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Broodstock Formation and Sterlet (*Acipenser ruthenus*) Reproduction in the West-Kazakhstan Region

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Abstract: A dramatic decline of sturgeons' stocks including the sterlet (*Acipenser ruthenus*) in the Caspian Sea including creates the need to establish aquaculture broodstock. Broodstock management can allow conserving the gene pool of the sterlet and the creation of captive broodstock further serve as a source of sterlet fry aimed at restoring natural populations of the sterlet in the Caspian Sea. In this regard, the study aims to develop the technology of sterlet broodstock formation in the pond-based sturgeon hatchery and recirculatory aquaculture systems in the West-Kazakhstan region. Captive sterlet broodstock formation was carried out by two methods-domestication and from the egg. Both methods were effective in sterlet broodstock management. It was the first sterlet brood stock program carried out in the sturgeon hatchery in Kazakhstan in which brood fish were taken from the natural populations of sterlet in the Kazakhstan parts of the Caspian Sea. The sterlet broodstock was genotyped using mitochondrial DNA markers. Sequencing of the control region of mitochondrial DNA in the studied individuals, for the purpose of species identification and detection of maternally inherited variability, reveals that captured broodstock from natural populations belongs to *Acipenser ruthenus*. As a result of artificial breeding in the hatchery 3.6 kg of fertilized eggs was obtained from 34 females and 20 males. Genetic analysis was carried out to create a genetic passport of sterlet for the Kazakhstan populations. It also revealed that at least 20 pairs of brood stock are required to avoid inbreeding and conserve the genetic diversity of the sterlet in the hatchery system. For this purpose, the structure of the recirculatory aquaculture system should provide the possibility for industrial breeding of sterlet in the region.

Keywords: Formation, Sterlet, Broodstock, Domestication, Reproduction, Circular System, Genetic Analysis, Eggs, Larvae, Sexual Products

Introduction

The Ural-Caspian basin is a unique aquatic fishery basin as the Ural River is the only river with an unregulated channel in the lower reaches where the main spawning grounds of sturgeon fish are located and where natural spawning of sturgeon is still recorded (Vasilyeva and Rabazonov, 2022; Bulgakova *et al*., 2016). Such an opportunity allows sturgeon farms in Kazakhstan to form broodstocks from sturgeon breeders that go to spawn in the Zhaiyk River (Ural) (Khodorevskaya *et al*., 2009).

Sterlet (*Acipenser ruthenus* (Linnaeus, 1758) belongs to the relict species of fish of the Caspian region with a habitat in the Zhayk River (Ural) and is the object of commercial demand for black caviar and meat. Anthropogenic factors, including poaching, affected the structure and size of its natural population (Sudakova *et al*., 2018; Bronzi *et al*., 1999). Under these conditions, there is a need to accelerate the formation of sterlet production herds in controlled conditions of detention for the purposes of artificial reproduction and commercial sturgeon breeding

(Stakėnas *et al.*, 2019). In the current economic and environmental conditions in the Republic of Kazakhstan, the only legal way to produce marketable products from sturgeon species of fish is their cultivation at fish farms, so commercial aquaculture of sturgeon is an urgent necessity (Kokoza, 2004).

Genetic researches of sturgeons are an integral part of monitoring artificial reproduction and preservation of natural populations (Birstein *et al*., 1993). It is generally known that fish brought from other regions and hybrid species are cultivated in aquaculture conditions which complicates their correct species identification (Miuge *et al*., 2008). The genetic identification is necessary to preserve and replenish the number of sturgeons before stocking natural water bodies and breeding the sterlet in aquaculture (Welsh *et al*., 2003). The genetic monitoring system allows us to assess the level of the genetic kinship of individuals involved in obtaining sexual products for artificial reproduction and identify offspring obtained from breeders genotyping (Pyatskowit *et al*., 2001). Genotyping allows for identifying and preventing closely related crosses which directly lead to inbreeding.

About sturgeons in Kazakhstan. Due to the reduction of natural reproduction of sturgeons and intensification of hydrocarbon production on the north-eastern coast of the Caspian Sea, Atyrau Sturgeon Hatchery (present Ural-Atyrau Sturgeon Hatchery Republican State Public enterprise) was built and commissioned in 1998 as a compensation facility located in the Ural River Delta. This Ural-Atyrau Sturgeon Hatchery RSPE was designed by Kazgiprorybhoz Institute (Almaty) in 1996. The Facility was built for compensation funds of Kazakhstan kaspi shelf by Glavbolgarstroy Construction Company (Sofia). The facility engages with replenishing stocks of sturgeon species in the Caspian Sea by releasing juvenile sturgeon species obtained and grown artificially into the Ural River. It is located 7 km from the cut of the Caspian Sea and 10 km from Atyrau city.

