Bulgarian Journal of Agricultural Science, 25 (Suppl. 1) 2019
Agricultural Academy

Development of female Russian sturgeon (*Acipenser gueldenstaedtii*) and hybrid (*Acipenser baerii x Acipenser gueldenstaedtii*) gonads reared in net cages

Stanimir Bonev*, Lyudmila Nikolova

Agricultural University, Faculty of Agronomy, Department "Animal science, Plovdiv 4000, Bulgaria
**Corresponding author:* dr_bonev@yahoo.com

Abstract

Bonev, St., & Nikolova, L. (2019). Development of female Russian sturgeon (*Acipenser gueldenstaedtii*) and hybrid (*Acipenser baerii x Acipenser gueldenstaedtii*) gonads reared in net cages. *Bulgarian. Journal of Agricultural Sci*ence, *25* (Suppl. 1), 62–68

The study was conducted with female individuals of Russian Sturgeon at the age of five and seven years old and hybrid of Siberian and Russian Sturgeon at the same age in the spring and the same groups in the winter. In hybrids of both age groups, there was a significant increase in gonads during the growing season. At 5-year-old, ovarian height increased by 69.8% ($p < 0.05$); the girth by 68.0% ($p < 0.05$); area – almost three times ($p < 0.001$). At 7-year-old differences are: 32.8% ($p < 0.05$); 29.5% ($p < 0.05$); 2.3 times ($p < 0.01$). In the spring, in 10% of the studied hybrids, an onset of oocyte formation was found. In December, oocytes are very well seen in 30% of the studied fish. In 5-year-old Russian Sturgeon, the height of the ovary increased by 64.1% ($p < 0.01$); area 64.3% ($p < 0.05$); but the girth has practically not changed. In the 7-year-old Russian Sturgeon, the height of the ovary increased by 78.5% ($p < 0.001$), the girth by 28.9% ($p < 0.001$) and the area by 95.08%. ($p < 0.01$). The height of the hybrid ovary during the spring was higher compared to the Russian Sturgeon by 51.1% ($p < 0.05$) in the 5-year-old fish and by 71% ($p < 0.001$) in the 7-year-old. However, the ovary girth in the 5-year-old Russian Sturgeon has higher values than in the same age hybrid – 13.6% ($p < 0.05$). There is no significant difference in ovarian girth and area in 7-year-old Russian Sturgeons and hybrids. A significant difference in area was found between Russian Sturgeon of different age, and in 7-year-old it was larger by 41.9% ($p < 0.05$). In winter with a 6-summer-old hybrid compared to Russian Sturgeon at the same age, the height of the ovary and its girth were higher 56.3% ($p < 0.001$) and 45.0% ($p < 0.05$) respectively, while at 8-summer-olds, at higher hybrid parameter values, the differences are not significant.

Key words: sturgeon; gonads; ultrasound techniques
Abbreviations: Ag_5 – 5-years old *A. gueldenstaedtii*; Ag_7 – 7-years old *A. gueldenstaedtii*; Ag_{5+} – 6-summers old *A. gueldenstaedtii*;Ag_{7+} – 8-summers old *A. gueldenstaedtii*; Hy_5 – 5-years old hybrid (F_1 *A. baerii x A. gueldenstaedtii*); Hy_7 – 7-years old hybrid (F_1 *A. baerii x A. gueldenstaedtii*); Hy_{5+} – 6-summers old hybrid (F_1 *A. baerii x A. gueldenstaedtii*); Hy_{7+} – 8-summers old hybrid (F_1 *A. baerii x A. gueldenstaedtii*)

Introduction

Natural populations of Sturgeon species are threatened (Boyadzhiev, 2002, etc.), and a number of measures are being taken to conserve and restore them (Bloesch et al., 2006; Kecse-Nagy, 2011). For the Bulgarian Danube and Black Sea aquatory of 2012, the catching, transport and sale of sturgeons, as well as products from them are prohibited. In 2016 the ban was prolonged by another 5 years (MZH, 2016). The primary task of rescuing endangered populations is the development of aquaculture production of Sturgeon fish.

Important for Sturgeon aquaculture in our country is the Russian Sturgeon. The species is often used for industrial hybridization in connection with increased production efficiency (Ponomarev & Ivanov, 2009, etc.). The Sturgeon fish cross and under natural conditions, hybrids are often found in sympatric populations (Chebanov & Galich, 2013).

