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Morphological And Biological Features Of Ship Sturgeon Replacement And Breeding Stock Of Ural-Caspian Population, Grown Under Conditions Of Controlled Systems.

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ABSTRACT

This article describes morphological and biological features of ship sturgeon replacement and breeding stock of Ural-Caspian population grown in a closed water supply. The object of the study was ship sturgeon replacement and breeding stock (Acipenser nudiventris) of Ural-Caspian population in closed water supply facilities, under 4+ age and with an average weight of 3.1 kg. Genetical and morphometrical analysis of ship sturgeon was also carried out. The basic parameters of blood were determined. The analysis of microsatellites was carried out. All the data are presented in tabular form.

Keywords: sturgeon fisheries, ship sturgeon (Acipenser nudiventris), morphological characters, blood analysis, microsatellite loci

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INTRODUCTION

The Caspian Sea is the richest reservoir by its size and population of sturgeon species in the world. The Caspian sea is home for 6 species of sturgeon out of 26 known, such as Beluga (Huso huso), Russian sturgeon (Acipenser güldenstädtii), Persian sturgeon (Acipenser persicus), Stellate sturgeon (Acipenser stellatus), Sterlet (Acipenser ruthenus), Ship (Acipenser nudiventris) (Korobochkina 1964). Sturgeons belong to an ancient group of highly valuable fish because of its black caviar and boneless meat (Fopp-Bayat et al. 2007). Sturgeon fish that are grown in the controlled systems (RAS) show rapid growth and reach sexual maturity earlier than in nature (Chebanov, Billard 2001).

Ship is one of the valuable sturgeon breeds, which is the most exposed to the negative effects of human activities. These species are characterized by low natural population in the whole area (Shilin 2001). Main stocks of ship are concentrated in the Caspian Sea reservoir; its population had never exceeded 3% of the total yield of sturgeon (Kozhin 1964). Ship is a diadromous fish, reaching more than 2 meters in length, 50-60 kg in weight, with a life span of 30 years. Sexual maturity occurs at 12-14 years. Ship spawns once every two or three years. The spawning migration in the river begins in the second half of the summer with immature spawn (Early Run). Ship hibernates in rivers in the pits. Spawning takes place from late March to June at pebble, rocky or coarse sand ground at a water temperature of 10-15 ° C. Fertility of the Aral ship sturgeon is 216-388 thousand spawns, Caspian ship sturgeon makes up to 280-1290 thousand spawns. (593 thousand spawns in average). Ship feeds on fish and lower invertebrates (Bartley, Leber 2004). Mollusks in the Aral Sea; fish and mollusks in the Caspian Sea are the main food sources of ship. Incubation period of ship is 5 days at a temperature of 15 ° C. Diameter of the spawn is 1.5-3.0 mm. After spawning breeders run into the sea. Some juveniles stay in the river for more than a year. Dimensions of juveniles in the river are 3,3-25 cm. They gain weight in the open sea, eating fish and mollusks; in the river juveniles eat dragonfly larvae. The growth rate in the Aral Sea is worse than in other reservoirs, in seven years ship gain up to 4-5 kg of weight, in seventeen years - 14-16 kg, in twenty-five - 20-30 kg (Zharkovsky 1950, Markova's 1969, Mitrofanov et al. 1986).

The current catastrophic decline in population of sturgeon in the Caspian Sea is a result of unsustainable fishing, reduction of migratory routes and natural reproduction; all this determines the need for effective conservation measures. These measures should take into account species-specific complex adaptations and mechanisms of sturgeon migration. Opening the consistent patterns of formation of sturgeon population that live in the Caspian Sea, is crucial not only for the preservation of natural reproduction, but also for the improvement of bio-industrial sturgeon breeding (Khodorevskaya et al. 2012).

Taking into account catastrophic decline in the population of ship in the natural reservoir, as well as its complete disappearance from the greater part of its historical habitat, preservation of this species will be determined by the state of its artificial reproduction (Kokoza, Erbulekov 2007, Ivanov's 2010).

The main goal of our research is to study and develop biotechnology of ship reproduction in the regulated conditions of aquatic environment, and formation of the productive stock.

Under the circumstances of reducing the volume of juvenile sturgeon production it is necessary to improve scientific research in the field of sturgeon breeding, in particular, to study the artificial generation of fish-breeding to clarify the existing regulatory indicators, as well as to improve the outdated biotechnology of artificial reproduction.

METHOD

The morphometric analysis

The object of the study was ship sturgeon replacement and breeding stock (Acipenser nudiventris) of the Ural-Caspian population in closed water supply facilities.

