Morphofunctional assessment of domesticated and aquacultured beluga females.

Aliya Akhmedzhanova 1,*, Sergey Ponomarev 1, Yulia Fedorovykh 1, Olga Levina1, Evgeniy Dutikov1, and Vladislav Vyatchin1

1 Astrakhan State Technical University, Tatishev str., 16, 414056 Astrakhan, Russia

Abstract. In the early 80-ies of the last century, there was a sharp decline in the population of the Caspian beluga in Russia. Its fishing was prohibited, except for catching for reproduction and scientific purposes. In the practice of sturgeon breeding, the method of repeated use of sturgeon females and males by obtaining sexual products in vivo, as well as growing breeders in artificial conditions, has become widely put into practice. Against the background of an acute shortage of wild breeders of this sturgeon species, in recent years, the main way to form sturgeon broodstock in the conditions of the Lower Volga region is domestication. However, in recent years, the catches of wild beluga females in the fishing areas of the Lower Volga are estimated as single specimens. The article studies the fish-breeding, biological and physiological parameters of domesticated and aquaculture-bred beluga females. The reproductive function of domesticated and artificially raised beluga females is evaluated. Some proposals for the intensification and priority areas of artificial reproduction in order to replenish natural populations are presented.

1 Introduction

The Caspian Sea is one of the unique internal reservoirs of the planet with an amazing variety of species composition of ichthyofauna and other representatives of the animal world. A special place in the biocenosis of this reservoir is occupied by sturgeon species of the oldest relict ichthyofauna [1-3].

Beluga (Huso huso Linnaeus, 1758) - the largest specie of the sturgeon family. It is a typical anadroumos fish. Previously, the beluga was relatively numerous. At present, however, its stock are in a critical state. The beluga population is represented by winter and spring forms. Spring forms of beluga spawn in the same year (usually in early summer), winter ones spend time in rivers on pits in winter and breed in the spring of the following year [4-7].

In the last century, despite the strong anthropogenic impact on the ecosystem of the Caspian basin and the negative environmental situation, the maximum catches of sturgeon in this reservoir reached 22-25 thousand tons.

* Corresponding author: aliyaakhmed14@gmail.com

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If earlier the state of the unique stocks of these fish species was influenced by such factors as the regulation of spawning rivers, the decline in the level and pollution of the Caspian Sea, then at the end of the last and at the beginning of this century, the dominant factor was an unprecedented outbreak of poaching in the sea and on the ways of spawning migrations.

Consequently, the instability of environmental factors, an unprecedented surge in poaching in the sea and in spawning rivers, the absence of an interstate agreement on rational fishing, reproduction and protection of biological resources of the Caspian Sea, has led to a total undermining of stocks and populations of sturgeon fish in the Caspian basin. Without proper conservation measures, the unique Caspian ichthyofauna is threatened with complete extinction.

It is believed that against the background of the total undermining of sturgeon stocks, artificial reproduction has become more important. However, due to the reduction in the number of spawning populations of sturgeon, there was a problem with breeders of natural generation for existing fish hatcheries. As an alternative, the question of forming productive stocks of endangered species in artificial conditions arose [8]. However, this direction in sturgeon breeding, primarily in sturgeon hatcheries (SH), is still sluggish in nature and is carried out without proper scientific support.

In recent years, to solve this problem in sturgeon breeding, two main directions have been formed – the domestication of wild fish and the cultivation of mature breeders in aquaculture.

This study is devoted to a comparative assessment of fish-breeding biological and morphophysiological indicators of breeders of domesticated beluga forms, as well as those grown in artificial conditions.

2 Research material and methods

The collection of experimental material was carried out during the fish-breeding season at the AFC “Beluga” and at the Sergievskiy Sturgeon Hatchery operating in the Volga River Delta. The object of the study was grown in artificial conditions and domesticated beluga females, taking into account their fish-breeding, biological and physiological indicators. The paper uses morphometric and physiological research methods with the use of mathematical statistics.

The physiological state of the studied fish was evaluated by biochemical parameters of protein, lipid and carbohydrate metabolism, according to the developed methods.

Blood was collected in vivo from the caudal vein in Eppendorf tubes. For hematological analysis (hemoglobin concentration, erythrocyte sedimentation rate), heparin was used as an anticoagulant.

The concentration of hemoglobin in the blood was determined photometrically using a set of reagents from “Agat-Med”, ESR was determined by the Panchenkov method. The content of whey protein was determined using reagent kits of the company "Agat-med", the level of cholesterol in the blood was determined by the enzymatic method. The concentration of glucose in the blood serum was determined by enzymatic colorimetric method without deproteinization (Trinder reaction). To measure the optical density of the obtained samples, a Unico 2100 spectrophotometer was used.