Burtsev (2013) points out that, at the level of Acipenseridae's threatening in natural ecosystems, the development of biotechnology for their cultivation is an innovative mechanism for their recovery. Representatives of the Acipenseridae family refer to species carrying out breeding migrations (Nikolyskiy, 1971). When grown in cages, Sturgeon fish are placed in an environment that is radically different from the conditions in natural ecosystems where there is a complex of factors and conditions necessary for successful reproduction. For an efficient reproduction in a full-aquaculture super-intensive farm, it is necessary to know the patterns of development of Sturgeon in unusual conditions. In this regard, we set our goal to study the development of ovary in Russian Sturgeon and hybrid (Siberian and Russian Sturgeon) at the age of five and seven years old in the spring and in the winter using ultrasound methods.

Materials and Methods

The study was conducted with female Russian Sturgeon (*Acipenser gueldenstaedtii*) at five- (Ag_5) and seven years of age (Ag_7); hybrid of Siberian and Russian Sturgeon (F1 *Acipenser baerii* x *Acipenser gueldenstaedtii*) at five- (Hy_5) and seven years of age (Hy_7) in the spring, and with the same groups during the winter. We performed the first scan in May 2017 and the second in December 2017 and the beginning of January 2018. From each group, 10 individuals (n = 40) were studied in each of the seasons. The average body weight of the fish was: Ag_5 – 4.952 ± 0.09 kg; Ag_7 – 5.200 ± 0.09 kg; Hy_5 – 4.200 ± 0.06 kg; Hy_7 – 5.575 ± 0.076 kg.

The fish have been grown on a farm for super intensive production in cages localized in the reservoir of Kardzhali since they were one summer old. By type, the reservoir refers to large, deep reservoirs – its area is 16.07 km^2, the volume 532.9 x 10^6.m^3. Kardzhali reservoir is located in Southeastern Bulgaria at 41° 37 'N latitude and 25°20' E longitude. It falls into the South-Bulgarian climate zone, the East Rhodopean climatic region. The average altitude is about 280 m.

Fish from different species and age groups are grown in separate 8x8 m cages with water depth 6 m from the surface. The net of the cages is double, of polyamide. Feeding is with specialized for sturgeon fish granulated feed.

Ovarian development was monitored by non-invasive herd monitoring, using sonographic methods in live fish not participating in the breeding campaign. A Mindray DP 50 echograph was used with a 75L38EA linear transducer (5-10 MHz). With specialized software originally installed on the ultrasound scanner, the thickness (cm) of the ovary was measured at the frontal view; the girth (cm) and area (cm²) in transverse view. Scanning was performed in the area of 3-4th abdominal bone scute, counting from the abdominal fin to the head on the left side of each fish. IBM SPSS Statistics 21 was used for statistical data processing.

Results and Discussion

As a result, a collection of ultrasound images of the Russian Sturgeon and the hybrid was collected, from which we chose the most characteristic of each group (Figures 1-9).

In sturgeon fish, sexual dimorphism is not well expressed. Chebanov & Galich (2010) note that until recently, the lack of appropriate methods for early diagnosis of the sex was the main limiting factor in the creation of uterine herds of Sturgeons for industrial rearing, and the problem has diminished after the introduction of ultrasound diagnostics. These methods allow non-invasive determination of gonad sizes and their individual characteristics (Du et al., 2017). The aim is to define gender as early as possible (Masoudifard et al., 2011).

On Figure 1 there is a frontal (1a) and a transverse (1b) photograph of the gonads of a Ag_5 in the spring. The heterogeneous structure of the generative part and the pronounced hipoechogenicity are well seen. The ovarian fat envelops most of the ovary. It has a pronounced anechogenicity, which is visualized in black in the picture. The generative tissue is with roughly unclear contours, and the upper part clearly shows the border between the ovary and the musculature – a serous membrane. The height of the ovary is 1.49 cm, the girth is 10.30 cm, and the area of the generative part is 1.97 cm. In the same species, at the age of 6 summers, during the winter period (Fig. 2), the heterogeneous structure of the generative part is not so well expressed. The height of the ovary is 1.88 cm. Ovarian fat takes a small part of the ovary at the expense of better-developed generative tissue. The ovary's generative part girth is 12.25 cm and the area is 4.67 cm².

In Ag_7, during the spring period (Fig. 3), the heterogeneous structure of the generative part is well visible. The hyperechoic serous membrane is clearly visible. The height of the ovary is 1.11 cm, the girth is 7.03 cm and the area is 1.76 cm². During the winter period (Fig. 4), the increased generative part of the gonads is clearly visible. On the frontal picture, the increased ovarian height is evident due to the

