Use of ultrasonic screening in aquaculture for the directed formation sturgeon and salmon fish mature stocks

Marina Korentovich[1*], *Alexander* Litvinenko[1], *Aial* Egorov[1], *Polina* Zenkovich[1], and *Liudmila* Litvinenko[1]

[1]State Agrarian University of Northen Trans Urals, Respubliki str., 7, 625003 Tyumen, Russia

Abstract. The results of research work for the period from 2018 to 2023 on the use of early non-invasive functional ultrasound screening for the purpose of targeted formation of highly productive mature-brood stocks of Siberian sturgeon of the Ob, Yenisei, Baikal, Lena populations, European sterlet of the Kama population, Siberian sterlet of the Ob, Yenisei, Irtysh populations on main full-cycle industrial fish-breeding farms of Eastern and Western Siberia using water recirculation systems are presented in the article. Biotechnological methods for the formation of highly productive mature stocks using ultrasound monitoring are described. The number of individuals with pathologies and anomalies in the development of the reproductive system, liver, gallbladder and other internal organs were estimated; treatment methods were proposed. For the first time studies on the use of ultrasound for visualizing the gonads, determining the stages of maturity, detecting diseases of the heart, liver, gallbladder in salmon fish (trout, Atlantic salmon) grown in aquaculture have been carried out. For the first time ultrasound and histological studies were carried out to study the nature of "ink spots" on the skin of Siberian sturgeon Lena population from natural habitat. It was found that in the skin of sturgeon during pathology there was a growth of epidermal cells. Melanin melanocytes increased in both epidermis and dermis. At the same time there were no elastic fibers of the epidermis after painted according to Van-Gison. All these changes may indicate melanoma.

1 Introduction

In over the past ten to fifteen years in Western and Eastern Siberia for the purpose of artificial reproduction of sturgeon fish for compensatory measures, as well as for commercial cultivation, old fish-breeding enterprises have been reconstructed and new ones have been built. Most of them are full-system basin complexes based on recirculating water supply installations (RWS). The existing technologies for artificial reproduction and formation of brood stocks, biotechnological standards for sturgeon rearing in the West Siberian and East Siberian fishery basins do not differ significantly [1-2] and they are aimed at the most efficient use of available production capacities.

* Corresponding author: marinachep@yandex.ru

At the same time, despite certain successes in organizing the planning of breeding and breeding production works at the fish farms, during the annual ultrasound monitoring of repair and brood stocks, a number of common problems associated with violations of the biotechnology of the formation, maintenance and operation of them were studied.

It should be noted that the non-invasive express method of early sex determination and gonad maturity stages with help of ultrasound diagnostics developed by M. Chebanov and E. Galich [3] is used in the practice of forming mature stocks of sturgeon fish. At the same time, it should be said that the ultrasound scanning method has been used relatively recently in the wide practice of sturgeon breeding [4-8]. Due to the lack of experience and literacy of operators, diagnostic errors are often observed, especially when assessing the abnormal state of the generative tissue and identifying pathologies of internal organs. Modern ultrasound technologies (dopplerography, elastography, panoramic scanning, tissue harmonic mode, etc.) can significantly improve the quality of visualization and examine the structure of animal tissue in order to quickly and accurately diagnose anomalies and diseases [9-12]. This will make it possible to fill the existing gaps and significantly improve the accuracy of diagnostics of the state of the reproductive system, liver, gallbladder, heart, gastrointestinal tract, muscle tissue and other internal organs.

The use of ultrasound diagnostics opens up new opportunities for detecting anomalies of the skin epithelium of sturgeon fish, for example, when so-called "ink spots" appear on the skin. The etiology of the appearance of "ink spots" on the skin of sturgeons of natural origin, which do not have a visible negative effect on the physiological state of the fish, has not yet been clarified. Various assumptions have been made about their nature but since the quantity of individuals with spots are small, a detailed study of this phenomenon has not been carried out before. According to the literature, in addition to the Siberian sturgeon of the Lena population [13, 14], cases of detection of "ink spots" in domesticated spawners of Siberian sturgeon from the Ob-Irtysh basin and Russian sturgeons domesticated on Volga sturgeon fish farms [15] have been recorded and described. A year after the discovery of the spots by specialists of the Abalak sturgeon hatchery farm, an increase in both their number on the body of the fish and the number of affected individuals was noted. The occurrence of fish with spots in the mature stock was 10-15 % [15]. Our researches were aimed at studying the nature of the “ink spots” with help of ultrasound and histological methods. They appeared on the skin epithelium in Siberian sturgeon individuals of the Lena population two years after the beginning of fish domestication in the conditions of the Chernyshevsky fish hatchery of the Republic of Sakha (Yakutia).

The purpose of ultrasound studies of the internal organs of salmon fish species (rainbow trout, Atlantic salmon) was to provide methodological and practical assistance in the formation of highly productive breeding stocks at some fish-hatchery farms in Siberia and the Kola Peninsula. It should be noted that these works were carried out for the first time in Russia and they were not described in the scientific and methodological literature.

2 Materials and Methods

The objects of researches were different age groups of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), Atlantic salmon (*Salmo salar* Linnaeus, 1758), Siberian sturgeon (*Acipenser baerii* Brandt, 1869) of the Ob, Baikal, Yenisei and Lena populations, European sterlet (*Acipenser ruthenus* Linnaeus, 1758) of the Kama population, Siberian sterlet (*Acipenser ruthenus marsiglii* Brandt, 1833) of the Ob, Irtysh, Yenisei populations grown at fish farms both as part of routine maintenance on artificial reproduction and for aquaculture.

Scientific and production works were carried out in 2018-2023 on the bases of different fish farms in Murmansk region, Eastern and Western Siberia: in Republic of Sakha - Yakutia, Irkutsk, Kemerovo, Novosibirsk, Tyumen regions, Krasnoyarsk Territory, Yamalo-Nenets and Khanty-Mansi Autonomous (KhMAO-Yugra) Area. Various sources of water supply were used on farms for growing fish: sea water (Barents Sea), river, artesian and (or) geothermal water using direct-flow or recirculation systems.

The grading and culling of sturgeons and salmon fish from mature stocks were carried out in different periods: before and after artificial wintering, before spawning, during or after active feeding of fish. The following portable ultrasound scanners were used for scanning: with gray-scale image - AcuVista, Mindray DP-50, 6600, 6900, SonoScape-A6; with color dopplerography - model SonoScape–S2N. During ultrasound examination of the internal organs of Atlantic salmon and trout, a moisture-proof portable ultrasound machine of model Easi-Scan:Go (IMV imaging) with wireless glasses in the package was used. For clear visualization of the gonads, muscle tissue standard high-frequency linear 2D probe with working surface of 40 mm and frequency of 5.5-12 MHz was used. For ultrasound scanning of heart, liver, gallbladder and other internal organs, low-frequency microconvex and convex 2D probes with a smaller aperture and scanning depth of 2.5-5.0 MHz were used (figure 1).

Fig. 1. A - Portable ultrasound machine of model Easi-Scan:Go with wireless glasses in the package; B - Using ultrasound machine of model Easi-Scan:Go to study the internal organs of Atlantic salmon, cages, Kola Peninsula; C - Portable ultrasound scanner of model Sono Scape S2N

The duration of the ultrasound examination ranged from 2 to 10 seconds per individual. The exceptions were complex cases, such as anomalies in the development of the reproductive system, pathologies of the liver and gallbladder, polycystic disease, hydrocele and other diseases which require additional time for more in-depth studies.

During the grading period, weighing and measurement of the commercial body length (L), individual marking with microchips, visual features and deviations from the norm, as well as internal anomalies in the development of fish, were performed. In just 5 years more than 90 thousand ultrasound studies were carried out. The total number of sturgeon and salmon fish in mature stocks was more than 10,000 specimens.

The operation to remove the epithelium of "ink spot" in Siberian sturgeon of natural origin was carried out at the Chernyshevsky Fish Hatchery Farm (Republic of Sakha - Yakutia) in April 2023 (figure 2).

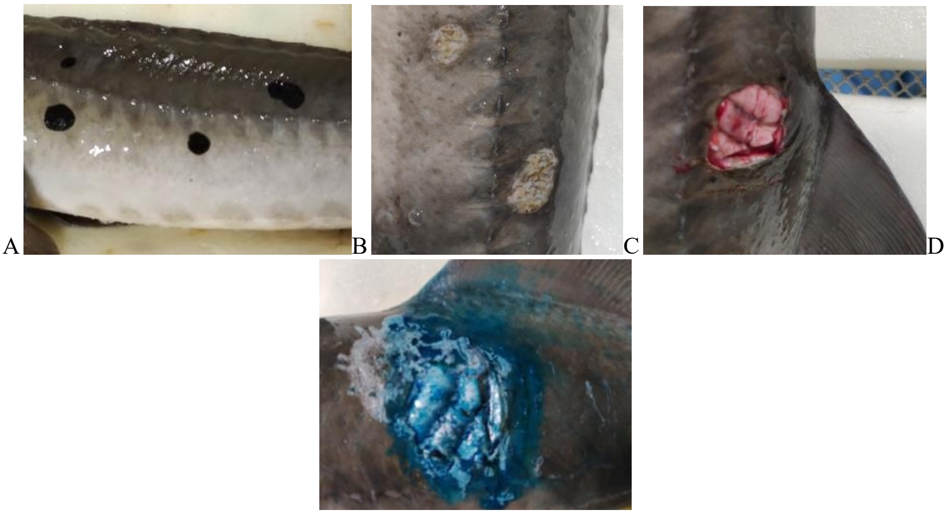


Fig. 2. The operation to remove the epithelium of the "ink spot" in Siberian sturgeon of natural origin: A – "Ink spots" before surgical operation; B - Skin epithelium after removal of "ink spots"; C - The area of the removed skin epithelium under the "ink spot"; D - an area of sturgeon skin after epithelial removal treated with antiseptic drugs

As anesthesia for sturgeon a solution of clove oil was used. It consisted of 10 ml of oil per 100 liters of water. The water temperature was 10.7 °C. Before the operation of the skin epithelium the "ink spot" was removed with a scalpel, making a scraping. The biopsy of the "ink spot" and the removed skin area measuring 1.1x1.3 cm were fixed with 10 % formalin solution. After the operation the sturgeon was injected intramuscularly with a solution of vikasol (vitamin K). The wound surface was treated by chlorhexidine bigluconate (0.05%), terramycin (spray) and antiseptic drug "Second hide super" with silver. The sturgeon wound was treated for 15 days.

