

## PROSPECTIVE AQUACULTURE TRENDS IN AZERBAIJAN

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### Abstract

The article presents the results of the formation of production schools of sterlet (*Acipenser ruthenus* Linnaeus, 1758), beluga (*Huso huso* Linnaeus) and bester (*Huso huso* x *Acipenser ruthenus*) bred at the "Samukh-fish" farm of the Azerbaijan Republic. At the Samukh fish farm in 2019, for the first time in the history of fishery research in Azerbaijan, a production school of sterlet was formed "from hard-ro" at the age of 4 years (3+) and offspring were obtained from them for fish farming. The fish were reared on artesian water with a controlled temperature regime. Obtaining of hard-roer from sterlet breeders was carried out in a lifetime method. In 2021, mature hard-roer will be re-obtained from these breeders. This farm has also formed a production school of beluga "from hard-roer" and in December 2020, at the age of 15, for the first time, mature hard-roer was obtained from them. As a result of fertilization of beluga hard-roes with sterlet sperm, hybrids - besters were obtained, which are successfully bred on this farm.

**Keywords:** broodstock breeders, sterlet, beluga, bester.

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### 1. Introduction

In the last decades of the 20th and early 21st centuries, serious changes occurred in the ecosystem of the Caspian Sea, caused by fluctuations in its level, the entry of pollutants into the reservoir, and the development of illegal fishing for valuable fish species, and, above all, sturgeon. In connection with this, sturgeon stocks throughout the Caspian region have sharply decreased. All types of livestock living in the Caspian basin have been included in Annex I and II of the CITES Convention (*The Convention on International Trade in Endangered Species of Wild Fauna and Flora*) since 1998, and all other species except the 2 species are included in various categories of the International Red Book (IUCN - The International Union for Conservation of Nature [20]). One species (*Acipenser nudiventris* Lovetsky) is included in the "Red Book" of the Republic of Azerbaijan. In this regard, preserving the gene pool and maintaining the number of sturgeons at a stable level is an urgent problem.

For many years, the issues of artificial rearing of sturgeon fish have been priority areas in various countries of the world. For the successful development of sturgeon farming, work is being carried out in the field of formation and operation of Broodstock Breeder Schools (BBS) of various sturgeon species. As a result of the research carried out, the biological foundations for the formation of BBS have been developed, which are being successfully introduced into the practice of fish farming [1, 3, 4, 5, 19, 21, 22, 24, 25]. The main goal of these works is the creation of full-system sturgeon farms of various technological types (cage, industrial, pond), focusing on two main areas of activity: 1) preserving and increasing sturgeon stocks in natural reservoirs through mass stocking of young fish; 2) an increase in the volume of commodity sturgeon production.

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The sterlet (*Acipenser ruthenus* Linnaeus, 1758) is one of the sturgeon species that are most important for commercial sturgeon rearing. The experience of fish rearing with sterlet has more than a century of history and now it can already be argued that the main features of the biotechnology of rearing with various technologies have been successfully solved [9, 14, 16, 23]. At the same time, work on artificial rearing of sterlet for reproduction in Azerbaijan has not previously been carried out, so the biotechnology of its rearing has not been developed. This is due, first of all, to the scarcity of its resources and the impossibility of procurement of breeders in the required quantity at the mouth of the Kura River.

The first experiments with commercial rearing of sturgeon hybrids were carried out in Azerbaijan back in Soviet times. For many years, Azerbaijan has carried out research work on obtaining sterlet hybrids - bester (♀ *Huso huso* x ♂ *Acipenser ruthenus*) and schister (♀ *Acipenser nudiventris* x ♂ *Acipenser ruthenus*), and also studied the ecological and physiological characteristics of intergeneric and interspecific hybrids manifestations of heterosis in early ontogenesis. In parallel, studies were carried out to study nutrition and commercial rearing of the beluga x sterlet hybrid in the conditions of Azerbaijan. Part of the juvenile bester was used for seeding the inland water bodies of Azerbaijan, first of all, the Mingechevir reservoir in order to obtain commodity products. When carrying out these works, hard-roe was obtained from the Kura beluga or thorn sturgeon (*Acipenser nudiventris*), and the hard-roes were fertilized with the sperm of sterlet brought from the Astrakhan region of the Russian Federation.

