Parasitic Diseases Identified at the *Cyprinus Carpio* (Carp) Population Reared in a Recirculating Pilot Aquaponic System (RAS) *(Elena Camelia Dragomir, Neculai Patriche, Magdalena Tenciu, Aurelia Țoțoiu, Maria Crivineanu)*	**"Cercetări Marine" Issue no. 50** **Pages 174 - 185**	**2020**

PARASITIC DISEASES IDENTIFIED AT THE *CYPRINUS CARPIO* (CARP) POPULATION REARED IN A RECIRCULATING PILOT AQUAPONIC SYSTEM (RAS)

Elena Camelia Dragomir[1*], Neculai Patriche[2], Magdalena Tenciu[3], Aurelia Țoțoiu[4], Maria Crivineanu[5]

*[1]*Institute for Research and Development for the Industrialization and Marketing of Horticultural Products, Gilau Street no. 5N , Bucharest, Romania*
[2,3] Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture Galați, Portului Street no. 54 Galați, Romania
[4]National Institute for Marine Research and Development "Grigore Antipa", 300 Mamaia Blvd, 900581, Constanta, Romania
[5]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, Independenţei Spl 105, District 5, Bucharest, Romania
**Corresponding author email: dragomirelenacamelia@gmail.com*

ABSTRACT

The study presents the results concerning the health status of the fish population reared in a recirculating pilot aquaponic system (RAS). The biological material used for the experiments was divided into eight tanks and was represented by 2 year old carp (*Cyprinus carpio*) and vegetable biological material represented by lettuce (*Lactuca sativa*), cultured in an aquaponics system, during 2018-2019. The experiment used in this study was carried out for a period of 42 days.

Parasitological analyzes for the identification of parasites were carried out by macroscopic and microscopic examinations on three fish samples from each experimental tank. The results of the pathological analyzes performed revealed the presence of both ectoparasites and endo parasites. Therefore, the research was oriented to underline the parasitosis which affects fishes reared in a RAS culture. Following the research, measures of preventing the spreading of the evaluated biological contaminants were recommended. Probiotics offer protection by creating a hostile environment for possible pathogens which compete for nutrients and which can affect the organism's immune system.

Key-Words: carp, reared, aquaponics system, parasitic diseases

AIMS AND BACKGROUND

Aquaponics represents the process of growing aquatic organisms and plants symbiotically, in which the effluent of aquaculture undergoes microbial transformations to be used as a source of nutrients for plant growth, while nutrient absorption from plants remediates water for aquaculture (Fig. 1).

Aquaponics is defined as the production system of aquatic resources and plants where the majority (>50%) of nutrients sustaining the optimal plant growth derives from waste originating from feeding the aquatic live resources' (Lennard, 2015; Palm et al., 2018). In an aquaponics system, the growth environment is suitable for both fish and for the nitrification process, which produces the necessary nutrients for plant growth (Filep et al., 2016).

The term "aquaponics" is a portmanteau of aquaculture and hydroponics. Aquaculture has been defined as "the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plant" (Food and Agriculture Organization of the United Nations, 1988), while hydroponics has been defined as "…the production of plants in a soilless medium where by all of the nutrients supplied to the crop are dissolved in water" (Diver, 2006).

In the mid 1980's, Mark McMurtry and Professor Doug Sanders created the first known closed loop aquaponic system. Effluent from fish tanks was used to trickle-irrigate tomatoes and cucumbers in sand grow beds which also functioned as bio-filters.

In the early 1990's, Missouri farmers Tom and Paula Speraneo modified the NCSU system and introduced their bioponics concept - grew herbs and vegetables in ebb and flow gravel grow beds irrigated by the nutrient rich water from a 2200 litters tank in which they raised Tilapia (Purkait et al., 2018).

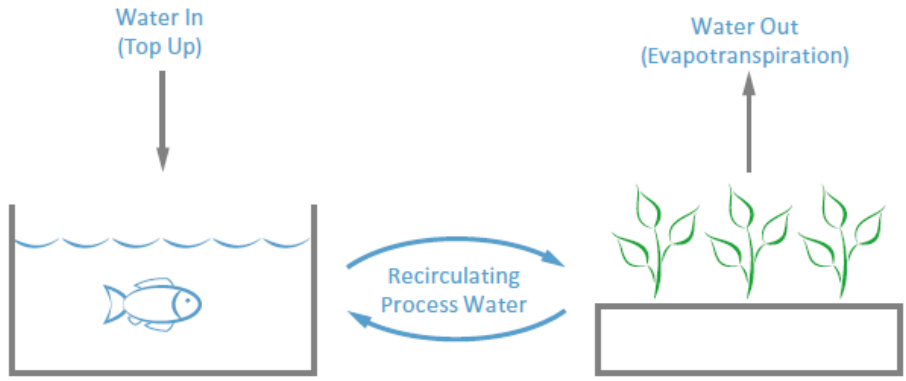

Fig. 1. Scheme of the water flow in an aquaponic system
(by Lennard & Goddek, 2019)

Diseases can be an important limiting factor in aquaculture but also in the natural environment, leading to reductions in populations and even to massive mortality, having a negative impact especially in intensive managed crops. (Rădulescu et al., 1976).