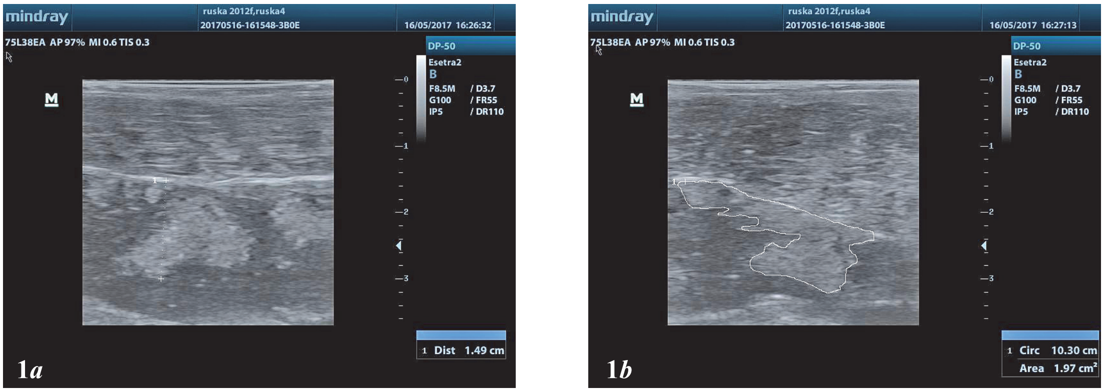


Fig. 1. Ultrasound images of a Ag$_5$ (spring season)
a) frontal view
b) transverse view

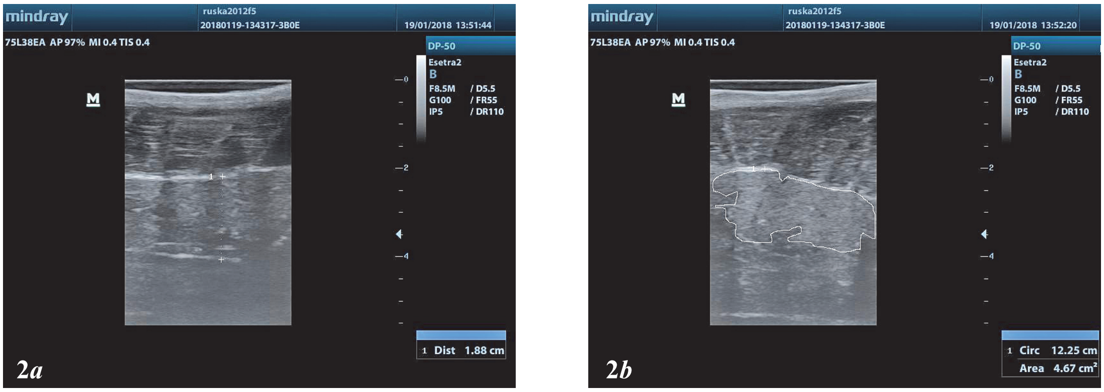


Fig. 2. Ultrasound images of a Ag$_{5+}$ (winter season)
a) frontal view
b) transverse view

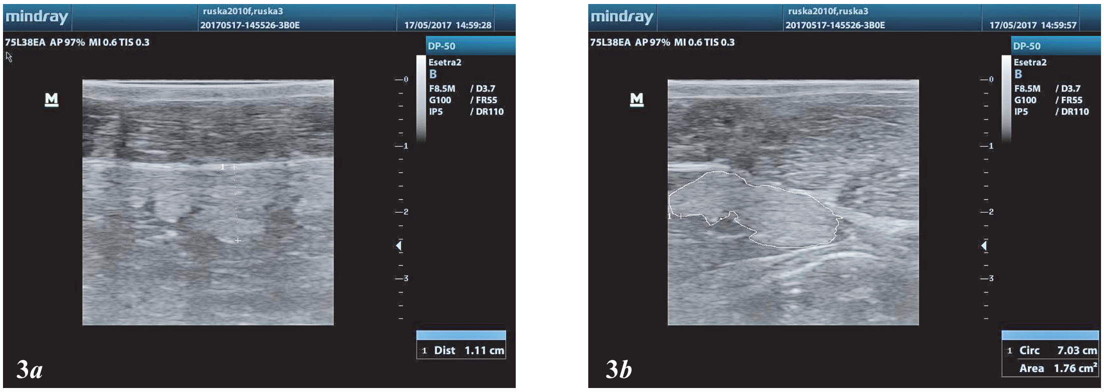


Fig. 3. Ultrasound images of a Ag$_7$ (spring season)
a) frontal view
b) transverse view

