AGRICULTURAL UNIVERSITY – PLOVDIV

FACULTY OF AGRONOMY
Department of "Animal sciences"

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MORPHOMETRIC AND REPRODUCTIVE CHARACTERISTICS OF ACIPENSERIDAE FISH IN THE CONDITIONS OF SUPERINTENSIVE BREEDING TECHNOLOGY

ABSTRACT

Of the dissertation for awarding educational and
scientific degree “DOCTOR” in a scientific field 6.3. "livestock breeding", scientific specialty "Breeding of agricultural animals, biology and biotechnics of reproduction"

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The dissertation is written on 208 pages and contains 35 tables and 56 figures. The references list contains 223 sources, of which 39 are in Cyrillic and 184 are in Latin.

The dissertation work has been discussed and directed for defense by the department council of the Department of "Animal Science", Faculty of Agronomy, Agricultural University - Plovdiv.

The defense of the dissertation will take place on ________ at _____ in the ___ hall of the Faculty of Agronomy at AU - Plovdiv at a meeting of the Specialized Scientific Jury, appointed by the Rector of the Agrarian University by Order No. RD-16-778/07/05/2022 in the composition :

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INTRODUCTION

Consumption of fish and fish products is constantly increasing, growing by more than 57% compared to the middle of the last century. Satisfaction of growing needs is associated with the development of aquaculture, as natural hydrobiont populations are depleted. The state of the world's natural sturgeon populations is of particular concern. Sturgeon bans have been introduced in a number of regions around the world. Their catching in Bulgaria, as well as transportation and sale of fish and fish products have been prohibited for the protection of Sturgeons since 2012. The ban was extended in 2016 for another 5 years, and in 2021 the period was increased by another 5 years. One of the modern approaches to the conservation of natural sturgeon populations is their introduction into new areas for industrial breeding. Aquaculture farms are in fact living genetic banks for in-vivo storage of the Acipenseridae family gene pool, in which endangered species are maintained. The sustainable development of European and Bulgarian Sturgeon farming can be realized only by building modern production based on scientific achievements in the field. At the same time, knowledge about the development of cultivated Sturgeon species in industrial production systems is extremely insufficient. In our country, researches on Sturgeon fishes are very scarce and mainly concern wild populations, and studies of the Acipenseridae family representatives, under the conditions of super-intensive cage technology, have not been done at all. In the present study, for the first time in Bulgaria, morphometric and reproductive characteristics of species and hybrids of the family Acipenseridae, cultivated in conditions of industrial super-intensive cage technology, have been studied.

1. PURPOSE AND TASKS

Considering the importance of the industrial Sturgeon breeding development in the world and the extremely insufficient knowledge about the ontogenesis of the Acipenseridae family species in cultivation, we set ourselves the goal to make morphometric and reproductive characteristics of Sturgeon fishes (Russian sturgeon and hybrid (F1 Ab x Ag), grown in the conditions of industrial super-intensive cage technology.

The following tasks were set to achieve this goal:

1. To make a morphometric characteristic of fish of the family Acipenseridae:
    a. Plastic, meristic signs and morphometric indices in Russian sturgeon and hybrid (F1 Ab x Ag).
    b. Comparative morphometric analysis.
2. To study the sexual development of Acipenseridae family female and male fish by ultrasound monitoring:
    a. Ovarian developement.
        i. Russian sturgeon, Siberian sturgeon and hybrid (F_1 *Ab* x *Ag*).

ii. Comparative analysis. Maturity stages.
b. Testicular development.
i. Russian sturgeon, Siberian sturgeon and hybrid (F_1 *Ab* x *Ag*).
ii. Comparative analysis. Maturity stages.
3. To characterize the sperm production of Acipenseridae family fish.
a. Russian sturgeon.
b. Hybrid (F_1 *Ab* x *Ag*).

2. MATERIAL AND METHODS.

The studies were conducted in the period 2017-2019, covering three complete vegetation periods.

2.1. Sturgeon species and hybrids subject to the study

Subject of the study are:

- Russian sturgeon (*Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833) (Fig. 2.1.1.);
- Siberian sturgeon (*Acipenser baeri* Brandt, 1869) (Fig. 2.1.2.);
- Siberian and Russian sturgeon hybrid (F_1 *A. baerii* x *A. gueldenstaedtii*) (Fig. 2.1.3.).

Fish of different sexes and ages were studied within each species and hybrid.

Fig. 2.1.1. Russian sturgeon

Fig. 2.1.2. Siberian sturgeon

Fig. 2.1.3. Hybrid (Siberian sturgeon x Russian sturgeon)

2.2. Characteristics of the farm.

The study was conducted on a cage superintensive sturgeon farm. The cages are located in the water area of the Kardzhali dam (Fig. 2.2.1.; 2.2.2.).

A **B**

Фиг. 2.2.1. The dam of Kurdzhali (A); Cage farms, located in the water area of the Kurdzhali dam (B).

The dam collects water from the Arda River and its tributaries and has a total volume of about 539.90 million m³. The catchment area of the dam is 1882 km². The farm has two production bases. The first one is located in the village of Brosh area, municipality of Kardzhali, with a capacity of 132 cages and coordinates N 41 ° 37′52,3 ″ E 25 ° 19′35,2 ″, and the second base is located in the village of Kamenartsi area, municipality of Kardzhali, with a capacity of 234 cages and coordinates N 41 ° 37′55,0 ″ E 25 ° 18′50,1 ″. The region falls within the South Bulgarian climate zone, East Rhodope climate region. The average altitude is about 280 m. Fish were kept under identical conditions throughout the study period. Full-feed specialized granular feed was used for feeding (Table 2.2.1.). A monitoring of hydrochemical and hydrophysical parameters was performed throughout the study period.

Table 2.2.1. Composition of the fish feed.

Indicators	Value
Crude protein, %	46
Crude Fats, %	15
Crude fibre, %	1.4
Ash, %	6.5
P, %	1.03
Ca, %	1.4
Na, %	0.3%
Vitamin A, $IU.kg^{-1}$	10 000
Vitamin C, $mg.kg^{-1}$	520
Vitamin E, $mg.kg^{-1}$	200
Vitamin D_3, $IU.kg^{-1}$	2 303
Gross energy, $MJ.kg^{-1}$	21.0
Digestible energy, $MJ.kg^{-1}$	19.2

Fig. 2.2.2. Sturgeon cage farm

The shape of the cages in the farm is square, one side is 8 m. Each cage is equipped with a double polyamide net, and the working depth is 6 m (Fig. 2.2.3.).

Fig. 2.2.3. Industrial cages.

Fishes of different origin and category are reared separately in different cages on the farm.

2.3. Morphometric analysis

Classical methods for studying living hydrobionts are applied. A morphological study scheme developed specifically for sturgeon species and hybrids was used (Table 2.3.1; Figure 2.3.1; 2.3.2).

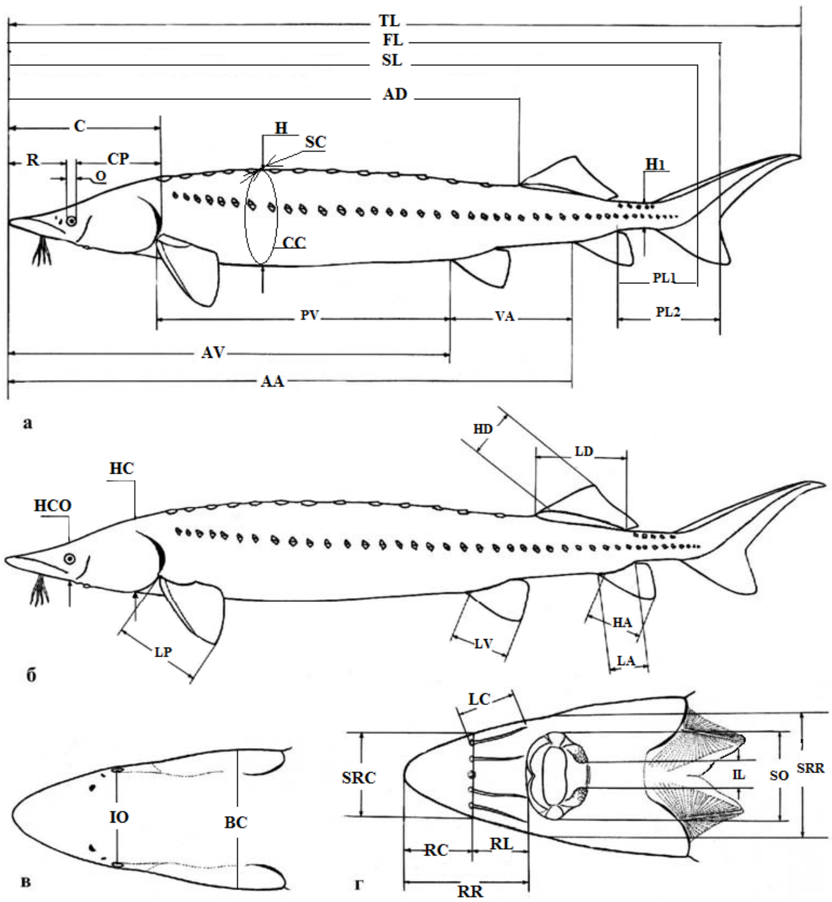
Fig. 2.3.1. Fish measurement.

An ichthyological 3D graduated board with an accuracy of 1 mm was made and used for morphometric measurements (lengths, thicknesses, body heights etc.). In addition to the board, a caliper with an accuracy of 0.1 mm and a graduated tape (to measure the girth of the body) with an accuracy of 1 mm were used for the individual measurements of the body, fins and head. The morphometric indicators characterizing the growth and development were studied by weight and linear methods in the dynamics, as each individual was measured a total of 36 plastic (20 on the body and 16 on the head) and 7 meristic signs.

Table 2.3.1. Metric and meristic features used in the study

Features	Code
1	***2***
Total body weight, g	**BW**
Metric body features	
1. Total length, cm	**TL**
2. Fork length, cm	**FL**
3. Standart length, cm	**SL**
4. Antidorsal distance, cm	**AD**

1	*2*
5. Antiventral distance, cm	**AV**
6. Antianal distance, cm	**AA**
7. Maximum body width, cm	**SC**
8. Maximum body height, cm	**H**
9. Minimum body height, cm	**H1**
10. Tail stalk length - from the end of the anal fin to the roots of the middle rays of the caudal fin, cm	**PL1**
11. Tail stalk length - from the end of anal fin to the end of the middle rays of the caudal fin, cm	**PL2**
12. Dorsal fin length, cm	**LD**
13. Dorsal fin height, cm	**HD**
14. Anal fin length, cm	**LA**
15. Anal fin height, cm	**HA**
16. Pectoral fin length, cm	**LP**
17. Abdominal fin length, cm	**LV**
18. Pecto – ventral distance, cm	**PV**
19. Ventro – anal distance, cm	**VA**
20. Maximum body girth, cm	**CC**
Metric head features	
21. Head lenght, cm	**C**
22. Snout length, cm	**R**
23. Maximum head height (before the 1st dorsal bony scute), cm	**HC**
24. Minimum head height (above the eye), cm	**HCO**
25. Behind eye area length, cm	**CP**
26. Horizontal eye diameter, cm	**O**
27. Inter orbital distance, cm	**IO**
28. Maximum head width, cm	**BC**
29. Distance from the beginning of the snout to a line passing through the middle of the front barbels` roots, cm	**RC**
30. Distance from the end of the snout to the mouth cartilaginous arch, cm	**RR**
31. Distance from the middle barbels` roots to the mouth cartilaginous arch, cm	**RL**
32. Longest / lateral / barbel`s length, cm	**LC**
33. Snout width at the middle barbels` roots, cm	**SRC**
34. Snout width at the mouth cartilaginous arch, cm	**SRR**
35. Mouth width, cm	**SO**
36. Lower lip`s break width, cm	**IL**
Meristic features	
1. Number of dorsal bony scutes	**SD**
2. Number of lateral bony scutes from the left side of the fish	**SL1**
3. Number of lateral bony scutes from the right side of the fish	**SL2**
4. Number of ventral bony scutes from the left side of the fish	**SV1**
5. Number of ventral bony scutes from the right side of the fish	**SV2**
6. Number of rays in the dorsal fin	**D**
7. Number of rays in the anal fin	**A**



|**Fig. 2.3.2. Sturgeon fish measurement scheme.**

Morphometric indices were calculated on the basis of the morphometric measurements (Table 2.3.2).

Table 2.3.2. Morphometric indices

Indices	
CFF	Fulton's coefficient ((BW/SL^3)*100), %
IC	Condition index (BW/(SL*H*CC) *100), %
ICR	Modified Fulton's coefficient by Jones et al., 1999 (acording Richter et al., 2000) ((BW/(SL^{2}H))*100)
IHB	High-backed index (SL/H)
IBB	Broad-backed index ((SC/SL)*100), %
ILH	Long-headed index ((C/SL)*100), %
IH	Hardness index ((CC/SL)*100)), %

Morphometric studies were performed with male and female individuals of Russian sturgeon and a hybrid of Siberian and Russian sturgeon of the same age (Table 2.3.3.)

Table 2.3.3. Characteristics of different fish groups subject to morphometric studies.

Species	Sex	Number	Age, years
Russian sturgeon (*A. gueldenstaedtii*)	Female	25	7
Russian sturgeon (*A. gueldenstaedtii*)	Male	25	7
Hybrid (F_1 *A. baerii* x *A. gueldenstaedtii*)	Female	25	7
Hybrid (F_1 *A. baerii* x *A. gueldenstaedtii*)	Male	25	7

2.4. Sexual developement

We monitored the sexual development of male and female individuals through non-invasive dynamic monitoring, using the methods of ultrasound examination of live fish.

We conducted ultrasound monitoring of randomly selected individuals from the respective flocks during the spring and autumn - winter seasons for each group of sturgeon (Table 2.4.1.).

We applied the methods developed and implemented by Chebanov and Galich (2010) to collect a collection of ultrasound images demonstrating the dynamics of the development of gonads of different species and hybrids grown under identical conditions in real production conditions in a super-intensive cage farm.

Table 2.4.1. Description of the individuals subject to ultrasound monitoring.

Species	Sex	Season		Age Years
		Spring	Autumn-winter	
Russian sturgeon (*A. gueldenstaedtii*)	Female	n= 10	n= 10	5
		n= 10	n= 10	6
		n= 10	n= 10	7
		n= 10	n= 10	8
	Male	n= 10	n= 10	5
		n= 10	n= 10	7
Hybrid (F1 *A. baerii* x *A. gueldenstaedtii*)	Female	n= 10	n= 10	5
		n= 10	n= 10	6
		n= 10	n= 10	7
		n= 10	n= 10	8
	Male	n= 10	n= 10	7
Siberian sturgeon (*A. baerii*)	Female	n= 10	n= 10	3
	Male	n= 10	n= 10	3

A portable ultrasound scanner type Mindray DP 50, with a linear transducer: 75L38EA (5.0-10.0 MHz) was used to perform the task (Fig. 3.3.1), and for the needs of the study we performed the scan with a frequency of 8.5 MHz and a depth of up to 4 cm.

