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Pathological and microbiological assessment of hemorrhagic septicemia in cultured juvenile Russian sturgeons (*Acipenser gueldenstaedtii*)

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ABSTRACT

Aquaculture has experienced sustained growth, now surpassing capture fisheries in global production. Among cultured species, sturgeon aquaculture has likewise expanded recently, prompting increased research and surveillance of diseases affecting this taxon. In this context, fifteen farmed Russian sturgeons (*Acipenser gueldenstaedtii*) were submitted for pathological investigation. A comprehensive study was conducted, encompassing autopsy, histopathology, parasitology, microbiology, and antimicrobial resistance analysis. Macroscopic examination revealed ulcerative lesions on the scutes, multiorgan hemorrhages, and multifocal black lesions in the liver. Histological analysis revealed a systemic granulocytic inflammatory response with associated melanomacrophage infiltration affecting the skin, gills, liver, spleen, heart, and digestive tract. Multifocal necrosis and hemorrhages were also present, consistent with hemorrhagic septicemia in fish. Microbiological identification confirmed the presence of *Aeromonas* spp., *Citrobacter* spp., and *Plesiomonas shigelloides*, all known pathogens associated with this condition in sturgeons. All three genera included isolates exhibiting resistance to at least one of the three antibiotics tested, with *C. braakii* displaying multidrug resistance. Additionally, *Flavobacterium succinicans* was also detected, representing the first report of this bacterium in sturgeons associated with skin ulcers. In conclusion, this study provides a detailed characterisation of hemorrhagic septicemia in Russian sturgeons, with pathological examination proving essential for identifying systemic lesions and validating microbiological findings. The detection of antimicrobial resistance raises concerns about the efficacy of current therapeutic options in potential future outbreaks, emphasising the importance of rapid and integrated diagnostic approaches, continued surveillance, and the implementation of effective preventive health management strategies in aquaculture.

1. Introduction

Aquaculture has been a growing sector for many years, with production surpassing capture fisheries since 2022 (FAO, 2024). This shift contributes to the sustainability of human activities by reducing pressure on wild species and their natural habitats (FAO, 2024). In the 1950s, sturgeonś caviar harvesting was primarily concentrated in the Soviet Union, with other countries playing a minor role, and it was mainly sourced from wild sturgeon populations. Over time, the development of sturgeon farming has led to a decline in fishing practices in favor of aquaculture, contributing to the recovery of wild sturgeon populations (Bronzi and Rosenthal, 2014). Although the Siberian sturgeon (*Acipenser baerii*) is the most widely cultivated species, other species are also commonly farmed in Europe, including the Russian sturgeon (*A. gueldenstaedtii*), White sturgeon (*A. transmontanus*), Adriatic sturgeon (*A. naccarii*), and Beluga sturgeon (*Huso huso*). On the other hand, China faces challenges with endangered sturgeon species, such as the Yangtze sturgeon (*A. dabryanus*), Chinese sturgeon (*A. sinensis*), and

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the Amur sturgeon (*A. schrenckii*). For this reason, sturgeons farming in China predominantly focuses on hybrid species (FAO, 2004, 2025; Qiwei, 2022).

With the expansion of sturgeon aquaculture, research and awareness of their major infectious diseases have likewise increased (Radosavljević et al., 2019). To date, no sturgeon diseases are officially listed by the World Organization for Animal Health (World Organisation for Animal Health WOAH, 2024). However, various factors may contribute to immunosuppression, increased disease susceptibility, and reduced welfare in sturgeons, particularly under intensive farming conditions. These include environmental stressors, suboptimal husbandry practices, and viral infections, among others.

In this context, water temperature, salinity, pH, oxygen levels, and ammonia concentration, along with feeding regimes and stocking densities, must be closely monitored, as deviations from optimal conditions may act as stressors, impairing immune function and favoring the proliferation of opportunistic pathogens (Guo and Dixon, 2021; Sneddon et al., 2016). Furthermore, viral agents such as herpesvirus (Soto et al., 2017) and iridovirus (Axén et al., 2018) have also been associated with immunosuppression in sturgeons, potentially triggering secondary bacterial infections.

Regardless of the underlying cause, these predisposing factors may lead to opportunistic bacterial infections. Under stress and suboptimal welfare conditions, commensal bacteria may become pathogenic, resulting in either chronic complications or acute outbreaks (Guo and Dixon, 2021; Ciulli et al., 2020; Santi et al., 2019). Gram-negative bacteria are the most frequently reported in sturgeons suffering from immunosuppression due to poor welfare conditions (Gholamhosseini et al., 2018; Kayiş et al., 2017).

For a comprehensive diagnosis, autopsy and thorough anatomopathological, parasitological, and microbiological examinations are essential to accurately determine the primary disease etiology and identify subclinical or associated alterations. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) facilitates both precise and rapid microbial identification. When necessary, additional complementary techniques such as genomic sequencing and Polymerase Chain Reaction (PCR) may be employed (Çağatay, 2024; Noga, 2010).

The emergence of antimicrobial-resistant pathogens in aquaculture facilities can adversely affect fish health and the sustainability of aquaculture systems. Therefore, surveillance represents the most effective tool to monitor and control the development of antimicrobial resistance (Pepi and Focardi, 2021). It is consequently critical that, regardless of the identification method employed, isolates undergo antimicrobial susceptibility testing to determine their resistance profiles and select the most appropriate treatment (Acharjee et al., 2021).

The aim of this study was to specifically describe both macroscopic and histological alterations associated with hemorrhagic septicemia in juvenile Russian sturgeons (*Acipenser gueldenstaedtii*) and identify the main causative pathogens and their antimicrobial resistance using microbiological analysis, MALDI-TOF MS, and antibiogram testing. This approach is crucial for enhancing disease surveillance in aquaculture, enabling timely detection and effective management of emerging pathogens, critical steps to mitigate economic losses, ensure animal welfare, and promote sustainable production in an industry facing increasing environmental and biological challenges.

2. Material and methods

2.1. Autopsy, macroscopic examination and sample collection

Fifteen Russian sturgeons (*Acipenser gueldenstaedtii*) were analyzed, originating from three different cages (1, 2, and 3), with mean individual weights of 46.6 g, 47.9 g, and 34.5 g, respectively. Systematic, orderly, and complete autopsies were randomly conducted to identify lesions and potential causal pathogens. The external evaluation of fish included the assessment of macroscopic lesions in the gills, skin and the internal organs for each fish. Organ samples were collected under sterile conditions for microbiological analysis and preserved in 10 % neutral-buffered formalin (Panreac AppliChem ITW Reagents, Barcelona, Spain) for at least 48 h for histological examination. The organs selected for both microbiological and histological analysis included the liver, spleen, kidney, and brain, while gills, skin, skeletal muscle, heart, and the digestive tract were only collected for histology.

2.2. Histological study

For the histopathological study, five fish per cage (n = 15) were analysed, resulting in a total of 135 organ samples. Fixed tissue samples collected during autopsy were processed using the Citadel 2000 Tissue Processor (Thermo Fisher Scientific, Waltham, Massachusetts, USA), dehydrated through graded ethanol solutions (70 %, 96 %, and 100 %) (PanReac AppliChem ITW Reagents, Barcelona, Spain), and cleared with a xylene substitute (Casa Álvarez, Madrid, Spain), prior to paraffin wax embedding using the HistoStar Embedding Workstation (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Five consecutive sections of 3–4 μm thickness were made using a rotary microtome (Epredia, New Hampshire, USA, Massachusetts, USA) and placed on charged slides. Each section was deparaffinized with Citrus Clearing Solvent (Thermo Fisher Scientific, Waltham, Massachusetts, USA), hydrated through decreasing ethanol concentrations (100º, 96º, 70º) (Panreac AppliChem ITW Reagents, Barcelona, Spain), and washed in running water.

One of the five sections was stained with Hematoxylin and Eosin (H-E) using an automatic stainer (Gemini AS Automated Slide Stainer, Thermo Fisher Scientific, Waltham, Massachusetts, USA) and used for histological examination under a light microscope with an integrated digital camera (Leica DM 1000/CP50).

A second section was processed for Alcian Blue–Periodic Acid–Schiff (AB-PAS) staining. Slides were stained with 1 % Alcian Blue in 3 % acetic acid (pH 2.5) for 15–20 min, rinsed in running tap water for 2 min, and washed with distilled water. Subsequently, the slides were immersed in 1 % Periodic Acid Solution (Panreac AppliChem ITW Reagents, Barcelona, Spain) for 5 min and washed several times with distilled water. Slides were then stained with Schiff Reagent (Bio Optica Milano SPA, Milan, Italy) for 10–20 min, rinsed with lukewarm running tap water for 10 min and washed with distilled water. Counterstaining was performed using Harris Hematoxylin (PanReac AppliChem) for 1 min. Slides were rinsed in running tap water, treated with 0.01 % hydrochloric acid in alcohol for 3–5 s, and washed again with distilled water.

All the slides were then dehydrated through increasing ethanol concentrations (PanReac AppliChem ITW Reagents, Barcelona, Spain) and cleared with Citrus Clearing Solvent. All hydration and dehydration steps were completed using the Gemini AS Automated Slide Stainer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Finally, adhesive was applied to the slides, and a coverslip was placed on top using CTM6 Coverslipper (Epredia, New Hampshire, USA) for long-term sample preservation.

