THE ROMANIAN ACADEMY INSTITUTE OF BIOLOGY BUCHAREST



PHD THESIS

SUMMARY

STUDIES ON THE ICHTHYOFAUNA OF PREAJBA VALLEY HYDROGAPHICAL BASIN AND ITS PARASITE LOAD

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KEY WORDS

ICHTHYOFAUNA PREAJBA VALLEY HYDROGRAPHICAL BASIN PARASITE LOAD PREAJBA-FĂCĂI LACUSTRINE COMPLEX ICHTHYOPARASITOLOGICAL LITHOLOGICAL SUBSTRATUM MORPHOMETRY OF THE RESERVOIRS ECTOPARASITE **ENDOPARASITE** LESIONS SCRAPING VISUALIZATION **INTERMEDIARY HOST** COMPLEMENTARY HOST FINAL HOST PARATENIC HOST ETIOLOGY PATHOLOGY SUBCLINICAL PSEUDOSCOLEX ICHTHYOPHAGOUS BIRDS

INTRODUCTION

The PhD thesis entitled *Studies on the ichthyofauna from Preajba Valley hydrographical basin and its parasite load* synthesized the results of my personal research related to the *knowledge of the composition of the fish populations* from the ten small reservoirs located within Oltenia Plain, reservoirs that are part of the site "*Preajba-Făcăi Lacustrine Complex*" (which according to the European and national legislation was designated protected aquatic area under Law 5/2000, G.U.D. 57/2007 and Law 49/2011), along with *the knowledge of the structure and distribution of parasites in fish* within Preajba Valley hydrographical basin.

The work contains 278 pages, 32 tables and 220 figures (50 maps, 56 graphs, 114 photos). The bibliography includes 237 titles of national and foreign studies.

The PhD thesis aimed at achieving important objectives related both to the theoretical aspects (known in the specialized literature), but also to the practical aspects (original research). The theoretical elements are rendered in the beginning of the study and they are represented by information including a brief history regarding ichthyologic and parasite research on freshwater fish in Romania, the research methods used for collecting fish samples, as well as for the ichthyoparasitological diagnosis, the physical-geographical, morphological and hydrological aspects of the Preajba Valley hydrographical basin.

The original aspects of the study make reference to the determination of fish population and seasonal parasite load of the fish present in the small reservoirs located along the Preajba Valley River in the period 2008-2014.

As a conclusion, my own research had as main objectives the correct identification of fish species in the reservoirs, as well as the seasonal presence of certain parasites and diagnosis of parasitoses in due time in order to adopt viable control measures. The approach of these two aspects in my PhD thesis was due to the accessibility provided by the specialists of SHFCA Dolj, for sampling the biological material from the reservoirs, but also to the professionalism of the specialists from the parasitology laboratory of the Sanitary Veterinary Directorate Dolj, who supported me to diagnose correctly and in a short time the parasitoses that evolved subclinically in the sampled fish.

The study represents an important contribution to the knowledge of Preajba Valley hydrographical basin taking into account the original tourism purposes (the reservoirs were built in the period 1976-1979), the poor maintenance of the lacustrine ecosystem starting with the '90s and the present interest of the authorities in maintaining its status of protected area.

The approached issues (ichthyofauna and parasite load) and the study area (Preajba Valley hydrographical basin) were chosen, on the one hand, due to my personal experience gained in the field of ichthyology at the Museum of Oltenia, and, on the other, due to the status of natural protected area of national interest and the challenges of the urban and rural environments from its proximity. The importance, novelty and originality the research brings to this field of study and the applied methodology can be extrapolated to other limnological areas as case studies.

The study is structured on six chapters. The **first chapter** is a brief overview of the previous research studies on fish parasites and ichthyofauna of Romania. There is rendered a short history of ichthyofaunistic studies where the most representative research on fish fauna of Romania is presented. Thus, in 1909, Gr. Antipa published *The ichthyologic fauna of Romania*, where there are presented the species of fish from Romania, followed by an informational material related to their biology and distribution; in 1916, the same author published *Fishery and fishing in Romania* and, in 1932, he set up the Institute of Biological Oceanography in Constanta (ANTIPA 1895; 1909; 1941). I. Borcea, founder of the Marine Zoological Station from Agigea, achieved numerous studies on the biology and migration of fish species in the Black Sea in the period 1926-1937 (BORCEA, 1930). In 1930, Th. Buşniţă published a study on the zonal distribution of fish in Romania.

