HAEMATOPHAGOUS PARASITES DETECTED ON FISH STOCKS FROM THE SMALL RESERVOIRS WITHIN THE OLTENIA PLAIN (ROMANIA)

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Abstract. *Piscicola geometra* and *Argulus foliaceus* are two haematophagous parasites collected through sporadic seasonal fishing carried out in the spring of 2009 and 2012, in the study area represented by ten reservoirs from the Oltenia Plain. These parasites are the most common in these lakes and affect a wide range of host fish, both in natural and artificial pools. In the spring of 2009, we sampled 42 specimens of fish and the etiological agent was the crustacean *Argulus foliaceus, which* was reported only for two fish species: *Perca fluviatilis* and *Cyprinus carpio*. In 2012, 47 fish specimens were captured belonging to the species – *Scardinius erythrophthalmus*, *Lepomis gibbosus*, *Abramis brama*, *Carassius gibelio*, *Perca fluviatilis*. The *Piscicola geometra* parasite was identified only in *Carassius gibelio*. It has been found out that an important common factor in causing these parasites is water temperature; consequently, the invasion decreases when water temperature increases. Although these parasitosis evolved subclinically without mortality, prevention and treatment measures were proposed to prevent their spreading. Thus, wood panels were installed into the water so that the *Argulus foliaceus* parasite could lay its eggs and, as measures of combat, the literature recommends the treatment with Trichlorfon at a dose of 1g/6m^3 of water. The destruction of the macrophyte vegetation and prevention of the penetration of fish species from one basin to another, given that they communicate via spillways, are some prophylactic measures to prevent fish infestation.

Keywords: parasites, *Piscicola geometra*, *Argulus foliaceus*, fish.

Rezumat. Paraziți hematofagi întâlniți la populații piscicole din lacurile mici de baraj din Câmpia Olteniei (România). *Piscicola geometra* și *Argulus foliaceus* sunt doi paraziți hematofagi colectați prin pescuiri sezoniere sporadice, efectuate în primăvara anului 2009 și 2012. Situl de studiu fiind cele zece lacuri de baraj din Câmpia Olteniei. Acești paraziți sunt cei mai frecvenți în aceste lacuri și afectează o gamă largă de pești gazdă, atât în cazul populațiilor naturale, cât și în cele din bazinele artificiale. În primăvara anului 2009 s-au prelevat 42 exemplare de pești, avand ca agent etiologic crustaceul *Argulus foliaceus* semnalat doar la două specii de pești: *Perca fluviatilis* și *Cyprinus carpio*. În 2012 au fost capturate 47 exemplare de peşti aparţinând speciilor: *Scardinius erythrophthalmus*, *Lepomis gibbosus*, *Abramis brama*, *Carassius gibelio*, *Perca fluviatilis,* parazitul *Piscicola geometra* fiind identificat numai la caras (*Carassius gibelio*). S-a constatat că un factor comun important în provocarea acestor parazitoze este temperatura apei, astfel invazia scade când temperatura apei crește. Chiar dacă aceste parazitoze au evoluat subclinic, fără a se produce mortalitate, pentru combaterea lor s-au propus măsuri de prevenție și tratament precum: instalarea în apa bazinelor a unor panouri din lemn pe care parazitul *Argulus foliaceus* să depună ouăle, iar ca măsuri de combatere, literatura de specialitate recomandă tratament cu Trichlorfon în doză de 1g/6m^3 de apă. Distrugerea vegetației macrofite și prevenirea pătrunderii speciilor de pești dintr-un bazin în altul, având în vedere că acestea comunică prin descarcatoare de prund, sunt câteva măsuri profilactice menite să prevină infestările.

Cuvinte cheie: paraziți, *Piscicola geometra*, *Argulus foliaceus*, pești.

INTRODUCTION

The basin of the Preajba river (15 sqkm / 1500 ha, Atlas of the Romanian Water Cadastre, 1992) is located in the Dolj county, 6 km South of Craiova, at the contact between the Getic Piedmont and the Oltenia Plain.

