DISEASES DETECTED AT STURGEON REARED IN FRESH AND SALT WATER

Tania ZAHARIA, Elena DUMITRESCU
National Institute for Marine Research and Development
"Grigore Antipa", Constanta, Romania
e-mail: zahar@alpha.rmri.ro

ABSTRACT

The paper presents the results of the experiments carried out between 2005-2008 within the "Scientific and Technological Partnership for the Promotion of the Sustainable Management of the Romanian Marine Fisheries, Based on an ecosystem approach"- CEEX project, the stage referring to "Pilot scale experiments for the determination of the technical-economical parameters in marine aquaculture for threatened and economically valuable species".
For the sturgeons reared experimentally in fresh and marine water a few diseases caused by bacteria and improper food/feeding were identified.
The most significant diseases affecting the sturgeons were infections. Thus, at the sturgeons reared in fresh water, we identified bacterial hemorrhagic septicemia, caused by species belonging to the *Aeromonas* and *Pseudomonas* genera, and, as far as the sturgeons reared in salt water are concerned, we identified vibriosis, caused by *Vibrio anguillarum.* At a lower intensity we also identified infections caused by species belonging to the C*ytofaga* genus.

KEY WORDS: disease, sturgeons, experimental and commercial rearing

INTRODUCTION

According to the FAO 2002 statistics, in the Romanian waters of the Black Sea, the dynamics of the sturgeons fishing dropped dramatically after 1970. In Romania, the management of wild sturgeon species that breed in the North-Western area of the Black Sea is the responsibility of the Ministry of the Environment and Sustainable Development. In this respect, for the restoration

of the populations of these species, in 2006 a common order of the Ministry of the Environment and Water Management and the Ministry of Agriculture, Forests and Rural Development was issued, through which commercial fishing of sturgeon species is prohibited for a period of 10 years and breeding and repopulation of the Danube with sturgeon juveniles programs are supported (Order No. 262/330 from 2006 of the Ministry of Agriculture, Forests and Rural Development and Ministry of the Environment and Water Management regarding the preservation of the sturgeon populations from natural waters and the development of sturgeon aquaculture in Romania).

In order to conserve and restore the sturgeons stock in the Danube, one of the important actions for reducing the fishing pressure on sturgeon populations (REINARTZ R. et al., 2003) is to promote aquaculture techniques for the production caviar and flesh (including possibilities for disseminating the resulted profits through the creation of new jobs for the local fishermen).

Sturgeon aquaculture for caviar and flesh consume was first developed in the ex-USSR. The hybrid named bester (*Huso huso* ♀ x *Acipenser ruthenus* ♂) was at the base of this development. Today, the development is based mainly on *A. baerii* and *A. gueldenstadtii*. Starting with 1990, sturgeons' breeding slowly developed in Europe and USA. In Western Europe, the interesting species for aquaculture are *A. transmontanus, A. baerii* and *A. nacarii*. Recently, after 1989, in Romania the following species aquaculture started to develop: *A. stellatus, A. gueldenstadtii, A. ruthenus, Huso huso*, in specialized farms in Galaţi, Isaccea, Tamadau, etc.

In Europe, the main producing countries are Italy, Germany and Spain. The European production was, in 1999, between 1.000 and 1.600 tons/year. For the same year, the European caviar production was of 5 - 6 tons, obtained from sturgeons reared in the countries mentioned above.

In this context our researches are being carried out on establishing the rearing possibilities of two important sturgeon species, the beluga *H. huso* and the Russian sturgeon *A. gueldenstaedti* on the Romanian littoral, using different conditions for rearing in the experimental and commercial capacities (from the National Institute for Marine Research and Development "Grigore Antipa" and SC "Kaviar House" SRL). The assessment of the health state was a continuous concern, representing one of the successful paths in aquaculture.

MATERIALS AND METHOD

The biological material was represented by beluga and Russian sturgeon individuals reared in freshwater (by SC "Kaviar House" SRL) and in marine water by the NIMRD "Grigore Antipa" in various facilities (ponds, fresh water cages, marine water cages, marine water concrete tanks) (Photos

no. 1-4). The monitoring of the experimental lots was done continuously during the experiences.

