THE STRUCTURE OF THE ECTOPARASITOPHAUNA OF FISH IN THE CYPRINIDAE FAMILY FROM THE SMALL DAM LAKES IN THE LOWER SECTOR OF THE JIU (DOLJ, ROMANIA)

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Abstract. Research on the ichthyoparasitic fauna of fish from the Cyprinidae family, taken from the ten dam lakes in the lower sector of Jiu, lakes that are part of the *"Preajba-Făcăi Lacustrine Complex"* site, revealed the following aspects: 9 species of parasites were identified: 2 cilophores (*Chilodonella cyprini*, *Ichthyophthirius multifiliis*), 1 oomycete *Saprolegnia* sp., 1 monogenous *Dactylogyrus* sp., 1 myxosporidia *Myxobolus* sp., 1 acanthocephalus *Pomphorhynchus laevis*, 2 crustaceans (*Lernaea cyprinacea*, *Argulus foliaceus*) and 1 annelid *Piscicola geometra*, in five of the seven host species studied. (6) species of parasites have been identified in crucian carp (*Saprolegnia* sp., *Ichthyophthirius multifiliis*, *Myxobolus* sp., *Dactylogyrus* sp., *Lernaea cyprinacea*, *Piscicola geometra*), in carp (5) (*Chilodonella cyprini*, *Dactylogyrus* sp., *Pomphorhynchus laevis*, *Lernaea cyprinacea*, *Argulus foliaceus*), bream (1) (*Saprolegnia parasitica*), bream (1) (*Myxobolus* sp.) and spotted murgoi (1) (*Saprolegnia parasitica*), followed by slugs and tomatoes in which no parasites were identified. Of the 9 species of parasites identified, 7 species are ectoparasitic. Clinical signs of ectoparasitosis have been identified in both ichthyophthyrosis of the crucian carp where hypoxia reactions and the presence of white punctate colonies have been found, as well as in the case of saprolegniosis due to the appearance of fine wool hyphae and scoliosis, argulosis and piscicolosis highlighted in crucian carp and carp by haemorrhagic wounds and obvious ulcerations but also by the presence of parasites.

Keywords: ectoparasitic fauna, Cyprinidae, Preajba Valley.

Rezumat. Structura ectoparazitofaunei la peşti din familia Cyprinidae din lacurile mici de baraj din sectorul inferior al Jiului (Dolj, România). Cercetările efectuate asupra ihtioparazitofaunei la peşti din familia Cyprinidae, prelevaţi din cele zece lacuri de baraj din sectorul inferior al Jiului, lacuri care fac parte din situl „*Complex Lacustru Preajba-Făcăi*", au relevat următoarele aspecte: au fost identificate 9 specii de paraziţi: 2 ciliofore (*Chilodonella cyprini*, *Ichthyophthirius multifiliis*), 1 oomicet *Saprolegnia* sp., 1 monogen *Dactylogyrus* sp., 1 mixosporidie *Myxobolus* sp., 1 acantocefal *Pomphorhynchus laevis*, 2 crustacee (*Lernaea cyprinacea*, *Argulus foliaceus*) şi 1 anelid *Piscicola geometra*, la cinci din cele şapte specii gazdă luate în studiu. (6) specii de paraziţi au fost identificate la caras (*Saprolegnia* sp., *Ichthyophthirius multifiliis, Myxobolus* sp., *Dactylogyrus* sp., *Lernaea cyprinacea*, *Piscicola geometra*), la crap (5) (*Chilodonella cyprini*, *Dactylogyrus* sp., *Pomphorhynchus laevis*, *Lernaea cyprinacea*, *Argulus foliaceus*), obleţ (1) (*Saprolegnia parasitica*), plătică (1) (*Myxobolus* sp.) şi murgoi bălţat (1) (*Saprolegnia parasitica*), urmate de babuşcă şi roşioară la care nu au fost identificaţi paraziţi. Dintre cele 9 specii de paraziţi deteriminaţi, 7 specii sunt ectoparazite. Semnele clinice manifestate de către ectoparazitoze au fost puse în evidenţă atât în cazul ihtioftiriozei la caras unde s-a constatat reacţii de hipoxie şi prezenţa coloniilor punctiforme de culoare albă, dar şi în cazul saprolegniozei prin aspectul hifelor de vată fină, precum şi al lerneozei, argulozei şi piscicolozei evidenţiate la caras şi crap prin răni hemoragice şi ulceraţii evidente dar și prin prezența paraziților.

