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PARASITISM WITH *ICHTHYOPHTHITIRIUS MULTIFILIIS* IN ROMANIA

PARAZITISMUL CU *ICHTHYOPHTHITIRIUS MULTIFILIIS* IN ROMANIA

G. DĂRĂBUȘ[1)], K.R. UJVARI[2)],
R.M. POPESCU[3)], Mirela IMRE[1),*)]

ABSTRACT

The world's growing demand for animal protein makes fish a solution for providing it. Therefore, the study of fish pathology, in general, and parasitic pathology, in particular, is an objective necessity given the economic damage they can cause. Among the parasitic diseases, ichthyophthyriasis is a protozoan that can cause significant damage. In the present study, whole fish samples from three categories of freshwaters were examined parasitologically using macro- and microscopic techniques. The samples were collected from rivers and their tributaries, lakes (natural, reservoir, and recreational), and breeding farms. From rivers, 22 fish species were examined, from lakes, 14 species, and from trout farms, three species. Parasitism with *I. multifiliis* was identified in river origin fishes in the native trout (*Salmo trutta fario*), schneider (*Alburnoides bipunctatus*), and European chub (*Squalius cephalus*), in lakes in the common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*), and from trout farms to brown trout (*Salmo trutta fario*), rainbow trout (*Oncorhynchus mykiss*), and brook trout (Salvelinus fontinalis). Lesions were located on the gills, integument, and fins. Parasitism was more intense in trout.

Keywords: *Ichthyophthirius multifiliis*, fish, rivers, lakes, trout

REZUMAT

Cerințele tot mai mari de proteină animală la nivel mondial fac ca peștii să reprezinte o soluție pentru asigurarea ei. De aceea, studiul patologiei peștilor, în general, și cel al patologiei parazitare, în special, reprezintă o necesitate obiectivă având în vedere pagubele economice pe care le pot produce. Dintre bolile parazitare ichtiophtirioza este o protozooză ce poate determina pagube importante. În prezentul studiu au fost examinați parazitologic, macro- și microscopic pești din trei categorii de ape dulci: râuri cu afluenții lor, lacuri (naturale, de acumulare și de agrement) și ferme piscicole. Din râuri au fost examinate 22 specii de pești, din lacuri 14 specii, iar din păstrăvarii trei specii. Parazitismul cu *I. multifiliis* a fost identificat în râuri la păstrăvul indigen (*Salmo trutta fario*), la beldiță (*Alburnoides bipunctatus*) și la clean (*Squalius cephalus*), în lacuri la crapul comun (*Cyprinus carpio*) și amur (*Ctenopharyngodon idella*), iar în păstrăvării la păstrăvul de munte (*Salmo trutta fario*), păstrăvul curcubeu (*Oncorhynchus mykiss*) și păstrăvul fântânel (*Salvelinus fontinalis*). Leziunile au fost localizate pe branhii, tegument și aripioare. Parazitismul a fost mai intens la peștii din păstrăvării.

Cuvinte cheie: *Ichthyophthirius multifiliis*, pești, râuri, lacuri, păstrăv

Globally, there is a growing need to provide more and more high-quality animal protein in correlation with the continuing growth of the human population. Fish is also known to be a dietetic food, as fish meat contains compounds important for human health: polyunsaturated fatty acids, omega-3 and omega-6 fatty acids, several essential minerals, antioxidants, and fat-soluble vitamins. In this context, the study of fish parasite pathology is an objective necessity given the economic damage caused to the fish industry in both natural and artificial systems. One of the protozoa that can cause morbidity and mortality is ichthyophyriasis. At the same time, besides the fact that the disease is found in fish from rivers, lakes, ponds, and fisheries, parasitism can also be found in fish in aquaria.

Ichthyophthiriasis is a disease of the tegument and gills found in various species of freshwater fish. The pathogen is *Ichthyophthirius multifiliis* (Fouchet, 1876) and can be located under the epithelium of the skin, fins, cornea, and gills. The protozoan has at least five different serotypes based on surface (Iag) antigens (10) or even more, as 17 Iag genes have been identified in the *I. multifiliis* genome (7).

Firstly, fish may exhibit signs of irritation, increased mucus, weakness, anaemia, loss of appetite, and decreased activity. A well-trained aquaculturist or aquarist will detect these changes before the fish's condition worsens and mortalities occur (6, 13). Because of the whitish lesions it produces under the fish's epidermis, ichthyophyriasis is also referred to as "white spot

1) University of Life Sciences "King Michael I", Faculty of Veterinary Medicine, Timișoara, Romania
2) DSVSA Harghita
3) DSVSA Gorj
*) *Corresponding author: mirela.imre@usvt.ro*

disease". When the spots have already appeared, the course of the disease is severe. Large infestations cause inflammation, irreversible changes in vital functions, and the death of the fish. The disease is widespread throughout the world and is one of the most dangerous parasites of freshwater, both flowing and standing (11).