In aquaculture, as in all sectors working with living organisms, control of biomass production is conditioned by the fish health. Certain physiological and biochemical indicators are used to obtain this kind of control in order to maintain the stocks in good conditions, in terms of nutrition and other elements such as density, flow, health (Anderson, 1991; Munteanu & Bogatu, 2003).

The term probiotics is generally used to denote bacteria that promote the health of other organisms. Lilley and Stillwell (1965) described them as substances secreted by one microorganism, which aim to stimulate the growth of another. An expert with the Joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), stated that probiotics are live microorganisms, which when consumed in adequate amounts, confer a health benefit for the host (FAO/WHO, 2001).

Bacterial antagonism is a common phenomenon in nature; therefore, microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic microorganisms. However, husbandry practices and environmental conditions that stimulate the proliferation of selected bacterial species can alter the composition of microbial community.

The microbiota found in the gastrointestinal tract of aquatic animals can be modified, for example by ingestion of other microorganisms; therefore, in order to reduce or eliminate the incidence of opportunist pathogens, the microbial manipulation constitutes a viable tool (Balcazar, 2002).

EXPERIMENTAL

The recirculating aquaponic system made for the research described in this paper, was prepared to support two sections: the fish breeding section represented by *C. carpio* (Linnaeus, 1758), and the plant growth represented by *L. sativa* L. The carp used in the experiments came from the Brateș Research and Development Base - Research and Development Institute for Aquatic Ecology, Fisheries and Aquaculture Galati.

For populating the aquaponic growth system with fish material, dimensions of the experimental tank and the volume of recirculated water were taken into consideration. Thus, the optimal population density was 5 kg of 2-year-old carp for each experimental tank. A total of 40 kg (172 specimens) of carp evenly distributed in 8 experimental tanks, with an average weight of 232.5 g/fish were monitored (Fig. 2).

Fig. 2. Recirculating aquaponic system (RAS) (original photo)

In the last stage of the experiment, to ensure the health of reared fish species in aquaponic systems and to comply with their physiological requirements, feed additives were added to fish feed.

In order to monitor the health of fish reared in the aquaponic recirculating pilot system, two experiments were performed by using for over 42 days ParaProteX probiotics and MCC mixture (grape marc, cinnamon and cloves) introduced as powder into the fish feed (Fig. 3). The components of the MCC mixture (grape marc, cinnamon, and cloves) were chosen for their antioxidant, antibacterial, antifungal, antiviral properties and because they stimulate the functioning of the immune system.

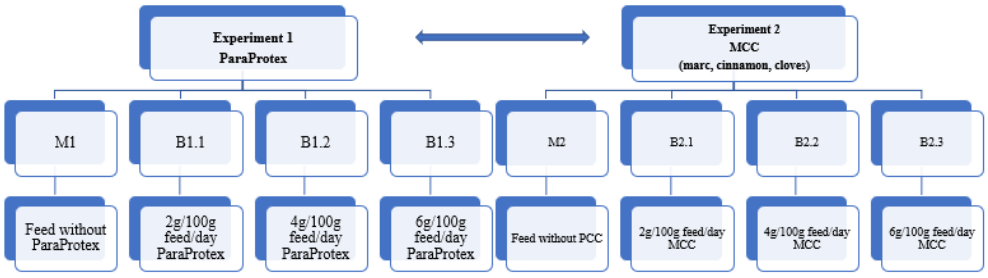

Fig. 3. Experiment scheme

To identify parasitic diseases, six carp specimens/tank were macroscopically and microscopically examined. The macroscopic examination was performed with the naked eye, using a magnifying glass, observing the body surface, eyes, gills. Then, with the help of surgery scissors, the fish was sectioned on the abdomen, analyzing each organ separately to highlight possible necrotic areas, cysts, parasites, color and other visible changes, being followed by microscopic examination (Fig. 4) (Amlacher, 1981).

