

DETECTION OF WHITE STURGEON IRIDOVIRUS (WSIV) IN STURGEONS (ACTINOPTERYGII: ACIPENSERIFORMES: ACIPENSERIDAE) FROM AQUACULTURE FACILITIES LOCATED IN POLAND, GERMANY, AND ITALY

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Background. White sturgeon iridovirus (WSIV) is one of the most serious threats for sturgeon aquaculture as the mortality in juvenile individuals can reach 95%. At the turn of the century WSIV was reported among hatchery-raised white sturgeon, *Acipenser transmontanus* Richardson, 1836, in North America, Russian sturgeon, *Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833, in northern Europe but also pallid sturgeon, *Scaphirhynchus albus* (Forbes et Richardson, 1905) and shovelnose sturgeon, *Scaphirhynchus platyrhynchus* (Rafinesque, 1820) in North and South Dakota. The clinical signs of infection include cessation of feeding and edematous and pale gills. Those pathological changes lead to respiratory problems of the juveniles and deterioration of their overall condition. Rapid detection of pathogen particles under aquaculture conditions is crucial, therefore the principal aim of this study was to establish a molecular method for rapid and effective detection of the WSIV. This is the first study thoroughly comparing WSIV detection methods in sturgeons cultured in Poland, Germany, and Italy.

Material and methods. A total of 244 samples were collected from 82 sturgeons representing six species: beluga, *Huso huso* (Linnaeus, 1758); sterlet, *Acipenser ruthenus* Linnaeus, 1758; Siberian sturgeon, *Acipenser baerii* Brandt, 1869; Russian sturgeon, *A. gueldenstaedtii*; Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill, 1815; and European sea sturgeon, *Acipenser sturio* Linnaeus, 1758, as well as five sturgeon hybrids: Siberian × Russian, Adriatic (*Acipenser naccarii* Bonaparte, 1836) × Siberian, Adriatic × beluga, Siberian × sterlet, and beluga × sterlet. The samples were screened for the WSIV using conventional PCR, real-time PCR, and in situ hybridization (ISH) methods.

Results. In total, positive results were obtained in 231 (94.67%) out of 244 analysed samples, except for *H. huso* where DNA of WSIV was not identified. DNA of WSIV was most frequently detected in the kidney (97.92%), intestine (97.50%), skin (94.97%), and gills (93.29%). The most effective method for detecting DNA of WSIV in sturgeons was real-time PCR (92.62% of), PCR (90.98%), and ISH (49.24%).

Conclusion. The host range of the WSIV seems to be wider than expected, however, further studies are necessary since no clinical symptoms were observed in sturgeons and their hybrids.

Keywords: white sturgeon iridovirus, WSIV, aquaculture, virus, infection, sturgeon

INTRODUCTION

Fish diseases are a major limiting factor for fish rearing, and viral diseases are responsible for the greatest economic loss (Shchelkunov et al. 2009). The knowledge of sturgeon diseases is insufficient and based primarily on the population of the white sturgeon, *Acipenser transmontanus* Richardson, 1836, commonly reared in North America (Hua and Wang

2005). A recent phylogenetic study showed that the viruses such as the white sturgeon iridovirus (WSIV), Missouri River sturgeon iridovirus (MRSIV), and shortnose sturgeon virus (SNSV) are only distantly related to Iridoviridae, and are included in a group actually referred to as sturgeon nucleocytoplasmic large DNA viruses (NCLDVs) in the order Megavirales, a new term not formally adopted by

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the International Committee of the Taxonomy of Viruses (Clouthier et al. 2015, 2018). To date, approximately 10 specific viruses have been found in sturgeons, the majority of which have been detected in fish originating from Canada and the USA. Viral diseases have also been observed in Europe (Italy), where acipenserids from North America have been introduced (Ravetry et al. 2003, Kelley et al. 2005). Among viruses specific for sturgeons are adenoviruses, papovaviruses, iridoviruses, and herpesviruses (Kelley et al. 2005). From an economic point of view, white sturgeon iridovirus (WSIV) and acipenserid herpesvirus-2 (AciHV-2) are of the greatest importance because of the economic loss they may cause. The mortality of WSIV infections in juvenile individuals may reach 95% (Watson et al. 1995, Georgiadis et al. 2001, Kelley et al. 2005). Iridoviruses cause infections mainly in lower invertebrates (Chinchar et al. 2009), but amphibians, insects, and fishes can be their hosts as well. According to the IUCN and CITES data, the viruses constitute a limiting factor also for species threatened with extinction (Mao et al. 1997).

Sturgeons are hosts to a group of specific nucleocytoplasmic large DNA viruses (NCLDV) initially classified in the family Iridoviridae based on the virion morphology (Pallandre et al. 2018). The first identified virus of this group was the white sturgeon iridovirus (WSIV). The WSIV was first isolated in the 1990s from white sturgeon (*A. transmontanus*) cell lines and then characterized molecularly (Hedrick et al. 1990, 1992). With the size of 120–200 nm, the virus replicates in the cytoplasm, and its genetic material is double-stranded genomic DNA. Among the primary problems facilitating the emergence of serious outbreaks of the disease are stressors such as high stock density, sudden changes in the environmental conditions (e.g., changing water temperature or oxygen content), or inadequately selected feed. Unfortunately, horizontal transmission is not the only route that can lead to infection. There is a possibility of transmitting the virus from adult individuals to offspring, i.e., vertical transmission. This transmission route is limited by the use of iodine used as a disinfectant to remove viruses from the egg sheath (LaPatra et al. 1996). The presently reported study was intended to elucidate whether after such treatment the WSIV will be sufficiently degraded to prevent its further transmission. The first significant sign of infection is the cessation of feeding by the fish, which leads to the deterioration of their overall condition. It is hypothesized that this is due to the infection of the sensory epithelium in the olfactory organ (Watson et al. 1998). Other clinical signs are edematous and pale gills, which lead to problems in oxygen uptake. A characteristic but less common sign of infection is reddening along the ventral plates which may subsequently turn into bloody petechiae (LaPatra et al. 1994).