In order to preserve the Ural-Caspian population of ship the research work was carried out on the development of biotechnology for growing the Ural-Caspian ship (Acipenser nudiventris) population under conditions of closed water supply facilities in the Institute of Biotechnology and Environmental Management of West Kazakhstan Agrarian Technical University named after Zhangir Khan.

Since 2009 in WKATU named after Zhangir Khan the works on the formation of ship sturgeon replacement and breeding stock of the Ural-Caspian population was conducted. Ship was introduced to the aquarium complex in the larval period of May 2012. Adaption to the artificial feed was successful. During the period of growing in RAS the results were obtained to assess the growth and development of fish (Fig.1).

Growing of ship was carried out in pools with the dimensions of 5x3x1,5 m. under controlled hydrological and hydro-chemical conditions. Temperature conditions during the growing period were stable - 20,0-22,0 ° C, oxygen varied between 8-12 mg / l. Fish feeding was carried out with combined feed made by "Coppens" firm.

The morphometric studies used common indications in the systematics of sturgeon: weight (G), total body length (TL), maximum body height (H), minimum body height (h), maximum body circumference (Ci), head length (C), snout length (ao), distance from the tip of the snout to the barbels (aB), width of the ventral part of the head at a level of barbels (wB), width of the mouth (wm), length of the pectoral fin (lP).

Genetic studies were conducted by the special molecular genetic methods (Zane et al. 2002, Welsh et al., Henderson-Arzapalo et al. 2002, Maniatis et al. 1984, Voinova 2004, Voinova et al.2003).

Fins of 17 ship species were selected for the genetic research. Samples were fixed in 96% ethanol at the place of collected material. Each sample was numbered with an identification number. All ship specimens were subjected to the parallel morphometric measurements and visual definition of age, size and weight for each specimen by fish-farmer. Samples were taken in order to extract and purify DNA. Extraction of DNA from the fins was carried by mechanical destruction of the material. Approximately 0.2 g. of fins were grinded by surgical blade into fragments with the size of 0.5-1 mm^2. Fragmented fin was mixed with 0.3 g of glass beads of 0.5 mm in size and 0.3 g of 0.1 mm, and 0.5 ml of lysing reagent for DNA extraction from AxyPrep kit (Axygen). The tubes were vortexed in the horizontal position for 5 minutes at maximum speed. Further selection was performed according to the manufacturer's instructions. DNA yield was 50 µl with a concentration of 20-50 ng/ µl. Primers were used for the species identification (Table 1).

Table 1. Primers for species identification of sturgeon fish

Name	Sequence (5'-3')	Use with primer	Length of the product (bps)	species specificity
AGF	GCACAGACTATGTGGTATCCGAA	AHR	420	Russian sturgeon
ABRM	TGTCTGTCTAGAACATAtG	ABF	182	Siberian sturgeon
NudF	TGTCTTTTCTGAAGGAGCTTTGC	AHR	329	Ship
RutF	GGGAATAACCGTTAATTTGG	AHR	190	Sterlet

PCR was performed with the following parameters: preheating at 95 ° C - 2 min, 35 cycles (92 ° C - 20 sec, 57 ° C - 30 sec, 72 ° C - 30 seconds...), And the final synthesis of 72. ° C - 10 minutes. All reactions were done by using Taq-polymerase (Evrogen, Moscow). The reaction products were separated by electrophoresis in 1.5% agarose, colored with ethidium bromide and photographed under ultraviolet light.

Analysis of microsatellite was performed using PCR. Primers and annealing temperature are shown in Table 2. Amplification was carried according to the following scheme: preheating DNA: 95 ° C - 10 minutes, synthesis of PCR products (35 cycles): Melting - 95 ° C - 20 sec, primer annealing - (Table 2) - 15 s, DNA synthesis - 72 ° C - 15 sec., final fill-in of chains: 72 ° C - 5 min.

Table 2. Primers for microsatellite analysis

Microsa tellite	Sequence of primers	Repeat	t annealing
AoxD16 1	Direct: 5’-GTTTGAAATGATTGAGAAAATGC-3’ Reverse:	(CTAT)n	58°C

Microsatellite	Sequence of primers	Repeat	t annealing
	5'-TGAGACAGACACTCTAGTTAAACAGC-3'		
AoxD165	Direct: 5'-TTTGACAGCTCCTAAGTGATACC-3' Reverse: 5'-AAAGCCCTACAACAAATGTCAC-3'	(CTAT)nCTAC(CTAT)n	60°C
AfuG41	Direct: 5'-TGACTCACAGTAGTATTATTTATG-3' Reverse: 5'-TGATGTTTGCTGAGGCTTTTC-3'	(GATA)nTA(GATA)n	56°C
AfuG51	Direct: 5'-ATAATAATGAGCGTGCTTTCTGTT-3' Reverse: 5'- ATTCCGCTTGCGACTTATTTA-3'	(CAAA)n	56°C

Separation of the amplification fragments was performed in the device for capillary electrophoresis MultiNA (Shimadzu). Cluster analysis was performed in the program Bionumerics 7.0.

