free in the abdominal cavity and abdominal wall muscles (Fig. 5D). The two species were considered secondary intermediate hosts. In fish, the characteristic symptoms of the disease are not precisely known, but it is estimated that there are growth disorders and reproduction difficulties in heavily parasitized individuals. In our country, *E. excisus* was reported for the first time by Ciurea (cited by ROMAN, 1955), who found it in the musculature of barbel, pike, perch and other fish. Much later, it was cited by Rădulescu in 1947 as a parasite on catfish and perch in the Danube Delta. In the ichthyofauna of Banat, 9 species of parasitic gastrointestinal nematodes were reported both in the adult stage and as larvae (COJOCARU, 2006). Water quality is a determining factor in the transmission of the parasite. An explanation for the presence of the parasite would be the abundance of ichthyophage birds in that period compared to the winter, the increased water temperature, the low oxygenation and the high content of organic substances.

Figure 9. The presence of parasites and encysted larvae (original).

The infection with the crustacean *Argulus foliaceus* was identified both in the sample with which the fish was brought, but also on the body surface of *Perca fluviatilis*. The parasite was cited for the first time in Romania by Borcea in 1915, on catfish, pike, carp, bream and perch; Dumitriu in 1937, signals him for pike, smooth and goldfish; Mark in 1929 for carp, crucian carp and tench; Zemianovski in 1946 for carp and Radulescu in 1948 for crucian carp, carp and walleye. In the hydrography of the Banat, COJOCARU (2006) identified 2 species of *Argulus*: *A. foliaceus* and *A. coregoni* for carp and bream, the species *A. coregoni* having not been reported in Romania until that time when the injuries caused by the mouth brace appeared as small necrotic ulcers. The action of the parasite on the host consists in damaging the gill epithelium as well as destroying the mucus layer on the skin surface. The parasite *A. foliaceus* was observed both ventrally and dorsally, mainly for the abdominal lobes to determine the species; in the present case, the lobes are rounded and less divided, which led to the species mentioned above. The parasite was cited for the first time in Romania by Borcea in 1915, on catfish, pike, carp, bream and perch; Dumitriu in 1937, signals him for pike, smooth and goldfish; Mark in 1929 for carp, crucian carp and flounder; Radulescu in 1948 for crucian carp, carp and bream. In the hydrography of Banat, COJOCARU (2006) identified 2 species of *Argulus*: *A. foliaceus* and *A. coregoni* for carp and bream, the species *A. coregoni* having not been reported in Romania until that time. From the point of view of localization, the ectoparasites have an increased intensity of over twenty individuals on the host fish, with localization at the level of the caudal peduncle but also at the level of the eyes and fins (Fig. 10).

Figure 10. Stereomicroscopic view (dorsal and ventral, original).

The infection with the crustacean *Lernaea cyprinacea* was identified in 8 specimens of perch (*Perca fluviatilis*) parasitizing at the base of the scales at the level of the lateral line, the muscles at the level of the eyeballs as well as at the base of the scales at the insertion of the caudal and dorsal fin. The high water temperature (over 28^0C) helps their metamorphosis. The identification of the ectoparasite was carried out following the general methods of ichthyoparasitological diagnosis, macroscopic through clinical examination and microscopic through scraping from the skin and fins and visualization with the optical microscope and stereomicroscope. Using the optical microscope with 20x, 40x lenses, the *L. cyprinacea* species was highlighted. The female's vermiform body, 12.5 – 16.5 mm long, presents, in the cephalothorax region, a segment provided with 2 pairs of anchor-like outgrowths, which form the apparatus with which the females attach themselves to the fish's body. The growths are thin, almost cylindrical and symmetrical, the dorsal ones being bifurcated with the appearance of the letter "T", while the ventral ones are much shorter (Figs. 11, 12). At the back of the body there are two cylindrical and elongated ovigerous sacs that reach 1/3 of the length of the entire body.

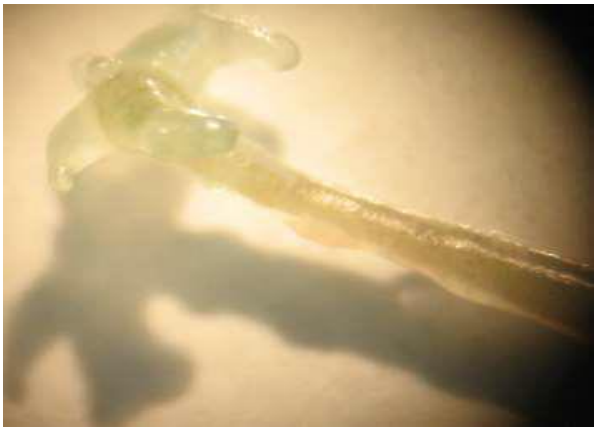


Figure 11. The front end of the body with the appearance of the letter "T" (original).

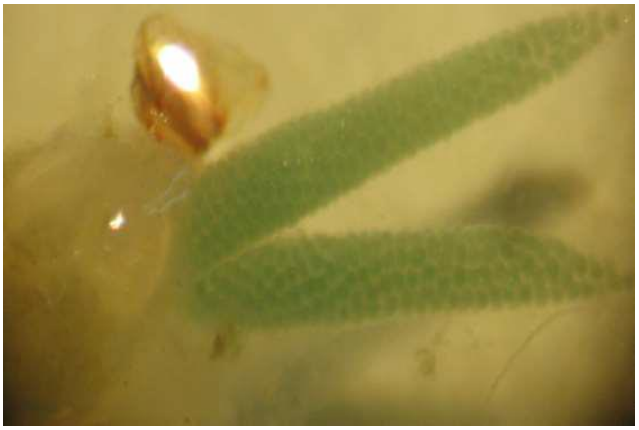


Figure 12. The posterior extremity of the body provided with cylindrical ovigerous sacs and elongate (original).