The main watercourse is the Preajba Valley having a length of 9.6 km (Atlas of the Romanian Water Cadastre, 1992), whose springs are located near the village of Cârcea, at the morphological contact between the Romanați Plain and the Jiu Plain. Its tributary on the right, the Bătrâna Valley (Ciliboaica), has a length of 6.8 km (GIS vectoring according to the topographic map 1: 50,000, 1991); the water level of the riverbed registers an important decrease due to the evaporation process. The basin is part of a protected area, "Preajba-Făcăi Lacustrine Complex" (28 ha) and is located in the area of Malu Mare, Preajba and Făcăi, southeast of Craiova, with a length of 3 km and a width of 40 -120 m. Different tourist facilities were built during 1976-1979, as this small tributary of the Jiu river was dammed. After this intervention on the minor riverbed, reservoirs appeared (with the role of separating lake depressions and surface spillway). The total surface of the reservoirs located along the Preajba Valley is 28 ha (GIS vectoring, ortophotos, 2009), but it is continuously reducing due to clogging with vegetal detritus and also due to the excessive development of the paludous macrophytes, especially in the area of the so-called "tail of the lake", which invades little by little the surface of the water. Between 1976 and 1979, 13 facilities were erected for touristic purposes along the Preajba Valley river and its tributary, the Bătrâna Valley (Ciliboaica), forming 13 reservoirs. The evolution of the reservoirs as indicated by the results of the researches carried out during 2008-2014 (by observations and ichthyologic collecting) revealed that, due to anthropogenic impact and intense eutrophication phenomena, only ten water accumulations still function. They vary in surface and depth and nine of them are on the Preajba Valley and one on the Bătrâna Valley (Fig. 1).

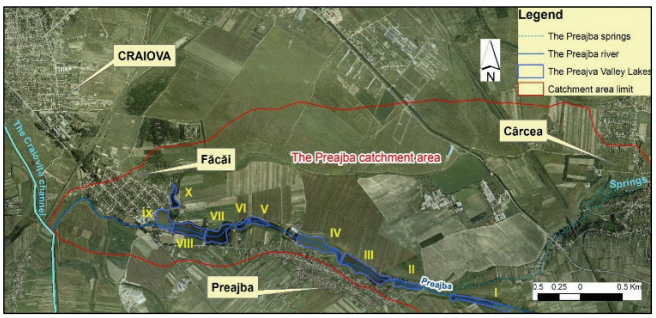


Figure 1. The Preajba River basin: springs, streams and reservoirs (GIS processing after ortophotos, 2009).

The study was conducted in the first quarter of 2012, covering the ten reservoirs, where sampling was carried out with the aim of obtaining ichthyologic material for subsequent parasitological studies (GOGA, 2009, 2010; GOGA & CODREANU - BĂLCESCU, 2011; GOGA & TUMBURESCU, 2011). Two parasites were identified; the first parasite, *Argulus foliaceus* L, 1758, is a hematophagous crustacean, which is called "fish louse", grayish-green, 5-10 mm in size, with the body well adapted to the parasitic life, present on the skin and gills of the fish *Perca fluviatilis* and *Cyprinus carpio*. The second haematophagous parasite is *Piscicola geometra* (Linnaeus, 1761), a common fish leech, which affects a wide range of host fish from both natural and cultured populations, causing hemorrhages and ulceration. This leech is part of the 60 species of freshwater and marine Hirudinea, referred to in the literature as fish parasites (GHITTINO, 1985), having a brown or green color. It was detected at *Carassius gibelio*, being up to 35 mm long and 3mm wide, with two well-shaped suckers (MUNTEANU & BOGATU, 2008). Generally, the color of these Hirudinea varies, depending on that of the host fish they parasite, the parasitation being carried out without intermediate hosts.

MATERIAL AND METHODS

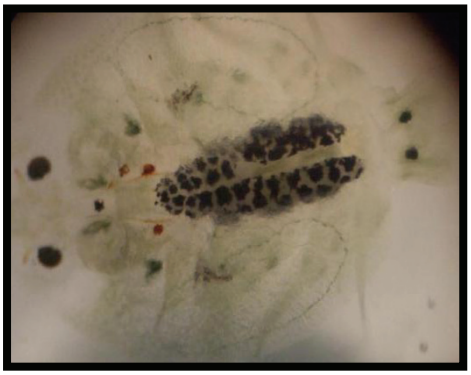
The collection of the material was carried out by sporadic seasonal fishing as a result of the monitoring of the fish populations in the small basin of the Preajba Valley, a tributary of the Jiu River. In the absence of the electronarcosis device, the three samplings were made with various monofilament nets with a length between 50 and 100 m and the mesh size of 40 - 60 mm, but also using fishing rod. Three samplings were carried out in May 2012 and 47 specimens of fish belonging to the species *Scardinius erythrophthalmus*, *Lepomis gibbosus*, *Abramis brama*, *Carassius gibelio*, *Perca fluviatilis* were caught. The parasite *Piscicola geometra* was identified only at *Carassius gibelio* from where 75 specimens fixed on different areas of the body were collected. Fish affected by piscicolosis showed bleeding lesions caused by suckers on the surface of the body. 42 specimens were sampled in the spring of 2009. The etiological agent, the crustacean *Argulus foliaceus*, was reported only to two fish species: *Perca fluviatilis* and *Cyprinus carpio*. The sampled fish material was subjected to a histological examination, respectively a clinical examination and a parasitological examination, at the laboratory of parasitology within Dolj Sanitary Veterinary. The fish were examined macroscopically, observing the parasitation place. The parasite was placed on a Petri dish in distilled water, being observed through transparency by means of the Olympus SZX7 stereomicroscope with 2x, 3.2x lenses, WHSZ 10x / 22 eyepiece and the optical microscope Olimpus BX 43 with 10x, 20x, 40x, WHN 10x / 22 lenses as a native blade – lamella preparation. After examination, the parasites were fixed in a 4% formaldehyde preservative liquid in a plastic container.