A semi-quantitative histological analysis was performed based on the lesions described in the histological study. Lesion severity was assessed using a semi-quantitative scale ranging from 0 to 3. Isolates with low scores (≤ 2) were excluded from the analysis. A Spearman correlation analysis was conducted in GraphPad Prism 8, with a significance level of $p < 0.05$.

2.3. Microbiology

Microbiological cultures were performed on organs from five sturgeons presenting external lesions (ulcers, hemorrhages, and gill lesions) using Blood Agar Base and Anacker-Ordal media. After 24 h of incubation, bacterial isolates were subjected to a formic acid–acetonitrile protein extraction protocol (Bruker Daltonik, Bremen, Germany) for MALDI-TOF MS identification. If distinct colonies appeared at later time

points, they were also identified using the same procedure. Identification of bacterial isolates was conducted using the MALDI Biotyper software (version 3.1.66; 11897 entries). *Aeromonas* spp. isolates underwent DNA extraction and were tested using a PCR protocol specific for *A. salmonicida* (Beaz-Hidalgo et al., 2008).

Antimicrobial susceptibility testing was performed using the disk diffusion method against antibiotics commonly used in European aquaculture (Rigos and Troisi, 2005), including florfenicol, flumequine, and tetracycline, following the Clinical and Laboratory Standards Institute (CLSI) guideline for aquatic animal bacteria VET03/VET04-S1 (CLSI, 2010). When specific interpretive criteria were unavailable (e.g., flumequine), breakpoints from the Comité de l'Antibiogramme de la Société Française de Microbiologie (CASFM) VET (2018) were applied. Results are summarized in Table 1. For *Flavobacterium succinicans* and *Haemophilus piscium*, inhibition zone diameters are presented as raw values due to the absence of established interpretive standards.

Samples that could not be identified or showed discordant results by MALDI-TOF MS were sent for sequencing. The resulting sequences were then analysed using BLAST against reference sequences in the National Center for Biotechnology Information (NCBI) database (2025) for definitive identification.

2.4. Parasitology

For each sturgeon, the operculum was carefully removed, and the gills were excised. All gill arches were collected and individually separated, then numbered sequentially from the outermost to the innermost arch relative to the operculum. Each gill arch was sectioned using a scalpel, and representative samples of gill filaments, including the secondary lamellae, were placed on a glass slide with a few drops of physiological saline solution. Fresh preparations were subsequently examined for the presence of parasites using brightfield microscopy.

3. Results

3.1. Macroscopic findings

Externally, the sturgeons presented multifocal, well-demarcated, irregular red lesions consistent with hyperaemia and hemorrhages, accompanied by a moderate amount of mucus. These lesions were distributed across the facial region, mouth (80 %), ventral scutes (73 %); ventral (67 %), pectoral (33 %) and dorsal (14 %) fins, perianal region (47 %), anterior orbital chamber (hyphema, 20 %), barbels (7 %) and operculum (3 %) (Fig. 1**A–C**). Ulcerative lesions were also observed on the ventral scutes (Fig. 1**A**). The gills showed abundant surface mucus (60 %) and diffuse colour changes, including either increased intensity (congestion, 13 %) or pallor indicative of anaemia (47 %) (white arrowhead, Fig. 1**D**).

Upon opening the coelomic cavity, the liver appeared pale with multifocal circular hemorrhages (20 %; white arrows, Fig. 1**D**). Black foci were also observed throughout the liver parenchyma, suggesting the presence of melanomacrophage infiltrates (100 %; Fig. 1**D**). The spleen exhibited reduced size, pale coloration, and a wrinkled appearance, indicative of exsanguination (40 %). The swim bladder presented a diffuse opaque appearance and, when incised, contained an amber-coloured fluid suggestive of aerocystitis (26 %; white asterisk, Fig. 1**D**). The kidney showed either a pale appearance, indicative of anemia (13 %), or a reddish coloration with blood oozing during incision, consistent with congestion (40 %). The digestive tract exhibited vascular congestion in the blood vessels of the serosa (27 %), while the anterior intestine contained a white mucous content mixed with blood as the sole abnormal finding. Examination of the cranial cavity showed diffuse vascular congestion in the telencephalon of two fish specimens (13 %).

Table 1
Breakpoints for the main antibiotics used in European aquaculture, determined by the disk diffusion method (CLSI, 2010; CASFM, 2018).

Antibiotic	Susceptible (mm)	Intermedium (mm)	Resistant (mm)
Florfenicol	≥ 27	-	≤ 26
Flumequine	≥ 25	-	< 21
Tetracycline	≥ 28	22–27	≤ 21

3.2. Histological findings

Microscopically, fish presented skin erosions and ulcers on the ventral scutes (73 %), with associated granulocytic inflammation (Fig. 2**A**). In the deeper dermal layers, clear spaces consistent with edema were observed within the connective tissue, alongside a predominantly granulocytic inflammatory infiltrate, with scattered melanomacrophages infiltrating the underlying muscle. Furthermore, basophilic formations compatible with bacterial colonies (inset, Fig. 2**A**) were also present (severe, subacute, multifocal ulcerative dermatitis with osteitis of the scute).

All examined sturgeons showed gill alterations characterized by marked epithelial hyperplasia (100 %), fusion of secondary lamellae (73 %), and occasional cell necrosis (60 %). These findings are consistent with severe hyperplastic and necrotizing branchitis (Fig. 2**B**). Necrotic cells exhibited nuclear pyknosis, karyorrhexis, and karyolysis. AB-PAS staining revealed increased numbers of violet goblet cells in the lamellae of affected fish (60 %), indicative of goblet cell hyperplasia (inset, Fig. 2**B**) and in correlation with the increased mucus production observed grossly. Additionally, in all sturgeons (100 %), the lamina propria of gills was expanded by an inflammatory infiltrate composed of lymphocytes, plasma cells, melanomacrophages, and, occasionally, well-demarcated clusters of granulocytes (microabscesses). Multifocal dilation of lamellar vessels due to intraluminal erythrocyte accumulation (congestion) was also observed in 53 % of the fish.

In the liver of all fish (100 %), multifocal inflammatory infiltrates, composed predominantly of lymphocytes and plasma cells with fewer granulocytes and melanomacrophages, were observed surrounding the portal spaces (arrows, Fig. 2**C**). In 53 % of samples, periportal hepatocytes displayed hypereosinophilic cytoplasm, nuclear pyknosis, karyorrhexis, and karyolysis, consistent with liquefactive necrosis. Additionally, an increased number of biliary ducts, characterized by tortuous and irregular morphology, was noted within the portal areas in 73 % of cases (bile ducts hyperplasia; inset, arrowheads, Fig. 2**C**). Multifocal, well-demarcated foci of erythrocyte extravasation (hemorrhage) were present in 33 % of samples, accompanied by ischemic necrosis of adjacent hepatocytes. Surrounding hepatocytes showed diffuse microvacuolar changes leading to cellular enlargement without nuclear displacement, compatible with hydropic degeneration. Collectively, these findings are consistent with severe, chronic-active, multifocal, periportal necrotizing hepatitis.

The spleen of all sturgeons (100 %) exhibited diffuse white pulp hyperplasia, characterized by a moderate infiltrate of granulocytes, lymphocytes, and plasma cells, indicative of moderate, subacute, diffuse granulocytic splenitis.

The kidneys exhibited moderate, subacute, multifocal tubulointerstitial nephritis. In most fish (60 %), lesions were characterized by inflammatory infiltrates primarily composed of granulocytes and melanomacrophages, accompanied by a marked increase in granulocytes within the hematopoietic tissue (100 %) (arrow, Fig. 2**D**). In a few cases (13 %), necrosis of blast cells was observed within hematopoietic zones.

The epicardial lympho-hematopoietic tissue exhibited a diffuse, moderate reduction in cellularity in 93 % of the fish, accompanied by multifocal cystic formations in 67 % of cases and a mild increase in granulocytes in 60 %, located inside and between myocardial vessels (arrow, Fig. 2**E**).

In the digestive tract, a mild inflammatory infiltrate was observed

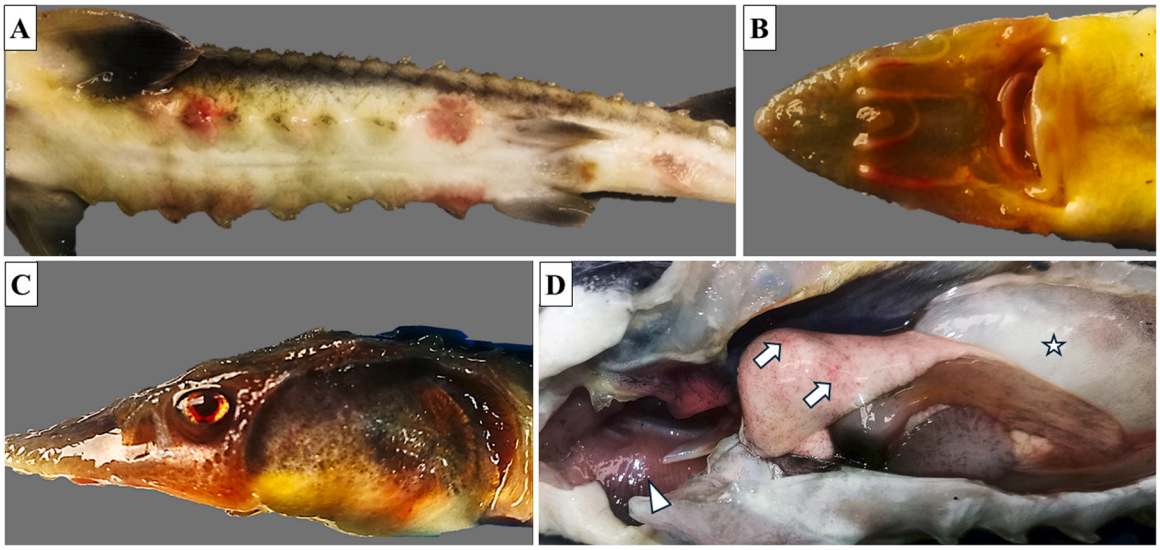

Fig. 1. Macroscopic lesions observed in sturgeons during the autopsy. A) Multifocal ulcerative lesions in the ventral scutes. B) Ventral view of a sturgeon showing multifocal hemorrhages in the barbels and the mouth. C) Marked hyperemia and multifocal hemorrhages on the head. Note the presence of blood in the anterior chamber of the eye (hyphema) and increased skin brightness due to moderate mucus accumulation. D) Multifocal hemorrhages (white arrows) and black foci of melanomacrophage infiltrates in the liver. The swim bladder appears opaque (white asterisk), and the gills are covered by abundant mucinous material (white arrowhead).

