INCIDENCE OF PARASITIC DISEASES ON STURGEON REARED IN POLYCULTURE FISH FARMS

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Abstract

In this study, the pathology of adult sturgeon reared in polyculture with different cyprinid species was investigated over a period of 60 days in the L.E.C.A.P. Brateș, Galați. Parasitic diseases were detected by microscopic examination and infectious diseases by clinical examination and history. Parasitosis intensity and disease prevalence assessment were associated with fish health status and water physicochemical parameters. The results suggest that the poor conservation status of some sturgeon and their feeding habits compared to cyprinids may be the cause of susceptibility to infectious diseases. Tegmental and gill parasitoses affected cyprinids more severely, while they affected sturgeons less severely, resulting in a decrease in their ectoparasitic diseases. Wild fish accidentally introduced into the pond showed increased resistance to infectious and parasitic diseases. Rearing sturgeon in polyculture with cyprinids resulted in a decrease in parasitic diseases in acipenserids

Key words: *infectious diseases, parasites, sturgeons, cyprinids, polyculture*

INTRODUCTION

One of the biggest problems of mankind is the search for new food sources for the growing world population [1, 2]. In recent years, the global production of fish from aquaculture has shown an increasing trend [3]. The key factor for high fish production is the health of the biological material, which in turn is influenced by the interaction of abiotic and biotic factors that affect the immune mechanism of fish and thus their resistance to diseases [4].

The climatic changes observed in recent years, which are in a continuous process of development worldwide and are expressed mainly in an increase in temperature and the degree of eutrophication of water in fish farms [5], have serious implications for the health and production of fish [6].

On the other hand, a large part of the substances used to treat ectoparasites and destroy harmful crustaceans in ponds have been banned by the legislation of the European Union without being replaced by new treatment substances [7], so that the resistance forms of parasitic organisms in ponds register a cumulative process that is constantly increasing.

It has been found that the ability of earthen pond parasites to adapt to climate change exceeds the ability of fish to adapt to the stress of new climate change [8].

Another important factor that contributes to the increase in fish production is polyculture, in which all trophic niches are used according to the food spectrum of the species and the age of the fish in a population [9]. In the earthen pond used for this experiment specially designed for sturgeon culture, the diseases of sturgeons reared in polyculture [10] with cyprinids were monitored over a-60-day summer period.

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MATERIAL AND METHOD

The experiment was conducted between 10.07-10.09.2023 in the earthen pond BP1 of the Brateș Experimental Agro-Piscicole Research Laboratory Galați and consisted in evaluating the incidence of bacterial and parasitic diseases in relation to the host fish species by monitoring diseases in acipenserids raised in polycultured with ciprinids [11, 12].

To avoid food competition from fish with the same nutritional spectrum, polyculture with adult sturgeon and two-summer-old cyprinids was practiced. The role of Chinese carps was to maintain water quality by controlling algae growth and the spread of reeds in the pond.

Pond BP1 with an area of 0,5 ha was stocked with 200 kg of adult sturgeon in a sturgeon: carp: Chinese carps of 2:1:1 ratio.

Sturgeons were represented by the species: sterlet (*Acipenser ruthenus* Linnaeus, 1758); starry sturgeon (*Acipenser stellatus* Pallas, 1771); Russian sturgeon (*Acipenser gueldenstaedtii* J. F. Brandt & Ratzeburg, 1833) and ciprinids by: carp (*Cyprinus carpio* Linnaeus, 1758); silver carp (*Hypophthalmichthys molitrix* Valenciennes, 1844); bighead carp (*Hypophthalmichthys nobilis* J. Richardson, 1845); grass carp (*Ctenopharyngodon idella* Valenciennes, 1844); common bream (*Abramis brama* Linnaeus, 1758).

Throughout the experimental period, the water in the pond was partially refreshed 8-10 hours per day at a flow rate of 1 litre per second, and additional aeration occurred 6 hours per 24 hours at night from 02-08 antemeridian.

