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ORIGINAL PAPERS

Evidence of ichthyophthiriasis in cultured *Acipenser stellatus* (*Pallas 1771*)

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Abstract

Although there is an old concern about sturgeon parasite research, few studies have delved into the histological changes produced by them on host organs. Gills, vital organs for the exchange of gases but also of ions, are targets for certain categories of ectoparasites.

During the histopathological analysis of gills in cultured *Acipenser stellatus* (stellate sturgeon), we identified the presence of *Ichthyophthirius multifiliis*, the causative agent of 'white-spot' in fresh-water fish. The species was identified histologically, without any exterior (skin) sign of disease. Different life cycle stages of *Ichthyophthirius multifiliis* were observed. Branchial changes as hypertrophy and proliferation of chloride and mucous cells were seen. To make a definitive diagnosis of ichthyophthiriasis, it is necessary to microscopically examine tissue from a gill, caudal fin or the body surface..

Keywords: stellate sturgeon, gill, *Ichthyophthirius multifiliis*, histopathology.

Introduction

Some of the most frequently encountered ectoparasites of fish are species of ciliates and of these the most important is the holotrich *Ichthyophthirius*

multifiliis, the causative agent of 'white-spot' in fresh-water fish. *Ichthyophthirius* is cosmopolitan, infecting most species of fresh-water fish, causing catastrophic epizootics in warm- and temperate-water fish culture and losses in wild fish on occasions [1].

Ich originated in Asia and spread throughout temperate regions via introductions largely linked with the aquarium and aquaculture trades [2]. Ich is a contagious disease of fishes, caused by *Ichthyophthirius multifiliis* that infect sturgeon species both in natural environment [3] and in aquaculture [4, 5].

With regard to decreasing of natural stocks of endangered sturgeons, cultured sturgeons farms are expanding, so infectious or noninfectious agents can cause problems in this industry. One of the most important infectious diseases, are parasitic diseases, like gill parasites.

Acipenser stellatus is one of the six sturgeon species, still existing in Danube River. It is currently considered to be extinct in the Upper Danube and the Upper Middle Danube Sector [6,7] and reproductive migrations in the Lower Danube considerably reduced [8].

Artificial culture of *A. stellatus* has been increased in romanian aquaculture. In-breeding and rearing of sturgeon aquaculture requires comprehensive information about health status and diseases, not only to conduce to the promotion of health and quality of sturgeon, but also to have an important role in the production and proliferation of endangered species [4]. So, early identification of different parasites could be helpful in culturing different sensitive sturgeon species, as *A. stellatus*.

Except in very severe infections, *I. multifiliis* is not usually uniformly distributed on the body of the fish. The parasite occurs most frequently on the dorsal surface, particularly the head and fins, but the gills are also important sites of *I. multifiliis* infection. Thus, the investigation of gills parasites is essential in protection of sturgeons from these parasites.

Materials and Methods

Acipenser stellatus juveniles were obtained from Brates Research Hatchery (fig.1) and transferred to the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture, Galati, Romania. The juveniles with a length of 26.9 ± 1.68 cm and a weight of 40.8 ± 6.05 g were obtained by artificial reproduction, from the parents caught in the Danube River. After the transfer, the juveniles were let to acclimate to laboratory conditions for 7 days.

The juveniles were fed with 2% of body weight with granulated food. Feeding was stopped 24 hours before sacrifice.

Histology analysis

The juveniles were captured and anesthetized by immersion to sedation in 2-phenoxyethanol solution. They were sacrificed by evisceration, the gills being immediately taken and fixed in 10% formalin for histopathological analysis.

The tissues were processed using the standard histological technique: dehydration in an ethanol series, embedding in paraffin, and serially sectioning at 7 μm. Sections were stained with hematoxylin and eosin (H/E). Microphotographs were taken with a Olympus microscope and camera.

The authors confirm that the ethical policies have been adhered to and the appropriate ethical review committee approval has been received. All experimental procedures and animal maintenance were in accordance with Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

Fig.1 Brates Research Hatchery

Results and discussions

During the histopathological analysis of stellate sturgeon (*Acipenser stellatus*) gills, we identified the presence of *Ichthyophthirius multifiliis* (fig.2).