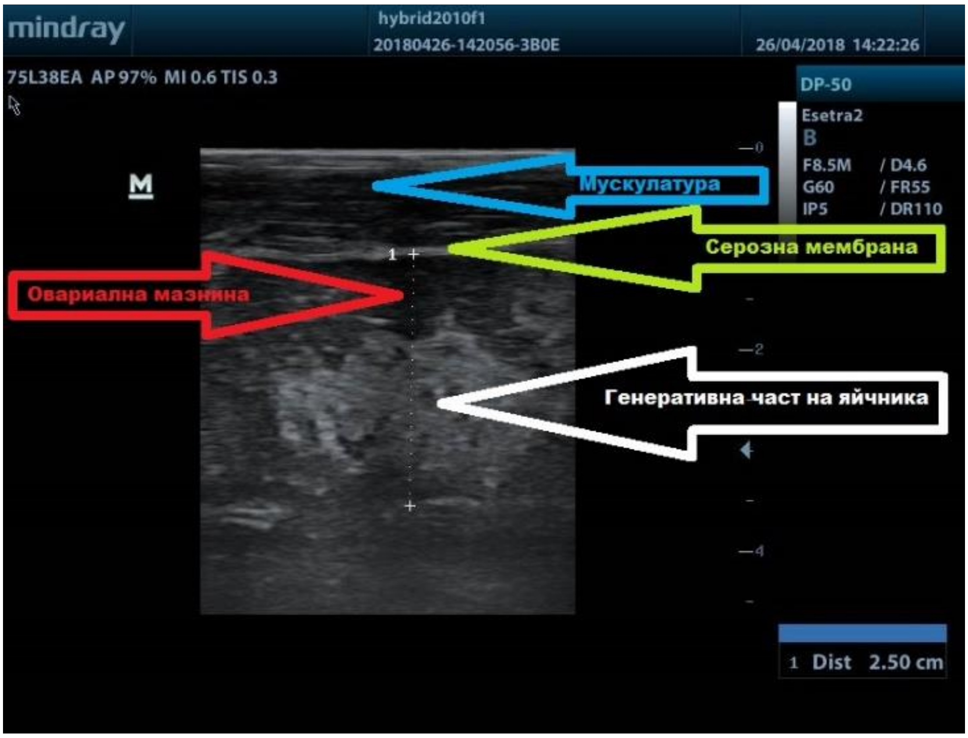
The ultrasound scan was performed on the left side of each individual, just above the line of the abdominal bony scutes in the area between the 3rd and 4th one from the tail to the head. The scan was performed in frontal and transverse directions (Fig. 2.4.2.).

Fig. 2.4.1. Portable ultrasound scanner Mindray DP 50, equipped with a linear transducer.

During the scan we took into account the condition of the gonads and performed their measurements: height, cm; girth, cm; area, cm^2 (Fig. 2.4.3; 2.4.4).

We determined the stages of maturity in all studied individuals, according to the classification developed by Trusov (1972) for the gonad stages of maturation in Russian sturgeon by ultrasound (Chebanov & Galich, 2013). According to the classification, phases M1, M2, M2sf, M2f, M3, M4 and M5 are reported in males, and F1, F2, F2sf, F2f, F2-3, F3, F4i, F4c, F5 and F6 in females, respectively.

Fig. 2.4.2. Ultrasound scan of sturgeon with marked directions of work with the transducer



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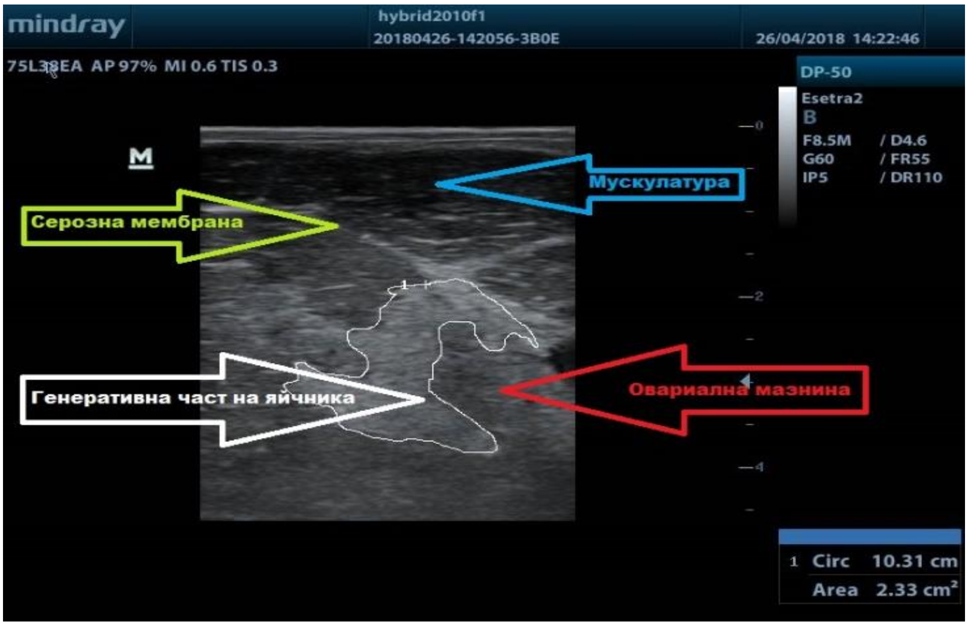

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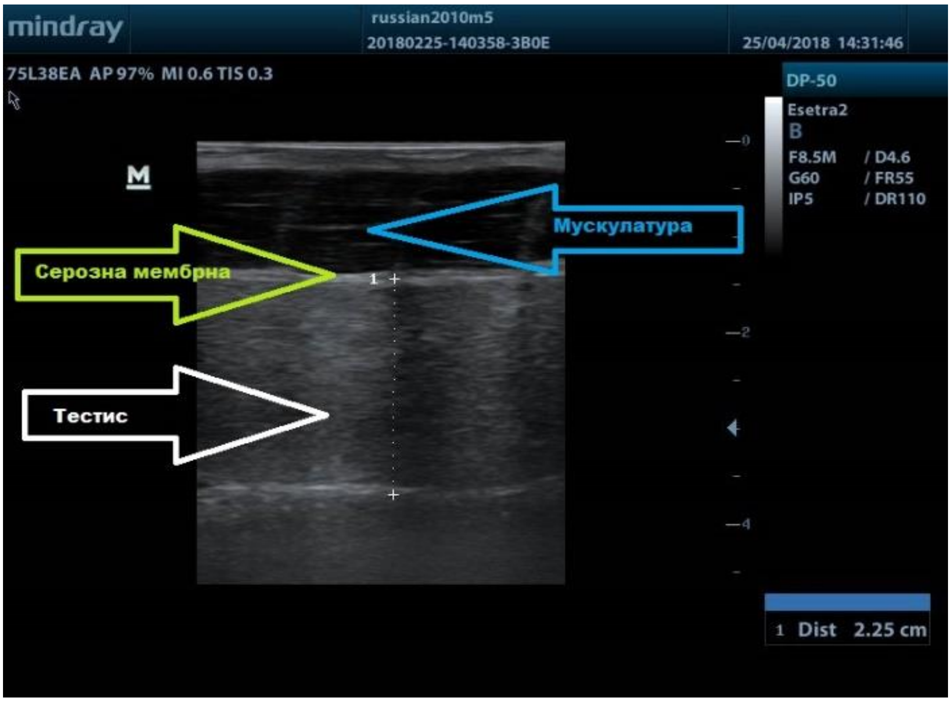
Fig. 2.4.3. Ultrasound examinations of female individuals: 1- Height of the ovary in frontal scanning; 2- Girth and area of the ovary in transverse scanning.



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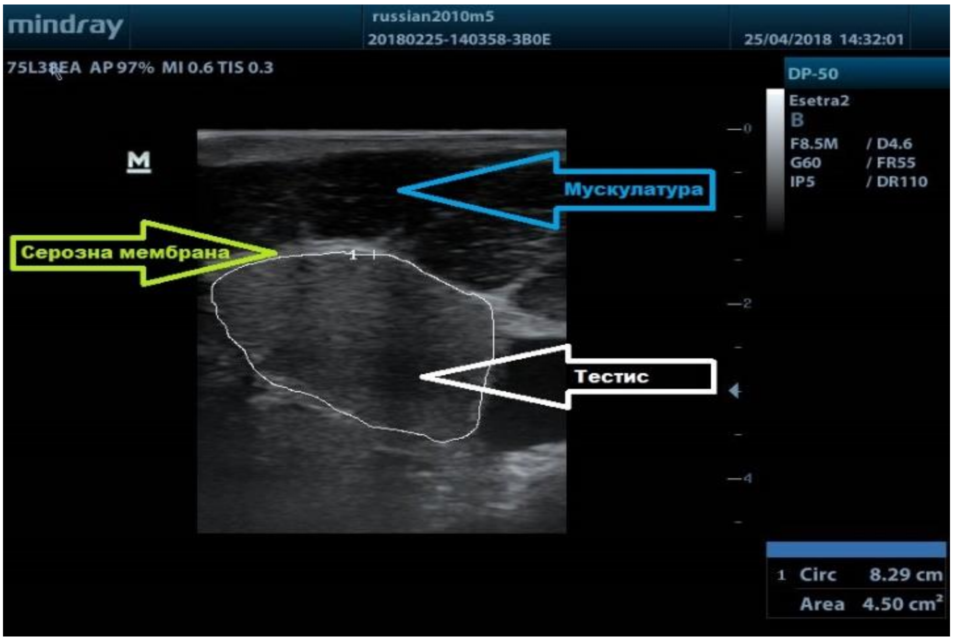

(2)

Fig. 2.4.4. Ultrasound examinations of male individuals: 1- Height of the testis in frontal scanning; 2- Girth and area of the testis during transverse scanning.

2.5. Sperm production analysis

The study was performed with Russian sturgeon (*Acipenser gueldenstaedtii*) at seven (n = 5), eight (n = 8) and nine (n = 15) years and a hybrid (F1 *Acipenser gueldenstaedtii* x *Acipenser baerii*) at seven (n = 5) , eight (n = 3) and nine (n = 18) years of age.

The fish are randomly selected from the flocks of producers grown on the farm.

The study was conducted over three consecutive years. An individual catheter for each fish was used to obtain the semen (Fig. 2.5.1.). Routine methodological approaches have been followed in obtaining semen. The volume of ejaculate was immediately measured in place, after which the semen was transported in cooler bags to a laboratory for biochemical analysis.

Фигура 2.5.1. Получаване на сперма от есетрови риби

Sperm concentrations were determined using a CASA system (Fig. 2.5.2.) as well as total motility; VCL – curvilinear velocity; VSL – straight line velocity; VAP – average path velocity; LIN - linearity; STR - straightness; WOB - wobble; ALH - amplitude of lateral head displacement; BCF – beat-cross frequency; the percentage of rapid, medium, slow and static sperm; sperm, with non-progressive and progressive motility.

Фигура 2.5.2. CASA system

The activity of LDH (lactate dehydrogenase), AP (alkaline phosphatase), GGT (gamma glutamyltransferase) and CK (creatine kinase) was analyzed. To remove the seminal plasma, ejaculate was centrifuged at 3000 rpm (Hermile Labor Technik Z326 K) for 10 min. Enzyme activity was performed by biochemical analyzer Mindray BA88A, using the following reagents:

1. GGT-Tris buffer 100 mM pH 8.25, glycyl glycine 100 mM, L-glutamyl-4-nitroanilide 4 mM, wavelength 405 nm;

2. AP-DEA buffer pH 9.8 1M, MgCl2 0.5 mM, 4-nitrophenylphosphate 10 mM, wavelength 405 nm;

3. CK-imidazole buffer 29 mM pH 6.50, creatine phosphate 30 mM, glucose 20 mM, N-acetyl-L-cysteine 20 mM, magnesium acetate 10 mM, EDTA 2 mM, ADP 2mM, NADP 2mM, AMP 5mM, Di (adenosine-5) pentaphosphate 12 MikroM, glucose-6-phosphate dehydrogenase> 3kU / l, hexokinase> 3kU / l, wavelength 340 nm;

4. LDH-phosphate buffer pH7.50 50 mM, sodium pyruvate 0.60 mM, NADH 0.18 mM, 340 nm wavelength.

Фигура 2.5.3. Biochemical analyzer Mindray BA88A

Sperm pellet, received after seminal plasma removal was resuspended with 0.9% saline and centrifuged at 3000 g for 10 min at 4°C. The procedure was repeated 3 times. After last centrifugation, the pellet was resuspended with distilled water and frozen at -20° C overnight. On the next day, the samples were sonicated by ultrasound 3 x 10 sec and centrifuged at 12000 g, 30 min. at 4°C. Received supernatant, containing water soluble proteins was removed and analyzed for enzyme activity. The pellet was resuspended with 1% triton X100 and centrifuged at the same condition. Supernatant was analyzed for membrane connected proteins.

2.6. Graphics and photos

Original ultrasound photos from a collection made with a Mindray DP 50 ultrasound scanner are used in the disertation; original photos of live fish from the studied flocks. The graphics for presenting the results are performed with MS Excel 10.

2.7. Statistical data processing.

One-way analysis of variance (ANOVA) was performed for statistical data processing, using specialized software - IBM SPSS Stasistics 21. The data in the individual sections are tabulated and presented as X ± Sx and CV, where X is the arithmetic mean for each sample, Sx is the error of the arithmetic mean, and CV is the sign coefficient of variation.

Differences between groups in the individual studies in all sections were determined by Fisher's LSD method, with a significant level of $P \leq 0.05$ (0.01 or 0.001).

3. RESULTS AND DISCUSSION

3.1. Morphometric characteristics of sexually mature fish of the Acipenseridae family, reared under conditions of super-intensive cage technology

Different methods are used to characterize fish, and to solve scientific and selection problems morphometric analysis, including plastic, meristic indicators and morphophysiological indices is mainly used. Morphometric indicators and morphophysiological indices are the subject of studies in different breeds and populations of fish.

3.1.1. Plastic, meristic signs and morphometric indices in Russian sturgeon of different sexes

Plastic signs in seven-year-old Russian sturgeons of different sexes are presented in Table 3.1.1.1. and 3.1.1.2.. We measured different body lengths used in ichthyological practice. The mean body weight of females was 5.1 ± 0.14 kg and that of males was 4.5 ± 0.10 kg. A total of 20 plastic signs of the body and 16 of the head are included in the measurement scheme.

The study of morphological variability allows to assess the rate of reaction in a particular species, its adaptive capacity. This is especially important for fish farmed in production conditions.

