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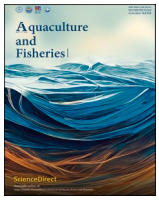

Original research article

Changes in *Acipenser ruthenus* liver structure during domestication: Preliminary data

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ABSTRACT

Wild sturgeon populations have declined dramatically worldwide due to habitat degradation, such as river fragmentation, caused by anthropogenic pressures including legal and illegal overfishing and pollution. Rearing and releasing juvenile fish into natural water bodies is one method to restore biological resources. This study aimed to: (1) examine the spectrum of conditionally pathogenic bacteria causing pathological processes in domesticated sterlet (*Acipenser ruthenus* Linnaeus, 1758); (2) investigate the effects of pathogenic bacteria on fish internal organs using combined histological techniques for tissue treatment; (3) identify tissues as biomarkers for assessing the impact of bacterial co-infection in sturgeon; and (4)evaluate the role of these infections in fish domestication. Liver damage was the primary clinical manifestation during co-bacterial infection in all tested fish. Additionally, shifts in liver cell functions and cytological characteristics were observed.

1. Introduction

The order *Acipenseriformes* includes 27 species naturally distributed in Eurasia and North America (Bronzi et al., 2019; Litvak, 2010). Wild sturgeon populations have declined dramatically due to legal and illegal overfishing, habitat degradation, river fragmentation (including damming) and anthropogenic pollution (Raymakers, 2002; Foster et al., 2016; Jarić et al., 2011; Poleksic et al., 2010). All sturgeon species have been listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora since 1998 (Raymakers, 2002; CITES-Convention, 1983; Foster et al., 2016). Since the late 20th century, aquaculture has partially replaced wild fish meat and caviar. If only one million tons of fish were farmed in the world sixty years ago, today this figure exceeds 82 million tons. In the last 25 years, aquaculture has grown by 67 million tons or 450 %, while fisheries have remained at a stable level. According to the Food and Agriculture Organization of the United Nations (FAO), aquaculture is the world's fastest-growing area of animal protein production (FAO, 2020a; 2020b, 2022).

Currently, fish from the family *Acipenseridae* are grown in aquaculture for various purposes, depending on the needs of the market (Saraiva et al., 2018; Wei et al., 2011). *A. ruthenus* is the most cultivated species among sturgeons grown under Recirculating Aquaculture System (RAS) conditions, because this species is characterized by the earliest period of sexual maturity compared to other sturgeons. However, there is a lack of both healthy and sexually mature individuals of sterlet from natural water bodies (Williot et al., 2005, 2018) and an adequate assessment of the functional status of this fish adapted to RAS conditions (Teletchea & Fontaine, 2012). According to the official report of the State Agency of Land Reclamation and Fisheries of Ukraine for 2021 (State Agency of Ukraine for Development of Land Reclamation, Fisheries and Food Programs, 2021), the reproduction of aquatic bioresources is assigned priority in Ukraine. The release of juveniles of valuable fish species into water bodies is aimed both at supporting aquatic ecosystems and formation of wild fish stocks.

For breeding and rearing in RAS, sturgeon fish are taken from natural water bodies, but the long-term intensive pollution of Ukrainian water bodies with various wastewater seasonally promotes the occurrence of

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infection foci (Koziy, 2011). All this is clearly reflected in the viability of individuals, the weakening of their reproductive functions and, consequently, their population size decline. There are many physiological and biochemical methods successfully used to assess the health status of fish and population dynamics (Li et al., 2010; Lorenzen et al., 2012; Koziy, 2014; Kozij & Matvienko, 2017; Frishtak et al., 2022; Matvienko et al., 2022). However, the use of traditional methods in ichthyological studies of sturgeon species can cause certain difficulties. On the one hand, there is a lack of reproduction in natural water bodies and the number of sexually mature individuals (as a potential object of domestication) is limited. On the other hand, the information available in literature on sturgeon domestication is based on limited, rather scattered data and is of theoretical character.

To address these challenges, several sub-tasks have been proposed, the adaptation of sterlets to RAS conditions being distinguished as the most important one. The difficulties inherent in the natural reproduction of sterlet, coupled with the necessity to restore rare fish stocks, have led to the emergence of fish farms or RASs as breeding and rearing sites for these fish, with the objective of further restoring wild sturgeon populations (Saraiva et al., 2018; Matviienko et al., 2021). However, during the adaptation of sturgeons to RAS, there can occur problems related to the emergence of bacterial infections (Price, 2002; Stachnik et al., 2021). These infections, mostly caused by bacteria of the genus *Aeromonas* and *Pseudomonas*, can lead to the disruption of internal organs' structure and/or function and even to the death of fish (Matvienko et al., 2015; Kozinska & Pezkala, 2004; Pękala et al., 2015; Gholamhosseini et al., 2018; Sebastião et al., 2019; Paździor et al., 2019; Stachnik et al., 2021). Another significant challenge arising from sterlet adaptation to RAS is the possibility of pathological changes in their organs, which are often observed in fish liver (Handy et al., 2002; Huang et al., 2022). However, no studies have yet confirmed the presence of structural changes in sturgeon organs during their adaptation to RAS conditions under the influence of bacterial co-infection. Consequently, it is of vital importance to investigate specific alterations in the cellular structure and function of organs occurring in response to bacterial co-infection. Furthermore, it is of vital importance to identify the tissue-based biomarkers that can be employed to detect and monitor the presence of bacterial agents. Despite the successful utilization of a multitude of monitoring methods, there is a scarcity of scientific research focused on this topic. It has been proved that the pathological process in fish primarily begins with the transformation of cellular structures, which in turn provokes certain morphological changes at the tissue level (Endmund, 1988; Matviishyn et al., 2014).

Therefore, the objective of this study was to investigate the adaptation process of the sterlet (*A. ruthenus*) collected from the lower reaches of the Dnieper River to the RAS conditions with special emphasis on microbiological and histological methods. This approach was employed to gain a deeper understanding of cellular changes in fish organs in order to prevent the onset of disease.

2. Methods

2.1. Study area, fish sampling and ichthyological analysis

Eight specimens of two-year-old sterlet (2+) individuals (*A. ruthenus* Linnaeus, 1758) (length 28 ± 3 cm, weight 260 ± 56 g) were collected using fixed nets, small fyke nets and seine nets from the Kakhovka Reservoir along the migration route of this species in the Dnieper estuary system (for about 120 km) in the summers of 2019–2021 at water temperatures of 20–24 °C (Fig. 1). All fishing operations were carried

Fig. 1. Sterlet (*A. ruthenus*) fishing sites on the Dnieper River in the Kherson region in summers 2019–2021.

out in accordance with the Rules of Fishing in the Black Sea Basin and the Annual Regime of Fishing (State Agency of Ukraine for Development of Land Reclamation, Fisheries and Food Programs, 2020; State Agency of Ukraine for Development of Land Reclamation, Fisheries and Food Programs, 2021) and Procedure for Special Use of Water Bioresources in Inland Fishery Water Bodies (their parts), Internal Sea Waters, Territorial Sea, Exclusive (Maritime) Economic Zone and in the Continental Shelf of Ukraine (Cabinet of Ministers of Ukraine, 2021:Rules, 2003). Fish experiment procedures were carried out in accordance with the requirements of the Directive 2010/63/EU on the protection of animals used for scientific purposes (European Commission, 2010). The experiment protocols used in this study were approved by the Laboratory Animal Ethics Commission of the Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine according to the protocol No. 6 (2019-03-03), Permit No. 17-15 of the Kyiv Regime Commission dated May 21, 2014, to conduct parasitological, bacterial and virological research on fish diseases. Euthanasia of all the tested fish was carried out according to the Council Regulation (EC) No 1099 (Council Regulation No. 1099/2009, 2009). After euthanasia of the fish using clove oil (450 mg/L), the chord of the fish was cut just behind the head, and the abdomen was dissected to collect organ samples for microbiological and histological studies.

Pectoral fins were collected to determine the age of the fish. Species and age identification of the studied specimens was made using modern taxonomy keys (Movchan, 2011). The collected fish were placed in a 20 m^3 RAS tank. Water temperature was maintained at 19 ± 1 °C, pH was 7.4, concentrations of dissolved oxygen were 8.0 ± 0.2 mg/L, NO_2, NO_3 and NH_4 were 0.01, 0.66 and < 0.001 mg/L, respectively. After fish acclimation, the temperature of water in the tanks was 18–20 °C.

The study assessed the physiological state of the sterlets (8 individuals) by examining their clinical signs immediately after transportation to RAS and after 14 days of acclimation. Fish were fed with commercial feed (Aller Metabolica, Poland) four times a day, at equal intervals, following a stepwise regime according to the schedule and norms specified by the feed manufacturer. The individuals with clearly visible clinical signs were dissected after 14 days. Clinical signs of bacterial infection included localized inflammation foci on the fish body surface, scutes and anus. A total of 8 individuals were dissected: 4 individuals with clinical signs and 4 individuals from the control group without clinical signs.

2.2. Hematological analysis

Hematological analysis was performed following a standardized procedure to ensure the consistency and reliability of results. After fish had been anesthetized, blood was collected from the caudal vein with a sterile syringe, transferred to a glass tube containing heparin, gently mixed to prevent clotting, and stored in a refrigerator. Hematocrit (Hct) was measured applying the standard microhematocrit method and expressed as a percentage according to the protocol of Sniezko (1960).

Hemoglobin (Hb) concentration was determined using the cyanomethemoglobin method. Uncoagulated blood (20 μL) was diluted with 5 ml of Drabkin's solution and allowed to react for 5 min under standard conditions (Blaxhall & Daisley, 1973). The absorbance of the resulting solution was measured at 540 nm.