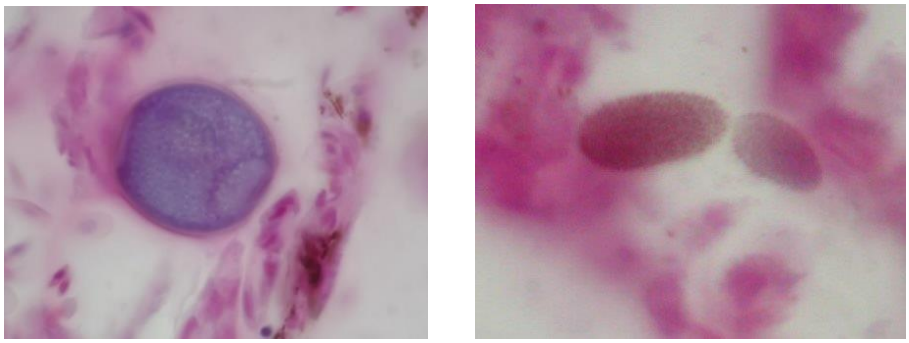

Fig.2 Different stages of *Ichthyophthirius multifiliis* observed in *Acipenser stellatus* gills original photos

Ich is a disease caused by this cosmopolitan, histophagous parasite of freshwater fishes. Outbreaks of *I. multifiliis* occur when conditions are favourable for rapid multiplication of the parasite, like a suitable environment (e.g. higher water temperature) and susceptible fishes [9].

Ichthyophthirius multifiliis is a ciliate, that possesses a large, reniform macronucleus and at least one small, round micronucleus. There are up to four micronuclei per theront, varying from one to four depending on temperature. The micronuclei of ciliates are transcriptionally inactive and play a role in genetic exchange [9].

Ichthyophthirius has direct life cycles (fig.3) but these involve trophont or feeding stages leaving the host fish and encysting within the environment [1].

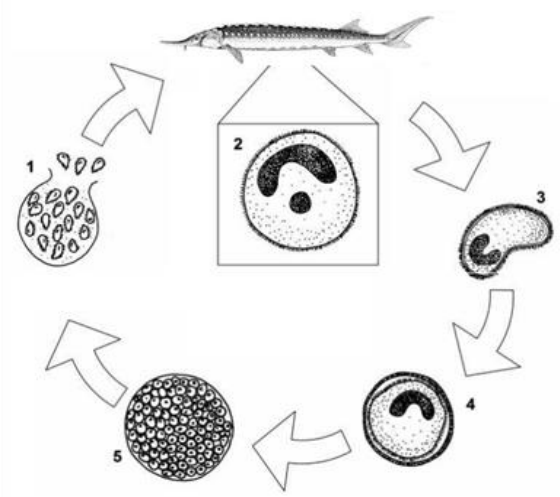

Fig.3 Lifecycle of *Ichthyophthirius multifiliis.* 1) Infective theronts released from cyst. 2) Parasitic trophont stage. 3) Exiting tomont. 4) Cyst. 5) Dividing tomites within cyst (after [11])

The trophont or feeding stage of *Ichthyophthirius* is found within the epidermis of the body surface and gills of fish where it may reach up to 1 mm in diameter and is seen as a characteristic white spot from which the disease takes its name [1]. When mature, the trophonts break out through the epidermis and into the water. The trophont is defined as a tomont a soon as it ceases to feed and extricates itself from the epithelium. This is the reproductive stage of the organism. The tomont divides nine to ten times to tomites. The daughter cells (tomites) of the tomont differentiate within a thin-walled cyst into free-swimming infective theronts [9].

The trophonts are readily identified in tissues sections of skin and gills by their large size (0.5-1 mm diameter in sections) and the horseshoe-shaped macronucleus (which may not be apparent in all sections). The trophont may be irregularly shaped in stained tissue sections, the H&E staining is useful, although cilia are not always visible [10].

During the present study, there were observed just a few branchial changes in stellate sturgeon juveniles, like a minor hyperplasia and hypertrophy of chloride and mucous cells, melanomacrophages aggregates (fig.4, fig.5). There weren't observed irreversible branchial lesions, that affect the normal functioning of respiratory epithelium.