Since this initial characterization, several viruses, related, although distinct from the WSIV, have been detected in North America and Europe, infecting various

farmed sturgeon species (Kurobe et al. 2011, Clouthier et al. 2013, Ciulli et al. 2016, Bigarré et al. 2017).

The presence of iridovirus, observed to date in the white sturgeon, has also been confirmed in other species. In 1993, WSIV arrived in Northern Europe and was identified as the cause of mass kills of the Russian sturgeon, *Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833 (see Adkinson et al. 1998). To date, the occurrence of a virus similar to the WSIV was detected in the shovelnose sturgeon, *Scaphirhynchus platyrhynchus* (Rafinesque, 1820), Russian sturgeon, *A. gueldenstaedtii*, and Siberian sturgeon, *Acipenser baerii* Brandt, 1869 (see Ciulli et al. 2016, Chesneau 2018), pallid sturgeon, *Scaphirhynchus albus* (Forbes et Richardson, 1905), and sturgeons originating from the Missouri River (Clouthier et al. 2013, MacConnell et al. unpublished*). From the mid-19th century, a rapid reduction in the population of the majority of Acipenseridae species occurred. It was caused by the developing industry, the spread of hydrotechnical installations in rivers, water pollution, intensive fishing (especially in the periods of spawning migrations), overfishing, as well as regulation of rivers associated with the deepening of riverbeds. The broadly understood anthropogenic impact and other adverse factors caused the destruction of spawning sites and an overall deterioration in the conditions of natural reproduction, resulting in the dramatic reduction and sometimes extinction of sturgeon populations (Bemis and Findeis 1994, Boreman 1997, Birstein et al. 1998). Currently, 26 species of the order Acipenseriformes are threatened with extinction. This was one of the reasons for including all sturgeon species in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1998 (Williot et al. 2001, Pikitch et al. 2005, Rosenthal et al. 2006).

Acipenseriformes play a significant role in world aquaculture production due to the increasing demand for their meat and caviar, but also due to the on-going protection and restitution programs (Jennings and Zigler 2000, Chebanov and Billard 2001, Williot et al. 2001, Geßner et al. 2010, Bronzi et al. 2011, Bronzi and Rosenthal 2014). Currently, 35 countries in the world specialize in sturgeon rearing, and the main European producers are Italy, France, Germany, Poland, and Spain (Bronzi et al. 2011, Anonymous unpublished**). With the increasing production of this species, there is no information regarding the current spread of the WSIV across Europe, particularly in aquaculture facilities where fish do not show signs of the disease. The overall epidemiological situation is poorly understood, with different iridoviruses infecting distinct specific species of sturgeon in various regions worldwide. In particular, no full-length iridovirus genome is available, except that of FV3 (Clouthier et al. 2013, Bigarré et al. 2017).

Therefore, in light of the risk of virus introduction into rearing centres and subsequently into open waters through reintroduction material, an attempt was made to determine the prevalence of WSIV in selected sturgeon farms located in Poland, Germany, and Italy.

* MacConnell E., Hedrick R.P., Hudson C., Speer C.A. 2001. Identification of an iridovirus in cultured pallid (*Scaphirhynchus albus*) and shovelnose sturgeon (*S. platyrhynchus*). *Fish Health Newsletter* 29: 102–105.

** Anonymous 2014. Federation of European Aquaculture Producers. Annual report 2014.

MATERIAL AND METHODS

Fish sampling. Within 2010–2014 a total of 244 samples were collected from 82 sturgeons that aged between 0+ and 20+ and originated from fish farms located in Poland, Germany, and Italy (Table 1). The material consisted of pieces of the spleen, gills, intestine, skin, kidney, brain samples, as well as gill swabs. The following sturgeon species were examined beluga, *Huso huso* (Linnaeus, 1758); sterlet, *Acipenser ruthenus* Linnaeus, 1758; Siberian sturgeon, *A. baerii*; Russian sturgeon, *A. gueldenstaedtii*; Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill, 1815; and European sturgeon, *Acipenser sturio* Linnaeus, 1758, as well as from five hybrids: *A. baerii* × *A. gueldenstaedtii*, Adriatic sturgeon (*Acipenser naccarii* Bonaparte, 1836) × *A. baerii*, *A. naccarii* × *H. huso*, *A. baerii* × *A. ruthenus*, *H. huso* × *A. ruthenus*. None of the fish included showed clinical symptoms typical of WSIV infection. Samples were kept at 4°C until arrival in the laboratory and then stored at –20°C or preserved in 70% isopropanol alcohol until analysed. Data concerning the names and locations of the rearing centres cannot be disclosed due to the confidentiality of such information.