The obtained data were processed by common methods of statistical analysis using Microsoft Excel program.

RESULTS

Morphometric parameters and mass indicator

Evaluation of fish was made during the entire period of growing ship in Recirculating aquaculture system (RAS), with the main indicator being the mass indicator (Kulachenko et al. 2011).

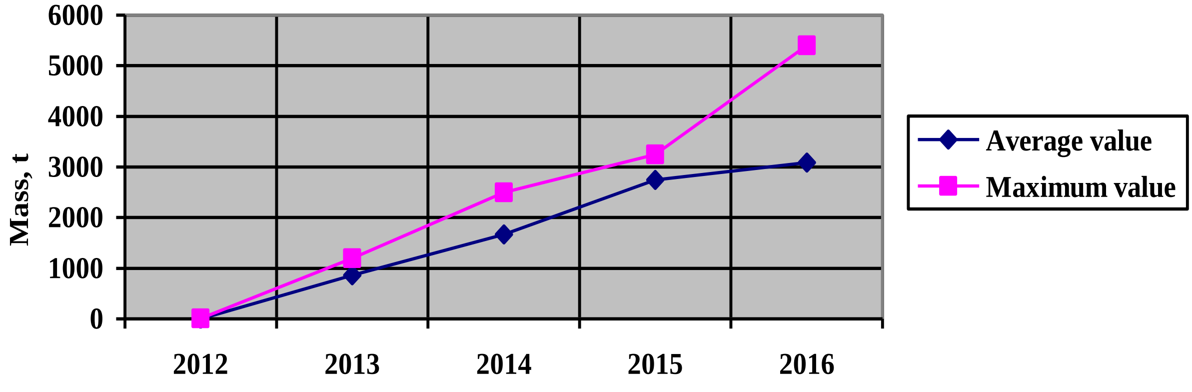

Figure 1. Indicators of dynamics of ship growth (Acipenser nudiventris) grown in RAS for the period of 5 years

According to the diagram 1 in 2012, the mass of the ship larvae ranged from 2 to 4 years, in 2013 the average value of the weight was 865 g, and the maximum 1200 g. In 2014, the average value of the mass reached 1670 g., the maximum 2500 g. With age, the rate of growth significantly reduced, in 2015 the average value of 2745 g. reached the maximum of 3250 g. In 2016, the average value is in the range of 3090 g., with the maximum value of 5400 g. The mass indicator of farmed fish has shown good results for five years, this indicator mainly depends on temperature, oxygen conditions and feeding rate. High mass indicator shows that the fish during the growing period was in a relatively favorable conditions (Kiselev 1999, Zhigin 2003)

A number of ship flexible attributes was investigated to study the morphological parameters, as well as fish mass indicators (Table 3).

Table 3. Several morphometric parameters of ship sturgeon (*Acipenser nudiventris)* 4+

Parameter	*Acipenser nudiventris (n=30)*		
	X±x	δ	CV%
G (g)	3176.67±391.56	470.24	14.8
TL (cm)	86.7±3.12	4.13	4.77
H (cm)	11.2±0.68	0.83	7.5
h (cm)	7.3±0.61	0.69	9.46
Ci (cm)	31.77±1.68	1.91	6.01
C (cm)	17.37±0.73	0.88	5.09
ao (cm)	8.55±0.4	0.47	5.52
aB (cm)	5.07±0.2	0.28	5.54
wB (cm)	6.07±0.19	0.33	5.52
wm (cm)	5.32±0.37	0.44	8.23
lP (cm)	16.27±1.1	1.34	8.24

Note. Designations of parameters: X±x - average value and mean linear deviation; δ - standard deviation; CV- coefficient of variation; G – weight; TL - total body length; H - maximum body height; h - minimum body height; Ci - maximum body circumference; C - head length; ao - snout length; aB - distance from the tip of the snout to the barbels; wB - width of the ventral part of the head at a level of barbels; wm - width of the mouth; lP - length of the pectoral fin.