Cuvinte cheie: ectoparazitofaună, Cyprinidae, Valea Preajba.

INTRODUCTION

Research in Romania on parasitic fauna in fish is often limited to biological studies, with a character, both in artificial growth systems and in natural conditions, more frequent in the Danube Delta (ROMAN, 1956; OŢEL & CONSTANTIN, 1989) and much rarer in other regions MIHALCA et al. (2003). These studies are intended to inform about issues related to the seasonal incidence of some sweet fish parasites being closely related to ecological issues. Parasitosis is also favoured by the degree of loading with stagnant organic matter of stagnant water where most often in summer there are algal blooms. The most common type of biological pollution is that with organic waste from human settlements and nearby farms. Our observations show that this type of pollution has a positive effect on the incidence of parasites, especially ciliated protozoa. The parasitism encountered in fish in the Preajba Valley lake ecosystem is an alarm signal due to mortality that makes seasonal sport fishing difficult. Among the target fish species, cyprinids are considered the most vulnerable species in the geoclimatic conditions of our country.

The present paper aims to inventory the ectoparasitic fauna of (7) species of fish from the Cyprinidae family, species present in the lakes along the Valea Preajba river, with the detection of some aspects of pathogenicity.

MATERIAL AND METHODS

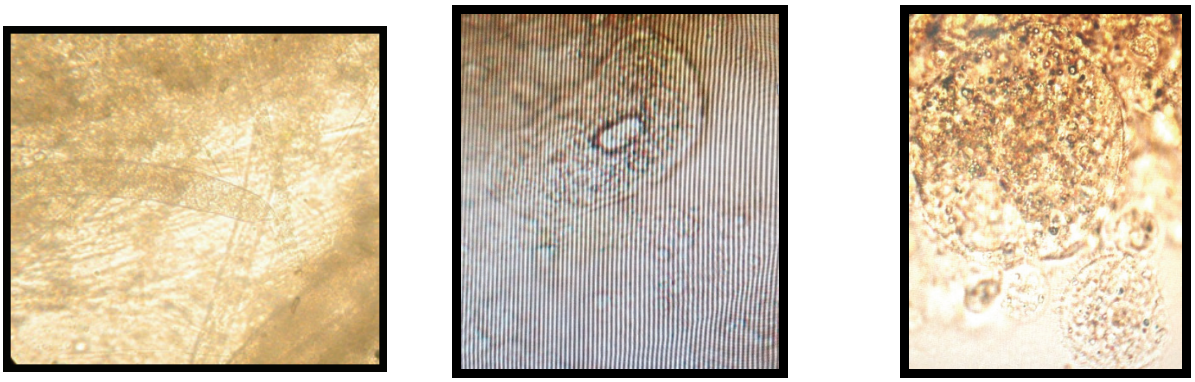
832 fish from the ten small dam lakes were examined, of which 560 belong to the Cyprinidae family: carp (*Cyprinus carpio*), prussian carp (*Carassius gibelio*), bleak (*Alburnus alburnus*), stone moroko (*Pseudorasbora parva*), roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*), common bream (*Abramis brama*) (BĂNĂRESCU, 1964; KOTTELAT & FREYHOF, 2007). The collection of ichthyological material was done seasonally, for each lake, except for periods when the weather was unfavourable (late autumn, winter). In the absence of electronarcosis equipment, the sampling was done with various monofilament nets with a length between 50-100 m and a mesh size of 40-60 mm, but

also by fishing with fishing rods by amateur fishermen in the area. The main collection points for fish material along the river have been established from the upstream to the downstream area. The collected fish were transported using plastic containers with live water, and the dead fish were transported in a refrigerated state. The transport was done quickly, and the examination of the fish was done in the parasitology laboratory within the Sanitary Veterinary and Food Safety Directorate of Dolj.

The macroscopic examination was done by observing the entire surface of the body, eyes and gills, to highlight external parasites, skin integrity and skin lesions (cysts, ulcerations, destroyed fins, lack of scales, etc.), and even the presence of parasites (*Argulus* sp., *Saprolegnia* sp.). The body surface, eyes and gills were examined with the naked eye or magnifying glass. From the level of the gills, by scraping, native preparations were made with the help of histological slides and examination under the Olimpus BX 43 optical microscope.