In 1952, S. Cărăuşu published a treatise on freshwater and marine fish in Romania. In 1957, C. S. Antonescu published *Fish from the waters of P.R.R.* (ANTONESCU, 1967). In 1957, Elena Dumitrescu, P. Bănărescu and A. Stoica described a new genus for Romania (*Romanichthys*), with the species *R. valsanicola* identified on the upper course of the Vâlsan River. In 1963, in collaboration with T. Alexandrescu, they published the *Fish Atlas of P.R.R.*, which was well promoted (BUŞNIŢĂ, 1967). Petru Bănărescu (1964) published a Romanian monograph of the ichtyofauna "fascicle Pisces - Osteichthyes (bony and ganoid fish)", Vol. XIII of the Fauna of Romania. Aurelia Nicolau, Gh. Brezeanu, Maria Caloianu - Iordăchel, A. Buşniţă published a study on *The artificial reproduction and development in fish*, which refers to the reproduction and development of the main freshwater and migratory fish species, with significant value for Romania (NICOLAU *et al.*, 1973). Several works with hydrobiological character contain data on the taxonomy, ecology and distribution of fish species of the Romanian fauna (OŢEL & BĂNĂRESCU, 1985; MANEA, 1985; OŢEL *et al.*, 1993, 1994; OŢEL, 1999; MEŞTER *et al.*, 2003; NALBANT, 2003; RANG & URECHE, 2003; BĂNĂRESCU, 2004; IFTIMIE, 2004; OŢEL *et al.*, 2004; URECHE *et al.*, 2004; GAVRILOAIE *et al.*, 2007; OŢEL, 2007).

The research on the parasites of freshwater fish and the development of parasitology as a science began in the nineteenth century, along with the research achieved by V. Babes in this field, who discovered Babesia. I. Ciurea is considered the founder of the Romanian ichthyoparasitology, but he made himself remarked especially due to the elucidation of the biological cycles of Trematoda: Heterophyidae, Opistorchiidae, Clinostomidae, Strigeidae. In 1960, ELENA ROMAN - CHIRIAC published the Fauna of P.R.R. Plathelminthes describing numerous parasites of fish. Between 1942 and 1944, within the framework of the Institute for Fisheries Research, under the guidance of I. Rădulescu, there are set up the foundations of the Romanian school of ichthyopathology. Among the researchers who have dealt in particular with the study of parasites and diseases of the fish from the Black Sea we mention: I. Borcea (1913), Aurelia Cărăușu Bosânceanu (1934-1959), M. Băcescu (1934-1936), Z. Popovici (1945), I. Rădulescu (1942) (ROMAN, 1955). În the period 1996-1998, Gabriela Munteanu, Veronica Cristea, I. Grecu parasitologically analysed the aquaculture fish species from certain farms from Romania. In 2007, V. Vulpe published the study Parasites and parasitoses of freshwater fish, where there are presented health issues of fish stocks and their habitat, but also the parasitic fauna of freshwater fish (VULPE, 2007); in 2008, D. Bogatu & Gabriela Munteanu published the *Ichthyopathology Treatise*, which lists general notions of pathology, morphology, parasitology and immunology necessary for understanding the diseases affecting fish (BOGATU & MUNTEANU, 2008). In 2014, as an objective of the activity of diagnosis, prevention and control of fish diseases. P. Dăscălescu and Mihaela Costea published the treatise Diseases of aquaculture fish species. Diagnosis, treatment and biosecurity methods (DASCALESCU & COSTEA, 2014).

The second chapter covers the research methods used both for collecting fish samples and ichthyoparasitologic diagnosis. During the research of Preajba Valley Hydrographical Basin, the sampling of the ichthyologic material was done seasonally, by means of various monofilament nets of different lengths, but also by fishing rods by amateur fishermen from the area. With regard to the methods of ichthyoparasitologic diagnosis, in order to correctly establish the ichthyoparasitologic diagnosis, besides the necessary equipments (ichthyoparasitologic kit, stereo microscope with Olympus photo camera, Olympus BX 43 digital microscope, analytical balance and reagents), it is also necessary to follow certain stages related to different *epidemiological, clinical* and *laboratory* aspects (naked eye *macroscopic examination* of fish, body surface, eyes and gills for highlighting cysts, ulcerations, damaged fins, lack of scales or parasites; *microscopic examination* of the fresh biological material, as well as of the coloured material sampled from the gills or skin, and *stereomicroscopic examination* in incident and reflected light of the parasitized anatomical parts).