The purpose of these studies was the formation of BBS of sterlet (*Acipenser ruthenus* Linnaeus, 1758) “from hard-roes” in Azerbaijan, as well as the study of the morphophysiological and fish-rearing characteristics of captive young of the year and adults. The tasks of our research at the first stage included the development of biotechnology for the formation of broodstock breeder and production school of sterlet (*Acipenser ruthenus* Linnaeus, 1758) in Azerbaijan, as well as monitoring the onset of their maturity and their use in fish farming on an industrial scale.

## 2. Material and Methods

The work was carried out in the period 2015-2021 at the fish farm “Samukh-fish” farm, located in the village of Samukh, Barda region of the Republic of Azerbaijan.

**Formation of broodstock breeders of sterlet - *Acipenser ruthenus* Linnaeus, 1758 in Azerbaijan.** Initially, a small batch of fertilized sterlet roe (*Acipenser ruthenus*) was brought to the Republic of Azerbaijan in May 2015 from the Astrakhan region of the Russian Federation. The pre-incubation of fertilized hard-roes was carried out in Weiss apparatus installed in the hatchery of the fish farm

After hatching, one day old free embryos obtained from one female were initially placed in plastic tanks with an area of 3.0 m<sup>2</sup>. Further rearing of larvae, fry, young fish and broodstock school of sterlet was carried out by the pool method. The water temperature during the rearing period was relatively stable and ranged from 18<sup>0</sup> to 22<sup>0</sup>C. A distinctive feature of the technology we used was that we used water from an artesian spring from a depth of 350 meters and with a stable temperature - in the winter months not lower than 18<sup>0</sup>C, and in the summer months - not higher than 22<sup>0</sup>C, which was the first time in the history of fishery research in Azerbaijan.

During the rearing process, constant control was also carried out over the stocking density, size groups of fish, as well as the hydrochemical parameters of water in each basin. For fry, the water consumption in the pools was 0.8-3.0 l/min per 1 kg of fish weight. As the juveniles grew, the water consumption was increased to 6.5-7.0 l / min. The dissolved oxygen level and pH of the water were 7.8-8.4 mg / l and 7.2-7.8, respectively.

Sterlet prelarvae were fed in the pools 2 days before their transition to exogenous feeding. During this period, the larvae were fed with live food - nauplii of *Artemia salina*. After the juveniles reached 60 mg weighed portions, they were gradually transferred to feeding with artificial food for sturgeon fishes of the Aller Aqua brand (SGP 493) with a fraction of 0.15 to 1.1 mm.

From one year of age, the fish were fed with Aller Aqua (45/15) production feed. We carried out rationing of feeding of groups of different age sterlet depending on the water temperature and body weight of the fish according to the following tables.

On the basis of the offspring of artificial generation, broodstock breeders were formed on the specified farm in order to obtain its own producers. The total number of breeders (males and females together) was over 500 individuals. The selection of fish for the broodstock school, the technology of keeping and feeding the broodstock and production schools of sterlet at the “Samukh-fish” farm during all these years was carried

out in accordance with the recommendations developed by specialists from Caspian Scientific research institute of fish industry and Krasnodar Scientific research institute of fish industry [19, 21, 22].

Ultrasound diagnostics of sex and stages of maturity of the gonads of sterlet individuals was carried out in the fall of 2019 according to the current method [6] using a PS-301V scanner. According to the results of the inspection, females and males were identified among the fish, whose gonads were at the III-IV stages of maturity.

At the age of 3 years (at the beginning of August 2019), sterlet breeders were transferred to cages at natural temperature. At the beginning of December, at a water temperature of 12<sup>0</sup>C, the producers were trained to obtain hard-roe.

For the purpose of injection, 15 female and male sterlets were initially selected from the cages with an average weight of 0.65 kg and 0.6 kg, respectively. After taking probe samples by biopsy [8] and determining the polarization coefficient of oocytes, female sterlet with gonads at maturity stage IV were placed in separate rectangular pools with an area of 3.0 m<sup>2</sup> for aging (Fig. 1).



**Fig.1.** Determination of the polarization coefficient of the sterlet oocyte nucleus by the biopsy method.

In the pools, a flow regime of water was created with an average speed of 0.5 m / s. The injection of female sterlet was started starting from December 9, 2019, at a water temperature of 11.3<sup>0</sup>C and a polarization coefficient of the oocyte nucleus <0.09. To prepare the suspension, dry acetone-pituitary glands of the carp (*Cyprinus carpio* Linnaeus) were used. The total dose of the pituitary gland of carp for female sterlet at a water temperature of 11.3<sup>0</sup>C was 6 mg/kg. Of these, 30% was the preliminary dose (1.8 mg/kg), and 70% was the final dose (4.2 mg/kg). The interval between preliminary and permissive injection at a water temperature of 11.8<sup>0</sup>C was 14 hours.