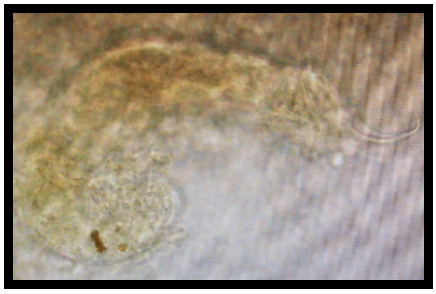
RESULTS AND DISCUSSION

In the period 2008-2014, ichthyological observations and collections highlighted the fact that, due to the anthropogenic impact and the intense eutrophication phenomena, the parasitic forms on the fish populations are more and more obvious. In the cyprinids collected, 9 species of parasites were highlighted (Fig. 1), of which 7 are ectoparasites: **A**. *Saprolegnia* sp., **B**. *Chilodonella cyprini*, **C**. *Ichthyophthirius multifiliis*, **D**. *Piscicola geometra*, **E**. *Argulus foliaceus*, **F**. *Lernaea cyprinacea*, **G**. *Dactylogyrus* sp.



A - *Saprolegnia* sp, **B** - *Chilodonella cyprini*, **C**- *Ichthyophthirius multifiliis*

D - *Piscicola geometra*, **E** - *Argulus foliaceus*, **F** - *Lernaea cyprinacea*



G - *Dactylogyrus* sp.

Figure 1. Ectoparasites identified in cyprinids from the dam lakes in the Valea Preajba river basin.

The etiological agent of this ectoparasitosis was ***Saprolegnia parasitica*** Coker, 1923 determined and subjected to parasitological examination, within the parasitology laboratory of the DSVSA The identification of the fungus was performed directly by macroscopic examination; the extramatric hyphae around the caudal peduncle looked like a dirty-white cotton-like fabric (GOGA, 2018). For more precise identification, a native blade was prepared - scraped lamella from the surface of the skin (cutaneous), fins, gills, followed by microscopic examination in order to differentiate it from other parasitic fungi (*Achlya*). Saprolegnia infection was reported in *Pseudorasbora parva* and *Carassius gibelio*. In most cases, the diagnosis of Saprolegnia infection can be made based on the anatomical and clinical features of the host fish, but microscopic examination is essential. The presence of the oomycete in the region of the caudal peduncle was also confirmed by the ulceration due to the damage of the layer of scales and protective mucus. In the literature it is mentioned that a bacterial infection can overlap with such lesions caused by fungi (PATRICHE & TALPEŞ, 1993; VULPE, 2007; ZAKI et al., 2010). After macroscopic observation of the external mycelium that in water acquires the appearance of a lump of cotton wool, the examination followed with the optical microscope, Olimpus BX 43 with 20x, 40x objectives; eyepiece WHN 10x / 22 but also with the Olympus SZX7 stereomicroscope (2x, 3.2x lenses; WHSZ 10x / 22 eyepiece), of the native preparations with the material scraped from the skin that allowed the highlighting of the hyphae. The thick extramatric hyphae showed mature tubular zoosporangia with formed zoospores, and much thicker than the rest of the hypha (OŢEL & CONSTANTIN, 1989). Zoospores released into the water reach the organic substrate (including fish) and germinate, forming a new mycelium. To prevent the manifestation of saprolegniosis, the literature recommends improving the water quality of fish ponds by reducing the amount of organic matter, performing unclogging, as well as controlling and avoiding favourable factors that may predispose fish and other aquatic life (amphibians, crustaceans, insects) infestation.

Chilodonella cyprini infection was identified in Lake IX in 7 mature specimens of *Cyprinus carpio* (500g). Every spring, amateur fishermen suspected the presence of such ciliophoran parasites in large fish in the lake by swimming agitated on the surface of the water and the skin with small portions without scales. The examination of the skin, fins and gills was done at the beginning with a magnifying glass (X 10), after the fish was spinalized, and the skin and gills were scraped from the mucus from the injured areas with the help of a blade, being afterwards placed in saline solution for microscopic examination (GOGA, 2018).

At least three parasites coming from the skin scraping could be identified in the field of view of the microscope, while the gill scraping did not present any parasites. Parasitosis has been reported with no clinical signs of disease. Following microscopic examination, the parasite with rectilinear motions in the field of view of the microscope was identified. The presence of a large slightly elongated macronucleus and two round micronuclei, as well as the cytopharynx certify the presence of the parasite in the collected sample. The severity of this parasitosis in the case of a massive infestation is high; in our case, the fact that this pool is not overcrowded resulted in the disease not being transmitted quickly and, thus, mortality is not present. In this basin system where samples were taken, where water enters from one lake to another, through pond and surface dischargers, the control of diseased fish is quite difficult to achieve. As a preventative measure, the only recommended antiparasitic treatment is lime chloride (1 mg / l water).