WSIV detection. Samples were used for DNA extraction by using the DNeasy Blood and Tissue Kit (Qiagen) according to the procedures outlined in the protocol. A polymerase chain reaction (PCR) assay was applied for detection of DNA amplicon from the WSIV genome that encodes the putative major capsid protein (MCP) gene using WS 229F/WS 245R primers and cycling conditions described by Kwak et al. (2006). Based on thermal gradient PCR, a primer annealing temperature of 55°C was established. The 25 µL PCR mixtures contained REDTaq® ReadyMix™ PCR Reaction Mix (Sigma-Aldrich, Germany), 0.2 mM of each primer pair and 1 µL of DNA template. In total, the PCR-based tests for the detection of WSIV were conducted in 6 species and 5 interspecific hybrids. The PCR products were resolved by gel electrophoresis and verified by comparison with a molecular size standard (GeneRuler 100 bp Plus DNA ladder, Thermo Fisher Scientific). Bidirectional sequencing of the obtained PCR products was ordered from Genomed, Warsaw, Poland. DNA sequence assembly and similarity analyses were completed using Geneious 8.0, BLAST and MEGA5 software (Altschul et al. 1990, Tamura et al. 2007, Kearse et al. 2012).

Detection of WSIV by real-time PCR. Primers and probe for detection 75 bp of WSIV genome were designed based on MCP consensus sequence obtained during the previous step. Real-time PCR was performed using 1 µL of DNA template, 5 µL of RT 2x HS PCR MasterMix (A&A Biotechnology), 0.2 µL of 0.2 mM PHofFor (5'-CSTCAACCCRCGTGCCRA-3'), 0.2 µL of 0.2 mM PHofRev (5'-TCCCYGATGCGGACAAGT-3'), 0.13 µL of 0.13 mM TaqMan probe (5'-FAM-CTTACTGGAYGCACCTATCTTCTCT-BHQ-3') and 3.47 µL of H₂O DEPC. The real-time PCR reaction was carried out in a Mastercycler ep realplex² (Eppendorf). The thermal profile consisted of polymerase activation at 95°C for 1 min followed by 40 cycles of denaturation at 95°C at 15 s

and annealing/elongation at 61°C for 1 min. In each run, positive control and negative control were included.

In situ hybridization (ISH). In-situ means of a molecular probe and virus detection was conducted using a kit of reagents from Roche by PCR (Eppendorf Mastercycler) in accordance to a certified methodology developed in the Institute of Infectology in Riems (Germany). Pieces of tissue were formalin fixed and paraffin embedded according to standard protocols. Organ specimens 5–7 µm were placed on Superfrost® microscope slides (Microm International) for 18 h at 62°C, dewaxed by 2 × Rotihistol (Roth) for 10 min, followed by 2 × 100% ethanol for 10 min, and air dried. Prepared slides were framed by PapPen (Merck), treated with proteinase K (100 µg proteinase K · mL⁻¹) in TE buffer (50 mM Tris, 10 mM EDTA) for 20 min at 37°C and fixed again by 95% ethanol followed by 100% ethanol for 1 min, respectively. After air drying sections were framed by Pap Pen again and, for equilibration, covered by hybridization mixture (ISH-M) containing 4 × standard saline citrate (SSC), 50% formamide, 1 × Denhardt's reagent, 250 µg yeast tRNA · mL⁻¹ and 10% dextran sulphate and incubated for 1 h at 42°C in a humid chamber. DIG-labelled probes prepared with the primer pair (Kwak et al. 2006) were added to the sections and covered by an ordinary coverslip, placed on the in-situ plate of a thermal cycler (Eppendorf Master Gradient), and heated to 95°C for 5 min for denaturation of DNAs. Then slides were cooled on ice for 2 min and incubated overnight at 42°C in a humid chamber for hybridization. The next day, coverslips were removed by washing in 2 × SSC twice for 10 min. For removal of unspecific bound probes, slides were incubated in 0.4 × SSC at 42°C for 10 min. Sections were counterstained with Bismarck-Brown Yellow (BBY) for sharpening and contrasting a possible positive signal which becomes visible as violet-black foci in infected cells. Analysis of in situ hybridization results was conducted using a fluorescence microscope (Nikon Eclipse E-600), and after all arithmetic data were calculated using MS Excel spreadsheet.

RESULTS

In total, positive results were obtained in 231 (94.67%) out of 244 analysed samples. Only for beluga (*H. huso*), no positive results were obtained. Fragments of WSIV DNA were in the majority of cases found in the kidney and the intestine, representing 97.92% and 97.50% of all tests, respectively. Other two types of samples in which WSIV was commonly detected were skin (94.97%) and gill (93.29%). As regards gill swabs, positive results were obtained in 80% of the samples. Sequencing and subsequent processing steps yielded in 79 assembled sequences identified in 34 sturgeon individuals. Sequence comparisons revealed 23 single nucleotide substitutions, i.e., 15 transitions and 8 transversions. Employing real-time PCR to detect WSIV yielded positive results in 226 (92.62%) out of 244 analysed isolates (Table 2). It was found that the threshold level was not exceeded (negative results) by a total of 17 samples, representing 6.97% of all tested DNA isolates. Detailed information on the number of positive and negative results in different organs is given in Table 3.