The total erythrocyte count (RBC) was done using the Goryaev chamber. RBC were counted in five selected large squares: four at the corners and one in the center of the chamber grid, corresponding to 80 small squares (Ivanova, 1983). This approach provided a statistically significant and accurate count of erythrocyte concentration in blood samples.

2.3. Microbiological analysis

Samples for research were taken from the fish with clear signs of disease. Sampling for microbiological analysis was done using sterile methods in order to avoid contamination. Samples were collected using a sterile incubation loop, scalpel, forceps and scissors.

All specimens were aseptically dissected for liver and kidney sample collection according to the fish necropsy protocol (Yanong, 2003) with some modifications. Before dissecting, the skin area of each fish, where the incision was to be made, was cleaned with ethanol (70 % solution). The liver was sampled as soon as it was exposed, and other internal organs were gently removed before kidney sampling. Before being used for making incisions in different tissues and different fish, necropsy tools were cleaned with distilled water and then dip-washed in sodium hypochlorite 6 %. Aseptic sample collection and avoidance of cross-contamination were the critical points in this study. Samples from external (gills, skin, mouth, and scutes and internal organs (liver, kidney, blood) of the fish were taken and immediately transferred to tryptone-soy agar (TSA; Oxoid, UK). Petri dishes were incubated at 20 °C for 24 h. Bacterial morphology was studied by Gram staining. Bacterial isolates were characterized morphologically, physiologically and biochemically using API 20 tests (BioMérieux, Inc., France) designed to identify Gram-negative bacteria based on 21 biochemical reactions mainly following the scheme proposed by Austin and Austin (2016). The biochemical characteristics of bacteria were studied applying the approach proposed by Kozinska and Pezkala (2004) and included the following biochemical tests: Catalase reaction (3 % H_2O_2), oxidase, indole, phosphatase, H_2S, lysine decarboxylase, ornithine decarboxylase, nitrate, sodium citrate; degradation of esculin, blood (hemolysis), gelatin, Tween 80, acid production from some sugars such as cellobiose, fructose, xylose, melecitose, lactose, arabinose, sucrose, galactose, raffinose, glucose, mannitol, sorbitol, mannitol, and sorbitol. Bergey's Manual of Systematic Bacteriology (Bergey, 2011) was used to identify bacteria and (LPSN, 2019).

2.4. Histological analysis

The liver tissue was stored in 10 % phosphate-buffered formalin, embedded in paraffin, sectioned (7 μm thick), which is necessary for obtaining a complete image of the nucleus, and stained with hematoxylin and eosin following the method suggested by Luna (1968). The microanatomy of the liver parenchyma was examined using magnifying endoscopy (Motic Instruments Inc., Canada) with magnification ranging from 10x to 100x. The analysis was performed using exclusive equipment and techniques improved by Koziy (2011) for the histological diagnosis of aquatic animals' tissue, using the optical equipment Leitz *Wetzlar* Microscope *Diaplan* (Germany) and the halogen illuminator Linvatec-2 (USA) with the nominal power 10–240 W. The best result was achieved using the original technique of combined tissue treatments. The proposed improvement makes it possible to reduce the time of technological tissue treatment 10 -fold, to reduce the total consumption of reagents 5-fold and to reduce the block compression factor by up to 20 %. Böhmer's hematoxylin, Hart's fuchselin (modified) (Bh,Hf) and Ehrlich's hematoxylin, Hart's fuchselin (modified) (Eh,Hf) were used to stain histologic preparations. Samples were contrasted using a MONOCHROM 2.5X correction filter (Russia). Microphotography of the tissue was performed using a Nikon D-60 digital camera (Japan) with a 1.6x trinocular lens (Russia) and a Minolta-EK computerized exposure detector (Japan). Morphometric studies of tissue structures were performed using a built-in ocular micrometer. To obtain reliable biometric data, 150–200 cells were analyzed, and the entire cell population in the field of view of the optical instruments was analyzed. The direction and specificity of parenchymal tissue rearrangements were determined using the method of plastic reconstruction, which involves creating spatially oriented histological 3 μm-thick sections with subsequent synthesis of volumetric images (*Micam* Ultima L type CMOS camera (Belgium)).

2.5. Statistical analysis

The data were first tested for normal distribution by performing the

Kolmogorov–Smirnov and Shapiro–Wilk tests, and for homogeneity of variances by performing the Levene test. The ANOVA test (F-statistics, one-way) followed by the Post-hoc Tukey's HSD test was used for normally distributed data and the Kruskal-Wallis test for non-parametric data (H-statistics). All analyses were performed using STATISTICA software (10.0 Software, Inc. PA, USA). For the comprehensive understanding of relationships among the tested variables, Principal Component Analysis (PCA) was employed. The data were normalized by sum and were log transformed. To ascertain the significance of differences in the parameters tested, we conducted a one-way Permutational Multivariate Analysis of Variance (PERMANOVA, 999 permutations).

3. Results

3.1. Clinical signs in sterlet under bacterial infections

On the first day after transportation, the fish began to adapt to new conditions: they huddled in the corners of the tank and did not move intensively. Almost all individuals had light-colored skin on the lateral and dorsal parts of the body, which is not characteristic of healthy fish. According to our observations, it was only on the third day that the sterlets began to show a pronounced morphological adaptation: their skin began to regain the color characteristic of this species, which indicated the normalization of the neurohumoral processes regulating the saturation of skin melanophorocytes with pigment. Also, the fish began to move around the RAS tank and to respond appropriately to feed.

Upon transportation to RAS, the sterlets did not exhibit any clinical signs. However, after 14 days, clinical signs appeared in external and internal organs of four individuals. The most abundant bacterial strains isolated from different organs of sterlets with visible clinical manifestations of infection, as well as from asymptomatic hosts, were determined. Clinical signs of bacterial infection included the appearance of inflammation foci on the surface of the fish body, on scutes and anus (Fig. 2A and B). There was a small amount of ascitic fluid found in the abdominal cavity of the sterlet (Fig. 2C and D), the structure of kidneys was loose (Fig. 2D), although flabby, the liver retained its shape (Fig. 2C). Changes in the microstructure of the liver were also observed. It should be noted that liver lesions were recorded in 90 % of the fish examined, and kidney lesions and superficial lesions were found in 35 % and 10 % of the fish respectively.

The internal organs of the studied sterlets were frequently found to have lesions caused by bacterial pathogens such as *Aeromonas* sp., *Pseudomonas* sp., and *Flavobacterium* sp. These lesions included ascites in the abdominal cavity and kidneys, concentration of bloody exudate in the liver and kidneys, reddening of the body surface in the area of scutes, changes in the structure of the liver (congestive necrosis), etc. (Fig. 2).

3.2. Identification of bacteria isolated from the internal organs of the sterlet

Bacterial isolates were obtained from the internal organs of the studied sterlets (Fig. 3, Table 1).

The identification of isolates showed that the pathogenic microflora of these fish was represented mainly by psychrophilic Gram-negative and oxidase-positive bacilli belonging to families Aeromonaceae, Pseudomonaceae and Flavobacteriacea

The first isolate was identified as Gram-negative with the

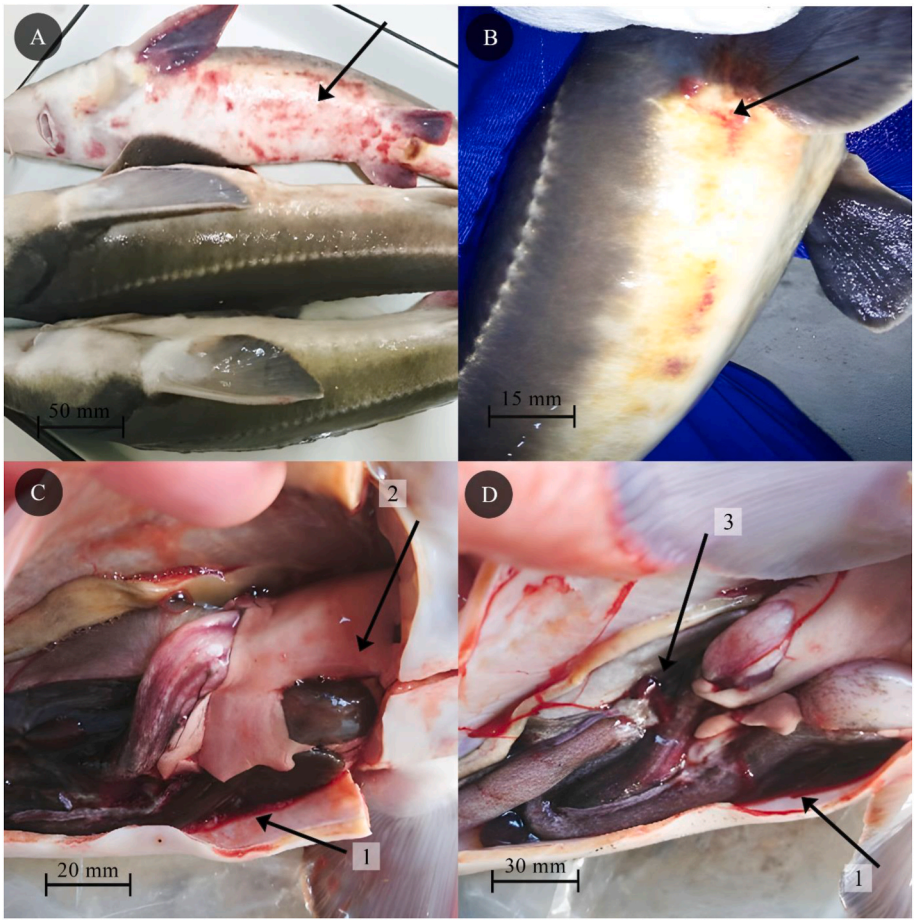

Fig. 2. Organs and tissues of sterlet (*A. ruthenus*) individuals under bacterial infection. A, B - external clinical signs, local hemorrhages on the body skin surface; C, D, - internal organs (1- bloody exudate, 2- liver, 3- kidneys).