The direct biological cycle has three phases: trophozoites, which can detach from the skin of the fish; cysts (tomonts), in the aquatic environment, whose nucleus divides and surrounds the cytoplasm, forming the third stage, the theronts or ciliospores (9, 25).

Juveniles are always more susceptible to *I. multifiliis* infection than other age groups. Once infected, fish acquire partial immunity, and on repeated exposure, infection is considerably weaker (12). The protective immune response occurs both at the affected site and systemically. IgT is one of the first adaptive immune molecules to react with the penetrating teront, having a central role in protection against the parasite (2).

Ichthyophyriasis can enter a lake, pond, or an aquarium with fish, various substrates, plants, decorations, structures, or equipment. Keeping substrates and live plants free of fish throughout the life cycle can prevent infection. Cleaning and disinfecting decorations or structures that come from another aquarium with fish is a useful prevention measure (13). Because large numbers of freshwater fish acquire this disease, the economic damage to aquaculture is high (20, 24). Based on the above, in this paper we aimed to highlight *I. multifiliis* parasitism in different aquatic locations in Romania.

MATERIALS AND METHODS

Location

Three categories of freshwaters were considered for the identification of *I. multifiliis* parasitism: rivers with their tributaries, lakes (natural, reservoirs, and recreational), and fish farms. Among the rivers, fish were collected from Olt (and 7 tributaries), Mures, Tarnava Mare, and Homorod (with 2 tributaries). From lakes, fish were collected from a natural lake (Red Lake), three reservoirs and a recreational lake. Fish from nine fish farms in the Transylvanian region and two fish farms in the Gorj area were collected for the identification of *I. multifiliis* parasitism.

Materials

Fish used to determine the prevalence of *I. multifiliis* parasitism were obtained randomly by angling and netting. For fish from rivers and lakes, the collection was done from different points in a completely random way. For fish from trout ponds, the sampling was done randomly following the detection of symptoms suggesting external parasitism: abnormal rubbing movements on the bottom or on the edge of the pond, refusal of food, and crowding in the stream.

A total of 147 fish of the following species were collected from the Olt River and its 7 tributaries: native trout (*Salmo trutta fario*), burbot (*Lota lota*), schneider (*Alburnoides bipunctatus*), the European chub (*Squalius cephalus*), rainbow trout (*Onchorhyncus mykiss*), brook trout (*Salvelinus fontinalis*), stone loach (*Barbatula barbatula*), spined loach (*Cobitis taenia*), gudgeon (*Gobio gobio*), common minnow (*Phoxinus phoxinus*), the Romanian barbel (*Barbus petenyi*), rutilus roach (*Rutilus rutilus*), gibel carp (*Carassius auratus gibelio*), perch (*Perca fluviatilis*), and the European bullhead (*Cottus gobio*) (Table 1).

From the Mures River, fish were cached from two areas: downstream of Izvoru Mures and in front of Remetea locality. A total of 88 fish of the following species were collected: the European chub (*Squalius cephalus*), common nase (*Chondrostoma nasus*), native trout (*Salmo trutta fario*), burbot (*Lota lota*), European grayling (*Thymallus thymallus*), gibel carp (*Carassius auratus gibelio*), common bleak (*Alburnus alburnus*), gudgeon (*Gobio gobio*), schneider (*Alburnoides bipunctatus*), rutilus roach (*Rutilus rutilus*), The Romanian barbel (*Barbus petenyi*), common barbel (*Barbus barbus*), common minnow (*Phoxinus phoxinus*), stone loach (*Noemacheilus barbatulus syn. Barbatula barbatula*), and European bullhead (*Cottus gobio*) (Table 1).