Sturgeon was fed pelleted Supreme 10 with a granulation of 4.5 mm from Coppens with a crude protein content of 49% at a daily feed ration of 5% of body mass, while ciprinids were fed feed with a protein content of 25% crude protein at a daily feed ration of 2.5% of body mass, at different locations specifically set up for separate feeding of sturgeon and cyprinids in the pond.

The physical parameters of the water were monitored with the multiparameter type HACH-HQ 40 d and the chemistry of the water was analyzed in the chemistry laboratory of I.C.D.E.A.P.A. Galati. The examined fishes were caught with a throwing fishing net (30 mm mesh size). Healthy specimens were returned to the pond after biometrics, weighing and collection of mucus samples from the skin and a small gill fragment.

The evaluation of the health status of the fish was performed after the macroscopic and microscopic examination. The macroscopic examination assessed the integument, fins, oral cavity, eyes, gills, general appearance of the organs in the abdominal cavity, and the presence of macroscopic parasites, cysts, or fungi.

Microscopic examination (using Olimpus SZ 61 stereomicroscope and Euromex Oxion microscope equipped with a Ccmex 3.0MP camera) used scrapings and fresh squash preparations to analyze the presence of parasites at the tissue and organ levels. Scraped samples of skin, gills, eyes, squash samples of liver, spleen, kidney, brain, muscle, and intestinal contents were examined. Intestinal sections were examined longitudinally for possible parasitosis, as well as physio pathological changes in the tissue. Blood and bile smear was used to identify possible parasitic flagellates [13]. Protozoan and metazoan ectoparasites were identified by microscopic examination of wet skin mucus preparations and gill biopsies [14]. The microscopic method was used to assess parasite infestation (identification and counting) and to determine by statistical analysis the prevalence, intensity of parasite infestation, incidence of parasites on skin and gills. Endo parasitism of myxosporidiosis was identified by microscopic examination of squash prepared from kidney tissue [15].

Prevalence was calculated by dividing the number of infected fish samples by the total number of examined fish and expressed as a percentage (Microsoft Excel, 2010). The relationship between intensity of parasite infection with length and weight or weight and infestation intensity of fish were compared with Pearson correlation coefficient (Excel, version 2010).

RESULTS AND DISCUSSIONS

Table 1 Taxonomic classification of identified parasites on sturgeons and cyprinids

Phylum subphylum	***Clasa subclasa***	***Ordinul***	***Genul***	***Species***
Ciliophora	Oligohymenophorea (Hymenostomatia)	Peritrichida	Trichodina	*Trichodina sp.*
Ciliophora	Oligohymenophorea (Hymenostomatia)	Peritrichida	Ambiphyra	*Ambiphyra sp.*
Ciliophora	Oligohymenophorea (Hymenostomatia)	Oligohymenophorea (Hymenostomatia)	Ichthyophthirius	*Ichthyophthirius multifiliis*
Plathelminthes	Monogenea (Monopisthocotylea)	Dactylogyrus	Dactylogyrus	*Dactylogyrus sp.*
Mixozoa	Myxosporea	Bivalvulida	Sphaerospora	*Sphaerospora renicola*

Photos 1-6 shows the parasites found on all fish species in pond BP1 (with taxonomic classification table 1). Photos 1-4 show external parasites on the skin and/or gills and photo 5 shows internal renal parasitosis in the form of myxosporidiosis.