In the **third chapter** there were rendered the physical-geographical, hydrological and morphological characteristics of Preajba Valley Hydrographical Basin. In the short description of

the geographical location, there are presented the elements according to the Atlas of Water Cadastre of Romania (1992). Thus, Preajba Valley hydrographical basin, with a surface of 15 sq. km, is located in Dolj County, 6 km south of Craiova, at the contact between the Getic Piedmont and Oltenia Plain. The main water course is represented by the Preajba Valley with a length of 9.6 km that springs in the proximity of Cârcea settlement, in the morphological contact area between Romanați Plain and the Jiu alluvial plain; its tributary on the right, Bătrâna Valley (Ciliboaica), has a length of 6.8 km (GIS vectorization after the topographical map 1: 50,000, 1991) and its bed considerably reduces due to evaporation. 1,200 meters before its confluence with the Jiu, the stream receives Craiovița sewer; thus, it crosses all the landforms located on the left of its collector on east-west direction. The basin is part of the protected area "*Preajba-Făcăi Lacustrine Complex*" (28 ha) and is located in the area of Malu Mare, Preajba and Făcăi localities, southeast of Craiova municipality.

Tourism facilities were built during 1976-1979 by damming this small tributary of the Jiu, and, through this intervention upon the minor stream bed, there appeared 13 reservoirs with dams (separating the lacustrine depressions) and surface weirs. The purpose of this complex was to create a recreational space for the residents of Craiova. In the period 2008-2014, due to human impact and intense phenomena of eutrophication, some of the reservoirs disappeared and, presently, there are only ten reservoirs, with a variable surface and depth, nine on the Preajba Valley and one on Bătrâna Valley. The lithologic substratum of the studied area, at the contact between Oltenia Plain and the Getic Piedmont, determines the presence of well-developed soils with different textures and predominantly agricultural use.

As agriculture developed, erosion intensity increased particularly because of deforestation and cultivation of slopes; thus, erosion became one of the main causes of agricultural and forest land degradation on the Preajba Valley River. Preajba-Făcăi Lacustrine complex presents a temperate continental climate. The prevailing climatic influences are the subtropical ones of Saharan or continental origin, causing extreme summer temperatures up to 35-38°C and very cold winters. Consequently, the annual thermal amplitude is 25°C, a value that directly influences the evolution of the morphometric elements of the lacustrine complex (VLĂDUȚ, 2003). In the study area, the climate is warmer than that characteristic to the northern half of the territory of Oltenia and is particularly favourable to biotic processes, especially in summer; the presence of water-covered areas plays an important role in moderating the local microclimate (MARINICĂ & CHIMIȘLIU, 2009; MARINICĂ & MARINICĂ, 2010; MARINICĂ *et al.*, 2013). Climate change occurred in this area by the increase of temperature values, the increase of the frequency of warm winters, earlier springs, heat wave, increased number of tropical days and nights.

The hydrographical network is represented by the Preajba Valley River, which receives only one tributary, Bătrâna Valley (Ciliboaica), and by the reservoirs numbered from I to X from upstream to downstream. The lacustrine complex is supplied by strong springs located at the morphological contact between Romanati Plain (Leu-Rotunda Field) and the high terrace of the Jiu, but also by other categories of springs, namely the valley springs emerging at the contact between the slope and reservoirs and by the limnocrene springs from the aquifers. Thus, the discharge is permanent and constant, especially because the reservoirs communicate with each other on the principle of communicating vessels by means of the weirs placed in the dam. The morphometrical elements of the lacustrine units are the perimeter, length and tortuosity coefficient. Reservoir III has the largest perimeter (1,510 m) compared to Reservoir VI with the smallest perimeter of 450 m. Reservoir I has the biggest length (706 m), while the lowest is registered in case of the same Reservoir VI (185 m). The tortuosity coefficient is maximum for Reservoir I (2.26) and minimum for Reservoir IX (1.14). Vegetation plays an important role in Preajba lacustrine system, favouring water infiltration and reducing surface runoff. Currently, the interfluve of the Preajba Valley is fully used as farmland, although the soil type and slope inclination attest that this area used to be initially covered by forest. Consequently, the current steppe is anthropogenic, resulting from people's need for the expansion of crops to ensure food resources; it is not about the need of the inhabitants of the nearby rural settlements but especially of those from Craiova. The primary micro and macrophytic producers, the planktonic and benthonic consumers are essential parts of biological production as an important element for the structure of ichtyofauna of the studied reservoirs. There are mentioned 36 species of paludous and aquatic macrophytes and the presence of phytoplankton (78 species) and zooplankton (65 species) represent the major component in the development of the ichtyofauna (ZINEVICI & PARPALĂ, 2007; BREZEANU *et al.*, 2011; CIOBOIU, 2014).

