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Sperm production of Russian sturgeon (*A. gueldenstaedtii*) raised on an industrial cage farm

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Abstract

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Sturgeons are in unusual conditions when farmed on industrial farms, which can affect their development. In this connection, the qualitative and quantitative characteristics of the semen of fish reared under similar conditions are of interest. The study covers 7, 8 and 9 year old Russian sturgeon raised on a super-intensive cage farm located in a large dam in southern Bulgaria. Sperm concentration, total motility, motility and movement characteristics were determined by using a CASA system. The levels of some enzymes were determined by spectrophotometric measurement. The average volume in the individual age groups ejaculate is from 10.50 ml (8-year-old), to 153 ml (7-year-old fish). The indicator has a very high variation, reaching 8.96% in 8-year-old fish. In this age group the variation in the concentration of sperm is the highest - from 56.89 to 2917.15 x 10^6 / ml. The highest sperm motility was found in 9-year-old fish (98.54%). VCL ranges 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 spermatozoa had the worst indicators in 8-year-old fish characterizing the movement, as the percentage of static ones reached 46.04%, which significantly exceeds ($p < 0.05$) the same in 7- and 9-year-olds by 15.35 and 31.53 times, respectively. In most of the studied enzymes, the semen of 8-year-old Russian sturgeon has significantly lower levels of AP, CK, LDH in water extract compared to 7 and 8-years old group. The differences of this group with 7- and 9-year-olds are significant ($p < 0.05$) respectively 2.36 and 2.44 times; 16.03 and 17.05 times; 2.68 and 2.59 times. Only on 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.

Keywords: Sturgeon; CASA; spermatozoa; motility; enzymes
Abbreviations: CASA – Computer-assisted sperm analysis; 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; AP – Alkaline Phosphatase; GGT – Gamma-glutamyl Transferase; CK – Creatine Kinase; LDH – Lactate Dehydrogenase.

Introduction

Acipenseridae species are valued for their delicacies, high quality products – black caviar and meat. Natural sturgeon populations are depleted, with a significant number of species on the verge of extinction. A number of measures have been taken to protect and restore them (Bloesch et al., 2006). The development of their aquaculture production has a special role (Vasileva, 2015).

The production of black caviar from aquaculture is constantly increasing, which contributes to the reduction of fishing pressure (Billard & Lecointre, 2005; Bronzi &

Rozenthal, 2014; Bronzi et al., 2019). The development of sturgeon aquaculture is related to the normal reproductive capacity of artificially reared populations. In this regard, the individual characteristics of semen in different species of fish are of interest (Cabrita et al., 2014; Gallego & Asturiano, 2019).

A number of quantitative and qualitative indicators have been studied to characterize sperm in fish (Fauvel et al., 2010). Among the important parameters of sperm is enzyme activity (Kowalski & Cejko, 2019). Enzymes in semen have been studied in Siberian sturgeon and Sterlet (Piros et al., 2002; Shaliutina-Kolešová et al., 2013); Russian sturgeon (Shaliutina-Kolešová et al., 2013; Sarosiek et al., 2004; Huang et al., 2014) and other sturgeon species. Despite a number of studies, Aramli et al. (2013) emphasize that the enzymology of fish gametes is little known when compared with mammalian semen.

Rurangwa et al. (2014) indicate that poor sperm quality may be a limiting factor for fish farming, with the characteristics of sperm being influenced by certain elements of breeding fish flocks.

Due to the lack of information on sperm quality and enzyme activity in sturgeon farmed in industrial aquaculture farms, we set out to study the main quantitative and qualitative characteristics of Russian sturgeon sperm farmed on a super-intensive cage farm.

Materials and Methods

The study was performed with Russian sturgeon (*Acipenser gueldenstaedtii*) at the age of seven, eight and nine years. The fish were randomly selected from industrial flocks of producers, raised on a super-intensive cage farm, situated in a large warm water dam, located in Southeastern Bulgaria at 41° 37′ N latitude and 25° 20′ E longitude. The region falls within the Southern Bulgarian climatic zone, Eastern Rhodopean climatic region. The average altitude is about 280 m. Fish were grown under identical conditions throughout the study period. Full-feed specialized granulated feed was used for feeding.