WSIV detection based on in situ hybridization (ISH) was conducted in samples obtained from 13 individuals belonging to four species, i.e., *A. ruthenus*, *A. baerii*, *A. gueldenstaedtii*, *A. oxyrinchus*, and two hybrids: *A. baerii* × *A. gueldenstaedtii* and *A. baerii* × *A. ruthenus*. Microscopic analyses of the specimens revealed a detection rate of 49.24%. Infected tissues were characterized by an altered structure apparent already in the histological analysis. The most noticeable changes occurred in the skin. A clear cytopathic effect (CPE) in the form of morphological changes was visible as the so-called ‘balloon-like’ cells (Fig. 1A, 1B). Within these structures, hybridization of the probe with WSIV DNA fragments occurred. The presence of the pathogen was also indicated by the specimen colour changed due to a reaction at the site of probe hybridization with the defined fragment of the viral genome in the tissue (Fig. 1C).

DISCUSSION

Viral infections in sturgeons and their interspecific hybrids pose a threat not only due to complicated

diagnostics but also especially due to the cessation of systematic monitoring in rearing centres. This situation favours uncontrolled carrying of viral diseases between rearing centres as constitutes a threat to open waters. Introduction of stocking material under the sturgeon restitution programs is not always preceded by mandatory diagnostic tests. As shown in this study, a high risk for farmers is posed not only by *A. oxyrinchus* or *A. sturio*, but also by sturgeon hybrids such as *A. naccarii* × *A. baerii* or *A. baerii* × *A. ruthenus*. In all the above-mentioned cases, a 100% level of WSIV infection was detected. In light of the fact that only samples collected from beluga and the beluga × *A. naccarii* hybrid were 100% free from the virus. Therefore, it can be presumed that there is a correlation between the resistance to WSIV and the genotype of beluga. WSIV probably penetrates the fish through the gill lamellae, as evidenced by the ‘balloon-like’ cells observed in the samples after in situ hybridization. The route of penetration of the virus in sturgeons is limited by the presence of scutes

Table 1

List of sturgeon species and hybrids obtained from rearing centres in Poland, Germany, and Italy

Species/hybrid	N	Poland		Germany		Italy	
		n	RC	n	RC	n	RC
<i>Huso huso</i>	3	—	—	—	—	3	1
<i>Acipenser ruthenus</i>	6	6	2	—	—	—	—
<i>Acipenser baerii</i>	14	2	1	—	—	12	1
<i>Acipenser gueldenstaedtii</i>	17	1	2	3	1	12	1
<i>Acipenser oxyrinchus</i>	2	2	2	—	—	—	—
<i>Acipenser sturio</i>	3	—	—	—	—	3	1
<i>A. baerii</i> × <i>A. gueldenstaedtii</i>	10	1	1	—	—	9	1
<i>A. naccarii</i> × <i>A. baerii</i>	15	—	—	3	1	12	1
<i>A. naccarii</i> × <i>Huso huso</i>	3	—	—	—	—	3	1
<i>A. baerii</i> × <i>A. ruthenus</i>	8	8	1	—	—	—	—
<i>Huso huso</i> × <i>A. ruthenus</i>	1	1	1	—	—	—	—

N = total number of individuals sampled, n = number of fish sampled in a given country, RC = number of rearing centres sampled.

Table 2

Summary of the results of WSIV detection in cultured acipenserid fishes by PCR (obtained in 222 samples) and by real-time PCR (obtained in 226 samples)

Species/hybrid	N	Positive isolates			
		PCR		Real-time PCR	
		n	[%]	n	[%]
<i>Huso huso</i>	3	0	0	0	0
<i>Acipenser ruthenus</i>	6	28	100	28	100
<i>Acipenser baerii</i>	14	28	93.33	25	83.33
<i>Acipenser gueldenstaedtii</i>	17	53	85.48	59	95.16
<i>Acipenser oxyrinchus</i>	2	7	77.78	9	100
<i>Acipenser sturio</i>	3	9	100	9	100
<i>A. baerii</i> × <i>A. gueldenstaedtii</i>	10	15	100	14	93.33
<i>A. naccarii</i> × <i>A. baerii</i>	15	30	100	30	100
<i>A. naccarii</i> × <i>Huso huso</i>	3	1	33.33	0	0
<i>A. baerii</i> × <i>A. ruthenus</i>	8	45	93.75	46	95.83
<i>Huso huso</i> × <i>A. ruthenus</i>	1	6	100	6	100

N = total number of individuals studied, n = number of positive isolates.

Table 3

Summary of the results of WSIV DNA detection (positive isolates) by real-time PCR and conventional PCR in various sturgeon organs analysed in this study