along its entire length, comprising mononuclear cells in 27 % of the fish and granulocytes in 44 %, with the latter occasionally migrating through the mucosa toward the lumen (Fig. 2**F**). In the pancreatic tissue, low numbers of granulocytes were consistently present in 93 % of the sturgeons.

A summary of all histological lesions described in this section is provided in the Supplementary Material (Table S1).

3.3. Microbiology

3.3.1. MALDI-TOF MS

After 24 h of incubation, different types of bacterial colonies grew on Blood Agar Base. All bacterial isolates were accurately identified to the genus level using the MALDI-TOF MS Biotyper. A highly reliable identification was defined as a log score $\geq$ 2.000 for both first and second-best matches (Table 2). Fifteen of the 26 isolates were accurately identified to the species level with MALDI-TOF MS Biotyper (Table 2). The genera identified in fish samples were *Aeromonas* spp. (Fig. 3**A**), *Citrobacter* spp. (Fig. 3**B**) and *Plesiomonas* spp. (Fig. 3**C**). However, seven fish samples showed discordant or inconclusive species-level identifications by the MALDI-TOF MS Biotyper and were therefore subjected to 16S rRNA gene sequencing for definitive identification (Table 3).

3.3.2. Identification of bacterial strains by 16S rRNA gene sequencing

Fish samples with discordant species-level identifications between the first and second-best MALDI-TOF MS Biotyper matches, or those not identified by this method, were subjected to 16S rRNA gene sequencing. The resulting sequences were analysed using the NCBI BLAST tool, yielding percentage similarities ranging from 98 % to 100 % with reference strains in the NCBI database (2025). These results are summarised in Table 3**.** Among the nine unreliably identified isolates, 33.3 % matched one of the two best by the MALDI-TOF MS Biotyper hits, 33.3 % showed no concordance with either (*C. portucalensis)*, and the remaining 33.3 % were newly identified via sequencing as *Cloacibacterium caeni* and *F. succinicans* (Fig. 3 **D**).

Spearman correlation analysis was performed for the presence of *A. veronii*, *Aeromonas* spp., and *Citrobacter* spp. No significant correlations were found between these bacterial isolates and histological lesions ($p > 0.05$).

3.3.3. Antimicrobial resistance

Antibiogram results demonstrated variable susceptibility patterns among the bacterial isolates to florfenicol, flumequine, and tetracycline (Table 4). *Aeromonas* spp. isolates recovered from cages 1 and 2 exhibited resistance to tetracycline. *P. shigelloides* was susceptible to all tested antimicrobials. In contrast, *Citrobacter* spp. displayed higher levels of resistance compared to *Aeromonas* and *Plesiomonas* isolates. Notably, *C. braakii* demonstrated resistance to all three antibiotics evaluated, whereas *C. gillenii* was resistant to two of the three antibiotics.

3.4. Gills parasitological findings

No parasites were identified upon microscopic examination.

4. Discussion

Aquaculture is the fastest-growing food production sector, reaching 130.9 million tons in 2022. It plays a crucial role in global food security by nourishing over 600 million people and supporting rural development (FAO, 2024). Sturgeons, particularly the Russian sturgeon (*Acipenser gueldenstaedtii*), are well-suited for aquaculture due to their resilience and adaptability, yielding premium caviar (Elhetawy et al., 2020) and high-value meat (Vilkova et al., 2022). Despite these advantages, sturgeons are classified as vulnerable to critically endangered (IUCN., SSG., 2022), rendering their farming both ecologically and economically significant and driving their global expansion (Bronzi et al., 2019). Rapid and effective diagnosis in aquaculture is grounded in population medicine principles, which prioritize group health over individual cases (Georgiadis et al., 2001). This approach is especially appropriate for intensive aquaculture, where pooled sampling and group-level histological analyses yield practical and representative data. Although this method restricts direct correlation between pathological and microbiological findings individually, it is consistent with standard health assessment practices in intensive care systems.

This study employs a population medicine framework to rapidly identify, characterize, and address hemorrhagic septicemia in juvenile Russian sturgeons (*Acipenser gueldenstaedtii*). Through the analysis of macroscopic and histological lesions, combined with the use of MALDI-TOF MS for pathogen identification and antimicrobial resistance