RESULTS AND DISCUSSIONS

In the 42 collected specimens belonging to 10 species of fish, the parasites are reported only for two species: *Perca fluviatilis* L, 1758 and *Cyprinus carpio* L. 1758, in the summer and autumn catch. The adult parasite *Argulus foliaceus* was identified in both the interior of the fish sample and on the body surface, where the lesions caused by the mouthpiece were presented as small necrosis ulcerations. On a Petri plate in distilled water, the crustacean was examined by transparency with the Olympus SZX7 stereomicroscope with 2x, 3.2x lenses, WHSZ 10x / 22 eyepiece, but also with the optical microscope Olympus BX 43, as a fresh blade – lamella preparation with 2x, 10x lenses and WHN 10x / 22 eyepiece, depending on the sample size. The characteristics of the abdominal lobes (their degree of division and appearance) were followed. The parasite was observed both ventrally and dorsally, especially the abdominal lobes for determining the species. In this case, the lobes are rounded and less divided, leading to the species mentioned above (Figs. 2, 3). The specialized literature mentions as a determining factor in parasite development, water temperature and pH. Being a thermophilic species, water samples were collected and measured at the time of collection. At a water temperature of 28 ° C and a maximum pH of 8, the crustacean has a quite rapid evolution, and when temperature falls below 16 °C, both adults and larvae slow down their metamorphosis (YILDIZI & KUMANTAS, 2002). Being a common parasite of freshwater fish, it was reported in the Danube Delta at Mila 23 on different species: common roach, asp, tench, bream, carp, pike, perch and sunfish, on rudd at Greaca, while at Calarasi, 1-3 specimens

were reported per host fish on ide, catfish and pike-perch. With a wide distribution, *A. foliaceus* Linnaeus is a parasite, which is found on many species of fish of different families. The parasite was first mentioned in Romania by Borcea in 1915, on catfish, pike, carp, plaice and perch; Dumitriu in 1937 noticed it on pike, tench and Prussian carp; Mark in 1929 on carp, Prussian carp and tench; Zemianovski in 1946 on carp and Radulescu in 1948 on Prussian 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 common bleak, the species *A. coregoni* not being noticed in Romania to that time. By locating them on the surface of the body, fins, gill arches, in host fish, parasites act mechanically and toxically, inoculating toxins and some infectious agents, which are considered the main vectors in the transmission of spring viremia virus of carp and bacteria that cause erythrodermatosis.

Although these parasites evolved subclinically without mortality, prevention and treatment measures were proposed to prevent their spreading. Thus, wood panels can be installed into the water so that the *Argulus foliaceus* parasite would lay its eggs and, as measures of combat, the literature recommends the treatment with Trichlorfon at a dose of 1g/6m^3 of water.



Figures 2, 3. *A. foliaceus* frontal, dorsal face image obtained by examination under optical microscope, 2x lens, 10x / 2 (Photo: Goga, 2009).