Species/hybrid	N	Organ	Poland			Germany			Italy		
			No. of analysed organs	real-time PCR	PCR	No. of analysed organs	real-time PCR	PCR	No. of analysed organs	real-time PCR	PCR
Beluga, <i>Huso huso</i>	3	Liver	—	—	—	—	—	—	—	NA	NA
		Gills	—	—	—	—	—	—	—	NA	NA
		Skin	—	—	—	—	—	—	—	NA	NA
		Kidney	—	—	—	—	—	—	—	NA	NA
		Gut	—	—	—	—	—	—	—	NA	NA
		Brain	—	—	—	—	—	—	—	NA	NA
		Swab	—	—	—	—	—	—	3	0	0
Sterlet, <i>Acipenser ruthenus</i>	6	Liver	6	6	6	—	—	—	—	—	—
		Gills	6	6	6	—	—	—	—	—	—
		Skin	6	6	6	—	—	—	—	—	—
		Kidney	6	6	6	—	—	—	—	—	—
		Gut	2	2	2	—	—	—	—	—	—
		Brain	2	2	2	—	—	—	—	—	—
		Swab	0	NA	NA	—	—	—	—	—	—
Siberian sturgeon, <i>Acipenser baerii</i>	14	Liver	1	1	1	—	—	—	3	3	3
		Gills	2	2	2	—	—	—	3	3	2
		Skin	2	2	2	—	—	—	3	3	3
		Kidney	2	2	2	—	—	—	0	NA	NA
		Gut	2	2	2	—	—	—	0	NA	NA
		Brain	0	NA	NA	—	—	—	0	NA	NA
		Swab	0	NA	NA	—	—	—	12	7	11
Russian sturgeon, <i>Acipenser gueldenstaedtii</i>	17	Liver	1	1	1	3	3	1	9	9	8
		Gills	1	1	1	3	3	3	9	9	8
		Skin	1	1	1	3	3	3	9	9	7
		Kidney	1	1	1	3	3	3	0	NA	NA
		Gut	1	1	1	3	3	3	0	NA	NA
		Brain	0	NA	NA	3	3	3	0	NA	NA
Atlantic sturgeon, <i>Acipenser oxyrinchus</i>	2	Liver	2	2	1	—	—	—	—	—	—
		Gills	2	2	1	—	—	—	—	—	—
		Skin	2	2	2	—	—	—	—	—	—
		Kidney	1	1	1	—	—	—	—	—	—
		Gut	1	1	1	—	—	—	—	—	—
		Brain	1	1	1	—	—	—	—	—	—
European sea sturgeon, <i>Acipenser sturio</i>	3	Liver	—	—	—	—	—	—	3	3	3
		Gills	—	—	—	—	—	—	3	3	3
		Skin	—	—	—	—	—	—	3	3	3
		Kidney	—	—	—	—	—	—	0	NA	NA
		Gut	—	—	—	—	—	—	0	NA	NA
		Brain	—	—	—	—	—	—	0	NA	NA
Siberian × Russian, <i>A. baerii</i> × <i>A. gueldenstaedtii</i>	10	Liver	1	1	1	—	—	—	0	NA	NA
		Gills	1	1	1	—	—	—	0	NA	NA
		Skin	1	1	1	—	—	—	0	NA	NA
		Kidney	1	1	1	—	—	—	0	NA	NA
		Gut	1	1	1	—	—	—	0	NA	NA
		Brain	1	1	1	—	—	—	0	NA	NA
Swab	0	NA	NA	—	—	—	9	8	9		

Table continues on next page.

Table 1 cont.

Species/hybrid	N	Organ	Poland			Germany			Italy		
			No. of analysed organs	real-time PCR	PCR	No. of analysed organs	real-time PCR	PCR	No. of analysed organs	real-time PCR	PCR
Adriatic × Siberian, <i>A. naccarii</i> × <i>A. baerii</i>	15	Liver	—	—	—	3	3	3	0	NA	NA
		Gills	—	—	—	3	3	3	0	NA	NA
		Skin	—	—	—	3	3	3	0	NA	NA
		Kidney	—	—	—	3	3	3	0	NA	NA
		Gut	—	—	—	3	3	3	0	NA	NA
		Brain	—	—	—	3	3	3	0	NA	NA
		Swab	—	—	—	0	NA	NA	12	12	12
Adriatic sturgeon × beluga, <i>A. naccarii</i> × <i>Huso huso</i>	3	Liver	—	—	—	—	—	—	0	NA	NA
		Gills	—	—	—	—	—	—	0	NA	NA
		Skin	—	—	—	—	—	—	0	NA	NA
		Kidney	—	—	—	—	—	—	0	NA	NA
		Gut	—	—	—	—	—	—	0	NA	NA
		Brain	—	—	—	—	—	—	0	NA	NA
		Swab	—	—	—	—	—	—	3	0	1
Siberian × sterlet, <i>A. baerii</i> × <i>A. ruthenus</i>	8	Liver	8	7	8	—	—	—	—	—	—
		Gills	8	7	7	—	—	—	—	—	—
		Skin	8	8	7	—	—	—	—	—	—
		Kidney	8	8	8	—	—	—	—	—	—
		Gut	8	8	8	—	—	—	—	—	—
		Brain	8	8	7	—	—	—	—	—	—
		Swab	0	NA	NA	—	—	—	—	—	—
Bester, <i>Huso huso</i> × <i>A. ruthenus</i>	1	Liver	1	1	1	—	—	—	—	—	—
		Gills	1	1	1	—	—	—	—	—	—
		Skin	1	1	1	—	—	—	—	—	—
		Kidney	1	1	1	—	—	—	—	—	—
		Gut	1	1	1	—	—	—	—	—	—
		Brain	1	1	1	—	—	—	—	—	—
		Swab	0	NA	NA	—	—	—	—	—	—

N = number of individuals studied, NA = not applicable.