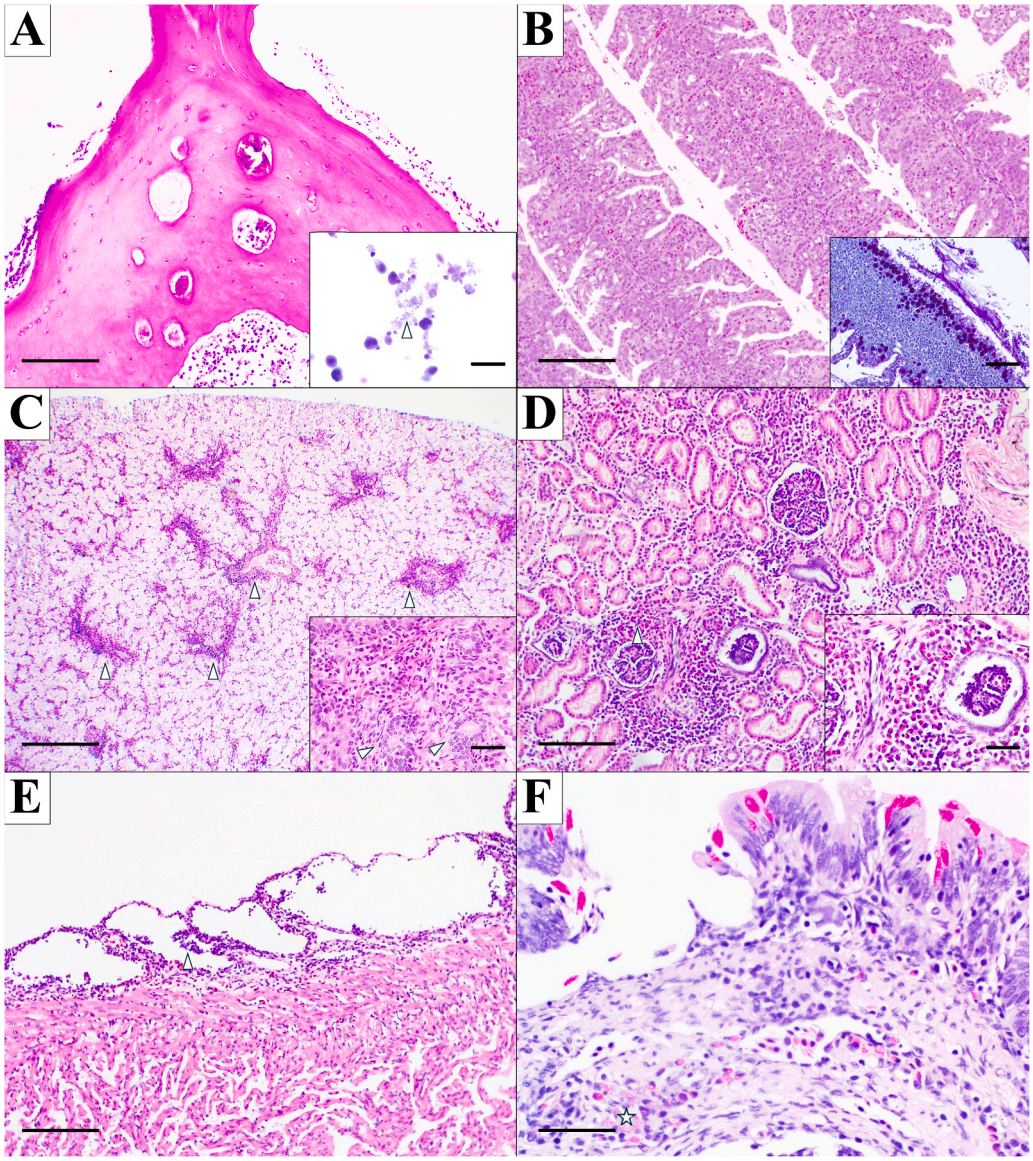

Fig. 2. Histological lesions observed in the examined sturgeons. A) Ventral scute dermis. Severe epidermal erosion (H-E, 100 ×; scale bar: 200 μm). *Inset:* granulocytic and mononuclear inflammatory infiltrates and bacterial colonies in the deep dermis (arrowhead; H-E, 1000 ×; scale bar: 20 μm). B) Gills, medial part of the filament. Severe, diffuse lamellar epithelial hyperplasia and lamellar fusion with inflammatory infiltrates (H-E, 100 ×; scale bar: 200 μm). *Inset:* marked violet staining indicating globet cell hyperplasia with interlamellar mucus and cellular debris (AB-PAS, 200 ×; scale bar: 100 μm). C) Liver. Panlobular ballooning degeneration associated with multifocal portal and periportal mixed granulocytic and lymphohistiocytic inflammatory infiltrates and moderate presence of melanomacrophages (arrowheads; H-E, 40 ×; scale bar: 500 μm). *Inset:* severe portal mixed inflammation with melanomacrophages and biliary duct hyperplasia (arrowheads; H-E, 400 ×; scale bar: 50 μm). D) Kidney. Hematopoietic tissue with moderate granulocyte infiltration (arrowhead; H-E, 100 ×; scale bar: 200 μm). *Inset:* increased number of granulocytes in the hematopoietic area (H-E, 400 ×; scale bar: 50 μm). E) Heart. Lymphoid tissue atrophy with cyst formation. The lumen of the cyst contains red blood cells and granulocytes (arrowhead; H-E, 100 ×; scale bar: 200 μm). F) Mid-intestine. Moderate granulocytic infiltrate in the lamina propria (asterisk) and epithelial exocytosis into the lumen (H-E, 200 ×; scale bar: 100 μm).

profiling, the study demonstrates that timely and cost-effective diagnostics can inform aquaculture management decisions (Çağatay, 2024).

Hemorrhagic septicemia is a prevalent fish disease characterized by external signs, including skin and fin hemorrhages, abdominal distension, exophthalmia, and internal lesions such as liver congestion and splenomegaly (Duman et al., 2023; Kayiş et al., 2017; Utegenova et al., 2024). The macroscopic lesions observed in the skin, liver, digestive tract, and respiratory system in the present study corresponded to those previously described for hemorrhagic septicemia. However, in sturgeon species, external lesions resulting from hemorrhagic septicemia are often indistinguishable among different bacterial isolates, complicating the identification of causative pathogens based solely on gross pathology (Radosavljević et al., 2019).

Histological examination of the sturgeons revealed skin ulceration, granulocytic inflammation, and hemorrhages. These findings closely resembled those previously reported in juvenile Russian sturgeons (*Acipenser gueldenstaedtii*) infected with *A. hydrophila* (Ture et al., 2018) and *A. salmonicida* (Vázquez-Fernández et al., 2023), as well as in juvenile Siberian sturgeons (*Acipenser baerii*) infected with *A. veronii* (Ma et al., 2009). Additionally, pronounced melanomacrophage hyperplasia was observed in both the liver and spleen, highlighting their significant immune function in sturgeons, a role well documented in Osteichthyes (Kasprzak et al., 2023; Stosik et al., 2019). Infection with *C. freundii* also elicited a granulocytic inflammatory response in multiple organs (Liu et al., 2024). The gill pathology suggested a chronic disease, potentially

Table 2
Identification of bacterial strains isolated from Russian sturgeons using MALDI-TOF MS. For each organ and fish, the best and second-best identification matches are provided along with their corresponding log-score values.

	Fish	Organ	Identification (best match)	Score	Identification (second best match)	Score
Cage 1	1	Brain	*Plesiomonas shigelloides*	2245 – A+ +	*Plesiomonas shigelloides*	*2215* – A++
		Brain	*Aeromonas veronii*	2381 – B+ ++	*Aeromonas veronii*	*2295* – B+++
	2	Spleen	*Aeromonas hydrophila*	2430 – B+ ++	*Aeromonas hydrophila*	*2337* – B+++
		Spleen†	*Citrobacter braakii*	2482 – B+ ++	*Citrobacter freundii*	*2388* – B+++
		Kidney	*Aeromonas veronii*	2323 – B+ ++	*Aeromonas veronii*	*2266* – B+++
		Kidney	*Citrobacter braakii*	2340 – B+ ++	*Citrobaccter braakii*	*2216* – B+++
	3	Kidney	*Aeromonas veronii*	2186 – B+ ++	*Aeromonas veronii*	*2177* – B++
		Kidney†	*Aeromonas sobria*	2332 – B+ ++	*Aeromonas hydrophila*	*2122* – B++
	4	Kidney	*Aeromonas hydrophila*	2316 – A+ ++	*Aeromonas hydrophila*	*2233* – A++
Cage 2	1	Liver	*Aeromonas veronii*	2321 – B+ ++	*Aeromonas veronii*	*2264* – B++
		Liver	*Citrobacter gilenii*	2246 – B+ +	*Citrobacter gillenii*	*2203* – B++
		Kidney	*Aeromonas veronii*	2297 – B+ +	*Aeromonas veronii*	*2278* – B++
		Kidney	*Citrobacter gilenii*	2191 – B+ +	*Citrobacter gillenii*	*2145* – B++
	2	Liver	*Aeromonas bestiarum*	2160 – B+ +	*Aeromonas bestiarum*	*2146* – B++
Cage 3	1	Kidney†	Unidentified	-	-	-
	2	Liver†	Unidentified	-	-	-
		Spleen	*Aeromonas veronii*	2279 – B+ +	*Aeromonas veronii*	*2146* – B++
		Spleen†	*Aeromonas sobria*	2202 – B+ +	*Aeromonas hydrophila*	*2077* – B++
	3	Liver†	*Aeromonas bestiarum*	2230 – B+ +	*Aeromonas salmonicida*	*2134* – B++
	4	Spleen†	*Aeromonas veronii*	2279 – B+ +	*Aeromonas hydrophila*	*2199* – B++
		Spleen†	*Citrobacter braakii*	2317 – B+ ++	*Citrobacter freundii*	*2288* – B++
		Skin†	Unidentified	-	-	-

Note: † Fish samples with discordant first and second-best matches at the species level or unidentified by MALDI-TOF MS Biotyper.

influenced by persistent bacterial infections (Ciulli et al., 2020), viral pathogens (Ciulli et al., 2016), and environmental factors such as elevated suspended solids, temperature fluctuations, and water quality (Banihashemi et al., 2016; Zhang et al., 2025).

The disease in sturgeons, typically caused by Gram-negative bacteria, is most commonly associated with species such as *Citrobacter* spp., *Pseudomonas* spp., *Streptococcus* spp., and *Aeromonas* spp. (Hu et al., 2025). Infections with *Aeromonas* spp. are particularly prevalent in aquaculture. Notably, furunculosis, caused by *A. salmonicida*, is distinguished from hemorrhagic septicemia, which is generally attributed to motile Aeromonas (MA) species such as *A. hydrophila*, *A. veronii,* or *A. bestiarum*. These species have been identified in the present study and have been previously linked to sturgeon pathology (Bakiyev et al., 2022; Utegenova et al., 2024; Zhu et al., 2023). In contrast, other MA species, such as *A. sobria*, have not been associated with pathological findings in sturgeons (Liu et al., 2025), but they are implicated in fish spoilage due to their proteolytic activity (Tan et al., 2023).

A Flavobacterium species was also isolated from a cutaneous ulcer in this study. In sturgeons, *F. psychrophilum* (Chinchilla et al., 2023) and *F. johnsoniae* (Karatas et al., 2010) have been reported in ulcerative lesions, demonstrating direct pathogenicity, particularly in juvenile fish. These species are not exclusive to sturgeons, as *F. psychrophilum*, *F. columnare*, and *F. branchiophilum* are also recognized as significant pathogens in rainbow trout production (Loch and Faisal, 2015). Other *Flavobacterium* species, such as *F. hydatis* and *F. succinicans*, have been described as opportunistic pathogens in fish (Loch and Faisal, 2015). Notably, *F. succinicans* has not been previously reported in sturgeons but has been isolated from rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*). In rainbow trout, *F. succinicans* has been found in gill lesions (Good et al., 2015), whereas in Chinook salmon, it was associated with a caudal erosive lesion (Anderson and Ordal, 1961). In both cases, other primary bacterial pathogens (*F. branchiophilum* and *A. salmonicida*, respectively) were also present. Bernadet and Bowman (2006) proposed that *F. succinicans* may act as an opportunistic pathogen, particularly under suboptimal farming conditions, where it may contribute to disease development. In the present study, *F. succinicans* was isolated from skin lesions but not from gills, consistent with previous findings in Chinook salmon (Anderson and Ordal, 1961). The presence of multiple bacterial isolates across different organs supports the hypothesis that *F. succinicans* is not a primary pathogen (Bernadet and Bowman, 2006).

Citrobacter spp. are Gram-negative bacteria implicated in hemorrhagic septicemia in fish. Although *C. freundii* is the species level most frequently associated with clinical disease in Chinese (Deng et al., 2022), Siberian (Kayiş et al., 2017) and Russian (Liu et al., 2024) sturgeons, this study detected *C. gilleni* in only a single cage, a species previously reported in Russian sturgeon (Türe et al., 2022). *C. braakii* was isolated from all cages but has not previously been linked to sturgeon pathology, although it induces systemic disease in rainbow trout (Altun et al., 2013). Additionally, *C. portucalensis*, which is genetically similar to *C. braakii* and *C. freundii* (Ribeiro et al., 2017), was detected but has not been implicated in fish infections to date, highlighting the need for further investigation.