Fig. 4. Specimen of carp used in experiments (original photo)

For the microscopic examination, full preparations were used (small portions of tissues and organs), as well as crushed preparations (squashes) between the blade and the lamella, making the film formed translucid and as thin as possible, allowing the sighting of potential parasites.

After identifying and counting the parasites for each fish specimen, a detailed analysis was made on the intensity (number of parasites / host) and the extent of parasitism (number of parasitized fish), assessing the influence of parasites reported on the carp populations health in the aquaponic systems.

Throughout the experiments, the following physico-chemical water parameters were monitored: temperature, pH, dissolved oxygen, and once a week, under the same conditions, the chemical oxygen consumption was determined. The parameters were monitored daily, at water supply and at discharge, by the following methods:

- Temperature, pH, and dissolved oxygen were determined using the portable multiparameter AZ 86031 (Fig. 5).
- Chemical oxygen consumption was determined by spectrophotometric method.

Biometric measurements performed at the beginning and at the end of the experiment helped to calculate the fish material technological performance indicators. The fish in each tank were weighed and measured (Fig. 6). The following technological parameters were calculated to establish the fish growth performance:

- the final biomass on each tank;
- the individual growth.

The individual growth represents the difference between the final average mass and the initial average mass per specimen, expressed in grams.

Fig. 5. Portable multiparameter AZ 86031 (original photo)

Fig. 6. Biometric measurements (original photo)

RESULTS AND DISCUSSION

At the beginning of the experiment, the fish material was examined macroscopically to determine the health state. This was necessary to avoid triggering of unknown diseases during the experiments.

The experiments were performed to evaluate the effect of probiotic mixtures ParaProteX and MCC on:

- Physico-chemical parameters of water in the aquaponic system.
- Health status and growth performance of carp *C. carpio* used in experiments with ParaProteX and MCC mixture.

Dynamics of physico-chemical water parameters in RAS system

The physico-chemical parameters of the supply / discharge water in the aquaponic system designed for experiments were determined daily. Samples were taken from water entering the system and from water leaving the system.

Temperature

The water temperature did not change significantly. At the water supply the aquaponic system, the recorded values varied between 22.1°C - 26.9°C, with an average of 24.4°C, and at discharge the values were between 22.3°C - 27.1°C, with an average of 24.7°C. The optimum temperature for growing carp is 22°C - 27°C.

Water pH

Fish grow in optimal conditions in waters with a pH between 7.2 - 7.8 pH units. At the water supply into the aquaponic system, the pH values were between 7.3 - 7.7 with an average of 7.5 and at discharge the pH recorded values

between 7.4 - 8 with an average of 7.6. The water pH did not change significantly.

Dissolved oxygen

In recirculating systems, it is very important to keep the dissolved oxygen value within normal limits. For short periods of time, carp can survive at low concentrations of dissolved oxygen. When using probiotics as food bio additives, for normal carp growth it is recommended the dissolved oxygen to be at least 5 mg/L.

In experimental aquaponic system, the concentration of dissolved oxygen varied between 5.24 - 9.37 mg/L at supply, with an average of 7.31 mg/L, and at discharge between 4.89 - 8.06 mg/L, with an average of 6.47 mg/L. There was a slight decrease in the oxygen value recorded at the discharge compared to the supply, of almost 1 mg/L.

Chemical oxygen consumption

Chemical oxygen consumption increased after water passed through the recirculating system. The values recorded at supply were between 20.9 - 25.7 mgO_2/L with an average of 23.3 mgO_2/L, and at the discharge between 26.3 - 31.2 mgO_2/L, with an average of 28.75 mgO_2/L.

The use of probiotics in recirculating systems improves water quality by accelerating the effluents bioremediation from fish intensive systems, probiotics being involved in the process of decomposition and mineralization of harmful substances in water, leading to improved water quality in aquaculture.

Health status and growth performance of carp *C. carpio* from experiments with ParaProteX and MCC mixture

The aim of these experiments was to evaluate the effect that the two types of probiotic mixtures have on the health and growth performance of fish material, so that the ratio between the growth rate and the efficiency of using different food and probiotic mixtures can lead to obtaining a healthy fish population and a higher production of fish meat.

At the beginning and end of the experiments, six specimens from each tank were evaluated in terms of health, with a total of 48 analyzed fish. Following the analyzes performed, the presence of ectoparasites and endoparasites was reported.

Ectoparasites are parasites that affect the fish body, especially the skin substrate, fins, eyes, and gills, being present in a small number. Three species have been identified, as follows:

• *Trichodina domerguei* (Wallengren,1897) is a ciliated protozoan that has been reported in very weak invasions on the skin and gills, without representing a major danger to the carp population (Fig. 7). The parasitic intensity was 5-6 parasites/host, and the parasitic extensiveness was 12 parasitic specimens from 48 studied fish.