The research study is to form sterlet broodstock and obtain viable juveniles in RAS located in the Caspian Region of Kazakhstan.

Materials and Methods

The study object is the sterlet breeders collected selected from the Zhaiyk (Ural) River by domestication into the broodstock on two sturgeons Farms-Ural-Atyrau Sturgeon Hatchery RSPE and Kazakh osseter research and production enterprise LLP (Private Sturgeon Hatchery) of sterlet juveniles obtained and cultivated in RAS at sturgeon farm of the Ural-Caspian basin of Aktau City.

We used the methods developed by Kazakh and international scientists while forming the sterlet broodstock (Bell *et al*., 2008; Aubakirova *et al*., 2023).

Chipping of broodstock sterlets was performed in order to identify accurately each individual while selecting parent-paid, track increments, and assess the reproductive qualities of each breeder (Fig. 1) (Chebanov and Galich, 2010).

In order to keep accounts and control over the condition of sturgeon species broodstock, identify the level of maturity and physiological condition of fish, predict ripening and possible volumes of fish breeding products, spring, and autumn valuation was performed (Tat'iana Antonovna Detlaf *et al*., 1993).

Fig. 1: Process of chipping and reading tags

While conducting a spawning campaign, females were assessed for working fertility by calculating relative fertility to fertilization percentage, relative fertility was calculated per 1 kg of fish weight (Kazansky, 1956).

A visual assessment of the ovulated unfertilized egg (color, consistency, and amount of ovarian fluid, appearance of eggs, and presence of foreign inclusions), simultaneous or prolonged ovulation, and egg filtering were carried out (Pravdin, 1966; Mylonas *et al*., 2010; Vasilyeva and Rabazonov, 2022). Daily samples of laid eggs and hatched preserves were taken and their quality was assessed, embryonic development was analyzed and the following indicators were determined:

- Percentage of fertilization
- Development of embryos at different stages
- Duration of pecking
- Percentage of larval pecking

The stages of embryonic development of the studied species were determined according to.

Mitochondrial DNA Analysis

Separation and further purification of DNA from starlet fins were performed by absorption on columns (PALL) and control of the quality on spectrophotometer SPECTRAmax PLUS 384. DNA was stored at-20°C prior to use.

Analysis of mitochondrial DNA polymorphism was carried out by PCR amplification using primers.

DL651 (ATCTTAACATCTTCAGTG).

M13AHR3(TCACACAGGAAACAGCTATGACA TACCATAATGTTTCATCTACC).

PCR reactions contained about 100 ng of DNA and were in the volume of 15 µL (70 mMTris-H Cl (pH 8.3), 16.6 mm $(NH_4)_2SO_4$, 2-3 mm $MgCl_2$, 100 µm each deoxyribonucleoside triphosphate, 1.5 pkM of each of the primers, 1-unit Color Taq polymerase). Amplification was carried out according to the following scheme: Preliminary DNA denaturation: 95°C-10 min, synthesis of PCR products (30 cycles): Melting-94°C-20 sec, annealing of primers -52°C-40 sec, DNA synthesis-72°C-60 sec, final completion of chains: 72°C-10 min. The amplification result was checked by agarose gel electrophoresis with ethidium bromide staining. Sequencing of the control region of mitochondrial DNA was carried out from one strand with universal primer M13R (TCACACAGGAAACAGCTATGA). Multiple sequence alignment was performed using the Seqman program of the LaserGene bioinformatics package (USA). For each individual, a mitochondrial haplotype was determined and compared with the database of mt haplotypes characteristic of sterlet.

Establishing the species affiliation of the test sample was carried out by comparing the obtained nucleotide sequence of the mitochondrial DNA control region with the reference sequences of sturgeon fish deposited in the international database of sequences of the mitochondrial DNA section by the method of searching for paired coincidences (BLAST). For this purpose, an open database NCBI-National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) is used.

Results and Discussion

Sterlet broodstock formation began in 2016. There are two methods to form the sterlet broodstock-domestication of wild breeders and cultivation of the fish until maturity from the early stages of ontogenesis. Over the past few years, the plant has tested and introduced modern methods of working with breeders, such as lifetime production of sexual products, electronic tagging of breeders, and diagnostics of gonadal maturity. The sexual structure and quantitative composition of the formed sterlet broodstock are presented in (Table 1).

Females prevail in the quantitative structure of the sterlet brood stock. An increase in the total biomass of the sterlet broodstock is provided in the graph (Fig. 2).

