FARMED CYPRINIDS DISEASES FROM THE PRUT RIVER BASIN

Adrian BĂLBĂRĂU[1], Vasile VULPE, Ramona GHIORGHIASA, Dumitru ACATRINEI, Larisa IVĂNESCU, Liviu MIRON

e-mail: dacatrinei@yahoo.com

Abstract

The current paper aims to outline the most common fish diseases that affect farmed cyprinids in ponds from the Prut river basin, in order to better understand and reduce fish health risks. After 2 field work expeditions to Rompescaris farm -Podu-Iloaiei from Iasi county and to Dracșani fish farm from Piscicola-Botoșani county, biological samples were collected, in May 2021. Using net fishing the following fish species were harvested: carp (Cyprinus carpio), silver carp (Hypophthalmichthys molitrix) and mirror carp (Cyprinus carpio var. specularis). the biological material, the moribund specimens with visible lesions were isolated and the apparently healthy specimens were released. The collected specimens were subjected to a clinical, a parasitological and a bacteriological investigation.

Key words: Cyprinus carpio, Hypophthalmichthys molitrix, Cyprinus carpio var. specularis, diseases

Fish consumption has been rapidly increasing from 5.2 kg per capita in 1961 to 19.4 kg in 2017. Even the less developed countries increased their consumption from 6.1 kg in 1961 to 12.6 kg in 2017.The Food and Agriculture Organization of the United Nations estimates that by 2030 aquaculture will produce 60% of all fish destined for human consumption (FAO 2020).

With the rapid growth of this sector farmers also have to cope with an increased demand and with more fish health risks. Studies carried out in the early stages of aquaculture focused mainly on new intensive fish farming systems, improved feed and an increased fish growth rate. In recent years aligning with the rapid growth of this industry worldwide there has also been an increase demand of fish diseases diagnostic and in order to find out the most common problems that have an impact on fish health in a certain region. Furthermore, bringing together large numbers of animals of a single species or closely related species increases the risk of disease outbreaks (Kibenge et al., 2012).

Aquaculture systems similar to some found in Moldova county in which farmed fish are kept at high population densities in close proximity with wild fish reservoirs is ideal for the spreading of wild type pathogens form the wild fish to the farmed ones and vice versa (Kibenge et. al 2012) thus ensuring a permanent supply of parasitic, bacterial, viral and fungi pathogens.

Adding to the problem in the last three decades global temperature near Earth's surface has been increasing at an unusually rapid rate (Stott et al., 2000). It is well known that temperature facilitates the multiplication of pathogens and the infection of new hosts as long as the other pathogen-specific conditions for transmission are met. The rising of temperature could also amplify the parasites metabolism resulting in a higher number of transmission stages being produced, which would lead to a higher parasite fitness and a more rapid spread of certain diseases (Karvonen et al.,2010).

That being said now more than ever in Moldova county aquaculture units face problems that are either the result of different pathogens or are a consequence of inappropriate water quality parameters and are in need of either a diagnostic or a water quality assessment. In this article we present our findings in order to better understand, and in the future, reduce fish health risks in Moldova county.

MATERIAL AND METHOD

All the sampled fish were first subjected to an external clinical examination. The fish were inspected and the skin was examined to determine if there are any ulcers, furuncles, skin hemorrhages, spots, skin darkening, tumor like lesions or macroscopical parasites. The mouth of the fish was inspected for hemorrhages or macroscopical parasites. The eyes were examined for hemorrhages, corneal opacity, sunken eye or exophthalmia.

[1] Iasi University of Life Science, Faculty of Veterinary Medicine

Following the same pattern of examination used on the skin, the gills were also inspected. After the external evaluation the fish were placed with the left lateral side towards the examiner and using an anatomical scissors a section was made along the abdomen, starting from the anal orifice upwards, on the right side of the body towards the spine and then continued parallel to it, up to the head.