L. cyprinacea is considered a 240 warm water crustacean. The development of the nauplii takes place during three days at a water temperature between 23-30 ^{0}C. After 72 hours they become copepods and enter the gill cavity of the fish transforming into cyclopoids, when sexual differentiation occurs (OŢEL & CONSTANTIN, 1989). Females switch to parasitic life, deeply embedding their anchor-like attachment organ in the skin of the host. The sources of parasites are infested fish, as well as the water in which they are found at larval stages. At the places of attachment on the hosts' body, the crustaceans produce erosions of the scales, haemorrhages, superficial or deep ulcerations, tissue destruction. The intensity of the parasite is variable, between 1 and 14 parasites per fish. The disease is difficult to combat in large ponds, and the prophylactic measures recommended by specialized literature in order to prevent it are: mandatory quarantine of the stocking material or preventive bathing in an antiparasitic solution, ensuring an optimal stocking density, parasitological examination of the fish at shorter time intervals, the exclusion of infested fish from the culture as well as from the supply water, the periodic vacuuming of the pools and their disinfection with quicklime. Lerneosis control is difficult to achieve in fish in pools with a very large surface area sea where permanent checks must be made regarding the health of the fish, and the appearance the parasite must be supervised by blocking with a screen the surface spillways that allow fish to pass from one pool to another.

CONCLUSIONS

• In the research carried out at the level of the Valea Preajba hydrographic basin, all 10 dam lakes were prospected starting from October 2008, so that the results obtained were representative.
• Following the obtained results (observations and research carried out during the years 2008-2014), a final conclusion is drawn regarding the fact that the presence and evolution of fish parasites is in close correlation with the diversity of plants and animals in the existing food chains. Although the Valea Preajba hydrographic basin is not affected by important sources of pollution, the lakes have a high degree of eutrophication due to the penetration of mineral nutrients from the use of chemical fertilizers on the neighbouring agricultural lands and the discharge of untreated wastewater.
• *Clinostomum complanatum* metacercariae have been reported in the species *Perca fluviatilis*, to which the parasite shows an increased receptivity at the level of an ecosystem that offers optimal conditions for development. *Perca fluviatilis* has a trophic regime consisting of aquatic invertebrates (chironomids, worms, amphipods, crustaceans, gastropods, copepods), but also ichthyophage (*Alburnus alburnus*, *Pseudorasbora parva*). The cercariae that have arrived in the body of these invertebrates (the first intermediate host), through ingestion, lose their tails and close, turning into metacercariae. These trematode worms use predatory fish as a second intermediate host (complementary host). From the point of view of pathogenesis, peaceful fish infested with metacercariae can be ingested by predatory fish species, in this case seabass, and the trematode can cover the skin (gill, muscle) or affect the internal organs.
• In the case of the parasites *Diphyllobothrium latum* and *Triaenophorus nodulosus* (cestode worms), the hosts are infested with plerocercoid worms. Their penetration and development in the body of the host is done simultaneously with the ingested food, respectively aquatic invertebrates (chironomids, worms, amphipods as well as copepod crustaceans) carrying the first parasitic stage, but also with peaceful fish species, especially cyprinids (*Carassius gibelio* and *Pseudorasbora parva*), which were found ingested in their stomachs.
• The endoparasite *Eustrongilides excisus* (larvae) was taken from three species of predatory fish (*Sander lucioperca*, *Silurus glanis* and *Perca fluviatilis*) whose trophic basis is represented by insects, crustaceans, worms, fish and amphibians. Adult specimens eventually become ichthyophages consuming fish species, such as: *Alburnus alburnus*, *Carassius gibelio*, *Pseudorasbora parva*. Fish infested with nematodes presents 4 developmental stages of the parasite that are interposed between the egg and adult phases. The first stage is ingested by an oligochaete of the genus Tubifex, at which level the larval stages (L2 and L3) develop. Small fish, such as *Alburnus alburnus* and *Pseudorasbora parva*, have been found ingested in the stomachs of infested fish, suggesting that they may be facultative hosts for this parasite.
• The two crustaceans *Lernaea cyprinacea* and *Argulus foliaceus* include in their evolutionary cycle the eggs laid on plants, shells and woody debris in the water, and the hatched larvae are considered sources of parasitism. The high temperature of the water (over 28^0C) helps their metamorphosis, the free larval evolutionary stages being carried out in water without changing hosts.
• The presence of ichthyophage birds, final hosts for many species of trematodes, can explain the occurrence of the Clinostomum complanatum parasite in the species *Perca fluviatilis* in the studied area.
• In conclusion, the chosen research method can be extrapolated to other study areas given the fact that all the parasites presented in the paper had a mildly pathogenic subclinical evolution. Under favourable conditions, their pathogenicity (*Clinostomum complanatum*) can increase greatly, having a potential zoonotic character, unlike the other identified species that have definite ichthyopathological significance.

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