A total of 61 fish of the following species were collected from the Târnava Mare River: the European chub (*Squalius cephalus*), common nase (*Chondrostoma nasus*), native trout (*Salmo trutta fario*), perch (*Perca fluviatilis*), European grayling (*Thymallus thymallus*), common bleak (*Alburnus alburnus*), gudgeon (*Gobio gobio*), schneider (*Alburnoides bipunctatus*), European bullhead (*Cottus gobio*), common barbel (*Barbus barbus*), and the Romanian barbel (*Barbus petenyi*). Nine fish were caught from the river Homorodul Mare: the European chub (*Squalius cephalus*), common barbel (*Barbus barbus*), gudgeon (*Gobio gobio*), and schneider (*Alburnoides bipunctatus*). Twentyseven fish of the following species were collected from the Little Homorod River: European chub (*Squalius cephalus*), common barbel (*Barbus barbus*), common dace (*Leuciscus leuciscus*), schneider (*Alburnoides bipunctatus*), Romanian barbel (*Barbus petenyi*), gudgeon (*Gobio gobio*), European bullhead (*Cottus gobio*), and stone loach (*Noemacheilus barbatulus syn. Barbatula barbatula*) (Table 1).

From Lake Mesteacănul, six fish of the following species were caught: perch (*Perca fluviatilis*) and rainbow trout (*Onchorhyncus mykiss*). From the Sub Cetate reservoir, 42 fish were harvested from the following species: perch (*Perca fluviatilis*), common bleak (*Alburnus alburnus*), pond perch (*Lepomis gibbosus*),

Table 1

Parasitism with *I. multifiliis* in Romanian rivers

River	Olt	Mures	TARNAVA MARE	HOMORODUL MARE	HOMORODUL MIC
Fish species	**Positive / total number (%)**				
Native trout (*Salmo trutta fario*)	**1/23 (4.34)**	0/5	0/3	-	-
Burbot (*Lota lota*)	0/5	-	-	-	-
Schneider (*Alburnoides bipunctatus*)	0/16	0/15	**2/13 (15.38)**	0/1	0/8
European chub (*Squalius cephalus*)	0/14	0/27	0/12	**2/4 (50)**	0/2
Brook trout (*Salvelinus fontinalis*)	0/1	-	-	-	-
Rainbow trout (*Onchorhyncus mykiss*)	0/1	-	-	-	-
Stone loach (*Barbatula barbatula*)	0/9	-	-	-	-
Spined loach (*Cobitis taenia*)	0/4	-	-	-	-
Gudgeon (*Gobio gobio*)	0/17	0/8	0/11	0/3	0/ 5
Common minnow (*Phoxinus phoxinus*)	0/20	0/5	-	-	-
The Romanian barbel (*Barbus petenyi*)	0/12	0/2	0/2	-	0/4
Rutilus roach (*Rutilus rutilus*)	0/10	0/10	-	-	-
Gibel carp (*Carassius auratus gibelio*)	0/1	0/1	-	-	-
Perch (*Perca fluviatilis*)	0/4	-	0/3	-	-
European bullhead (*Cottus gobio*)	0/10	0/7	0/5	-	0/3
Common nase (*Chondrostoma nasus*)	-	0/4	0/5	-	-
Burbot (*Lota lota*)	-	0/2	-	-	-
European grayling (*Thymallus thymallus*)	-	0/1	0/4	-	-
Common bleak (*Alburnus alburnus*)	-	0/5	0/2	-	-
Common barbel (*Barbus barbus*)	-	0/1	0/1	0/1	0/1
Stone loach (*Noemacheilus barbatulus*)	-	0/1	-	-	0/3
Common dace (*Leuciscus leuciscus*)	-	-	-	-	0/1

and schneider (*Alburnoides bipunctatus*). From Frumoasa Reservoir, 21 perch (*Perca fluviatilis*), 76 rutilus roaches (*Rutilus rutilus*), and one pike (*Esox lucius*) were angled. Twelve fish of the species of rutilus roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), and pike (*Essox lucius*) were caught in the Red Lake. At the Sânpaul pond, 57 specimens of the following species were examined: common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), silver carp (Hypophthalmichthys molitrix syn. *Aristichtys nobilis*), zander (*Stizostedion lucioperca*), perch (*Perca fluviatilis*), gibel carp (*Carassius auratus gibelio*), carp bream (*Abramis brama*), rutilus roach (*Rutilus rutilus*), and pond perch (*Lepomis gibbosus*) (Table 2).

In the fish farms, the fish material was harvested by net, as follows: from Lacu Roșu Trout Hatchery: 36 rainbow trout (Oncorhynchus mykiss); from Bălan Trout Hatchery: 40 rainbow trout (*Oncorhynchus mykiss*) and 20 brook trout (*Salvelinus fontinalis*); 50 rainbow trout (*Oncorhynchus mykiss*) from the Sândominic - Bălan Trout Farm; 40 rainbow trout (*Oncorhynchus mykiss*) from the Ciaracio Trout Farm; from Vlahița Fishpond: 4 rainbow trout (*Oncorhynchus mykiss*); from Armaseni Fishpond 5 brook trout (*Salvelinus fontinalis*); Tismana Fishpond 1: 62 rainbow trout (*Oncorhynchus mykiss*); and Tismana Fishpond 2: 32 brown trout (*Salmo trutta fario*) (Table 3).

