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## Risk of contamination of sturgeon species along the Lower Danube with AcIV-E virus from sturgeons raised in aquaculture systems

Gy Deák<sup>1</sup>, R Matache<sup>1,\*</sup>, R Prangate<sup>1</sup>, G Dumitrescu<sup>1</sup>, E Holban<sup>1</sup>, L Lupea<sup>1</sup>, Nurliza Rahim Norlia<sup>2</sup>, Mohamad Ibrahim<sup>2</sup>

<sup>1</sup>National Research and Development Institute for Environmental Protection, Splaiul Independenței 294, Bucharest, 060031 Romania,

<sup>2</sup>Sustainable Environment Research Group (SERG), Centre of Excellence Geopolymer and Green Technology (CEGeoGTech), Universiti Malaysia Perlis, Arau 02600 Perlis, Malaysia

\*Email: matache.razvan@incdpm.ro

**Abstract.** It is considered that most infections caused by pathogen agents in fish species raised in the closed medium like fishing farms originate from the natural environment, thus there are few information on virus transmission from species raised in aquaculture programs to the wild ones. This issue is important taking into account that the wild sturgeon populations are critically endangerment, and regarding to the IUCN Red List, two of these are already declared extinct. Within Danube Basin, environmental and anthropic factors series (reproduction habitats lost, hydrotechnical works) have caused the sturgeon distribution area to be restricted mainly in the lower segment of the river. In addition, intensive fishing or the introduction of invasive species have produced negative effects and hence the decrease in the population effectiveness. As result, from six native sturgeon species within Danube Basin, in our days, only four are still found. This review shall include an analysis of the status of knowledge, regarding the possible influence which the transmission of some viruses within sNCLDV from aquaculture species to the wild ones, also it shall propose an assessment of the risk of infection within Danube ichthyofaunal.

### 1. Introduction

At the global level, starting with the middle of the XIX century, sturgeon population numbers have started to decrease [1, 2]. One of the causes of these population declines is the loss of reproduction habitats, as an effect of hydrotechnical constructions [3]. Within the Danube Basin, the hydrotechnical structures made in the last century have generated a great impact on the sturgeon population as partially blocked their migration from the Black Sea to reproduction habitats situated in the river's upper part [3, 4].

Excessive and uncontrolled catches, corroborated with over-exploitation of sturgeon species as a source of caviar and meat, have led to a drastic decrease of wild sturgeon populations in the Danube River [5]. In addition, the lack of legislative clear fishing regulations [6], water pollution [7] and the introduction of allochthone species affected thus changes in the trophic structure – competition for food and reproductions habitats, also the introduction of a pathogen of a viral, bacterial or parasite nature, also a higher risk of transmission, in synergy with the significant decline in the abundance of indigenous species [5], [8].



From a taxonomical point of view, the Acipenseridae family includes 25 sturgeon species [9], six of which are native to the Danube River Basin: *Acipenser sturio*, *Acipenser nudiventris*, *Acipenser ruthenus*, *Acipenser gueldenstaedtii*, *Acipenser stellatus* and *Huso huso* [10], [11]. Nowadays, the sturgeon population's distribution is restricted to the lower segment of the Danube River [10], [12]. According to within the Danube Basin, the wild sturgeon population is currently represented by only four of the six native species, as *A. sturio* and *A. nudiventris* are declared extinct. This fact is also confirmed by the National Institute for Research & Development in Environmental Protection in Bucharest researcher's team, which have conducted in the last decade in situ monitoring campaigns by using ultrasonic telemetry methods [13], [14]. In the upper and middle segments of Danube, only isolated *A. ruthenus* populations remain, sustained by repopulation programs [15].