P. shigelloides, a Gram-negative bacterium, is associated with hemorrhagic septicemia in rainbow trout (Duman et al., 2023; Salgado-Miranda et al., 2010), carp (Hu et al., 2014), and sturgeon (Jiang et al., 2021). It is typically part of the commensal microbiota and generally causes opportunistic rather than primary infections (Duman et al., 2023).

Europe currently lacks comprehensive surveillance databases documenting the antimicrobial resistance patterns of the bacterial species identified in the present study. Consequently, research on uncommon bacterial species, as well as broader investigations, are of significant importance for comparing results and establishing resistance profile monitoring.

MA species in this study demonstrated susceptibility to enrofloxacin and florfenicol; however, resistance to tetracycline was observed. In MA, resistance to tetracycline, florfenicol, and quinolones has been reported, primarily associated with resistance genes. The most prevalent resistance genes identified to date include tetE and tet34, although slight resistance to florfenicol, conferred by floR, has been observed (Agersø et al., 2007; Duman et al., 2020; Hayatgheib et al., 2021; Tekedar et al., 2020). Previous research has identified virulence genes associated with enrofloxacin, tetracycline, and florfenicol resistance in *A. hydrophila* isolates from sturgeons (Bakiyev et al., 2022) and in other strains lacking the *Cfr* and *FexA* genes, which are involved in florfenicol resistance (Hu et al., 2025). In other studies, these resistance genes have been mainly related to *tetA*, *tetE*, and *tetD* (Agersø et al., 2007). In contrast, *A. veronii* infections in sturgeons have shown susceptibility to florfenicol, supporting its use as a recommended treatment (Zhu et al., 2023).

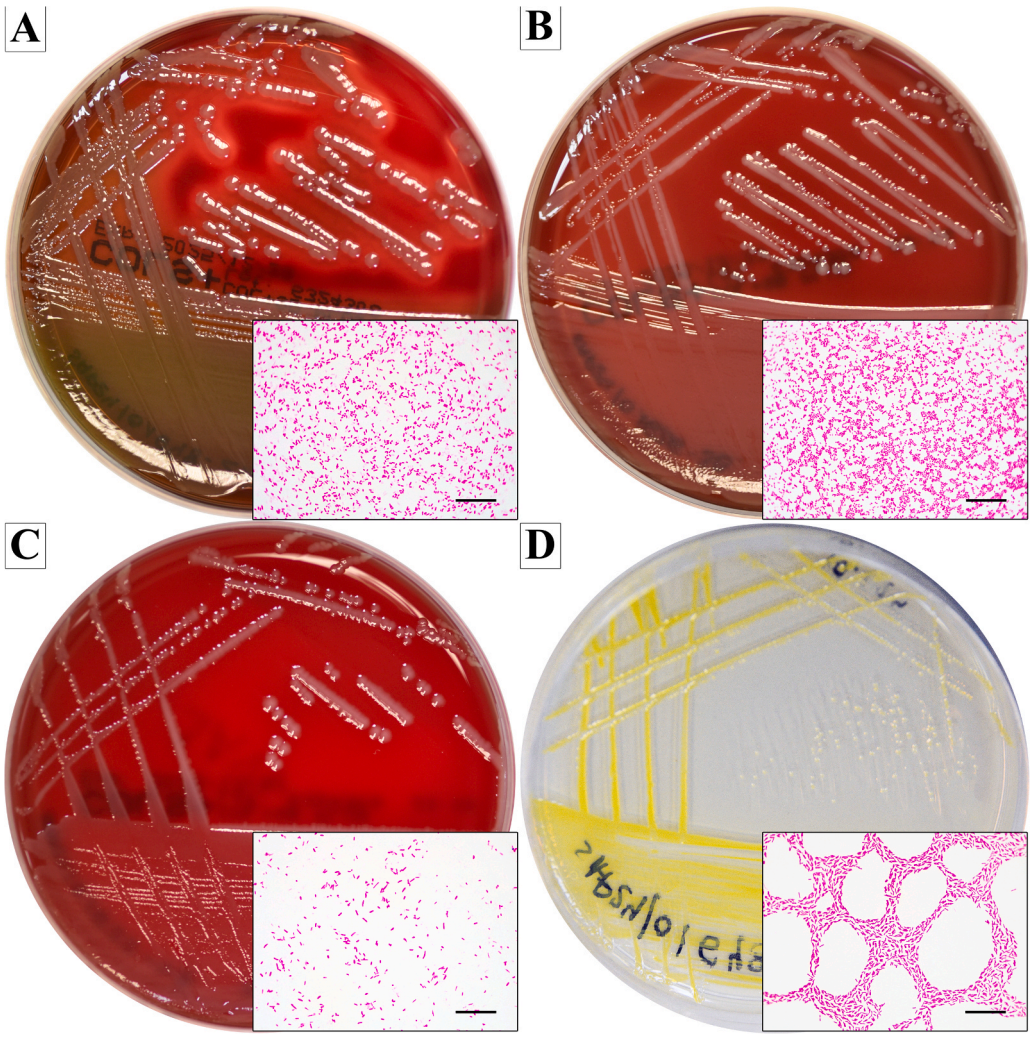

Fig. 3. Bacterial isolates identified in sturgeons. A) Cage 3, sturgeon 4, spleen. Growth of *A. veronii* on blood agar base demonstrates substantial proliferation, with colonies approximately 2 mm in diameter, rounded, shiny, and exhibiting characteristic beta hemolysis. *Inset*: Bacillus, gram-negative, slightly curved, approximately 3 μm in length (Gram 1000x, scale bar: 20 μm). B) Cage 1, sturgeon 2, kidney. Growth of *C. braakii* on blood agar base, presenting small, rounded, grayish colonies measuring 1–2 mm. *Inset*: Straight, gram-negative bacilli approximately 2 μm (Gram 1000x, scale bar: 20 μm). C) Cage 1, sturgeon 1, brain. Growth of *P. shigelloides* on blood agar base, characterized by opaque colonies, 1–2 mm in size, with a shiny gray appearance. *Inset*: Slightly curved bacilli, approximately 3 μm long, gram-negative (Gram 1000x, scale bar: 20 μm). D) Cage 3, sturgeon 4, skin. Growth of *F. succinicans* on Anacker-Ordal media, exhibiting small yellow colonies of 1 mm diameter that tend to merge. *Inset*: Elongated bacilli, slender bacteria about 7 μm long, gram-negative, with a propensity for chain formation (Gram 1000x, scale bar: 20 μm).

Table 3
Bacterial strains identification by 16S rRNA gene sequencing.

Cage	Fish	Organ	Identification
1	2	Spleen	*Citrobacter portucalensis*
	3	Kidney	*Aeromonas sobria*
3	1	Kidney	*Cloacibacterium caeni*
	2	Liver	*Cloacibacterium caeni*
		Spleen	*Aeromonas sobria*
	3	Liver	*Haemophilus piscium*
	4	Spleen	*Aeromonas veronii*
		Spleen	*Citrobacter portucalensis*
		Skin	*Flavobacterium succinicans*

Specifically, *A. veronii* isolates from other fish species exhibited resistance to tetracycline, primarily involving genes such as *tet34* and *tetE*, whereas genes such as *tet57* or *tetD*, although reported, are less common (Agersø et al., 2007; Hayatgheib et al., 2021; Tekedar et al., 2020). Additionally, resistance to florfenicol remains low in *Aeromonas* spp., making detection of the *florR* gene challenging (Tekedar et al., 2020).

Table 4
Susceptibility of bacterial isolates to commonly used antimicrobials in aquaculture.

	Isolate	Florfenicol	Flumequine	Tetracycline
Cage 1	*Aeromonas* spp.	S (40 mm)	S (41 mm)	R (17 mm)
	Pleisomonas shigelloides	S (36 mm)	S (37 mm)	S (30 mm)
	Citrobacter braakii	R (13 mm)	R (24 mm)	R (19 mm)
Cage 2	*Citrobacter gillenii*	R (18 mm)	S (25 mm)	I (23 mm)
	Aeromonas spp.	S (30 mm)	S (40 mm)	R (14 mm)
Cage 3	*Aeromonas* spp.	S (37 mm)	S (41 mm)	S (32 mm)
	Citrobacter braakii	R (12 mm)	S (28 mm)	I (22 mm)
	Haemophilus piscium	25 mm	38 mm	12 mm
	Flavobacterium succinicans	35 mm	45 mm	44 mm

Note: "R" for resistant, "S" for susceptible, and "I" for intermediate.

However, no studies have specifically addressed enrofloxacin resistance in this species. Resistance patterns of *A. sobria* in sturgeons are not yet documented. However, in rainbow trout, resistance to tetracycline and susceptibility to phenicols have been previously reported (Liu et al.,

2025; Kronvall et al., 2019), with associated resistance genes being highly variable, including *tetA*, *tetC*, *tetD*, *tetE*, *tetH*, and *floR* (Duman et al., 2020). In contrast, *A. bestiarum* isolated from sturgeons showed susceptibility to both florfenicol and tetracycline (Utegenova et al., 2024), although tetracycline resistance genes such as tetE have occasionally been detected (Duman et al., 2020). Resistance to enrofloxacin in this species has been documented in rainbow trout, but this has not yet been observed in sturgeons (Naviner et al., 2011).