Sterlet males were injected with 1/3 of the hormonal preparation dose used for females with single injections (2 mg / kg). The injection of males was carried out during the period of permissive injection of females. The quality and activity of spermatozoa were determined according to the scale of G.M. Persov [15].

When obtaining hard-roe from sterlet breeders, the method of incision of the oviduct was mainly used [17, 18]. Fertilization of the obtained sterlet hard-roes was carried out by a semi-dry method, and the debonding of the fertilized hard-roes was carried out according to the current method [7, 12, 13].

Volcanic clay was used as a debonding agent [18]. The incubation of fertilized hard-roes was carried out by the method developed by us [10].

#### **Formation of broodstock breeders of beluga – (*Huso huso* Linnaeus, 1758) in Azerbaijan and bester (♀ *Huso huso* x ♂ *Acipenser ruthenus*).**

The formation of a beluga broodstock at the “Samukh-fish” farm began in August 2019. Initially, two-year-old beluga sturgeon (*Huso huso*) were brought from Iran in 2007 and until 2015 they were raised in a pond method on the basis of a fish farm located in the Sabirabad region of the Republic of Azerbaijan. When feeding beluga, sprat was mainly used as feed. At the end of 2015, these belugas were transported to the Khyilly sturgeon fish hatchery (Neftchala), where they were kept in rectangular concrete pools with an area of 72 m<sup>2</sup> (18 x 4 m) until August 2019. In August 2019, these belugas were delivered to the “Samukh-fish” fish farm and were placed in round concrete pools with an area of 178 m<sup>2</sup>, where artesian water was supplied, the temperature of which does not fall below 16<sup>0</sup>C in winter and does not rise above 22<sup>0</sup>C in summer time. The fish were fed partly with sprat, but then they were gradually switched to Aller Aqua granular feed. Under these conditions, the belugas were kept until reproductive products were obtained (December 5, 2020).

For the purpose of injection, 5 individual females and males of beluga with an average weight of 30 kg were initially selected from the cages. After taking probe samples and determining the polarization coefficient of the oocyte nucleus, female beluga sturgeon with gonads at stage IV of maturity were placed in separate round pools with an area of 6.0 m<sup>2</sup> for aging. The nuclear polarization coefficient of the oocytes of one female beluga was 0.07 (Fig. 2).

### 3. Results ad discussion

Sterlet breeders, whose genital products were obtained in vivo, were transplanted into special cages for their further aging. These breeders were bred in a fish farm with the aim of re-maturing.

The first injected sterlet females matured on December 13, 2019. Since these females matured for the first time, their gonado-somatic index was relatively low and ranged from 15.4 to 18.5%. The number of hard-roes per gram ranged from 128 to 132 hard-roes. The percentage of normal development of embryos at the stage of the small yolk plug (17th stage) ranged from 44 to 53% in different individuals. The yield of prelarvae from live hard-roes as a whole was 55%. Sterlet breeders, whose sex products were obtained in vivo, were transferred to special cages for their further aging.

The use of the sterlet production stock at the “Samukh-fish” continued until March 2020. In total, hard-roes were received from 10 batches of fish, 15 individuals each. Some of the hard-roes was used for food purposes.



**Fig.2.** Taking oocytes by biopsy from female beluga

In February 2021, a repeat ultrasound diagnosis of reproductive female sterlet, from which mature eggs were first obtained in 2019, was carried out. As a result of the ultrasonic method of analysis, 87 individuals of sterlet breeders were identified, of which 39 individuals turned out to be males (♂), and 48 individuals were females (♀). Of these, 35 males and 40 females had gonad maturity at the fourth stage of development. In February 2021, mature hard-roes of high fish breeding quality was again obtained from them (Table 1). The amount of hard-roes was 83 pcs. /g. The amount of hard-roes was 83 pcs. / g.

#### **Formation of broodstock breeders of beluga – (*Huso huso* Linnaeus) in Azerbaijan and bester (♀ *Huso huso* x ♂ *Acipenser ruthenus*).**

On December 7, 2020, mature eggs with a total weight of 3 kg were obtained from one female beluga at the age of 15 years (first spawning beluga). The gonadosomatic index (GSI) of the female beluga was 10%. However, of the 5 injected beluga males, none produced sperm. Therefore, beluga eggs were fertilized with sterlet sperm. As a result, on December 7, 2020, beluga eggs were fertilized with sterlet sperm and for the first time in the conditions of Azerbaijan we received bester (♀ *Huso huso* x ♂ *Acipenser ruthenus*) from factory-formed beluga and sterlet breeders.