Histological studies of the skin of healthy sturgeon (control), biopsy samples of the "ink spot" and epithelium of the affected part of the skin were performed by employee of the laboratory of the Institute of Biotechnology and Veterinary Medicine of the State Agrarian University of the Northern Trans-Urals Krasnolobova E.P. in June 2023. Skin samples were carried out through alcohols of different strength and poured into paraffin according to the generally accepted method. Sections with a thickness of 5 microns were stained with hematoxylin and eosin, as well as by Van Gieson according to standard methods. Histopreparations were studied using light microscope Micros equipped with a video camera at small, medium and large magnifications (increase of 100, 200 and 400), assessing the condition of cells and other structures. Later taking picture was carried out.

3 Research results

3.1 Sturgeon fish

3.1.1 Methods of formation of repair and mature stocks using ultrasound

It is known that the most optimal period for determining sex and maturity stages of sturgeon gonads is after artificial wintering with a gradual increase in water temperature from 2-3 ºC to 8-12 ºC [3]. For Siberian sturgeons of the Lena, Yenisei, Ob, Baikal populations and Siberian sterlet the wintering period, starting from the second stage of gonad maturity should be at least 4-5 months [2]. Abundant feeding and water temperature above 20 ° C negatively affect the results of ultrasound examinations. Despite the higher growth rates, sex identification of fish kept at high temperatures is significantly more difficult due to the accumulation of a fair amount of fat and the predominance of mainly somatic growth. The first assessment of Siberian sturgeons is carried out after a year and a half of rearing in mature stock, guided by the following [2, 16]:

• conducting ultrasound after a short wintering (two to three weeks);
• minimum weight for ultrasound study: Siberian sturgeon of the Ob (Baikal, Yenisei) populations - 2.0-2.5 kg, Lena population - 0.8-1.0 kg; sterlet (Ob, Yenisei) - 0.5-0.6 kg, Irtysh - 0.3-0.4 kg;
• early determination of sex and maturity stages of gonads (1-2, 2nd stages of maturity) (figure 3);
• identification of individuals with underdevelopment of generative tissue (figure 4);
• adjustment for rearing biotechnics.

A B C

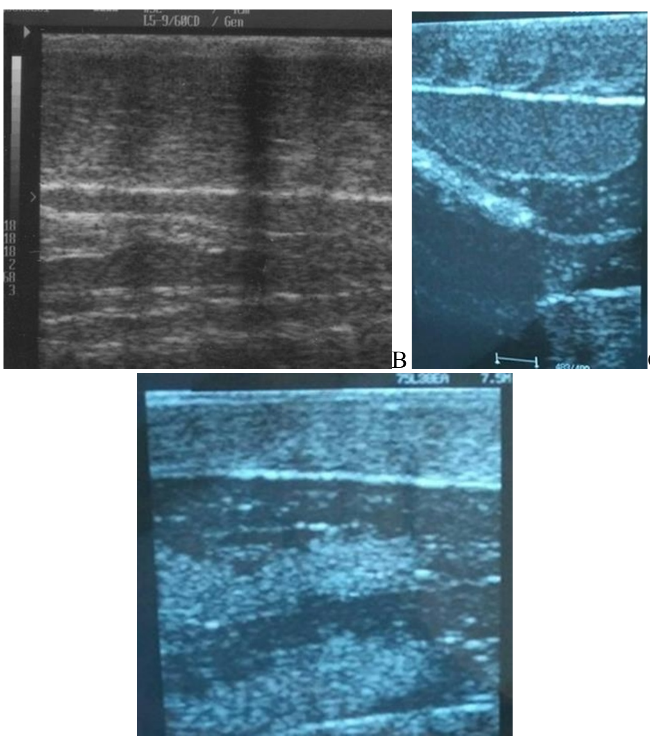


Fig. 3. Echograms of the gonads, longitudinal scanning (**normal**): A - Sterlet of the Irtysh population, M2, 1+; B - Sturgeon of the Ob population, M2, 2-yearling; C - Sterlet of the Irtysh population, F2sf, 1+

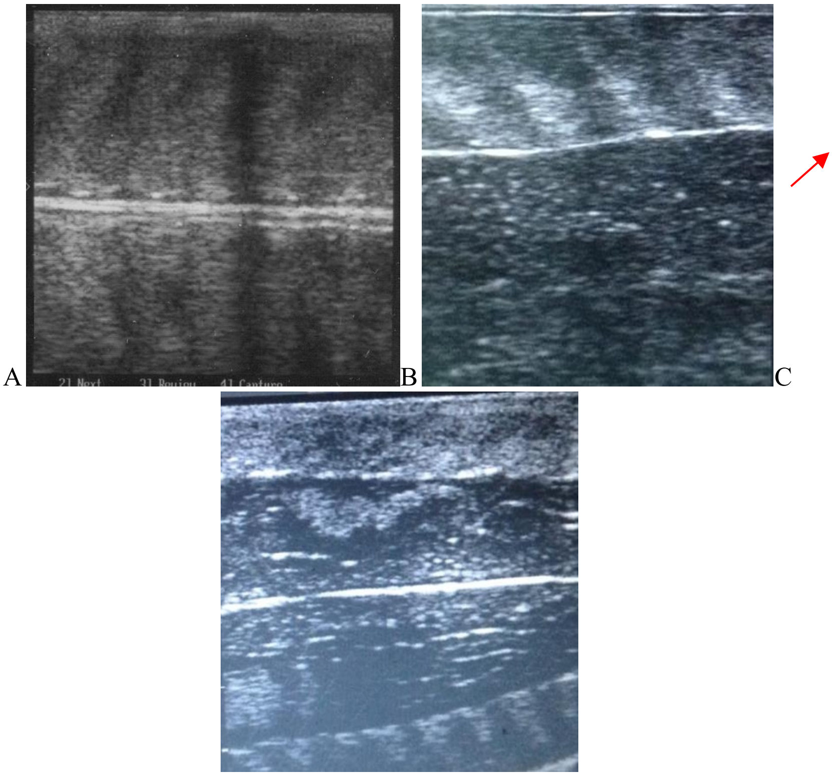


Fig. 4. Echograms of internal organs of sturgeon fish, longitudinal scanning (**pathology**): A - Sterlet of the Irtysh population, two-yaerling, the gonads are undeveloped; B – Siberian sturgeon of Lena population, weight 2.5 kg, three-yearling, after feeding, visualization of generative tissue is impossible due to fat; C - Sterlet of the Irtysh population, F2f, 2+, generative tissue is visualized only on the left gonad (arrow)

During the second and subsequent ultrasonic evaluations (until the maturation of the fish), the following types of work are performed [2, 16]:
• determination of sex and stages of maturity of the gonads;
• individual labeling with microchips of sturgeons with developed gonads (3-4, 4 stages of maturity);
detection of diseases, anomalies of internal organs;
• once in a half of the year ultrasonic monitoring of maturing individuals for the first time;
• forecast of maturation of spawners and determination of the amount of eggs for reproduction (3-4, 4 stages):
• recommendations for treatment or culling;
• adjustment of growing biotechnics.

It should be noted that due to timely adjustments in the biotechnics of growing Siberian sturgeons, individuals with scoliosis (curvature of the cartilaginous structure of the chord) and exhaustion were rarely encountered during the repare mature stocks bonification.

During ultrasound bonitation individuals with the following developmental abnormalities can be identified:
• cystic (polycystic) generative tissue and liver;
• cholecystitis;
• acute hepatitis;
• fatty degeneration of the gonads;
• atrophy of the testes (ovaries);

• synchronous (asynchronous) hermaphroditism;
• neoplasms in the generative tissue;
• foreign bodies in the gastrointestinal tract, etc.

3.1.2 Ultrasound examination of gonad pathology

With help of ultrasound examination of testes (*testis*) and ovaries (*ovarium*) at different stages of maturation cystic and polycystic cysts were visualized in 1.4% of individuals (figure 5).

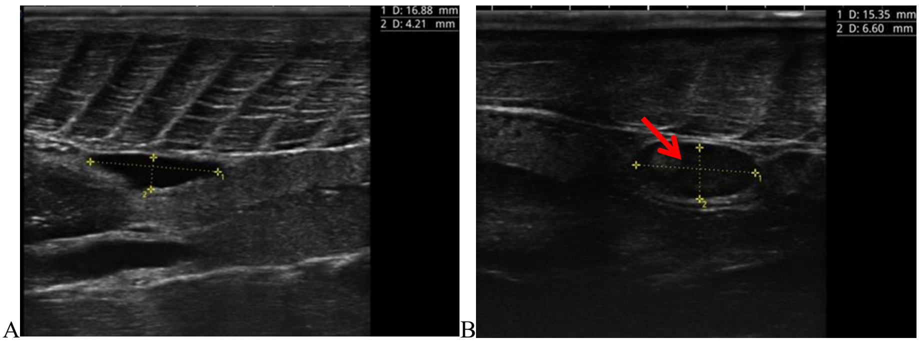


Fig. 5. Echograms of testes (A) and ovaries (B) of Siberian sturgeon of Lena population with cysts measuring 16,88x4,21 mm and 15,35x6,60 mm (the arrow marks the neoplasm); longitudinal scanning

In polycystic cysts small cysts had uneven fuzzy walls. The effect of distal pseudo-amplification and lateral shadows was observed behind the cysts. Occasionally, hyperechoic solid neoplasms of rounded shape with coarse-grained echotexture were visualized in large anechoic cysts (see figure 5B, arrow). If the size of such formations increases, studies should be carried out using contrast agents (for example, Sonovue, iodine-dipamide ethyl ether), aspiration biopsy under ultrasound control to identify pathogenic microorganisms, clarify the diagnosis of the disease and determine treatment methods.