Citrobacter spp. isolates showed higher resistance to the antibiotics tested in this study, with *C. braakii* displaying the greatest resistance level. Previous studies have identified this strain as highly resistant to tetracycline, flumequine, and florfenicol (Altun et al., 2013; Nawaz et al., 2008), representing a potential challenge for aquaculture. In Türkiye, *C. gillenii* isolated from reared Russian Sturgeon demonstrated resistance to tetracycline, associated with the presence of *tetA* and *tetB*, as well as resistance to florfenicol conferred by *floR* (Türe et al., 2022). In Mediterranean Sea regions, rainbow trout were found to harbor bacteria carrying resistance genes such as *tetA*, *tetB*, and *tetD*, although these bacterial strains did not show resistance to tetracycline in minimum inhibitory concentration (MIC) assays (Duman et al., 2017). Similarly, *florR*, the gene conferring resistance to florfenicol, was not detected in resistant strains through MIC analysis (Duman et al., 2017). Furthermore, in Poland, among *C. freundii* isolates from ornamental fish, 60 % of samples exhibited tetracycline resistance, with *tetA*, *tetB*, and *tetG* found in 40 %, 10 %, and 40 % of *Citrobacter* spp. samples, respectively (Pastuszka et al., 2025). Collectively, these findings suggest that resistance in *Citrobacter* spp. is increasing in European aquaculture.

P. shigelloides showed susceptibility to all three antibiotics tested. While previous studies on sturgeons have reported tetracycline resistance, no studies have assessed *P. shigelloides* susceptibility to flumequine and florfenicol in farmed sturgeons (Deng et al., 2022; Jiang et al., 2021). A study conducted on rainbow trout in Turkey found *P. shigelloides* to be susceptible to tetracycline and flumequine but resistant to florfenicol, which contrasts with our findings (Duman et al., 2023). These discrepancies highlight the impact of environmental and host-specific factors, including management practices and the indiscriminate use of antibiotics, on antimicrobial susceptibility.

Another bacterium isolated without a clear pathological correlation was *H. piscium*, previously described as the etiological agent of ulcer disease in salmonids (Paterson et al., 1980). However, it was later reclassified as an atypical strain of *A. salmonicida* lacking known virulence factors and exotoxins (Gudmundsdóttir et al., 2003). In one sturgeon sample, MALDI-TOF MS initially identified an isolate from the liver as *A. bestiarum* (and *A. salmonicida*), whereas 16S rRNA sequencing subsequently confirmed it as *H. piscium.* This discrepancy is attributable to the close phylogenetic relationship between these species, as both *A. bestiarum* and *A. salmonicida* are highly related to *H. piscium* (Hunter and Kuykendall, 2006)

Finally, it is hypothesized that, under stress-induced conditions, commensal bacteria within the sturgeon microbiota may undergo functional and behavioral modifications that increase their pathogenicity, consistent with the “Rasputin effect” proposed by Hurst (2016). Investigating this hypothesis could help elucidate the mechanisms underlying opportunistic infections in aquaculture environments. Furthermore, implementing comprehensive diagnostic protocols, enhancing husbandry practices, and adopting preventive health strategies are expected to mitigate these microbial alterations, thereby improving sturgeon welfare and reducing the incidence of disease outbreaks. Future research should also explore how stress-related microbial changes influence antimicrobial resistance patterns, highlighting the importance of responsible antimicrobial use in the development of resistance in aquaculture systems.

5. Conclusions

This study constitutes the first comprehensive macroscopic and histological description of an outbreak of hemorrhagic septicemia caused by *Aeromonas spp*., *Citrobacter spp*., and *P. shigelloides* in juvenile Russian sturgeon (*Acipenser gueldenstaedtii*). It provides updated insights into the antibiotic resistance profiles of these bacterial species and underscores the importance of implementing effective management strategies in sturgeon aquaculture. Additionally, this study reports, for the first time, the presence of *F. succinicans* in sturgeons, providing new evidence of a limited or indirect pathogenic role. Lastly, these findings are valuable information for advancing current knowledge on the histopathological features and disease processes in this fish species.

CRediT authorship contribution statement

Antonio J. García-Saorín: Investigation. **Sergio Santos-López:** Investigation. **Antonio Rodríguez-Bertos:** Writing – review & editing, Supervision, Resources, Conceptualization. **Blanca Chinchilla:** Writing – review & editing, Validation, Methodology, Data curation, Conceptualization. **Javier M. De Pablo-Moreno:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nicolás Aradilla:** Writing – review & editing, Investigation. **José A. Blázquez-Rangel:** Investigation.

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Appendix A. Supporting Information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2025.103346.

Data Availability

Data will be made available on request. All data generated or analyzed during this study are included in this published article and its supplementary information files (see Supplementary Table S1).

References

Acharjee, M., Hoque, R., Shreya, S.S., Tabassum, N., Acharjee, M.R., Rezanujjaman, M., Rahman, M., Amin, A., Mahmud, M.R., 2021. Antibiotic susceptibility pattern of fish pathogens: a new approach of emerging the bacterial resistance through biofilm formation in in-vitro condition. Saudi J. Biol. Sci. 28 (12), 6933–6938. https://doi.org/10.1016/j.sjbs.2021.07.063.

Agersø, Y., Bruun, M.S., Dalsgaard, I., Larsen, J.L., 2007. The tetracycline resistance gene tet(E) is frequently occurring and present on large horizontally transferable plasmids in Aeromonas spp. From fish farms. Aquac 266 (1), 47–52. https://doi.org/10.1016/j.aquaculture.2007.01.012.

Altun, S., Duman, M., Buyukekiz, A.G., Ozyigit, M.O., Steinum, S.K., Turgay, E., 2013. Isolation of *Citrobacter braakii* from Rainbow Trout (*Oncorhynchus mykiss*). Isr. J. Aquac. ⟨https://www.researchgate.net/publication/286688151_Isolation_of_Citrobacter_braakii_from_Rainbow_Trout_Oncorhynchus_mykiss⟩.

Anderson, R.L., Ordal, E.J., 1961. *Cytophaga succinicans* sp. n., a facultatively anaerobic, aquatic myxobacterium. J. Bacteriol. 81 (1), 130–138. https://doi.org/10.1128/jb.81.1.130-138.1961.

Axén, C., Vendramin, N., Toffan, A., 2018. Outbreak of mortality associated with Acipenser Iridovirus European (AcIV-E) detection in Siberian sturgeon (*Acipenser baerii*) farmed in Sweden. Fishes 3 (4), 42. https://doi.org/10.3390/fishes3040042.

Bakiyev, S., Smekenov, I., Zharkova, I., Kobegenova, S., Sergaliyev, N., Absatirov, G., Bissenbaev, A., 2022. Isolation, identification, and characterization of pathogenic *Aeromonas hydrophila* from critically endangered *Acipenser baerii*. Aquac. Rep. 26 (101293), 101293. https://doi.org/10.1016/j.aqrep.2022.101293.

Banihashemi, E.S., Khara, H., Pajand, Z., Rahnandeh, M., 2016. Histopathological study of gill, kidney and liver of Persian Sturgeon (*Acipenser persicus* Borodin, 1897) and Stellate (*Acipenser stellatus* Pallas, 1811) exposed to sublethal concentration of un-ionised ammonia UAN. J. Parasit. Dis. Off. Organ Indian Soc. Parasitol. 40 (4), 1443–1450. https://doi.org/10.1007/s12639-015-0710-0.

Beaz-Hidalgo, R., Magi, G.E., Balboa, S., Barja, J.L., Romalde, J.L., 2008. Development of a PCR protocol for the detection of *Aeromonas salmonicida* in fish by amplification of the fstA (ferric siderophore receptor) gene. Vet. Microbiol 128 (3–4), 386–394. https://doi.org/10.1016/j.vetmic.2007.10.004.

Bernadet, J.F., Bowman, J.P., 2006. The Genus Flavobacterium. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E. (Eds.), The Prokaryotes: a handbook on the biology of bacteria, third Edition, 7. Springer, Berlin, pp. 481–531.

Bronzi, P., Rosenthal, H., 2014. Present and future sturgeon and caviar production and marketing: global market overview. J. Appl. Ichthyol. 30 (6), 1536–1546. https://doi.org/10.1111/jai.12628.

Bronzi, Paolo, Chebanov, M., Michaels, J.T., Wei, Q., Rosenthal, H., Gessner, J., 2019. Sturgeon meat and caviar production: global update 2017. J. Appl. Ichthyol. 35 (1), 257–266. https://doi.org/10.1111/jai.13870.

Çağatay, İ.T., 2024. Use of proteomic-based MALDI-TOF mass spectra for identification of bacterial pathogens in aquaculture: a review. Aquac. Int. J. Eur. Aquac. Soc. https://doi.org/10.1007/s10499-024-01544-x.

CASFM, 2018. Recommandations du Comité de l'Antibiogramme de la Société Française de Microbiologie (CASFM): V1.0 Février 2018. Société Française de Microbiologie, Paris, France. ⟨https://www.sfm-microbiologie.org/wp-content/uploads/2019/02/CASFM-V1_0-FEV_2018.pdf.CLSI⟩, 2010. Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Approved Guideline. CLSI document VET03/VET04-S1. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Chinchilla, B., Vázquez-Fernández, E., Rebollada-Merino, A., Pérez-Sancho, M., Domínguez, L., Rodríguez-Bertos, A., 2023. First detection of *Flavobacterium psychrophilum* in juvenile Siberian sturgeons (*Acipenser baerii*) and description of the pathological findings. J. Fish. Dis. 46 (8), 887–894. https://doi.org/10.1111/jfd.13801.