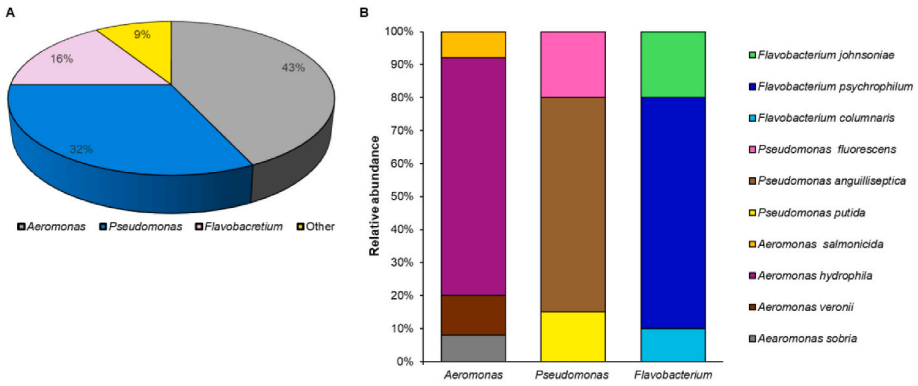

Fig. 3. Identification of the genus (A) and distribution of bacteria isolated from the internal organs of the *A. ruthenus* by species (B) after 14 days of acclimation.

Table 1
The most important phenotypic and biochemical properties of bacterial isolates from the internal organs of the studied sterlet (*A. ruthenus*) individuals.

Characteristics	*Isolate-1*	*Isolate-2*	*Isolate-3*
Gram type (±)	–	–	–
Morphology	Bacilli short t	Bacilli long	Long rods
Oxidase	+	+	–
Motility	gliding	gliding	gliding
Catalase	+	+	W
O/F test		–	–
b-galactosidase	+	–	–
Arginine dihydrolase	+	–	–
Ornithine decarboxylase	–	–	–
Tryoptophan deaminase	–	–	–
Gelatin hydrolysis	+	–	+
Production of indole	+	–	–
Production of urease	–	–	–
Reduction of nitrates	+	+	–
Production of acid from:			
adonitol	–	–	–
arabinose	+	–	–
fructose	+	+	+
glucose	+	–	–
inositol	–	–	–
xyloses	–	+	–
lactose	–	–	–
maltose	+	–	+
trehalose	+	–	+
Belonging to the species	*Aeromonas hydrophila*	*Pseudomonas anguilliseptica*	*Flavobacterium psychrophilum*

Positive (+); Negative (−), and W (weakly positive).

morphology of short bacilli. It tested positive for oxidase and catalase and exhibited gliding motility. Significant biochemical characteristics included positive reactions for β-galactosidase, arginine dihydrolase, and gelatin hydrolysis. Additionally, this isolate produced indole, reduced nitrates, and produced acid from arabinose, fructose, glucose, maltose, and trehalose. These properties allowed this isolate to be identified as *Aeromonas hydrophila.*

The second isolate, also Gram-negative, had long bacilli morphology and was oxidase-positive and catalase-positive, demonstrating gliding motility. It tested negative for β-galactosidase, arginine dihydrolase, indole production, and gelatin hydrolysis. This isolate did not produce acid from glucose or trehalose but did produce acid from fructose and xylose. Based on these phenotypic and biochemical properties, the second isolate was identified as *Pseudomonas anguilliseptica.*

The third isolate, characterized by long-rod morphology, was Gram-negative and oxidase-negative, with weak positive catalase activity and gliding motility. It tested negative for β-galactosidase, arginine dihydrolase, indole production, and gelatin hydrolysis. However, it produces acid from fructose, maltose, and trehalose. Based on these characteristics, the third isolate was identified as *Flavobacterium psychrophilum.*

In summary, the results of this study indicate that the conditionally pathogenic and pathogenic microflora of the fish is primarily composed *of Aeromonas hydrophila, Pseudomonas anguilliseptica, and Flavobacterium psychrophilum*. These species were identified through the comprehensive analysis of the phenotypic and biochemical properties of each isolate.

3.3. Hematological parameters of sterlet during acclimation

Monitoring of hematological parameters during broodstock development is a mandatory procedure in sturgeon farming. A comprehensive blood test allows drawing conclusions about the adequacy of feeding and housing conditions.

After the acclimation, the hematological parameters of *A. ruthenus* (Fig. 4) were found to have significantly changed. The 14-day acclimation caused an increase in hematocrit ($F_{2.13} = 11.76$, $P = 0.001$) and hemoglobin levels ($F_{2.13} = 3.92$, $P = 0.047$), erythrocyte count ($F_{2.13} = 30.29$, $P < 0.001$) and erythrocyte sedimentation rate ($F_{2.13} = 20.24$, $P < 0.001$) in *A. ruthenus* individuals with clinical signs compared to these parameters of the pre-acclimation period ($P < 0.05$) and to those in the fish exhibiting no clinical signs ($P < 0.05$). After the 14-day acclimation period, fish without clinical signs did not show statistically significant differences ($P > 0.05$) in these parameters, except for the erythrocyte count ($P < 0.05$) and erythrocyte sedimentation rate ($P = 0.02$), when compared to their pre-acclimation values (Fig. 4). Principal Component Analysis (PCA) was performed to provide an overview of data clustering. The PCA-based analysis of potential relationships between different hematological parameters of *A. ruthenus* with and without clinical signs before and after the 14-day acclimation yielded two principal components (PCs), which explained more than 90 % of the total variance. The Principal Component Analysis showed statistically significant differences (PERMANOVA, $F = 70.73$, $P < 0.001$) in the tested parameters of *A. ruthenus* during the acclimation (Fig. 4F). The PCA score and 3 D plots demonstrated that the pre-acclimation fish was clearly separated from the post-acclimation fish with and without clinical sign in terms of their hematological parameters (in all cases $P = 0.002$) (Fig. 4F). Furthermore, our results showed a significant difference between the responses elicited by the fish with and without clinical signs ($P = 0.027$) (Fig. 4F).

3.4. Histological examination of sterlet liver

Structure of the studied sterlets' liver was found to be identical to that of the representatives of the Cartilaginous Ganoids Subclass and quite complex (Fig. 5 A). The liver tissues presented in Fig. 6 were

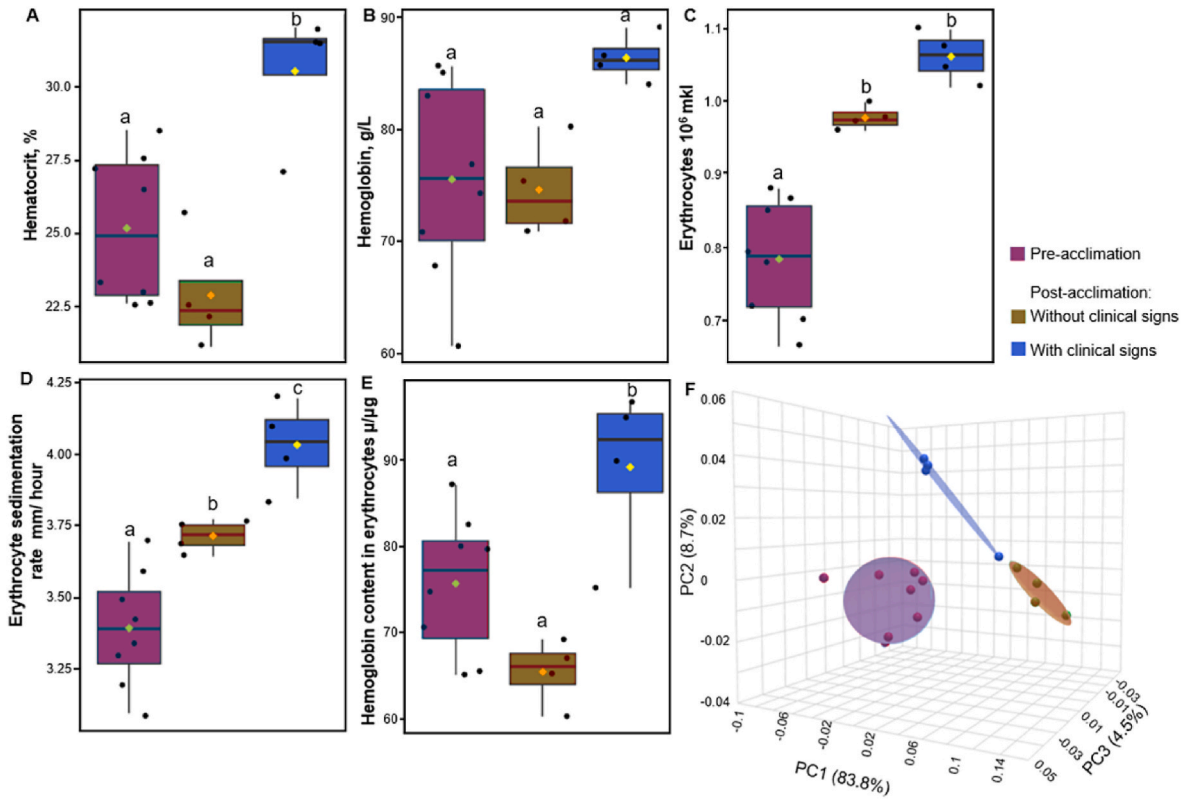

Fig. 4. Changes in hematocrit (A), hemoglobin (B), erythrocytes (C), erythrocyte sedimentation rate (D) and hemoglobin content in erythrocytes (E) in the blood of *A. ruthenus* individuals with and without clinical signs before and after the14-day acclimation (N = 8, $P < 0.05$, mean ± SD), scores of the Principal Component Analysis (PCA) and 3D plots (F). The colored ellipses in the plots represent 95 % confidence regions for each group.

obtained from fish without clinical signs of bacterial infection.