The study was conducted over three consecutive years. Upon receipt of the semen, the volume of ejaculate was immediately measured on the spot, after which the semen was transported in refrigerated bags to a laboratory for biochemical analysis. Sperm concentration was determined by a CASA system 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 spermatozoa; spermatozoa with progressive and non-progressive motile.

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:

GGT-Tris buffer – 100 mM pH 8.25, glycil-glycine 100 mM, L-Glutamyl-4-nitroanilide 4mM, wavelength 405 nm.

AP-DEA buffer – pH 9.8 1M, $MgCl_2$ 0.5 mM, 4-Nitrophenilphosphate 10 mM, wavelength 405 nm.

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.

LDH-phosphate buffer – pH7.50 50 mM, sodium pyruvate 0.60 mM, NADH 0.18 mM, 340 nm wave length.

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 freeze at -20°C overnight. On the next day, the samples were sonicated by ultrasound 3 × 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.

IBM SPSS Stasistics 21 was used for statistical processing.

Results and Discussion

The main indicators of Russian sturgeon semen of different ages are presented in table 1. The studied fish show a very high variation, as in the group of 8-year-olds it reaches 76.96% for the volume. The maximum volume for the study was 153 ml (7-year Ag) and the minimum was 10.50 ml (8-year Ag). Generally seven-year-old fish have the largest ejaculate. The difference in the indicator with 8-year-old individuals reaches over 2.5 times ($p < 0.05$), and with 9-year-olds – 28.9%, but is insignificant. 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 was reported in 9-year-old fish (98.54%), and in 7-year-olds it was lower by 1.56%, with an insignificant difference. In 8-year-old Russian sturgeon

Table 1. Indicators characterizing the Russian sturgeon sperm quality

Parameters	Indicators		
	Volume, ml	Motility, %	Concentration, 1.10^6/ml
7 years old (n = 5)			
X	114.80 c	97.00 c	723.46
±SE	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 old (n = 8)			
X	44.81 cd	53.98 cd	1128.63
±SE	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 old (n = 15)			
X	89.07 d	98.54 d	983.82
±SE	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

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

the motility was significantly lower, as the difference with 9-year-olds was 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 semen with the lowest sperm motility at the smallest volume. Like the volume, the spermatozoa motility variation of these fish, is very high – 65.33%.

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

A significant difference was found in VCL between the three studied groups of Russian sturgeon ($p < 0.05$). 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 2).

The results in terms of straight line velocity (VSL) show that 9-year-olds significantly exceed 7- (2.5 times; $p < 0.05$) and 8-year-olds (4.3 times; $p < 0.05$).

The same applies to most of the motility indicators, which are significantly ($p < 0.05$) higher in 9-year-old fish

Table 2. Sperm motility in 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
±SE	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	15.90d	25.08 d	32.34 d	56.28 d	48.65 d	1.613 cd	2.750 d
±SE	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	128.90c	69.11 cd	95.00 cd	53.69 cd	72.05 cd	73.87cd	3.559 d	6.502 cd
±SE	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 – $p < 0.05$.

Table 3. Sperm distribution according to the characteristics of their movement and speed in the Russian sturgeon semen, %

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
±SE	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
±SE	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	67.12 d	21.61	9.81 c	1.46 d	63.76 d	34.78 cd
±SE	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

The differences between the values marked with the same symbols in each column are significant: c, d – $p<0.05$ are reported here, but the differences between the different groups are not significant.

compared to 7- and 8-year-olds – the average path velocity (VAP), respectively, 95.15% and 3.8 times; linearity (LIN) by 71.5% and 66.02%, respectively; straightness (STR) of 29.45 and 28.02%, respectively; the wobble (WOB) by 32.0 and 51.83%, respectively; beat/cross frequency (BCF) by 51.56% and 2.36 times, respectively.

The only indicator by which the oldest individuals do not outperform younger fish is the amplitude of lateral head displacement (ALH).

The highest value of the indicator was found 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 is significant ($p < 0.05$) and is 2.2 times.