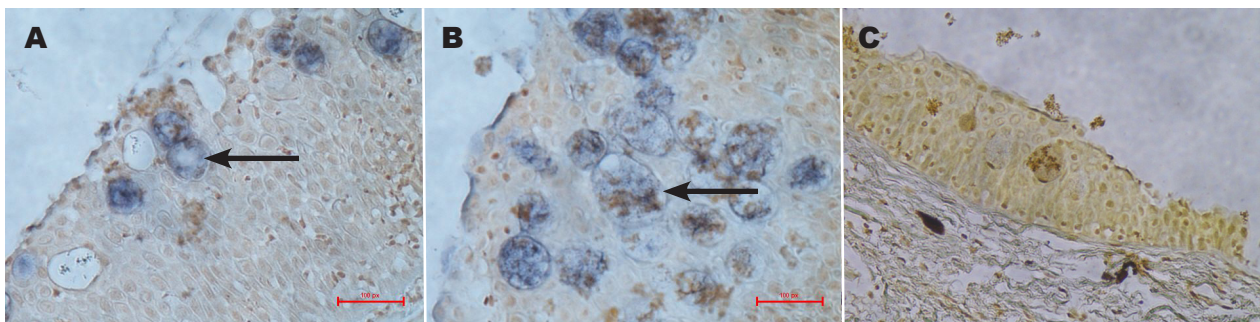


Fig. 1. *Acipenser baerii*; baloon-like skin structures with WSIV probe attached (A, B); A skin transection of the specimen without the characteristic colour and enlarged cells due to disruption of homeostasis; WSIV not detected (C)

that hinder the direct penetration through the skin. This means, however, that juvenile individuals are particularly vulnerable, and their anatomical features, including skin thickness, may not be a sufficient barrier to protect them against the penetration of the virus directly from the water. Because of the high stock density in the rearing centres, and particularly the absence of natural immune mechanisms before the end of the third month of life, it can

be presumed that infection originating from other fishes—the carriers (including spawning individuals)—may be permanent. As indicated by Drennan et al. (2006), vertical transmission of WSIV is possible from adult sturgeons of the *A. transmontanus* species to their offspring, which indicates that wild individuals are carriers of this virus. Therefore, it is not only diagnostics that poses a problem, but also the feasibility of obtaining virus-free offspring.

To this end, iodophor preparations of limited efficiency were used. The study demonstrated that the offspring of not all wild sturgeons of the *A. transmontanus* species showed WSIV-positive results after a bath in iodophor preparations. For the practice of restitution of sturgeons and stocking initiatives, this means that there is currently no method that would guarantee to obtain virus-free material. Russian researchers (Bauer et al. 2002) indicate that no viruses have been found in the health status monitoring of sturgeons. The authors state, however, that WSIV detection in Russia is only a matter of time, increasing the frequency of virological monitoring and implementation of more effective detection methods. According to the results of observations associated with programs of restitution and stocking of various sturgeon species into natural waters, this issue poses a threat, as currently there is no information about the possible WSIV transmission by other species. Moreover, the possible scenarios for the evolution of the WSIV genome in its adoption of new niches, i.e., replication capabilities in other species than sturgeons, are not known. This constitutes a threat to the local ichthyocoenoses and species that have not had previous contact with viruses of the family Iridoviridae.

