ABSTRACT

Key words: *salmonids, parasitosis, reservoirs*

Aquaculture is the most fast growing sector from all the domains of food producing biotechnologies. For Romania there is a bio potential that has known a significant development in the last few decades. For this reason we have chosen, Izvoru-Mount Bicaz reservoir as case study where was patented and implemented in the 80's, an intensive breeding in the floating cage systems, that was later used on Brădişor Vâlcea reservoir. With the development of aquaculture in floating cage on mountain reservoires in Romania, a series of problems related to the growing biotechnology, emerged from the high density conditions, so there were some pathology aspects with economical implications.

In the Potoci slamonides floating farm, in the last decade have been diagnosed some parasitic diseases with sporadic or enzootic evolution: 7 protozoosis, 1 monogenoidosis and 2 crustaceosis. Therefore we considered necessary to updated study on the morphoclinical, diagnosis and treatment aspects.

In these circumstances the targets of the present study have been the following:

- evaluation of the quality of biotics and abiotics factors of Bicaz lake in order to correlate them with the evolution of parasites in trout bred intensively;

- qualitative evaluation of the ichthyofauna in order to appreciate the health degree of varous fish groups in relation to the environment and parasatises cycles, and also to determine the current ichthyofauna composition;

- evaluation of the parasitosis evolution in trout bred in floating cages in the dynamic of past decades once the new flotable farms were opened as a result of patent no. 123419/1986 (Miron I. *et al*, 1988).

- description of parasite entities that evolved in salmonid farm during this research (2008-2012).

- from the frequently encountered mycoses in salmoniculture, saprolegniasis with all its morbid characteristics was observed and described.

- producing a scheme containing the therapeutical management of parasitoses and mycoses that evolved in trout bred in floating cages in Bicaz lake.

To understand and analyze the development of the pathological entities, it was used as a work frame the diagram proposed by Kinkelin şi Ghittino (1985), that describe the interactions, succession and the effects of natural and biotechnological induced causes on the evolution of fish

diseases. Also it was used the diagram of Balbir (1989), under the aspect the importance of microclimate through its biotic components (ichtyofauna) and abiotic (physical and chemical factors of water) in the development and evolution of the disease.

This thesis was divided into two parts.

Part I contains bibliographic studys of the limnophysical, limnobiological characteristics of the studied aquatic ecosystem (Izvoru-Muntelui Bicaz reservoir) and of parasitic/fungal pathology in trout bred in floating cages.

Part II was divided into 4 chapters as follows:

In Chapter VI our study was conducted on the importance of microclimate changes in salmonids growth and pathology as an expression of the interaction between pathogen, host and microclimate, resulting a multifactorial interference which was expanded to control the intensive breed systems. For this reason, in order to characterise the salmonids diseases, we proceeded to the identification and analyses of abiotic components of the aquatic environment.

Monitoring physic-chemical parameters of the Bicaz reservoir water, we aimed to establish the periods of time with negative influences on trout health and also the correlation between seasonal climate variations and the incidence of parasitic/fungal diseases. To obtain this, 61 sampling expeditions were made, in which it was used a monitoring system **Environmental monitoring system 6600 V2, Multiparameter Water Quality Sonde YSI 2**, approved and used in international laboratory for water quality expertise.

From the analysis of the results we distinguish three important periods in trout breeds in floating cages: the ***first period***-that includes the summer months when the increased water temperature from the surface horizons favours the development of several groups of bacteria, protozoa, helminth, parasitic artropods and fungal, and so the parasitic pathology is encountered with a maximum incidence where the temperature is the highest; ***the second period*** during autumn -winter when the decrease in water temperature leads to a decrease in disease incidence. ***Third period*** includes autumn and spring homotermia with the uniformity of the water physico-chemical characteristics due to the mixture of water from the hipolimnion layer and the epilimnion layer. This process enriches in nutrients the water mass and promote parasites development together with the explosion of the zooplanckton since May.

Analyzing the degree of water oxygenation and the pH level we notice an optimal level for trout development in all horizons of depth. The existence of a sufficient quantity of oxygen in all horizons and the epiliminon extreme temperatures from the summer period (superficial layer) suggest the idea of biohandling by moving the fish in deep horizons without requiring them to live in terms of hypoxia, anoxia and limiting the parasite development by disrupting their life cycle.

Chapter VII covers the study of **ichthyofauna structure Bicaz lake**. The reef effect of salmonid farm, favours the development of pelagic fishes through the additional source of nutrients provided by periphyton attached to cage nets. Pelagial fish contact with fish in floating cages in search for food, favours interspecific transmition of parasite: For example the protozoan *Ichthyophthirius multifiliis*, the only species of the genus, have no host specificity and can infest many fish species.

