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THE DEVELOPMENT OF METHODS OF HORMONAL STIMULATION OF STURGEONS BY N.L. GERBYLSKY AND HIS FOLLOWERS

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Abstract. The main discovery of the 20th century in the field of sturgeon breeding is "method of pituitary injections". In earlier experiments, fish breeders needed to catch spawning sturgeons in nature because final stages of maturation in these fishes were blocked at hatcheries. The Leningrad ichthyologist N.L. Gerbilsky. Proposed methods of mass extraction of sturgeon pituitaries, their dehydration by acetone for later storage and use, testing of pituitary gonadotropic activity on different objects. The effective doses of dried pituitaries for sturgeons were determined in milligrams and loach units or frog units. This method of hormonal stimulation of final stages of maturation was used for functioning of sturgeon breeding facilities at Volga, Don, Kuban and Kura and other rivers. For guaranteed answer fish, breeders used hyperdoses of dry pituitary powder because its gonadotropic activity varied in dependence on stage of maturity of fish-"donors". The followers of N.L. Gerbilsky led by Prof. I.A. Barannikova developed the technique of production of special glycerol hypophyseal extract (GHE) with determined gonadotropic activity. The main role in formulation of the recipe of GHE belongs to A.A. Boyev. The use of GHE has removed the problem of hyperdosing leading to worsening quality of mature germinal cells of sturgeons. Later A.A. Boyev on the basis of this recipe with replacement of glycerol to propylene glycol created stabilizing solution of analogue of mammal LH-RH ("surphagon") of long-term storage with concentration of active substance from 20 to 500 micrograms/ml.

Keywords: *Sturgeons, hormonal stimulation, hatcheries, glycerol hypophyseal extract.*

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1. Introduction

First experiments on artificial propagation of sturgeons were carried out in 1869 in Russia. Ichthyologist F.V. Ovsyannikov fertilized the sterlet (*Acipenser ruthenus* L.) eggs at Volga River and received viable fingerlings of hybrids *A.ruthenus x A.stellatus*. After this pioneer experiment, further works with sturgeon breeding in Russia had irregular character before creation of special Ichthyological Laboratory in Baku in 1912. The head of this Laboratory A.N. Derzhavin was considered as Father of Russian and Azerbaijan sturgeon breeding (Trenkler & Mamedov, 2019).

In 1875, Seth Green succeeded in reproducing *A. oxyrinchus* on the Hudson River in USA. Later such works were conducted with lake sturgeon *A. fulvescens*. From 1920 before end of 1970s, any attempts to breed sturgeons in USA were absent (Conte *et al*., 1988).

The first German experiments with artificial fertilization and incubation of eggs of

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European sturgeon *A. sturio* were carried out on the Elbe in 1870-s and in 1877 hatchery sturgeon breeding started in Germany. The releases of larvae exceeded 1 million individuals between 1886 and 1896 but feeding and growing of sturgeon larvae and fry was carried out at experimental scale only in 1891 on the Oste River (Gessner *et al*., 2011).

Main reason of stopping of sturgeon breeding in USA and Germany was collapse of sturgeon populations caused by overfishing. Due to a lack of methods of hormonal stimulation, it was impossible to induce ovulation in females because final stages of maturation in these fishes were blocked at hatcheries. The only solution was to catch ovulating females on presumed spawning sites. To catch such females in beginning of 20th century in USA or Western Europe was almost or completely impossible.

In Russia, great sturgeon stocks allowed to carry out commercial fishing and artificial propagation with using of ovulating females caught at spawning sites. In 1920-s A.N. Derzhavin (Derzhavin, 1947) proposed to hold sturgeon females in pre-maturing state in round tanks with flowing water. This "ecological" method of inducing of ovulation increased the efficacy of sturgeon breeding and the number of larvae released from Soviet "sturgeon-breeding points" was measured in millions individuals.

The "method of pituitary injections"

The first works on using of pituitary tissue for ovulation induction in teleost fish were carried out in Brazil (Von Ihering, 1935; Ihering & Azevedo, 1936; Pereira & Cardoso, 1934) but creation of methodic of practical application of acetonic pituitaries for sturgeon breeding was associated with the name of Leningrad ichthyologist Nikolai Lvovich Gerbilsky.

N.L. Gerbilsky began his work with pituitary preparations on different teleost including the Neva smelt *Osmerus eperlanus* (Gerbilsky & Kaschenko, 1937), but in 1938, he conducted successful experiments with stellate sturgeon *A. stellatus* (Gerbilski, 1938a; 1938b) and in the same year founded the Laboratory of Basics of Fish Breeding for development of new "method of pituitary injections".

One of the most important technical tasks of new Laboratory was to find a methodic of mass collection of sturgeon pituitary glands because cartilaginous skull of sturgeon fish was protected by very hard integumentary bones. Initially, the operation was performed via cutting off upper bones of skull with a sharp knife. Later fish breeders began using special instrument (trepan) for extraction cartilaginous plug containing pituitary gland (Figure 1).

The water suspension of fresh pituitary tissue was injected directly to sturgeon skull using Lumbal or Beer needles. Later intracranial injections were replaced by intramuscular ones. The injected fish were held in the river being tied by a rope through mouth and gills.

To facilitate the process of extracting the pituitary glands, special trepans were constructed. The art of fish breeder was to find the site and direction of drilling sturgeon skull. It was especially difficult to get the pituitary gland from giant sturgeon *Huso huso*. Later, trepans were supplied with whirlpools, which made possible to reduce the time of drilling out the cartilaginous plug.