Ichthyophthirius multifiliis infection was found subclinically in 10 crucian carp (*Carassius gibelio*), reported in March 2011 in Lake IX, with small whitish nodules on the skin located uniformly like meridians in the head and pectoral fins (GOGA, 2018). The arrangement of these nodules on the body surface is mentioned in the literature (RĂDULESCU, 1983). The pathogenic action of the parasite is mechanical, irritating and spoiling, and the scale-free portions of the skin have confirmed this. Thus, whitish nodules spread mainly on the head on a hypersecretion of mucus, fins and caps and less on the body, without the appearance of necrotic foci, induced visible disturbances in fish. Examination with a magnifying glass confirmed the presence of whitish colonies of *I. multifiliis*. The receptivity of fish to Ichthyophthirius invasion also depends on the amount of oxygen dissolved in the water. The decrease in the amount of oxygen (at 3mg / l) determines physiological changes in the body, characteristic of a state of stress, which will lead to a decrease in the body's resistance to the attack of this parasite (VULPE, 2007). Successive scraping and washing in distilled water were performed on Petri dishes from the affected areas, and microscopic and stereomicroscopic examination revealed the parasite (Fig. 1). Crucian infestation is thought to have been due to infested tomites, resulting from the division of the trapped parasite on various water substrates, especially on aquatic plants overdeveloped due to the eutrophication process, inducing hypoxia in fish. The tomites that reach the skin feed on the scales caused throughout the growing period, up to the trophon stage (appearance of grains) forming small cavities (WAHLIT et al., 1997; MATTHEWS, 2005). It is considered that the infection develops most frequently asymptomatically, and the effective measures to control the disease in the dam lake system include: intensification of water flow in infested basins, reduction of population density, increase of water temperature up to 28 ^{0}C in basins where there is a possibility (OŢEL & CONSTANTIN, 1989; MATTHEW, 2005; VULPE, 2007; DĂSCĂLESCU & COSTEA, 2014).

Infection with ***Piscicola geometra*** was detected in May 2012 in *Carassius gibelio* (ARSLAN & EMIROGLU, 2010), the parasite being up to 35mm long and 3mm wide with two well-shaped suction cups (MUNTEANU & BOGATU, 2008. The fish were examined macroscopically, observing the place of parasitism. The parasite was placed on a petri dish in distilled water, being observed through transparency on the Olympus SZX7 stereomicroscope with 2x, 3.2x, WHSZ 10x / 22 eyepiece lenses, but also on the Olimpus BX 43 optical microscope, as a native slide-slide preparation with 10x, 20x, 40x lenses, WHN 10x / 22 eyepiece. 75 specimens were taken, which were fixed on different areas of the body (gills, buccal mucosa, pectoral fins, caudal fin and scales on the ventral part of the body), with lengths

between 15 mm and 35 mm. Fish affected by piscicolosis had bleeding sores on their bodies (GOGA, 2018). The diagnosis of parasitosis was established quite simply, by macroscopic examination highlighting the leeches. The parasitosis evolved subclinically given the small number of parasitized fish, but also by its non-existence in the following catches. In our country, *P. geometra* was also cited by ANTIPA (1909) for carp, DUMITRU (1937) for pike, barbel and bream, ZEMIANKOVSKI (1946) for carp (quoted by ROMAN, 1955). By destroying macrophyte vegetation and preventing fish species from entering one basin to another, given that they communicate with each other and the excess water passes from one basin to another through surface dischargers, several preventive measures can be taken on the appearance of piscicolosis.