Sporadically, multiple small cysts penetrate the testis tissue (figure 6A, red arrows) were studied in sturgeon males aged 10+ and older. On the echogram oval pyloric department of the stomach (length - about 2 cm) of mixed echogenicity was visualized (yellow arrow). If the stomach is empty, the folds of the mucous membrane have the shape of a wheel with spokes. The lumen of the pyloric gland is small and narrowed. Ultrasound examination revealed cases of torsion and atrophy of the testes (figure 6B), as well as fatty degeneration of generative tissue (figure 6C).

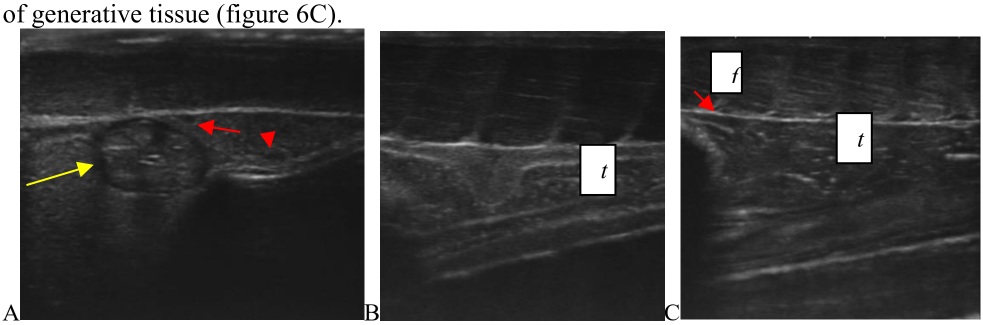


Fig.6. Echograms of testes of male sturgeon, longitudinal scan: A - small cysts (red arrow), pyloric gland (D=2 cm, yellow arrow); B - torsion and atrophy of the testis; C – fatty degeneration of the testis; testis (*t*) - generative tissue, fat (*f*) – fat

Testicular atrophy is a decrease in the size of the testis after its normal development due to a violation of blood circulation in it [11]. Atrophy may be associated with a neoplasm in the opposite testis. The atrophied testes are small in size but their structure remains normal. Visualizing gonads have shown there were hypoechoic inclusions with areas of reduced echogenicity.

With fatty degeneration the generative tissue of the testis does not develop, adipose tissue covers the generative from the medial and lateral sides. On the echograms adipose tissue was visualized as a heterogeneous structure of mixed echogenicity (figure 7 A, B).

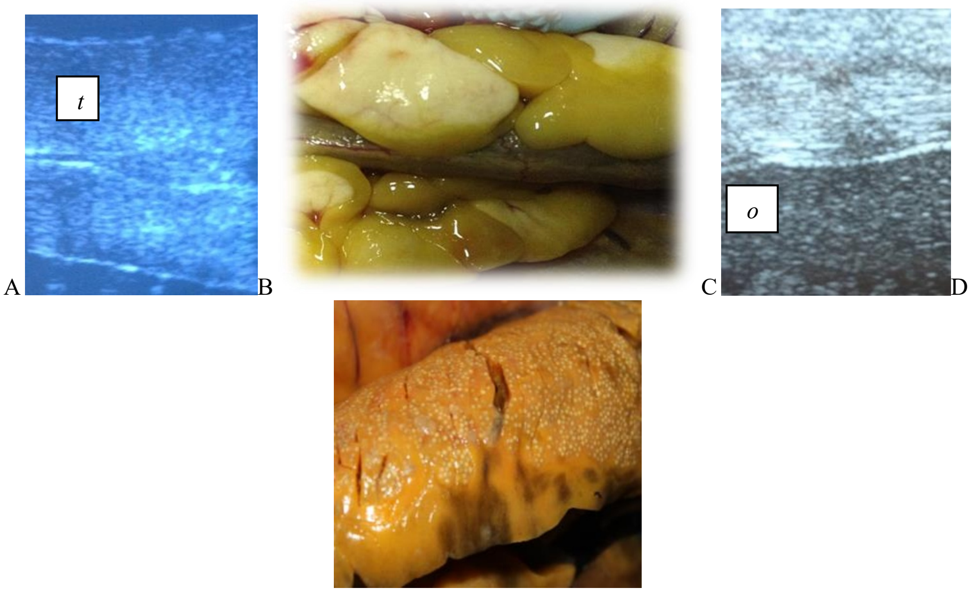


Fig. 7. A, B - Testes of sterlet male of Kama population, age – 8 years: A - Echogram of testes (*t*), longitudinal scan; B – The condition of the testes after cutting the fish; C, D - Fatty degeneration of the ovaries, Siberian sturgeon, Ob population, age – 11 years: C - Echogram of ovaries (*o*), longitudinal scan; D - The condition of ovaries after cutting the fish

If the technological regime of growing sturgeon fish is not observed in order to obtain sexual products of high fish breeding quality, violations of gametogenesis are observed. The annual absence of wintering period with food deprivation in eight-year-old sterlets of the Kama population led to synchronous and asynchronous hermaphroditism in 12% of individuals from studied fish (figure 8).

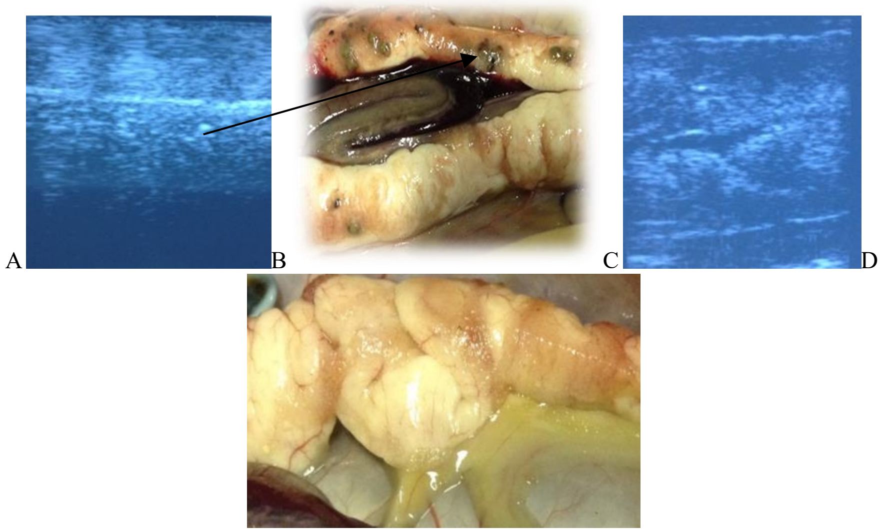


Fig. 8. Ovotestis of sterlet (8 years old) with asynchronous (A, B) and synchronous (C, D) hermaphroditism: A, C - Echograms of ovotestis; B, D – A view of ovotestis after cutting fish

On the echograms ovotestises of such fish are visualized as structures of mixed echogenicity with blurred edges, having similar features to both testes and ovaries.

In rare cases (less than 0.5%) serous anechoic liquid (hydrocele) was visualized in males between paired testes (figure 9). This is usually facilitated by a bacterial disease, injuries, bumps and falls. In case of occurrence the hydrocele does not disappear on its own and tends to progress.

If this disease is detected in sturgeon fish such specimens are subject to culling. If there is a need to save an individual for reproduction (especially domesticated sturgeons from natural populations) it is possible to apply a minimally invasive method of treatment, for example, sclerotherapy.

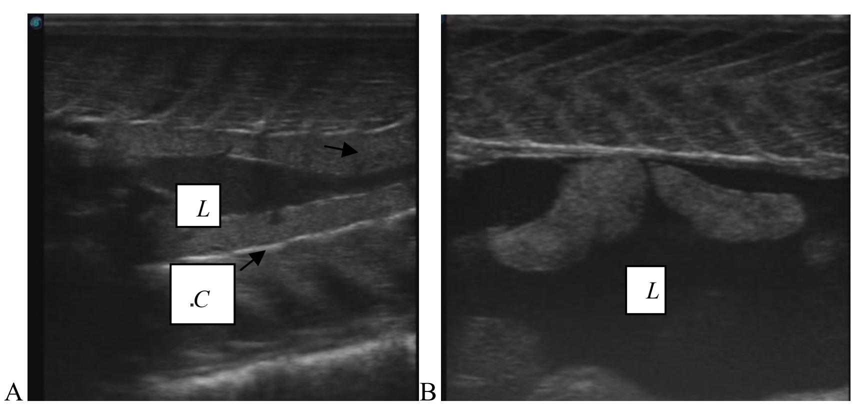


Fig. 9. Echograms of testes of male Siberian sturgeon of Lena (A) and Ob (B) populations: anechoic liquid (*L*) accumulates between the lobes of the testes; there are some small cysts (*C*) in the testes

The serous fluid is sucked off with a syringe under the control of ultrasound, for this purpose a puncture is performed. After removing the fluid sclerosing drugs are injected into the cavity. The mechanism of their action is based on the stimulation of the inflammatory cellular response to the introduction of a chemical substance [12]. For sclerotherapy with hydrocele various substances are used such as tetracycline, betadine (polyvidone-iodine), polydocanol, ethanolamine, phenol, 96% ethyl alcohol.

3.1.3 Pathology of the liver and gallbladder

In sturgeon fish, when visualizing the liver (*hepar*), as a rule the edges are smooth, pointed; the liver parenchyma is homogeneous, hypoechoic, medium granularity, vascularization is moderately pronounced. The liver index is normally 8-10% of the body weight of the fish.

In the detection of liver pathologies single and multiple cysts were visualized (1.9 % of individuals of sturgeon fish from mature stocks) (figure 10).