The comparative analysis of the individual parameters to the total length of the body ratio (Table 3.1.1.1.) shows that there are significant differences between Russian sturgeons of different sexes. In female fish the ratios - AV / TL ($P < 0.001$) ; SC / TL ($P < 0.01$); PV / TL ($P < 0.001$) and CC / TL ($P < 0.001$) are larger but C / TL ($P < 0.01$); PL1 / TL ($P < 0.001$); PL2 / TL ($P < 0.001$); HA / TL ($P < 0.001$); LV / TL ($P < 0.05$); VA / TL ($P < 0.001$) are smaller.

Significant differences were found between the sexes and in the relative values of the individual features of the head to its length in the Russian sturgeon object of our study, (Table 3.1.1.2.).

Females have a greater (p <0.001) value at the smallest head height (HCO), a greater (p <0.001) length of the behind eye area (CP) and a larger (p <0.05) horizontal diameter of the eye (O).

Table 3.1.1.1. Individual measurements to the total body length ratio in Russian sturgeon,%

Features	Sex	X	Min	Max	±Sx	CV
FL/TL	**F**	86.88	83.05	96.44	0.57	3.00
	M	87.02	84.42	96.13	0.50	2.62
SL/TL	**F**	81.32	78.48	84.84	0.32	1.83
	M	82.20	79.47	89.28	0.44	2.44
AD/TL	**F**	62.54	59.05	65.68	0.37	2.72
	M	61.96	58.82	70.17	0.51	3.75
AV/TL	**F**	54.28***	51.46	58.21	0.39	3.33
	M	52.23***	49.95	58.01	0.42	3.67
AA/TL	**F**	67.69	64.00	72.21	0.39	2.62
	M	67.06	64.51	73.81	0.43	2.97
SC/TL	**F**	9.92**	9.14	10.84	0.10	4.72
	M	9.46**	8.45	10.50	0.14	6.72
H/TL	**F**	12.36	11.46	13.89	0.12	4.53
	M	12.28	11.45	15.11	0.19	7.04
H1/TL	**F**	3.62	3.17	4.18	0.05	6.27
	M	3.66	3.13	4.14	0.05	6.09
C/TL	**F**	16.61**	15.80	18.05	0.12	3.25
	M	17.22**	15.66	19.34	0.18	4.83
PL1/TL	**F**	8.45***	7.42	9.36	0.12	6.34
	M	9.92***	8.67	11.86	0.18	8.40
PL2/TL	**F**	13.78***	12.65	15.22	0.15	4.96
	M	15.50***	12.83	17.24	0.21	6.08
LD/TL	**F**	10.12	9.26	10.95	0.11	4.90
	M	10.40	9.11	11.75	0.18	8.12
HD/TL	**F**	9.24	7.14	10.57	0.19	9.46
	M	9.60	6.98	12.11	0.29	13.75
LA/TL	**F**	5.59	4.01	7.09	0.16	12.75
	M	5.88	4.24	12.33	0.34	26.81
HA/TL	**F**	9.10***	7.29	10.38	0.20	9.83
	M	10.30***	8.51	11.93	0.20	8.82
LP/TL	**F**	11.83	10.62	13.05	0.17	6.63
	M	11.94	8.58	15.00	0.39	15.04
LV/TL	**F**	7.73*	6.48	8.88	0.15	9.16
	M	8.56*	6.30	10.36	0.29	15.31
PV/TL	**F**	36.75***	33.57	40.48	0.42	5.23
	M	34.15***	31.92	40.10	0.45	6.10
VA/TL	**F**	13.98**	11.67	15.54	0.23	7.60
	M	15.40**	12.57	21.13	0.41	12.19
CC/TL	**F**	37.60***	35.30	41.01	0.32	3.85
	M	34.75***	31.44	39.23	0.41	5.40

Differences between the values within the feature are significant: ****P<0.001, **P<0.01, *P<0.05*

In turn, male fish have higher values for the different lengths around the rostrum and mouth - such as the length of the rostrum (R) (p <0.001), the length from the beginning of the rostrum to a line passing through the middle of the front barbels` roots (RC) (p <0.001), length from the end of the rostrum to the cartilaginous arch of the mouth (RR) (p <0.001) and length from the roots of the middle barbels to the cartilaginous arch of the mouth (RL) (p <0.001).

Table 3.1.1.2. Head metric features to head length ratio of seven years old Russian sturgeon, %

Features	Sex	X	Min	Max	±Sx	CV
% of the head length						
R/C	**F**	31.84***	28.91	37.88	0.48	6.91
	M	38.08***	29.81	42.90	0.67	8.04
HC/C	**F**	53.25	48.48	61.30	0.80	6.88
	M	51.20	44.13	82.35	1.71	15.29
HCO/C	**F**	34.51***	31.89	41.36	0.47	6.27
	M	31.96***	28.70	37.74	0.42	6.01
CP/C	**F**	62.73***	59.15	67.92	0.46	3.36
	M	56.74***	51.40	63.83	0.71	5.75
O/C	**F**	9.67*	7.41	12.80	0.31	14.72
	M	8.77*	6.73	14.69	0.35	18.47
IO/C	**F**	36.84	34.03	38.51	0.24	3.01
	M	37.36	35.48	39.42	0.24	2.93
BC/C	**F**	47.03	44.90	49.11	0.29	2.81
	M	47.08	45.41	49.12	0.27	2.64
RC/C	**F**	9.25***	7.46	11.31	0.24	11.97
	M	15.57***	9.01	19.69	0.54	15.89
RR/C	**F**	33.42***	28.85	36.19	0.50	6.85
	M	40.89***	30.68	44.41	0.78	8.72
RL/C	**F**	23.68***	20.37	28.48	0.51	9.95
	M	26.81***	23.33	33.21	0.51	8.80
LC/C	**F**	17.21	12.34	19.87	0.43	11.52
	M	17.78	10.97	22.17	0.61	15.71
SRC/C	**F**	28.83	24.40	34.35	0.52	8.32
	M	29.05	26.02	34.21	0.43	6.84
SRR/C	**F**	48.92***	45.56	53.92	0.40	3.75
	M	46.43***	43.52	49.60	0.36	3.58
SO/C	**F**	36.13***	32.07	39.88	0.37	4.66
	M	31.09***	27.94	34.69	0.39	5.82
IL/C	**F**	8.84	4.90	12.49	0.39	20.36
	M	9.63	6.88	12.96	0.33	15.68
в % of the mouth width						
IL/SO	**F**	24.48***	13.01	34.40	1.06	19.79
	M	30.94***	21.67	37.37	0.89	13.23

Differences between the values within the feature are significant: ****P<0.001*, ***P<0.01*, **P<0.05*

The comparison of the results obtained in individual morphometric studies of sturgeon shows that individual populations can differ significantly in characteristics and proportions of the body. Exterior profiles are used to assess the physique of animals, and in fish they are built on the basis of each feature and the total body length ratio.

We made separate body and head profiles. The body profiles of Russian sturgeon of both sexes are presented in Fig. 3.1.1.1. The ratios in female fish - AV / TL ($P < 0.001$); SC / TL ($P < 0.01$); PV / TL ($P < 0.001$) and CC / TL ($P < 0.001$) are higher and C / TL ($P < 0.01$); PL1 / TL ($P < 0.001$); PL2 / TL ($P < 0.001$); HA / TL ($P < 0.001$); LV / TL ($P < 0.05$); VA / TL ($P < 0.001$) are smaller, as well as the ratios HCO / C ($P < 0.001$); CP / C ($P < 0.001$); O / C ($P < 0.05$); SRR / C ($P < 0.001$); SO / C ($P < 0.001$) are higher and R / C ($P < 0.001$); RC / C ($P < 0.001$); RR / C ($P < 0.001$) (Fig. 3.1.1.2) are smaller. The ratio of lower lip brake to mouth width is smaller ($P < 0.001$) in female Russian sturgeons.

Based on the measurements, we calculated 7 exterior indices (Table 3.1.1.3.). Different scientific studies related to the exterior of fish use different coefficients of fatness, which is why we calculated several basic indices used in fish farming - CFF, IC and ICR. This gives a good opportunity to compare the results obtained by us with those obtained by other authors.

Using the Fulton's body condition factor (CFF) is a classic method for non-invasive determination of fish fatness by determining body weight and standard body length. This condition factor is studied not only in different species, but also in different breeds of fish.

A number of coefficients have been developed in order to improve the accuracy of establishing the fatness of live fish, which include more exterior indicators. Thus, ICR takes into account body height, and IC takes into account body height and girth.

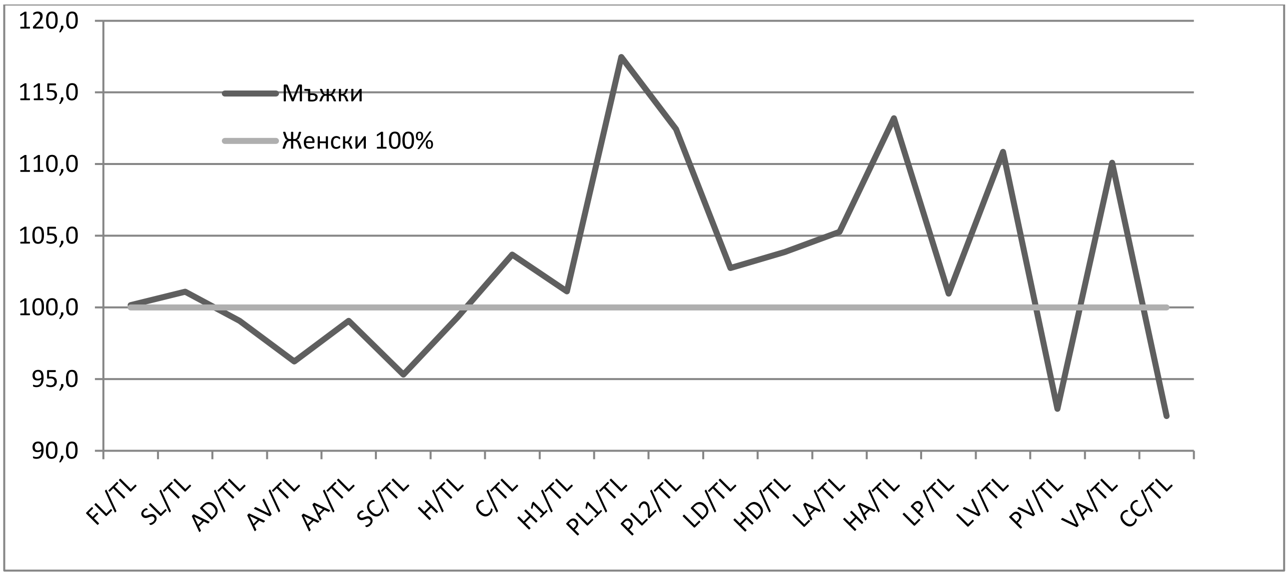

Fig. 3.1.1.1. Exterior body profiles of Russian sturgeon of different sex (100% - females).

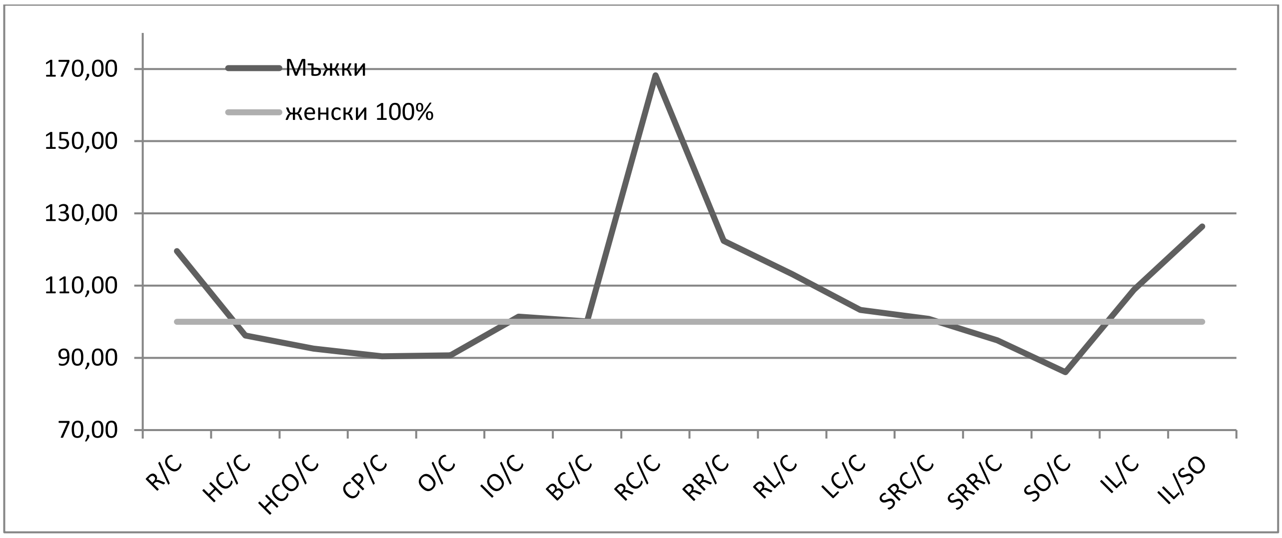

Fig. 3.1.1.2. Exterior head profiles of Russian sturgeon of different sex (100% - females).

Table 3.1.1.3. Morphometric indices in Russian sturgeon.

Features	Sex	X	Min	Max	±Sx	CV
CFF	**F**	0.97***	0.86	1.11	0.01	6.49
	M	0.87***	0.73	1.05	0.02	10.3
IC	**F**	13.8	12.80	16.20	0.16	5.38
	M	13.9	11.60	15.40	0.21	7.01
ICR	**F**	6.40***	5.75	7.28	0.08	5.90
	M	5.85***	5.09	6.87	0.10	8.21
IHB	**F**	6.59	6.11	7.08	0.06	4.29
	M	6.72	5.44	7.15	0.10	6.49
IBB	**F**	12.20**	11.00	13.20	0.12	4.44
	M	11.50**	10.10	12.60	0.17	6.68
ILH	**F**	20.40*	19.10	21.80	0.15	3.33
	M	21.00*	18.60	22.30	0.22	4.77
IH	**F**	46.20***	43.10	50.10	0.39	3.83
	M	42.3***	37.60	45.40	0.50	5.46

The differences between the values within the feature are significant: ****P<0.001, **P<0.01, *P<0.05*

The data in table. 3.1.1.3. show similar trends in CFF and ICR comparison in both male and female Russian sturgeons. These indices show that female Russian sturgeons have significantly (P <0.001) better fattening than males of the same age under the same breeding conditions. Regarding the IC and the high backed index, no significant differences were found between the sexes, and the difference in body thickness (P <0.01) is in favor of female fish. The long-headed index is higher (P <0.05) in male fish and the hardness index is higher in female fish (P <0.001).