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-cytoplasmatic large DNA virus. Vet. Microbiol 191, 27–34. https://doi.org/10.1016/j.vetmic.2016.05.012.

Ciulli, S., Volpe, E., Sirri, R., Tura, G., Errani, F., Zamperin, G., Toffan, A., Silvi, M., Renzi, A., Abbadi, M., Biasini, L., Pretto, T., Emmanuele, P., Casalini, A., Sarli, G., Serratore, P., Mordenti, O., Mandrioli, L., 2020. Multifactorial causes of chronic mortality in juvenile sturgeon (*Huso huso*). Anim 10 (10), 1866. https://doi.org/10.3390/ani10101866.

CLSI, 2010. Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Approved Guideline. CLSI document VET03/VET04-S1. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Deng, D., Mu, Z., Lv, X., Jiang, X., Zhou, J., Guo, H., Zhang, W., Wang, B., Lu, Y., Wu, J., Du, H., Zhang, S., Xu, Q., Wei, Q., 2022. Pathogenicity of *Plesiomonas shigelloides* and *Citrobacter freundii* isolated from the endangered Chinese sturgeon (*Acipenser sinensis*). Microb. Pathog. 173 (Pt A), 105818. https://doi.org/10.1016/j.micpath.2022.105818.

Duman, M., Saticioglu, I.B., Buyukekiz, A.G., Balta, F., Altun, S., 2017. Molecular characterization and antimicrobial resistance profile of atypical *Citrobacter gillenii* and *Citrobacter* sp. isolated from diseased rainbow trout (*Oncorhynchus mykiss*). J. Glob. Antimicrob. Resist 10, 136–142. https://doi.org/10.1016/j.jgar.2017.05.014.

Duman, M., Saticioglu, I.B., Altun, S., 2020. The determination of antimicrobial susceptibility by MIC and epidemiological cut-off values and the detection of resistance genes in Aeromonas species isolated from cultured fish. Lett. Appl. Microbiol 71 (5), 531–541. https://doi.org/10.1111/lam.13363.

Duman, M., García Valdés, E., Ay, H., Altun, S., Saticioglu, I., 2023. Description of a novel fish pathogen, *Plesiomonas shigelloides* subsp. *oncorhynchi*, isolated from rainbow trout (*Oncorhynchus mykiss*): first genome analysis and comparative genomics. Fishes 8 (4), 179. https://doi.org/10.3390/fishes8040179.

FAO, 2004. Sturgeons (Acipenseriformes). Food and Agriculture Organization of the United Nations. Available at ⟨https://www.fao.org/4/y5261e/y5261e06.htm⟩.

FAO, 2024. The state of world fisheries and aquaculture 2024. Food and Agriculture Organization of the United Nations. Available at ⟨https://www.fao.org/publications/sofia⟩.

Elhetawy, A.I.G., Vasilyeva, L.M., Lotfy, A.M., Emelianova, N.A., Abdel-Rahim, M.M., Helal, A.M., Sudakova, N.V., 2020. Effects of the rearing system of the Russian sturgeon (Acipenser gueldenstaedtii) on growth, maturity, and the quality of produced caviar. Aquac. Aquar. Conserv. Legislat. (AACL Bioflux). 13 (6), 3798–3809. http://www.bioflux.com.ro/aacl.

FAO, 2025. Aquatic species search. Food and Agriculture Organization of the United Nations. Available at ⟨https://www.fao.org/fishery/en/aqspecies/search⟩. Available at ⟨https://www.fao.org/fishery/en/aqspecies/search⟩.

Georgiadis, M.P., Gardner, I.A., Hedrick, R.P., 2001. The role of epidemiology in the prevention, diagnosis, and control of infectious diseases of fish. Prev. Vet. Med. 48 (4), 287–302. https://doi.org/10.1016/s0167-5877(00)00202-6.

Gholamhosseini, A., Taghadosi, V., Shiry, N., Akhlaghi, M., Sharifiyazdi, H., Soltanian, S., Ahmadi, N., 2018. First isolation and identification of *Aeromonas veronii* and *Chryseobacterium joostei* from reared sturgeons in Fars province, Iran. Vet. Res. Forum 9 (2), 113–119. https://doi.org/10.30466/VRF.2018.30826.

Good, C., Davidson, J., Wiens, G.D., Welch, T.J., Summerfelt, S., 2015. *Flavobacterium branchiophilum* and *F. succinicans* associated with bacterial gill disease in rainbow trout *Oncorhynchus mykiss* (Walbaum) in water recirculation aquaculture systems. J. Fish. Dis. 38 (4), 409–413. https://doi.org/10.1111/jfd.12249.

Gudmundsdóttir, B.K., Hvanndal, Í., Björnsdóttir, B., Wagner, U., 2003. Analysis of exotoxins produced by atypical isolates of *Aeromonas salmonicida*, by enzymatic and serological methods. J. Fish. Dis. 26 (1), 15–29. https://doi.org/10.1046/j.1365-2761.2003.00433.x.

Guo, H., Dixon, B., 2021. Understanding acute stress-mediated immunity in teleost fish. Fish. Shellfish Immunol. Rep. 2 (100010), 100010. https://doi.org/10.1016/j.fsirep.2021.100010.

Hayatgheib, N., Calvez, S., Fournel, C., Pineau, L., Pouliquen, H., Moreau, E., 2021. Antimicrobial susceptibility profiles and resistance Genes in Genus Aeromonas spp. Isolated from the environment and rainbow Trout of Two Fish Farms in France. Microorg 9 (6), 1201. https://doi.org/10.3390/microorganisms9061201.

Hu, H., Weng, X., Pang, G., Li, X., Xia, J., Gao, X., He, J., Li, J., Qian, D., 2025. Identification, Pathogenicity, and antimicrobial resistance analysis of bacterial pathogenesis *Aeromonas hydrophila* from hybrid sturgeon (*Huso dauricus* ♀ × *A. schrenckii* ♂) in Zhejiang, China. Microor 13 (2). https://doi.org/10.3390/microorganisms13020278.

Hu Q, Lin Q, Shi C, Fu X, Li N, Liu L, Wu S. [Isolation and identification of a pathogenic Plesiomonas shigelloides from diseased grass carp]. Wei Sheng Wu Xue Bao. 2014 Feb 4;54(2):229-35. Chinese. PMID: 24818472.

Hunter, W.J., Kuykendall, L.D., 2006. Identification and Characterization of an Aeromonas salmonicida (syn Haemophilus piscium) strain that reduces selenite to elemental red selenium. Curr. Microbiol 52 (4), 305–309. https://doi.org/10.1007/s00284-005-0303-8.

Hurst, C.J., 2016. The Rasputin effect: When commensals and symbionts become parasitic, First ed. Springer International Publishing, Cham, Suiza (Ed.).

IUCN., SSG, 2022. The new assessment of the sturgeons and paddlefishes of the IUCN-SSG. International Union for the Conservation of Nature. Sturgeon Specialist Group. Available at ⟨https://nasps-sturgeon.org/iucn-assessment-2022/⟩.

Jiang, J.Z., Liu, Y., Yan, L.H., Yan, Q.G., Wen, X.T., Cao, S.J., Huang, Y., Huang, X.B., Ma, X.P., Han, X.F., Zhao, Q., Wen, Y.P., Wu, R., Lin, J.C., Bai, Y., 2021. Identification and pathogenicity of *Plesiomonas shigelloides* from *Acipenser dabryanus* in China. Aquac. Res. 52 (5), 2286–2293. https://doi.org/10.1111/are.15080.

Karatas, S., Ercan, D., Steinum, T.M., Turgay, E., Memis, D., Candan, A., 2010. First isolation of a *Flavobacterium johnsoniae* like bacteria from cultured Russian sturgeon in turkey. J. Anim. Vet. Adv. JAVA 9 (14), 1943–1946. https://doi.org/10.3923/javaa.2010.1943.1946.

Kasprzak, R., Zakęś, Z., Kamaszewski, M., Szudrowicz, H., Wiechetek, W., Janusz, J.R., Ostaszewska, T., Korzelecka-Orkisz, A., Formicki, K., 2023. Histomorphometric evaluation of melanomacrophage centers (MMCs) and CD3+ T cells of two morphs of brown trout (*Salmo trutta*) fed diets with immunostimulants. Fish. Shellfish Immunol. 141 (109020), 109020. https://doi.org/10.1016/j.fsi.2023.109020.

Kayiş, Ş., Er, A., Kangel, P., Kurtoğlu, I.Z., 2017. Bacterial pathogens and health problems of *Acipenser gueldenstaedtii* and *Acipenser baerii* sturgeons reared in the eastern Black Sea region of Turkey. Iran. J. Vet. Res. 18 (1), 18–24.

Kronvall, G., Baron, S., Larvor, E., Rudas-Villarreal, C., Hobson, J., Smith, P., 2019. Single-strain regression analysis evaluating disc potencies of flumequine and enrofloxacin for testing *Aeromonas sobria* and *Vibrio anguillarum*: disc content evaluated using SRA. APMIS 127 (8), 570–576. https://doi.org/10.1111/apm.12972.