If in 1960, when the lake was formed, ichtyofauna composition comprised 25 species of fishes, in the next decade 19 species and as time goes, it drops to 15 species in the 4th decade. Making a comparation with the previous decade, we have identified the existence of 13 species of fish in Lake Bicaz, indicating the presence of *Hucho Hucho* L., an endemic salmonid of the Carpatho-danubian areal, *L. phoxinus phoxinus*, and *Acipenser stellatus*, the last, probably escaped from the farm.

Chapter VIII covers the study of **parasitic diseases of trout in Bicaz reservoir.** All parasitic diseases reported in this paper were treated emphasizing the aetiologic agent in the current nomenclature, morphometric features of parasites, morphoclinical and lesional aspectes and preventive or curative measures required, all these being useful for practitioners.

To diagnose these diseases both classical and molecular diagnostics methods were used being added to the previous descriptions of pathological entities according to a diagram propouse by us with three stages.

We consider that after the first stage we can put a primary diagnosis that can guide us to select the next diagnostic techniques needed to confirm it. In some diseases, such as saprolegniasis or argulosis when clinical signs are obvious, the final diagnosis can be made at the end of the first stage in which case we skip to establish the therapeutic measures.

After the second stage was completed, more often the primary diagnosis was confirmed and we passed to remove the cause by medical, ecological or management measures without further investigation.

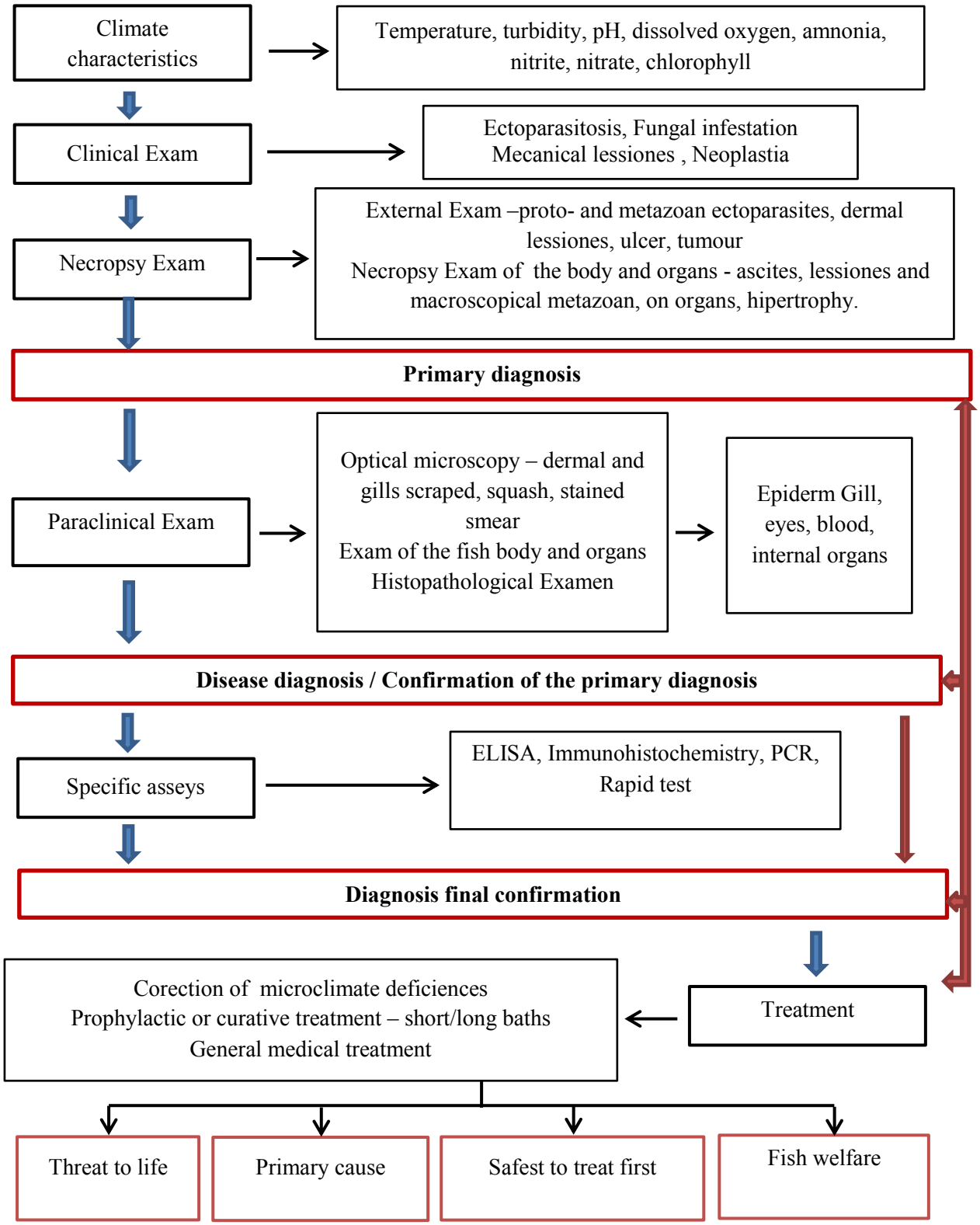
If at this level of research we could not identify the cause, we proceeded to the third stage III: Immunological diagnostic phase: ELISA immunoassay or methods of immunohistochemistry, and molecular diagnosis. These techniques and also the rapid diagnostic tests (available for some bacterial or viral diseases) are expensive and require a special training of the salmonid farms staff. Unfortunately, this training is lacking, and the final diagnosis is sometime incorrectly made after the end of phase II.