Based on the graph of the total increase in biomass of the sterlet species, we can conclude that during the period of maintaining sterlets at Ural-Atyrau Sturgeon Hatchery, the fish has a stable weight gain which is slightly low in the spring and apparently associated with reproduction processes and losing weight from the gonads.

In 2021, 19 sterlets (*Acipenser ruthenus*) breeders were caught in the Ural River and transported to the base by special live-fish containers. All caught individuals were placed for adaptation and domestication to the Kurinsky-type tanks (Kazansky-type ponds). The feeding diet was constituted of a 50/50 feeding mixture of minced fish and artificial feed. Sterlet domestication began with the adaptation of wild females to maintain them in tanks. During the work with breeders, special attention was paid to creating favorable conditions for their maintenance (regular fish feeding with high-quality, full-fledged feed, favorable sanitary and epizootic conditions, and compliance with biotechnology). The adaptation period was 6 months, over the past period, all breeders switched to 100% artificial feeds and all breeders were transferred to the RAS. The survival rate of breeders during adaptation is 100%. Characteristics of the sterlet specimens caught are presented in (Table 2). The number of females caught in 2021 was 12, all sexually mature, and the number of males was 7.

Table 1: Structure of the formed sterlet broodstock at Ural-Atyrau Sturgeon Hatchery

Structure of the formed sterlet broodstock		
Year of caught	Females	Males
2016	2	4
2017	4	5
2018	5	3
2019	9	15
2020	14	8
2021	12	8
Total	46	43

Table 2: Characteristics of the sterlet (*Acipenser ruthenus*) specimens caught in 2021

Indicators		
Gender	Length, cm	Weight, kg
♀	50	0,9
♀	60	1,5
♀	58	1,3
♀	48	1,5
♀	50	1,0
♀	67	2,1
♀	62	2,2
♀	52	1,3
♀	57	1,6
♀	60	1,7
♀	62	2,0
♀	60	2,0
♂	48	1,8
♂	59	1,5
♂	50	0,7
♂	65	1,3
♂	56	1,2
♂	52	1,0
♂	62	2,0

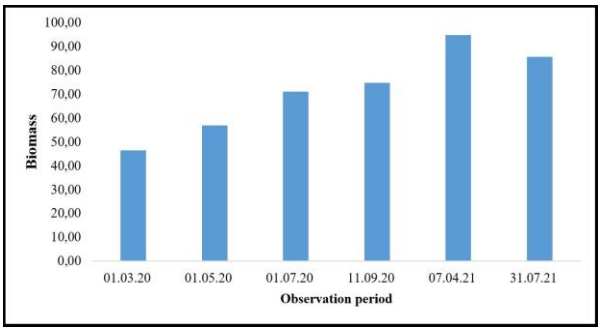

Fig. 2: Increase in the total biomass of sterlet broodstock species formed at Ural-Atyrau sturgeon

The average weight of the sterlet female caught in 2021 was 1.6 kg with a length of 57.2 cm., minimum weight of the females was 900 g at a length of 48 cm and the maximal weight of the female sterlet was 2.2 kg with a body length of 67 cm. The average weight of a male sterlet caught in 2021 was 1.36 kg with a length of 55.7 cm, the minimum weight in male sterlet was 700 g with a length of 48 commands maximum weight of male sterlets was 2 kg with a body length of 65 cm. During the adaptation of wild sterlet breeders to maintaining in artificial conditions, special attention was paid to switching them to unusual feeds. During the first month of placing wild breeders in the ponds, they were not fed during the first week as they had been adapting to the new living conditions. From the second week, the feeding was started at a temperature not lower than 10°C, the feed was provided at the rate of 1% sterlet biomass controlling its digestibility daily. In case of unsatisfactory feed intake, adjustments were made to feeding, and the daily ration was reduced. Feed mixtures consisting of 50% dry loose feed and 50% minced fish were used.

Such feed contains 39% protein and 10% fat and in terms of feed mixture 27% protein and 7% fat. Feeding of fish in tanks was carried out at feeding places or feeding grounds. The average feeding rate for breeders was 2% per day of fish biomass. With normal feed intake, the feeding rate in the ponds was increased to 3% per day. Using these daily feeding rates of breeders allows the most rational use of the feed used. Received fish breeding standards for the domestication of "wild" sterlet breeders are given in (Table 3).

Maintaining Sterlet Broodstock at Ural-Atyrau Sturgeon Hatchery

RAS has a favorable temperature for the growth of individuals of the Siberian sturgeon, it was 22.6°C on average varying between 18.5-25.3°C (Figs. 3-6).

Oxygen regime ranged from 7.5-9.1 mg/dm³ and on average amounted to 8.43 mg/dm³ which does not go beyond the standard limits.