Meristic features in Russian sturgeon of different sexes are presented in the table 3.1.1.4.. The number of dorsal scutes in our study was from 9 to 13 in females, from 8 to 13 in males (Table 3.1.1.4.) and no significant difference in the indicator was found in fish of different sex. The coefficient of variation is medium, slightly exceeding the maximum level for a low degree of variation.

The number of lateral bony scutes on the left side of the fish varies from 24 to 30 in females and from 25 to 32 in males. There is a low degree of variation in the indicator. A difference was found between the number of lateral scutes on the left and on the right. On the right, their number varies from 22 to 31 in females and from 25 to 32 in males.

Female fish have significantly fewer ventral scutes on both the left ($P < 0.05$) and right ($P < 0.01$) side. The maximum number of ventral scutes in both male and female fish on both sides of the body is the same - 11. The minimum number of ventral scutes on the left side in female fish is 7 and in males 8. Eight is the minimum number of scutes on the right side in fish of both sexes.

Table 3.1.1.4. Meristic features in Russian sturgeon

Features	Sex	X	Min	Max	±Sx	CV
SD	**F**	11.30	9	13	0.25	10.30
	M	11.20	8	13	0.26	10.60
SL1	**F**	27.50	24	30	0.46	7.66
	M	28.10	25	32	0.51	8.23
SL2	**F**	27.10	22	31	0.46	7.66
	M	27.30	25	34	0.96	16.20
SV1	**F**	9.14*	7	11	0.25	12.30
	M	9.73*	8	11	0.18	8.50
SV2	**F**	8.86**	8	11	0.19	10.00
	M	9.68**	8	11	0.20	9.23
D	**F**	32.20	21	39	0.90	12.80
	M	34.50	23	45	1.12	15.00
A	**F**	19.90	16	25	0.44	10.10
	M	19.60	16	23	0.44	10.40

The differences between the values within the feature are significant: ***$P<0.01$*, *$P<0.05$*

There are no significant differences between the sexes of Russian sturgeon in the amount of rays in the dorsal and anal fins. The amount of rays in the dorsal fin varies from 21 to 39, and in the anal - from 16 to 25 in female fishes. In males, respectively, from 23 to 45 and from 16 to 23. The highest variation, within the average regarding the rays number in the fins of the studied fish was found to be the dorsal fin in the male individuals.

3.1.2. Plastic, meristic features and morphometric indices for hybrids (F1 *A. baeri* x *A. gueldenstaedtii*) of different sex.

Morphometric studies usually calculate the ratio of individual measurements to the length of the fish body.

Table 3.1.2.1. Individual measurements to total body length ratio in hybrids (F1 Ab x Ag),%

Features	Sex	X	Min	Max	±Sx	CV
FL/TL	**F**	87.20	85.30	87.90	0.20	1.11
	M	86.60	75.10	89.80	0.58	3.31
SL/TL	**F**	81.70	78.80	84.20	0.27	1.60
	M	81.10	70.90	83.80	0.54	3.24
AD/TL	**F**	62.40*	60.00	64.60	0.27	2.10
	M	61.30*	58.40	63.90	0.30	2.37
AV/TL	**F**	54.00*	49.30	70.40	0.80	7.26
	M	51.90*	49.40	54.20	0.26	2.44
AA/TL	**F**	67.70*	63.90	70.10	0.29	2.09
	M	66.50*	60.60	69.90	0.41	3.01
SC/TL	**F**	10.30	9.08	11.00	0.10	4.71
	M	10.30	9.10	11.10	0.11	5.08
H/TL	**F**	11.60**	11.00	13.20	0.11	4.47
	M	12.30**	10.70	14.10	0.16	6.37
H1/TL	**F**	3.23*	2.91	3.85	0.04	5.75
	M	3.37*	2.91	3.83	0.04	6.35
C/TL	**F**	18.40	16.40	20.10	0.20	5.25
	M	18.60	16.50	21.0	0.18	4.83
PL1/TL	**F**	8.64	7.64	10.10	0.13	7.09
	M	8.68	7.48	10.80	0.14	7.94
PL2/TL	**F**	14.40	12.80	16.60	0.18	5.98
	M	14.30	13.00	15.20	0.13	4.39
LD/TL	**F**	11.24	9.72	12.62	0.12	5.26
	M	11.02	9.71	12.18	0.14	6.01
HD/TL	**F**	9.63*	8.02	10.70	0.13	6.77
	M	9.20*	8.22	10.60	0.13	6.76
LA/TL	**F**	5.43**	4.27	6.02	0.09	7.98
	M	5.89**	4.73	6.80	0.10	8.41
HA/TL	**F**	10.00*	8.21	11.10	0.14	6.96
	M	9.63*	7.99	10.40	0.12	6.20
LP/TL	**F**	12.90***	11.10	14.60	0.19	7.17
	M	11.60***	9.93	13.10	0.18	7.61
LV/TL	**F**	8.29*	5.09	9.71	0.18	10.50
	M	7.93*	6.99	8.83	0.10	5.89
PV/TL	**F**	34.80**	31.40	36.10	0.25	3.52
	M	33.70**	31.80	36.00	0.29	4.15
VA/TL	**F**	14.90	13.80	16.00	0.11	3.69
	M	15.00	13.40	17.10	0.15	4.89
CC/TL	**F**	36.60	34.10	40.60	0.32	4.35
	M	36.50	32.90	40.00	0.34	4.56

The differences between the values within the feature are significant: ****P<0.001, **P<0.01, *P<0.05*

Table 3.1.2.2. Plastic features of the head to its length ratio in hybrids (Ab x Ag),%

Features	Sex	X	Min	Max	±Sx	CV
% of head length						
R/C	**F**	37.30***	34.30	39.50	0.27	3.61
	M	42.00***	36.60	46.50	0.51	5.97
HC/C	**F**	44.80	39.00	53.60	0.72	7.89
	M	43.50	37.70	49.10	0.52	5.84
HCO/C	**F**	30.60*	26.60	33.70	0.36	5.83
	M	29.50*	26.50	32.50	0.38	6.32
CP/C	**F**	57.20***	51.70	60.10	0.38	3.27
	M	53.20***	47.80	58.30	0.57	5.25
O/C	**F**	7.74**	7.10	8.63	0.09	6.00
	M	7.26**	6.60	8.77	0.10	6.84
IO/C	**F**	34.20	30.80	38.3	0.33	4.80
	M	33.60	29.90	35.90	0.33	4.83
BC/C	**F**	45.00***	39.50	48.70	0.55	6.00
	M	41.40***	38.10	46.30	0.38	4.50
RC/C	**F**	16.00***	13.70	17.90	0.22	6.73
	M	20.70***	15.00	26.50	0.64	15.10
RR/C	**F**	38.90***	35.60	41.70	0.29	3.60
	M	43.30***	37.10	48.20	0.64	7.24
RL/C	**F**	23.60	20.90	25.30	0.23	4.83
	M	23.00	20.80	26.40	0.32	6.73
LC/C	**F**	21.00	16.20	25.90	0.50	11.60
	M	19.90	13.30	23.20	0.49	12.00
SRC/C	**F**	31.20	27.60	35.20	0.39	6.11
	M	31.30	27.10	35.20	0.40	6.23
SRR/C	**F**	47.00***	42.40	50.30	0.44	4.61
	M	43.60***	38.30	48.30	0.47	5.23
SO/C	**F**	35.80***	33.20	38.10	0.26	3.53
	M	32.20***	29.40	36.90	0.39	5.90
IL/C	**F**	5.58*	2.13	8.13	0.28	24.70
	M	6.43*	4.11	9.64	0.31	23.50
% of mouth width						
IL/SO	**F**	15.50***	9.54	22.10	0.75	23.70
	M	20.00***	14.00	28.70	0.91	22.40

The differences between the values within the feature are significant: ****P<0.001, **P<0.01, *P<0.05*

The analysis of the ratio of the individual measurements to the total body length showed a significant difference between individuals of different sex on several indicators (Table 3.1.2.1.). The values of H / TL (P <0.01); H1 / TL (P <0.05); LA / TL (P <0.01) are higher in male individuals and lower in AD / TL (P <0.05); AV / TL

(P <0.05); AA / TL (P <0.05); HD / TL (P <0.05); HA / TL (P <0.05); LP / TL (P <0.001); LV / TL (P <0.05); PV / TL (P <0.01). In sturgeon breeding, hybridization is usually used to produce hybrids grown for meat. The body shape of fish is closely related to meat-producing characteristics. The plastic features of the head to its length ratio are presented in table 3.1.2.2.. The rostrum occupies a larger share of the head (P <0.001) in male fish than in females.

The rostrum occupies from 34.3 to 39.50% of the head length in female fish, and from 36.60 to 46.50% in males respectively.

There is no significant difference in the maximum head height to its length ratio between female and male hybrids. A significant difference in favor of female fish (P <0.05) was found with respect to the minimum head height to head length ratio.

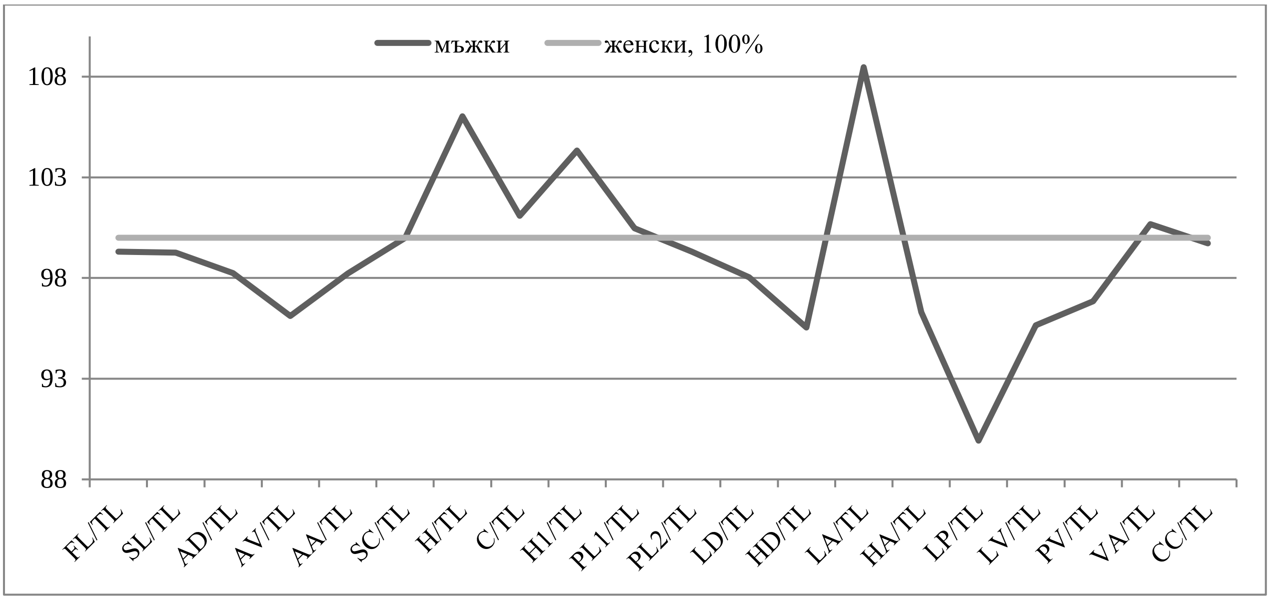

Fig. 3.1.2.1. Exterior body profiles in hybrids of different sex (100% - females).

The length behind the eye to the head length ratio in female fish was significantly higher (P <0.001) as well as eye diameter (P <0.01), the head width (P <0.001), mouth width (P <0.001) and rostrum at the cartilaginous arch (P <0.001) and in males, respectively, the distance from the beginning of the snout to a line passing through the middle of the front barbels` roots (P <0.001) the distance from the end of the snout to the mouth cartilaginous arch (P <0.001). The lower lip brake to the mouth width ratio is significantly higher in male fish (P <0.001).

The exterior profiles of the hybrid body are presented in Fig. 3.1.2.1., And those of the head of Fig. 3.1.2.2.

Morphometric indices of the hybrid are presented in table 3.1.2.3. Exterior indices are taken as a basis for conducting selection work with fish. The high-backed index and the hardness index are especially important. They characterize producers and are directly related to productivity indicators.

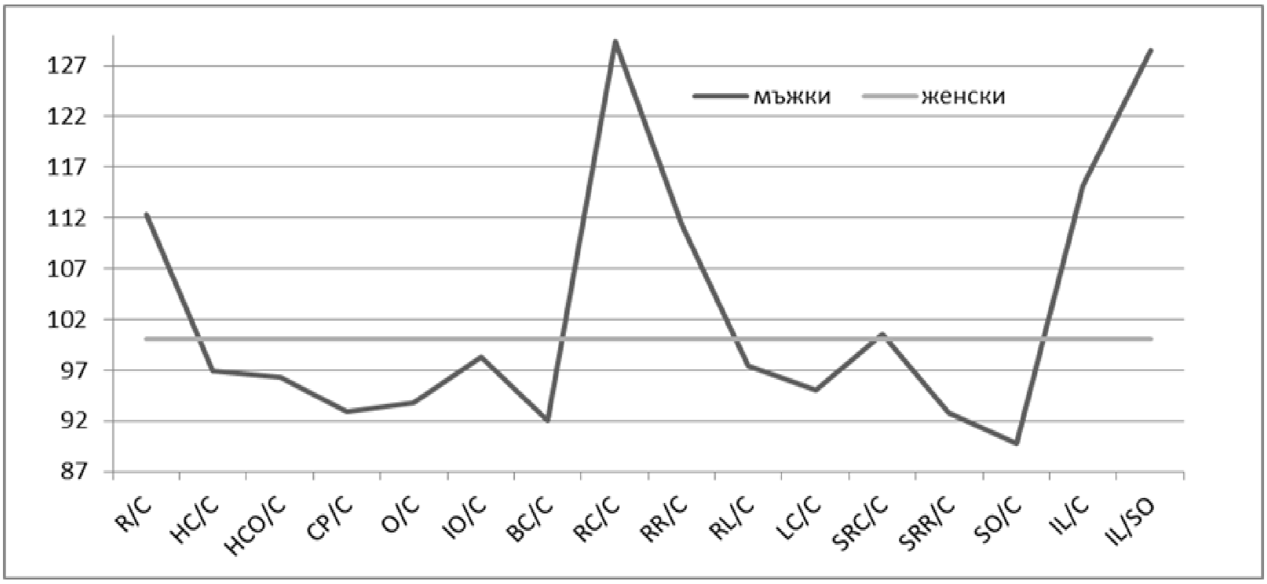

Fig. 3.1.2.2. Head profiles in hybrids of different sex (100% - females).

Table 3.1.2.3. Morphometric indices in hybrids (Ab x Ag).