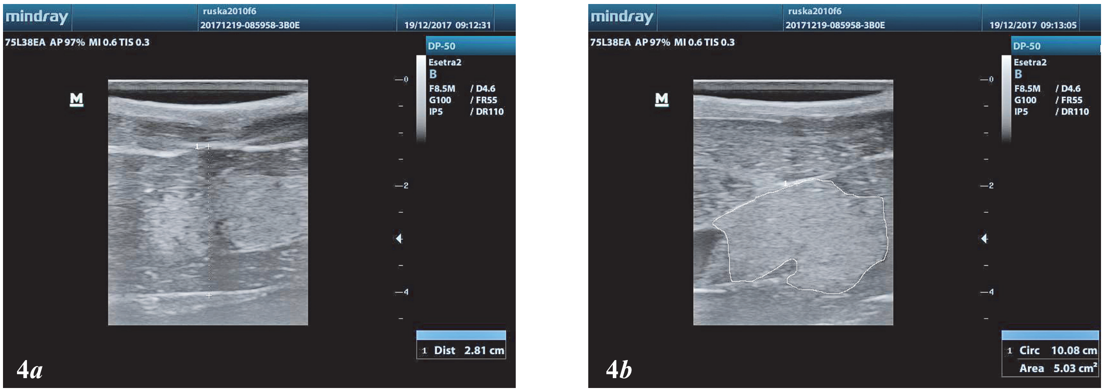


Fig. 4. Ultrasound images of a Ag$_{7+}$ (winter season)
a) frontal view
b) transverse view

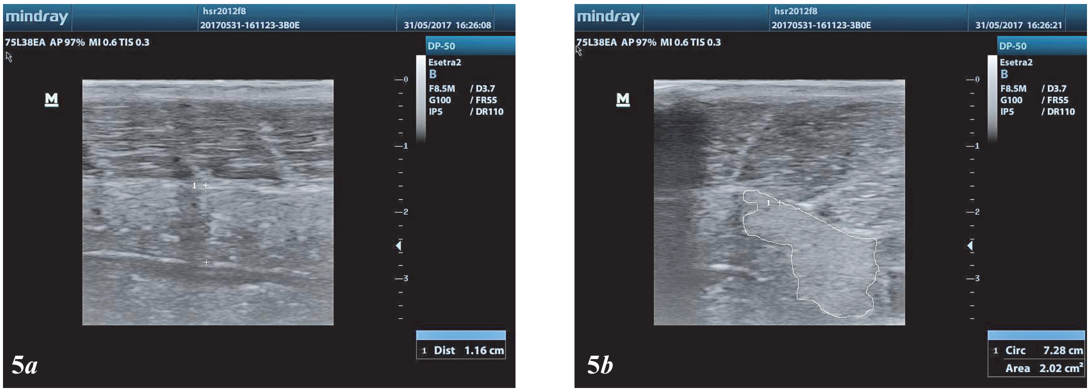


Fig. 5. Ultrasound images of a Hy$_5$ (spring season)
a) frontal view
b) transverse view

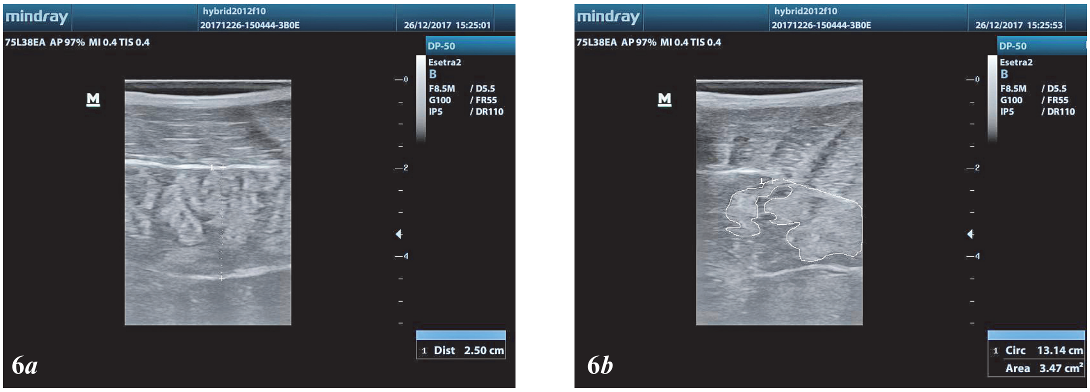


Fig. 6. Ultrasound images of a Hy$_{5+}$ (winter season)
a) frontal view
b) transverse view