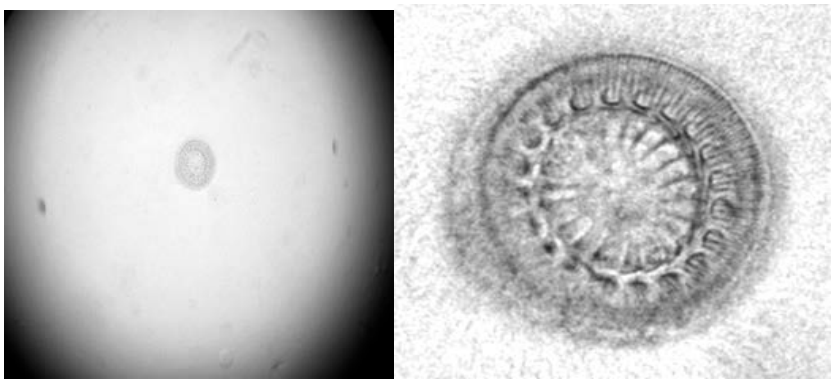

Fig. 7. *T. domerguei* (original photo)

The parasite attaches to the fish skin and gills and feeds on mucus, the bacteria it contains and dead cells. The transmission is made by direct contact of the fish. The clinical manifestations of trichodynia's are poorly visible, almost imperceptible in weak invasions.

• *Dactylogyrus vastator* (Nybelin, 1924) is a monogenic worm, specific to carp fry, preferring water temperatures of 22 - 24^{o}C. It was reported on the gills of eight fish out of 48 specimens analyzed. The number of parasites was very low, with an intensity of 3 - 4 parasites / host. Being present in a small number, it did not represent a danger for the carp population (Fig. 8).

Fig. 8. *D. vastator* (original photo)

• *Gyrodactylus* sp. it is a monogenic worm, identified after analyzing the scrapes at the base of the fins and on the gills (Fig. 9). The parasitic intensity was 3 - 4 parasites / host, the parasitic intensity being recorded at 10 specimens out of 48 analyzed. This parasite feeds on mucus and scaly cells on host's skin or gills**.**

Following the analyzes performed, only one species of endoparasite was reported:

• *Ligula intestinalis* (Linnaeus, 1758) is a Cestode worm that affects freshwater fish species, present mainly in cyprinids (Fig. 10).

It was identified in ten fish specimens from the total studied, in the body cavity, among the viscera. Its parasitic intensity was very low, 1 - 2 parasites/host.

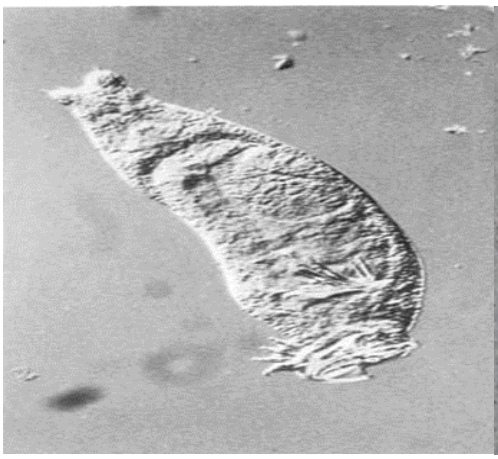

Fig. 9. *Gyrodactylus* sp. (original photo)

Fig. 10. *L. intestinalis* (original photo)

The parasitological analyzes at the end of the experiment showed a lower parasitic intensity and extensiveness, in the groups where probiotics were used as natural additives in the administered feed. They had a strong beneficial effect on fish reared in the aquaponic system by eliminating existing endo and ectoparasites. Probiotics create a special and effective protection against various parasites, bacteria, fungi, viruses. The use of probiotics to prevent fish diseases is relatively recent. Studies have shown the beneficial effect of probiotics introduced in the fish diet, manifested by improving health and increasing immunity.

The initial biomass on each tank was 5000g, after 42 days of experiment and it varied both in the experiment with ParaProteX and in the one with MCC mixture. The highest final biomass was obtained in B.1.3 where ParaProteX was added at a concentration of 6g/100g feed /day (Fig. 11). This led to weight gain of fish in the B.1.3 experimental basin where the individual growth increase was 69 g/ex (Fig. 12).