Features	Sex	X	Min	Max	±Sx	CV
CFF	**F**	0.91	0.84	1.13	0.01	7.90
	M	0.93	0.81	1.33	0.02	12.50
IC	**F**	14.30*	12.50	15.60	0.14	4.65
	M	13.70*	11.60	15.90	0.23	8.08
ICR	**F**	6.39	5.43	7.06	0.08	6.00
	M	6.18	5.00	8.12	0.13	10.30
IHB	**F**	7.03**	6.14	7.40	0.06	4.02
	M	6.64**	11.30	7.73	0.09	6.60
IBB	**F**	12.50	11.90	13.70	0.11	4.40
	M	12.70	11.30	13.90	0.15	5.63
ILH	**F**	22.50	20.10	24.40	0.18	4.02
	M	22.90	20.60	25.60	0.20	4.31
IH	**F**	44.80	41.70	49.90	0.39	4.23
	M	45.10	40.20	51.10	0.48	5.24

The differences between the values within the feature are significant: ****P<0.001, **P<0.01, *P<0.05*

We did not find significant differences between female and male fish in the CFF and ICR obesity indices, but in female fish the IC values were significantly higher (P <0.05). The IC index shows the girth of the body, with the bulkier abdominal area usually associated with more massive gonads in females. The values of the high backed index are higher in female fish (P <0.01). According to the other indices, the differences between the two sexes are not significant.

The variation of the indices in the hybrid was from low to medium in our study, and in most of the indices the variation was low (<10%).

Table 3.1.2.4. Meristic features in hybrids (Ab x Ag),

Features	Sex	X	Min	Max	±Sx	CV
SD	**F**	13.10***	10	16	0.26	9.67
	M	11.70***	9	14	0.23	9.70
SL1	**F**	35.40*	29	41	0.62	8.58
	M	33.40*	27	40	0.68	10.00
SL2	**F**	35.2*	27	39	0.52	7.20
	M	33.60*	27	39	0.72	10.50
SV1	**F**	9.88	8	8	0.23	11.40
	M	9.60	7	13	0.26	13.40
SV2	**F**	9.96**	8	12	0.23	11.40
	M	9.32**	7	11	0.23	12.30
D	**F**	38.40*	28	45	0.75	9.60
	M	36.10*	31	44	0.68	9.19
A	**F**	21.00*	13	26	0.70	16.30
	M	18.90*	15	24	0.45	11.80

The differences between the values within the feature are significant: ****P<0.001, **P<0.01, *P<0.05*

Higher to average variation values were obtained for the CFF and ICR index for male fish. Both indices are related to fish fattening.

The results of the meristic features analysis in hybrids of different sex are presented in the table. 3.1.2.4.

Most morphometric studies do not indicate the sex of the studied fish. There is no significant difference between fish of different sex on the number of ventral scutes on the left side in our study, and female fish have more on the right side ($P \leq 0.01$) and their number varies from 8 to 12. The number of dorsal bony scutes is larger in female fish ($P \leq 0.001$), ranging from 10 to 16. The indicator varies from 9 to 14 in male fish. The difference in favor of female fish regarding the number of lateral scutes on the left and right is significant ($P < 0.05$).

Female fish have a significantly higher ($P \leq 0.05$) number of rays in the dorsal and anal fins. The analysis generally shows a higher variation in the number of rays in the anal fin and in the number of ventral scutes.

The results obtained in our study on the characteristics of the morphometric features of the Siberian and Russian sturgeon hybrid can be useful not only for aquaculture, but also when working with natural populations. Issues of "genetic pollution" are particularly relevant for sturgeon species in the wild. The development of sturgeon farming is one of the tools aimed at reducing anthropogenic pressure on endangered natural populations, while sturgeon aquaculture carries potential risks.

3.2. Sexual development of female and male fish of the Acipenseridae family, reared under conditions of super-intensive cage technology.

Ultrasound methods provide a very good opportunity for non-invasive sex determination. Attempts are also being made to use them to determine the development of gonads in sturgeon. However, applying this method requires the accumulation of a large amount of knowledge about the sexual development of different sturgeon species. The visual structure of the scanned object can be homogeneous or heterogeneous, depending on the echogenicity of the various structures that make up the organ or tissue. For example, a homogeneous structure is characteristic of mature testes, while the ovaries are characterized by a heterogeneous one.

3.2.1. Comparative analysis of ovarian development in the studied fish. Maturity stages.

The ovaries were seen as heterogeneous structures with mixed echogenicity with uneven borders without a tunic in all examined individuals on the ultrasound image. The ovarian fat was visualized in the form of darker areas, clearly different from the brighter generative tissue.

The characteristics of the gonads in female individuals from all studied groups (genotype and age within each genotype) at the beginning (spring period) and the end (autumn-winter period) of the vegetation period are presented in table 3.2.1.1..

There was a significant difference between 5 and 7 year old Russian sturgeons (41.8% (p <0.05)) in favor of older fish during the spring regarding the gonads area in a transverse view. Changes are observed with more features with a greater difference in age. Thus, there is a significant increase in the height (over 2 times; p <0.001) and the area (2 times; p <0.001) of the ovary between 5 and 8-year-old female Russian sturgeons. There is a significant difference (p <0.01) only in the area of the ovary between 6 and 8 years old, where the difference in age is only 2 years, the difference is 62.5% in favor of older fish, and there was more than a twofold increase (p <0.001) with age in ovarian height and area increase by 44.8% (p <0.05) between 7 and 8 years old. Russian sturgeons of different ages have similar trends as in spring at the end of the vegetation period. There was a 68.4% (p <0.05) increase in the area of the gonads with age between 5 and 7 year old fish. Ovarian height increased by 50% (p <0.01) and area by 45.6% (p <0.001) between 6 and 7 years of age. The differences are significant for all the three features between 6 and 8 years old fish. In older fish, the height of the ovary was higher by 46% (p <0.001), the girth by 33.8% (p <0.001) and the area by 45.6% (p <0.05).

There is a seasonally significant gonad increase in 5 and 7 year old fish, as in 5 year olds the ovarian height during the vegetation period increased by 64% (p <0.01), and the height and area increased by 78.5% (p <0.001), and almost double (p <0.001) in 7 year olds.

Табл. 3.2.1.1. Характеристика на гонадите при женски индивиди през пролетен и есенно-зимен период. Table 4.2.1.1. Female gonads characteristics in spring and autumn-winter period.

Season	**Features**								
	Height, cm			**Girth, cm**			**Area, cm**		
	□X	**±Sx**	**CV**	**□X**	**±Sx**	**CV**	**□X**	**±Sx**	**CV**
Ag_5 *									
Spring	0.92 **at**	0.29	35.40	7.03	2.22	25,36	1,29 **ad**	0.41	33.43
Autumn	1.51 **t**	0.48	27.85	7.17	2.27	42,60	2,12 **j**	0.67	64.85
Ag_6									
Sprin	1.78	0.56	12.58	6.29	1.99	21.92	1.63 **c**	0.52	30.08
Autumn	1.27 **gi**	0.40	16.93	6.48 **h**	2.05	13.78	1.84 **fk**	0.58	28.25
Ag_7									
Spring	1.07 **bu**	0.34	17.39	7.19	2.27	13.76	1.83 **deu**	0.58	28.03
Autumn	1.91 **iu**	0.61	24.90	9.27	2.93	7.16	3.57 **fju**	1.13	31.72
Ag_8									
Spring	2.32 **ab**	0.73	39.50	7.78	2.46	29.96	2.65 **ace**	0.84	33.41
Autumn	1.86 **g**	0.59	18.10	8.67 **h**	2.74	12.53	2.68 **k**	0.85	35.48
Hy_5									
Spring	1.39 **lw**	0.49	20.01	6.19	2.19	15.89	1.57 **nv**	0.56	30.09
Autumn	2.43 **w**	0.92	30.10	9.44 **q**	3.57	8.65	4.31 **sv**	1.63	40.49
Hy_6									
Spring	1.93 **lo**	0.61	19.57	7.83	2.48	27.54	2.38 **nz**	0.75	34.03
Autumn	1.96	0.62	27.80	8.80	2.78	25.34	3.73 **z**	1.18	46.64
Hy_7									
Spring	1.83 **x**	0.61	18.15	7.29 **w**	2.43	26.11	1.84	0.61	36.83
Autumn	2.36 **x**	0.89	8.70	10.40 **rw**	3.93	17.69	4.35 **p**	1.64	17.39
Hy_8									
Spring	2.36 **o**	0.75	16.16	7.83	2.48	26.93	2.28	0.72	44.87
Autumn	2.04	0.72	17.19	7.52 **qr**	2.66	15.57	2.67 **ps**	0.94	17.34
Ab_3									
Spring	1.82	0.57	30.94	6.86	2.17	23.82	1.45	0.46	35.98
Autumn	2.17	0.69	11.49	8.23	2.60	22.42	2.56	0.81	43.28

* - The index means the age of the fish in years. Differences between values marked with the same symbols are significant: *a, b, f, g, h, p, v, u - P<0.001; c, i, l, q, r, t, w, x - P<0.01; d, e, j, k, n, o, s, z - P<0.05*

There is a significant difference in favor of older fish between 5 and 6 year old hybrid individuals at the beginning of the vegetation period in terms of ovarian size, as the height is higher by 38.8% (p <0.01) and the area with 51.6% (p <0.05).

The difference between 5 and 7 year old fish in ovarian height was 31.6% (p <0.01) in favor of older fish. The gonads height in 8-year-old female hybrids was higher by 22.2% (p <0.05) compared to 6-year-olds.

We found lower values at the end of the vegetation period, in terms of girth and area for 8-year-old hybrids compared to 5-year-olds, older fish having 25.5% (p <0.01) smaller girth and 61.4% (p<0.05) smaller area. This is probably due to the accumulation of more ovarian fat at the expense of the generative part of the ovary. A similar trend is observed between 7 and 8 year old fish, where the girth of adults is lower by 38.3% (p <0.01) and the area by 62.9% (p <0.001).

There is a seasonal increase in some indicators in 5, 6 and 7 year old hybrids characterizing the gonads, as an example in 5 year olds the height from the beginning to the end of the vegetation period increased by 53.2% (p <0.01) and the area almost three times (p <0.001). The area increased significantly (p <0.05) by 56.7% at 6 years of age. The height increased by 28.9% (p <0.01) and the girth by 42.6% (p <0.01) at the age of 7.

The height of the ovaries was 0.92 cm in 5-year-old Russian sturgeons in our study during the spring, and this indicator in 8-year-old individuals of the same species is 2.32 cm, which clearly shows much better development of the gonads in older fish (Table 3.2.1.1.).

Table 3.2.1.2. presents the state of the gonads with respect to the maturity stages in groups of different origin and age. It is clear that most of the older fish have moved to phase F3 and F4 (F4i and F4c). It can be seen that all individuals have developed at most by the end of the 2nd phase in the case of Russian sturgeons at the age of 5, and 30% of the fish were in this phase during the spring. Fifty percents of the fish reached phase F2sf, with no fish in phase F2f in 6-year-old fish during the spring.

Ten percent of the individuals in both the spring and autumn-winter seasons have already passed into phase F3 in 7-year-old Russian sturgeons with the age increasing.

There is not a single individual in the F4 phase during either spring or autumn in the 5-year-old hybrids. Thirty percent of the fish have reached phase F3 during the autumn in this group. Thirty six percents of the 6-year-old hybrid were found in phase F3, 24% in phase F4i and 4% in phase F4c in the autumn. It should be noted that, neither in spring nor in autumn, individuals with ovarian development in the fourth phase were identified in 7-year-old hybrids. The maximum phase of development was F3, found in autumn in 30% of the fish.

A better ovarian development is already seen in 8-year-old hybrids. The fish from this group were developed to phase F2-3 in the spring season, while in autumn 5% of the fish were in this phase, 45% were in F3, 5% in F4i and 15% reached phase F4c.

The analysis of the obtained data shows that when cultivated in the studied conditions, female hybrids are significantly ahead of Russian sturgeons of the same sex and age in terms of sexual development.

During the autumn period 30 % of the analysed Siberian sturgeon individuals were in phase F2-3 (Table 3.2.1.2.), which is intermediate and shows the transition from F2f to F3. In comparison, during the spring season only 10% of the individuals were in phase F2-3. Most females were in F2sf and F2f, as in both spring and autumn-winter seasons 20% of the fish were in F2sf, and during the spring 70 % of the individuals were in F2f, in autumn - 50%.

Table 3.2.1.2. Maturity stages in female fish of different ages according to the classification of Trusov (1972), by ultrasound examination by Chebanov and Galich (2013),% of the flock

Species	Age, years	Season	Maturity stages									
			F1	F2	F2sf	F2f	F2-3	F3	F4i	F4c	F5	F6
Ag	5	Spring	-	-	70	30	-	-	-	-	-	-
		Autumn	-	50	40	10	-	-	-	-	-	-
	6	Spring	-	50	50	-	-	-	-	-	-	-
		Autumn	-	50	20	30	-	-	-	-	-	-
	7	Spring	-	10	60	20	-	10	-	-	-	-
		Autumn	-	10	60	20	-	10	-	-	-	-
	8	Spring	-	-	60	40	-	-	-	-	-	-
		Autumn	-	-	50	50	-	-	-	-	-	-
Hy	5	Spring	-	-	30	50	20	-	-	-	-	-
		Autumn	-	-	10	10	50	30	-	-	-	-
	6	Spring	-	20	40	20	20	-	-	-	-	-
		Autumn	-	-	4	16	16	36	24	4	-	-
	7	Spring	-	-	50	30	20	-	-	-	-	-
		Autumn	-	-	10	40	20	30	-	-	-	-
	8	Spring	-	-	10	80	10	-	-	-	-	-
		Autumn	-	-	10	20	5	45	5	15	-	-
Ab	3	Spring	-	-	20	70	10	-	-	-	-	-
		Autumn	-	-	20	50	30	-	-	-	-	-

3.2.2. Dynamics of testicular development

There are no significant differences in the measured indicators between the studied Russian sturgeon male individual groups (Table 3.2.2.1), neither between the different ages, nor in the different seasons within one age.

A significant increase was obtained from spring to the end of the vegetation period (autumn-winter season) regarding all the three measured indicators in the hybrid, in contrast to the Russian sturgeon. The height of the testes increased by 40.5% ($p < 0.05$), the girth by 28% ($p < 0.01$), and the area practically doubled ($p < 0.01$).

In the Siberian sturgeon at the age of 3 from the beginning to the end of the vegetation period the testicular girth increased by 16% ($p < 0.01$) and the area by 75% ($p < 0.001$).

Table 3.2.2.2 presents the state of the gonads with respect to the maturity stages in the individual age and species groups of male sturgeon and hybrids. The gonads are less developed than the Russian sturgeon and hybrid in the Siberian sturgeon, which is the youngest studied group.