There were identified 18 species of gastropods, intermediary hosts in the evolutionary cycle of platyhelminth worms, parasites encountered in lake fish (CIOBOIU 2002, 2014; CIOBOIU & BREZEANU, 2009). With regard to the qualitative aspects of the water, by taking into account the seasonal analysis of the main 11 physico - chemical indicators and the specificities of biotopes and biocoenoses (sandy-oozy bottom, rich in organic substances resulted from the degradation of the excessively developed macrophytes and of the phytoplankton in summer) and the sources of the springs from the shore and bottom of the reservoirs, we obtained a projection on the environment that determines water chemistry.

According to the quality standards for surface water, the ten reservoirs located on the Preajba Valley fall into the category of mezosaprobe quality II, being suitable for fishing but also for tourism and leisure. Over time, the water chemistry of this basin has been influenced by the poor management of the maintenance works related to clogging and limiting the expansion of the macrophyte vegetation, which abundantly developed in the area called the "end of the lake." Most of the times, the main factor in the transmission of the diseases from sick to healthy but sensitive fish is represented by water and the substratum of these basins, where parasites find conservation conditions and an optimal climate for multiplication. In order to protect and maintain biological diversity and natural resources, the site "*Preajba-Făcăi Lacustrine Complex*" was designated natural protected area under Law No. 5/2000, G.U.D. 57/2007 and Law No. 49/2011 for species of flora and fauna that are vulnerable (according to File of E.P.A. Dolj and the in Red Book of Vertebrates of Romania (2005), IUCN Red List, 2008).

In the **fourth chapter**, there is presented the original research on fish fauna of the small reservoirs along the Preajba Valley River (2008-2014). Although it is located in Dolj County, 6 km south of Craiova, within Oltenia Plain, it is an area that was less studied in terms of ichthyofauna and, thus, the research in this field tries to cover the existing gap. The monitoring of the ichtyofauna from the ten reservoirs is important because this lacustrine complex built in the immediate proximity of Craiova is a tourist area where sport fishing is practiced. The 832 sampled individuals weighing between 90 g and 422 g were personally caught by means of monofilament nets, but also by amateur fishermen. The ichthyologic material was determined from the taxonomic point of view, each species being graphically represented according to the sampling location (reservoirs). There were identified 14 fish species belonging to four orders and six families. The determination of the sampled species was made based on the comparison with the monograph of the ichtyofauna "Pisces Fascicle - Osteichthyes (bony and ganoid fish)", Vol. XIII of the Fauna of Romania, author Petru Bănărescu (1964), actualized according to the current nomenclature, valid according to the latest systematic reviews (NALBANT, 2003; OTEL, 2007; NELSON, 2006; www. Fish Base, version 2004; www. Catalogue of fishes, version May 2006; Catalogue of Fishes - Eschmeyer, William N., 1998; Fauna Europaea). Each species is systematically described, namely: scientific name, popular name, size and weight, geographical distribution (in Oltenia), parasites mentioned in the consulted literature and in the research area and briefings on the ecology of each species in relation to the aquatic habitat and conservation status under the IUCN Red List (2008). The ichthyologic material was also ecologically analysed with regard to the biocoenotic quantitative indexes (frequency and abundance). Of the fourteen determined species, 10 present a phytophagous, detritophagous, benthophagous trophic regime and only 4 an ichthyophagous trophic regime (predators).

According to achieved research it resulted that the distribution of fish species is characteristic to small reservoirs formed by damming a river course. Once this watercourse was

dammed for tourist purposes and to encourage sport fishing, SHFCA Dolj populated the reservoirs with mainly stagnophilous fish stocks, which are mostly consumers of vegetarian food and characterized by an increased prolificacy and limited requirements on oxygen consumption such as euryoxibiont species (*Carassius gibelio*).

The number of species varies from one reservoir to another and gradually increases in the reservoirs located downstream, because they pass with the water surplus from one reservoir to another through surges and surface weirs, which are at the edge of each reservoir. The 14 fish species were classified into 4 orders and 6 families: Ord. Esociformes, Fam. *Esocidae* (1 species *Esox lucius*); Ord. Cypriniformes, Fam. *Cyprinidae* (7 species *Cyprinus carpio, Carassius gibelio, Alburnus alburnus, Pseudorasbora parva, Rutilus rutilus, Scardinius erythrophthalmus, Abramis brama*), Fam. *Cobitidae* (1 species *Cobitis taenia*); Ord. Siluriformes, Fam. *Siluridae* (1 species *Silurus glanis*); Ord. Perciformes, Fam. *Percidae* (3 species *Perca fluviatilis, Sander lucioperca, Gymnocephalus cernuus*), Fam. *Centrarchidae* (1 species *Lepomis gibbosus*).