In the case of the second parasite, the literature (MUNTEANU & BOGATU, 2008) mentions a possible preference of the leech for the Cyprinidae species, especially for the fish populations in basins invaded by vegetation, without the participation of any intermediate host (VULPE , 2007). Regarding the pathogenicity of the disease, parasitic sources are elder fish, water, macrophytic vegetation and the substrate that leeches lay eggs (summer). An important factor in triggering parasitosis is water temperature; the invasion decreases in intensity when the water begins to heat up. 75 specimens were sampled from different body areas (gills, mucous membranes, pectoral fins, caudal fin, and ventral body scales), with lengths ranging from 15 mm to 35 mm. Fish affected by piscicolosis showed bleeding lesions caused by suckers on the surface of the body. Several Prussian carp specimens had at the level of the dorsal scales of the body, portions of tegument destroyed by the disappearance of the mucus layer, which determined the penetration of other pathogens, in this case *Saprolegnia* sp. Oomycetes. The diagnosis of parasitosis was performed relatively simply, by macroscopic examination revealing leeches (Fig. 4). Microscopically, the anterior extremity of the parasite is provided with a distinct sucker with four ocular black eyes (two pairs of eyes), the first two of which are larger, and the posterior extremity provided with a much larger and stronger sucker, with pigmentary spots arranged radially (VULPE, 2007). In the superficial layer of the body wall, small brown pigment cells are observed in the form of a light brown star, while darker brown cells are found in deeper layers.

The parasitosis evolved subclinically, given the small number of parasitized fish, but also its absence in subsequent catches. In the book "Research on the Parasite Fauna of the Danube Fish" (ROMAN, 1955), the leech was mentioned on the gills and on the skin of rudd and perch. In the Danube basin, it was also identified in pike, nase, chub and perch. In our country, *P. geometra* was also quoted by ANTIPA (1909) on carp, DUMITRU (1937) on pike, barbel and plaice, ZEMIANKOVSKI (1946) on carp (quoted by ROMAN, 1955). Frequently, by detaching from one fish and attaching to another, leeches are vectors of blood parasites such as *Trypanosoma* and *Cryptobia*.

In the Banat area, scientific literature (MUNTEANU & BOGATU, 2008) mentions prophylactic measures for the prevention of piscicolosis: the destruction of the macrophyte vegetation and the avoidance of the penetration of fish species from one basin into another, considering that the basins communicate with each other, as the surplus water flows from one basin to another through some surface dischargers.

Figure 4. *Piscicola geometra* highlighting to stereomicroscope (original).

CONCLUSIONS

• Through macroscopic and microscopic examinations, we were able to identify the crustacean *Argulus foliaceus* L, 1758 at *Perca fluviatilis* L, 1758 and *Cyprinus carpio* L. 1758.

• In terms of localization, ectoparasites have an increased intensity of over twenty individuals on the host fish, localized at the caudal peduncle, but also in the eyes and fins.

• We consider that water quality and temperature are factors that favour the parasite evolution.

• To prevent eggs from being deposited, it is advisable to remove the submerged vegetation from the substrate or to introduce wooden beams as an artificial substrate for egg deposition or the full drainage of basins and drying them for 24 hours for the destruction of eggs, larvae and adults.

• Another measure to combat this parasite is to eliminate the dead fish from the pools, and increase the water level to lower the temperature and stop the parasite.

• The control of this parasite must be based on preventive measures by avoiding to populate these basins with infected fish and periodic drainage of basins to prevent the development of new parasites.

• The collecting of the second hematophagous *Piscicola geometra* parasite was carried out in May 2012, the study site being the reservoirs along the Preajba Valley River.

• Parasitosis evolved subclinically, given the small number of parasitized fish, as well as the absence of leeches in the following catches.

• The sampled fish material was subjected to a histopathological examination, respectively a clinical examination and a parasitological examination, within the laboratory of parasitology of Dolj Sanitary Veterinary Directorate.

• The fish were examined macroscopically and the diagnosis of parasitosis was made relatively simple, observing with naked eye the parasite site of the leeches, but also microscopically and stereomicroscopically.

• Fish affected by piscicolosis present on the surface of the body bleeding wounds caused by the suckers of the leeches and the disappearance of the mucus layer caused the penetration of other pathogens, in the present case, *Saprolegnia* sp. Oomycetes.

• An important factor in triggering parasitosis is water temperature, but also the abundant submerged macrophyte vegetation.

• Destruction of over-developed macrophyte vegetation, avoidance of fish species penetration from one basin to another and treatment with Trichlorfon 1g / 6m3apa, are some recommended measures that can stop parasitosis.

Based on the performed researches, it was found that the two ectoparasites produced by crustaceans and Hirudinea were most easily diagnosed, especially due to the size of the parasites and the obvious lesions produced by them. We can conclude that although all the parasites presented in the paper had a poor pathogenic subclinical evolution, the absence or presence of parasite groups in these highly eutrophic small reservoirs can also be expressed by the specificity of the micro-habitat, submerged macrophyte vegetation and even mud and water temperature. Although the Valea Preajba river basin is not affected by significant 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 fields and the impurified waste water spill.

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