Eighty percents of the individuals were in the M3 phase during the spring season and 20% in the M4 phase among five-year-old Russian sturgeons, and vice versa in the autumn-winter season, 20% in the M3 phase and 80% in the M4 phase. Thirty percents were in the M3 phase and 70% in the M4 phase during the spring in the seven-year-olds, 70% in the M3 phase and 30% in the M4 phase during the autumn. Most of the seven-year hybrid individuals were in the M3 phase - 80%, while in the autumn-winter 70% of the fish were in the M4 during the spring season.

Table 3.2.2.1. Male gonads characteristics at the beginning and the end of the vegetation period.

Season	Features								
	Height, cm			Girth, cm			Area, cm		
	X	±Sx	CV	X	±Sx	CV	X	±Sx	CV
Ag_5 *									
Spring	1.32	0.42	15.79	6.86	2.17	14.30	2.52	0.80	26.97
Autumn	1.31	0.42	9.96	6.96	2.20	12.74	2.68	0.85	30.05
Ag_7									
Spring	1.45	0.46	22.38	6.96	2.20	9.08	2.51	0.79	20.80
Autumn	1.52	0.48	19.36	6.81	2.15	12.27	2.64	0.83	28.01
Hy_7									
Spring	1.16 **d**	0.37	30.45	5.69 **b**	1.80	11.04	1.63 **b**	0.52	25.89
Autumn	1.63 **d**	0.51	27.34	7.29 **b**	2.30	19.29	3.17 **b**	1.00	40.16
Ab_3									
Spring	0.96	0.30	22.09	5.25 **c**	1.66	9.63	1.54 **a**	0.49	17.92
Autumn	1.61	0.51	21.84	6.14 **c**	1.94	10.83	2.70 **a**	0.85	30.42

Differences between values marked with the same letters in rows are significant: *a – P<0.001; b, c – P<0.01; d - P<0.05*

Table 3.2.2.2. Male fish maturity stages of different ages,% of the herd (according to Trusov's classification (1972), by ultrasound examination by Chebanov and Galich (2013))

Species	Age, years	Season	Maturity stages						
			M1	M2	M2sf	M2f	M3	M4	M5
Ag	**5**	**Spring**	-	-	-	-	80	20	-
		Autumn	-	-	-	-	20	80	-
	7	**Spring**	-	-	-	-	30	70	-
		Autumn	-	-	-	-	70	30	-
Hy	**7**	**Spring**	-	-	-	10	80	10	-
		Autumn	-	-	-	-	30	70	-
Ab	**3**	**Spring**	-	-	-	30	70	-	-
		Autumn	-	-	-	70	30	-	-

3.3. Sperm production characteristics of fish from the Acipenseridae family, grown under conditions of super-intensive cage technology.

Fish are found in unusual conditions when raised on industrial farms, which affects their growth and development. In this regard, the qualitative characteristics of sturgeon semen reared in the conditions of super-intensive cage technology are among the most important characteristics determining the efficiency of production. Some characteristics of semen of Russian sturgeon of different ages are presented in section 4.3.1., and in section 4.3.2. - the hybrid of Siberian and Russian sturgeon of different ages. The most modern method for sperm analysis - CASA system is applied in the present study.

3.3.1. Russian sturgeon.

The main indicators of the Russian sturgeon sperm at different ages are presented in the table. 3.3.1.1.

Table 3.3.1.1. Indicators characterizing the quality of sperm in Russian sturgeon

Parameters	**Показатели**		
	Volume, ml	**Motility, %**	**Concentration, 1.10^6/ml**
7-years of age (n=5)			
X	114.80 **c**	97.00 **c**	723.46
±Sx	14.53	8.41	270.65
CV	33.64	5.44	67.48
min	60.00	87.80	113.77
max	153.00	100.00	1450.09
8-years of age (n=8)			
X	44.81 c**d**	53.98 **cd**	1128.63
±Sx	11.49	6.65	213.97
CV	76.96	65.33	84.01
min	10.50	11.90	56.89
max	113.00	99.60	2917.15
9-years of age (n=15)			
X	89.07 **d**	98.54 **d**	983.82
±Sx	8.40	4.85	158.26
CV	32.99	1.12	37.54
min	16.00	96.10	136.73
max	120.00	100.00	1510.97

Differences between values marked with the same symbols in each column are significant: c,d - $p<0.05$

The Russian sturgeon ejaculate volume in our study ranged from 10.5 to 153.0 ml; the concentration and sperm motility from 56.89 to 2917.15 x 10^6 / ml and from 11.90 to 100%, respectively. Ejaculate volume is one of the most important macroscopic characteristics. The volume of the Russian sturgeon ejaculate is very high in our study, with the group of 8-year CV reaching 76.96%. The maximum volume for the study was 153 ml (7-year-old Ag) and the minimum was 10.50 ml (8-year-old Ag). Seven-year-old fish generally have the largest ejaculate volume. The difference in the indicator with 8-year-old individuals reached over 2.5 times ($p <0.05$), and with

9-year-olds - 28.9%, but it is not significant. The difference in volume between 8- and 9-year-old individuals is significant, in favor of the latter (98.8%; p <0.05).

The highest sperm motility in our study was reported in 9-year-old fish (98.54%), and it was lower by 1.56% in 7-year-olds, with an insignificant difference. The motility is significantly lower in 8-year-old Russian sturgeon, as the difference with 9-year-olds is 82.55% (p <0.05), and with 7-year-olds - 79.69% (p <0.05). Motility ranges from 11.90 (8-year-olds) to 100% (7- and 9-year-olds). It is noteworthy that 8-year-old fish have sperm with the lowest motility at the lowest volume. The coefficient of variation of the volume and motility of these fish is very high - 65.33%.

The maximum and minimum values of sperm concentration in Russian sturgeon were reported in 8-year-old sturgeons in our study, 56.89 and 2917.15 x 10^6 / ml, respectively, with the CV again being the highest in this group (84.01%). The average concentration is the highest in the study in this group, with the difference with 7- and 9-year-old Russian sturgeon was 1.6 times and 14.7%, respectively.

Table 3.3.1.2. Sperm motility in the Russian sturgeon semen.

Parameters	**Indicators**							
	VCL, µm/s	**VSL, µm/s**	**VAP, µm/s**	**LIN, %**	**STR, %**	**WOB, %**	**ALH, µm**	**BCF, Hz**
7-years old								
X	88.00 **c**	27.16 **c**	48.68 **c**	31.30 **c**	55.66 c	55.96 c	4.054 c	4.290 **c**
±Sx	15.52	8.59	10.68	7,01	6,22	6,46	0.649	0.879
CV	34.55	31.59	30.89	18.00	6.57	11.77	30.24	29.94
min	62.30	18.50	34.00	26.70	51.10	52.20	3.00	3.10
max	139.50	40.00	73.30	41.00	60.70	67.60	6.10	6.20
8-years old								
X	46.06 **c**	15.90**d**	25.08 **d**	32.34 **d**	56.28 **d**	48.65 **d**	1.613 c**d**	2.750 **d**
±Sx	12.27	6.79	8.44	5,54	4,92	5.11	0.513	0.695
CV	95.18	88.27	96.36	78.39	43.62	50.09	132.82	105.18
min	14.40	0.50	1.80	3.40	28.00	12.20	0	0
max	143.90	34.80	69.90	73.90	93.90	78.70	6.30	7.30
9-years old								
X	128.90**c**	69.11 c**d**	95.00 c**d**	53.69 **cd**	72.05 c**d**	73.87c**d**	3.559 **d**	6.502 c**d**
±Sx	8.96	4.96	6.17	4.05	1.65	3.73	0.375	0.508
CV	23.61	33.61	27.08	19.39	8.86	10.74	28.58	23.08
min	29.05	16.85	22.89	36.92	60.64	60.22	0.68	1.21
max	160.03	116.24	140.17	72.64	82.93	87.59	5.14	7.56

** VCL – curvilinear velocity; VSL – straight line velocity; VAP – average path velocity; LIN - linearity; STR - straightness; WOB - wobble; ALH – amplitude of lateral head displacement; BCF – beat/cross frequency. The differences between the values marked with the same symbols in each column are significant: c, d - p<0.05*

Although the percentage of fully motile spermatozoa and those with progressive motility are the most used parameters in the analysis of motility, it is believed that the speed and its individual parameters (VCL, VSL, VAP, LIN, STR, WOB) can characterize sperm quality most accurately. A significant difference was obtained between the three studied groups of Russian sturgeon ($p < 0.05$) on VCL in our study. The indicator ranges from 14.40 (8-year-olds) to 160.03 μm/s (9-year-olds). The curvilinear velocity (VCL) is highest in 9-year-old Russian sturgeon, as the difference with 7- and 8-year-old sturgeon is 85.3% and over 2.7 times, respectively (Table 3.3.1.2.).

The results in terms of straight line velocity (VSL) show that 9-year-olds significantly exceeded 7- (2.5 times; $p < 0.05$) and 8-year-olds (4.3 times; $p < 0.05$). The same is for most of the motility indicators, which are significantly ($p < 0.05$) higher in 9-year-old fish compared to 7- and 8-year-olds - the average path velocity (VAP) by 95.15% and 3.8 times, respectively; linearity (LIN) - by 71.5% and 66.02%; straightness (STR) - 29.45 and 28.02%; wobble (WOB) with 32.0 and 51.83%; beat/cross frequency (BCF) by 51.56% and 2.36 times. The only indicator that the oldest individuals do not exceed all younger fish is the amplitude of lateral head displacement (ALH). The highest value of this indicator was obtained in 7-year-old fish, as the difference with 8- and 9-year-olds was 2.5 times ($p < 0.05$) and 13.9%, respectively. The difference in ALH between 8- and 9-year-old fish was significant ($p < 0.05$) and was 2.2 times.

Table 3.3.1.3. Distribution of sperm according to the characteristics of their movement and speed in the Russian sturgeon sperm,%

Parameters	**Indicators**					
	Rapid	**Medium**	**Slow**	**Static**	**Non-progressive motile**	**Progressive motile**
7-years old						
X	42.84 c	35.14 **c**	19.02	3.00 **c**	85.92 **cd**	11.08 c
±Sx	15.53	8.11	8.86	2.64	2.41	2.59
CV	72.51	46.15	93.12	175.89	5.61	46.74
min	14.80	16.20	0.30	0	80.20	6.50
max	83.50	60.50	41.40	12.30	90.10	19.80
8-years old						
X	13.15 cd	11.24 **c**	29.55 c	46.04 **cd**	48.97 **c**	4.99 **d**
±Sx	10.36	3.96	7.15	12.47	11.47	1.70
CV	222.56	99.59	68.48	76.55	66.21	96.40
min	0	0	0.80	0.40	11.90	0
max	85.00	27.60	58.60	88.10	92.20	12.50
9-years old						
X	67.12d	21.61	9.81 **c**	1.46 **d**	63.76 d	34.78 c**d**
±Sx	4.97	3.87	1.35	0.28	2.90	2.63
CV	28.69	69.28	53.21	75.18	17.60	29.24
min	37.39	5.54	0.36	0	38.60	24.94
max	90.80	43.62	17.67	3.90	75.00	57.49

Differences between values marked with the same symbols in each column are significant: c, d - $p<0.05$

Nine-year-old fish had semen with better sperm motility, with the oldest individuals outperforming the younger ones in 7 of the 8 of the studied indicators in our study. It can be stated summarizing the data on the movement of sperm in Russian sturgeon that the VCL varies from 14.40 to 160.03 µm / s; VSL from 0.50 to 116.24 µm / s; VAP from 1.80 to 140.17 µm / s; LIN from 3.40 to 73.90%; STR from 28.0 to 93.90%; WOB from 12.20 to 87.59%; ALH from 0 to 5.14 µm; BCF from 0 to 7.56 Hz.

The oldest fish also have better indicators characterizing the speed and characteristics of sperms movement (Table 4.3.1.3). They have the highest amount of rapid sperms (67.01%), the lowest static (1.46%). The lowest amount of rapid sperms was obtained in 8-year-old fish, with the difference in the indicator with 7- and 9-year-olds being 3.26 ($p < 0.05$) and 5.10 times ($p < 0.05$), respectively. Medium sperms are most common in the youngest fish, and the difference in the indicator with 8-year-olds is significant (3.13 times; $p < 0.05$). The largest number of progressive motility sperms was obtained in the oldest fish, and the difference was significant ($p < 0.05$) with 7- and 8-year-old fish 3.14 and 6.97 times, respectively.

Table 3.3.1.4. Enzymatic activity of Russian sturgeon sperm in water and triton x100 extract, UI / L

Parameters	Indicators*							
	Water extract				Triton x 100			
	AP	GGT	CK	LDH	AP	GGT	CK	LDH
	7-years old (n=5)							
X	732.00**c**	19.39	598.04**c**	929.8**c**	604.13**c**	4.75 **c**	82.74**c**	465.67
±Sx	93.11	3.96	37.08	84.79	55.32	1.07	10.08	92.00
CV	31.13	46.77	28.35	20.05	43.16	40.39	27.47	47.64
min	435.00	10.93	421.00	744.30	293.00	2.33	52.00	211.30
max	998.00	33.04	813.00	1211.00	933.20	7.62	113.3	766.20
	8-years old (n= 3)							
X	310.67c**d**	25.07	37.08**cd**	346.42**cd**	56.67**cd**	23.02**cd**	27.65**cd**	535.38
±Sx	120.20	5.08	72.84	109.46	71.41	1.38	13.02	118.77
CV	152.93	71.03	91.10	82.72	78.77	21.94	26.65	75.58
min	21.00	4.82	1.60	175.77	27.00	17.74	23.15	108.98
max	859.00	38.29	68.87	677.27	108.00	27.81	36.15	914.05
	9-years old (n=15)							
X	758.40**d**	18.94	636.00**d**	896.33**d**	601.27**d**	5.80**d**	72.80**d**	471.07
±Sx	53.76	2.27	32.57	48.95	31.94	0.62	5.82	53.12
CV	16.06	34.04	18.84	19.22	7.71	31.77	32.82	32.19
min	548.00	11.54	428.00	677.00	487.00	3.56	49.00	280.00
max	934.00	32.43	874.00	1210.00	673.00	9.62	116.00	720.00

AP – alkaline phosphatase; GGT – gamma glutamyltransferase; CK – creatine kinase; LDH – lactate dehydrogenase. The differences between the values marked with the same symbols in each column are significant: c, d - $p<0.05$

It can be stated in general that in 8-year-old fish the sperms have the worst indicators of movement, with the percentage of static ones reaching 46.04%, which significantly exceeds ($p < 0.05$) the same in 7- and 9-year-olds, 15.35 and 31.53 times respectively.