The last restocking of these reservoirs was done in 2006 with fish (Prussian carp, carp, perch, roach and perch sunfish) brought from the ponds from the Danube Alluvial Plain and fisheries.

According to the interpretation of the qualitative biocoenotic indexes we may conclude that the species *Carassius gibelio* is **euconstant** in the reservoirs where (**F-86.8%**), followed by *Perca fluviatilis* (**F-37.75%**) and *Lepomis gibbosus* (F-30.2%) as accessory species and accidental species: *Scardinius erythrophthalmus*, *Lepomis gibbosus*, *Pseudorasbora parva*, *Cobitis taenia*, *Rutilus rutilus*,*Gymnocephalus cernuus*,*Alburnus alburnus*, *Cyprinus carpio*, *Abramis brama*, *Silurus glanis*, *Sander lucioperca*, *Esox Lucius* characterized by a reduced percentage of the biocoenotic indexes (**F**<25%).

Although the structural and functional characteristics of this area are similar lithological substrate, (hydrographical network, water chemistry, climatic potential. morphometric characteristics trophic structure), the composition of fish stocks is different. There are species that were identified in only one reservoir, such as Silurus glanis and Esox lucius (reservoir X), Alburnus alburnus and Rutilus rutilus (reservoir VII); in two reservoirs (reservoirs VII and X) Gymnocephalus cernuus and Sander lucioperca (reservoirs VII and VIII) or in all ten reservoirs, such as the species Carassius gibelio. The frequency and abundance of the species in these reservoirs is induced by the specificity of the microhabitat as these species prefer the reservoirs where they were identified. The distribution of the species is more or less uniform, except for the species Silurus glanis, Esox lucius, Alburnus alburnus, Rutilus rutilus, Gymnocephalus cernuus with preference for certain reservoirs (VII, VIII, X). Taking into account their previously rendered ecological preferences, most species present in the reservoirs are specific to stagnant and reophilous ecosystems, cosmopolitan, with high resistance to the variations of an eutrophic environment.

In **chapter five**, there are presented original research on the parasites identified at the fish populating the Preajba Valley reservoirs. The research of the fish parasites was done by macroscopic examination (dissection), according to the standards set in ecological parasitology following: detection of parasites on the surface / inside of the fresh biological material, the number of all parasites detected in each host fish, as well as the correlation of parasites with their living environment. The samples were examined microscopically, as fresh and coloured smear (Giemsa, malachite green) and stereomicroscopically using transparent casts.

The 13 identified parasites are part of taxonomic groups belonging to 8 phylums, 11 classes and 12 orders, such as: Oomyceta, Ciliata, Myxosporidia, Monogenea, Trematoda, Cestoda, Acanthocephala, Nematoda, Annelida and Crustacea. The taxonomic classification of the parasites was achieved by consulting specialized guides elaborated by the authors Paperna (1979, 1980), Elena - Roman Chiriac (1955, 1960) Dăscălescu & Costea (2014), along with the treatises of Schäperclaus (1979), Rădulescu (1976) and Munteanu & Bogatu (2008) and by accessing online web files, which refer to the taxonomy and biology of freshwater fish parasites.

The parasites are presented systematically according to their taxonomic classification. Thus, **Oomyceta** (Class Oomycota, Order Saprolegniales) are represented by *Saprolegnia* *parasitica*, species considered to have a significant ichthyopatological importance. It develops on skin, gills and rarely internal organs. It parasitizes freshwater fish and other vertebrates. The macroscopic and microscopic examination revealed the presence of hyphae with cotton-like appearance in two fish species (*Pseudorasbora parva* and *Carassius gibelio*).

Ciliophora (Class Oligohymenophorea, Order Ophryoglenina) *Ichthyophthirius multifiliis* and (Class Phyllopharyngea, Order Chlamydodontida) *Chilodonella cyprini* are cosmopolite ectoparasites on the skin, gills and fins of the freshwater and aquarium fish. The presence of whitish nodules allowed the microscopic visualization of trophonts from the tegumentary scrapings. *Ichthyophthirius multifiliis* was reported in the species *Carassius gibelio* and *Chilodonella cyprini* observed in *Cyprinus carpio* by microscopic examination of the mucus sampled from the skin and gill scrapings.