The growth of sturgeons has become an important aquaculture sector [16], and currently, approximately 95% of the world's annual production is based on three species: *A. transmontanus*, *A. baerii* and *A. naccarii* [17]. However, intensive aquaculture and worldwide trade with infected organisms expose fish to diseases of an infectious nature [17]. Several assumptions have been made, including that these problems may be generated by disregard of sturgeon growing protocols, established at the international level. The lack of regular screening of viral diseases also leads to pathogens spread through subclinically infected individuals [18]. This aspect has been reinforced by *in vitro* observation of some disease which individuals grown in artificial environments have developed [7]. These diseases include mycosis, tegument sensitivity in synergy with bacterial infections, morphological changes, lethargy, or even the death of the individuals by viral infections [19]. Infection caused by viruses from NCLDV superfamily on sturgeons grown in aquaculture systems were recorded first in North America on *A. transmontanus* species, infected with WSIV (White Sturgeon Iridovirus) [20]. Subsequently, several cases of infection with AcIV-E (Acipenser-Iridovirus European) on sturgeons grown on farms were reported in Europe. From a phylogenetic point of view, ACIV-E is slightly similar to WSIV, but over time it has suffered different mutations, caused by medium factors and host species [18], [21]–[23]. From an economic perspective, viral infections of sturgeons with sNCLDV viruses, require particular attention because of the high economic losses they cause. The mortality rate for AcIV-E infection range from 50% (*A. baerii*) to 90% (*A. gueldenstaedtii*) [18], while for WSIV infection of juveniles (*A. transmontanus*) can reach about 95% [24]. At the same time, from an ecological point of view, viruses are a limiting factor for species threatened with extinction [5].

This article summarizes the evidence of sturgeon infections with AcIV-E virus at the European level, analyzes their impact on individuals raised on aquaculture farms and the potential routes of transmission of the disease. Outbreaks of infection on aquaculture farms may be a potential pathway for the virus to spread to wild sturgeons. This research aims to create the framework for a scenario on the interspecific and intraspecific routes of the AcIV-E transmission and the possibility for infected individuals to enter the Danube Basin.

## 2. NCDLV: Taxonomy aspects and relationship with infectious origin within sturgeon species

### 2.1 Taxonomy aspects

Sturgeon Nucleocytoplasmatic Large DNA Viruses (sNCLDV) is a large group of viral agents that cause some of the most serious diseases of sturgeon [7]. sNCLDV include the following: White sturgeon iridovirus (WSIV), Shortnose sturgeon virus (SNSV), Missouri River sturgeon iridovirus (MRSIV), British Columbia white sturgeon virus (BCWSV) and Namao virus (NV). Even if sNCLDV are not officially recognized by the ICTV (International Committee on Taxonomy of Viruses) [25], it has shown closer similarity with Mimiviridae, as Acipenser iridovirus-European (AcIV-E) [26]. Outbreaks of viral diseases pose a potential risk to strategies designed to help restore natural sturgeon populations [22].

In Europe, WSIV first record was made in years 90' and it was associated with a high level of mortality within *A. gueldenstaedtii* species individuals. Thereafter, a new form of WSIV called temporally Acipenser iridovirus-European (AcIV-E), has been discovered [22]. This form has affected four sturgeon species raised in aquaculture systems. (*A. gueldenstaedtii*, *A. baerii*, *A. naccarii*, *H. huso*) [27]. The mimivirus, also called AcIV-E induces clinical symptoms such as pale gills and liver, hemorrhages on the skin, abnormal behavior and mortality rate growth, which have been observed in several European countries such France, Italy, Ukraine and Sweden [7], [18], [23], [26], now in Romania

as well.

### 2.2 *AcIV-E transmission. Infections origin*

First reports of an emerging pathogen in sturgeons growth, AcIV-E, date back between 2012-2015, in studies carried in Western European countries and involving several species of sturgeons: *A. gueldenstaedtii*, *A. baerii*, *A. naccarii*, *H. huso* [7], [27]. Further creating difficulties in prevention and control in these diseases is the lack of essential information on the AcIV-E like mechanism of infection for the development of infections in sturgeons or their transmission [18].

Relevant aspects for the prevention of infection with AcIV-E on sturgeons, such as testing of individuals who may have subclinical viral infections, but also the trade restrictions are also a lack of legislation, are contributing to pathogen agents intra/interspecific spreading [18]. Therefore, the risk of AcIV-E infected individuals entering the centers for the rearing of the juvenile and later in natural habitats as a result of repopulation actions is high and constitutes a serious threat to populations of wild sturgeons threatened with extinction.