Table 3: Fish breeding standards of domestication of «wild» sterlet breeders at UASH RSPE

Fish breeding standards of domestication of «wild» sterlet breeders	
Indicator	Meaning
Place of preparation	Ural (Zhaiyk) river
Transportation	Live fish slots
Used feed at the beginning of adaptation feed	Feeding mixtures from minced fish and artificial in the proportion of 50/50
Used feed at the end of adaptation	Artificial feed
Adaptation period, month	6
The survival rate, %	100

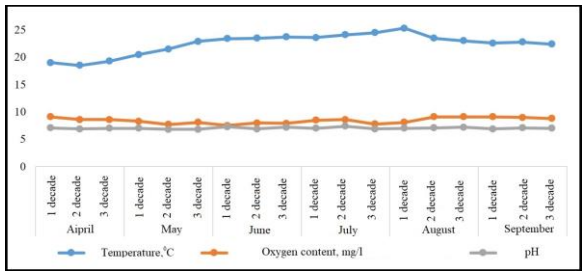

Fig. 3: Temperature and oxygen regimes of RAS where the sterlet broodstock species are maintained

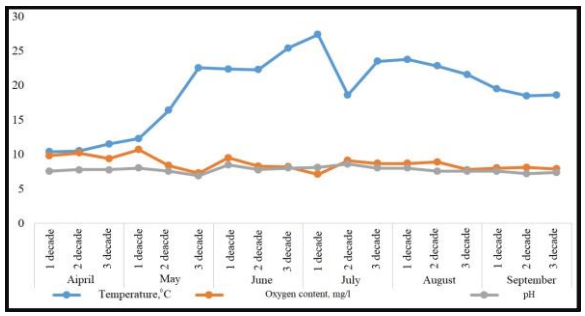

Fig. 4: Temperature and oxygen regimes of ponds with the sterlet broodstock species at Ural-Atyrau Sturgeon Hatchery

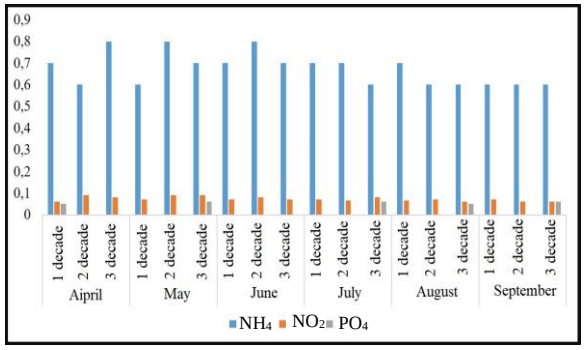

Fig. 5: Monitoring of biogenic elements content in RAS where the sterlet broodstock species are stored at the Ural-Atyrau Sturgeon Hatchery

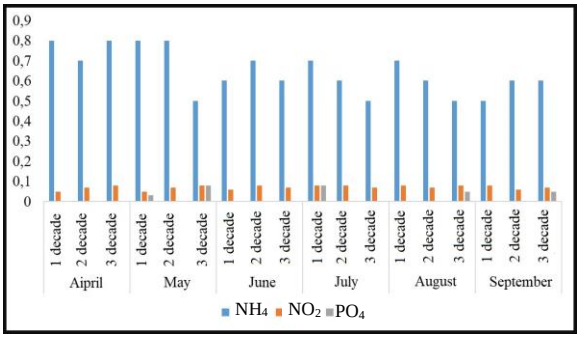

Fig. 6: Monitoring of biogenic elements content in the ponds where the sterlet brood stock species are stored at Ural-Atyrau Sturgeon Hatchery

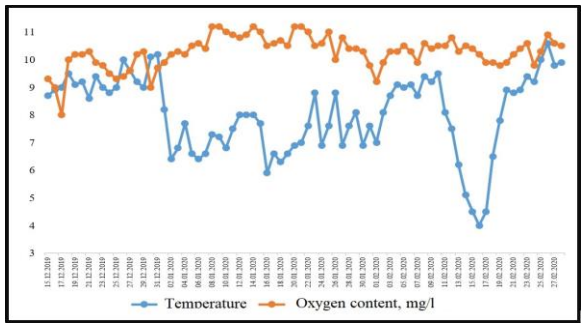

Fig. 7: Graph of temperature and oxygen regimes during wintering of the sterlet breeders

In ponds, the temperature was also favorable for the growth of individuals of the Siberian sturgeon, it was 19.33°C on average varying quite strongly in the range of 10.4-27.4°C (Fig. 7). The oxygen regime varied between 7.1-10.7 mg/dm^3 and averaged 8.67 mg/dm^3.