Argulus foliaceus infection has been found in summer and autumn catches in *Cyprinus carpio*. A common parasite of freshwater fish, it has been reported in the Danube Delta at Mile 23 in species such as: roach, asp, flax, bream, carp, pike, perch and perch sun, in Greaca, and in Calarasi in a number of 1-3 specimens per host for the following species: widow, catfish and pikeperch. It was first cited in Romania by Borcea in 1915, in pike, carp, catfish, bream and perch; Dumitriu in 1937 points it out for pike, flax and crucian; Mark in 1929 for carp, crucian and flax; Zemianovski in 1946 for carp and Radulescu in 1948 for crucian carp, carp and three-spined stickleback. In the hydrography of Banat, COJOCARU (2006) identified 2 species of *Argulus*: *A. foliaceus* and *A. coregoni* in carp and bream, the species *A. coregoni* not being reported in Romania until that moment. The adult parasite was identified both in the water sample with which the fish was brought, but also on the surface of the body, where the lesions caused by the buccal armature presented as small necrotic ulcerations (GOGA, 2018). The macroscopic evaluation was performed on a Petri dish in distilled water, following the examination of the crustacean by transparency on the Olympus SZX7 stereomicroscope with 2x, 3.2x objectives; eyepiece WHSZ 10x / 22, but also with the Olimpus BX 43 optical microscope, as a fresh slide-slide preparation with 2x, 10x objectives; ocular WHN 10x / 22 (YILDIZI & KUMANTAS, 2002; MOLNAR & SZEKEL, 1998). The characteristics of the abdominal lobes (degree of division and their appearance) were followed. The parasite was observed both ventrally and dorsally, especially the abdominal lobes to determine the species; In this case, the lobes are rounded and less divided, which led to the above-mentioned specie. The literature mentions as a determining factor in the development of the parasite, the temperature and pH of the water. Being a thermophilic species, water samples were collected and measured at the time of collection. Even if this parasitosis has evolved subclinically, without causing mortality, preventive measures are very important.

Infection with the copepod ***Lernaea cyprinacea*** was reported in 2009 and 2011 (May-August) in *Carassius gibelio* and *Cyprinus carpio* parasitizing at the base of the scales at the level of the lateral line (OŢEL & CONSTANTIN, 1989). After examination of the skin and gills, the parasites present were extracted with tweezers and spread on a slide in a drop of distilled water, examined under an optical microscope and then stereomicroscopic and photographed. Using the optical microscope with 20x, 40x lenses the species *Lernaea cyprinacea* was highlighted. The body of the vermiform female, 12.5 - 16.5 mm long, has a segment in the region of the cephalothorax with 2 pairs of anchor-like growths, which form the apparatus with which the females attach themselves to the body of the fish. At the back of the body there are two elongated cylindrical sacs. At the sites of attachment to the body of the hosts, crustaceans cause scale erosion, bleeding, superficial or deep ulceration, tissue damage. The intensity of the parasite being variable, highlighting between 1 and 14 parasites in a fish sample. The diagnosis was established by clinical observations with the naked eye of the crustaceans, but also the microscopic visualization of the parasites (GOGA, 2018). The disease is difficult to control in large pools, and prophylactic measures are recommended.

Infection with the monogenous ***Dactylogyrus* sp**. was revealed by macroscopic examination of the gill cavity and caudal fin in two species of cyprinidae (*Carassius gibelio* and *Cyprinus carpio*) by the presence of a significant amount of mucus and small portions of haemorrhagic lesions, as a result of actions chem of the parasite. ROMAN (1955) in his work "Research on the parasitofauna of fish in the Danube", reported several species of *Dactylogyrus* parasitizing the gills of roach, carp, barbel, widow, redfish, crucian and bream in the Danube. (COJOCARU, 2006) mentions that over 40 species of *Dactylogyrus* have been identified in Romania, out of which 11 species have been identified in Banat. Intensive fishing has not been practiced in the research area for a long time, so the infection with *Dactylogyrus* sp. identified by us in carp and crucian carp during the summer months evolved subclinically, without mortality, most of the fish collected near the shore of the basin showed obvious signs of hypoxia. Due to the fact that the obtained microscopic images did not show morphological details very clearly, the species could not be identified accurately (RĂDULESCU et al., 1976; MEHLHORN, 1998; WOO, 2006; GOGA, 2010, 2012; GOGA & CODREANU-BĂLCESCU, 2011, 2013; GOGA & TÎMBURESCU, 2011, 2012, 2013, 2015; GOGA et al., 2014; IONUŞ et al., 2014). Rising water temperatures, excess aquatic macrophytes, the process of eutrophication, and the fact that fish species can enter their parasitic loads upstream and downstream from one lake to another through pond dischargers are just a few of the causes that can cause and parasite development.

CONCLUSIONS

In the area studied during 2008-2014 we identified: 1 oomycete (*Saprolegnia parasitica*), 2 species of ciliophorans (*Ichthyophthirius multifiliis* and *Chilodonella cyprini*), 1 annelid (*Piscicola geometra*), 1 monogenous (*Dactylogyrus* sp.) and 2 species of crustaceans (*Lernaea cyprinacea*, *Argulus foliaceus*).