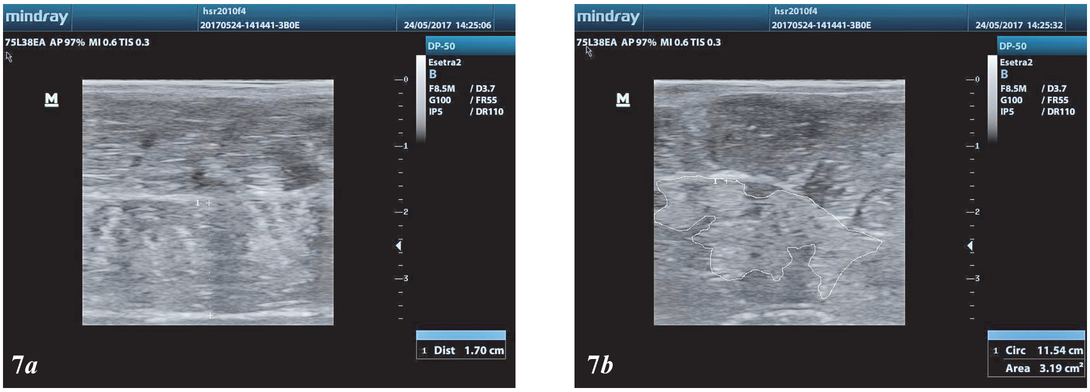


Fig. 7. Ultrasound images of a Hy$_7$ (spring season)
a) frontal view
b) transverse view

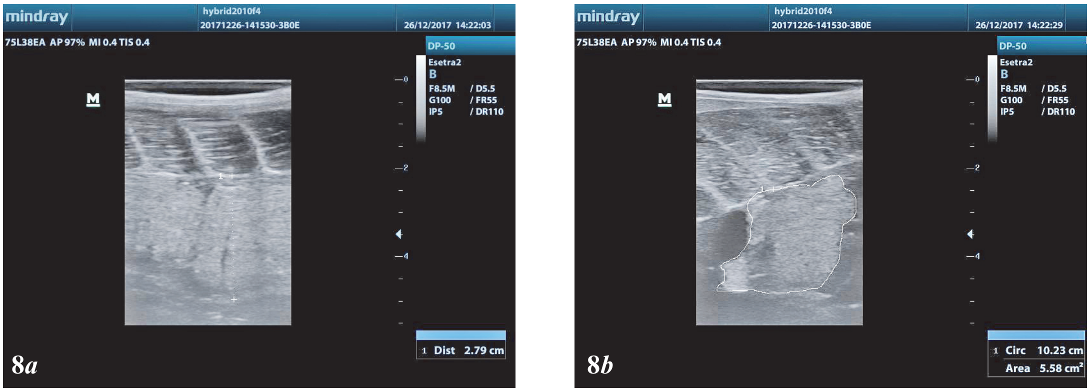
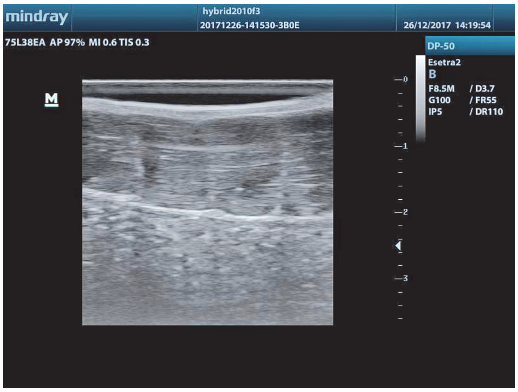


Fig. 8. Ultrasound images of a Hy$_{7+}$ (winter season)
a) frontal view
b) transverse view

9*a*



9*b*

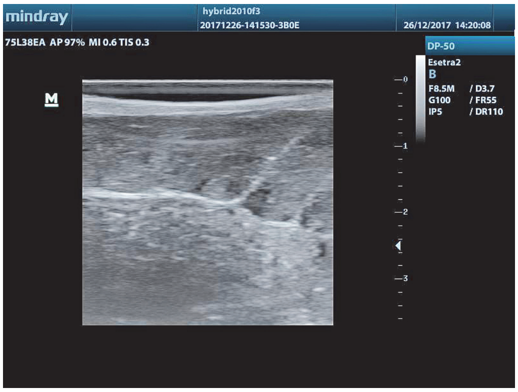


Fig. 9. Ultrasound images of a Hy$_{7+}$ with oocyte forming processes (winter season)
a) frontal view *b*) transverse view

accumulation of ovarian fat, clearly expressed by its anechogenicity. The height of the ovary is 2.81 cm. The ovarian fat envelops almost entirely the generative tissue, which in this case is visualized with uncleared rough contours. The serous membrane is even more distinguishable, as it borders with anechoic fat area. The girth of the ovarian generative part is 10.08 cm and the area is 5.03 cm².

On Figure 5 there is a frontal (5a) and a transverse (5b) photograph of a Hy$_5$ in the spring season. The height of the ovary is 1.16 cm. Muscle stands out with its hypoechoic structure, as well as the generative part of the ovary, but it is darker in color. The serous membrane clearly stands out with its hyperechogenicity. The generative part has a clearly expressed heterogeneous structure. Ovarian fat occupies a noticeably smaller part of the gonads. The girth of the ovarian generative part is 7.28 cm and the area is 2.02 cm². At Hy$_{5+}$ during winter season the most pronounced heterogenicity of the ovarian generative part is observed, which occupies a significantly smaller part at the expense of the ovarian fat, and has a hypoechoic structure with a light hue (Fig. 6) The measured ovary height is 2.50 cm. The girth of the ovarian generative part is 13.14 cm and the area is 3.47 cm².