A lid was outlined that was removed with the help of an anatomical forceps revealing the visceral mass. The aspect of the abdominal cavity was examined as a whole and then the presence color and position of the internal organs was examined. The liver, spleen and kidney were examined for hemorrhages, nodules or changes in color and in case of the kidney the texture of the organ was also assessed.

The digestive tract was unrolled and using scissors was divided into three sections (anterior, middle and posterior) then opened along its entire length. The content and scrapes of the intestinal mucosa were placed between a slide and a cover slip and were examined microscopically without any staining.

The bacteriological examinations were performed by inoculation on specific media of bacterial strains sampled from the injured tissue and uninjured organs. Samples were taken from the spleen, kidney, liver, gills, skin and from areas adjacent to the injured tissue.

Using a sterile Pasteur pipette and a sterile loop, the organs were deeply pierced and biological material was sampled. The samples were deposited on the surface of a non-selective medium (TSA agar, nourishing agar, BHI agar) and incubated at 25ºC for 24 to 48 hours (Kimberly A. Whitman, 2004).

After preforming the cultural examination, morphological identification was performed by Gram staining. Inoculating the bacterial strains on biochemical media and with the use of API diagnostic tests the morphological characteristics were also identified.

In order to identify the species within the genus *Aeromonas*, mass spectrometry was used (MALDI-TOF MS bioMérieux system).

The parasitological examination began with the inspection of the skin and gills. The skin was examined for macroscopical parasites, and after that the gills were exposed by removing the gill covers with a pair of scissors. After examining the gills, they were removed from the body and placed in a Petri dish. The mouth was visually inspected for the presence of parasites and the eyes were removed using a curved scissor and then cut open using a scalpel in order to examine the vitreous humor.

RESULTS AND DISCUSSIONS

At the external clinical examination hemorrhagic lesions located on the abdomen (figure 1) and on the left lateral side immediately next to the gill cover (figure 2) were observed in common carp (*Cyprinus carpio*). Skin ulcers with local shedding of the scales (figure 3), fringing of the anal fin (figure 4) and congested gills with areas of necrosis (figure 5) were also observed in the case three other individuals of the same species.

Figure 1. **Abdominal hemorrhage (*Cyprinus carpio*)**

Figure 2. **Skin hemorrhage next to the gill cover (*Cyprinus carpio*)**

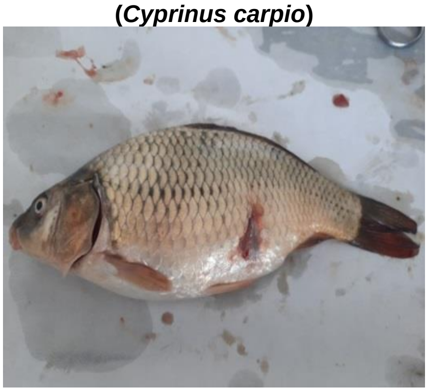

Figure 3. **Skin ulcer with local shedding of the scales (*Cyprinus carpio*)**

Figure 4. **Fringing of the anal fin (*Cyprinus carpio*)**

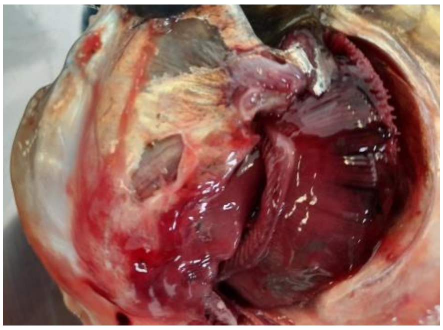

Figure 5. **Gill congestion and necrosis (*Cyprinus carpio*)**

The observed modifications can be caused by both external abiotic factors such as water quality criteria, different objects capable of harming the fish and by biotic factors such as starvation, stocking density or different types of pathogens. A variety of aethiological factors may cause lesions in the skin, gills, eyes under farming conditions. A poor aquaculture system and rapid changes in environmental conditions may lead to health problems and diseases that will have an impact on fish welfare. Handling and grading may impair the mucus layer and the skin if not done in a gentle way. Lesions of mechanical origin may improper tanks and cages as well as natural predators like birds (Tørud, B., & Håstein, T., 2008).