Formation of Sterlet Broodstock at Kazakh Osseter LLP

Immature fish was used to form the broodstock in the RAS and for selecting breeding groups. As the main criteria for selection, morphometric features were used: Body weight, fatness coefficient (KF), body thickness index (B/L), and body girth index for females (O/L) (Table 4).

An effective number of broodstock is determined by the number of interbreeding individuals. In order to reduce the number of individuals of identical origin, it is necessary to ensure the equal contribution of females and males to each subsequent generation. Therefore, to replenish the brood stock per breeder, on average, it is necessary to grow an appropriate number of replacers (Table 5).

30 thousand species weighing up to 2 kg are required in order to fill the RAS. This condition is feasible in the presence of 10-15 female sterlet or 3-5 female Siberian sturgeon and a corresponding number of males for this; in this regard, it is necessary to receive up to 120 thousand eggs annually, this amount can be obtained from 3 kilograms of eggs.

Table 4: Characteristics of the broodstock of sturgeon fish

Main characteristics	
Indicator	Sterlet
General quantitative composition, pcs	131
Breeding stock, pcs	25
Senior breeding stock, pcs	25
Junior breeding stock, pcs	81
Age structure, year (quantity of species)	2+(81)/ 3+, 4+(50)
Sexual structure, females/males	94/25 Non-defined-12
Degree of maturity of the gonads, stages of maturity	II-IV

Table 5: Recommended number of replacement herds needed to replenish the broodstock

Main indicators	
Age of replaces, year	Necessary quantity, pls
Juveniles 1.5-3 g	160-200
Yearlings	16-24
Two-year-old fish	8-12
Three-year-old fish	4

Table 6: Statistical parameters of the body weight (Q) of the sterlet replacement stock

Indicators	Meaning
Average value of the feature (X ± m), g	1100,89±28,58
Variation coefficient (Cv), %	23,37
Median (Me), g	1066,29
Stat (Mo), g	935,73
Asymmetry (As)	1,72
Excess (Ex)	1,410

Table 7: Statistical parameters of commercial body length Indicator (L) of the sterlet replacement stock

Indicators	Meaning
Average value of the feature (X ± m), cm	59,13±0,81
Variation coefficient (Cv), %	12,40
Median (Me), cm	58,89
Stat (Mo), cm	57,49
Asymmetry (As)	-0,039
Excess (Ex)	2,810

Table 8: Statistical parameters of the body length indicator to the end of middle rays of the caudal fin (l) of the sterlet replacement stock

Indicators	Meaning
Average value of the feature (X ± m), cm	53,16±0,71
Variation coefficient (Cv), %	6,25
Median (Me), cm	52,88
Stat (Mo), cm	56,01
Asymmetry (As)	-1,006
Excess (Ex)	4,990

Table 9: Statistical parameters of the fatness index according to Fulton (FCF) of the sterlet replacement stock

Indicators	Meaning
Average value of the feature (X ± m), pcs	0,82±0,05
Variation coefficient (Cv), %	26,52
Median (Me), pcs	0,78
Stat (Mo), pcs	0,76
Asymmetry (As)	1,283
Excess (Ex)	1,353

Data on body weight, commercial body length, body length to the end of the middle rays of the caudal fin, and Fulton fatness of individuals of the sterlet replacement stock are presented in (Tables 6-9).

Body weight is characterized by an average variation, predominance of individuals with values below the average, and a "single-peak" values distribution curve. The proportion of small individuals due to body weight is 55.56%, medium 38.27%, and large 6.17%.

Commercial body length is characterized by an average variation, predominance of individuals with values above the average, and a "single-peak" values distribution curve. The proportion of small individuals due to commercial body length is 18.52%, medium 61.73%, and large 19.75%.

Body length to the end of the middle rays of the caudal fin is characterized by a small variation, predominance of individuals with values below the average, and a "single-peak" values distribution curve. The proportion of small individuals according to the body length to the end of middle rays of the caudal fin is 36.40%, medium 36.49%, and large 27.20%.

Fulton's condition factor is characterized by an average variation, predominance of individuals with values below the average, and a "single-peak" values distribution curve. The proportion of small individuals according to Fulton's condition factor is 54.55%, medium 27.27%, and large 8.18%.

Reproduction

Artificial reproduction of the sterlet was divided into several production processes:

- Winter maintenance of sturgeon fish breeders with food deprivation
- End of wintering for breeders and spring valuation
- Obtaining sexual products, insemination, degumming, and eggs incubation

In early December, the sterlet broodstock underwent valuation in the RAS. An ultrasound scan was performed of the sterlet from replacement stock from which it is planned to obtain sexual products in the spring of this year, a total of 60 individuals where 39 are females (where 4 females participated in the spawning campaign last year) and 21 males. Sterlet breeders with gonads in stages 3-4 of maturity were selected for the 2020 spawning campaign. The breeders selected were transferred to the tanks for wintering.