Summarizing the data on sperm motility in Russian sturgeon, it can be stated that 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 related to the speed and characteristics of sperm movement (Table 3). They have the highest amount of rapid sperm (67.12%), the least of static (1.46%). The lowest amount of rapid sperm was found in 8-year-old fish, and the difference in the indicator with 7- and 9-year-olds was 3.26 ($p < 0.05$) and 5.10 times ($p < 0.05$), respectively. Medium-fast sperm are most in the youngest fish, and the difference in the indicator with 8-year-olds is significant (3.13 times; $p < 0.05$). The highest number of progressive motile spermatozoa was found in the oldest fish, and the difference was significant ($p < 0.05$) with 7- and 8-year-old fish, respectively 3.14 and 6.97 times.

In general, it can be pointed out that the 8-year-old fish sperm have the worst indicators characterizing the movement, as the percentage of static ones reaches 46.04%, which significantly exceeds ($p < 0.05$) the same in 7- and 9-year-olds, respectively 15.35 and 31.53 times.

Levels of major enzymes (AP – Alkaline Phosphatase; GGT – Gamma-glutamyl Transferase; CK – Creatine Kinase; LDH – Lactate Dehydrogenase) in water and triton X 100 extract were examined in our study (Table 4). Lactate dehydrogenase is known to be involved in sperm metabolism by catalyzing the conversion from pyruvate (the end product of glycolysis) to lactate, which is essential for ATP production through glycolysis (Miki, 2007).

Scientific publications related to alkaline phosphatase and creatine kinase levels in fish, especially sturgeon, are very limited (Aramli et al., 2013). Alkaline phosphatase is thought to be related to capacitation in some species (Bucci et al., 2013). Despite the unclear role of alkaline phosphatase in male reproduction, Bucci et al. (2013) suggest that AP is still present on the surface of sperm even after induction of capacitation. According to the authors, this fact confirms the hypothesis that AP plays a role in sperm capacitation. According to other authors the concentration of AP in semen is a good indicator of sperm quality, as this enzyme is an indicator of the stability of the sperm membrane (Pace & Graham 1970) and reflects the secretory activity of additional gonads (Aramli et al., 2013).

Creatine kinase provides the energy for phosphate hydrolysis needed to drive the normal function of many cellular systems, including sperm, thus maintaining an immediately available energy supply in the cell (Aramli et al., 2013). High-energy cells such as sperm are characterized by high CK (Walliman & Hemmer, 1994).

The function of many enzymes in semen is poorly defined, such as the function of gamma glutamyl transferase (Awadallah et al., 2003). However, it has been reported that this enzyme protects spermatozoa from oxidative stress (Hinton et al., 1998).

In most of the studied enzymes, the semen of 8-year-old Russian sturgeon has significantly lower levels compared to the other two groups. The water extract of AP, CK, LDH has a significant difference ($p < 0.05$) of this group with 7- and 9-year-olds and is respectively: 2.36 and 2.44 times; 16.03 and 17.05 times; 2.68 and 2.59 times.

Only on water extract of GGT in 8-year-old individuals the level of the enzyme is the highest, but the differences between the groups are not significant. However, this is not the case with regard to the level of GGT in the triton X 100 extract. Here again, the GGT content is highest in 8-year-old fish and 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 X 100 extract, the trend observed for these enzymes in water extract is the same – 8-year-old fish have significantly ($p < 0.05$) lower levels, respectively 10.66 and 10.61 times; and 2.99 and 2.63 times. There is a particularly big difference in AP content. There is variability in the range from medium (CV 16.06%) to strong (CV 91.10%) in most enzyme parameters in our study. The CV level for AP in water extract at 8 years of age was 152.93%, and it should be noted that the number of the studied fish was small.