### 2.3 *Inter and intraspecific transfer*

D. Mugetti et al. have carried out studies in the past on the possibility of transfer of viral genetic material between different individuals. In this way, it has been noted that infectivity is transferred from contaminated individuals of *Scaphirhynchus albus* species to the new inserted juveniles [28]. Restored the experiment, subjecting individuals of different species to cohabitation (healthy juveniles within *S. albus* species and uninfected mature one within *Scaphirhynchus platyrhynchus*). This experiment showed that this virus has overcome species-specific barriers causing mortality between 30% and 90% within *S. albus* species [28]. Mugetti, also confirms the horizontal transfer capacity of the AcIV-E between different sturgeon species [7], [27].

### 2.4 *Pathology*

The infections provoked by viruses from sturgeon Nucleocytoplasmic Large DNA Viruses (sNCLDV), may cause major injuries within epithelial tissue and over gills [20]. Thus, in infected sturgeon, the appearance of pale color of the skin and gills was observed to indicate viral infection [18], [21]. It has also been demonstrated that epithelial cells of the infected skin and gills grow in dimensions [18]. These may often include occasional hyperplasia, predominantly on the respiratory epithelium, with the formation of necrosis in the proximity of blood vessels [21]. Thus, this may lead to the emergence of point hemorrhages [18], [20], [29]. Many viral inclusions were revealed through molecular and microscopic examination in the cytoplasm [18], [20]. Infection with sNCLDV tends to occur predominantly in juvenile sturgeons (less than 1 year) and it has been observed that the severity of the disease is decreasing inversely proportional to the stage of development [18], [29].

### 2.5 *Infection diagnosis*

Much of the current understanding of the viruses pathogenic to sturgeons is based on the study of hosts with obvious clinical symptoms. A recent research aimed at determining the presence of the AcIV-E from *A. ruthenus*, *A. stellatus*, *A. gueldenstaedtii*, it consisted of the determination of a major protein fragment of the viral capsid (MCP) isolated from gills [7], [27]. Thanks to the stronger signal of the AcIV-E virus at the level of gills, the use of such samples as model tissues is chosen [18], [22]. In addition, for the same MCP determination of the AcIV-E, on *A. baerii* and *A. gueldenstaedtii* species, some authors used HRM (High Resolution Melting), followed by Sanger sequencing methods [23]. This same protocol for detection of the viral capsid major protein gene (MCP), has been used in the past for *A. baerii*, *A. naccarii*, *H. huso* species [22], with efficacy in the detection of AcIV-E infection.

### 3. AcIV-E dispersion scenarios in the natural environment

Sturgeons, like many other aquatic species, are exposed to a large number of diseases [30], and studies on viral infections in Acipenseridae are generally rare [31] and non-existent for the lower segment of the Danube and the Black Sea. Although initially, the origin of sNCLDV detected in sturgeons was unknown [20], further studies have identified infected sturgeons in both the aquaculture-controlled environment and the natural environment. At European level, the situation is equally uncertain, due to the lack of data on life cycle, ecology and prevalence of sturgeon NCLDV infection [30].

The movement of living aquatic vertebrates between different geographical regions or from aquaculture conditions in the natural environment can be key processes in the emergence and spread of viral pathogens [23]. Water, a common vehicle for transmitting viruses, can often allow them to survive [32], and by association with certain environmental factors, such as pH, salinity 12-17% [33], temperature 10°C [32], dissolved oxygen level, turbidity and sediment load [20], may lead to quantitative changes in populations of pathogenic virus in sturgeons, present in the wild.

Some authors have observed *in vitro*, the fact that a low water temperature [32], with an optimum around 10°C [20], leads to an increase in the number of infected with NCLDV juvenile individuals [29] and the long-term survival of viruses [32]. Turbidity and depth of penetration of UV radiation into the water mass appear to be other limiting factors [32], with a major impact on the inactivation of sturgeon viruses. It was found in the sense that increased turbidity of the water, limits the ability of light to penetrate deep, thus facilitating the survival of viruses present especially on the bottom of the water [32]. Moreover, it seems that in association with a lower temperature of approx. 10°C and climate changes, viruses survive a longer period in the depth of the water, compared to the same temperature, but at a higher light exposure [32]. Salinity, on the other hand, seems to negatively influence viral activity [32], thus, a higher salinity of water, similar to that of the Black Sea (13-23%) is likely to lead to an increase in the inactivation of sturgeon viruses. It is important to ensure environmental sustainability, including the improvement of natural resources [34]. Sturgeons are normally present throughout the body of water, but spend most of their life in the lower segment, where they feed, winter or lay their eggs [35].