Stocking of wintering tanks was carried out at an average daily water temperature of 16°C with a gradual decrease to 8°C for 10 days. The optimal temperature range for keeping fish during wintering is 4-5°C. At the same time, a short-term increase in temperature to 7°C and its decrease to 2°C are allowed.

The temperature regime graph in the tanks for wintering is provided in (Fig. 4). We can see from the graph that the average temperature fluctuated between 4-10.6°C, the average value was 7.9°C which does not correspond to the optimal indicators. However, it should be noted that the survival rate during this period was

100% and 33 females were selected for the spawning campaign where 30 females gave high-quality fish eggs.

During the entire period of wintering in the tanks, we maintained optimal water exchange and constant flow and controlled permanently oxygen and hydrochemical (oxygen content, iron oxides, ammonia, oxidizability, pH) regimes in the tanks. Besides we monitored the fish's behavior. The oxygen regime in the tanks during the entire period of wintering was favorable and did not decrease below the optimal values and averaged 10.27 mg/LWintering lasted 2.5 months and when the water temperature in the workshop increased to the optimal spawning values (15.7°C), the breeders were taken from wintering tanks for spring valuation after maintaining the breeders in the RAS workshop for 1 day and the spawning campaign started. The survival rate of sturgeon fish breeders (sterlet and Siberian sturgeon) during wintering was 100%.

The spawning campaign was divided into two rounds:

- For the first round of the spawning campaign, 13 females with eggs at the IV stage of maturity were selected, the group of males included 8 individuals of sterlet, a total of 21 individuals
- For the second round of the spawning campaign, 21 females with eggs at the IV stage of maturity were selected, as well as 12 males, a total of 33 individuals

During valuation, females who had not reached the IV stage maturity of the gonads during the wintering period and with oocyte resorption were rejected and placed for fattening (a total of 6 females).

Conducting the First and Second Rounds of the Spawning Campaign

During the first round, 1830 gr eggs were obtained, the eggs were fertilized with milt from 5 male sterlets, degummed with a clay solution, and placed in the Weiss apparatus (Total 3 apparatuses).

The second round of the spawning campaign was held after the females were aged for 2 days. During the second round, 1820 gr. eggs were obtained and the eggs were fertilized with milt from 7 male sterlets, degummed with clay and eggs were laid for incubation in Weiss apparatuses.

Hormonal Stimulation of Breeders Spawning

Acetonated pituitary gland of cyprinid fish was used from gonadotropic drugs the use of which is possible to stimulate the maturation of sterlets. To obtain sterlet's sexual products, a gradual injection with acetonated cyprinid pituitary gland was chosen. Standard medical syringes were used for injections. When preparing a solution with an acetonated cyprinid pituitary gland we complied with the term that the volume of the ready prepared for the fish weighing up to 5 kg did not exceed 2 mL. The injection was made into the dorsal muscle between the dorsal and side bony plates at the level of 2-4 dorsal bony plates (Fig. 8). A preliminary injection was made in the right side of the sterlet's back, the second injection was made in the left side of the back to avoid loss of the preparation through the hole remaining after the first injection.

Use of Pituitary Preparation

The total dose of the preparation was calculated depending on the fish's temperature and weight and the proportion of pre-injection from the degree of maturity of the oocytes estimated by the relative value of the polarization coefficient. The injection was carried out on special stretchers.

Examination of Females, Signs of Maturation

The examination was performed according to the estimated maturation time of the first females. When the fish was bent, a stream of ovarian fluid was observed with a large number of eggs. Frequency of examinations during maintaining the sterlets:

- The first Examination was necessarily carried out at the estimated time of maturation of the first females
- During the first examination, females producing ovarian fluid, separate eggs, and a stream of eggs were noted
- Next Examination of the fish, in which no signs of maturation were found except for the soft abdomen, was carried out no earlier than after 2-3 h