In order to determine the actual cause of the discovered lesions further investigations were required.

The mirror carp (*Cyprinus carpio* var. *specularis)* showed lesions characterized by ulcers that occasionally reached the muscle tissue and were surrounded inconstantly by a whitish halo, erythema, hemorrhagic infiltrations located in areas devoid of scales (figure 6 and 7).

Figure 6. **Skin ulcer that reached the muscle tissue (*Cyprinus carpio* var. *specularis*)**

Figure 7. **Skin ulcer surrounded by a whitish halo (*Cyprinus carpio* var. *specularis*)**

Examination of the gills revealed an abundant whitish mucus deposit at the base of the gill arch, gill hemorrhages and areas of gill necrosis (figure 8).

Figure 8. **Gill necrosis (*Cyprinus carpio* var. *specularis*)**

The clinical examination of fish belonging to the silver carp (*Hypophthalmichthys molitrix*) species revealed the presence of parasites belonging to the *Lernaea* genus imbedded in the skin (figure 9) and parasites belonging to the *Sinergasilus* genus fixed on the gill lamellae (figure 10). For proper identification of the parasite species further investigations will be required.

Figure 9. **Copepod crustaceans (genus *Lernaea*) imbedded in the skin, scale erosions and hemorrhagic ulcerations (*Hypophthalmichthys molitrix*)**

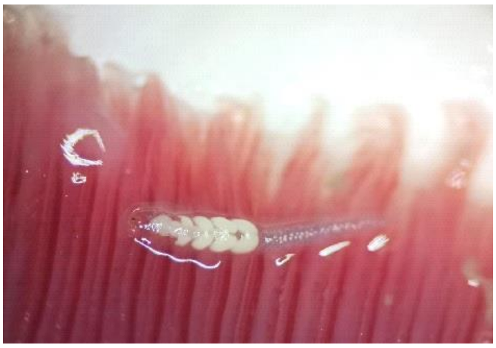

Figure 10. **Copepod crustaceans (genus *Sinergasilus*) fixed on the gill lamellae 4x magnification (*Hypophthalmichthys molitrix*)**

Parasites found on the gills were sampled and examined under the microscope after being fixed between a slide and a cover slip (figure 11) and the parasites found imbedded in the skin were examined under a magnifying glass (figure 12).

The most common indicators of poor health in fish include tumors, hemorrhage, necrosis, fin and skin damage, deformities, discoloration of organs or tissue, excessive mucous and heavy infestation with parasites (Karr J. R., 1981).

In addition to osmoregulatory problems associated with the ulcerations caused by the parasites secondary bacterial infections may hinder the welfare of affected fish and treating them properly is a major challenge since a relatively high number of opportunistic bacterial species are usually present in every water body.

Figure 12. **Crustacean copepod of the genus *Lernaea* examined at a 4x magnification (*Hypophthalmichthys molitrix*)**

Crustacean copepods like of the genus *Lernaea* and *Sinergasilus* can survive a wide range of conditions but thrive when the water comprising the ecosystem has a low oxygen concentration and a high mass of stagnant organic matter.

Even if we were to give the fish the best environmental conditions, gentle handling or an adequate treatment, if necessary, some members of such large populations will still suffer because of skin and gill lesions. To get these particular individuals out of production may be a great challenge (Tørud, B., & Håstein, T., 2008).

Very few treatments are available worldwide that are effective on crustacean parasites and there are major difficulties in their application as well as serious concerns regarding the environmental impact. The risk of developing a resistance to the limited range of effective therapeutants is also very high (Tørud, B., & Håstein, T., 2008).

Even if the water quality is high and prevention methods are tried under farming conditions where fish are kept in high densities the ability of a parasite to find a host is greatly increased along with the level of parasitic infestation (Tørud, B., & Håstein, T., 2008).