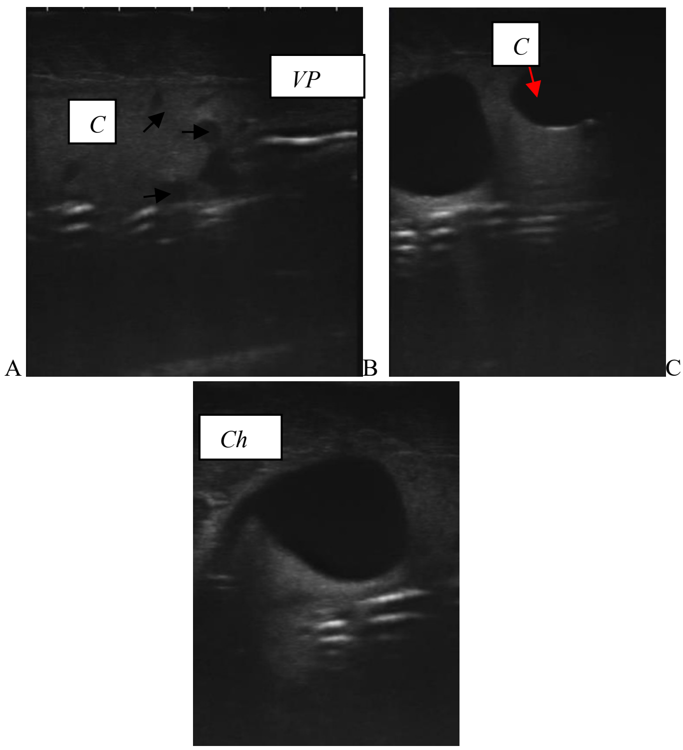


Fig. 10. Echograms of the liver of Siberian sturgeon of Lena (A) and Ob populations (B, C) with polycystic (*C*), dilated portal vein (*VP*) and choledochus (*Ch*); longitudinal and saggital scanning

Liver cysts are cavities filled with fluid, lined from the inside with a layer of cylindrical or cubic epithelium. Most often cysts are filled with a clear liquid that has neither color nor smell. With hemorrhages in the cavity of the liver cyst, the content becomes hemorrhagic; with infection it is creamy, purulent [12].

In polycystic disease cysts compress parenchyma and liver vessels and can become re-infected. Large cysts on ultrasound are characterized by clear contours, rounded shape, anechoic contents, distal enhancement (figure 10B). In small cystic formations the contours are indistinct, there are partitions, the internal contents are anechoic (figure 10A).

The cysts do not pose any serious threat to the health and life of the fish, however, in the event of a ruptured large cysts, hepatomegaly may begin, infection, suppuration in the cavity, peritonitis, liver abscess, massive bleeding in the abdominal cavity, liver failure, malignant degeneration and death [12].

Thus, the liver cysts in fish are completely treatable. The main thing is not to miss the moment of their appearance and control the condition and size of the neoplasm in order to prevent serious consequences.

Cysts in sturgeon fish are most often acquired after injury, stroke, falls or inflammatory processes, infection and antibiotic treatment. The largest number of individuals with polycystic disease (up to 30%) was noted on farms where plastic boxes without water were used for transplantation and transportation spawners.

Sclerosing therapy can also be used to treat individuals with large growing cysts (figure 10B), as well as when detecting hydroceles. The shell of the cyst sticks together under the action of special potent solutions, as a result it significantly reduces its size. The drug is injected into the cavity of the cyst. The procedure is carried out under the obligatory control of ultrasonic device. Some of the pathologies associated with diffuse hemodynamic disorders (the development of congestive and dystrophic changes in the liver) are the expansion of the portal hepatic vein, as well as choledochus (common bile duct) (figure 10C).

An increase the diameter of choledochus more than 1 mm may be one of the signs of obstruction (blockage or blocking) of the common bile duct. One of the first signs of biliary obstruction is an increase in the size of the gallbladder - *vesica fellea* (*vf*). The wall of the gallbladder expands and becomes more winding than normal [12].

To find out the cause of choledochus obstruction it is advisable to examine the liver additionally, pancreas, gastrointestinal tract (*GIT*), lymph nodes (*ln*) of the liver gate. Thickening of the gallbladder walls was observed in some individuals with increased volume of gallbladder (figure 11).

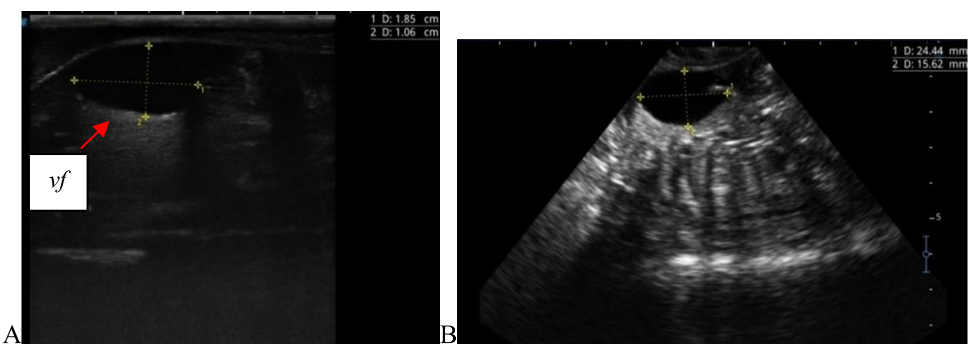


Fig. 11. Liver echograms of the Siberian sturgeon of the Lena population with an enlarged gallbladder of 1.85x1.06 cm (A); longitudinal scan, linear sensor and 2.44x1.56 cm (B); sagittal scan, microconvex sensor

In order to find out whether the increased accumulation of bile with the subsequent stretching and compaction of the walls of this organ is a pathology and the cause of mucocele (dropsy) or other diseases of the gallbladder, it is necessary to conduct a repeated ultrasound examination of this organ during the period of active feeding of fish.

In two sturgeon individuals aged 10 + nodules were visualized in the gallbladder: hyper- and hypoechoic inclusions (figure 12). It should be noted that brightness depends on the chemical composition of the stone, soft cholesterol inclusions do not give an acoustic shadow.

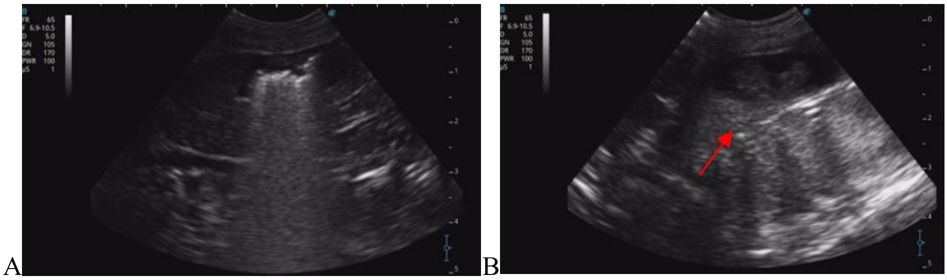


Fig. 12. Nodules in the gallbladder: sterlet of the Kama population (10 +), hyperechoic inclusions of various shapes and sizes (A); Siberian sturgeon of the Ob population (10 +), hypoechogenic inclusions (B); arrow

3.1.4 Heart studies

In 2022, during the spring bonitization period, ultrasound examinations of the heart were performed for the first time in 50 three-year-olds (Lena population) and 50 spawners of the Ob population of Siberian sturgeon (random sample) using pulse wave, tissue and color Doppler in motion mode (M-mode) (figure 13).

Atrium and ventricle, arterial cone and venous sinus were examined. It should be noted that pathological changes in experimental fish were not detected. The number of heart beats was 25±3 beats per minute which corresponded to the physiological norm at a water temperature of 10-12 ºC and deprivation.

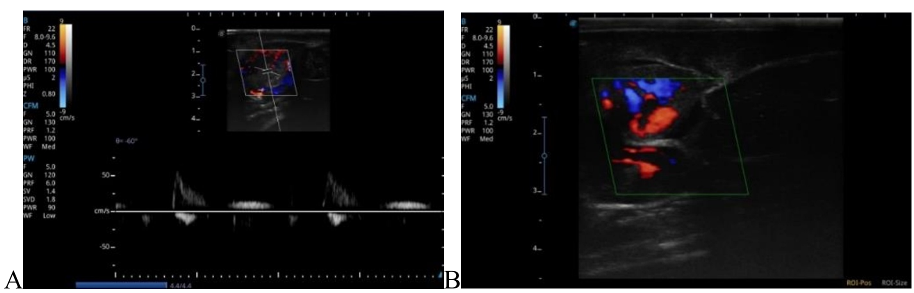


Fig. 13. Echograms of the heart of the Siberian sturgeon of the Lena (A) and Ob (B) populations; longitudinal scanning; color mapping of aortic flow

3.1.5 Random finds

During examining the anterior part of the middle intestine - duodenum with help of high-frequency microconvex sensor in two individuals of the Siberian sturgeon (0.02%) hyperechoic formations resembling foreign bodies were visualized. On the echogram they give a characteristic glow and acoustic shadow (figure 14).

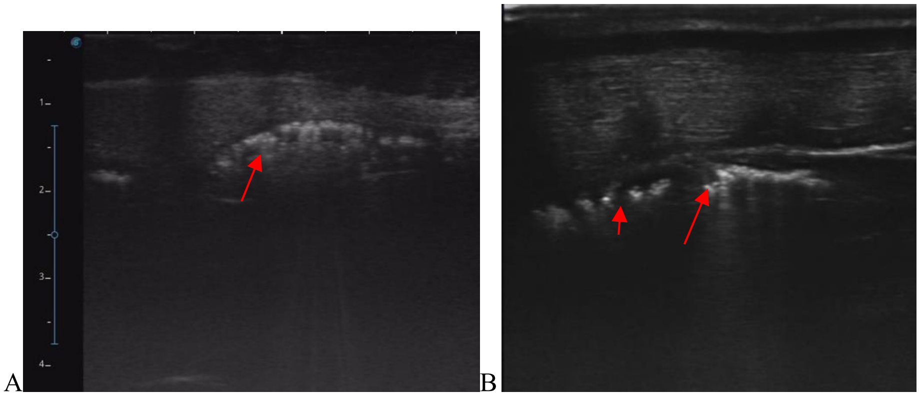


Fig. 14. Echograms of the gastrointestinal tract of the Siberian sturgeon of the Lena population (spawners from natural environment); longitudinal scanning; foreign bodies (arrows)

The foreign body of the gastrointestinal tract is an object that entered the digestive tract from the outside or formed in it itself, but it is not a kind of food in composition [12].