Myxosporidia (Class Myxosporea, Order Bivalvulida) are represented by a single species *Myxobolus* sp., which parasitizes a wide range of host fish. The parasite was reported in the species *Abramis brama* and *Carassius gibelio* by microscopic examination of the suspension obtained by grinding the cranial and gill cartilage in distilled water. May Grünwald - Giemsa and malachite green staining of the preparation allowed the microscopic visualization of at least two oval spores with two equal capsules and of the amoeboid germ with the iodophilous vacuole less highlighted, so we could not determine the species.

The **Monogenea** (Class Monogenea, Order Monopisthocotylea) of freshwater fish are mostly ectoparasites on fish gills and skin. The clinical examination revealed mucus hypersecretion, bruises and ulcerations at the level of gills and skin. The monogenean *Dactylogyrus* sp., signalled by us at two fish species *Carassius gibelio* and *Cyprinus carpio* by laboratory examination, revealed the morphology of the opisthaptor. The life cycle of the parasite is direct, without intermediary hosts.

Trematoda (Class Trematoda, Order Strigeidida) are represented by *Clinostomum complanatum*, which, in their biological cycle, present as a first intermediary host a gastropod; infested fish can be intermediary or definitive hosts. The clinical examination allowed the observation of some ovoid white-yellowish formations in the branchial arches and lamellae of the species *Perca fluviatilis*, showing preference for well-vascularized areas with intense activity. Rolled metacercariae were examined microscopically highlighting the presence of two suction cups, one being large and located ventrally. Trematoda worms use predatory fish as a second intermediary host (complementary host). The Cercariae that reach their body using gastropods or bivalves (first intermediary host), trophic base for these fish, lose their tail, form a cyst and turn into metacercariae.

In our case, the definitive host is represented by ichthyophagous birds that are present in the study area. When consuming fish infested with metacercariae, the parasites attach to the larynx-tracheal mucosa causing an acute dyspneic syndrome.

Cestodes (Class Cestoda, Order Pseudophyllidea) present in fish hosts from the studied reservoirs parasitize both as adults (*Triaenophorus nodulosus*) and as larvae (*Diphyllobothrium latum*). As adults, they have as host numerous vertebrates, while as larvae both invertebrates and vertebrates. In the biological cycle, there are involved one or many intermediary hosts and there is also an accumulation host. The first intermediary host is usually a crustacean or an Oligochaeta. In the studied reservoirs, the cestodes were sampled from only one species *Perca fluviatilis*, from the intestine (*Triaenophorus nodulosus*) and musculature (*Diphyllobothrium latum*).

Acanthocephala (Class Palaeacanthocephala, Order Echinorhynchida) are represented by *Pomphorhynchus laevisla* at the species *Cyprinus carpio*. Fish parasitation is made by eating crustaceans together with acanthella. In our case, *Cyprinus carpio* is only an accumulation host; the infested larvae of acanthella containing all the organs of an adult cross the intestinal wall, enter the body cavity and form a cyst into the internal organs (liver). They transform in adult worms when they reach the intestine of the definitive host (predatory fish, ichthyophagous birds, mammals).

The identification of the endoparasitic **nematodes** (Class Secernentea, Order Ascaridida) was achieved directly by macroscopic examination, observing the larvae puncturing the

abdominal wall, but also by microscopic examination analysing fresh preparations. The parasite *Eustrongylides excisus* was sampled from three fish species *Sander lucioperca*, *Silurus glanis* and *Perca fluviatilis*. The fish infected with this nematode show four stages of the parasite development that interpose between the egg and adult stages. The eggs laid by the adult parasites in the body of the definitive host (in this case ichthyophagous birds present in the area: little egret, herons, cormorants, swans), get into the water with the host excrements, hatch and release a swimming larva, which is ingested by an Oligochaeta of the genus Tubifex, where the larval stages (L2 and L3) develop. Small fish such as *Alburnus alburnus* (bleak) and *Pseudorasbora parva* (stone maroko) were found ingested in the stomach of the infested fish; thus, we concluded that they could be facultative hosts for this parasite. At the level of the three predatory fish species, there develops the invasive larvae stage L4, waiting to be ingested by the final host (inthyophagous birds).