Table 4. Russian sturgeon sperm enzyme activity in water and triton X 100 extract, UI/L

Parameters	Indicators*							
	Water extract				Triton X 100			
	AP	GGT	CK	LDH	AP	GGT	CK	LDH
7 years old (n = 5)								
X	732.00c	19.39	598.04c	929.8c	604.13c	4.75 c	82.74c	465.67
±SE	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	310.67cd	25.07	37.08cd	346.42cd	56.67cd	23.02cd	27.65cd	535.38
±SE	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	758.40d	18.94	636.00d	896.33d	601.27d	5.80d	72.80d	471.07
±SE	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 – $p<0.05$

An increasing number of species and hybrids are involved in sturgeon farming. As an endangered species, the Russian sturgeon is widely introduced in aquaculture (Hurvitz et al., 2008) and today it is one of the most important aquaculture species in a number of countries around the world (Kim et al., 2019; Nikolova, 2019; Sergeev, 2020). According to the latest data, the share of Russian sturgeon in the production of black caviar is about 20% (Bronzi et al., 2019).

When farmed on industrial farms, fish live in unusual conditions, which affects their growth and development (Agnese et al., 1995) as well as a deterioration in sperm quality is often observed (Fauvel et al., 2010; Zohar, 1996). In connection to this, the quality characteristics of the semen of sturgeon reared in the conditions of super-intensive cage aquaculture are of interest.

Sperm quality indicators in sturgeon obtained in different studies differ significantly.

The most modern method for analyzing sperm quality is the CASA system. It has only been used extensively in the analysis of fish semen for the last two decades (Gallego &Asturiano, 2018). According to Sieczynski et al. (2012) the use of the CASA system allows an accurate assessment of sperm cell motility in individual species (Siberian sturgeon, Sterlet), which in turn allows to determine the potential suitability of sperm for sturgeon reproduction.

In fish, particular reproductive conditions have formed evolutionarily diverse and complex physiological mechanisms in both females and males. Thus Ginzburg (1968) points out that in natural conditions the volume of ejaculate in fish depends on many factors – the size of the male fish, the duration of participation of individuals in the reproduction process and the amount of ejaculate produced, the number of caviar grains simultaneously discarded by the female fish, relative number of male and female fish involved, spawning conditions (whether small or large groups are involved; the speed of water flow in the hatcheries, related to the speed of sperm distribution in the water, etc.).

Ejaculate volume is one of the important macroscopic characteristics. In sturgeons, sperm volume can reach 1-1.5 l in large individuals (Horvath et al., 2011).

In our study, in adult Russian sturgeons raised in cages, ejaculate volume ranged from 10.5 to 153.0 ml; sperm concentration from 56.89 to 2917.15 x 10^6 / ml; sperm motility – from 11.90 to 100%.

Halimi et al. (2014) in Russian sturgeon obtained a volume of 86.3 ml. and total motility – 69.6%. Yamaner et al. (2017) in a study of sperm production of Russian sturgeon obtained a volume of 151.3 – 188.0 ml, with sperm motility 66.2 – 76%.

Sperm motility is one of the most important indicators of sperm quality (Rurangwa et al., 2014; Horton & Ott, 2011).

Motility in sturgeon species can vary considerably. Psenicka et al. (2008b) when analyzing sperm on which they obtained 76.6% motility, Sarosiek et al. (2004b) – 67%, Sieczynski et al. (2012) – 44.8%. Horvath et al. (2011) in Atlantic sturgeon from two individuals obtained motility values of 90 and 85%, respectively. In Stelate sturgeon, sperm motility varies from 80.63 (Sadeghi et al., 2020) to 81.16% (Sadeghi et al., 2013).

Comparing our results with those obtained in Russian sturgeon by Yamaner et al. (2017), it is evident that the motility of sperm in our study is higher than the maximum values indicated by the authors, in 9- and 7-year-old fish by 29.7% and 27.6%, respectively, and in 8-year-olds is lower with 28.9%.

Aydin et al. (2012) in a study of sperm quality in Russian sturgeon in the two individuals studied at the age of 7 years obtained ejaculate volume of 225 ml and 155 ml, with a total sperm motility of 90% and 100%, respectively. The total sperm motility in 7-year-old individuals in our study approximates the values obtained by the authors, and the maximum sperm volume is 32% less than the maximum indicated by the authors.

Huang et al. (2014) in Russian sturgeon obtained a total sperm motility – 95%, and this value is close to that of 7- and 9-year-old individuals subject of our study.