To clarify the nature of echographic artifacts (wood, glass, metal, plastic, etc.) during repeated ultrasound examination, it is necessary to determine the nature of the acoustic shadow and conduct more detailed study of the gastrointestinal tract of these individuals, especially during the period of active feeding of fish.

If foreign bodies remain in place, they are not clinically manifest or they are difficult to remove due to their small size, then they can be left in place.

3.1.6 Research of sturgeons with "Ink spots"

During the domestication of Siberian sturgeon of natural origin in two individuals after a year of domestication in the tanks with recirculation water system at the Chernyshevsky fish hatchery (Yakutia), the appearance of "ink spots" on the skin was revealed (figure 15 A). A year later the number of spots in one individual increased by more than 3 times (figure 15 B).

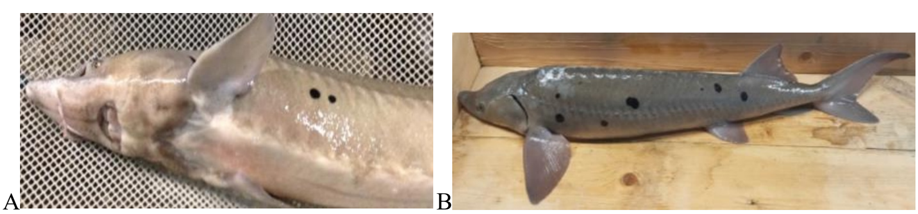
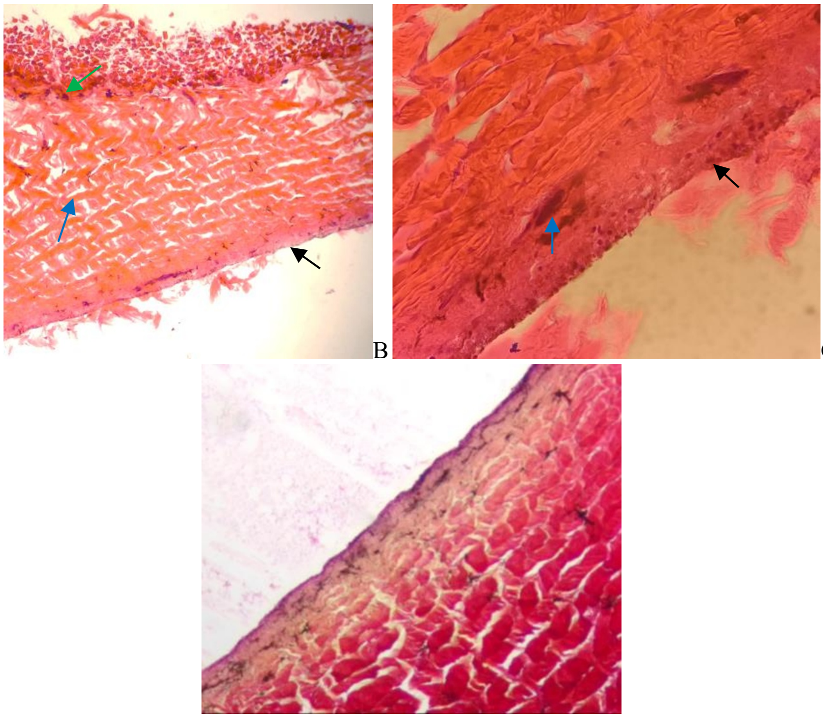


Fig. 15. "Ink spots" on the skin of Siberian sturgeon Lena population of natural origin: A – after one year of domestication; B – after two years of domestication

Sturgeon skin in normal. Sturgeon skin (figure 16A) consists of epidermis (black arrow), dermis (blue arrow), hypoderma (green arrow). The epidermis (figure 16B) is represented by three rows of cells of a flat non-threshold epithelium of cubic shape (black arrow), oval nuclei located in the center of the cell. The epidermis contains melanocytes in the cytoplasm which contains the pigment melanin (blue arrow).

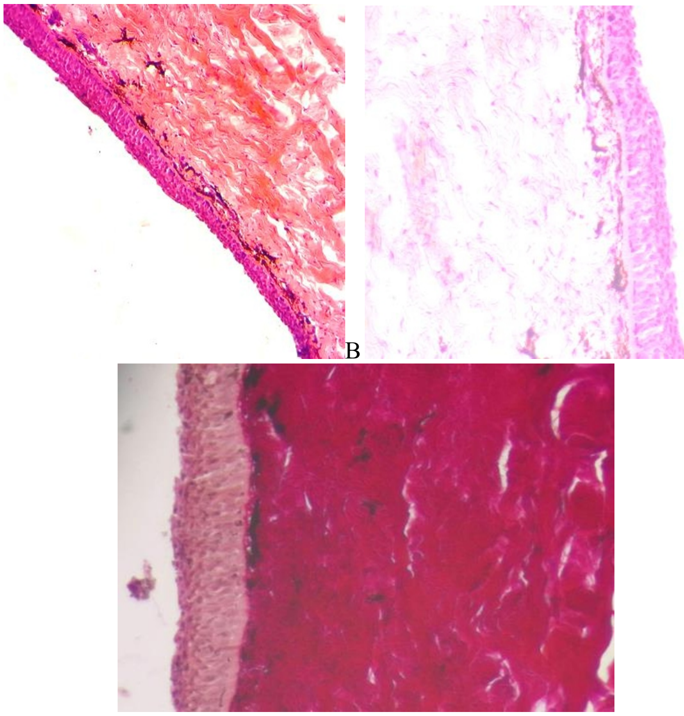


A B C

Fig. 16. The histological structure of Siberian sturgeon skin is in **normal** condition: A - Hematoxylin-eosin coloration, magnification 100; B – Hematoxylin-eosin coloration, magnification 400; C – Coloration according to Van Gison, magnification 100, orange part is the elastic fibers of the epidermis, red one is the collagen fibers of the dermis

They are well organized and grouped in dense groups throughout the dermis under the basement membrane of the epidermis. At the base of the epidermis there are elastic fibers. In the figure 16C the elastic fibers of the epidermis are light orange color, the collagen fibers of the dermis are dark red ones. The dermis is represented by collagen fibers. Fibers spread in different directions in several rows and alternate with each other.

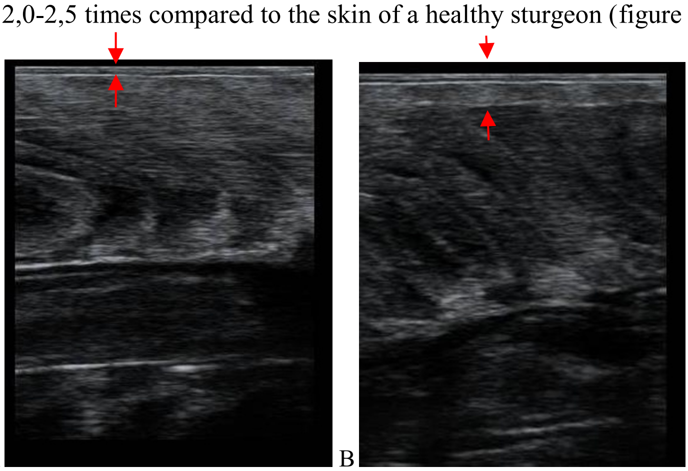
Sturgeon skin in pathology. In the skin with pathology (figure 17 A, B) the cells of the epidermis grow. The cells are initially elongated rectangular shape. The nuclei are shifted to the periphery, the remaining layers are represented by a cubic shape with small areas of erosion. The number of melanocytes with melanin are increased both in the epidermis and in the dermis. They are located in clusters and contain pigment in the lesions. At the same time there are no elastic fibers of the epidermis after coloration according to Van Gieson staining (figure 17 C).



A B C

Fig. 17. Histological pattern of Siberian sturgeon skin with "ink-spot": A - Increase in the epidermis of the sturgeon skin, hematoxylin-eosin staining, magnification 100; B - Increased amount of melanin in the skin, hematoxylin-eosin staining, magnification 400; C - Increase in the epidermis of skin, red color – collagen fibers of the dermis, Van Gieson coloring, magnification 400

Ultrasound research of sturgeon skin in the area of "ink spots" showed a thickening of the epidermis in 2,0-2,5 times compared to the skin of a healthy sturgeon (figure 18).

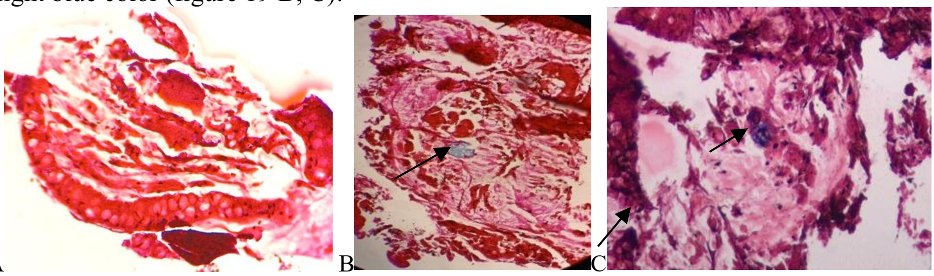


A B

Fig. 18. Echograms of skin and muscle tissue of Siberian sturgeon Lena population; longitudinal scanning; linear sensor: A – Healthy individual; B - Epidermal thickening in the individual with "ink spot"

On the echogram the skin epithelium under the "ink spot" is visualized as a double stripe with clear light hyperechoic borders and internal hypoechoic structure.

Histological study of sturgeon "ink spots" showed the follow. The "ink spot" consists of round-shape cells with light cytoplasm (figure 19A). The cells are arranged in 2-3 rows. The ring-shape nuclei are shifted to the periphery. After staining the biopsy by Van Gieson it was revealed that the central part of “ink spot” consists of collagen fibers and lumps of melanin of light blue color (figure 19 B, C).