In Hy$_7$, in the spring (Fig. 7), the generative part is with roughly unclear contours and a heightened heterogeneous structure and the pronounced hypoechogenicity with different shades. Ovarian fat occupies more than 50% of the whole ovary. The height of the ovary is 1.70 cm. The girth of the generative part is 11.54 cm and the area is 3.19 cm². The ultrasound images of Hy$_{7+}$ during the winter season (Fig. 8) show a better developed ovary with less ovarian fat compared to spring photographs of individuals in the same group. The height of the ovary is 2.79 cm, the girth is 10.23 cm and the area 5.58 cm². The generative part has a heterogeneous structure and a marked hypoechoic character, but it has lighter shades. Ovarian fat visibly occupies a smaller part of the ovary. The muscle fibers are visualized with moderate brightness, and alternating walls separated by connective tissue appear on the screen as narrow sloping, almost vertical stripes and have a brighter hue of the muscles.

In the ultrasonographic studies in the spring, 10% of the individuals were found to start oocyte formation. In December, in 30% of the studied females the oocytes are well seen. (Fig. 9). The growth of gonads and the deposition of yolk in oocytes leads to swallowing of ultrasound waves, thus unable to visualize the ventral border of the ovary and to distinguish the generative part of ovarian fat. The serous membrane at the dorsal end stands out well.

In general, it can be concluded that using ultrasound imaging the ovaries are seen as heterogeneous structures with mixed echogenicity, uneven borders, and without a tunic. The fat part of the ovary is visualized in the form of darker areas, distinct from brighter ovarian tissue.

The two parental forms involved in the hybridization – the Russian and Siberian Sturgeons, differ significantly in biological features. The Russian Sturgeon is a typical anadromous species. Chebanov & Galich (2013) note a complex intra-species structure in natural populations of the species (local populations, subspecies, summer and autumn forms). The authors point out that females of this species mature at about 10-16 years of age; Vasileva et al. (2006) – 12-15 years; Filipova & Zuevskiy (2009) in different populations from 8-10 to 14-15 years. Sevryukov et al. (2001) establish mass maturation of female Russian Sturgeons grown under industrial conditions at 6-7 years. The Siberian sturgeon is a monotypical species (Ruban, 1999), represented by isolated populations, and within the natural areas there are river, lake-river forms that carry out postmodromal migrations or are not anadromous at all. In northern regions, female individuals mature at 11-30 years of age (Makeyeva et al., 2011). Konchits & Savonchik (2010) indicate that the age of sexual maturation in the Siberian Sturgeon can vary considerably depending on the conditions of breeding, whereas in warm-water farms the female fish mature at the age of 5-7 years. Similar age (4-7 years) is indicated by Filipova & Zuevskiy (2009). According to Ponomarev & Ivanov (2009) Siberian Sturgeon matures earlier than Russian, and is better adapted to artificial cultivation conditions. Its use as a parental form is conditioned by the good characteristics of the species as a subject of cultivation. For the first time a hybrid between Russian and Siberian Sturgeon was obtained in the second half of the last century (Filipova & Zuevskiy, 2009).

Our studies show that in the spring period in the Russian Sturgeon compared to the hybrid of the same age, the ovaries are smaller in height (Table 1). In 5-year old hybrids the height was higher by 51.1% ($P < 0.05$) and by the age of 7 years by 71% ($p < 0.001$). The individual variability of the indicator is broad, the most significant being for Ag_5, and the lowest for Ag_7. The variation in the ovarian girth is also relatively high, with final variants being the 5 and 7-year old Russian Sturgeon in the above-mentioned directions. However, in the ovary girth, for Ag_5, the value is higher, compared to the hybrid at the same age by 13.6% ($p < 0.05$). In the seven-year-old individuals, of both genotypes, no significant difference was found in the ovary girth and area. A significant difference in ovarian area was found among Russian Sturgeons of different age, and in the seven-year-olds it is higher with 41.9% ($p < 0.05$). The ovary area is the most variable of the three studied parameters.