Urbanyi et al. (2004) reported high values of sperm motility in Sterlet (90% in one individual and 80% in the other). The authors obtained a motility of 50%, in Russian sturgeon in the same study, which approximates the average value (53.98%) obtained in our study in Russian sturgeon at 8 years of age, but is significantly lower than sperm motility in fish at 7 and 9 years old.

It is known for Sturgeons that when the ejaculate volume is big of, the concentration of sperm per unit volume ranges from one tenth to one fifth of that in most other fish (Ginsburg, 1968). In general, the spermatozoa in the different Acipenseridae species are similar, with small differences in morphology (Psenicka et al., 2007). Horvath et al. (2011) note that the sperm concentration in Sturgeon is significantly lower than that of teleosts and ranges between 0.5 – 2 x 10^9 sperm / ml.

A number of authors (Ginsburg, 1968; Detlaf & Ginzburg, 1968; Detlaf et al., 1981) point out that the quality of sperm in different individuals, and even individual portions of sperm obtained at different times in the same individual, in sturgeons can differ significantly. It is emphasized that sperm concentration is particularly variable, but low concentration in Sturgeon is not always associated with poor sperm activity and low fertility.

Data on sperm concentration in sturgeons varies considerably in various scientific studies. This is due to the significant influence of a number of genetic and environmental factors and their interaction. The sperm concentration in Sturgeon species is known to be lower than in teleosts (Psenicka et al., 2008b). According to the results of various authors cited by Ginsburg (1968), sperm concentration in Beluga varies in the range 0.58 – 6.40 x 10^9 sperm / ml; in Sterlet – 0.59 – 2.41 x 10^9 sperm / ml; in Russian sturgeon – 1.07 – 3.16 x 10^9 sperm / ml; in Persian sturgeon – 0.6 – 1.5 x 10^9 sperm / ml; in Stelate – from 0.9 – 10.37 x 10^9 sperm / ml.

Yamaner et al. (2017) in Russian sturgeon reported a sperm concentration of 1.3 x 10^9 / ml (1300 x 10^6 / ml), which is 13% higher than the highest reported in our studies – in 8-year-old fish (Table 4.3.1.1.).

Sadeghi et al. (2020) in Stelate sturgeon received a concentration of 2.4 x 10^9 / ml. at a sperm motility rate of 80.63%.

Shaluei et al. (2017) conducted two experiments with Persian sturgeons, in March and April. The concentration in the samples from March is 1.880 x 10^9 / ml (1880 x 10^6 / ml), and from April – 2.09 x 10^9 / ml (2090 x 10^6 / ml). Li et al. (2010) when working with Beluga, obtain a concentration of 0.28 x 10^9 / ml. Psenicka et al. (2008a) obtained a concentration of 0.37 x 10^9 / ml in Sterlet.

Although the percentage of total 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 most accurately characterize sperm quality (Gallego et al., 2017). In this regard, Barulin and Shumsky (2018) note the prospects for the use of CASA systems in aquaculture, especially in the cultivation of valuable species. The authors obtained sperm motility 84.80 ± 4.02% and VCL – 57.3 ± 1.39 µm/s in a study of semen from a 7-year-old Siberian (Lena) sturgeon raised in aquaculture. In another study of Lena sturgeon semen at the age of 5 years, the indicators characterizing the movement were as follows VCL – 173.37 ± 1.59; VSL – 164.19 ± 1.82; VAP – 171.22 ± 1.20; WOB – 0.99 ± 0.01; LIN – 0.95 ± 0.01; STR – 0.96 ± 0.10 (Barulin et al., 2013).

Yamaner et al. (2017) when tracking the value of VCL for Russian sturgeon, obtained 103.95 µm / s, and this value is 24% lower than the highest average value of the indicator in our study (at 9 years), but and more than twice as high as the lowest obtained with us (8 years old).

Shaliutina-Kolesova et al. (2011) obtained a higher value for VCL – 181.12 µm / s in Sterlet, which is 28% more than the highest value measured by us in Russian sturgeon.

Sieczynski et al. (2012) in the analysis of Sterlet semen semen with CASA system obtained the following values: VCL (119.9 µm / s), VSL (86.8 µm / s), VAP (102.4 µm / s), ALH (12.8 µm / s), BCF (3.7 Hz), LIN (55.1%), STR (72.5%).