The implementation of the treatment regime consist of either correction of microclimate or biotechnological deficiency, or by applying prophylactic/curative medicinal substances.

Based on this scheme, the analysis of the pathological condition in trouts from two

floating farms, and free trout led to diagnosis of 5 parasites, which evolved with increased incidence in caged fishes compared to those in freedom.

Diagnostic Scheme in fish diseases



Eimeriosis was diagnosed after clinical examination even if clinical signs such as enteric, and so very shallow or even absent. In some cases signs were masked by respiratory disorders.

The necropsy examination was able to highlight issues such as weakness and anorexia,

haemorrhagic spots located on caeca, inflammation of the posterior intestinal wall to the back of the bowel with reddish aspects, a small amount of faeces, diarrheic, yellowish-white in appearance show. They can direct the examiner to a presumptive diagnosis. If the first stage results were indicative, at the end of the usual paraclinical diagnosis we were able to establish a diagnosis of certainty, by identifying the following stages of the parasite: schizont with merozoites, macrogametes, microgametocytes with microgametes, immature oocytes, matured oocytes with 4 sporocytes with 2 sporozoites.

Drug treatment in **eimeriosis** consists in the use of potentiated sulphonamides sulfametoxazol and trimethoprim-20 mg/kg, diet administered for 10 days. Also administration of coccidiostats 33% in water (1ml/32l) weekly, showed the experimental efficiency.

Ihtioftiriosis was diagnosed in three of the five age groups examined, most affected were 7-month old trout with an incidence of 71%, followed by those of 18 months with an incidence of 66.6%, and the 4-month with an incidence of 24%. Cases of illness occurred between June and September when water temperature was above 20 ° C and favourable to parasites rapid multiplication. In our research the peak incidence occurred to the 7 months old, also the identification the trophontes in 18 months old trout does not exclude the existence of an acquired immunity, but rather we consider that poor sanitary and hygienic measures, poor farm management and central disposition of the cages in the perimeter of the farm favours the increase of the capacity for invasion of the parasite. At the clinical examination of the fish it was observed the presence of respiratory disorders, associated with ciliates aggression, especially since parasitic co-infections were identified at the examined fish. So, association between *Eragsilus sieboldi* and *Ichthyophtirius multifiliis* was identified in 50% of the 4 months old trout 12.5% and 33 3% in 7 months respectively 18 months. In this case the clinical signs and the gill lesions were more obvious but difficult to differentiate by aetiology. At the clinical examination it was seen fish swimming on the surface of the water and their effort to breathe, effort shown by aerophagia and excessive movements in opening and closing the operculum. These respiratory symptoms can be found in other ectoparasitosis or in reduction of the water dissolved oxygen.

Anatomopathological examination, revealed the gill lesions: white spots, gray-yellow mucus hypersecretion consecutive to gill necrosis with ruptured edges. This can be aggravated by the flowering of microalgae that submit to the gills or parasitic associations with other species. Diagnosis of certainty was established in the stage II, when by optical microscopy it when parasites stages were observed: trophont, tomite and tomont. Investigations continued by performing ELISA method, both in captive and free living trouts. So after this specific exam we noted the existence of anti-*Ichthyophtirius* antibodies in free trouts even if they were not initially diagnosed as positive.

General preventive measures aimed at: preventing fish exposure to parasites, swift identification of the disease and when clinical manifestations appear, drug treatment of infected fish and immunization is required. Physical treatments intended to destroy free swimming terontes, interrupting the life cycle and thus preventing reinfection. This was done by continuously circulating the water and by UV treatments.