The results of the experiments were analyzed by methods of biological statistics using computer programs. The level of differences was assessed using the Student's confidence criterion.

3 Research results
Against the background of an acute shortage of wild breeders of this sturgeon species, in recent years, the main way to form productive stocks of sturgeon in the conditions of sturgeon hatcheries in the Lower Volga region is domestication [8]. However, in recent years, the catches of wild beluga females in the fishing areas of the Lower Volga are estimated as single specimens. The dynamics of beluga spawning in the Volga River is shown in figures 1 and 2.

**Fig. 1.** Dynamics of the beluga spawning run in the Volga spawning grounds

**Fig. 2.** The intensity of beluga reproduction in the Volga spawning grounds

Based on these data, a number setting spawn Beluga breeders, starting around the end of the last century, it is not necessary to count even on the minimum supporting of the Volga SH required of breeders to obtain reproductive eggs.

It follows that the preservation of the gene pool of this endangered species of the Caspian relict ichthyofauna is possible due to the domestication of wild fish and the cultivation of breeders in artificial conditions. However, the opportunities for catching beluga breeders are extremely limited due to the low number of the species in the Caspian Sea.

Along with quotas for the beluga catch for reproduction use in the existing SH, part of the quotas was also allocated for some commodity farms operating in the Lower Volga region. Naturally, larger females were selected to produce caviar. A certain number of wild females and males were also allocated for the commodity farm of LLC AFC "Beluga" created in the sluice channel of the Volga water divider. From these breeders, at present, along with obtaining caviar, offspring are obtained for the formation of a broodstock. Currently, a small number of domesticated beluga breeders are also found in the operating SH of the Lower Volga engaged in artificial reproduction of sturgeon fish. Fish-breeding and biological
indicators of beluga females after their re-maturation were studied at the Sergievsky SH, shown in table 1. In the studied group, females (AFC "Beluga") have a body weight of 106.8 kg, while the working fecundity reaches 14.4 kg/kg of female weight or 511 thousand pcs, the relative working fecundity is 13.78 %.

Table 1. Fish-breeding and biological indicators of mature domesticated beluga females

| Indicator                     | Females body weight, kg | Females body length, sm | Working fecundity, thousand pcs | Relative working fecundity, % | Number of eggs in 1 g, in pcs. | Working fecundity of females, thousand pcs. |
|-------------------------------|-------------------------|-------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------------------|
| Domesticated beluga females at AFC “Beluga LLC” (n=14) | M±m 106.8±5.6 | 218.5±4.1 | 14.4±0.9 | 13.78±1.3 | 35.8±1.5 | 511.3±29.3 |
| Domesticated beluga females at “Sergievsky” hatchery (n=14) | M±m 123.0±1.1 | 222.5±8 | 17.2±0.4 | 13.98±1.2 | 40.0±0.4 | 701.0±21.3 |

The coefficient of eggs’ polarization before stimulation of these females with the hormone preparation "Surfagon" averaged 12.1±1.3%. Fertilization of eggs reached a value of 83.0±1.1%.

Before the hormone injections is the polarization coefficient of the oocytes in females of beluga (Sergievsky SH) amounted to an average of 8.7 %. It should be noted that only 8.2 kg of reproductive eggs were obtained from one female beluga. At the same time, one of the females had an exceptionally high fecundity, amounting to more than 1 million, which indicates the grinding of oocytes. According to specialists of fish hatcheries, this is not an isolated case that requires further study.

Thus, the mass of domesticated, re-matured beluga females, in both variants of studies, was more than 100 kg: 106.8±5.6 kg and 123.0±1.1 kg, respectively. The yield of eggs per kg of fish body weight is approximately the same 13.78±1.3% and 13.74±0.2 % (p>0.05). The indicators of fertilization of eggs are similar in their value - 83.0±1.1 % and 82.3±0.1% (p>0.05). At the same time, one of the noted features is the crushing of oocytes in domesticated females, so the number of eggs in 1 gram reached 40.0±0.4 pieces (Sergievsky SH).

We studied the fish-breeding, biological and physiological parameters of the first matured beluga females raised in aquaculture, against the background of the annual dynamics of the natural temperature of the aquatic environment at the Sergievsky SH. These females had the following fish-breeding and biological indicators data are presented in table 2. The time to reach sexual maturity of females was approximately 16 years. At the same time, males mature 2-3 years earlier than females. The oocyte polarization coefficient was on average 9 %, which is within the normal range [8].