Researchers within National Institute for Research & Development in Environmental Protection, Bucharest Romania (INCDPM Bucharest) have detected in 2018, an *A. gueldenstaedtii* juvenile individual within Lower Course of Danube (figure 1), showing abnormalities over the rostrum, more specifically at the opercula and gills. These components are of a yellow-reddish color, a physical characteristic considered abnormal for this sturgeon species. Thus, it may be compared and correlated with the AcIV-E findings on *A. gueldenstaedtii* by Bigarre [22]. It is thus considered that there is a high possibility of AcIV-E virus spreading over Lower Danube Course.



**Figure 1.** *A. gueldenstaedtii* juvenile individual identified on the Lower Course of the Danube, during on-site monitoring in 2018, which presents different physiological aspect than a healthy one, all images are of the same individual. It may be considered suspect for AcIV-E infection. (Photographs from Deak Gyorgy).

In addition, besides the juvenile specimen of *A. gueldenstaedtii* identified by INCDPM Bucharest researchers and considered as possibly infected, it seems that attempts to avoid the spread of the virus have failed and diverse aquaculture farms from Romania, have recorded a high mortality rate among fingerlings/juvenile's sturgeons (*A. baerii*) raised in controlled environments. A concrete example is represented by the situation with a Romanian aquaculture farm, specialized in sturgeon breeding. Between November 2019 and March 2020, four batches of eggs were purchased from different distributors within Italy, Germany and the Czech Republic, a total of approximately 1.026.360 eggs. We only will discuss about just two batches (November and December 2019), as partial data of this study (Table 1). Therefore, in November 2019, the aquaculture farms from Romania, have bought *A. baerii* eggs, but the hatching efficiency was only 20%, of which 53.348% from hatched eggs died during the incubation stage (1-30 days), only 9.29% (of the total eggs purchased in November) have hatched and reached the pre-feeding stage.

**Table 1.** Total number of sturgeon (*A. baerii*) eggs divided in 4 lots; Sturgeon eggs – total eggs bought from distributors; Hatched eggs – total eggs which hatched from total bought; Mortality during incubation – total amount of individuals which recorded mortality during incubation; Feeding individuals – total amount of individuals which reached the pre-feeding stage for growing; Hatching efficiency – the percentage of individuals which hatched from the total eggs; Average mortality rate speed – the value of mortality rate speed taking into account the total amount of feeding individuals and the last age (days) in which was recorded individuals death (deaths/day)

	<i>A. baerii</i> eggs	Hatched eggs	Mortality during incubation	Feeding individuals	Hatching efficiency	Average mortality rate speed [deaths/day]
<b>LOT NOV 2019</b>	250.000	50.000	26.674	23.224	20%	≅ 195
<b>LOT DEC 2019</b>	276.360	276.360	172.790	103.570	100%	≅ 950

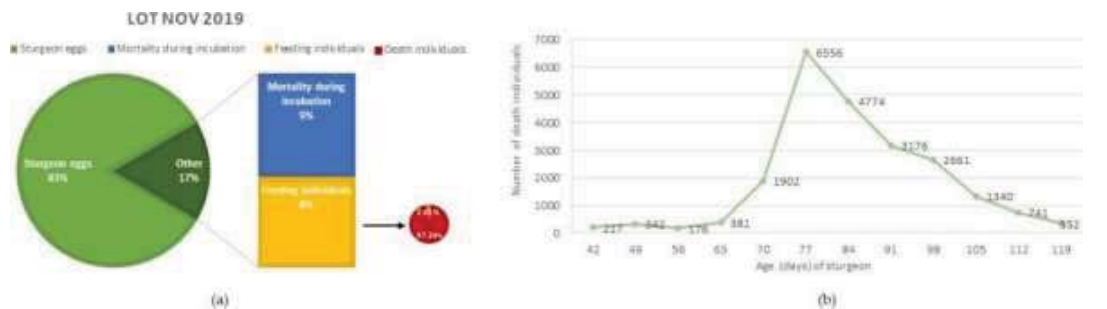
The fingerlings began to show various anomalies (figure 2), and in the proximity of the age of 70 days, the number of deceased individuals increases exponentially.