Photo no. 1 - Cages placed in the Horia pond
(SC "Kaviar House" SRL - photo T. Zaharia)

Photos no. 2 - Sturgeon rearing cage - marine water (NIMRD - photo T. Zaharia)


Photos no. 3 - 4 - Tanks for the marine water rearing of sturgeons
(left - exterior concrete tanks, right - interior fiber glass tanks - photo T. Zaharia)

For the identification of the pathogen agents, dissections of the ill individuals were made, clinical examinations, parasitic examinations and the technique of usual and selective culture environment impregnations, in order to trace the infections caused by bacteria and fungi. In addition, antibiograms were carried out, in order to establish the most adequate treatments.

RESULTS AND DISSCUSSIONS

For the sturgeons experimentally and commercially bread in fresh and marine waters several diseases caused by bacteria and improper food/feeding were identified.

The most significant diseases affecting the sturgeons were infections. Thus, at the sturgeons reared in fresh water, we identified ***bacterial hemorrhagic septicemia***, caused by species belonging to the *Aeromonas* and *Pseudomonas* genera, and, as far as the sturgeons reared in marine water are concerned, we identified vibriosis, caused by *Vibrio anguillarum.* At a lower intensity, we also identified infections caused by species belonging to the C*ytofaga* genus.

► **Hemorrhagic bacterial septicemia** is a fresh water fish disease with a complex and not sufficiently cleared etiology. The disease is widely spread worldwide, affecting especially culture fish species (NOGA E.H., 2000, PREARO et al.., 2009).

The etiological agents of the disease are species belonging to the *Aeromonas* - *A. hydrophila* and *Pseudomonas* - *P. flurorescens* genera.

Aeromonas hydrophila is a Gram negative bacteria, shaped as a straight 0.3 -1.0 x 1.0 -3.5 um cane, mobile, monotrichous (Photo no. 5). It is an extremely unpretentious microorganism, it grows on the usual and selective

nutritive environment (Mac Conkey), on which it develops round, creamy colonies, white, neat, convex, up to 5 mm in diameter. It muds the broth with or without pigment (orange yellowish), it is oxydaso-positive and ferments glucose, maltose and lactose.

Pseudomonas flurorescens is a Gram negative bacteria, shaped as a 0.3 -0.6 x 0.8 -2 µm bacillus, it develops 2-3 mm round colonies, neat, mucoid, green or yellow (on Meitert Istrati agar). It is oxydaso-positive, produces pigments, does not ferment alcohols and some sugars, it hydrolizes jelly.

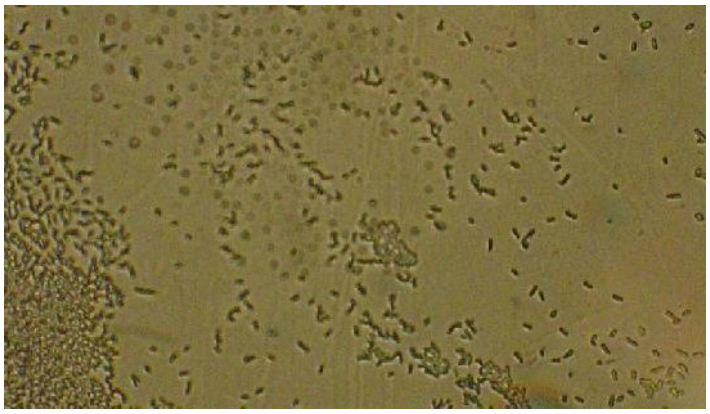

Photo no. 5 - *Aeromonas hidrophila* isolated on the diseased fish
(Photo E. Dumitrescu)

The natural sources of the infection are represented by the tank water, the bursting of the disease being favored by an improper water quality. The infection occurs by means of the digestive canal. The bacteria normally present in the tank water and in the digestive system of the fish invade their organism, under the conditions of stress caused by improper environmental conditions.

Another source of infection was represented by the natural food provided (*Tubifex*), contaminated with the bacteria causing this disease.