REFERENCES

- Adkinson M.A., Cambré M., Hedrick R.P.** 1998. Identification of an iridovirus in Russian sturgeon (*Acipenser gueldenstaedtii*) from northern Europe. *Bulletin of the European Association of Fish Pathologists* **18** (1): 29–32.
- Altschul S.F., Gish W., Miller W., Myers E.W., Lipman D.J.** 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215** (3): 403–410. DOI: [10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Bauer O.N., Pugachev O.N., Voronin V.N.** 2002. Study of parasites and diseases of sturgeons in Russia: A review. *Journal of Applied Ichthyology* **18** (4–6): 420–429. DOI: [10.1046/j.1439-0426.2002.00422.x](https://doi.org/10.1046/j.1439-0426.2002.00422.x)
- Bemis W.E., Findeis E.K.** 1994. The sturgeons' plight. *Nature* **370** (6491): 602. DOI: [10.1038/370602a0](https://doi.org/10.1038/370602a0)
- Bigarré L., Lesne M., Lautraite A., Chesneau V., Leroux A., Jamin M., Boitard P.M., Toffan A., Prearo M., Labrut S., Daniel P.** 2017. Molecular identification of iridoviruses infecting various sturgeon species in Europe. *Journal of Fish Diseases* **40** (1): 105–118. DOI: [10.1111/jfd.12498](https://doi.org/10.1111/jfd.12498)
- Birstein V.J., Doukakis P., Sorkin B., DeSalle R.** 1998. Population aggregation analysis of three caviar-producing species of sturgeons and implications for the species identification of black caviar. *Conservation Biology* **12** (4): 766–775. DOI: [10.1111/j.1523-1739.1998.97081.x](https://doi.org/10.1111/j.1523-1739.1998.97081.x)
- Boreman J.** 1997. Sensitivity of North American sturgeons and paddlefish to fishing mortality. *Environmental Biology of Fishes* **48** (1–4): 399–405. DOI: [10.1023/A:1007345806559](https://doi.org/10.1023/A:1007345806559)
- Bronzi P., Rosenthal H.** 2014. Present and future sturgeon and caviar production and marketing: A global market overview. *Journal of Applied Ichthyology* **30** (6): 1536–1546. DOI: [10.1111/jai.12628](https://doi.org/10.1111/jai.12628)
- Bronzi P., Rosenthal H., Gessner J.** 2011. Global sturgeon aquaculture production: An overview. *Journal of Applied Ichthyology* **27** (2): 169–175. DOI: [10.1111/j.1439-0426.2011.01757.x](https://doi.org/10.1111/j.1439-0426.2011.01757.x)
- Chebanov M., Billard R.** 2001. The culture of sturgeons in Russia: Production of juveniles for stocking and meat for human consumption. *Aquatic Living Resources* **14** (6): 375–381. DOI: [10.1016/S0990-7440\(01\)01122-6](https://doi.org/10.1016/S0990-7440(01)01122-6)
- Chesneau V.** 2018. [43] Immunology in sturgeons with a focus on the Siberian sturgeon mechanisms, responses to stress and stimulation. Pp. 373–402. DOI: [10.1007/978-3-319-61676-6_18](https://doi.org/10.1007/978-3-319-61676-6_18) In: Williot P., Nonnotte G., Chebanov M. (eds.) *The Siberian sturgeon (Acipenser baerii, Brandt, 1869) Volume 2—Farming*. Springer, Cham, Switzerland. DOI: [10.1007/978-3-319-61676-6](https://doi.org/10.1007/978-3-319-61676-6)
- Chinchar V.G., Hyatt A., Miyazaki T., Williams T.** 2009. Family Iridoviridae: Poor viral relations no longer. Pp. 123–170. In: Van Etten J.L. (ed.) *Lesser known large dsDNA viruses. Current Topics in Microbiology and Immunology*. Vol. 328. Springer, Berlin, Heidelberg. DOI: [10.1007/978-3-540-68618-7_4](https://doi.org/10.1007/978-3-540-68618-7_4)
- Ciulli S., Volpe E., Sirri R., Passalacqua P.L., Cesa Bianchi F., Serratore P., Mandrioli L.** 2016. Outbreak of mortality in Russian (*Acipenser gueldenstaedtii*) and Siberian (*Acipenser baerii*) sturgeons associated with sturgeon nucleo-cytoplasmic large DNA virus. *Veterinary Microbiology* **191**: 27–34. DOI: [10.1016/j.vetmic.2016.05.012](https://doi.org/10.1016/j.vetmic.2016.05.012)
- Clouthier S.C., Anderson E., Kurath G., Breyta R.** 2018. Molecular systematics of sturgeon nucleocytoplasmic large DNA viruses. *Molecular Phylogenetics and Evolution* **128**: 26–37. DOI: [10.1016/j.ympev.2018.07.019](https://doi.org/10.1016/j.ympev.2018.07.019)
- Clouthier S.C., VanWalleghem E., Anderson E.D.** 2015. Sturgeon nucleo-cytoplasmic large DNA virus phylogeny and PCR tests. *Diseases of Aquatic Organisms* **117** (2): 93–106. DOI: [10.3354/dao02937](https://doi.org/10.3354/dao02937)
- Clouthier S.C., VanWalleghem E., Copeland S., Klassen C., Hobbs G., Nielsen O., Anderson E.D.** 2013. A new species of nucleo-cytoplasmic large DNA virus (NCLDV) associated with mortalities in Manitoba Lake sturgeon *Acipenser fulvescens*. *Diseases of Aquatic Organisms* **102** (3): 195–209. DOI: [10.3354/dao02548](https://doi.org/10.3354/dao02548)
- Drennan J.D., LaPatra S.E., Siple J.T., Ireland S., Cain K.D.** 2006. Transmission of white sturgeon iridovirus in Kootenai River white sturgeon *Acipenser transmontanus*. *Diseases of Aquatic Organisms* **70** (1–2): 37–45. DOI: [10.3354/dao070037](https://doi.org/10.3354/dao070037)
- Georgiadis M.P., Hedrick R.P., Carpenter T.E., Gardner I.A.** 2001. Factors influencing transmission, onset and severity of outbreaks due to white sturgeon iridovirus in a commercial hatchery. *Aquaculture* **194** (1–2): 21–35. DOI: [10.1016/S0044-8486\(00\)00503-2](https://doi.org/10.1016/S0044-8486(00)00503-2)
- Geßner J., Tautenhahn M., von Nordheim H., Borchers T.** 2010. German Action Plan for the conservation