One of the important signs of sperm function is enzyme activity (Kowalski & Cejko, 2019).

Piros et al. (2002) note the importance of sperm and plasma enzymatic activity studies in Sturgeon in connection with the improvement of methods of short and long-term storage of sperm. An increase in the activity of lactate dehydrogenase (LDH), arylsulfatase (AS) and acid phosphatase (AcP) has been found in the semen of Siberian sturgeon (*Acipenser baerii*) and Sterlet (*Acipenser ruthenus*) as a result of cryopreservation (Piros et al., 2002).

Sarosiek et al. (2004a) made a biochemical characterization of arylsulfatase (AS) found in the sperm plasma and sperm of the Russian sturgeon (*Acipenser gueldenstaedtii*), believing that due to the similarity of this enzyme in sperm and sperm plasma, it can be pointed that AS owes its presence in the plasma due to sperm destruction.

According to Huang et al. (2014) a number of enzymes (LDH; CK) are essential for metabolic processes that provide energy for sperm survival, motility and fertility.

Characteristics of sperm in different systematic fish groups and species can differ significantly (Piros et al., 2002; Horvath et al., 2011). Comparing the results obtained by us with those of other studies in Russian sturgeon and other sturgeon species, also makes a big difference between the obtained values.

Thus Huang et al. (2014) obtained for CK – 3 IU / ml (3000 IU / L) in a study of some enzymes in Russian sturgeon, more than four times higher value than the highest measured by us (in the 9-year group of individuals). Regarding the levels of LDH, the authors get a value of 2000 IU / L, which is again higher than the highest in our study (in 7-year-old fish), and the difference is more than twice.

Aramli (2014) in a study of Beluga sperm (*Huso huso*) obtained values for LDH – 234.4 IU / L.

Piros et al., (2002) in a study of sperm quality obtained: for LDH 278.4 and 400.9 IU / L in Siberian sturgeon, 71.0 and 761.7 IU / L in Sterlet; for AP, 1.0 – 1.21 IU / L, and 0.90 and 1.85 IU / L, respectively

Aramli et al. (2014) in Persian sturgeon (*Acipenser persicus*) studied the levels of total protein and the concentration of some enzymes, indicating data for LDH – 484.4 IU / L. In another study of the concentration of various enzymes in the semen of a Persian sturgeon, Aramli et al. (2013) obtained values for LDH – 472 IU / L, CK – 553.8 IU / L and AP-12.23 IU / L. The values of the enzymes mentioned above are higher. In CK the difference is the smallest – 14.84%, and in LDH and AP – significantly higher, respectively 49.2% and 62 times.

Enzyme levels in semen are studied in different teleost fish species. Thus, Ciereszko and Dabrowski (1994) found AP – 8.76 IU / L for rainbow trout (*Onchorhyncus mykiss*) and 4.77 IU / L for lake whitefish (*Coregonus clupeaformis*).

Conclusions

Despite the unusual conditions for Sturgeons when farmed on industrial farms, Russian sturgeon individuals of three different age groups showed good results regarding Sperm production. The ejaculate volume is not bigger in older fish and can be concluded that it is rather an individual trait that has no connection with age. The average volume in the individual age groups ejaculate is from 10.50 ml (8-year-old), to 153 ml (7-year-old fish). Age also doesn`t affect the sperm concentration as there are no any significant differences on this indicator between the different age groups. In the 8 years old group of fish the variation in the concentration of sperm is the highest – from 56.89 to 2917.15 x 10^6 / ml.

Regarding the sperm movement characterization, the worst values were obtained in 8-year-old fish, as the percentage of static ones reached 46.04, which significantly exceeds ($p < 0.05$) the same in 7- and 9-year-olds by 15.35 and 31.53 times, respectively. In most of the studied enzymes, the semen of 8-year-old Russian sturgeon has significantly lower levels compared to two other groups of AP, CK, LDH in water extract, the difference of this group with 7- and 9-year-olds is significant ($p < 0.05$) and is respectively: 2.36 and 2.44 times; 16.03 and 17.05 times; 2.68 and 2.59 times. Only on 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.

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