Hirudinea (Class Clitellata, Order Rynchobdellida) represented by the species *Piscicola gemetra*, the most common leech affecting a wide range of host fish in natural and aquaculture populations, cause bleeding and ulcerations. It may also act as vectors in the transmission of certain diseases such as spring viraemia of carp and cryptobiosis. The preference for stagnant waters full of vegetation makes Preajba Valley hydrographical system be its favourite habitat. *Piscicola geometra* was detected by clinical examination at the species *Carassius gibelio*. By performing native preparations there were revealed the two suction cups with the four ocular spots and the pigmentation fields radially disposed. They are hermaphrodite without intermediary hosts and the cocoons are deposited on the bottom of the reservoirs or on aquatic plants.

Crustaceans parasitize the fish tegument and gills. The two species of crustacean that we sampled from the fish species *Cyprinus carpio*, *Carassius gibelio*, *Perca fluviatilis* and *Carassius auratus auratus* are *Argulus foliaceus* (Class Maxillopoda, Order Arguloida) and *Lernaea cyprinacea* (Class Maxillopoda, Order Cyclopoida). At the species *Argulus foliaceus* both sexes parasitize or only female in the case of the crustacean *Lernaea cyprinacea*, the male being a free form. The shape of the body, the character of the fixation device, and the shape of the ovigerous sacs represents taxonomic criteria in determining species. They feed on mucus, damaged epithelial cells, blood, causing bleeding, inflammation and ulcerations of the affected tissue. The most intense parasitation with *Lernaea cyprinacea* was found in the ornamental species *Carassius auratus auratus* from the reservoirs.

In **chapter six** there are rendered the ecological peculiarities regarding the ichthyofauna of Preajba Valley hydrographical basin and its parasitic load. There are issued taxonomic considerations on ichtyofauna and distribution of parasites and parasitized host species and the dependence among the parasite, host and type of food.

The 14 described species are cited in the specialized literature both in Oltenia and Romania. The greatest share is held by the order *Cypriniformes*, family *Cyprinidae* (7 species) and family *Cobitidae* (1 species), followed by the order *Perciformes* with two families: family *Percidae* (3 species) and family *Centrarchidae* (1 species) and the orders *Esociformes* and *Siluriformes* (with 1 species each).

Ectoparasites are predominant at the fish species from Preajba Valley reservoirs and they are represented by seven species (*Saprolegnia parasitica*, *Ichthyophtirius multifiliis*, *Chilodonella cyprini*, *Dactylogyrus* sp., *Piscicola geometra*, *Argulus foliaceus*, *Lernaea cyprinacea*); most commonly, they fix on the tegument and gills. They are followed by endoparasites found in the intestines (3 species: *Pomphorhynchus laevis*, *Eustrongylides excisus*, *Triaenophorus nodulosus*), viscera: mesentery (one species *Eustrongylides excisus* - larvae) and liver (one species: *Pomphorhynchus laevis*), muscles (two species: *Eustrongylides excisus* - larvae), cranial and branchial cartilage (one species: *Myxobolus* sp.).