The outer part of the liver was covered by a thin envelope of fibrous connective tissues, which is a species-specific characteristic. The parenchyma was made up of narrow plates, which in turn were formed of radial cords of hepatocytes branching from the central vein. The cytoplasm of the hepatic cells was moderately acidophilic. Hepatocyte nuclei were round or elliptical, located predominantly in the center of the cell, with clearly distinguishable clumps of chromatin (Fig. 5A). Fig. 5A also shows that the hepatic cords were closely intertwined with sinusoid capillaries having the shape of narrow (or moderately dilating) gaps between hepatocyte strands. The endothelial nuclei of sinusoids were elongated, sometimes rod-shaped, in some places protruding into the lumen of vessels. On the one side, hepatocytes were directed to sinusoids and on the other, to bile capillaries, which fully corresponded to the general plan of the histological structure of the liver of Chordate representatives (Fig. 5A). The interlobular bile ducts with branches of the portal vein and hepatic artery formed a triad between lobules. The bile duct was internally lined with low prismatic, weakly acidophilic epithelium with sparse granulation of the cytoplasm. The epithelial cell nuclei were round and located in the center of the cell.

A number of specific changes were identified in the marginal zone of the liver, as outlined below (Fig. 5B). The cells of the low epithelium visible directly under the connective capsule became difficult to distinguish due to their hyperchromaticity (in the form of intense oxyphilicity) (Fig. 5B). It was clearly visible that in deeper parts of the liver, hepatocytes acquired a cuboid shape (Fig. 5B), which differed from the shape of the normal structure. In addition, irregular vacuolization of cytoplasm or its complete absence was detected in these cells (Fig. 5C). As can be seen in Fig. 5C, the hepatocytes of the liver marginal zone were almost round, less frequently they were cuboid-shaped, and practically did not differ from the cells of the peripheral layers of the medial zone. The cell nuclei were relatively small and moderately hyperchromic. Extensive regeneration foci were often found in the conglomerate of cells of the deeper areas of the liver marginal zone (Fig. 5D). It is noteworthy that visually, these cells differed from the cells of the surrounding parenchyma areas in clusters of small, densely arranged cells with relatively large, strongly hyperchromic nuclei. However, with increasing distance from the center of the regeneration foci, the above-mentioned signs became less discernible. As can be seen in Fig. 6, the enhancement of cell synthesis function can be visually determined from the appearance of specific granularity and "saturation" of the cytoplasm. It is also noteworthy that the absence of nucleoli in the hepatocytes of the marginal zone of the liver indicated the presence of some hepatocytes at the prophase (or middle telophase) stage (Fig. 5E). Changes in medium- and small-sized hepatic blood vessels, which manifested themselves as local blood stasis, were also recorded. (Fig. 5F). As shown in Fig. 5F, the lumen of the hepatic vein contains local accumulations of leached erythrocytes and macrophages, indicating the presence of inflammation in the organ. As can be seen, the cells around the blood vessel are inflamed and edematous.

The method of plastic reconstruction used in the study showed that the histological structure of the sterlet liver is heterogeneous, without clear boundaries, conditionally divided into three zones (marginal, medial and caudal). Additionally, it was found that depending on zonal localization, hepatocytes differed in their cytological characteristics (Supplement 1).

As can be seen from the results presented in Supplement 1, bacterial pathogens can change the size of liver cells, which occurred mainly in the marginal zone of the sterlet liver.

The cytological examination of the liver marginal zone revealed a statistically significant decrease in both the nuclear diameter ($H = 5.33$, $df = 1$, $P = 0.021$) and the number of intracellular cavities ($H = 5.33$, $df = 1$, $P = 0.020$) in *A. ruthenus* with clinical signs compared with the fish without clinical signs after the acclimation period (Fig. 6 B and E).

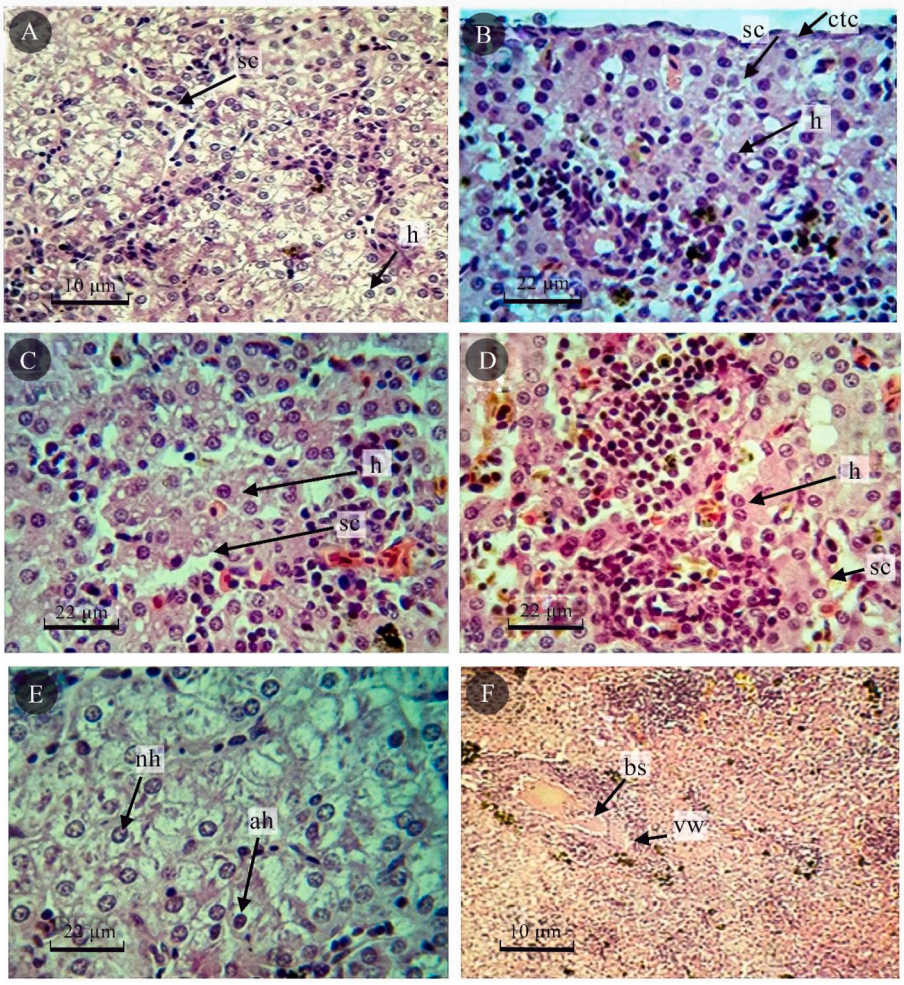

Fig. 5. Liver obtained from two-year-old sterlet (*A. ruthenus*) individuals without and with the clinical signs of bacterial infection. A. Liver without clinical signs of bacterial infection (sc - sinusoidal capillary; h - hepatocyte). (Böhmer hematoxylin, Hart's fuchseline (modified) (Bh, Hf), "MONOCHROM 2.5×" correction filter, 200× magnification were used for image production). B. Marginal zone of the liver with the clinical signs of bacterial infection (ctc - connective tissue capsule; sc - sinusoidal capillary; h – hepatocyte). Ehrlich's hematoxylin, Hart's fuchselin (modified) (Eh, Hf), Multiform filter "FGPM-2.5×"· 450× magnification were used for image production. C. Uneven vacuolization of the cytoplasm of the hepatocytes in the marginal zone (changes in cells are shown with arrows) of the liver (sc – sinusoidal capillary; h – hepatocyte) Eh, Hf, Multiform filter "FGPM-2.5×", ×450. D. Foci of regeneration (shown with arrows) in the marginal zone of the liver (sc – sinusoidal capillary; h – hepatocyte). Eh, Hf. Multiform filter "FGPM-2.5×", ×450. E. Absence of nucleoli in hepatocytes of the marginal zone of the liver (nh - normal hepatocyte; ah - abnormal hepatocyte). Bh, Hf. Correction filter "MONOCHROM 2.5×", ×600. F. Marginal zone of the liver. Stagnation of blood in the vessels (indicated by/with arrows): bs - blood stasis; vw - vessel wall. Bh, Hf. Correction filter "MONOCHROM 2.5×", ×200.

Conversely, our analysis revealed no significant differences in cell diameter ($H = 3.00$, $df = 1$, $P = 0.083$), the ratio of nucleus to cell diameter ($H = 0.76$, $df = 1$, $P = 0.38$), or the ratio of nucleus-to-nucleus diameter ($H = 0.34$, $df = 1$, $P = 0.56$) (Fig. 6A–C and D) among fish regardless of the presence of clinical signs. The PCA analysis revealed a clear separation between parameters of the liver marginal zone in fish with and without clinical signs (PERMANOVA, $F = 8.36$, $P = 0.029$) (Fig. 6F).