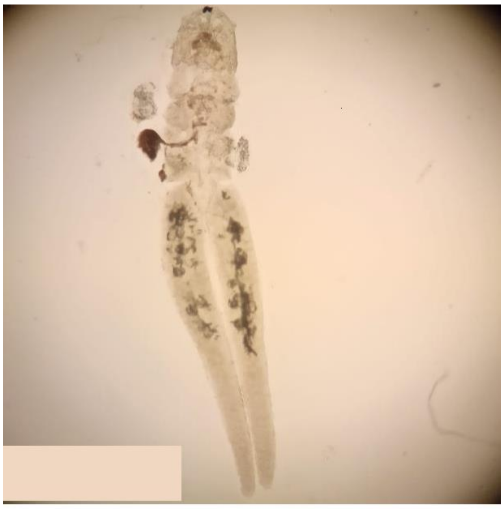

Figure 11. **Copepod crustaceans of the genus *Sinergasilus* examined between slide and cover slip at a 10x magnification (*Hypophthalmichthys molitrix*)**

After the gills were examined macroscopically scraping were taken and analyzed between a slide and a cover slip. The exam of such a sample from a silver carp (*Hypophthalmichthys molitrix*) revealed the presence of a nematode belonging to the *Dactylogyrus* genus that was identified based on the following morphological characters, the presence of 4 pigmented spots in in the anterior extremity (figure 13) and the presence of the haptor fixing organ (figure 14) at the posterior extremity.

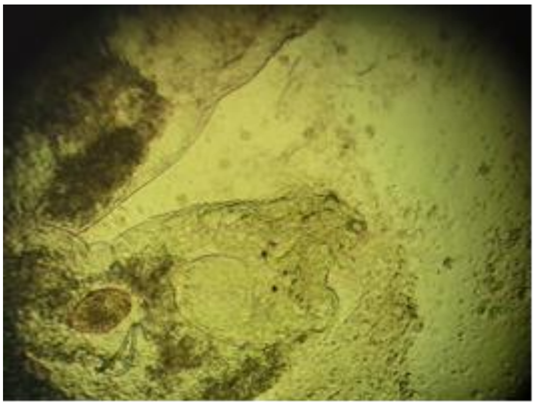

Figure 13. **Dactylogyrus spp. - anterior extremity with 4 pigment spots**

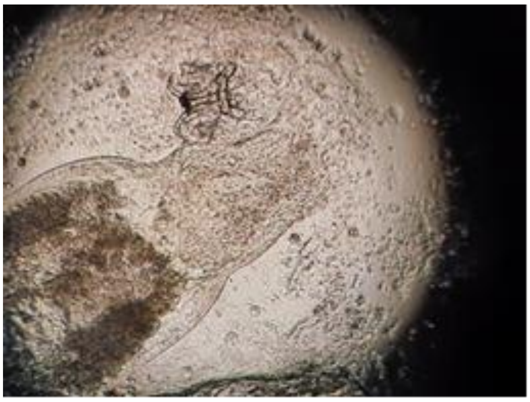

Figure 14. **Dactylogyrus spp. - posterior extremity with the haptor fixing organ**

Parasites causing little apparent damage in feral fish populations, may, become causative agents of major diseases in farmed fish, leading to a decrease of fitness or reduction of the market value of the fish (Scholz T., 1999). Parasites affecting farmed fish will alter the host's weight, sometimes substantially even if apparently there are no signs of disease (Jakob et al., 1996).

Despite considerable progress in fish parasitology in the last decades, major gaps still exist in the knowledge of taxonomy, biology, epizootiology and control of fish parasites (Scholz T., 1999).

Fish parasites are an integral part of water ecosystems and are present in natural and cultured fish populations alike. In natural conditions, most parasites do not tend to severely injure their host and cause mortalities which affect the population size at detectable levels (Scholz T., 1999), this is because feral fish are more resistant than farmed ones and because natural selection greatly reduces the spreading of parasites in wild fish populations.

If an individual with a low resistance dies when taking contact with a parasite the life cycle of the pathogen will not be completed.

It should also be emphasized that the presence of a parasite does not necessarily imply manifestation of a disease. (Scholz T., 1999).