Liu, J., Ji, K., Pang, X., Jin, S., Zheng, Y., Xu, J., Houyong, F., Wang, Y., Hu, M., 2024. Effects of *Citrobacter freundii* on sturgeon: Insights from haematological and intestinal-liver immunity. Aquac 586 (740811), 740811. https://doi.org/10.1016/j.aquaculture.2024.740811.

Liu, L.P., Fang, Y.D., Kang, P.T., Gao, X.Y., Zhang, G.W., Pan, J., Lu, J., Liu, J.X., Zhang, W.D., 2025. Isolation, identification and characteristics of *Aeromonas sobria* from diseased rainbow trout (*Oncorhynchus mykiss*). Front. Microbiol 15, 1499126. https://doi.org/10.3389/fmicb.2024.1499126.

Loch, T.P., Faisal, M., 2015. Emerging flavobacterial infections in fish: a review. J. Adv. Res. 6 (3), 283–300. https://doi.org/10.1016/j.jare.2014.10.009.

Ma Z, Yang H, Li T, Luo L, Gao J. [Isolation and identification of pathogenic Aeromonas veronii isolated from infected Siberian sturgeon (Acipenser baerii)]. Wei Sheng Wu Xue Bao. 2009 Oct;49(10):1289-94. Chinese. PMID: 20069873.

Naviner, M., Gordon, L., Giraud, E., Denis, M., Mangion, C., Le Bris, H., Ganière, J.P., 2011. Antimicrobial resistance of *Aeromonas* spp. isolated from the growth pond to the commercial product in a rainbow trout farm following a flumequine treatment. Aquac 315 (3–4), 236–241. https://doi.org/10.1016/j.aquaculture.2011.03.006.

Nawaz, M., Khan, A.A., Khan, S., Sung, K., Steele, R., 2008. Isolation and characterization of tetracycline-resistant *Citrobacter* spp. from catfish. Food Microbiol 25 (1), 85–91. https://doi.org/10.1016/j.fm.2007.07.008.

Noga, E.J., 2010. Fish Disease: Diagnosis and Treatment. Wiley-Blackwell, New Jersey, United States of America.

Pastuszka, A., Guz, L., Puk, K., Pietras-Ożga, D., 2025. Occurrence of virulence factors and antimicrobial susceptibility of *Citrobacter freundii* isolated from diseased ornamental fish in Poland. J. Vet. Res. 69 (1), 17–26. https://doi.org/10.2478/jvetres-2025-0017.

Paterson, W.D., Douey, D., Desautels, D., 1980. Relationships between selected strains of typical and atypical *Aeromonas salmonicida*, *Aeromonas hydrophila*, and *Haemophilus piscium*. Can. J. Microbiol 26 (5), 588–598. https://doi.org/10.1139/m80-104.

Pepi, M., Focardi, S., 2021. Antibiotic-resistant bacteria in aquaculture and climate change: a challenge for health in the Mediterranean area. Int. J. Environ. Res. Public Health 18 (11), 5723. https://doi.org/10.3390/ijerph18115723.

Qiwei, W., 2022. Acipenser sinensis (amended version of 2022 assessment). The IUCN Red List of Threatened Species 2022: e.T236A219152605. Available at https://dx.doi.org/10.2305/IUCN.UK.2022- 2.RLTS.T236A219152605.en.

Radosavljević, V., Milićević, V., Maksimović-Zorić, J., Veljović, L., Nešić, K., Pavlović, M., Pelić, D.L., Marković, Z., 2019. Sturgeon diseases in aquaculture. Arch. Vet. Med. 12 (1), 5–20. https://doi.org/10.46784/e-avm.v12i1.34.

Ribeiro, T.G., Gonçalves, B.R., da Silva, M.S., Novais, Â., Machado, E., Carriço, J.A., Peixe, L., 2017. *Citrobacter portucalensis* sp. nov., isolated from an aquatic sample. Int. J. Syst. Evol. Microbiol 67 (9), 3513–3517. https://doi.org/10.1099/ijsem.0.002154.

Rigos, G., Troisi, G.M., 2005. Antibacterial agents in Mediterranean finfish farming: a synopsis of drug pharmacokinetics in important euryhaline fish species and possible environmental implications. Rev. Fish. Biol. Fish. 15 (1–2), 53–73. https://doi.org/10.1007/s11160-005-7850-8.

Salgado-Miranda, C., Palomares, E., Jurado, M., Marín, A., Vega, F., Soriano-Vargas, E., 2010. Isolation and distribution of bacterial flora in farmed rainbow trout from Mexico. J. Aquat. Anim. Health 22 (4), 244–247. https://doi.org/10.1577/H09-004.1.

Santi, M., Pastorino, P., Foglini, C., Righetti, M., Pedron, C., Prearo, M., 2019. A survey of bacterial infections in sturgeon farming in Italy. J. Appl. Ichthyol. 35 (1), 275–282. https://doi.org/10.1111/jai.13802.

Sneddon, L.U., Wolfenden, D.C.C., Thomson, J.S., 2016. Stress Management and Welfare. In: Carl, B.S., Tort, L., Farrel, A.P., Brauner, C.J. (Eds.), Fish Physiology. Academic Press, San Diego, United States of America, pp. 463–539.

Soto, E., Richey, C., Stevens, B., Yun, S., Kenelty, K., Reichley, S., Griffin, M., Kurobe, T., Camus, A., 2017. Co-infection of Acipenserid herpesvirus 2 (AciHV-2) and *Streptococcus iniae* in cultured white sturgeon *Acipenser transmontanus*. Dis. Aquat. Org. 124 (1), 11–20. https://doi.org/10.3354/dao03108.

Stosik, M.P., Tokarz-Deptuła, B., Deptuła, W., 2019. Melanomacrophages and melanomacrophage centres in Osteichthyes. Cent. Eur. J. Immunol. 44 (2), 201–205. https://doi.org/10.5114/ceji.2019.87072.

Tan, C., Hu, J., Gao, B., Zhang, B., Li, P., Shang, N., 2023. Effects of the interaction between *Aeromonas sobria* and *Macrococcus caseolyticus* on protein degradation of refrigerated sturgeon fillets: novel perspective on fish spoilage. Lebensm. Wiss. Technol. 183 (114908), 114908. https://doi.org/10.1016/j.lwt.2023.114908.

Tekedar, H.C., Arick, M.A., Hsu, C.Y., Thrash, A., Blom, J., Lawrence, M.L., Abdelhamed, H., 2020. Identification of antimicrobial resistance determinants in *Aeromonas veronii* strain MS-17-88 recovered from channel catfish (*Ictalurus punctatus*). Front. Cell Infect. Microbiol 10, 348. https://doi.org/10.3389/fcimb.2020.00348.

Türe, M., Cebeci, A., Özcelep, T., 2022. The first outbreak of citrobacteriosis caused by *Citrobacter gillenii* in reared Russian sturgeon (*Acipenser gueldenstaedtii*) in Turkiye. Vet. Res. Forum 13 (3), 323–329. https://doi.org/10.30466/vrf.2021.137808.3076.

Ture, M., Ozcelep, T., Akbulut, B., Kutlu, I., 2018. Disease of Russian sturgeon (Acipenser gueldenstaedtii) caused by Aeromonas sp. Genet. of Aquac. Organ. 2 (2), 43–47. https://doi.org/10.4194/2459-1831-v2_2_03.

Utegenova, K.S., Bakiyev, S.S., Mambetova, D.S., Kauysbekov, A.Z., Bissenbaev, A.K., 2024. Biochemical and molecular genetic identification of the bacterial pathogen - *Aeromonas bestiarum* from a diseased Siberian sturgeon (*Acipenser baerii*). Int. J. Biol. Chem. 17 (2), 37–44. https://doi.org/10.26577/ijbch2024v17.i2.4.

Vázquez-Fernández, E., Chinchilla, B., Rebollada-Merino, A., Domínguez, L., Rodríguez-Bertos, A., 2023. An Outbreak of *Aeromonas salmonicida* in Juvenile Siberian Sturgeons (*Acipenser baerii*). Animals 13, 2697. https://doi.org/10.3390/ani13172697.

Vilkova, D., Chèné, C., Kondratenko, E., Karoui, R., 2022. A comprehensive review on the assessment of the quality and authenticity of the sturgeon species by different analytical techniques. Food Control 133 (108648), 108648. https://doi.org/10.1016/j.foodcont.2021.108648.

World Organisation for Animal Health (WOAH), 2024. Manual of diagnostic tests for aquatic animals (11th ed.). ⟨https://www.oie.int/en/standard-setting/aquatic-manual/⟩.

Zhang, K., Ye, Z., Qi, M., Cai, W., Saraiva, J.L., Wen, Y., Liu, G., Zhu, Z., Zhu, S., Zhao, J., 2025. Water quality impact on fish behavior: A review from an aquaculture perspective. Rev. Aquac. 17 (1). https://doi.org/10.1111/raq.12985.

Zhu, C., Yang, C., Li, F., Lv, G., Ren, C., Chu, P., Zhu, L., 2023. Isolation, identification of an *Aeromonas veronii* strain, and the immune response of hybrid sturgeon (*Acipenser baerii* ♀ × *Acipenser schrenckii* ♂) to the bacteria. Aquac 577 (739966), 739966. https://doi.org/10.1016/j.aquaculture.2023.739966.