A complex analysis of the *Tubifex* worms, provided as natural food, emphasized the following potentially pathogen agents charge:

- Heterotrophic Germs (NTG) - 2,500,000 /g;
- *Aeromonas* - 120,000/g;
- *Pseudomonas* - 200/g;
- *Vibrio* - 100,000/g;
- Sulphyde-reducing clostridia - 200/g;
- Microfungi and yeasts - 2,000/g
- Total coliforms > 1,609,000/dm^3

According to this data, we appreciate that the use of *Tubifex* worms represented a major source of germ contamination of the sturgeon juveniles reared in fresh water.

As far as the symptoms are concerned, the fish affected by this disease presented bleeding lacerations, ulcerations, exophtalmia, hyperemia and anal prolabation. Internally, there were hemorrhages (Photos nr. 6-7), enlarged spleen and kidneys, swollen intestines, hyperemiated livers, with areas touched by necrosis.


Photos nr. 6 - 7 - Hemorrhages on Russian sturgeon affected by septicemia (photos E. Dumitrescu)

The diagnosis was given as a follow up of the clinical examination, combined with the microbiological one. The bacteria were isolated from the liver, kidneys and blood of the affected fish.

In order to establish an efficient treatment, antibiograms were performed (Table no. 1).

Tabel no. 1 - The results of the antibiogram for the septicemia causing agents on sturgeons

Nr. crt.	**Antibiotics**	***Aeromonas***	***Pseudomonas***	**Total bacterian flora**
1.	Kanamicine	S	S	S
2.	Peniciline	SS	S	SS
3.	Streptomicine	S	S	S
4.	Tetracicline	S	S	S
5.	Oxacyline	R	R	R
6.	Neomicine	SS	SS	SS
7.	Neoxazol	R	R	R
8.	Bacitracine	R	SS	SS
9.	Cloramfenicol	S	S	S
10.	Oxitetracicline	S	S	S
11.	Methylic Blue	S	SS	SS

Based on their results, efficient treatments were established, using powder oxytetracicline (98%), in 50 mg/ fish kg doses (5 g/food kg), for 7 days. Also, treatments with tetracicline and cloramfenicol were efficient, the dosage and treatment duration being similar with the oxytetracicline ones. In order to prevent the *Aeromonas* infection from the *Aeromonas* infected *Tubifex* worms provided as natural food, we immersed the worms in methylic blue baths, in 200mg/food kg doses.

► **Marine fish vibriosis**, caused by bacteria belonging to the Vibrio genus, infectious disease largely spread on Earth at various fish species (Anguilidae, Gadidae, Salmonidae) (AUSTIN &AUSTIN, 2007), was detected at sturgeon juveniles also (beluga and Russian sturgeon) experimentally reared in marine water.

From the fish affected by vibriosis *Vibrio anguillarum* and *Vibrio* spp were isolated, Gram-negative, asporulate, acapsulate, monotrichous bacteria, measuring 0,5X1,2 - 2µm (Photo no.8).

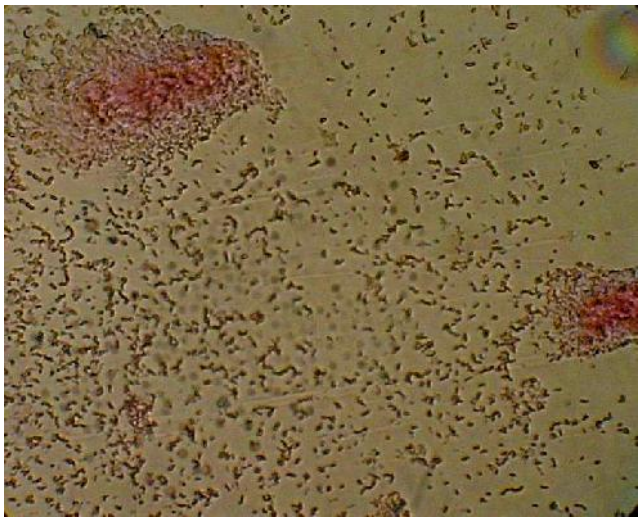

Photo no. 8 - *Vibrio anguillarum* isolated on the fish affected by vibriosis
(photo - E.Dumitrescu)

The disease manifested itself cronically, at certain fish individuals with a low degree of resistance, natural or gained due to improper environmental conditions, as the sudden temperature variations, high organic matter concentrations, high bacterial contamination of the marine environment.