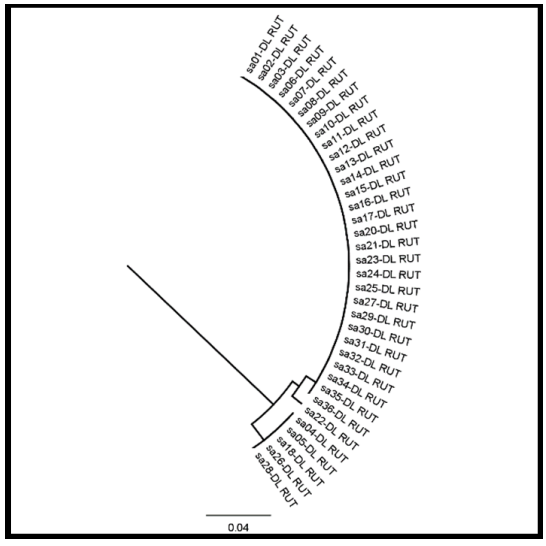

Fig. 8: Cladogram of mitochondrial haplotypes of the control area of the region D-loops of sterlet mtDNA (*Acipenser ruthenus*) in Kazakh Osseter LLP

Obtaining Sperm

In order to collect the sperm, plastic jars for analysis will be needed, as well as rags, a standard set of male urethral PVC catheters of different sizes, Janet plastic syringe, and a suitable catheter will be put on Janet syringe.

The male was laid on the back in the special stretchers with the belly towards the edge side. The genital opening and nearby area are wiped dry with rags. After performing these procedures, a free end of the catheter was inserted into the genital opening so that the end entered one of the spermatic vessels by 1-3 cm, the syringe plunger was slowly removed collecting sperm, observing that the catheter did not stick to the walls of the spermatic vessel. After selecting the required amount of sperm, the catheter was carefully removed and taken away from the syringe with sperm and transferred to the cool dark place. The sperm is mixed only before fertilization.

Insemination of Eggs

The insemination was performed by a dry method. The basic principle of this method is that a solution of the sperm mixed with the water will be added to eggs, the concentration of which will provide the greatest probability of monosperm fertilization (Tables 10-11). To achieve the required concentration, the optimal ratio of sperm and water is 1: 200. The same technique avoids a prolonged stay of the eggs in water without sperm (as in the "wet" method) because the eggs immediately enter the sperm solution in water where it is very quickly fertilized.

The fertilization time of the sterlet was from 3-5 min which ensured maximum fulfillment of the fertilizing potential of the sperm, meanwhile, almost all full-fledged eggs capable of fertilization were fertilized within the first 20-60 sec.

Degumming of Eggs

Methods of degumming sturgeon fish eggs proposed in current instructions are based on the mixing of newly fertilized eggs in the clay suspension, the particles of which will be glued to the sticky covers and deprive the egg of stickiness.

Egg Incubation

Degummed eggs were placed in the Weiss apparatuses. Hatched sac fry are carried out by the current of water from the apparatuses in which incubation took place and through a special channel enter the pool for further maintenance and cultivation.

During the egg's incubation, daily cleaning of dead eggs was carried out. Besides, the uninterrupted water supply was monitored round-the-clock.

Table 10: Average values of absolute indicators of morphometric traits of breeding stock and senior replacement stock of the starlet

	Body features, % of body length L, cm				
Indicators	L	C	H	h	pl
Min	67,63	14,15	13,77	3,39	2,16
Max	93,85	21,57	18,20	5,88	7,84
Average	79,96	17,54	16,21	4,64	5,30
Head features, % of head length C, cm					
	R	HC	BC	SO	
	25,00	38,46	36,36	16,19	
	44,44	75,00	81,25	33,33	
	32,18	55,88	55,20	24,01	
	Meristic features				
Indicators	Number of dorsal bony plates (Sl), pcs			Number of bony plates (Sd), pcs	
Min	12			34	
Max	21			67	
Average	15			56	

Table 11: Average indicators of exterior features of the sterlet broodstock

Indicators	L/H	O/L	KF
Min	4.50	36.96	0.67
Max	5.74	56.86	1.98
Average	4.94	49.97	1.17

In order to assess the fish-breeding quality of the eggs, the percentage of fertilization and share of the embryos developed regularly were defined. To determine the percentage of fertilization the eggs are mixed in the apparatus, a sample of 200-300 eggs is taken, and the proportion of normally developing embryos in the total number of eggs in the sample is calculated (Tables 3, 6, 7, 9, 10).

Obtaining Larvae

The start of pecking is characterized by appearing a single floating sac yolk in the incubation apparatus. Gradually, their number increases, and the time when several hundred prelarves appear in the apparatus can be considered the start of mass pecking. The prelarves were carried into the tanks by the current of water through a special channel. The density of planting prelarves in the tanks is shown in (Table 12).

Conditions for obtaining sexual products and egg incubation were optimal, as daily monitoring of hydrochemical parameters and temperature regimes in the incubation apparatus was carried out. Besides, the eggs were seen from the apparatuses for determination of the embryonic development status and compliance with the standards. The length of embryonic development of sterlet eggs at such temperature was 6 days from incubation to mass hatching. Eggs incubation was conducted in the Weiss apparatuses.