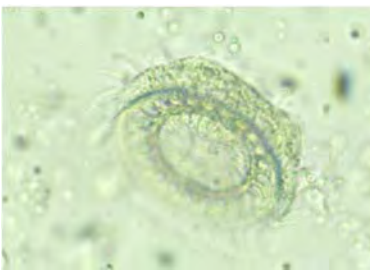

Foto nr.1-*Trichodina sp.*

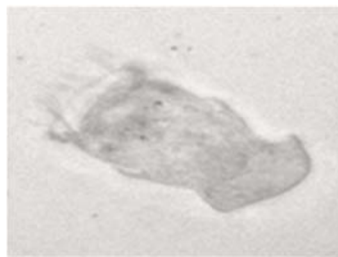

Foto nr.2-*Ambiphyra sp.*

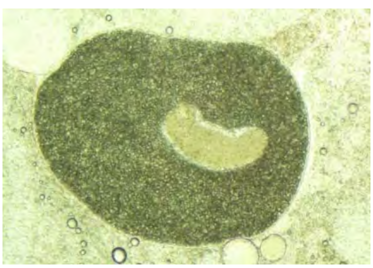

Foto nr.3-*Ichthyophthirius multifiliis*

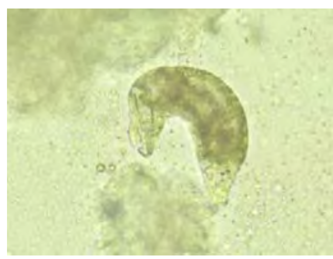

Foto nr.4-*Dactylogyrus sp.*

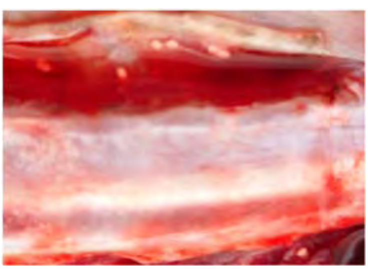

Photo no.5-Kidney cystic fibrosis

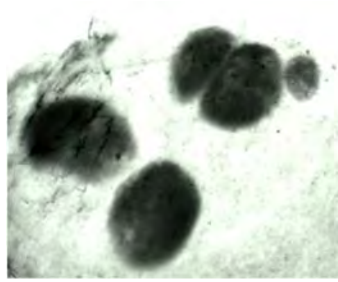

Photo no.6-*Sphaeospora renicola* isolated from kidney cysts

Photo 7-10 shows the specific symptomatology of a bacterial disease caused by *Aeromonas hydrophila* in association with *Pseudomnonas fluorescens* in Russian sturgeon and carp.

Photo no. 7-Russian sturgeon no.1 with subcutaneous abdominal haemorrhage

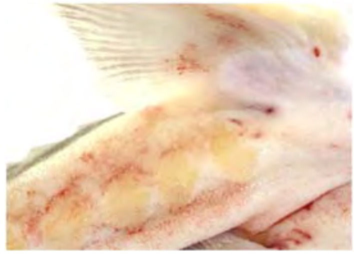

Photo no. 8- Russian sturgeon no. 2 with ventral subcutaneous haemorrhages and at the base of paired fins

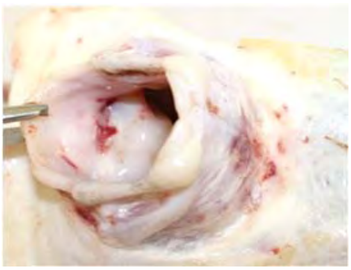

Photo no. 9 - External peribuccal and internal oral haemorrhages (Russian sturgeon no. 2)

Photo no.10 - Carp with subcutaneous petechial haemorrhages

Table 2 Data on the fish species with different parasitoses, their intensity, skin, and gill frequency in correlation with the standard length and body mass of the fish on the day of sampling

No.	Fish species	Parasite name	Intensity of parasite infestation	Standard body length (cm)	Body mass (g)	Tegumentary incidence (%)	Branhial incidence (%)
Date of sample collection 10.07.2023							
1	Starry sturgeon	*Trichodina sp.*	accidentally	33	407	100	-
2	Sterlet	*Trichodina sp.*	weak-medium	34	586	20	80
3	Common carp	*Argulus foliaceus*	accidentally	20	126	100	-
		Trichodina sp.	medium			70	30
4	Bighead carp	*Trichodina sp.*	massive	11	13	-	-
Date of sample collection 24.07.2023							
1	Russian sturgeon	*Trichodina sp.*	accidentaly	66,5	705	100	-
		Ambiphyra sp.	weak			40	60
		*Sphaeospora renicola**	weak			-	-
2	Starry sturgeon	-	-	88	2510	-	-
3	Common carp	-	-	23,5	192	-	-
4	Silver carp	*Ichthyophthirius multifiliis*	weak	14,5	16	33,33	66,67