Vibriosis was characterized by infections localized on the skin and muscles, defined through skin bleeding lesions, superficial or more profound

(Photos no. 9 - 10). Blood-red lesions were observed around the anus and at the base of the fins. Extremely rare, we identified fish individuals in which the pathological modifications of the internal organs reflected an acute form of disease: strong intestinal swelling, swollen spleen and kidney, congestions and hemorrhages in the peritoneum and internal organs.


Photos no. 9 - 10 - Vibriosis on the beluga - bleeding lesions in the head area (A) and liver with hemorrhagic infiltrations (B) - (photos - E. Dumitrescu)

As the disease was identified at a small number of fish individuals, we estimated that contracting it did not represent a danger for the fish experimentally reared in marine water.

When this disease affects a large number of fish, with an abrupt bursts, it is extremely dangerous. Under these circumstances, the mortality rate reaches 80% of the affected fish, the juveniles being most sensitive. In this case, even under treatment, 10-20% losses are registered, in a few days from the bursting of the disease.

The treatments were performed by administrating in the fodder antibiotics at which the agents causing the disease were sensible (detail established by the antibiograms), oxytetracicline and cloramfenicol. The daily doses where of 50 mg/fodder kg, for a period of 5 days. Periodically, the fish were immersed in methylic blue baths, in doses of 30 mg/l water, for 30 minutes.

For the chronic cases, with intestinal localization, furanic products can be used for therapy (furazolidon, furaxon), administered in the food, in a daily dose of 100 mg/ fish kg, for 6 days.

As the drugs are administered orally, in the food, anorexic fish do not accept them. Under these circumstances, vaccination is an efficient measure. A series of polivalent vaccines can be used, produced and distributed in various countries (USA, Germany, Norway). They can be administered through injections, baths or orally.

► **Bacterial gill disease:** is an infectious disease with a secondary character, caused by bacterial germs of the *Cytophaga* genus.

The bacteria belonging from this genus are Gram-negative, shaped as 0.9 -1.1x3.0-8.5 μm canes, asporulate, acapsulate, which on solid ground moves through slipping. They are oxydaso-positive, they liquefy jelly, attack glucose forming acids, does not form indol and H_2S. They are widely spread in nature, in the water and edges of tanks, on the gills and skin of healthy fish (Photo no.11). At the fish weakened by stress factors (lack of food, overpopulation, oxygen deficit, excess of suspensions etc.), they penetrate the gill tegument, generally causing an infection localized on the gills.

The disease manifests itself through the progressive swelling of the gills, mucus hypersecretion, necrosis and ruptures of the necrotic tissue.

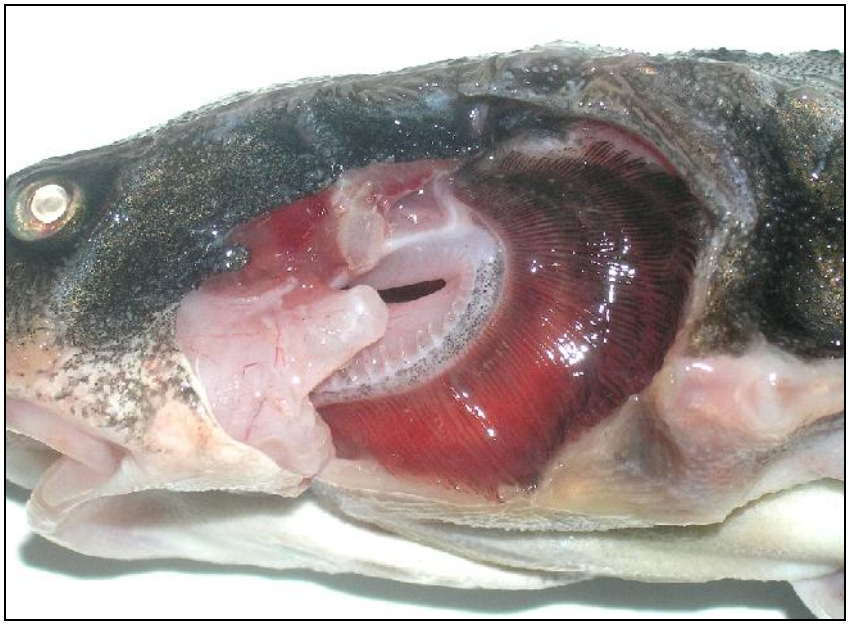

Photo no. 11 - Sturgeon gills affected by *Cytophaga spp.* (photo - E. Dumitrescu)