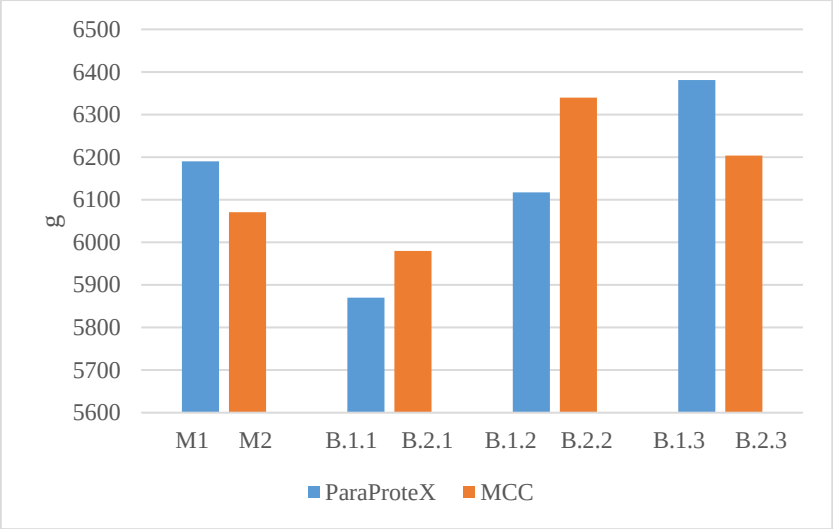

Fig. 11. Final biomass on each tank in the RAS system

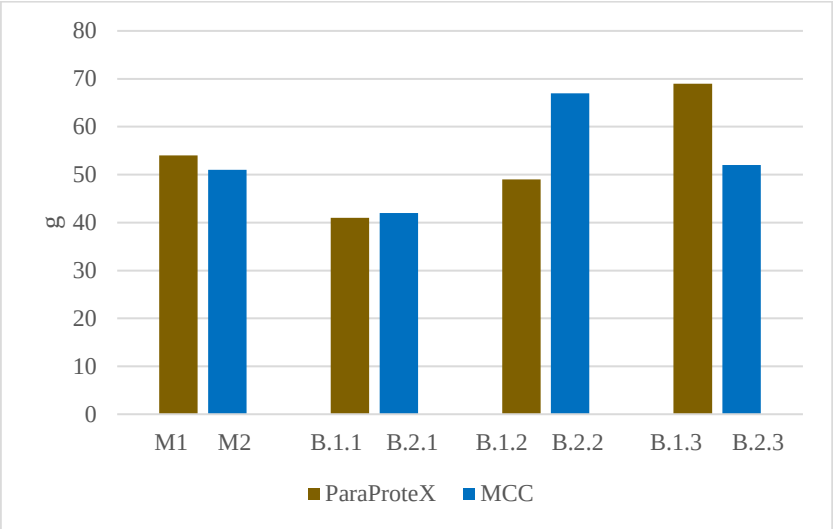

Fig. 12. Individual growth of carp reared in the RAS system

From the data obtained, it is observed that the MCC mixture had a homogeneous beneficial action on the fish in the RAS system. In tank B.2.2, the MCC mixture that was administered in a concentration of 4g grape marc, 4g cinnamon and 4g cloves/100g of feed/day, lead to a growth increase of 69 g/ex, which proves that this feed-probiotic mixture was the most balanced.

The monitoring of carp population's health status reared in the aquaponic system showed a favorable evolution and a permanent balance between the fish living environment, physico-chemical water parameters, breeding and feeding technology.

CONCLUSIONS

Following the morpho-pathological examination, four species of parasites were determined, reported in small numbers, and not endangering the carp population reared in the RAS system.

It was noted that the ParaProteX mixture represents an effective means of treatment against parasites without affecting the fish meat quality, as in the case of conventional antibiotic treatments. ParaProteX inhibits and kills over 1000 different species of parasites, from protozoa, to worms and mites, which end up in the recirculating system together with the supply water.

The MCC mixture, by its antibacterial, antiviral, antifungal and antiseptic action maintained within normal limits the organic load of the aquaponic recirculating system and did not favor the overgrowth of bacteria and fungi that reach the recirculating system with the water supply, as demonstrated by the fish good condition.

The analysis of physico-chemical water quality parameters in the RAS system discharge highlighted that the use of feed in which a natural mixture of ParaProteX or MCC type was added did not negatively influence the technological water quality. The parameters monitored for water supply and discharge did not register significant differences and were in optimal conditions for the development of the studied species (*C. carpio* and *L. sativa*).

The ParaProteX administrated in a concentration of 6g/ 100g feed / day and the MCC mixture in a concentration of 4g grape marc, 4g cinnamon and 4g cloves/100g feed/day had a strong beneficial action on the fish health in the RAS system. Their use led to an increase in the fish immunity system by maintaining the health of the intestinal flora.

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