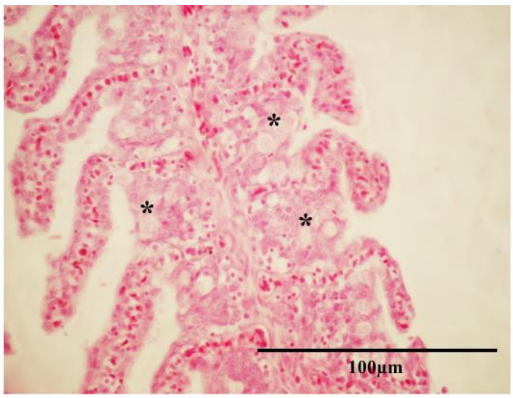

Fig.4 Gill of *Acipenser stellatus*, H&E staining: hypertrophy and proliferation of mucous and chloride cells (*), x40 (original photo)

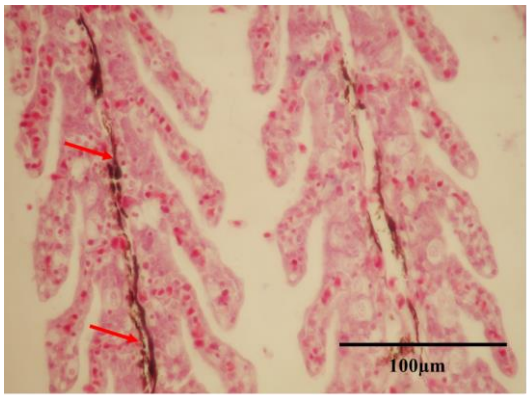

Fig.5 Gill of *Acipenser stellatus*, H&E staning: melanomacrophages agreggates (red arrow), x40 (original photo)

Less severe changes observed in the gills of stellate sturgeon, can be explained by a mild infection, that elicited minor cellular reactions. According to [9], the extensive histopathological changes reported to occur in *I. multifiliis* infections are only seen in severe epizootics or in experimental infections with large numbers of parasites. Fishes infected with small numbers of *I. multifiliis* show few signs of infection other than the development of white spots [9].

Hyperplasia of gill epithelial cells is a common lesion observed in ich infections. As a result, the gill interlamellar spaces are reduced, to interfere with

penetration of theronts, but that significantly limits the surface area available for oxygen interchange. Severe infections thus cause fishes to become oxygen-starved [9].

Hypertrophy of mucous cells means an intense activity of these cells. Surface mucus is the fish's first line of defence against infection. To infect, theronts must penetrate this mucous layer and burrow into the epithelium. Usually, mucus-secreting cells increase in the skin of infected fishes [9]. This change interferes with osmoregulatory functions as fish mucus is relatively impermeable to water and ions.

Hyperplasia between chloride cells, adhesions of the secondary lamellae, hyperaemia, necrosis and telangiectasia, proliferation of the mucous cells of the branchial arch and operculum, during high infestations were observed by [13] in *Gymnocorymbus ternetzi.* In *Danio rerio,* the parasite induced hyperplasia, an increase of mucous cells, adhesion and shortening of the secondary lamellae, narrowing of water channels [14].

In very mild *I. multifiliis* infections, the only detectable pathological change in the skin is the presence of a few white spots on the surface of the fishes. Each spot represents a developing trophont within an epithelial capsule or vesicle. In more severe cases there are usually large numbers of spots on the skin. On the stellate sturgeons, there weren't seen any white spot on the skin. This is not abnormal, since occasionally, *I. multifiliis* only infects the gills, with no obvious gross lesions on the body surface, as mentioned by [9]. According to [12] if the parasite is only present on the gills, white spots will not be seen at all, but fish will die in large numbers.

Conclusions

In stellate sturgeon, *Ichthyophthirius multifiliis* determined gill histopathological changes like a minor hyperplasia and hypertrophy of chloride and mucous cells, melanomacrophages aggregates which indicated a mild gill infection. Histology proved to be a reliable marker for identification of *Ichthyophthirius multifiliis* before severe infections can occur, and when control methods can prevent catastrophic outbreaks. Histological examination of this parasite is useful in prevention of financial losses, due to fish mortality, in rearing these rare sturgeon species, as *Acipenser stellatus.*

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