Analysis of Table 3 shows that the mass of ship (Acipenser nudiventris) varied in a rather large range (2500-4300 g). The lowest variability characterized by such features as the distance from the tip of the snout to the barbels, width of the ventral part of the head at a level of barbells, width of the mouth. The coefficient of these indicators variation was more than 5%.

Blood analysis

Ship is constantly being monitored for physiological state during the entire growing period in artificial conditions. An analysis of ship physiological state showed that the main parameters of blood, such as hemoglobin and protein were within normal limits. The results of the blood analysis indicators are presented in table 4.

Table 4. Blood indicators of ship sturgeon (Acipenser nudiventris)

Parameter	Acipenser nudiventris (n=30)		
G (g)	X<2500	2500<X<3000	X>3000
TL (cm)	X<80	80<X<85	X>85
HGB (mg/l)	63.6±0.17	68.5±0.13	72.8±0.2
TP (mg/l)	35.5±0.2	39.4±0.1	44.7±0.16

Note. Designations of indicators: G – weight; TL - total body length; X - average value; HGB - hemoglobin; TP - total protein.

Table 4 shows that the main blood indicators (HGB, TP) are within normal limits. The mass of fish that was below 2500 (g) have the following indicators: Hemoglobin 63.6 (mg / l), total protein 35.5 (mg / l). The mass of fish that was within 2500-3000 (g) have the following indicators: Hemoglobin 68.5 (mg / L), total protein 39.4 (mg / l). The mass of fish that was above 3000 (g) have the following indicators: Hemoglobin 72.8 (mg / l), total protein 44.7 (mg / l). We can see here that there is a direct relationship between the size and mass of fish and blood indicators.

Analysis of microsatellite

During identification of the species it was established that all ship was clean in genetic terms (Fig.2).

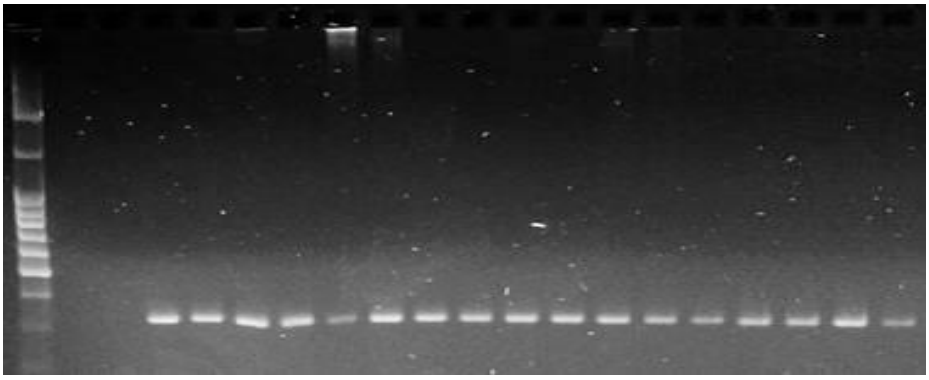

Figure 2. Results of PCR on ship identification of the species

Ship identification of the species grown in RAS conditions showed that the fish was clean-bred without any mixed genes from the other species of sturgeons (Table 5).

Table 5. Ship identification of the species

Sample №	Russian sturgeon	Siberian sturgeon	Ship	Sterlet
1	-	-	+	-
2	-	-	+	-
3	-	-	+	-
4	-	-	+	-
5	-	-	+	-
6	-	-	+	-
7	-	-	+	-
8	-	-	+	-
9	-	-	+	-
10	-	-	+	-
11	-	-	+	-
12	-	-	+	-
13	-	-	+	-
14	-	-	+	-
15	-	-	+	-
16	-	-	+	-
17	-	-	+	-

The use of molecular biology methods demonstrates that it is necessary to use these methods in the preparation of ship sturgeon replacement and breeding stock characterized by the purity of species that are highly heterogeneous and unique in intraspecific structure of sturgeon.

Analysis of the results showed that according to microsatellite we can distinguish 3 reliable clusters combining related genotypes. Table 6 shows the data on cluster analysis, indicating the reference number of the cluster to which the species belong.

Table 6. The results of microsatellite cluster analysis

Sample№	AfuG41	AfuG51	AoxD161	AoxD165	All loci
1	3	1	1	3	2
2	1	1	1	3	2
3	1	1	1	1	1
4	1	1	1	1	1
5	3	3	2	3	3
6	3	3	1	1	1
7	3	3	1	1	1
8	3	3	1	1	1
9	3	3	1	1	1
10	3	3	1	1	1
11	3	2	1	1	1
12	3	3	1	1	1
13	3	3	1	1	1
14	3	3	1	1	1
15	3	3	1	2	1
16	1	2	1	1	1
17	3	3	2	1	3

Cluster analysis of the four microsatellite gives a clear idea of the relationship degree of ship species (Fig.3).