Date of sample collection 31.07.2023							
1	Russian sturgeon	*Trichodina sp.*	accidentaly	72,5	1014	-	100
2	Common carp	*Ichthyophthirius multifiliis*	medium	16	102	37,5	62,5
3	Silver carp	*Ichthyophthirius multifiliis*	massive	11,2	13	21,05	78,95
	Grass carp	*Trichodina sp.*	weak	18	98	-	-
Date of sample collection 08.08.2023							
1	Russian sturgeon	*Ichthyophthirius multifiliis*	weak	79	1454	-	100
2	Russian sturgeon	-	-	80	1878	-	-
3	Russian sturgeon	-	-	73	1660	-	-
4	Common carp	*Dactylogyrus sp.*	medium	17	105	-	100
		Ichthyophthirius multifiliis	medium			11,12	88,88
5	Common carp	*Ichthyophthirius multifiliis*	massive	20,5	114	20	80
6	Common carp	*Dactylogyrus sp.*	medium	22,8	162	-	100
7	Common carp	*Ichthyophthirius multifiliis*	medium	21,5	138	30	70
8	Common bream	-	-	13,4	37	-	-
9	Silver carp	-	-	14	11,5	-	-
Date of sample collection 10.09.2023							
1	Russian sturgeon	*Trichodina sp.*	weak	71	1312	50	50
2	Common carp	*Dactylogyrus sp.*	weak	19	121	-	100
		Ichthyophthirius multifiliis	massive			27,7	72,3
3	Common carp	-	-	31	446	-	-
4	Silver carp	*Dactylogyrus sp.*	medium	6	8,5	-	100
		Ichthyophthirius multifiliis	medium			28,58	71,42

Legegend: week<5 specimens of parasites in the microscopic field, medium between 5-10 parasites in the microscopic field and massive with more than 10 specimens of parasites in the microscopic field (*)-on kidney

The species of ectoparasites found in sturgeon (table 2) in the BP1 basin were also identified in previous studies according to the literature [16, 17, 18].

Table 3 Experimentally determined parasite prevalence calculated on fish species

No.	Fish species	Parasite name	Prevalence /parasitic species/pond (%)
1.	Russian sturgeon	*Trichodina sp.*	50
		Ambiphyra sp.	16,6
		Sphaeospora renicola	16,6
		Ichthyophthirius multifiliis	16,6
2.	Starry sturgeon	*Trichodina sp.*	irrelevant
3.	Sterlet	*Trichodina sp.*	irrelevant
3.	Carp	*Argulus foliaceus*	11,1
		Trichodina sp.	11,1
		Ichthyophthirius multifiliis	55,5
		Dactylogyrus sp.	33,33
4.	Silver carp	*Ichthyophthirius multifiliis*	75
5.	Bighead carp	*Trichodina sp.*	irrelevant
6.	Grass carp	*Trichodina sp.*	irrelevant
7.	Common bream	-	-

The highest prevalence (table 3) is observed in ichthyophthiriasis in silver carp (70%) and common carp (55%), which is due to the explosive multiplication of the parasite during the period when increased water temperatures negatively affected the fish and lowered their immune mechanisms to adapt to the new environmental conditions. Trichodiniosis occurs in 50% of sturgeon and only 11.1% of carp and can be associated with periods of slight warming of water temperature and increased levels of organic matter [19].

The ectoparasites *Ambiphyra sp*., *Ichthyophthirius multifiliis*, and the endoparasite *Sphaeospora renicola* also had low prevalence in sturgeon (16.6%). Among fish species, silver carp was most parasitized with the ciliate *Ichthyophthirius multifiliis* (75%), followed by carp infested with *Ichthyophthirius multifiliis* (55.5%) and the monogenic trematode *Dactylogyrus sp*. Fish introduced with the pond water supply showed no parasites.