A B C

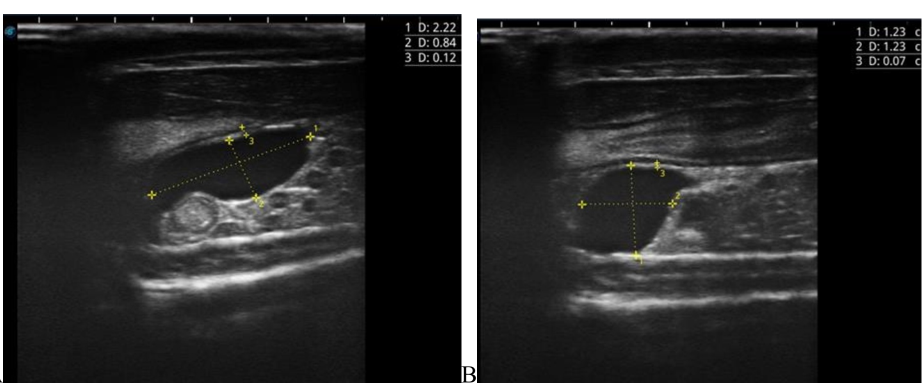
Fig. 19. Histological pattern of "ink-spot" from Siberian sturgeon skin: A - Hematoxylin-eosin coloration, magnification 100; B - Van Gison coloration, magnification 200; C - Hematoxylin-eosin coloration, magnification 200, arrows - melanocytes with melanin

All these changes may indicate melanoma. Usually there is a slight difference between precancerous melanosis and melanoma, while in the first case the number of pigmented cells (melanophores) are increased but limited to the dermis (most lesions in this study). In the second case the amount of pigmented cells (melanophores) increased, melanophores penetrate into the underlying tissues. In the future monitoring of the condition of the fish should be continued. If its health deteriorates, appropriate treatment must be carried out.

3.2 Salmon fish

3.2.1 Ultrasound of the liver and biliary system

The gallbladder. During ultrasound examination the gallbladder of trout yearlings is normally visualized as an anechoic formation with thin walls. In longitudinal scanning the shape of the gallbladder is pear-shaped or oval with narrowing in the neck area. The wall of the gallbladder is represented by a uniform thin line of increased echogenicity. The length of the gallbladder is normal 1.2-2.5 cm, width (in the most expanded place) is up to 0.8-1.2 cm; wall thickness is 0.07-0.12 cm (figure 20).



A B

Fig. 20. Visualization of the gallbladder in trout yearlings (during the feeding period); **norm:** A – the size of oval gallbladder is 2.22x0,84x0,12 cm; B - the size of pear-shaped gallbladder is 1.23x1,23x0,07 cm

https://doi.org/10.1051/bioconf/20248401052

In industrial aquaculture with help of using Coppens production feeds the number of individuals with a normally developed gallbladder was 83.3% after a year of growing rainbow trout. At the same time during ultrasound diagnostic of gallbladder it was noted that there were some pathologies in individuals (16.7%): wall thickening (in 1,3-1,7 times), reducing the size of the gallbladder (in 1,5-2,0 times), mineralization, calcinates as characteristic of chronic cholecystitis were noted (figure 21).

The gallbladder wall was uneven, asymmetrically thickened, with increased echogenicity. The color of bile can range from bright yellow to dark green. It should be noted that the number of trout and Atlantic salmon with gallbladder pathologies during commercial cultivation increases sharply depending on the age of the fish - for two-yearlings their number increases to 25.5%, for three-yearlings - to 46.8%.

Sediment and echogenic bile in the gallbladder are found during ultrasound in all age groups of salmon fish both during feeding and food deprivation (up to 15.5% of individuals). The causes and clinical significance of the sediment are not yet clear. In most cases, if a disease of the hepatobiliary system is not detected, it can be considered as a random finding. More often, the sediment does not give an acoustic shadow.

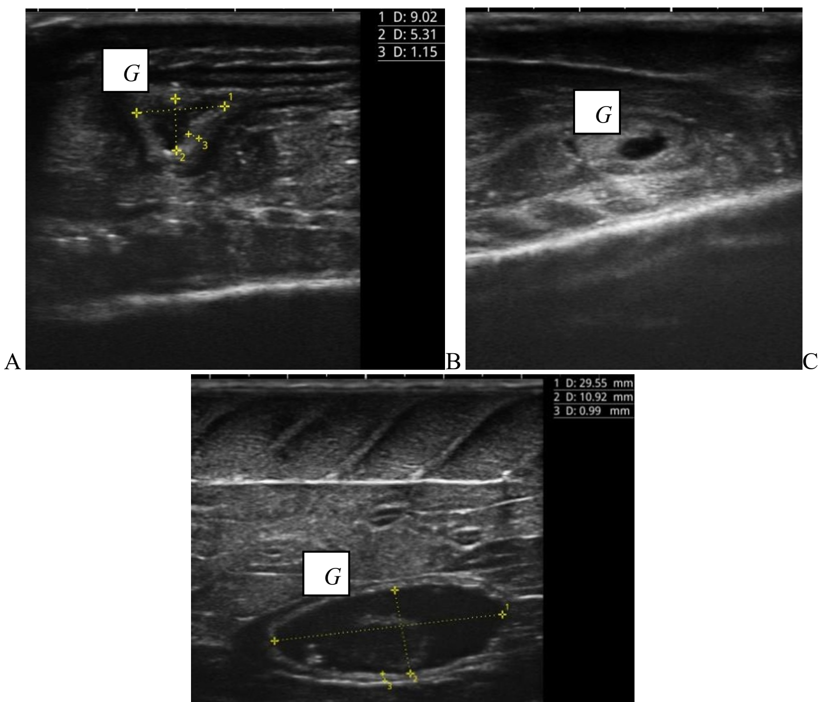


Fig. 21. Gallbladder *(G)* echograms in trout (during feeding); **pathology** - signs of chronic cholecystitis: A – one-yearling fish, the size of gallbladder is 0,9x0,53x0,12 cm; B – two-yearling, the walls of the gallbladder are strongly thickened and mineralized; C – three-yearling fish, the size of the gallbladder is enlarged (2,96x1,09x0,01 cm), there are calcifications

Liver. When examining the liver of salmon fish, the cranial part of the belly of the fish from the right pectoral fin was placed on its back and examined with a transducer, moving the sensor towards the caudal and dorsal parts of the body using a transverse-longitudinal scan. Ultrasound longitudinal scanning of trout and Atlantic salmon revealed that the liver (*L*) is located cranially, on the echogram between the muscles of the back and the pyloric appendages (*PA*) (figure 22).

https://doi.org/10.1051/bioconf/20248401052

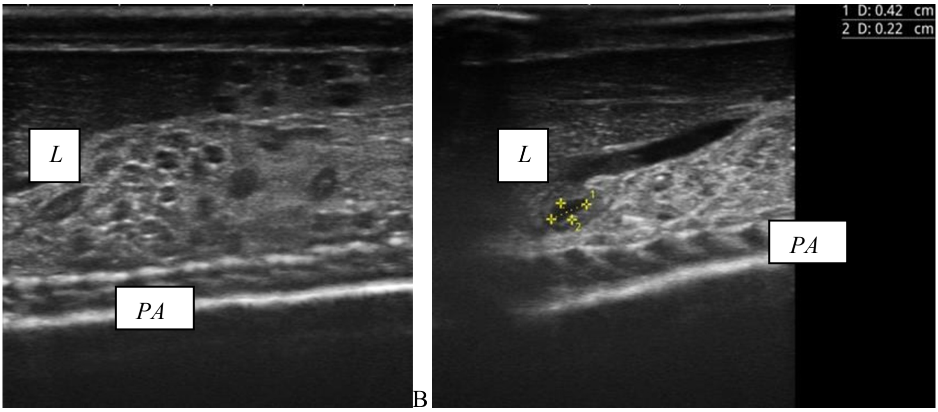


Fig. 22. Echograms of liver (*L*) and pyloric appendages (*PA*) in rainbow trout yearlings: A - normal, B – pathology

Before puberty the hepatic gland is well visualized. In adult females before spawning generative ovarian tissue can almost completely cover the lobes of the liver. Only a small part of the gland is visible on the echogram (figure 23).

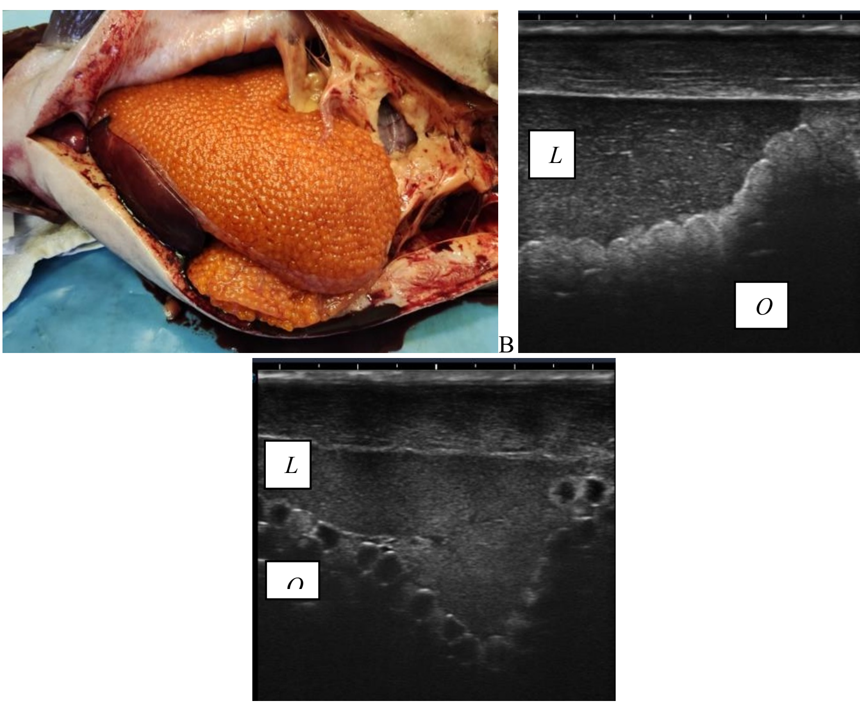


Fig. 23. Three-yearling trouts reared in cages, Barents Sea, Kola Peninsula: A - Localization of internal organs; B - Echogram of liver (*L*) and ovarian (*O*), female, IV incomplete stage of maturity (F4i); C - Echogram of liver (*L*) and ovarian (*O*), female, IV complete stage of maturity (F4)

In normal parenchyma of salmon fish liver is homogeneous, fine-grained, medium echogenicity, acute edges; vascularization is moderately pronounced. The caudal margin of the liver is not always visible, especially in large individuals due to developed numerous pyloric appendages.