Table 2. Fish-breeding and biological indicators of first matured beluga females raised in artificial conditions

| Indicator                     | Females body weight, kg | Females body length, sm | Working fecundity, thousand pcs | Relative working fecundity, % | Number of eggs in 1 g, in pcs. | Working fecundity of females, thousand pcs. |
|-------------------------------|-------------------------|-------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------------------|
| M±m                           | 61.2±1.4               | 4.3±2.3                 | 6.9 ±2.1                      | 46.0±0.3                      | 61.3±1.2                      | 202.5±18.3                               |
| CV %                          | 5.2                    | 8.6                    | 8.4                            | 2.1                           | 7.1                           | 67.5                                      |
| σ                             | 29.0                   | 10.1                   | 9.8                            | 6.7                           | 14.3                          | 37.2                                      |
Let's compare the number of eggs in females grown in artificial conditions, this indicator was 46.0±0.3, and in domesticated females (Sergievsky SH) - 40.0±0.4 pcs, which indicates the decrement of eggs in females grown in Sergievsky SH (p<0.05). The same differences were found in the mass of oocytes. Thus, the weight of unfertilized eggs in grown females averaged 20.1±0.01 mg and 23.1±0.02 mg in domesticated females, respectively. Fertilization of eggs in beluga whales grown under aquaculture conditions averaged 61.3 %, in domesticated females at the same SH, fertilization of eggs reached a value of 83.0±1.1%.

At the same time, it can be noted that a common feature for both first-matured beluga females raised in artificial conditions is relatively low values of fertilization of eggs.

A complex of physiological and biochemical blood parameters was studied, reflecting the functional state of beluga females raised in artificial conditions, as well as in domesticated females at the Sergievsky SH. The data are presented in figure 3 and table 3.

**Fig. 3. Hematological parameters of beluga females**

As a result of the conducted studies, differences in the level of total hemoglobin in the blood were revealed, while it was noted that in domesticated females this indicator is higher by 1.23 than in grown ones. Most likely, this is due to the lack of divalent iron and ascorbic acid in the feed during their cultivation, and possibly with a decrease in motor activity during artificial cultivation [9-11]. According to the content of total whey protein, there were no significant differences between the groups of females. At the same time, domesticated females showed an increase in blood lipids by about two times (p<0.05), data within the reference values.

**Table 3. Hematological parameters of beluga females**

| Indicators                  | Cholesterol, mmol / l | Glucose, mmol / l | ESR, mm / h |
|-----------------------------|-----------------------|-------------------|-------------|
| Domesticated beluga females (n=14) |                       |                   |             |
| M±m                         | 4.2±0.6               | 5.0±0.1           | 4.8±0.4     |
| Aquacultured females (n=14)  |                       |                   |             |
| M±m                         | 3.8±0.6               | 4.3±0.1           | 5.2±0.4     |

The concentration of cholesterol, the consumption of which in the blood is necessary for the structure of oocyte membranes and participation in the generative process, was 4.2±0.6 mmol/l in domesticated females and 3.8±0.5 mmol/l in artificially grown breeders, values of approximately the same order (p>0.05). The concentration of glucose in the blood serum was low in females raised in artificial conditions compared to domesticated ones and amounted to 4.3±0.1 mmol/l. This value is lower than the reference values, but is not extremely low.
At the same time, the rate of erythrocyte sedimentation in these females is approximately the same.

The rate of erythrocyte and cholesterol sedimentation in the study groups remained within the reference values, which is also consistent with the literature data [12,13,14,15] and indicates a constant protein composition of blood plasma.

4 Conclusion

Thus, it was found that a common feature for the first matured beluga females raised in aquaculture is relatively low values of fertilization of eggs - 61.3±1.2 %, with the number of eggs in 1 gram of 46.0 pcs. In this case, it is recommended to leave the first matured females in artificial conditions for re-maturation, since re-matured females have the best fish-breeding indicators.

The mass of domesticated, re-matured beluga females, in both versions of the study, was more than 100 kg. The yield of eggs per kg of fish body weight and the indicators of fertilization of eggs are approximately the same in their value (p>0.05). At the same time, one of the noted features is the reduction of oocytes in domesticated females. So the number of eggs in 1 gram reached 35.8±1.5 pcs. (AFC "Beluga") and 40.0±0.4 pcs. (Sergievsky SH). As for hematological parameters, a low concentration of glucose in the blood serum was found in females raised in artificial conditions, in comparison with domesticated ones. This value is lower than the reference values, but is not extremely low. The remaining physiological and biochemical parameters of sturgeon breeders were within the optimal reference values.

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