The evolution of the temperature and physicochemical parameters of the water (table 4) had a direct influence on the development of parasitosis and caused lack of appetite in the fish, which was reflected in a reduced resistance to diseases in the observed summer months.

Table 4 The evolution of water temperatures and dissolved oxygen during the experimental period

The measured monthly period	Water temperature (0C) at 12,00 p. m.			Dissolved oxygen (mg/l) at 12,00 p. m.		
	Med.	**Min.**	**Max.**	**Med.**	**Min.**	**Max.**
July	26.86	25.10	28.90	6.11	4.10	7.91
August	26.93	25.2	29.4	6.94	3.02	10.29
September	26.12	24.1	26.8	6.95	4.9	10.1

There is a direct relationship between water temperature, the amount of organic matter and the rate of parasite reproduction, which is reflected in the intensity of parasitosis. The aetiology of parasitic diseases for the ciliate protozoan *Trichodina sp.* is the source of the water supply, which has increasing rates of eutrophication in recent years of climate change and from the farm's bottom ponds used for cyprinid rearing. Ichthyophthiriasis and mixosporidiosis also correlate with the residues in the pond sludge, the condition of the fish stock (table 5), sick fish, the main source of infestation in the case of dactylogyriasis are the resistance eggs from the ponds.

Water chemistry showed values in the range of 7.11-8,73 for pH, with optimal values of 6.5-8.5 upH, according to Ord.nr. 161/2006 for quality class a- II [20, 21], mainly due to the overgrowth of algae and macro phytic vegetation on the pond, organic matter content between 77.86-107.22 (with optimal values of 60 mg $KMnO_4$/l) and chlorides in a range of 9.57-82.9 (with maximum permissible values of 40mg/l) due to the strong evaporation of water during the hot periods. During the period from 10/07 to 24/07/2023, the increase in water temperature to a maximum value of 28.1^{0}C (recorded at 12,00 p.m.) was associated with a massive infestation of *Trichodina sp*. in sturgeon and carp, which was also detected in the source of water supply.

The second peak of parasite development was recorded in the period 31.07.-08.08.2023. The increase in water temperature to a value of 29.4^{0}C (12,00 p.m.) was associated with massive *Ichthyophthirius multifiliis* infestation in carp and silver carp. In the period 08/08-10/09/2023 the increase in water temperature to a peak of 28.3^{0}C at midday was correlated with the intensity of ichthyophthiriasis, which showed a stagnant to decreasing trend, and was associated with dactylogyriasis which occurred mainly in carp and silver carp.

Table 5 Relationship between standard length and body mass of fish

No.	Fish species	Mean standard length (Ls)± standard deviation (cm)	Mean body Mass(W)± standard deviation (g)	Mean Fulton's condition factor) ± standard deviation	Fulton's condition factor		Ls (cm)		W (g)	
					Min.	Max.	Min.	Max.	Min.	Max.
1	Russian sturgeon	73,67±5,08	1337,17±427,8	0,003±0,001	0,002	0,004	66,5	80	705	1878
2	Starry sturgeon	60,5±19,45	1458,5±743,52	0,008±0,005	0,004	0,011	33	88	407	2510
3	Common carp	21,26±4,41	167,33±108,4	0,017±0,004	0,016	0,021	16	31	102	446
4	Silver carp	11,43±3,89	12,0±3,08	0,014±0,017	0,005	0,039	6	14,5	8,5	16

Fulton's condition factor, $k_c = 100*W/L^3$ where: W is the total weight of the fish and L is its total length

The correlation between weight and standard length for the five cyprinid species and the three sturgeon species are shown in Figure 1, and the correlation between fish weight and parasite infestation intensity according to the values in the legend of table 2 are shown in Figure 2.