It should be noted that in the majority of studied trout yearlings (93.3% of individuals), the hepatic gland was normal. Liver index, on average, was 1,44±0,21%, minimum - 0.94%, maximum - 1.90%, which corresponded to the physiological norm of trout. In two-year-old trout and Atlantic salmon, the number of individuals without liver gland pathologies was 86.5%, for three-year-old fish - 64.1%.

It should be said that after using production feeds in biennials, unlike yearlings, dystrophy of the hepatic gland is observed. So, for trout yearlings, the average liver index is 1,44±0,21%; after a year of cultivation for biennials this index decreased by 2.06 times - 0,70±0,2%.

Pathology of liver. In 6,7 % of individuals cystosis, increase in echogenicity, dilation of the hepatic veins and caudal vena cava were noted (figure 24). Ultrasonic signs of cystic disease are follow: thin wall, anechoic contents, rounded shape. Acquired cysts can be the result of bumps, injuries or inflammation.

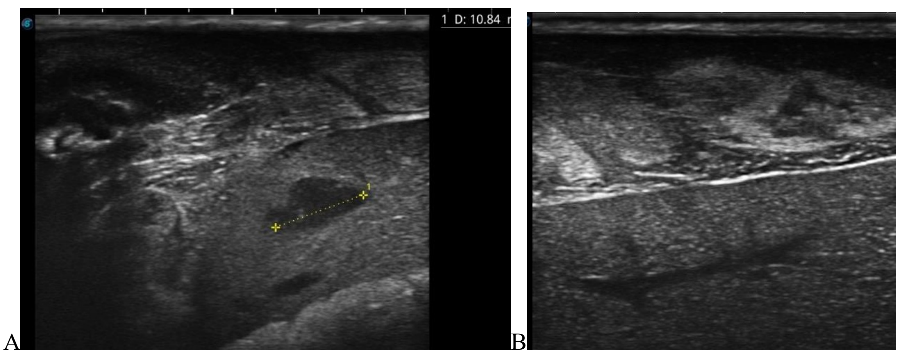


Fig. 24. Liver echograms, **pathology**: A - Polycystic, two-yearling Atlantic salmon: B - Dilation of the hepatic vein; three-yearling trout reared in cages, Barents Sea

3.2.2 Ultrasound of gonads

Topography of the genital glands. The gonads of males and females salmon fish in the first stage of maturity are quite difficult to visualize due to their small size. Topographically, the ovaries and testes are located bilaterally in the cranial area of the abdominal cavity, along dorsal wall. In fact, the genital glands are located outside the coelomic cavity, but only hang, protrude into it, being suspended at the folds of the peritoneum - mesovaria in females and mesorchia in males. Ovaries are "open" type. On the echogram the ovary is visualized only at the second stage of maturity (figure 25A). The cranial margin of the ovaries is extended, rounded, caudal - rounded.

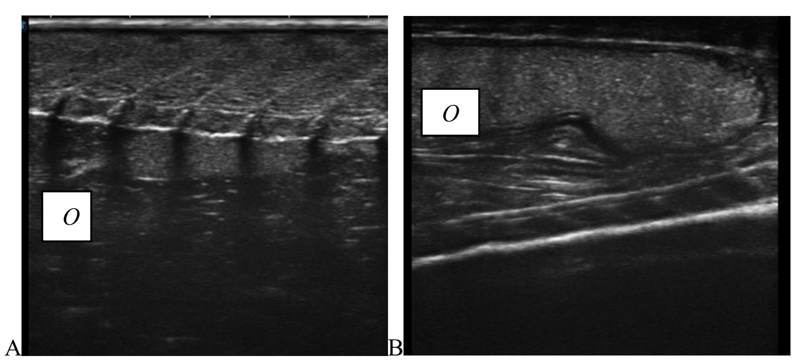


Fig. 25. Ovarian (*O*) echograms of rainbow trout yearlings: A - Stage 2 (F2), B - Stage 3 (F3)

On the echogram, the generative ovarian tissue at the 2nd stage of maturity is visualized as a hypoechoic granular loose structure with uneven edges inside the hyperechoic shell. Unlike sturgeon fish the gonads of salmon at stage 2 are located dorsally and cranially. The ovaries are visualized below the spinal muscle but above the lateral line. In large salmon weighing more than 5-6 kg due to dense thickened muscle tissue and developed pyloric appendages occupying the entire body cavity, a microconvex or convex sensor should be used to visualize the gonads.

In the second stage of maturity the generative tissue is not developed, hypoechogenic, poorly visualized due to the nearby organs that close it (liver, pyloric appendages). Transient stages 1-2 and 2 of ovarian maturity were noted in 50 % of trout yearlings and 68 % of Atlantic salmon two-yearlings. The gonado-somatic index (GSI) was 0.11-0.20 % of body weight.

At the third stage of maturity (F3) the generative tissue of females of mixed echogenicity is visualized as finely granular structure with uneven edges (figure 25 B). In contrast to the 2nd stage of maturity, the generative tissue of females grows in the dorsal, medial and lateral directions. The ovaries of females on the echogram are visualized as a loose cloud-shaped structure of increased echogenicity. Separate heterogeneous oocytes of lighter color are visible. The third stage was detected in 7.1 % of trout females at the age one year; GSI - 2.99 %.

Female trout maturation grown in recycling systems with use of artificial feed was noted at the age of one year (3,5 % individuals). In the echogram (figure 26A) ovaries at the 4th incomplete stage of maturity are visualized as coarse-grained cellular structure of increased echogenicity. In trout yearlings the gonads increase slightly, they are adjacent to the upper edge of the muscle tissue. Gonado-somatic index is 14.1%.

It should be noted that for three years old trout females grown in cages of the Kola Peninsula with seawater, the ovaries are visualized as hyperechoic structures of rounded or oval shape with a diameter of 3.36-3.86 mm; GSI is 20.5 % (figure 26 B). For some large females weighing more than 6 kg the diameter of oocytes reached from 3,9 to 4,4 mm (figure 26 C). The oocytes arrange in dense rows, occupying the entire internal cavity of the body.

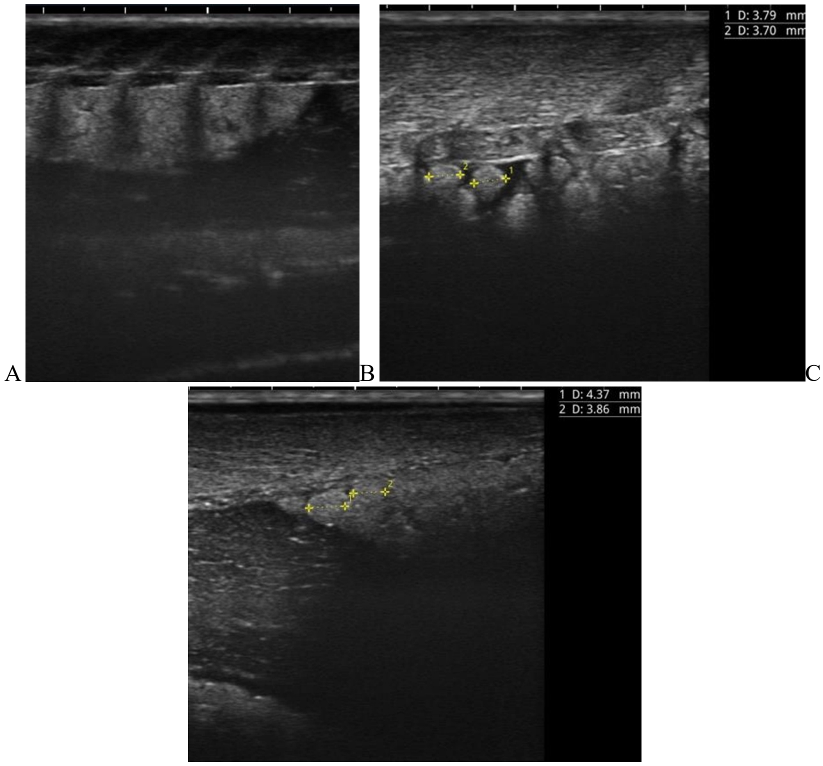


Fig. 26. Echograms of ovaries of rainbow trout at the 4th incomplete stage of maturity (F4i): A – one-yearling female, RUS, tanks, fresh water, Tyumen region; B, C - three-year-old females, cages, seawater, Barents Sea

The first two or three rows are hyperechoic light gray in color. In the underlying rows the echo signal is lost; the structure is anechoic.

At the 5th stage of maturation the ovaries resemble "honey-combs" with anechogenic content, they have clear hyperechogenic boundaries with dark anechoic content (figure 27). On the echogram individual eggs are arranged in rows, occupying the entire body cavity from the dorsal part. Usually the first 2-4 rows are well visualized, the echo signal does not pass below if scanning with a linear transducer takes place. The diameter of mature oocytes before ovulation is from 2.3 to 3.4 mm. Of the diseases and pathologies in gonads, ovarian cysts in 1.6 % of individuals should be distinguished (figure 28 A) and underdevelopment of generative tissue (figure 28 B) due to fatty degeneration.