The fish affected by this disease show respiratory ailments, reduction of appetite, slow swimming. The diagnosis was established through the association of the clinical signs with the isolation of the bacteria. The prognosis was not alarming, the disease being present on a small number of fish. The therapy and prophylactic measures were limited to the elimination of the stress factors. In addition, the fish were immersed in antimicrobian solutions, methylic blue (30 minutes short baths, in doses of 10-30 mg/l of water, and 3-5 days long baths, in 3-5 mg/l of water).

► **Lipoid liver degeneration**

The disease is caused by the consumption of rancid fodder, as a follow up of improper storage (long periods of time at high temperature and light). Oxydative rancidation is considered to be one of the most deteriorating modifications occurring at stored fodder. Rancid lipids are toxic. They also react with proteins, leading to the reduction of their biological value. Improper storage of fodder also leads to their contamination with micro-fungi and moulds.

The disease manifested itself through loss of appetite, pale colored gills, swollen, enlarged, greasy and discolored liver, sometimes with brownish-yellow spots (ceroid deposits), thickened kidneys in the posterior area and with oedemas (Photo no. 12).

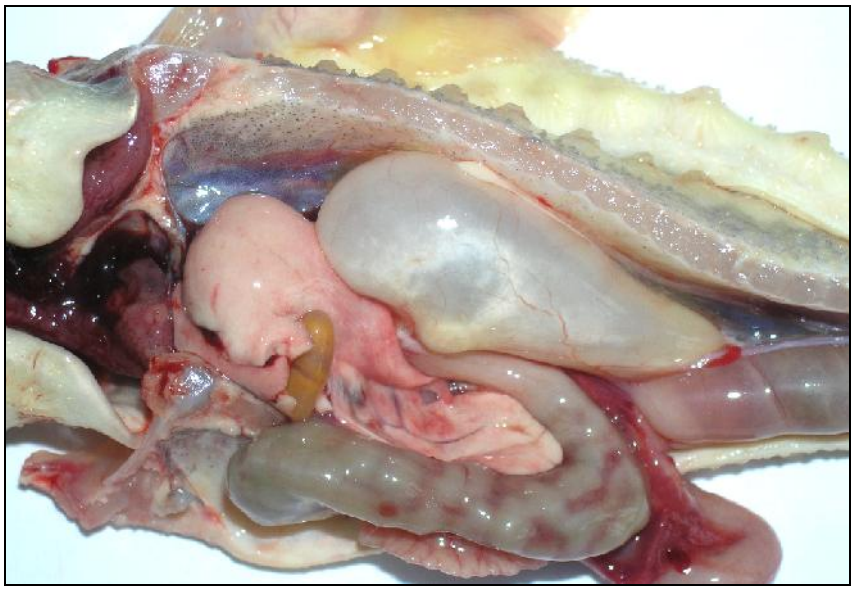

Photo no.12 - Lipoid liver degeneration on the beluga (photo - E. Dumitrescu)

At the fish affected by this disease we observed a growth of unsaturated fat acids, susceptible to auto-oxydation and ceroid formation. Ceroid is a brown-yellow pigment which accumulates in the liver of the fish. The ceroid degeneration of the liver usually appears under the circumstances of poor quality grains provided as fodder, rich in oxydable fats. Granulated fodder, rich in fats, actually contains a large quantity of peroxydes of the unsaturated fat acids which destroy vitamins A and E in the body of the fish. These vitamins, especially E, are extremely strong natural antioxydants,

which, under normal circumstances, ensure an important protection of fat acids in the cellular membranes.

The disease manifested itself at a small number of individuals, reared in fresh water, as well as in marine water, without affecting the sturgeon lot under experiment. After the identification of the disease, the replacement of the fodder measure was immediately taken.