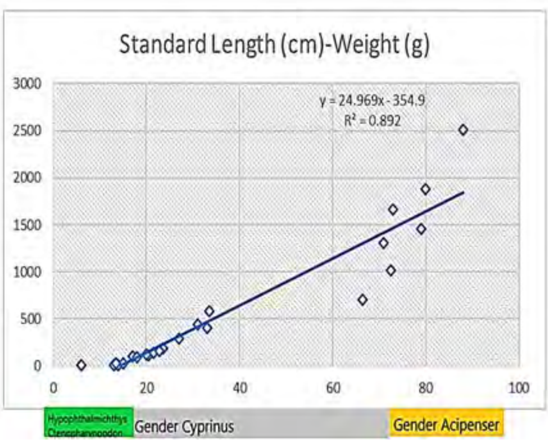

Fig. 1 Positive correlation between length and weight of two-summer-old cyprinids and adult sturgeon

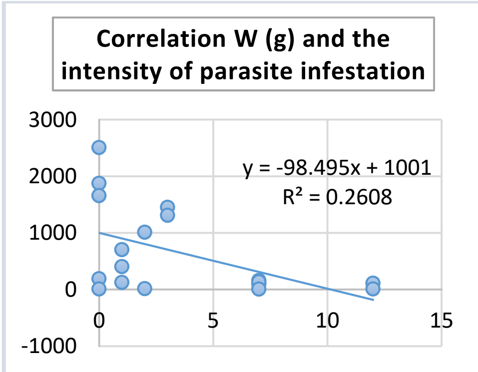

Figure 2 Negative correlation between the weight of fish and the intensity of parasite infestation

Bacterial disease caused by a bacterial association (*Aeromonas hydrophila* and *Pseudomonas fluorescens*) and detected in two Russian sturgeon and one carp (photos 7-10) on 10/08/2023 was examined microbiologically. Susceptibility testing revealed that fluorfenicol (Enrodem 50) should be administered in the feed at a dose of 15 mg/kg body mass for 14 days. The disease occurred in these species during periods of rising water temperatures in poorly fed fish and may be since both species, as bentophagous fish, are at the highest risk of contact with these ubiquitous bacteria that live in the slime on the pond bottom.

A positive correlation (r=0.892) was found between standard length and fish body mass (figure 1) and a negative correlation (r=0.26-figure 2) between fish body mass and parasite intensity.

The results show that *Trichodina sp.* infestation is directly proportional to the increase in water temperature and organic matter content (which favour rapid multiplication of the parasite, mostly by binary division) and inversely proportional to the physiological condition of fish in the pond, regardless of fish species, although lower parasitism was observed in sturgeons compared to cyprinids [18]. *Dactylogyrus sp. and I. multifiliis* were more abundant in silver carp and common carp, while *I. multifiliis* was found in a weak infestation on the gills of sturgeon.

Trichodiniasis may also be associated with other parasitoses, resulting in increased mortality due to asphyxia, anaemia, and risk of secondary viral, bacterial, and fungal infection when associated with gill ichthyophthiriasis and dactylogyriasis.

CONCLUSIONS

1 - The incidence of bacterial diseases is higher in sturgeon, lower in carp, and absent in Chinese cyprinids and bream (introduced via water supply). One explanation for this could be differences in disease resistance among species combined with the conservation status of individual specimens. However, it is also possible that benthophagous fish are more susceptible to disease because they are in direct contact with the mud, which has a higher susceptibility to bacteria.

2 - The ectoparasite *Argulus foliaceus* has not been found in sturgeon, but only in carp. It is possible that the bony scutes (ganoid modified scales) of the sturgeon and the intense mucus secretions of the tegument protect these fish species from infestation by parasitic crustaceans.

3 - *Dactylogyrus sp.* infestation occurred with a medium frequency in cyprinids, and *Ichthyophthirius multifiliis* infestation was weak in acipenserids and massive in cyprinids. *Trichodina sp.* was found in water, on acipenserids, carps and Chinese carps.

4 - Diseases in fish depend on the immune system, environmental stress, diet,

and physiological condition of the fish. Different species of fish raised in the same pond have different susceptibility to disease.

5 - Wild fish accidentally introduced from pond water supply are the most resistant to infectious and parasitic diseases.

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