Methods

For examination, the fish were brought alive to the

Table 2

Parasitism with *I. multifiliis* in Romanian lakes

Lake	LACUL ROSU	MESTEAC ANUL	SUB CETATE	FRUMOASA	HELESTEU SANPAUL
Fish specie	**Positive /total number (%)**				
Schneider (*Alburnoides bipunctatus*)	-	-	0/6	-	-
Rainbow trout (*Onchorhyncus mykiss*)	-	0/1	-	-	-
Rutilus roach (*Rutilus rutilus*)	0/7	-	-	0/76	0/1
Gibel carp (*Carassius auratus gibelio*)	-	-	-	-	0/4
Perch (*Perca fluviatilis*)	0/4	0/5	0/30	0/21	0/1
Common bleak (*Alburnus alburnus*)	-	-	0/4	-	-
Pike (*Esox lucius*)	0/1	-	-	0/1	-
Pond perch (*Lepomis gibbosus*)	-	-	0/2	-	0/2
Common carp (*Cyprinus carpio*)	-	-	-	-	**3/21 (14.28)**
Grass carp (*Ctenopharyngodon idella*)	-	-	-	-	**1/3 (33.33)**
Silver carp (Hypophthalmichthys molitrix)	-	-	-	-	0/6
Silver carp (*Aristichtys nobilis*)	-	-	-	-	0/4
Zander (*Stizostedion lucioperca*)	-	-	-	-	0/11
Carp bream (*Abramis brama*)	-	-	-	-	0/4

Table 3

Parasitism with *I. multifiliis* in trout farms in Romania

Fish specie	*Oncorhynchus mykiss*	*Salvelinus fontinalis*	*Salmo trutta fario*
Fishery	**Positive /total number (%)**		
Păstrăvăria Lacu Roşu	**36/36 (100)**	-	-
Păstrăvăria Bălan	0/40	0/20	-
Păstrăvăria Sândominic Bălan	**50/50 (100)**	-	-
Păstrăvăria Ciaracio	**40/40 (100)**	-	-
Păstrăvăria Vlăhiţa	**4/4 (100)**	-	-
Păstrăvăria Armăşeni	**-**	**5/5 (100)**	-
Pescăria Tismana 1	**17/62 (27.41)**	-	-
Păstrăvăria Tismana 2	**-**	-	**9/32 (28.12)**

laboratory. Firstly, the fish were examined macroscopically for any external changes and lesions: excess mucus, spots, congestion, haemorrhages, necrosis, skin, fin, and gill sloughing. Then, microscopic examination of the pathological material collected from the regions or areas with macroscopic lesions was performed. For this, mucus and tissues were collected by scraping with a scalpel from the gills, fins, or tegument. From the collected material, a preparation was made between slides, with clarification using a drop of saline solution. The preparation was examined under a microscope with 10x, 20x, and 40x objectives.

RESULTS AND DISCUSSIONS

In the five rivers of Romania, 22 fish species were collected. Infection with *I. multifiliis* was identified in three rivers, namely the Olt River for native trout, the Tarnava Mare River for schneider, and the Homorodul Mare River for European chub (Table 1). Fourteen fish species were collected in the five lakes studied. *I. multifiliis* was found only in the Sanpaul Heliport, in two species: common carp and grass carp (Table 2). Concerning parasitism in Transylvanian fisheries, out of the six fisheries from which trout were collected, para-

sitism with *I. multifiliis* was identified in five. Two fisheries from the Gorj area (south-western Romania) were found infected with *I. multifiliis* (Table 3). *I. multifiliis* parasitism was diagnosed in fish of all three trout species farmed in Romania. From the results presented, a higher prevalence of *I. multifiliis* infection in fisheries can be observed, which can be explained by the higher concentration of fish per unit area compared to natural flowing or stagnant waters. However, it was difficult to make comparisons between the different situations encountered, as the number of fish harvested for some species was small. However, this presentation provides an evaluation of *I. multifiliis* parasitism in Romania in different rearing systems and locations, and the epidemiological situation found can provide data for eventual parasitological control.