- and restoration of the European Sturgeon (*Acipenser sturio*). Federal Ministry for the Environment, Nature Conservation and Nuclear Safety. Bonn, 2010. http://awsassets.panda.org/downloads/5_2010_german_ap_a_sturio_germany_1.pdf
- Hedrick R.P., Groff J.M., McDowell T., Wingfield W.H.** 1990. An iridovirus infection of the integument of the white sturgeon *Acipenser transmontanus*. *Diseases of Aquatic Organisms* **8** (1): 39–44. DOI: [10.3354/dao008039](https://doi.org/10.3354/dao008039)
- Hedrick R.P., McDowell T., Groff J.M., Wingfield W.H.** 1992. Isolation and some properties of an iridovirus-like agent from white sturgeon *Acipenser transmontanus*. *Diseases of Aquatic Organisms* **12**: 75–81. DOI: [10.3354/dao012075](https://doi.org/10.3354/dao012075)
- Hua Y.-p., Wang D.** 2005. A review of sturgeon virosis. *Journal of Forestry Research* **16** (1): 79–82. DOI: [10.1007/BF02856862](https://doi.org/10.1007/BF02856862)
- Jennings C.A., Zigler S.J.** 2000. Ecology and biology of paddlefish in North America: Historical perspectives, management approaches, and research priorities. *Revision of Fish Biology and Fisheries* **10** (2): 167–181. DOI: [10.1023/A:1016633604301](https://doi.org/10.1023/A:1016633604301)
- Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Mentjies P., Drummond A.** 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28** (12): 1647–1649. DOI: [10.1093/bioinformatics/bts199](https://doi.org/10.1093/bioinformatics/bts199)
- Kelley G.O., Waltzek T.B., McDowell T.S., Yun S.C., LaPatra S.E., Hedrick R.P.** 2005. Genetic relationships among herpes-like viruses isolated from sturgeon. *Journal of Aquatic Animal Health* **17** (4): 297–303. DOI: [10.1577/H05-002.1](https://doi.org/10.1577/H05-002.1)
- Kurobe T., MacConnell E., Hudson C., McDowell T.S., Mardones F.O., Hedrick R.P.** 2011. Iridovirus infections among Missouri River sturgeon: Initial characterization, transmission, and evidence for establishment of a carrier state. *Journal of Aquatic Animal Health* **23** (1): 9–18. DOI: [10.1080/08997659.2011.545697](https://doi.org/10.1080/08997659.2011.545697)
- Kwak K.T., Gardner I.A., Farver T.B., Hedrick R.P.** 2006. Rapid detection of white sturgeon iridovirus (WSIV) using a polymerase chain reaction (PCR) assay. *Aquaculture* **254** (1–4): 92–101. DOI: [10.1016/j.aquaculture.2005.10.037](https://doi.org/10.1016/j.aquaculture.2005.10.037)
- LaPatra R.P., Groff J.M., Jones G.R., Munn B., Patterson T.L., Holt R.A., Hauck A.K., Hedrick R.P.** 1994. Occurrence of white sturgeon iridovirus infections among cultured white sturgeon in the Pacific Northwest. *Aquaculture* **126** (3–4): 201–210. DOI: [10.1016/0044-8486\(94\)90036-1](https://doi.org/10.1016/0044-8486(94)90036-1)
- LaPatra S.E., Groff J.M., Patterson T.L., Shewmaker W.D., Casten M., Siple J., Hauck A.K.** 1996. Preliminary evidence of sturgeon density and other stressors on manifestation of white sturgeon iridovirus disease. *Journal of Applied Aquaculture* **6** (3): 51–58. DOI: [10.1300/J028v06n03_05](https://doi.org/10.1300/J028v06n03_05)
- Mao J., Hedrick R.P., Chinchar V.G.** 1997. Molecular characterization, sequence analysis, and taxonomic position of newly isolated fish iridoviruses. *Virology* **229** (1): 212–220. DOI: [10.1006/viro.1996.8435](https://doi.org/10.1006/viro.1996.8435)
- Pallandre L., Lesne M., de Boisséson C., Briand F.-E., Charrier A., Waltzek T., Daniel P., Tragnan A., Debeuf B., Chesneau V., Bigarré L.** 2018. *Acipenser iridovirus*-European encodes a replication factor C (RFC) sub-unit. *Archives of Virology* **163** (11): 2985–2995. DOI: [10.1007/s00705-018-3963-y](https://doi.org/10.1007/s00705-018-3963-y)
- Pikitch E.K., Doukakis P.L., Lauck P., Chakrabarty P., Erickson D.L.** 2005. Status, trends and management of sturgeon and paddlefish fisheries. *Fisheries* **6** (3): 233–265. DOI: [10.1111/j.1467-2979.2005.00190.x](https://doi.org/10.1111/j.1467-2979.2005.00190.x)
- Raverty S., Hedrick R., Justine H.** 2003. Diagnosis of sturgeon iridovirus infection in farmed white sturgeon in British Columbia. *Canadian Veterinary Journal* **44** (4): 327–328.
- Rosenthal H., Pourkazemi M., Bruch R.M.** 2006. The 5th International Symposium on sturgeons: A conference with major emphasis on conservation, environmental mitigation and sustainable use of the sturgeon resources. *Journal of Applied Ichthyology* **22** (s1): 1–4. DOI: [10.1111/j.1439-0426.2007.00921.x](https://doi.org/10.1111/j.1439-0426.2007.00921.x)
- Shchelkunov I.S., Shchelkunova T.I., Shchelkunov A.I., Kolbassova Y.P., Didenko L.V., Bykovsky A.P.** 2009. First detection of a viral agent causing disease in farmed sturgeon in Russia. *Diseases of Aquatic Organisms* **86** (3): 193–203. DOI: [10.3354/dao02124](https://doi.org/10.3354/dao02124)
- Tamura K., Dudley J., Nei M., Kumar S.** 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology Evolution* **24** (8): 1596–1599. DOI: [10.1093/molbev/msm092](https://doi.org/10.1093/molbev/msm092)
- Watson L.R., Groff J.M., Hedrick R.P.** 1998. Replication and pathogenesis of white sturgeon iridovirus (WSIV) in experimentally infected white sturgeon *Acipenser transmontanus* juveniles and sturgeon cell lines. *Diseases of Aquatic Organisms* **32** (3): 173–184. DOI: [10.3354/dao032173](https://doi.org/10.3354/dao032173)
- Watson L.R., Yun S.C., Groff J.M., Hedrick R.P.** 1995. Characteristics and pathogenicity of a novel herpesvirus isolated from adult and subadult white sturgeon *Acipenser transmontanus*. *Diseases of Aquatic Organisms* **22** (3): 199–210. DOI: [10.3354/dao022199](https://doi.org/10.3354/dao022199)
- Williot P., Sabeau L., Gessner J., Arlati G., Bronzi P., Gulyas T., Berni P.** 2001. Sturgeon farming in western Europe: Recent developments and perspectives. *Aquatic Living Resources* **14** (6): 367–374. DOI: [10.1016/S0990-7440\(01\)01136-6](https://doi.org/10.1016/S0990-7440(01)01136-6)

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