The levels of basic enzymes in both water and triton x 100 extract were examined in our study, (Table 3.3.1.4). It is noteworthy that the semen of 8-year-old Russian sturgeon has significantly lower levels in most of the studied enzymes. The difference of this group in AP, CK, LDH in water extract with 7- and 9-year-olds was significant ($p < 0.05$) and was: 2.36 and 2.44 times, respectively; 16.03 and 17.05 times; 2.68 and 2.59 times. Only on GGT in water extract the level is highest in 8-year-old individuals, but the differences between the groups are not significant. However, this is not the case with regard to the level of GGT in Triton extract. The GGT content is highest in 8-year-old fish here again, but the difference between the groups of 7- and 9-year-olds is significant ($p < 0.05$) and is 4.85 and 3.97 times, respectively. There is also a difference with regard to LDH. If in the water extract of this enzyme 8-year-old fish have significantly lower levels, then in the triton x 100 extract on the contrary, the highest levels are reported here, but the differences between the different groups are not significant.

With regard to AP and CK in triton extract, the trend observed for these enzymes in water extract continues - 8-year-old fish have significantly ($p < 0.05$) lower levels, 10.66 and 10.61 times respectively; and 2.99 and 2.63 times. The big difference in AP content is especially impressive. There was variability from medium (CV 16.06%) to strong (CV 91.10%) for most enzyme indicators in our study. The CV level is 152.93% for AP in water extract at 8 years of age but it should be noted that the number of the studied fish is small.

3.3.2. Hybrid (F_1 Ab x Ag).

Indicators of the sperm production quality in different age groups of hybrid are presented in table. 3.3.2.1

Ejaculate volume in the studied hybrid ranged from 36 to 150 ml; sperm concentration from 749.60 to 2787.36 x 10^6 / ml and motility - from 16 to 100%. No significant differences were found between age groups in ejaculate volume and sperm concentration. The highest average volume was obtained in 8-year-old fish (104 ml), with differences in the indicator with 7- and 9-year-old fish being 23.81 and 14.49%, respectively. The highest concentration is in 7-year-old fish that have the smallest ejaculate volume. The difference in concentration between the youngest fish and those at 8- and 9-year-olds is 26.56 and 23.21%, respectively, and the difference between 8- and 9-year-old fish is only 2.7%. Maximum sperm motility was found in 9-year-old fish (over 99%), with differences with other age groups being significant, with 7-year-olds - 28.77% ($p < 0.05$), and with 8-year-olds reaching 53.44% ($p < 0.05$) respectively. The highest motility in the oldest fish is combined with relatively good ejaculate volume and sperm concentration. The lowest level of variation of motility was obtained in the same group, as the minimum motility is high enough - 97.8%.

Table 3.3.2.1. Indicators characterizing the hybrid sperm quality.

Parameters	Indicators		
	Volume, ml	**Motility, %**	**Concentration, 1.10^6/ml**
7-years of age (n=5)			
X	84.00	77.36 **c**	1956.08
±Sx	15.26	8.37	223.09
CV	27.73	44.84	47.81
min	45.00	16.00	749.60
max	100.00	98.70	2787.36
8-years of age (n=3)			
X	104.00	64.93 **d**	1545.60
±Sx	19.70	10.81	288.01
CV	55.66	62.07	18.45
min	39.00	18.40	1216.56
max	150.00	88.40	1721.55
9-years of age (n=18)			
X	90.83	99.63 **cd**	1587.54
±Sx	8.04	4.41	117.58
CV	35.73	0.65	21.94
min	36.00	97.80	1147.70
max	142.00	100.00	2057.88

Differences between values marked with the same symbols in each column are significant: c, d - $p<0.05$;

Table 3.3.2.2. shows that the sperm in the oldest individuals have higher values ($p<0.05$) of VCL, VSL and VAP, as the differences with 7-year-old fish are 3.7, 2.49, 3.22 times, respectively; and with 8-year-olds 5.19, 3.32, 4.24 times, respectively. The differences in the above indicators are not significant between hybrids aged 7 and 8 years. The differences on LIN and STR are not significant between the groups, with the highest values obtained in 8-year-old hybrids. The highest WOB value was reported in 7-year-old fish, and the differences between the studied groups are not significant.

The highest values of ALH and BCF were found in 9-year-old fish, with the difference with 7- and 8-year-old fish in the first indicator being 1.73 ($p<0.05$) and 2.08 times ($p<0.05$), respectively; and the second 1.38 and 1.27 ($p<0.05$) times, respectively. It can be stated by summarizing the data on hybrid (F1 Ab x Ag) sperm movement that VCL varies from 9.22 to 160.59 µm / s; VSL from 1.90 to 67.50 µm / s; VAP from 3.90 to 101.60 µm / s; LIN from 14.90 to 64.30%; STR from 49 to 79.90%; WOB from 30.50 to 81.50%; ALH from 2.0 to 6.50 µm; BCF from 1.80 to 7.90 Hz.

The relative proportion of rapid sperms in the hybrid semen varies from 0 to 96.90%; of medium from 1.0 to 46.74%; of slow from 0.28 to 75.30%; of static from 0 to 84% (Table 3.3.2.3.). The relative share of sperm with non-progressive motility varies from 15.30 to 89.70%, and those with progressive motility – from 0.10 to 29.85%.

Table 3.3.2.2. Sperm motility in hybrid semen

Parameters	Indicators							
	VCL, μm/s	**VSL, μm/s**	**VAP, μm/s**	**LIN, %**	**STR, %**	**WOB, %**	**ALH, μm**	**BCF, Hz**
7-years old								
X	35.90**c**	19.20**c**	24.80**c**	43.10	62.10	68.70	2.80**c**	4.50**c**
±Sx	5.31	4.73	4.30	5.17	3.54	4.87	0.35	0.57
CV	27.63	76.08	31.85	24.79	14.42	9.40	24.93	48.17
min	21.60	8.90	14.70	35.20	56.30	62.50	2.30	2.20
max	44.90	44.90	35.70	61.80	77.80	79.50	3.90	7.90
8-years old								
X	25.80**d**	14.47**d**	18.83**d**	47.73	68.90	64.43	2.33**d**	4.90
±Sx	6.85	6.11	5.55	6.68	4.56	6.29	0.449	0.74
CV	44.07	75.25	68.69	59.57	25.01	45.61	12.37	54.80
min	12.70	1.90	3.90	14.90	49.00	30.50	2.00	1.80
max	32.80	21.00	26.60	64.30	78.90	81.50	2.50	6.50
9-years old								
X	133.82**cd**	47.97**cd**	79.88**cd**	36.09	59.65	59.91	4.85**cd**	6.22**c**
±Sx	2.80	2.49	2.27	2.73	1.86	2.56	0.18	0.30
CV	9.22	19.46	11.93	21.30	9.29	11.68	17.13	8.51
min	115.92	35.87	64.84	25.97	52.43	49.53	3.59	4.86
max	160.59	67.50	101.60	51.89	71.40	72.68	6.50	7.04

** VCL – curvilinear velocity; VSL – straight line velocity; VAP – average path velocity; LIN - linearity; STR - straightness; WOB - wobble; ALH – amplitude of lateral head displacement; BCF – beat/cross frequency.*

The differences between the values marked with the same symbols in each column are significant: c, d - $p<0.05$

The oldest fish (9-year-olds) have the best indicators of sperms speed. The rapid sperms have the highest percentage in this group, with the difference with 7- and 8-year-olds being 30 ($p <0.05$) and 41.8 ($p <0.05$) times, respectively. The slow and static ones in the 9-year-old hybrid are the least in number, the differences with the 7-year-olds being 8.4 ($p <0.05$) and 59.6 ($p <0.05$) times, respectively, and with the 8-year-olds by 9.3 ($p <0.05$) and 92.4 ($p <0.05$) times, respectively.

No significant difference was found in the relative share of medium sperms in the individual groups.

No significant difference was found between the individual groups and the relative share of sperms with non-progressive motility, as the average values of the indicator vary from 61.87 (8-year-old) to 78.75% (9-year-old). The share of sperms with progressive motility is highest in the oldest fish (9 years old), as the difference in the indicator with 7-year-olds is 2.7 times ($p <0.05$), and with 8-year-olds 6.96 times ($p < 0.05$).

In summary, it can be pointed out that in the hybrid (F1 *A. baerii* x *A. gueldenstaedtii*) the oldest fish had the best characteristics of sperms movement - the highest share of rapid, the lowest of slow and static; the highest percentage of sperm with progressive motility.

Table 3.3.2.3. Sperm distribution according to the characteristics of their movement and speed in the hybrid semen, %

Parameters	**Indicators**					
	Rapid	**Medium**	**Slow**	**Static**	**Non-progressive motile**	**Progressive motile**
7-years old						
X	2.36**c**	24.26	50.72**c**	22.66**c**	69.74	7.60**c**
±Sx	6.13	7.17	6.46	8,37	8.00	2.95
CV	40.82	67.70	42.79	153.01	44.73	136.23
min	1.10	1.30	13.60	1.40	15.30	0.70
max	3.70	46.60	68.00	84.00	89.70	25.90
8-years old						
X	1.70**d**	7.17	56.00**c**	35.13**d**	61.87	3.00**d**
±Sx	7,91	7.32	8.34	10,81	10.33	3.81
CV	86.65	74.55	59.69	114.73	60.97	83.79
min	0	1.00	17.40	11.70	18.30	0.10
max	2.60	10.40	75.30	81.60	83.80	4.60
9-years old						
X	71.00**cd**	22.61	6.01**cd**	0.38**cd**	78.75	20.87**cd**
±Sx	3.23	2.99	3.41	4.14	4.212	1.56
CV	22.43	54.28	105.50	177.92	7.73	27.47
min	45.50	2.82	0.28	0	69.74	10.70
max	96.90	46.74	21.95	2.19	89.30	29.85

The differences between the values marked with the same symbols in each column are significant: c, d - $p<0.05$

Sperm enzyme activity was studied in all age groups, but in the 8-year-old group only two samples were obtained, making it impossible to perform statistical data processing. The results for the individual enzymes in this group are as follows: in water extract AP - 53.0 - 68.0 UI / L, GGT - 11.98-14.29 UI / L, CK - 39.31-56.92 UI / L, LDH - 219.42 - 268.29 UI / L; in triton x 100 extract - AP - 28.0 - 64.0 UI / L, GGT - 10.64 - 31.10 UI / L, CK - 9.21 - 20.12 UI / L, LDH - 46.74 - 95.18 UI / L. It is noteworthy that in the case of the 8-year-old hybrid the values obtained for AP, SC in both water and triton x 100 extract are significantly lower compared to 7- and 9-year-old fish (Table 3.3.2.4).

The comparative analysis of the enzyme activity of the semen of 7 - and 9 - year - old fish shows that there is a significant difference between the groups of all studied enzymes in water extract, and only LDH in Triton. There are significantly higher levels of AP (7 times; $p <0.05$) and GGT (3.2 times; $p <0.05$) in the water extract of older fish, while the values of CK are higher in younger fish – 2.3 times ($p <0.05$).

AP levels are also higher (over 30%) in the triton x 100 extract of 9-year-old fish, but the difference is not significant. Higher levels were found in younger individuals, 4.6%; 22.1% and 2.9 times (p <0.05) regarding GGT, CK and LDH,.

Table 3.3.2.4. Hybrid sperm enzyme activity in water and triton X 100 extract

Parameters	Indicators*							
	Water extract				Triton x 100			
	AP	GGT	CK	LDH	AP	GGT	CK	LDH
7-years old (n=5)								
X	117.40c	6.40c	1515.30c	1224.20	409.60	8.54	113.68	1531.10c
±Sx	49.62	2.14	311.56	230.43	139.75	2.45	15.09	268.86
CV	175.30	67.24	106.52	95.74	175.66	114.14	59.81	91.22
min	7.00	2.80	107.40	262.70	30.00	1.14	21.05	98.40
max	485.00	13.40	4268.60	3245.90	1695.00	19.30	191.20	3267.90
9-years old (n=18)								
X	821.28c	20.16c	660.28c	823.72	586.56	8.15	88.61	523.83c
±Sx	26.51	1.13	164.21	121.45	73.65	1.29	7.95	141.70
CV	9.40	24.89	18.62	17.29	11.46	28.17	22.07	17.83
min	679.00	12.23	488.00	580.00	492.00	4.87	56.00	390.00
max	935.00	30.25	873.00	1050.00	724.00	12.87	121.00	720.00

**AP - алкална фосфатаза; GGT - гамаглутамилтрансфераза; CK - креатин киназа; LDH - лактат дехидрогеназа. AP – alkaline phosphatase; GGT – gamma glutamyltransferase; CK – creatine kinase; LDH – lactate dehydrogenase.* The differences between the values marked with the same symbols in each column are significant: c - p<0.05

4. CONCLUSION

MORPHOMETRIC CHARACTERISTICS

Comparative analysis of female and male individuals of Russian sturgeon (Acipenser gueldenstaedtii) and hybrid (F 1 Acipenser baerii x Acipenser gueldenstaedtii) at the age of seven shows that there are differences between the sexes in a number of morphometric characteristics.

Female Russian sturgeons have significantly better fattening than males (P <0.001), their body is more compact (P <0.001) and thicker (P <0.01). They have larger ratio to the total length of the body at an anti-ventral distance, the thickness of the back and the girth of the body. The proportions of the head in female individuals differ by greater height and length of the distance behind the eye, wider mouth, bigger eye and wider rostrum at the cartilaginous arch of the mouth. The number of dorsal bony scutes varies from 9 to 13. The lateral ones on the left vary from 24 to 30, and on the right - from 22 to 31. The ventral ones on the left are from 7 to 11, and on the right from 8 to 11. The number of rays in the dorsal fin it is from 21 to 39, and in the anal from 16 to 25. Female fish have a significantly lower number of ventral bony scutes compared to males on both the left (P <0.05) and right (P <0.01) sides on the body.

Male Russian sturgeons , compared to females have bigger head. The ratio to the total length of the body of the head length, the caudal stalk, the ventral fin, the height of the anal fin and the ventro - anal distance is higher. In males, the long-headed index is higher (P <0.05). The rostrum and the distance from its end to the mouth are longer and the lower lip break is bigger. The number of dorsal bony scutes varies from 8 to 13. The lateral ones on the left side are from 25 - 32, and from the right from 25 to 32. Ventral ones on both sides of the body vary from 8 to 11. The number of rays in the dorsal fin is from 23 to 45, and in the anal from 16 to 23.

Female hybrids have a higher high-backed index and have a higher degree of fatening, expressed by the IC index. They have a greater anti-ventral and pectoventral distance. The head of the female hybrids, compared to the male ones, is wider, higher in the area above the eye and has a relatively larger space behind the eye. Female fish have a relatively larger eye diameter; relatively wider mouth and wider rostrum in the cartilaginous arch. The dorsal and anal fins are higher and the pectoral and abdominal fins are longer.