4. Discussion

Biodiversity loss is a serious manifestation of the global environmental crisis. Decades of significant anthropogenic pressure on the aquatic environment have reduced the efficiency of the natural reproduction of sturgeon species to almost zero (Jarić et al., 2011). Artificial reproduction (Saraiva et al., 2018) of sturgeon species, carried out since the third quarter of the 20th century, has helped to slow down the disappearance of their population in the Black Sea. One of the main problems facing the artificial reproduction of sturgeons is an acute shortage of reproducers due to the reduction in the total number of sexually mature individuals in the sturgeon population(State Agency of Ukraine for Development of Land Reclamation, Fisheries and Food Programs, 2020). This has led to a steady decline in numbers of the fish stocked since 2008 in Ukraine (Conservation of Sturgeon Fish of the Azov-Black Sea Basin, 2020). The domestication of sturgeon fish is one of the possible solutions to this problem. However, domestication is an anthropogenic process that gradually modifies the farmed organism. Modifications are intergenerational and involve changes in the development of each generation, culminating, as a rule, in genetic changes through generations (Price, 2002). These developmental alterations are particularly important for fish as they exhibit remarkably high levels of phenotypic plasticity, much higher than terrestrial vertebrates (Lorenzen, Beveridge, & Marc, 2012; Thorpe, 2004). It has been found that fish raised in well-managed artificial systems can grow close to their physiological maximum size, but problems of excess mortality due to infectious diseases remain (Lorenzen, Beveridge, & Marc, 2012).

The negative impact of pathogenic microflora on the physiological state of fish manifests itself in the form of metabolic and functional disorders of vital organs (Austin & Austin, 2016; Chen et al., 2012). There is little data on bacterial pathogens in sturgeons, which is

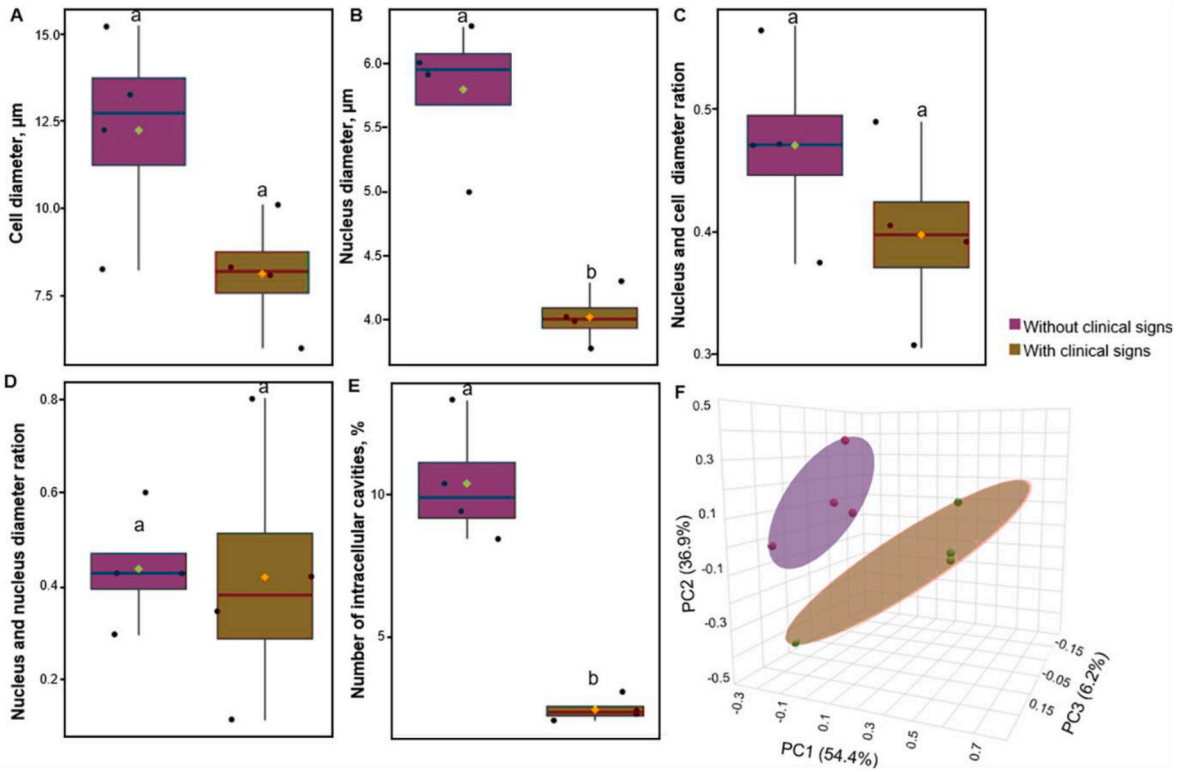

Fig. 6. Cytological structure of the marginal zone of the liver A. cell diameter, B. nucleus diameter, C. nucleus and cell diameter ration, D. nucleus and nucleus diameter ratio, E. number of intracellular cavities. Obtained from *A. ruthenus* with and without clinical signs under bacterial infection and Principal Component Analysis (PCA) scores and 3D plots (F). The colored ellipses in the plots represent 95 % confidence regions for each group.

probably because these fish seem to be resistant to infections once they reach the mature stage (Kayiş et al., 2017). Outbreaks of disease associated with bacteria such as *Vibrio vulnificus*, *Aeromonas hydrophila* and *A. veronii*, *Lactococcus lactis* and *Yersinia ruckeri* in populations of great sturgeon and its hybrids have been reported (Chen et al., 2012; Ciulli et al., 2020; Handy, Runnalls, & Russell, 2002; Safari et al., 2015; van der Oost, Beyer, & Vermeulen, 2003; Yáñez, Catalán, Apráiz, Figueras, & Martínez-Murcia, 2003). During a three-year-long observation period, Kayis et al. (2017) isolated and profiled several bacteria to which RAS-reared sturgeons are susceptible. However, most of the bacteria isolated from sturgeon are opportunistic, and are often reported as a source of secondary infections due to the high stress or high density of farmed fish (Handy, Runnalls, & Russell, 2002; Radosavljević et al., 2019). According to (Pierazan et al., 2020), *Staphylococcus iniae* cocci were found scattered across the surface of the affected muscles of the cultured sturgeon, indicating the occurrence of necrotic and heterophilic myositis. In addition, the authors stated that increased density of farmed fish and elevated water temperatures can also provoke this process, so they are also considered to be important epidemiological factors.

Therefore, we studied the problems arising after the transference of sturgeons from the natural environment to RAS, specifically, how fish adapt to the new living conditions and what changes occur in their organs and tissues. The analysis of clinical data revealed that it is in the internal organs of sturgeons that changes occur primarily. It was found that almost in all infected fish, the color of the liver changed (became pale), in some of them there was spleen enlargement detected, while in others the appearance of ascitic fluid in the abdominal cavity was recorded. It should be noted that our results clearly correlate with those of other authors (Sebastião et al., 2019; Soto et al., 2017; Stachnik, Matras, Borzym, Maj-Paluch, & Reichert, 2021) who have studied sturgeon diseases. Bacteriological studies revealed the presence of bacteria (*Aeromonas hydrophila, Shewanella putrefaciens and Flavobacterium* sp.) on the skin and in tissues of Siberian sturgeon (Stachnik, Matras, Borzym, Maj-Paluch, & Reichert, 2021).

In our study, we observed the predominance of *Aeromonas* lesions in sterlets with characteristic symptoms such as haemorrhages on the body surface, focal inflammations under the scutes and pathological changes in internal organs (Fig. 4). Santi et al. (2019) stated that *A. veronii*, *S. putrefaciens*, *Citrobacter freundii* and *Chryseobacterium* sp. were identified in sturgeon and noted their variable pathogenicity to this fish. These authors also showed that the presence of ulcerative dermatitis and serohemorrhagic coelomic effusions in sturgeons correlated with bacteriological findings indicating bacterial septicemia caused by several species such as *Citrobacter* and *Aeromonas*. A few isolations of *A. veronii* from diseased sturgeons have been reported (Gholamhosseini et al., 2018; Ma et al., 2009), but *Aeromonas* infection is claimed to be one of the most common ones in sturgeons (Santi et al., 2019). Co-infection caused by *A. veronii* and *Chryseobacterium joostei* has also been reported in starred sturgeon (*A. stellatus*) (Gholamhosseini et al., 2018). In addition, *Chryseobacterium* sp. has been isolated from diseased sturgeons, including the species *H. huso* (great sturgeon), and clinical manifestations of the disease with skin ulcerative lesions, ascites and increased mortality were revealed (Gholamhosseini et al., 2018; Sebastião et al., 2019).

However, experimental infections with *Chryseobacterium* sp. isolated from fish did not cause any clinical symptoms or serious pathological changes in great sturgeon (Sebastião et al., 2019), in contrast to what had been previously observed in other fish species with other strains of *Chryseobacterium* sp. (Loch & Faisal, 2015). In addition, *Citrobacter freundii* has been isolated from the internal organs of sick sturgeons previously, but a detailed description of these infections has not been provided (Kayiş et al., 2017). The bacterium *S. putrefaciens* has not been associated with diseased sturgeons, but the bacterium has often been isolated from diseased fish during outbreaks and has been linked to septicemia (Sebastião et al., 2019).

In our study we detected the co-infection that was caused by three

species of bacteria, namely *Aeromonas hydrophila, Pseudomonas anguilliseptica* and *Flavobacterium psychrophilum*. The presence of co-infection, which was determined in our study, and the general fish condition, which was assessed based on clinical and pathological signs, indicate the role of bacterial co-infection in the alteration of internal organs (Fig. 5). Based on our results, we can conclude that most often it is the liver of infected fish that is affected.