Due to the fact that these lakes are not fished industrially, but only sporadically by amateur fishermen, the parasites were not reported before our research in the lake ecosystem, so, as they are of low intensity, they are not obvious and do not generate a mass of pathological fish.

We can say that ectoparasites represented by 7 species (*Saprolegnia parasitica*, *Ichthyophtirius multifiliis*, *Chilodonella cyprini*, *Dactylogyrus* sp., *Piscicola geometra*, *Argulus foliaceus*, *Lernaea cyprinacea*) predominate in the cyprinid fish collected from the Valea Preajba river basin, being most commonly located on the skin and gills, along with 2 species of endoparasites: *Myxobolu*s sp. and *Pomphorhynchus laevis.*

Research has shown that ectoparasitosis is the easiest to diagnose, especially in crustaceans and hirudines, due to the size of the parasites and the obvious damage they cause. Regarding the organic distribution of the detected ectoparasites, it is observed that most ectoparasites are present (7 species) in the three species of the Cyprinidae family: *Carassius gibelio*, *Cyprinus carpio* and *Pseudorasbora parva*,.

An important role in the appearance and spread of parasites in fish is played by their close relationships with other aquatic organisms, as well as the degree of pollution of their living environment. The more elements in the aquatic environment, plants or animals, are present in the aquatic environment, the greater the chances that fish will encounter intermediate or accumulation hosts of parasites. Parasites such as the monogenous *Dactylogyrus* sp. and *Argulus foliaceus* crustaceans, *Lernaea cyprinacea* do not need intermediate or accumulation hosts in their evolutionary cycle.

In the case of ciliophorans (*Ichthyophtirius multifiliis* and *Chilodonella cyprini*), weak and malnourished fish were a favourable substrate for rapid multiplication. Cyprinids (*Carassius gibelio* and *Pseudorasbora parva*), affected by *Saprolegnia parasitica*, have as a source of disease the oomycete zoospores, which are found in pools with a rich content of decomposing organic substances, such as, in our case, the studied lakes, and which are found in an intense clogging process, precisely due to the slow decomposition of excess marshy and aquatic macrophytes among other factors and the water temperature between 3-28^{0}C.

In the case of the monogenous *Dactylogyrus* sp., present in *Carassius gibelio* and *Cyprinus carpio*, through our observations and consulting the literature, the development of this parasite is direct, without intermediate hosts, ciliated larvae can be swallowed with water, located in the gills. The two crustaceans *Lernaea cyprinacea* and *Argulus foliaceus,* found in the species *Cyprinus carpio* and *Carassius gibelio*, include in their evolutionary cycle the eggs laid by the adult on plants, gastropods and wood fragments from the water, and the hatched larvae are considered sources of parasites, in close connection high water temperature.

Piscicola geometra, a hematophagous ectoparasitic hirudine, parasitizes on the body of fish in the spring. In search of food (submerged macrophyte vegetation), species with increased receptivity in the lakes of the Preajba Valley (crucian carp and carp) may come into direct contact with the parasite, which, depending on water temperature, may parasitize for a shorter or longer time.

In the case of crustaceans *Argulus foliaceus* and *Lernaea cyprinacea*, they need in their metamorphosis an increased water temperature above 28 ^{0}C, the free larval evolutionary stages being realized in water without change of hosts, with receptivity especially for cyprinids (*Cyprinus carpio*, *Carassius gibelio*).

The ten anthropogenic accumulations formed by the dam of the main watercourse on the Preajba Valley and the tributary of the Old Valley, represent a determining factor in the appearance and distribution of parasites in the fish populations in these lakes.

Although the ichthyoparasitofauna is dependent on the diversity of plants and animals in the existing food chains, the area is not affected by major sources of pollution, the lakes still have a high degree of eutrophication, due to the penetration of mineral nutrients from the use of chemical fertilizers of untreated wastewater.

We can conclude that all the parasites presented in the paper had a weakly pathogenic subclinical evolution. At the same time, the presence of ciliophoran protozoa in lakes is an indicator of pollution of these waters. Referring to the size of the study area, the distribution of ichthyofauna and the anthropogenic factor, we can say that the number and diversity of parasitic species are directly proportional to the complexity of the studied lake ecosystems. The absence or presence of groups of parasites in these small, highly eutrophic dam lakes can also be expressed by the specificity of the microhabitat.

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