For the prevention and treatment of the disease, good quality fodder was provided as a measure, as well as the administration, in the fodder, of vitamin E, which has an important antioxydant role. The dose we used was of 10 ml/ food kg, for 1 week every month of intense growth.

The fodders charged with yeasts and moulds were themselves a contamination source. The administration of contaminated fodder led to the development of yeasts and moulds in the culture water of the fish. Under the circumstances of the apparition of tegumentary and gill lesions on the fish, they installed there, affecting the fish directly.

From the fodder, the water and the contaminated fish various yeast and mould species were isolated.

An obvious contamination with moulds and micro-fungi was identified in 2006, at two young Russian sturgeon individuals reared in fresh water. From the skin lesions visibly affected by *Saprolegnia* and from the gills some micro-fungi and mould species were isolated (*Trichophyton, Aspergillus, Penicillium, Geotrichum Cryptococcus, Rodothorula*). All these species can cause severe dermic aliments (Photo no.13).

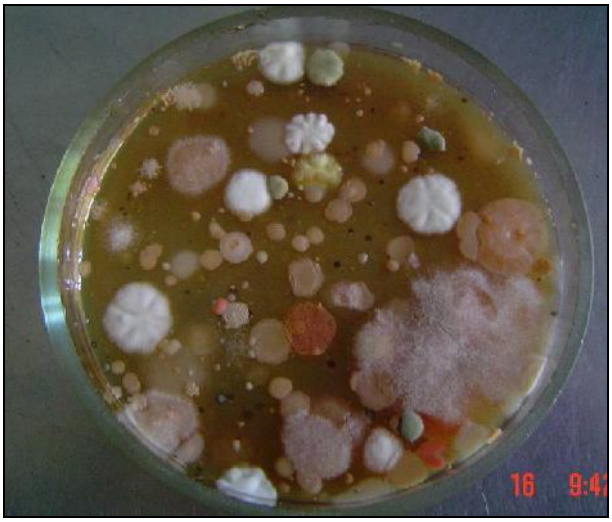

Photo no. 13 - Micro-fungi and moulds identified on a skin lesion
at the Russian sturgeon (photo - E. Dumitrescu)

The most numerous were the micro-fungi belonging to the *Trichophyton* genus and the *Cryptococcus* and *Rodothorula* moulds.

Trichophyton spp. is a micro-fungus that develops white, pink or yellow granular or powdery colonies, sometimes puffy, with radial indentations. It causes skin micoses.

Aspergillus spp was isolated in rather reduced proportions. It develops green yellowish colonies. It is toxic

Penicillium spp. was also identified in a rather reduced proportions. The *Penicillium* culture develops on the surface of the environment shaped as circumscribed colonies, adhering to the substratum, with a fine, granular aspect of the white, blue-green, white-yellow-violet conidia

Criptococcus spp. is a yeast with a brown-yellow creamy-mucous honey-like aspect, dripping on the environment.

Rodothorula spp. develops Sabouraud-type agar, red, yellow-orange circumscribed colonies.

The total contamination with these species was under 10,000 germs/g fodder, which is a low contamination. The micro-fungi and moulds identified, associated with the oxydated fodder, nevertheless produce obvious disturbances to the fish: tegumentary lesions, fish anemia, degenerated liver.

► General prophylactic and therapeutic measures

As it is easier to prevent than to treat a disease, generally in aquaculture and in sturgeon aquaculture especially, fighting diseases through prophylaxy has a great importance. As a whole, the prophylactic measures aim to:

- to ensure a higher resistance of the fish;
- to identify the natural disease sources and stop the penetration of the pathogen agents;
- to preserve the bio-pathologic balance.

Genetic factors, as well as ecological ones contribute to the triggering of the diseases. Consequently, prevention measures must have in view the organism (its genetic structure), as well as the physico-chemical characteristics of the aquatic environment. The general prophylactic measures aim at: creating through selection lines or families with natural resistance, ensuring the best maintenance conditions and imuno-prophylaxy.

Together with the physico-chemical parameters, the food of the fish represents the source of several diseases, for which reason it must contain all the nutritive elements required by the growth of sturgeons, it must by administered in proper ratios and not be oxydated (in order top revent lipoid liver degeneration). The periodic administration of vitamin E (10ml/Kg of

food, for 10 days/month, during the intensive growth period) is also and important measure of preventing fish lipoid liver degeneration.