To elucidate the main processes occurring in the liver of infected sterlet, we studied the transformations in the liver parenchyma, which, according to our previous studies (Koziy, 2014), are most contrasting in early postnatal ontogenesis in the form of age-related restructuring of liver functional zones. Sturgeon resistance to infections under stressful conditions is significantly reduced due to impaired differentiation of liver cells (Koziy, 2011), which in turn leads to a sharp increase in fish mortality and, consequently, a decrease in sturgeon population (Stachnik, Matras, Borzym, Maj-Paluch, & Reichert, 2021). The detailed histological examination of the samples revealed both general and specific patterns of tissue response to domestication as a stressor and resulting bacterial infection in sterlets. The results obtained in our study showed that under the impact of bacterial pathogens, the most pronounced changes occurred in cells of the marginal zone of the liver (Supplement 1). In healthy sturgeons, cells of the marginal zone were predominantly polygonal and contained lipid vacuoles, with the cells of the medial zone predominantly synthesizing glycogen and the cells of the caudal zone intensively producing and secreting bile. Their cytoplasm was optically homogeneous, but most often contained a certain number of inclusions, which corresponded to different phases of lipid accumulation (Koziy, 2009). During the pathological process, certain changes occurred in cells of the marginal zone of the liver. Characteristics of these changes are presented in Supplement 1.

Analysis of the data presented in Fig. 6 showed that a statistically significant decrease ($P < 0.01$) in the value of linear cell size leads to a significant decrease in the value of nucleus parameters. This process indicates a change in the pattern of intracellular metabolism. A statistically significant decrease ($P < 0.001$) in the number of intracellular cavities is direct evidence of impaired lipostatic function of hepatocytes (Fig. 6). A slight change in the value of the nucleolus/nucleus diameter ratio (1.02 times) (Fig. 6) indicated a cell function alteration such as activation of glycogen synthesis. The disappearance of the typical cell monochromasy and specific granularity of the cytoplasm also confirmed the cell function alteration (Fig. 6B). All the above-mentioned processes indicated a partial failure of metabolic processes. The value of Hertwig's constant (nuclear and cytoplasmic ratio) of glycogen-containing and lipid-containing hepatocytes in healthy and infected sterlets practically coincided, which indicated the absence of cellular pathology signs (Fig. 6). From our point of view, the presence of certain hepatocytes at the prophase (or middle telophase) stage is of interest (Fig. 6E). We hypothesize that the parallel presence of hepatocytes at different stages of formation in conditions of organ cytostructure fluctuations indicates a relative stabilization of the cell function alteration process at the organ locus and reflects the species-specific variability in the adaptive capabilities of an organism. It is possible to premise that the co-existence of hepatocytes of different development levels in response to the organ's cytostructural dynamics shows that the process of liver cell function alteration in the organ itself has stabilized to a certain degree. This phenomenon can also reflect the species-specific adaptability of an organism. The results of our studies are consistent with the data of other authors (Braz-Mota, Sadauskas-Henrique, Duarte, Almeida-Val, & Adalberto, 2015; Endmund, 1988). According to several authors (Matviishyn, Kubrak, Husak, Storey, & Lushchak, 2014; Kozij & Matvienko, 2017), the intensity of the bacterial infection-induced fish intoxication most likely reached critical values, causing the cellular function to shift towards lipostasis, indicating a weakened immune condition and, consequently, a decreased adaptive potential of older fish. The data presented in previous studies (Koziy, 2009, 2011, 2014; (Kozii, Sherman, & Lyanzberg, 2018); Kozij & Matvienko, 2017) showed that lipid accumulation, glycogen synthesis and bile secretion in the liver of healthy sterlets was of varying intensity, causing changes in specific liver functions, and, as a result, narrow specialization of cells in particular areas of the organ. Additionally, locally occurring loosely packed cell clusters indicated the violation of cell contact integrity (Fig. 5D). It must be noted that there was no bile stasis in the liver of the tested sterlets accompanied by bile duct dilation, which, according to Soto et al. (2017), is characteristic of some microbial and viral infections. Also, there was no mechanical deformation of intact hepatocytes with the displacement of nuclei, separate hepatic cords, excessive enlargement of sinusoidal spaces or detachment of the basal membrane recorded (Fig. 5E). According to the classification proposed by Ostapenko et al. (2011), the liver damage in the tested fish can be evaluated as moderate severity.

It should be noted that the fish immune system is not clearly differentiated and there are no well-defined specific immune defense functions, which does not allow adequate protection of the organism during bacterial infection (Monsefi et al., 2010; Höhne et al., 2021).

In sterlet, the general phagocytic-tissue effect, the presence of which manifests itself in the elimination of the pathogen from the organism, is contrastingly pronounced (Iwanowicz et al., 2012). As a result of a gradual decrease in the level of bacterial pathogens (I step), the number and size of intracellular cavities decreased, which, in accordance with the law of the nuclear-cytoplasmic ratio, is accompanied by a decrease in the value of the linear size of cells and nuclei (II step). The most likely explanation for the mechanisms of the second stage of infection is a decrease in cavity size and cytoplasmic and nuclear volumes, which may be partly due to the role of lipids in the functional activity and DNA biosynthesis (Athanassopoulou, Billinis, & Prapas, 2004; Kayiş et al., 2017).

The results of our research revealed the possibility for using the integrated results of clinical and morphological studies in ichthyological and fish breeding practices involved in the domestication of sturgeons as a valuable aquaculture species.

Our study has investigated the main diseases of sturgeon in RAS and identified pathogens of the genera *Aeromonas, Pseudomonas and Flavobacterium*. In addition, the mechanism underlying effects of these infections on the organs and tissues of sturgeon were elucidated. The findings indicated that conducting external examinations of fish and performing microbiological studies can contribute to the reduction of invasive methods during the acclimation process of sturgeon in a RAS system.The issue of sturgeon health maintenance during domestication is relevant since the problem of the shortage of healthy and mature sturgeon individuals in natural water bodies in Ukraine is currently a rather serious deterrent to the recovery of their population. Artificial reproduction of sturgeon species in RAS can partially solve the issue of the wild sturgeon's population restoration in natural water bodies.

Summarizing the data obtained we can state that more attention should be paid to the risk associated with bacterial co-infection of fish during their cultivation in RAS, which can impair their health and reproductive function, especially if domestication is associated with the restoration of rare fish species.

5. Conclusions

Data from clinical and bacteriological studies on sterlet (*A. ruthenus*) during its domestication in conditions of Recirculating Aquaculture System (RAS) are presented. The role of conditionally pathogenic bacteria *Aeromonas, Pseudomonas*, and *Flavobacterium* in the pathological process development in sterlet was established. Liver damage was the main clinical manifestation during the co-bacterial infection in all the fish tested. The study of these lesions employed innovative methods of fixation of histological specimens. Changes in cell generations of the medial zone of the liver were shown. Additionally, a shift in cell function was found, which is naturally accompanied by shifts in cytological characteristics. The adaptive liver response to bacterial infection has

been shown to reduce the number of lipid-dependent cavities and to prevent the premitotic formation of excessive concentrations of lipid-soluble toxins caused by the bacterial infection. The complex data obtained can be useful in clinical morphology, ichthyology and fish breeding practice for disease prevention and for improving the health of valuable domesticated fish and evaluation of the role of bacterial co-infection in the domestication process.

CRediT authorship contribution statement

Nataliia Matviienko: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Danguole Montvydienė:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Conceptualization. **Nijole Kazlauskienė:** Writing – review & editing, Visualization, Supervision, Methodology. **Živilė Jurgelėnė:** Writing – review & editing, Visualization, Formal analysis. **Alexander Didenko:** Validation, Resources, Investigation, Formal analysis, Data curation. **Mykhailo Koziy:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Ethic statements

The work was carried out within the framework of the research program plan of the State Higher Educational Institution “Kherson Agrarian University” on the topic “Microanatomical Characteristics of the Ichthyofauna of Inland Water Bodies of Various Origins and Designated Purposes” (state registration number 0117U002809). The work was carried out in accordance with the Declaration of Helsinki (World Medical Assembly, 1964), the international principles of the European convention for the protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 1986), the Declaration of Principles of Tolerance (28th session of UNESCO, 1995), the Universal Declaration on Bioethics and Human Rights (UN, 1997), the norms of the Convention on the Protection of Human Rights in Connection with the Introduction of New Biomedical Technologies, adopted in 1997 in Oviedo (Spain) and signed by the Verkhovna Rada of Ukraine in 2002, as well as the Law of Ukraine No. 3447 IV "On the Protection of Animals from Cruelty."

The fish were collected according to permits from the Institute of Fisheries of the National Academy of Sciences of Ukraine (IF NAAS) and processed in the Laboratory of Histology, Cytology and Embryology of the Petro Mohyla Black Sea National University (Ukraine), as well as in the Laboratory of Ecotoxicology of Nature Research Centre (Vilnius, Lithuania) and the Department of Ichthyopathology of the IF NAAS. All fishing operations were carried out in accordance with the Rules of Fishing in the Black Sea Basin, the Annual Regime of Fishing (2020, 2021) and the Procedure for Special Use of Water Bioresources in Inland Fishery Water Bodies (their parts) (Cabinet of Ministers of Ukraine, 2015), Internal Sea Waters, Territorial Sea, Exclusive (Maritime) Economic Zone and on the Continental Shelf of Ukraine (Rules, 2003). Experimental procedures involving fish were carried out according to the requirements of the Directive 2010/63/EU on the protection of animals used for scientific purposes(European Commission, 2010)

Ethics declaration for manuscript submission

1. **Authorship Approval**: The corresponding author has obtained approval from all other authors for the submission and publication of all versions of this manuscript. All authors have made significant and independent contributions to the work, and no one who qualifies for authorship has been omitted.
2. O**riginality**: This work has not been published nor is it under consideration for publication elsewhere (outside of oral, poster, or abstract formats). All materials reproduced from other sources, including previous publications by the authors, are appropriately cited and attributed.
3. **Ethical Compliance**: The materials and data presented in the manuscript have been acquired in accordance with contemporary ethical standards and have received approval from the relevant ethical committee(s).
4. **Conflict of Interest**: All material conflicts of interest have been disclosed, including the sources of funding for the research.
5. **Ongoing Validity**: The manuscript will continue to be valid as a published work by the journal only as long as the statements in this declaration are true. The authors commit to promptly notifying the journal editors if any of these statements ceases to be accurate.