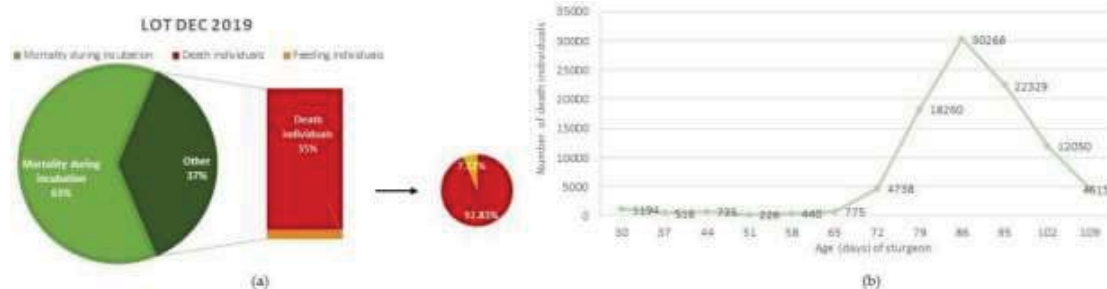
**Figure 2.** Symptoms observed in sturgeons (*A. baerii*) infected with AcIV-E, in an aquaculture farm in Romania; (a) can be observed specimens of juvenile sturgeons, which show infection with AcIV-E, being able to observe the abnormal dimensions of the head compared to the trunk, respectively the deformation at the level of the opercules; (b) the mentioned deformations can be observed more clearly as well as the reddish color of the opercules; (c) exemplifies 3 specimens that show a circular body motion, abnormal to the behavior of this species; (d) is same with (a) but in a close view of the individual analysed; (e) it is taken from [18] in order to realize a correlation between individuals; (a, b, c, d, Photographs from Lupea Lucia)

Even in this stage, the fingerlings have begun to manifest specific symptoms as pale color, circular body motion inappropriate to their behaviour. Thus, recording a 97.39% (of those who hatched and reached the pre-feeding stage) mortality rate, in the next 12 weeks (figure 3). In December 2019, another batch of eggs was purchased, which hatched completely, but only 37.47% of them reached the pre-

feeding stage. As in the previous case, fingerlings began to show symptoms, also in proximity of the age of 70 days and in the next 12 weeks, 92.83% of individuals died (figure 4). The mortality trend is variable, taking into account the interval of days in which the young mortality rates were recorded. For the analysed lots, Dec 2019, respectively Nov 2019, it can be observed that the maximum mortality was registered at the age of the young of approximately 80 days.



**Figure 3.** (a) Mortality rate within LOT NOV 2019 from hatched eggs; (b) Number of death individuals depending on the age of sturgeon by days



**Figure 4.** (a) Mortality rate within LOT DEC 2019 from hatched eggs; (b) Number of death individuals depending on the age of sturgeon by days

**4. Conclusions**

The data currently available in the literature do not provide a solid basis for a correlation between the factors reported at the Danube level that have over time decreased population numbers (hyrotechnical constructions, intensive fishing, introduction of non-native species, water pollution, climate change) of the species – *Acipenser sturio*, *Acipenser nudiventris*, *Acipenser ruthenus*, *Acipenser gueldenstaedtii*, *Acipenser stellatus*, respectively *Huso huso* – and virus infections belonging to the NCLDV superfamily. Future studies should focus on identifying new, cost-effective, and affordable methods for detecting and monitoring AcIV-E infection. Next, it is necessary to clarify the aspects related to the persistence and infectivity of the virus in relation to environmental factors, in the context in which sturgeons migrate from the Danube to the Black Sea. In addition, the possibility of infecting sturgeon species from the Danube with this virus must be considered. Finally, the implementation of programs which purpose is monitoring NCLDVs dispersion and specific AcIV-E, from aquaculture systems to the natural environment, constitutes important measures of conservation and restoration of the Lower Danube sturgeon populations, thus determining an improvement of the current state of conservation.

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