An important aspect of prophylaxy is represented by imuno-prophylaxy, ensured by vaccines that substantially contribute to the limitation of infectious diseases.

The measures for establishing the natural disease sources and for stopping the penetration of pathogen agents in the fish rearing tanks represents a highly important prophylactic measure for the health of the fish. Pathogen agents can penetrate into the rearing tanks together with the water flow and/or with the fish transferred from other locations. Under these circumstances, the water flow must be provided from unpolluted sources, and the fish transferred from other locations must be put into special quarantine tanks, for 20-30 days. When using natural food consisting in *Tubifex* worms, they will be administered as food to the sturgeon juveniles after a previous methylic blue bathing (200 mg/kg of food).

Maintaining the bio-pathologic balance is very important for the prevention of diseases. In case of prolonged contact of the fish with pathogen agents, at a certain point a balance between the agent and the host is registered. Under these circumstances, pathogen agents continue to live and breed in the tanks, as well as in the organism of the fish, without the latter suffering any harm. Introducing new fish coming from other units, mixed with the fish reared at a certain point, causes the breaking of the bio-pathologic balance created and the triggering of a severe disease for at the reared fish, as well as the ones newly introduced. The cause of breaking the bio-pathologic balance is represented by the introduction of new agents to which the reared fish have no resistance and the presence of the newly introduced fish being receptive to the pathogen agents already existing in the rearing tanks. For these reasons, mixing the fish coming from different environments is contra-indicated, as it should be done after the setting of balance for the newly introducted fish as well.

Disease therapy is realized when the prophylactic measures do not ensure disease prevention and bursts of disease are registered, affecting a large number of the reared fish. The treatment is done with medicines that are efficient in killing the pathogen agents, do not deteriorate the quality of the rearing environment and do not harm the health of the fish and of the other aquatic living resources in the tanks.

CONCLUSIONS

For the sturgeons (Russian sturgeon and beluga) reared experimentally (in marine water) and commercially (in fresh water) a few diseases caused by bacteria and improper food/feeding were identified.

The most significant diseases affecting the sturgeons were infections. Thus, at the sturgeons reared in fresh water, we identified bacterial hemorrhagic septicemia, caused by species belonging to the *Aeromonas* and *Pseudomonas* genera and, as far as the sturgeons reared in marine water are concerned, we identified vibriosis, caused by *Vibrio anguillarum.* At a lower intensity we also identified infections caused by species belonging to the C*ytofaga* genus.

Prophylactic and treatment measures were established and applied for each particular case.

Acknowledgements

The experiments were carried out within the Scientific and Technological Partnership for the Promotion of the Sustainable Management of the Romanian Marine Fisheries, Based on an ecosystem approach" CEEX project, the stage referring to "*Pilot scale experiments for the determination of the technical-economical parameters in marine aquaculture for threatened and economically valuable species*". We would like to thank SC "Kaviar House" SRL, Mrs. Marilena Maereanu, for the support given during the entire period during which the project was carried out.

SELECTIVE BIBLIOGRAPHY

AUSTIN B., AUSTIN D., 2007 - Bacterial Fish Pathogens - *Diseases of Farmed and Wild Fish*, ed. Springer: 575 - www.books.google.com/

NOGA E.J., 2000 – Fish Disease – *Diagnosis and Treatment*, Iowa University Press, 395 p - www.books.google.com/

PREARO MARINO, STEFANIA SQUADRONE, MARIA LETIZIA FIORAVANTI, ILARIA GIORGI, STEFANO MARTURANO, FILIPPO GASPARRI, MARIA CESARINA ABETE, RENATO GIULIO ZANONI, 2009 - Italian farmed sturgeon: mortality events during 2004-2008, *WAS 2009*, Veracruz, Mexico: 265

REINARTZ R., BLOESCH J., RING T., STEIN H., 2003 - Sturgeons are more than caviar: A plea for the revival of sturgeons in the Danube River (Literature review): *Large Rivers* Vol.14, no.3-4, Arch.Hydrobiol.suppl.147/3-4: 387-403