Declaration of generative AI and AI-assisted technologies in the Writing process

During the preparation of this manuscript " CHANGES IN *Acipenser ruthenus* LIVER STRUCTURE DURING DOMESTICATION: PRELIMINARY DATA”

the author(s) *Nataliia Matviienko, Danguolė Montvydienė, Živilė Jurgelėnė, Nijolė Kazlauskienė, Alexander Didenko, Mykhailo Koziy*, non used service AI, and take(s) full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aaf.2025.03.002.

References

Athanassopoulou, F., Billinis, C., & Prapas, T. (2004). Important disease conditions of newly cultured species in intensive freshwater farms in Greece: First incidence of nodavirus infection in *Acipenser* sp. *Diseases of Aquatic Organisms, 60*(3), 247–252. https://doi.org/10.3354/dao060247.

Austin, B., & Austin, D. A. (2016). *Bacterial fish pathogens*. Cham: Springer International Publishing.

Bergey. (2011). Bergey's manual of systematic bacteriology. *The major handbook with descriptions of species and higher taxa of prokaryotes, providing a systematic framework for their classification – the taxonomic outline of bacteria and archaea*.

Blaxhall, P. C., & Daisley, K. W. (1973). Routine hematological methods for use with fish blood. *Journal of Fish Biology, 5*(6), 771–781.

Braz-Mota, S., Sadauskas-Henrique, H., Duarte, R. M., Almeida-Val, V. M. F., & Adalberto, L. V. (2015). Roundup® exposure promotes gills and liver impairments, DNA damage, and inhibition of brain cholinergic activity in the Amazon teleost fish *Colossoma macropomum*. *Chemosphere, 135*, 53–60. https://doi.org/10.1016/j.chemosphere.2015.03.042.

Bronzi, P., Chebanov, M., Michaels, J. T., Wei, Q., Rosenthal, H., & Gessner, J. (2019). Sturgeon meat and caviar production: Global update 2017. *Journal of Applied Ichthyology, 35*(1), 257–266. https://doi.org/10.1111/jai.13870

Cabinet of Ministers of Ukraine. (2015). *On the procedure for special use of aquatic bioresources in internal fisheries water bodies (parts thereof), internal sea waters, territorial sea, exclusive (marine) economic zone, and on the continental shelf of Ukraine*. https://zakon.rada.gov.ua/laws/show/992-2015-%D0%B.

Cabinet of Ministers of Ukraine. (2021). *About the approval of the Order for the implementation of the special use of aquatic bioresources in inland fisheries water objects (parts), inland sea waters, territorial sea, exclusive (marine) economic zone and on the continental shelf of Ukraine*. https://zakon.rada.gov.ua/laws/show/992-2015-%D0%BF#Text.

Chen, M. H., Hung, S. W., Shyu, C. L., Lin, C. C., Liu, P. C., Chang, C. H., … Wang, W. S. (2012). *Lactococcus lactis* subsp. *lactis* infection in Bester sturgeon, a cultured hybrid of *Huso huso* × *Acipenser ruthenus*, in Taiwan. *Research in Veterinary Science, 93*(2), 581–588. https://doi.org/10.1016/j.rvsc.2011.10.007.

CITES-Convention. (1983). CITES-Convention. Retrieved from https://cites.org/sites/default/files/eng/disc/CITES-Convention-EN.pdf.

Ciulli, S., Volpe, E., Sirri, R., Tura, G., Errani, F., Zamperin, G., … Mandrioli, L. (2020). Multifactorial causes of chronic mortality in juvenile sturgeon (*Huso huso*). *Animals, 10*(10), 1866. https://doi.org/10.3390/ani10101866.

Conservation of sturgeon fish of the azov-black Sea Basin. Retrieved from https://fishindustry.com.ua/soxranenie-osetrovyx-ryb-azovo-chernomorskogo-bassejna/, (2020).

Council Regulation (EC) No. 1099/2009. (2009). On the protection of animals at the time of killing (Text with EEA relevance). Retrieved from http://data.europa.eu/eli/reg/2009/1099/oj.

Endmund, L. (1988). Cell Cycle Clocks. In *Cellular and Molecular Bases of Biological Clocks*. New York, NY: Springer. https://doi.org/10.1007/978-1-4612-3742-6_3.

European Commission (EC). (2010). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Retrieved from https://www.legislation.gov.uk/eudr/2010/63/contents.

FAO. (2020a). *The state of world fisheries and aquaculture 2020*. FAO. Retrieved from http://www.fao.org/documents/card/en/c/ca9229en.

FAO. (2020b). *The state of world fisheries and aquaculture 2020: In brief*. FAO. Retrieved from http://www.fao.org/documents/card/en/c/ca9231en.

FAO. (2022). *The state of world fisheries and aquaculture 2022*. FAO. Retrieved from http://www.fao.org/documents/card/en/c/ca9231en.

Foster, S., Wiswedel, S., & Vincent, A. (2016). Opportunities and challenges for analysis of wildlife trade using CITES data—seahorses as a case study. *Aquatic Conservation: Marine and Freshwater Ecosystems, 26*(1), 154–172. https://doi.org/10.1002/aqc.2493.

Frishtak, O., Koryliak, M., & Dobrianska, O. (2022). Antioxidant status of fish with inflammation of the swim bladder in yearling carp. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, 24*(106), 172–176. https://doi.org/10.32718/nvlvet10626.

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. *Veterinary Research Forum, 9*(2). https://doi.org/10.30466/vrf.2018.30826.

Handy, R. D., Runnalls, T., & Russell, P. M. (2002). Histopathologic biomarkers in three-spined sticklebacks (*Gasterosteus aculeatus*) from several rivers in Southern England that meet the Freshwater Fisheries Directive. *Ecotoxicology, 11*(6), 467–479. https://doi.org/10.1023/A:1021061402491.

Höhne, C., Prokopov, D., Kuhl, H., Du, K., Klopp, C., Wuertz, S., … Stöck, M. (2021). The immune system of sturgeons and paddlefish (*Acipenseriformes*): A review with new data from a chromosome-scale sturgeon genome. *Reviews in Aquaculture, 13*(3), 1709–1729. https://doi.org/10.1111/raq.12542.

Huang, X., Liu, S., Zhang, H., Yao, J., Geng, Y., Ou, Y., … Luo, W. (2022). Pathological characterization and cause of a novel liver disease in largemouth bass (*Micropterus salmoides*). *Aquaculture Reports, 23*, 101028. https://doi.org/10.1016/j.aqrep.2022.101028.

Ivanova, N. T. (1983). *Atlas kletok krovi ryb: sravnitel'naya morfologiya i klassifikatsiya formennykh elementov krovi ryb (Atlas of fish blood cells: Comparative morphology and classification of fish blood formed elements)*. Moscow: Legkaya i Pishchevaya Promyshlennost'.

Iwanowicz, L. R., Blazer, V. S., Hitt, N. P., McCormick, S. D., DeVault, D. S., & Ottinger, C. A. (2012). Histologic, immunologic and endocrine biomarkers indicate contaminant effects in fishes of the Ashtabula River. *Ecotoxicology, 21*(1), 165–182. https://doi.org/10.1007/s10646-011-0776-0.

Jarić, I., Višnjić-Jeftić, Ž., Cvijanović, G., Gačić, Z., Jovanović, L., Skorić, S., & Lenhardt, M. (2011). Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (*Acipenser ruthenus*) from the Danube River in Serbia by ICP-OES. *Microchemical Journal, 98*(1), 77–81. https://doi.org/10.1016/j.microc.2010.11.008.

Kayiş, Ş., Kangel, A., & 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. *Iranian Journal of Veterinary Research, 18*(1), 18–24.

Kozii, M. S., Sherman, I. M., & Lyanzberg, O. V. (2018). *Atlas of histology and embryology of industrial fish*. Kherson: Oldie plus.404.

Kozij, M. S., & Matvienko, N. N. (2017). Bioindication of natural waters using a histological analysis of the *Carassius auratus* mesonephros structure. *Journal of Water Chemistry and Technology, 39*(3), 177–180. https://doi.org/10.3103/S1063455X17030109.

Kozinska, A., & Pezkala, A. (2004). First isolation of *Shewanella putrefaciens* from freshwater fish – a potential new pathogen. *Bulletin of the European Association of Fish Pathologists, 24*(24), 199–203.

Koziy, M. S. (2009). *Assessment of the current state of histological technology and ways to improve the study of ichthyofauna. 310*. Kherson: Oldie Plus.

Koziy, M. S. (2011). Histomorphological features of the ichthyofauna of the South of Ukraine. *Kherson: Oldie Plus*, 324.

Koziy, M. S. (2014). Morphofunctional adaptation of the renal tissue of *Acipenser ruthenus* (*Actinopterygii, Acipenseriformes*) under transformed habitation conditions. *Vestnik Zoologii, 48*(4), 365–370. https://doi.org/10.2478/vzoo-2014-0044.

Li, L., Zheng, B., & Liu, L. (2010). Biomonitoring and bioindicators used for river ecosystems: Definitions, approaches, and trends. *Procedia Environmental Sciences, 2*, 1510–1524. https://doi.org/10.1016/j.proenv.2010.10.164.