The bacteriological examination started with the analysis of the Petri dishes after the incubation period ended. On TSA agar species of *Aeromonas spp., Shewanella spp.* and *Pseudomonas spp.* developed. The *Aeromonas spp*. colonies were characterized by round "S" type colonies with a diameter of 2-5 mm, with a regular shape, opaque and unpigmented edges. The *Pseudomonas spp*. developed "S" type colonies, slightly yellow pigmented, fluorescent, with a tendency to confluence. The *Schewanella spp.* colonies were circular, convex had a regular outline a diameter of 1-4 mm and were pigmented in orange.

Some of the lesions observed on the fish were similar to those seen in case of erythrodermatitis which can be caused in fish by several species of conditionally pathogenic bacteria as fallows: *Aeromonas hydrophila, Aeromonas caviae, Aeromonas sobria, Pseudomonas aeruginosa, Shewanella putrefaciens or Plesiomonas shigelloides*. (Brain Austin and Dawn Austin, 2007).

CONCLUSIONS

We are not entirely sure if some of the skin lesions found on the common carp (*Cyprinus carpio*) are the result of poor management system and have been colonized by bacteria afterworlds or are the direct result of a bacterial pathogen but we believe the former to be the case.

Adding to the problem mechanical lesions that result from a poor farming system or lesions which are a result of parasite infestations greatly facilitate the development of opportunistic bacteria or fungi that are normally present in the water.

Of all the specimens examined the silver carp (*Hypophthalmichthys molitrix*) was the most relevant from a parasitological point of view.

Each silver carp (*Hypophthalmichthys molitrix*) was found caring at least one species of parasite form the genus *Sinergasillus, Lernaea,* or *Dactylogyrus spp*. and one particular individual had both crustacean copepods at the same time.

The common carp (*Cyprinus carpio*) and the mirror carp (*Cyprinus carpio* var. *specullaris*) after the clinical examination were the fish with the most ulcer like lesions on the skin and were the most relevant from a bacteriological point of view. Of all the specimens examined only two common carps (*Cyprinus carpio*) after the samples were taken and investigated showed signs of *Saprolegnia spp.*

Control of diseases when it comes to farmed fish is far from being satisfactory and further investigation will be needed. Use of chemotherapy is very limited and new methods of treatment that do not pose the technical difficulties of standard ones will have to be developed. At the same time, it is worth noting that any new treatment has to be environmentally safe.

Fish farmers must also improve the conditions in which fish are farmed in order to reduce the fish health risks. Overcrowding should be avoided as much as possible and the providing and after that maintaining of good quality water is mandatory.

High densities of fish help spread diseases and the if the water is also improper the fish's natural resistance will decrease and at the same time the parasites and other opportunistic pathogens will increase in number.

Until a proper treatment is developed aquaculture units in the area need to make improvements on the farming systems, avoid overcrowding and regularly check the water quality parameters and the fish's health status.

Further investigations will be required in order to determine as many pathogens as possible that are affecting aquaculture in Moldova county and devise a viable solution that can reduce fish health risk as much as possible and improve their welfare.

ACKNOWLEGMENTS

We would like to thank the team of researches involved in the project "Team up for healthy fish in aquaculture systems of the Prut River basin 2SOFT / 1.2 / 47", researchers from the Institute of Zoology (IZ) of the Academy of Sciences in Chișinău (Republic of Moldova) and the University of Life Sciences " Ion Ionescu de la Brad" from Iași (USV Iași).

REFERENCES

Austin Brain and Austin Dawn, 2007 - Bacterial fish pathogens. Diseases of fish farmed fish and wild fish Ed. 4

Barber I, 2007 - Parasites, behavior and welfare in fish.

Eissa Alaa Eldin, 2016 – Clinical and laboratory manual of fish diseases

FAO. 2020 - The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

Håstein Tørud and Tore, **2008** - Skin lesions in fish: causes and solutions warming

Jakob, E. M., Marshall, S. D. and Uetz, G. W. 1996 - Estimating fitness: a comparison of body conditions indices