In the Hy_{5+} in winter, compared to Russian Sturgeons of the same age, the ovary height and its girth were higher by 56.3% ($p < 0.001$) and 45.0% ($p < 0.05$) respectively, while at 8 summer-olds, at higher hybrid parameter values, the differences are not significant. In Ag_{7+}, the height of the ovary is 26.5% higher ($p < 0.05$), compared to individuals at the age of 6 summers. The variation of the indicator in the individual groups is quite heterogeneous – from relatively low in the hybrids at the age of five to significant in the other groups. A significant difference in individual variability is also seen in the ovarian girth. As with the Russian Sturgeon and the hybrid, at the 6 summers of age, the variation is significantly higher than at the 8 summer-olds. Ovarian area in hybrids of different ages is similar, whereas in Russian Sturgeon the ovary area of the 8-summer-old fish is 68.4% ($p < 0.001$) larger than the 6-summer-old. In the Ag_{5+}, the ovarian area variation is extremely high. The smallest is the coefficient of variation in Hy_{5+}.

A significant increase in the size of the gonads from spring to winter was found in the hybrids of both age groups (Fig. 10). In five-year-olds, ovarian height increased by 69.8% ($p < 0.05$); the girth by 68.0% ($p < 0.05$); area – almost three times ($p < 0.001$). In the seven-year-olds differences are: 32.8% ($P < 0.05$); 29.5% ($P < 0.05$); 2.3 times ($p < 0.01$) respectively. In the Russian Sturgeon at the age of 7 years the height increased by 78.5% ($p < 0.001$), the girth by

Table 1. Ovarian characteristics of Russian Sturgeon and Siberian and Russian Sturgeon hybrid, of different ages during different seasons

Indices	Genotype / Age							
	Ag_5 (n = 10)	Ag_{5+} (n = 10)	Ag_7 (n = 10)	Ag_{7+} (n = 10)	Hy_5 (n = 10)	Hy_{5+} (n = 10)	Hy_7 (n = 10)	Hy_{7+} (n = 10)
	Season							
	Spring	Winter	Spring	Winter	Spring	Winter	Spring	Winter
Height, cm								
ΔX	0.92ci	1.51dfi	1.07ah	1.91fh	1.39bce	2.36de	1.83abg	2.43g
±Sx	0.11	0.14	0.07	0.17	0.10	0.08	0.12	0.30
CV	35.40	27.85	17.39	24.90	20.01	8.70	18.15	30.10
Girth, cm								
ΔX	7.03c	7.17af	7.19h	9.27ah	6.19ce	10.40fe	7.29g	9.44g
±Sx	0.59	1.02	0.35	0.23	0.37	0.75	0.67	0.33
CV	25.36	42.60	13.76	7.16	15.89	17.69	26.11	8.65
Area, cm^2								
ΔX	1.29cf	2.12def	1.83ci	3.57di	1.57a	4.35ea	1.84b	4.31b
±Sx	0.14	0.46	0.18	0.40	0.18	0.31	0.24	0.71
CV	33.43	64.85	28.03	31.72	30.09	17.39	36.83	40.49

Differences between the values denoted by the same symbols in the rows are significant: a; d; e – $p < 0.001$; b; i – $p < 0.01$; c; f; g – $p < 0.05$

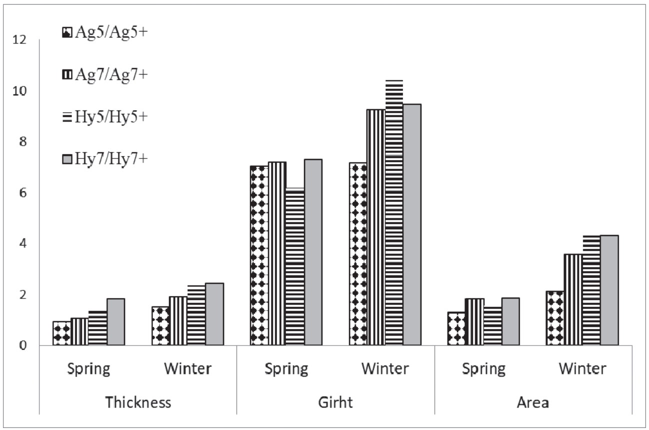


Fig. 10. Ovarian development of Russian Sturgeon *(Acipenser gueldenstaedtii)* and hybrid of Siberian and Russian Sturgeon *(F1 Acipenser baerii x Acipenser gueldenstaedtii),* at different ages, through different seasons (height, cm; girth, cm; area, cm²)

28.9% ($p < 0.001$) and the area by 95.08%. ($p < 0.01$); while in the case of five-year-olds, the girth did not change significantly with a significant height increase of 64.1% ($p < 0.01$) and the area of 64.3% ($p < 0.05$).

Conclusions

Ultrasound monitoring of live individuals provides a good opportunity to track ovarian development in Sturgeon fish. On the ultrasound image, they are seen as heterogeneous structures with mixed echogenicity, uneven borders, and without a tunic. The ovarian fat is visualized in the form of darker areas.