Table 12: Conditions for maintaining sterlet prelarves in tanks

Indicators	Measurement unit	Standard value
Square of fish breeding tanks	m^2	1-2
The density of prelarves planting	thous. pcs/m^2	6-8
Depth of water in the tank	cm	20
Oxygen content	mg/L	10,3-11,5
Water consumption	l/min	8-14

Table 13: Conditions for maintaining sterlet prelarves in tanks

Indicators	First round	Second round
Fertilization rate, %	88	59
The incubation period, days	6	6
Pathology, %	8	12
Prelarvae hatching, duration, hour	8	10

Fish breeding and biological indicators of the sterlet eggs during the spawning campaign were provided in (Table 13).

As a result, Kazakh Osseter LLP has conducted the sterlet spawning campaign and organized wintering, adaptation, and maintenance of breeders, obtaining sexual products, insemination, degumming, and incubation of the sterlet fertilized eggs. Own viable sterlet larvae were obtained.

Species Identification of Sterlet Broodstock

Sequencing of the hypervariable area of the controlled region of the mitochondrial DNA of 15 sterlet species in order to species identification and detection of maternally inherited variability revealed no features, the entire sample is related to the species *Acipenser ruthenus*. Analysis of the obtained mtDNA sequence data indicates a reduced genetic polymorphism in the sterlet herd in the broodstock. There are only two types of nucleotide sequences of the highly polymorphic region (2 haplotypes) (Fig. 8).

In figure shows that all individuals of the sterlet in the broodstock belong to the offspring of two maternal lines that are often found in the artificial rearing of fish in aquaculture.

There have been several pieces of research done on freshwater fish domestication of freshwater fish for the purpose of developing aquaculture and releasing fry into their habitats to support natural populations of endangered or threatened species over the years (Van Eenennaam *et al*., 1996). The results of much research provided evidence of the possibility of breeding and growing sturgeon fish in farming conditions (Pavel *et al*., 2022). Our obtained results also demonstrated the captive breeding and domestication of sturgeon fish particularly sterlet in controlled artificial waters. Genetic screening and monitoring of the released fish into natural reservoirs are important to assess the effectiveness of supportive stocking practices in restoring natural populations of species. In parentage analysis, the mitochondrial DNA haplotype markers of females can be used to distinguish stocked fish from naturally produced fish (Rożyński *et al*., 2015; Holostenco *et al*., 2021). One of the significant aspects of this research was that for the first time sterlet brood stock was created from individuals, 34 females, and 20 males, captured from the Kazakhstan part of the Caspian Sea. As a result of captive breeding, 3.6 kg of fertilized eggs was taken from those parent pairs from which viable sterlet larva was hatched in the farming conditions (Mamedov *et al*., 2020). Conserving the gene pool of rare and endangered sturgeon species through captive broodstock programs has been proven to be effective by global practice (Bronzi *et al*., 1999; Williot *et al*., 2005; 2007; Holčik *et al*., 2006; Chebanov and Savelyeva, 1999).

Conclusion

For the first time, artificial propagation of sterlet populations that inhibit in the Kazakhstan part of the Caspian Sea was carried out at a sturgeon fish farm, and genotyping of sterlet broodstock using DNA markers. Sequencing of the control region of mitochondrial DNA in the studied individuals for the purpose of species identification and detection of maternal hereditary variability did not find any signs of introgression or hybridization: All the captured broodstock from natural populations belong to the species *Acipenser ruthenus*. As a result of the artificial propagation, 3.6 kg of fertilized eggs were obtained from 34 females and 20 males, from which their own viable sterlet larvae were hatched. Our work also revealed that conserving and maintaining the genetic diversity of broodstock in the hatchery system in the future require 20 pairs of females and males. For the same purpose, the genetic structure of the broodstock should provide for the possibility of industrial crossing. Thus, our studies have confirmed the possibility of forming a productive broodstock of endangered species such as *Acipenser ruthenus* in different aquaculture practices to conserve their gene pool and increase the natural population size of those species in the natural reservoirs.

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Author's Contributions

Guldana Maratova: Conceived the original idea, data analysis, and manuscript written.

Kuanysh Isbekov: Reviewed and coordinated.

Shokhan Alpeisov: Managment and manuscript are written.

Nailya Bulavina: Designed research methodology and data in interpretation.

Saule Assylbekova: Materials and equipment engagement.

Kamila Adyrbekova: Genetic methods and literature search.

Bekzhan Barbol: Abstract and discussion.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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