CONCLUSIONS

- 1. The sampling of the ichthyologic material was made seasonally during the period 2008 2014 (except for the hiemal season) being investigated **832 fish specimens** from the ten reservoirs.
- 2. The ichthyofauna of Preajba Valley hydrographical basin includes **14 species** belonging to **four orders** and **six families**.
- 3. The 14 described species are mentioned in the specialized literature both in Oltenia and Romania. The greatest share is registered by the order *Cypriniformes* with the family *Cyprinidae* (seven species) and the family *Cobitidae* (one species), followed by the order *Perciformes* with two families: *Percidae* (three species) and *Centrarchidae* (one species) and the orders *Esociformes* and *Siluriformes* (one species each).
- 4. According to the interpretation of the qualitative biocoenotic indexes, we may conclude that the species *Carassius gibelio* is **euconstant** in the reservoirs having (**F-86.8%**) and prefers the oozy substratum rich in organic detritus, typical for eutrophic lacustrine ecosystems, the studied reservoirs belong to.
- 5. This species is closely followed by *Perca fluviatilis* (**F-37.75%**) and *Lepomis gibbosus* (F-**30.2%**) as **accessory species**, which prefer the areas with detritic facies that alternate with the sandy facies, but also the sunny areas located close to the shores covered by concrete panels where they find abundant sources of aquatic invertebrates, mainly gastropods.
- 6. With a lower percentage of the biocoenotic indexes (**F**< 25%) we mention the accidental species: *Scardinius erythrophthalmus, Lepomis gibbosus, Pseudorasbora parva, Cobitis taenia, Rutilus rutilus, Gymnocephalus cernuus, Alburnus alburnus, Cyprinus carpio, Abramis brama, Silurus glanis, Sander lucioperca, Esox lucius.*
- 7. Most of the species comes from the pre-existent reophilous ecosystem, but there appeared other new species such as *Esox lucius* and *Silurus glanis*, which were introduced by the fishermen in reservoir X in 2006, the species being brought from the natural ponds located within the Danube Alluvial Plain or from fisheries.
- 8. We may conclude that the distribution of the species in the ten reservoirs, characterized by a slight agglomeration in the reservoirs VII and IX, is induced by environmental factors, mainly by those related to feeding; a lower concentration is characteristic to reservoirs VI and X, where the values of the environmental factors are far from being optimum for the studied species.
- 9. As the reservoirs from the Preajba Valley are not used in intensive fishing but only by amateur fishermen, the presence of the parasites was weak and there were not signalled massive pathological states. Of the 14 fish species, parasites were identified only at the species belonging to the families *Cyprinidae*, *Percidae* and *Siluridae*.
- 10. Consequently, in the period 2008-2014, there were seasonally identified 13 parasites belonging to **8** phylums, **11** classes and **12** orders: **one species of imperfect fungi** (*Saprolegnia parasitica*), **two species of Ciliophora** (*Ichthyophthirius multifiliis* and *Chilodonella cyprini*), **one of Myxosporidia** (*Myxobolus* sp.), **one of Monogenea** (*Dactylogyrus* sp.), **one of Trematoda** (*Clinostomum complanatum*), **two of Cestoda** (*Diphyllobothrium latum, Triaenophorus nodulosus*), **one of Acanthocephala** (*Pomphorhynchus laevis*), **one of Nematoda** (*Eustrongylides excisus*), **one of Annelida** (*Piscicola geometra*) and **two species of Crustacea** (*Lernaea cyprinacea, Argulus foliaceus*).
- 11. According to the achieved research, it was noticed that the ectoparasites were the easiest to diagnose, especially those caused by crustaceans and Hirudinea, due to the dimensions of the parasites and the obvious lesions they produce. It could be also noticed that the species *Carassius gibelio* and *Perca fluviatilis* were the most affected by parasites as there were identified six species of parasites, followed by *Cyprinus carpio* with four species of parasites

and the other fish species, respectively *Abramis brama*, *Sander lucioperca*, *Silurus glanis* and *Carassius auratus auratus* with only one species.

- 12. From my personal observations correlated with the specialized literature, it results a close connection between the trophic base represented by macrophytes and algae of the periphyton, Cladocera and Copepoda rotifers of the zooplankton, larvae of Chironomida, Nematoda, Oligochaeta, adult and larvae insects, mollusks from the zoobenthos and parasites.
- 13. The obtained results emphasize that the presence and evolution of fish parasites is closely correlated with the diversity of plants and animals of the present trophic levels.
- 14. The appearance of certain groups of parasites (Ciliophora and Trematoda protozoa) is triggered also by biological pollution induced by daily domestic activities, namely domestic wastewater discharged directly into the reservoirs (for example, Reservoirs VII, X).
- 15. The achieved study represents an important contribution to the knowledge, identification and distribution of the parasites of the ichthyofauna from small reservoirs, taking into account the concrete results obtained with regard to the 14 fish species of which seven are hosts for 13 groups of parasites.
- 16. The chosen research methods may be extrapolated to other areas taking into account that the pathogeny of the parasites from the studied reservoirs may increase a lot displaying a potentially zoonotic character.
- 17. The proposed measures aimed at controlling ichthyoparasitologically these reservoirs are especially related to prevention and consist in preventing sick fish from passing from one reservoir to another through weirs; creating a healthy ecosystem through periodic desilting and increasing the stream water pH; stopping accidental discharges into the reservoirs due to anthropogenic activities; seasonal control fishing for ichthyoparasitological examinations; limiting the presence of ichthyophagous birds (final host for trematodes: *Clinostomum complanatum*, nematodes: *Eustrongylides excisus*, Acanthocephala: *Pomphorhynchus laevis*) destroying excessively developed macrophyte vegetation.
- 18. The proposed chemical treatment is limited, given that sport fishing is practiced in the area. The treatment with bleaching powder 600 Kg / ha and copper sulfate 0.5 Kg/ha directly into the reservoirs for destroying the parasitic free-living stages of the copepod crustaceans, Annelida cocoons and gastropods as well as of the intermediary hosts are some of the measures that can prevent the occurrence and spread of parasites.

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