Litvak, M. (2010). The sturgeons (family: Acipenseridae). In F. N L, M. Jobling, C. Carter, & P. Blier (Eds.), *Finfish aquaculture diversification* (pp. 178–199). UK: CABI.

Loch, T., & Faisal, M. (2015). Polyphasic characterization reveals the presence of novel fish-associated Chryseobacterium spp. in the Great Lakes of North America. *Diseases of Aquatic Organisms, 113*(2), 113–125. https://doi.org/10.3354/dao02819.

Lorenzen, K., Beveridge, M. C. M., & Marc, M. (2012). Cultured fish: Integrative biology and management of domestication and interactions with wild fish. *Biological Reviews, 87*(3), 639–660. https://doi.org/10.1111/j.1469-185X.2011.00215.x.

LPSN. (2019). Classification of domains and phyla - hierarchical classification of prokaryotes (bacteria). Retrieved from Version 2.2. https://lpsn-dev.dsmz.de/archive/-classifphyla.html.

Luna, L. G. (1968). *Manual of histologic staining methods of the armed forces Institute of pathology*. New York: McGraw-Hill.

Ma, Z., Yang, H., Li, T., Luo, L., & Gao, J. (2009). Isolation and identification of pathogenic *Aeromonas veronii* isolated from infected Siberian sturgeon (*Acipenser baerii*). *Acta Microbiologica Sinica, 49*(10), 1289–1294.

Matvienko, N., Nedosekov, V., Koziy, M., & Kravchenko, A. (2022). Fish welfare in Ukraine. *Zoology and Ecology, 32*(1), 84–89. https://doi.org/10.35513/21658005.2022.1.10.

Matvienko, N., Vashchenko, A., Tsiganok, I., & Buchatsky, L. (2015). Results of surveillance studies of infectious fish diseases in freshwater aquaculture of Ukraine. *Agricultural Science and Practice, 2*(2), 32–38. https://doi.org/10.15407/agrisp2.02.032.

Matviienko, N., Koziy, A., Didenko, A., & Sherman, I. (2021). Assessment of the effect of Catosal on the stimulation of ovogenesis in sterlet during pre-spawning period. *Aquaculture Research, 22*(1). https://doi.org/10.4194/AQUAST583.

Matviishyn, T. M., Kubrak, O. I., Husak, V. V., Storey, K. B., & Lushchak, V. I. (2014). Tissue-specific induction of oxidative stress in goldfish by 2,4-dichlorophenoxyacetic acid: Mild in brain and moderate in liver and kidney. *Environmental Toxicology and Pharmacology, 37*(2), 861–869. https://doi.org/10.1016/j.etap.2014.02.007.

Monsefi, M., Gholami, Z., & Esmaeili, H. (2010). Histological and morphological studies of the digestive tube and liver of the Persian tooth-carp, *Aphanius persicus* (Actinopterygii: Cyprinodontidae). *European Journal of Biology, 69*(1), 57–64.

Movchan, Y. V. (2011). *Fish of Ukraine*. Kyiv.

Ostapenko, Y. N., Brusin, K. M., Zobnin, Y. V., Shchupak, A. Y., Vishnevetskiy, M. K., Sentsov, V. G., … Puchkov, Y. B. (2011). Acute cholestatic liver injury caused by polyhexamethyleneguanidine hydrochloride admixed to ethyl alcohol. *Clinical Toxicology, 49*(6), 471–477. https://doi.org/10.3109/15563650.2011.592837.

Paździor, E., Pękala-Safińska, A., & Wasyl, D. (2019). Phenotypic diversity and potential virulence factors of the Shewanella putrefaciens group isolated from freshwater fish. *Journal of Veterinary Research, 63*(3), 321–332. https://doi.org/10.2478/jvetres-2019-0046

Pękala, A., Kozińska, A., Paździor, E., & Głowacka, H. (2015). Phenotypical and genotypical characterization of Shewanella putrefaciens strains isolated from diseased freshwater fish. *Journal of Fish Diseases, 38*(3), 283–293. https://doi.org/10.1111/jfd.12231.

Pierezan, F., Shahin, K., Heckman, T. I., Ang, J., Byrne, B. A., & Soto, E. (2020). Outbreaks of severe myositis in cultured white sturgeon (*Acipenser transmontanus* L.) associated with *Streptococcus iniae*. *Journal of Fish Diseases, 43*(4), 485–490. https://doi.org/10.1111/jfd.13145.

Poleksic, V., Lenhardt, M., Jarić, I., Djordjevic, D., Gačić, Z., Cvijanović, G., & Rašković, B. (2010). Liver, gills, and skin histopathology and heavy metal content of the Danube sterlet (Acipenser ruthenus Linnaeus, 1758). *Environmental Toxicology and Chemistry, 29*(3), 515–521. https://doi.org/10.1002/etc.82.

Price, E. O. (2002). *Animal domestication and behavior*. CAB International.

Radosavljević, V., Milićević, V., Maksimović-Zorić, J., Veljović, L., Nešić, K., Pavlović, M., … Marković, Z. (2019). Sturgeon diseases in aquaculture. *Archives of Veterinary Science, 12*(1), 5–20. https://doi.org/10.46784/e-avm.v12i1.34.

Raymakers, C. (2002). International trade in sturgeon and paddlefish species: The effect of CITES listing. *International Review of Hydrobiology, 87*, 525–537.

Stachnik, M., Matras, M., Borzym, E., Maj-Paluch, J., & Reichert, M. (2021). Emerging viral pathogens in sturgeon aquaculture in Poland: Focus on herpesviruses and mimivirus detection. *Viruses, 13*(8), 1496. https://doi.org/10.3390/v13081496.

State Agency of Ukraine for Development of Land Reclamation, Fisheries and Food Programs. (2020). *Report of head of state agency for Land reclamation and fisheries of Ukraine for 2020*. https://darg.gov.ua/_publichnij_zvit_golovi_0_0_0_10694_1.html.

Cabinet of Ministers of Ukraine. (2004). *Rules of industrial fishing Black Sea basin*. https://zakon.rada.gov.ua/laws/show/z0147-99#Text.

Safari, R. A. M., Ghiasi, M., Asl, M. R. S., & Khalili, E. (2015). First isolation and identification of *Vibrio vulnificus* (biotype 2) from cultured beluga (*Huso huso*) in Iran. *Caspian Journal of Environmental Sciences, 3*(13), 275–281.

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

Saraiva, J. L., Castanheira, M. F., Arechavala-López, P., Volstorf, J., & Studer, B. H. (2018). Domestication and welfare in farmed fish. In F. Teletchea (Ed.), *Animal domestication*. IntechOpen.

Sebastião, F. A., Loch, T., Marancik, D., Griffin, M., Maret, J., Richey, C., & Soto, E. (2019). Identification of *Chryseobacterium* spp. isolated from clinically affected fish in California. *USA. Diseases of Aquatic Organisms, 136*(3), 227–234. https://doi.org/10.3354/dao03409.

Snieszko, S. F. (1960). *Microhaematocrit as a tool in fisheries management (Special Scientific Report No. 314)*. Washington, DC: U.S. Department of the Interior, Fish and Wildlife Service.

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

State Agency of Ukraine for Development of Land Reclamation, Fisheries and Food Programs. (2021). *Report of head of state agency for Land reclamation and fisheries of Ukraine for 2021*. https://darg.gov.ua/files/21/02_18_zvit.pdf.

Teletchea, F., & Fontaine, P. (2012). Levels of domestication in fish: Implications for the sustainable future of aquaculture. *Fish and Fisheries, 15*. https://doi.org/10.1111/faf.12006

Cabinet of Ministers of Ukraine. (1999). The rules of fishing in the Black Sea basin and the annual fishing regime. https://zakon.rada.gov.ua/laws/show/z0147-99.

Thorpe, J. E. (2004). Life history responses of fishes to culture. *Journal of Fish Biology, 65* (1), 263–285. https://doi.org/10.1111/j.0022-1112.2004.00556.x.

van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology, 13*(2), 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6.

Wei, Q. W., Zou, Y., Li, P., & Li, L. (2011). Sturgeon aquaculture in China: Progress, strategies, and prospects assessed on the basis of nation-wide surveys (2007–2009). *Journal of Applied Ichthyology, 27*(2), 162–168. https://doi.org/10.1111/j.1439-0426.2011.01669.x.

Williot, P., Brun, R., Rouault, T., Pelard, M., Mercier, D., & Ludwig, A. (2005). Artificial spawning in cultured sterlet sturgeon (*Acipenser ruthenus* L.), with special emphasis on hermaphrodites. *Aquaculture, 246*(1–4), 263–273. https://doi.org/10.1016/j.aquaculture.2005.02.048.

Williot, P., Chebanov, M., & Nonnotte, G. (2018). Welfare in the cultured siberian sturgeon (*Acipenser baerii* Brandt): State of the art. In P. Williot, G. Nonnotte, & M. Chebanov (Eds.), *The siberian sturgeon (Acipenser baerii, Brandt, 1869) volume 2 - farming* (pp. 403–450). Springer International Publishing.

Yáñez, M. A., Catalán, V., Apráiz, D., Figueras, M. J., & Martínez-Murcia, A. J. (2003). Phylogenetic analysis of members of the genus *Aeromonas* based on *gyrB* gene sequences. *International Journal of Systematic and Evolutionary Microbiology, 53*(3), 875–883. https://doi.org/10.1099/ijs.0.02443-0.

Yanong, R. P. E. (2003). Necropsy techniques for fish. *Seminars in Avian and Exotic Pet Medicine, 12*, 89–105.