**Table.** Fish-rearing indicators of sterlet when receiving ovulated hard-roes in 2021.

N: ♀	Space of fish	Sex of fish ♂/♀	N: of tags	Length, cm		Weigh t, kg	Polari- zation coeffi- cient of oocytes	Total amount of hard- roe received, g
				L	l			
1	<i>Acipenser ruthenus</i>	♀	4846	55	49	0,8	0,08	55
2		♀	4818	53	51	1,0	0,09	55
3		♀	4845	49	45	0,7	0,08	65
4		♀	4852	58	54	1,0	0,08	75
5		♀	4849	73	66	2,5	0,11	215
6		♀	4721	56	53	1,0	0,09	50
7		♀	4717	55	51	1,0	0,08	70
8		♀	4712	56	52	0,9	0,09	135
9		♀	5397	67	62	2,0	0,06	130
10		♀	5438	61	58	1,3	0,08	70
11		♀	5475	56	51	0,9	0,09	120
12		♀	4742	63	57	1,6	0,16	215
13		♀	5418	55	51	0,8	0,08	90
14		♀	5449	57	52	0,9	0,09	no hard- roe produced
15		♀	4870	55	50	0,9	0,09	no hard- roe produced
16		♀	4890	54	50	0,9	0,06	125
17		♀	5429	76	68	3,0	0,08	495
18		♀	5467	61	58	1,3	0,10	205
19		♀	5444	53	49	0,8	0,10	55
20		♀	4803	48	44	0,6	0,10	65

The growing of the larvae and fry of bester was carried out by the basin method on artesian water, and at present the average weight of fry of bester (17.01.2021 - at the age of 35 days from the moment of hatching) is 1.4 g.

#### 4. Conclusions

The principle of completing broodstock sturgeon fish from "hard-roe to hard-roe" is based on the selection of elite offspring with subsequent rearing to mature producers [5, 19, 21, 22]. In the conditions of the Republic of Azerbaijan, scientific research on the formation of broodstock of sturgeon fish for fish rearing purposes has been carried out since 2005 [12].

Aquaculture farms in Azerbaijan at the present stage operate in two directions. The first direction is the artificial reproduction of juveniles of valuable commercial fish species, which are then released to feed in natural reservoirs (Caspian Sea, Kura River, reservoirs) to replenish the stocks of these fish. This area is also referred to as pasture-based aquaculture; they are under government control. The second direction is farms growing commercial fish and fish seeding material. The first private farms in Azerbaijan appeared relatively recently. In particular, a commercial fish farm capable of producing 15-20 tons of sturgeon per year began operating in the city of Mingachevir of the Azerbaijan Republic in 2013. In subsequent years, a cage farm was put into operation on the Mingachevir Reservoir and a fish farm using a RAS (recirculated water supply installation) in Nerekent (Pirallahi village, a suburb of Baku) with a design capacity of 100 tons of Russian sturgeon and sterlet per year [2, 25, 26, 27, 28].

In recent years, aquaculture has been intensively developing in Azerbaijan, and many other private fish farms are functioning, where broodstock breeders and reproductive schools of many valuable commercial

fish species are formed. One of such private farms in Azerbaijan is the “Samukh-fish” farm, located in the village of Samukh, Barda region. The foundation of this farm was laid back in the 90s of the XX century, but since 2015 it has been functioning as a private fish farm. It is noteworthy that when breeding sturgeon fish on this farm, unlike other farms, geothermal water from an artesian source is mainly used, which is the first time in the conditions of the Republic of Azerbaijan.

At present, sterlet is one of the most common aquaculture objects in various countries of the world and is bred mainly for commercial purposes. In addition to the Samukh fish farm, commercial sterlet farming is carried out in some other fish farms in Azerbaijan. However, the uniqueness of our research on the basis of the “Samukh-fish” farm and the presented work lies in the fact that in the history of sturgeon rearing in Azerbaijan, for the first time on this farm, broodstock schools of sterlet “from hard-roe” was formed and offspring were obtained from them for further fish farming use. In parallel, the biotechnology of sterlet rearing at various stages of development by the basin method in a controlled thermal regime of the fish farm has been developed. Breeding of broodstock in a controlled temperature regime with year-round feeding with balanced feed allowed us to accelerate the maturation of sterlet producers.

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