Following studies carried out in 2001–2002, Cojocaru (2003) identifies *Ichthyophthirius multifiliis* parasitism in several fish species: comoun carp (*Cyprinus carpio*) and grass carp (*Carassius auratus gibelio*) in the Sacosu Turcesc and Timișoara fish accumulations; European bitterling (*Rhodeus sericeus amarus*); common rudd (*Scardinius erythrophtalmus*); and rutilus roach (*Rutilus rutilus*), fish collected from pools located in the Bega and Timiș river meadows (5).

Goga and Timburescu (2011) identify parasitism with *I. multifiliis* in a single gibel carp in one lake of the 13, formed by damming the Preajba Valley river, taken in the study (14). While Cojocaru and Munteanu (2002), in Romania, identify parasitism with *I. multifiliis* only in Ciprinidae (4), in the recent study, parasitism is also found in other species.

In trout farms in Romania, Călescu et al. (2011) identify six parasitic species, including *I. multifiliis*, with variable prevalence depending on farm, season, and age (3). In Romania, Vasile et al. (2019) identified by histopathological examination infection *with I. multifiliis* in one sturgeon species, namely *Acipenser stellatus*. They conclude that histological examination of the gills can be a good method of examination in mild infections before a severe disease outbreak (23). In a study from Brazil, the authors find that *I. multifiliis* is the dominant parasite in the fish *Astronotus ocellatus* (Perciformes, Cichlidae), with a prevalence of parasitic infection of 51.5% (21).

Another study conducted in southeastern Brazil on several species of juvenile freshwater fish (*Oreochromis niloticus, Ictalurus punctatus, Ctenopharyngodon idella, Cyprinus carpio, Astyanax bimaculatus,* and *Brycon amazonicus*) from a farm identified at least one parasite species based on macro- and microscopic gill lesions. *Ichthyophthirius multifiliis* was also found among them. The presence of these parasites on farms was attributed to predisposing factors such as stress, low water quality, temperature, life stage, or contact with other animals (birds and snails). The presence of the parasite in all fish species examined was due to a lack of sanitary and biosecurity control and the absence of good overall management practices on the farm (18). In Egypt, a 4% prevalence of *I. multifiliis* parasitism was identified in 300 *Oreochromis niloticus* fish collected from the Nile River (1).

In the literature, it is emphasised that the susceptibility of fish to parasitic infections may increase due to environmental stress generated by fluctuations in some water quality parameters (temperature, oxygen, pH, and nitrogen compounds) (15). Thus, these differences in *I. multifiliis* infection prevalence could be due to the varying environmental conditions in which they live. It is suggested that hatchery fish may represent a source of *I. multifiliis* for wild fish populations.

Table 4 presents the location of lesions in the seven fish species identified as being parasitized with *I. multifiliis*. Infected fish swam at the surface of the water, and because of itching, they rubbed against the walls of the tanks, were agitated, flocked to the banks, and

Table 4

Location of lesions in *I. multifiliis* parasitism

Specie	Location	Gills	Fins	Tegument
	Positive Fish/fish tested (%)			
Native trout (*Salmo trutta fario*)	River	1/1	0/1	0/1
	Fishery	9/9	7/9	9/9
Rainbow trout (*Onchorhyncus mykiss*)	Fishery	95/97	90/97	93/97
Brook trout (*Salvelinus fontinalis*)	Fishery	5/5	3/5	4/5
Schneider (*Alburnoides bipunctatus*)	River	2/2	0/2	1/2
European chub (*Squalius cephalus*)	River	1/2	0/2	2/2
Common carp (*Cyprinus carpio*)	Lake	3/3	3/3	1/3
Grass carp (*Ctenopharyngodon idella*)	Lake	1/1	0/1	1/1
TOTAL		117/120 (97.5)	105/120 (87.5)	111/120 (92.5)

in more severe cases, had white spots on the integument or even tegument sloughing and gill necrosis. From Table 4, we can see a wider localization of lesions in the fish in all three main areas of choice, i.e., in descending order: gills, tegument, fins. Gill localization can lead to asphyxia and death. The same locations are found in *Cyprinidae* by other authors (8, 16, 19).

CONCLUSIONS

Parasitism with *I. multifiliis*, in Romania, has been identified in fish from all aquatic environments studied: rivers, lakes, and artificial environments. In rivers, parasitism was found in native trout, schneider, and European chub; in lakes, in common carp and grass carp; and in fisheries, in native brown trout, brook trout, and rainbow trout. The prevalence of parasitism was higher in trout. In decreasing order, parasite lesions were found on the gills, tegument, and fins.

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