During the growing season, in hybrids, the size of the gonads grew significantly and by five-year-olds the increase was substantial compared to the seven-year-olds. The Russian Sturgeon has the same regularity, with the dynamics of seven-year-olds being more substantial. In hybrids, the height of the ovary is higher than that of the Russian Sturgeon, while the differences in girth and area vary across seasons and age groups.

During the spring season, 10% of the hybrids are found to begin oocyte formation. In December, oocytes are very well seen in 30% of the studied females.

References

Bloesch, J. J., Jones, T., Reinartz, R., & Striebel, **B.** (2006). An action plan for the conservation of sturgeons (Acipenseridae) in the Danube River Basin. **Österreichische** *Wasser- und Abfallwirtschaft*, *58*(5-6), 81-88.

Boyadzhiev, N. (2002). State of sturgeon stocks and perspective of their futurity exploitation. *The Journal of Management and Sustainable Development*, *3-4***,** 31-35 (Bg).

Burtsev, I. A. (2013). Biological bases of full-cyclic cultivation of sturgeon and creation of new breeds by methods of hybridization and selection. Doctoral dissertation abstract, Moscow, pp. 47 (Ru).

Chebanov, M. S., & Galich, **E. V.** (2010). Ultrasound diagnostics of sturgeons. FSGTSR, *Izdatel`stvo Prosveshenie-Yug*, Krasnodar pp.135 (Ru).

Chebanov, M. S., & Galich, E. V. (2013). Sturgeon Hatchery Manual. FAO. Ankara. pp. 303.

Du, H., Zhang, X., Leng, X., Zhang, S., Luo, J., Liu, Z., Qiao, X., Kynard, B., & Wei, Q. (2017). Gender and gonadal maturity stage identification of captive Chinese sturgeon, *Acipenser sinensis*, using ultrasound imagery and sex steroids. *General and Comparative Endocrinology*, *245***,** 36-43. doi: 10.1016/j.ygcen.2016.08.004.

Filipova, O. P., & Zuevskiy, **S. E.** (2009). The prospects of cultivation of a hybrid of the Russian sturgeon with the Siberian sturgeon in Russia, Proceeding of conference, 23.12.2008-15.02. 2009, M.: MGTU Publishing, pp. 56-66 (Ru).

Kecse-Nagy, K. (2011). Trade in Sturgeon Caviar in Bulgaria and Romania overview of reported trade in caviar, 1998-2008. A TRAFFIC report for WWF Austria. Budapest. Hungary, pp. 17.

Konchits, V. V., & Savonchik,A. L. (2010). Lenski sturgeon (*Acipenser baeri* Brandt) – a perspective object of fish farming in Belarus. *Current problems of intensive development of livestock production*, pp. 366-371 (Ru).

Makeyeva, A. P., Pavlov, D. S., & Pavlov, **D. A.** (2011). Atlas of larvae and juveniles of freshwater fishes of Russia, *Moscow*: *KMK Scientific Press*, pp. 383 (Ru).

Masoudifard, M., Vajhi, A. R., Moghim, M., Nazari, R. M., Naghavi, A. R., & Sohrabnejad, M. (2011). High validity sex determination of three years old cultured beluga sturgeon (*Huso huso*) using ultrasonography. *Journal of Applied Ichthyology*, *27*(2), 643-647. doi.org/10.1111/j.1439-0426.2011.01726.x

MZH, 2016. Annual report on the state and development of agriculture, pp. 240 (Bg).

Nikolyskiy, G. V., 1971. Special ichthyology, *Visshaya shkola*, Moscow, pp. 472 (Ru).

Ponomarev, S. V., & Ivanov, D. I. (2009). Sturgens farming on an intensive basis, *Kolos*. Moscow, 312 pp (Ru).

Ruban, G. I. (1999). The Siberian Sturgeon *Acipenser baeri* Brandt (Species Structure and Ecology), *GEOS Press*, pp. 230 (Ru).

Sevryukov, V. N., Semjanihin,V. V., Ustinov, A. S., & Podushka, **S. B.** (2001). Maturing of female Russian sturgeon in the conditions of industrial fishfarming, *Scientific – Technical Bulletin of Laboratory of Ichthyology INENKO*, *5,* 15-20 (Ru).

Vasileva, L. M., Yakovleva, A. P., Scherbatova, T. G., Petrushina, T. N., Tyapugin, V. V., Kitanov, A. A., Arkhangelskiy, V.V., Sudakova, N. V., Astafieva, S. S., & Fedoseyeva, **E.A.** (2006) Technologies and biological norms for sturgeon farming in the VIth climate fish farming area, N.V. Sudakova (Ed.), *VNIRO Publishing*, pp. 100 (Ru).