Male hybrids have a higher body relative to its total length and a higher caudal stalk. The anal fin is longer in them. The head of male fish in comparison with female fish has a more massive and longer rostrum, a greater distance from the end of the snout to the mouth and a greater width of the break of the lower lip. In male fish, the ratio of the lower lip break to the mouth width is also higher.

SEXUAL DEVELOPMENT

In sturgeon fish cultivated under conditions of super-intensive cage technology, ultrasound methods not only successfully determine the sex of fish, but can, in both sexes, track the age dynamics of gonadal development by identifying the individual stages of maturity.

In all studied individuals of ultrasonic image **ovaries** can be seen as heterogeneous structures with mixed echogenicity with uneven borders without tunic. The fatty part of the ovary is visualized in the form of darker areas, differing clearly from the brighter generative tissue.

In all examined individuals on the ultrasound image, the **testes** are easily differentiated. Testicular tissue is hyperechoic. The borders of the testes are smooth and the bright hyperechoic tunic is clearly visible. The testes are oval and almond-shaped. Unlike ovarian tissue, testis is located just below the muscle tissue. The fatty part is insufficiently or poorly developed on the medial side and is practically difficult to see.

In **female Russian sturgeon** age and seasonal dynamics in the development of gonads has been established. In the **spring** in fish with a difference in age of 2 years significantly differs, in favor of older fish, the area of the ovary. The difference in the indicator was 41.8% (p <0.05) between 5 and 7 year old individuals; and 62.5% (p <0.01) between 6 and 8 years of age, respectively. With a larger age difference (5 and 8 years old) a significant increase was found not only in the area (2 times ; p <0.001) , but also the height (over 2 times ; p <0.001) of the ovary. In older fish, even with a difference of 1 year (7 and 8 years), with age there was an increase in both area and height of the ovary, respectively, by 44.8% (p <0.05) and more than twice (p <0.001) . During the **autumn-winter** period, similar trends are observed in Russian sturgeons of different ages as in spring. Between 5 and 7 year old fish with age there was a 68.4% (p <0.05) increase in the area of the gonads. Between 6 and 7 years of age, the height of the ovary was increased by 50% (p <0.01) and the area by 45.6 % (p <0.001). A comparison of 6- and 8-year-old fish showed a significant increase in all ovarian parameters: ovarian height increased by 46% (p <0.001), girth by 33.8 % (p <0.001) and area by 45.6% (p <0.05). In 5 and 7 year old fish there was a significant increase in gonads during the vegetation period, as in 5 year olds the ovarian height increased by 64% (p <0.01) , and in 7 year olds the height and area increased by 7 %. 78.5% (p <0.001), and double (p <0.001) .

It was found that in female sturgeon at the age of 5 the ovaries develop to the maximum by the end of the 2nd stage, and in the spring 30% of the studied fish were in this stage. In 6-year-old fish in the spring, 50% of the fish reached stage F2sf , with no fish in stage F2f. With increasing age in 7-year-old Russian sturgeons, 10% of individuals in both the spring and autumn-winter seasons are already in stage F3. In 8-year-old fish in the spring, 60% of the individuals were in the F2sf stage and the remaining 40% in the F2f stage. In the fall, 50% of the fish in the same group had ovaries in the F2sf stage, and the others in stage F2f.

In **female hybrids**, there are trends similar to those found in female Russian sturgeons for age differences in gonad size in the spring. In older fish, the ovaries are better developed. In 5 -year- old fish the ovarian height is higher by 38.8% (p<0.01) and the area by 51.6% (p<0.05) compared to 6-year-olds. The difference between 5 and 7 year old fish in ovarian height was 31.6% (p <0.01); and between 6 and 8 years old - 22.2% (p <0.05). During the autumn-winter period, however, in female hybrids at the age of 8 years, we found smaller values in terms of girth and area, compared to 5-year-olds, respectively 25.5% (p <0.01) by the girth and 61.4% (p <0.05) by the area. The obtained results show that at the end of the vegetation period in the ovaries of 8-year-old hybrids more ovarian fat accumulates at the expense of the generative part of the gonad. The same was found in 7- and 8-year- old fish, where the ovarian girth in the elderly was lower by 38.3% (p <0.01) and the area by 62.9% (p <0.001). A study of the seasonal dynamics of ovarian development within each age group found that the gonads increase towards the end of the vegetation period. In 5-year old hybrids, ovarian height increased by 53.2% (p <0.01) and area almost three times (p <0.001). At 6 years of age, 56.7% (p <0.05) was increased the area. At the age of 7 the height increased by 28.9% (p <0.01), and the girth with 42.6 % (p <0.01).

The 5-year - old hybrid, in the autmun 30% of the studied fish reached stage F3 and in both seasons there was not a single individual in stage F4. In a 6-year-old hybrid in the autumn, 36% of the fish were in stage F3, 24% in stage F4i and 4% in stage F4c. In 7-year-old hybrids in both seasons, no individuals with ovarian development in the fourth stage were identified. The maximum stage of development was F3, found in autumn in 30% of fish. In the 8-year-old hybrids, in the spring the fish were developing to stage F 2-3, while in the autumn of the studied fish in this stage were 5%, in F3 - 45%, in F4i - 5% and in F4c - 15 % .

Under the conditions of the studied technology **Siberian sturgeon** at a relatively early age (three years) has very well-formed ovaries, which allows the successful application of ultrasound diagnostics of the sex. In the Siberian sturgeon in the autumn period, 30 % of the analyzed individuals are in stage F 2-3, which is intermediate and shows the transition from F2f to F3; 20% of the fish were in F2sf and 50% in F2f . During the spring period only 10% of the individuals are in stage F2-3 and in F2f - 70% of the individuals.

In **male Russian** sturgeon no significant differences in the measured indicators were found between different studied groups - neither between the different ages, nor in the different seasons within the same age. Regarding the degree of maturity, it was found that in five-year- old individuals in the spring season 80% were in stage M3 and 20% in stage M4, and in the autumn-winter vice versa, 20% in stage M3 and 80% in stage M4. In the seven-year-olds , 30% were in the M3 stage and 70% in the M4 stage in the spring, and 70% in the M3 stage and 30% in the M4 stage in the autumn .

In **male hybrids**, in contrast to the Russian sturgeon, a significant increase was found from spring to the end of the vegetation period (autumn-winter season) for all three measured indicators. Testicular height increased by 40.5% ($p < 0.05$), the girth of 28% ($p < 0.01$), and the area has practically doubled ($p < 0.01$). In the seven-year-old hybrid, in the spring season most individuals are in the M3 stage - 80%, while in the autumn-winter 70% of the fish are in the M4.

In the **Siberian sturgeon** at the age of 3 years from the beginning to the end of the vegetation period the testicular girth increased by 16% ($p < 0.01$) and the area by 75% ($p < 0.001$).

The study results of the maturity satges in the studied groups show that in the Siberian sturgeon, which is the youngest studied group, the gonads are less developed than studied groups of Russian sturgeon and hybrid.

CHARACTERISTICS OF SPERM PRODUCTION

Despite the unusual conditions for sturgeon farming in super-intensive cage technology, Russian sturgeons and hybrids from the three age studied groups had good sperm production. There is no clear pattern of influence by the age on ejaculate volume.

In **Russian sturgeon**, the average ejaculate volume varies from 10.50 ml (8-year-old) to 153 ml (7-year-old fish). Age does not have a significant effect on sperm concentration. In 8-year-old fish, the variation in sperm concentration is greatest - from 56.89 to 2917.15 x 10^6 / ml. Regarding the characteristics of sperm movement, poorest indicators were obtained in 8-year-old fish, as the share of static ones in this group reached 46.04%, which significantly exceeds ($p < 0.05$) the established values for the indicator in 7- and 9-year-old fish, respectively by 15.35% and 31.53 times. For most of the studied enzymes, the semen of an 8-year-old Russian sturgeon has significantly lower levels than the other two groups. Regarding AP, CK, LDH in water extract, the difference of this group with 7- and 9-year-olds was significant ($p < 0.05$) and was respectively: 2.36 and 2.44 times; 16.03 and 17.05 times; 2.68 and 2.59 times.

Only in GGT in water extract in 8-year-old individuals the level of the enzyme is the highest, but the differences between the groups are not significant.

Hybrids of all ages had good sperm production. The difference in age does not significantly affect the volume of the ejaculate. In terms of motility, the oldest individuals have the highest values ($p < 0.05$) of VCL, VSL, VAP, ALH and BCF, as well as the best ($p < 0.05$) characteristics of sperm movement - the highest share of the rapid ones and the ones with progressive motility, also the lowest of the slow and static ones. Age affects the enzyme activity of semen, with higher levels of AP (7 times; $p < 0.05$) and GGT (3.2 times; $p < 0.05$) in the water extract of older fish and younger fish values of the enzyme CK are 2.3 times higher ($p < 0.05$). The values of LDH in triton x 100 extract was 2.9 times ($p < 0.05$) higher in the younger fish.

5. INFERENCES

1. Based on a morphometric analysis of Russian sturgeon and hybrid (F_1 Ab x Ag) reared under super-intensive cage technology, significant differences were found in a number of indicators, both between genotypes and between sexes within the genotype, allowing morphometric analysis to be used to assess fish responses to farming conditions as well as a tool for species and gender identification.
2. Ultrasound monitoring of the ovaries and testicles of live sturgeon allows to monitor the development and degree of maturity of the gonads.
3. When raised in the conditions of super-intensive cage technology, using ultrasound diagnostics, the sex of the Siberian sturgeon can be successfully determined from the age of three.
4. When grown in conditions of super - intensive cage technology, female hybrids (F_1 Ab x Ag) are sooner mature than female Russian sturgeons. At the same age, hybrids are significantly ahead of Russian sturgeons in ovarian development.
5. Male sturgeon and hybrid reared on industrial farms (F_1 Ab x Ag) can be successfully used for reproduction, as the studied technology does not adversely affect sperm quality.

6. RECOMMENDATIONS

1. The established morphometric parameters of Russian sturgeon and hybrid (F_1 Ab x Ag) , when grown in the conditions of super-intensive cage technology, to be used as basic information in future activities related to technology improvement or determining the directions of breeding and improvement activities.
2. Taking into account the urgency of the problem of "genetic pollution" of natural populations of aquatic organisms, the database of morphometric features of Russian sturgeon and hybrids (F_1 Ab x Ag) should be used in the identification of fish from natural sturgeon populations.

3. The data obtained from morphometric analyzes should be used in the development of specialized software for sex determination in sturgeon.
4. To increase the efficiency of caviar farms, use ultrasound surveys for early sex determination in order to exclude males from industrial herds as early as possible.
5. When raising sturgeon fish in the conditions of super-intensive cage technology, to increase the efficiency of production by sorting fish in different stages of their sexual development, use ultrasound to calculate the gonadosomatic index, monitor the development of gonads and the degree of maturity.
6. For reproduction both in Russian sturgeon and in hybrid (F 1 Ab x Ag) over 9 years of age males to be used.

7. MAIN SCIENTIFIC CONTRIBUTIONS IN THE DISSERTATION

1. For the first time a complex study of morphometric and reproductive characteristics of sturgeon fishes (Russian sturgeon and hybrid (F1 *Ab* x *Ag*)) of different sex and age, cultivated under conditions of super-intensive cage technology was performed. **Original contribution.**
2. Morphometric differences have been demonstrated on a number of plastic and meristic features, between Russian sturgeon and hybrid (F1 *Ab* x *Ag*), and between the sexes within the genotype, grown in conditions of super-intensive cage technology. The possibility to use the morphometric analysis as a tool for species and sex identification, to assess the reaction of fish to the conditions of cultivation, as well as to determine the possibility and directions of breeding and improvement activities has been established. **Original contribution.**
3. The parameters of the main indicators characterizing the economic qualities of Russian sturgeon and hybrid (F1 *Ab* x *Ag*) (fatness, compactness, thickness and width of the body, etc.) grown in conditions of super-intensive cage technology are established, as we as the influence of genotype and sex on them. **Original contribution.**
4. The possibility of ultrasound diagnosis of ovaries and testes to be used for monitoring the gonad development and stages of maturity in live sturgeon is proven, as well as for early sex determination, which is particularly important to increase the efficiency of farms for caviar production. **Affirmative contribution with elements of originality.**
5. For the first time a study was conducted in which age and seasonal dynamics of gonad development in Russian sturgeon and hybrid (F1 *Ab* x *Ag*), cultivated in the conditions of super-intensive cage technology, were established. A comparative analysis of the sexual development of male and female Russina sturgeon and hybrid individuals was performed. **Original contribution.**
6. It has been found that, when reared under super-intensive cage technology, female hybrids (F1 *Ab* x *Ag*) are more mature than female Russian sturgeons, and at the same age are significantly ahead of their ovaries development. **Original contribution.**

7. It has been established that in the conditions of super-intensive cage technology, the sex of the Siberian sturgeon can be determined as early as 3 years of age. **Affirmative contribution with elements of originality.**

8. For the first time, seasonal dynamics of ovarian and testicular development in Siberian sturgeon, cultivated in the conditions of super-intensive cage technology, has been established. **Original contribution.**

9. For the first time a study of sperm production complex indicators of Russian sturgeon and hybrid (F1 *Ab* x *Ag*) of different age groups was conducted. It was found that when cultivated in conditions of super-intensive cage technology, Russian sturgeon and hybrid have good sperm production and can be successfully used for reproduction. It is recommended to use males over 9 years of age for breeding. **Original contribution.**

List of scientific publications in connection with the dissertation

1. **Bonev St**., 2018. Use of echographic methods for sex determination of three-year-old Siberian sturgeon, reared in a cage farm. *Conference proceedings. VIII International conference "Water & Fish", 13-15 June 2018, Faculty of Agriculture, Belgrade-Zemun, Serbia*, 306-311. ISBN 978-86-7834-308-7 7

2. **Bonev S.**, L. Nikolova, 2019. Development of female Russian sturgeon (*Acipenser gueldenstaedtii*) and hybrid (*Acipenser baerii x Acipenser gueldenstaedtii*) gonads reared in net cages. *Bulgarian Journal of Agricultural Science, 25 (Suppl. 1): 62-68.*

3. **Bonev S.**, L. Nikolova, 2021. Development of male Russian sturgeon (*Acipenser gueldenstaedtii*) and hybrid (*Acipenser baerii x Acipenser gueldenstaedtii*) gonads reared in net cages. *Agricultural Sciences, 1: 202113(28):* 65-74.

4. Nikolova L., **St. Bonev**, 2021. Comparative morphometric analysis of male and female hybrids (F1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) at the age of seven years. Scientific Papers. Series D. Animal Science. Vol. LXIV, No. 1, 2021 ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

5. Nikolova L., **St. Bonev**, 2021. Comparative morphometric analysis of Russian sturgeon (*Acipenser gueldenstaedtii*) male and female individuals at the age of seven years. Scientific Papers. Series D. Animal Science. Vol. LXIV, No. 2: 471-478. ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750.