The treatment in Ihtioftiriosis is required by the identification of a single *Ichthyophtirius multifiliis* trophont and consists in breaking the parasites life cycle by environmental manipulation and by modifying the physico-chemical parameters of water to prevent the development of the parasitic stages. Also drug therapy should not be excluded especially for heavy infestations, by short or long term baths with formalin, depending on the degree of infestation, on the chosen concentration and not least on the farm structural possibilities.

Tricodinosis was found in October, by examination of the stained smear from mucus inside the nostrils of farmed trout. Because we managed to collect a single specimen and the incomplete staining of the oral appliance, we could not identify the species. Clinical diagnosis provides evident data in massive infestations, but usual paraclinical diagnosis leads to the identification of weak infestation. Being a saprophytic species, occurrence of sickness is not solely due to parasitic development, fish health and immune status having important influences.

In terms of pathology, due to parasites superficial attachment to the tissue, diagnosis based on histological examination may be wrong, they being washed when fixing. Preventive treatment is to maintain the optimum microclimate conditions and to avoid technological gaps that can weaken the fish, and also regular parasite checks. Curative treatment, costs in administering formalin, potassium permanganate, copper sulfate, sodium chloride, or fresh water for marine species. In heavy infestations with serious, pyridyl mercuric acetate 1: 500000/30 min or 1 hour or potassium permanganate 1: 500000/30 min provides good results.

Clinical signs in diplostomosis consisted in fish swimming at the water surface, unilateral or bilateral exophthalmos, unilateral or bilateral deformation of the shape of the head, and the lack of reactivity at the food administration and eye enucleation when fish were removed from the water due to elevated intraocular pressure. Through our research it was found that cercariae invading the ocular structures are present in the water when the temperature is higher than 9° C (mid-April). Invasion and penetration through the skin, migration to the target organ, the eye, and transformation in metacercaria is carried out quickly in June-July when water temperatures averaging around 22°C.

Diplostomosis diagnosis was made by microscopic examination of the lens and by identifying the metacercariae stages in the aqueous humour. Differentiation between *Diplostomum pseudosphataceum*, and *Diplostomum sphataceum* was performed by

morphometric studies of metacercariae and because of the great similarities between species we have resorted to molecular biology exams-PCR which did not confirme the development of *Diplostomum pseudosphataceum*.

Preventive treatment consists mainly in the interruption of the parasites life cycle either by eliminating the first intermediate hosts -aquatic gastropods, or by eliminating the definitive host represented by piscivorous birds. In removing in a high percentage (100%) of *Diplostomum sphataceum* metacercaria, paraziquantel is successfully, if used as bathing, orally administered or by injection. Use of the poduct - Drontal Bayer in dose of 330 mg / kg fish / day for 6 consecutive days may result in removal metacercariae.

Ergasilosis - was also diagnosed at the caged trouts by the usual laboratory tests, the most affected being those of 24 months old with an 100% incidence, followed by those of 4 months old (50%), 18 months old (33.30%) and those of 7 months old (28.5%). Clinical signs are, as in ihtioftiriosis, represented by respiratory dysfunction and at the histoptologic examination of gill sections, capillary congestion, thrombosis and hyperplastia of the chloride cells from the secondary lamella, were observed.

On free trout two parasitic species were identified, the copepods *Ergasilus sieboldi* and the digenetic trematode *Diplostomomum sphathaceum* on the same fish. Compared to the above, on the caged cultured trout we find the presence of parasitic copepods on the gills, metacercariaea stages in the eyes and tomites of *Ichthyophthirius multifiliis* on the gills. This mode of parasitic evolution, with a significant intensity and three species of parasites on the same fish makes impossible trout survival.

It is possible that lake trout infestation is made from the caged trout and not the opposite, since evolution, at least for the parasitic copepods is cited to occure in conditions of poor hygiene.

Chapter IX includes the study of **fungal diseases of trout in Bicaz reservoir.** Saprolegniasis has not evolved as an independent disease, but cases have been identified especially in the cold season (November-December). Fungal infections were characterized by the appearance on the surface of the body, near the lateral line and posterior to the dorsal fins of scales bare areas where we found an increased secretion of mucus.

From the ulcerative areas, tissue collection was made by scraping and the inseminations ware performed on PDA medium with addition of chloramphenicol 1%. So after a period of incubation of 5-30 days at room temperature, in liquid medium we observed the development of of dirty-looking or gray-white colonies. Microscopic examination led to the identification *Saprolegnia parasitica* stages like zoosporangii and oospores.