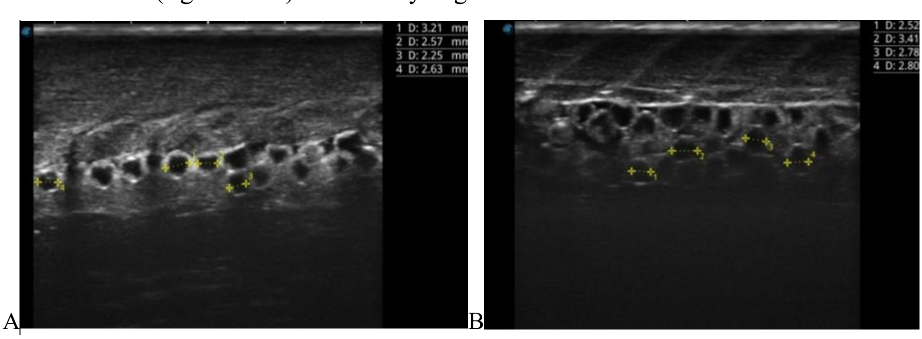


Fig. 27. Echograms of rainbow trout ovaries, cages, seawater, Barents Sea: A, B – F5

Males. The testes of salmon fish are scanned in the sagittal and transversal planes in the cranial region behind the dorsal muscle. It is necessary to withdraw the lower edge of the back muscle and move the transducer caudally to the pyloric appendages.

The testes at the 1st stage of development are thin, white or pinkish-gray color strands, they are not practically visualized on the echogram. Like sturgeon determining the sex of salmon fish at this stage with use of ultrasound diagnostics is difficult.

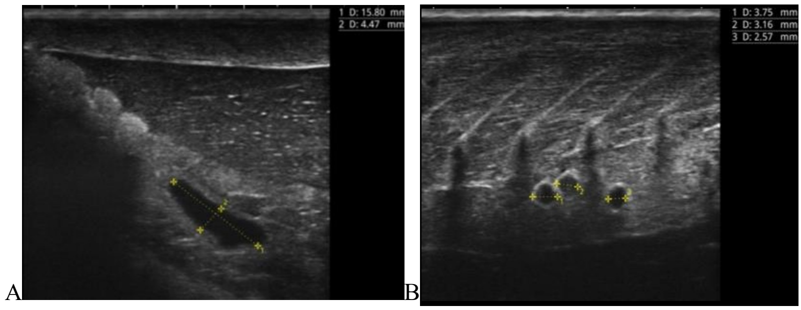


Fig. 28. Echograms of rainbow trout three-year-old females, cages, seawater, Barents Sea: A - ovarian cyst, size 15,80x4,47 mm; B – F5, underdevelopment of generative tissue due to fatty degeneration

The testes at the 2nd (M2) stage are visualized as a hypoechoic fine-grained structure covered with a hyperechoic shell. Narrow gray strands are located by the muscles of the back below the urinary system topographically closer to the head of the fish (figure 29 A). Sometimes the testes on the 2nd stage are not visible on the echogram due to the developed pyloric appendages. In this case it is necessary to use microconvex or convexic transducer.

Testes at the 3d (M3) stage of three-year-old trout salmon males are large, lobed, occupy a significant area of the cranial part of the body. At the 3d stage (figure 29 B) the testes are visualized as a hypoechoic lobular homogeneous fine-grained structure. On the echogram the shells of the lobules are lighter in contrast to the main tissue of the testes, which looks like a dark gray structure.

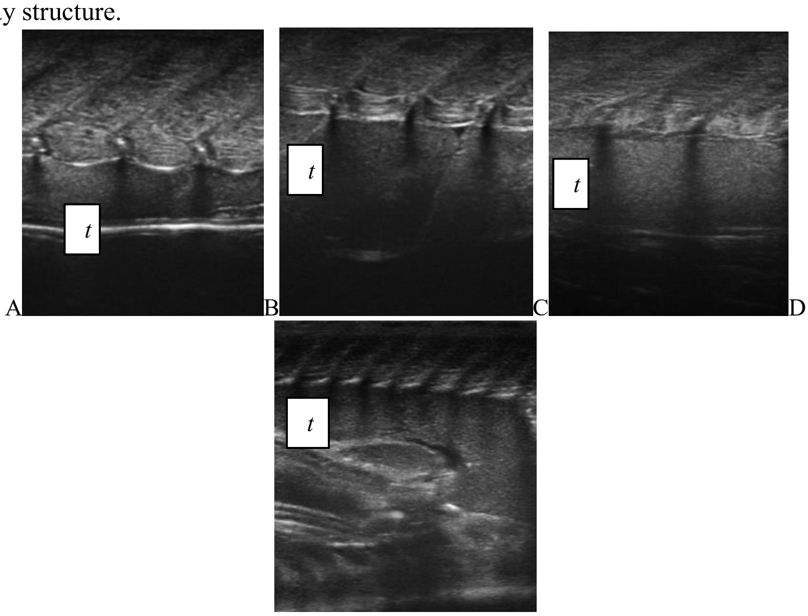


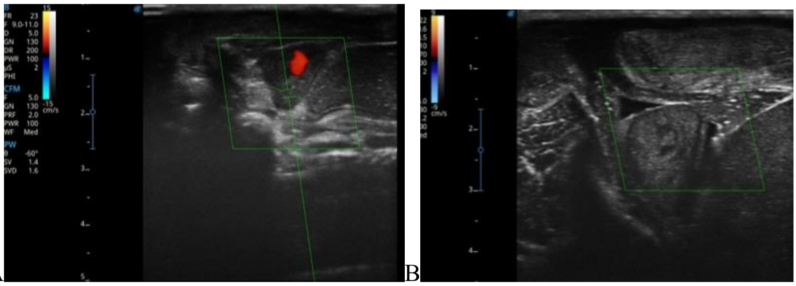
Fig. 29. Echograms of testes *(t)* of rainbow trout three-yearling males; cages, seawater, Kola Peninsula (A, B, C): A - M2; B – M3, C– M4; D - one-yearling trout, M5, tanks, RWS, Tyumen region

At stage 4 (M4) the testes are visualized as hyperechoic fine-grained structure. On the echogram they are light gray color; there is no lobulation. The shells are lighter in color than the tissue of the testes (figure 29 C).

Testes at stage 5th (M5, "fluid" males) were hypoechoic strands of dark gray color, the edges were even (figure 29 D); the GSI was 1.54%.

3.2.3 Ultrasound of heart

There were no pathological abnormalities in the ultrasound study of the heart of trout yearlings (figure 30 A). On the echogram, the heart is hypoechoic, triangular form, the dimensions are not enlarged. Topographically the heart is located cranially, in the lateral-caudal part it adjoins the hepatic gland. Studying three-year-old trout fish grown in cages of the Kola Peninsula with use of artificial feed (figure 30 B) had been shown that 78 % of individuals had increased heart muscle; wall thickening was visualized; arrhythmia was observed.



A B

Fig. 30. Echograms of rainbow trout heart muscle: A - age of one year old (RWS, tanks; Tyumen region); B - three-years-old fish (cages, seawater, Kola Peninsula)

4 Conclusion

Ultrasonography in modern realities is a fairly fast, safe, non-invasive and informative method of visual diagnostics. The method of determining the sex and maturity stages of gonads described in sufficient detail and used in the practice of sturgeon breeding [2] allows not only to continuously improve this biotechnology for sturgeon fish species, but also to develop a new methodology for other valuable aquaculture objects (in particular, salmon).

With the help of comprehensive studies of fish pathologies: the high diagnostic information content of the ultrasound method and laboratory histological analysis, it is possible to identify such dangerous diseases as fish skin cancer at early stages and to outline ways of treatment. The probable cause of the appearance of "ink spots" is the stress state of the adult sturgeon from the natural habitat during the period of forced domestication to the maintenance and feeding in artificial conditions.

The use of ultrasound diagnostics to assess the condition of the internal organs of sturgeon and salmon fish opens up new opportunities for determining the optimal temperature regime, feeding norms, feed recipes with early detection of violations of the process of maturation of sexual products and anomalies of internal organs. This is due to the food security of commercial fish grown in industrial conditions and contributes to the sustainable development of the Russian fisheries.

References

1. M. Korentovich, A. Litvinenko, Siberian sturgeon, **2**, 181-216 (2018) doi: 10.1007/978-3-319-61676-6_12.
2. M. Korentovich, A. Egorov, A. Sheveleva, Proceedings of the national scientific and practical conference "Integration of science and education in agricultural universities to ensure food security in Russia", ISBN 978-5-98346-109-3, 25-37 (2022)
3. M. Chebanov, Elena Galich, Sturgeon Hatchery Manual, 240-291 (2013)
4. J. Merkulov, I. Markov, Practical Guideline for Ultrasound Diagnostic of Sturgeon. Atlas, 6-42 (2017)
5. M. Chebanov, P. Williot, Siberian sturgeon, **2**, 217-286 (2018) doi: 10.1007/978-3-319-61676-6.
6. M. Chebanov, E. Galich, Siberian sturgeon, **2**, 529-567 (2018) doi: 10.1007/978-3-319-61676-6.
7. M. Chebanov, E. Galich, Topical issues of fishing, fish farming (aquaculture) and ecological monitoring of aquatic ecosystem, **3-4**, 84-89 (2018)
8. M. Chebanov, E. Galich, Fish farming, **3-4**, 10-17 (2019)
9. G. R. Lisciandro, Focused Ultrasound Techniques for the Small Animal Practitioner, 44-64 (2014)
10. D. S. Bucy, D. Sanchez-Migallon Guzman, A. L. Zwingenberger, Scientific Reports, **246-8**, 868-873 (2015)
11. D. Pennick, M. Andre Anjou, Atlas of Small Animal Ultrasonography, 250-380 (2015)
12. J. S. Matton, T. G. Nyland, Small Animal Diagnostic Ultrasound, 94-211 (2015) doi: 978-1-4160-4867-1
13. E. Danilova, Fish farming, 3-4, 46-49 (2019)
14. E. Danilova, Fish farming, 3-4, 63-64 (2020)
15. E. Sirotkina, V. Khrisanfov, S. Kiknadze, Fish farming, **1-2**, 53-54 (2019)
16. E. Ermyshova, M. Korentovich, Topical issues of science and economy: new challenges and solutions, 313-317 (2019)