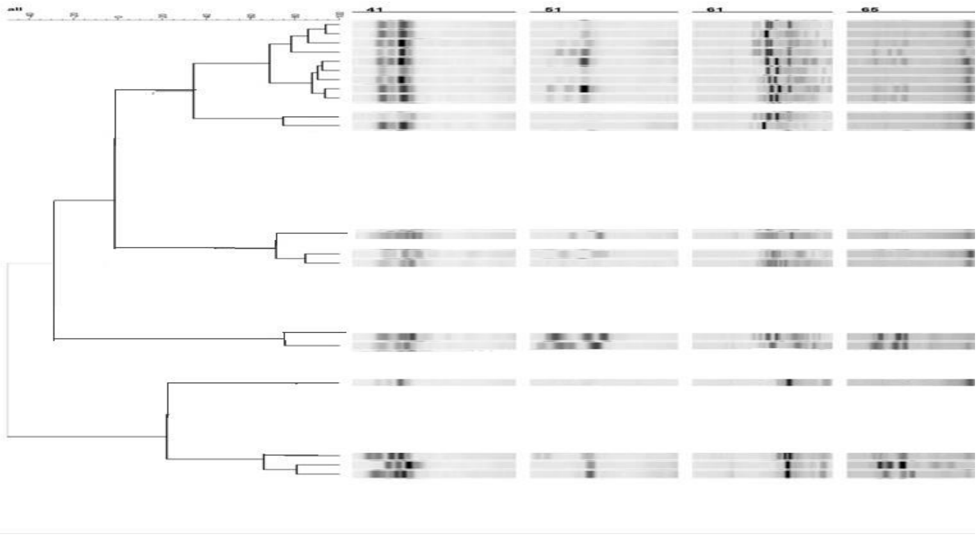

Figure 3. The results of cluster analysis on four microsatellite AoxD165, AoxD161, AfuG41 и AfuG51.

DISCUSSION

Today, the experience of Ural-Caspian population of ship sturgeon artificial breeding is at insignificant level of development, but the main stages of the biotechnical process are developing quite successfully. At this stage of development in biotechnology ship breeding it is very important to study reproductive organs and technology for determining the stage of maturity by ultrasonic scanning method in the early stages of generative system development. This will accurately determine sex composition, and in the subsequent development stages of reproductive organs for replacement and breeding stock. Due to high annual level of deficit of natural generation it is efficient to form the ship breeding stock of Ural-Caspian population for subsequent breed. Almost all stages of biotechnological process (at embryonic, post-embryonic development stages, as well as at the stage of growing juveniles in ponds) have considerable room for improving the quantitative and qualitative enhancement of fish-breeding production in the factories of the Ural-Caspian region. Achieving this goal is possible through introduction of more advanced reproductive technologies and in-depth studies of the biology of this relict fish fauna species. The results of these studies outlined in this article may serve as a basis for further improvement of biological standards and technologies of artificial breeding of this unique Caspian sturgeon species.

Ship is constantly being monitored for physiological state during the entire growing period in artificial conditions. An analysis of ship physiological state is determined by the main blood indicators: hemoglobin, total protein. The data obtained from the analysis of ship blood showed that the main blood parameters were within normal limits, which characterizes normal physiological state of the examined fish.

The use of microsatellite analysis will allow us to be more informed and directed to carry out the breeding work, track genetic diversity within the stock in order to avoid weakening of genetic body composition and phenodeviant appearance.

CONCLUSION

The studied Ural-Caspian population of ship grown in artificial conditions of closed water supply had revealed high rates of growth and a fairly good adaptation of this sturgeon species in artificial conditions. When optimizing the feeding rates, as well as stabilizing of the temperature and oxygen that were within 20,0-22,0ºC and oxygen in the range of 8-12 mg/l, it is possible to achieve accelerated growth and development of mass indicators and organs of reproductive system of ship sturgeon replacement and breeding stock. Today, in the current difficult situation, which happened to the Caspian sturgeon it is necessary not only to maintain this type of fish (Acipenser nudiventris) but also to develop new biotechnological methods of reproduction for the next breed. But for the realization of these goals it is necessary to have a considerable amount of time and effort by the scientific-research team in the field of aquaculture, ichthyology and fish physiology.

As a result of the study it has been established, that ship grown in a closed water supply systems are clean species. According to the results of microsatellite analysis it can be previously said that in this sample there are three distinct clusters belonging to three different parents.

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