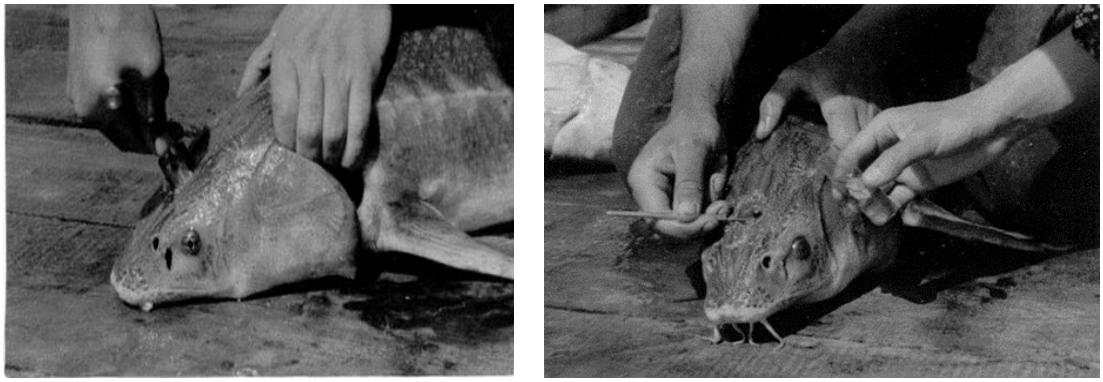

Figure 1. Pituitary extraction in Russian sturgeon *Acipenser gueldenstaedti*: a – skull trepanation by simple instrument (trepan) without handle, b – removing the cartilaginous plug. The photograph from archive of Central Laboratory on reproduction of fish stocks (former Laboratory of Basics of fish breening)

The second task of N.L. Gerbilsky was the search for effective methods of preserving of pituitary glands. Sturgeon breeders experienced with fresh, frozen and dehydrated pituitaries. The best results were obtained with acetone-dried pituitaries, because acetone could be easily evaporated. The acetonated pituitary glands were well stored in sealed jars even in the absence of refrigerators and could be introduced intramuscularly in form of water suspension. The injection was carried out in simplest wooden cages, lifting the sturgeon on a stretcher (Figure 2).

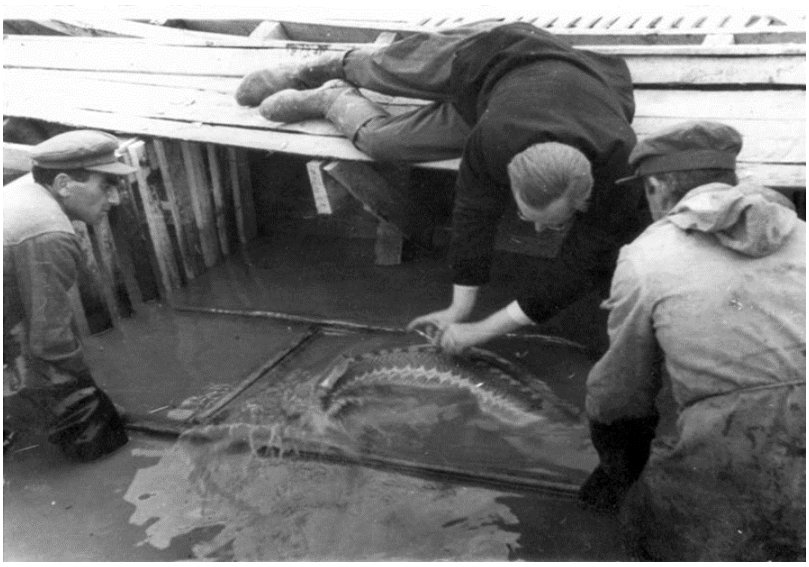

Figure 2. The injection of Russian sturgeon by ichthyologist N.L.Gerbilsky. The photograph from archive of Central Laboratory on reproduction of fish stocks

In pre-war experiments, 26 female sturgeons out of 36 injected ones ovulated and mature eggs at Bogdanovsky fish breeding station on Kura River; it was a great achievement for 1941 year. After the end of Second World War, experiments on hormonal stimulation of sturgeons were continued (Gerbilsky, 1941).

Determination of gonadotropic activity of pituitary tissue

The important task of N.L. Gerbilsky's team was to test the pituitary glands, since already in first experiments high fluctuations in the gonadotropic activity of fresh and acetonated glands were detected (Gerbilsly, 1940; 1947; 1949). So it was necessary to calculate the dosage not in milligrams of dry pituitary tissue but in units of activity. B.N. Kazansky (Kazansky, 1949; Kazansky & Nusenbaum, 1947) proposed using of "loach units". One loach unit corresponded to pituitary activity that could induce ovulation in a medium-sized female loach *Misgurnus fossilis* in a pre-spawning state (caught in early spring) under artificial conditions. Testing showed the specificity of pituitary glands at the level of orders and families of fish and a clear dependence of gonadotropic activity on the state of maturity of fish-"donors". Another method of testing gonadotropic activity of pituitary glands was the Galli-Mainini spermiation reaction in male frogs (*Rana ridibunda* or *R. temporaria*), which allowed to get a result much faster than testing on loaches (Alpatov & Stroganov, 1950).

However, the testing pituitary activity was not actual procedure in the 1950s because at initial stage of sturgeon breeding the needs for pituitary preparation were relatively small. So fish breeders had no problems with extraction of pituitary glands only from pre-spawning sturgeons controlling the state of the gonads of "donor" fish. For the same reason, the first experiments with the preparation of glycerole extract of sturgeon pituitaries by I.A. Barannikova under guidance of N.L. Gerbilsky (Kazansky, 1949) were stopped.

In the 1950-60s, many new sturgeon hatcheries were built on the Kura, Volga, Don and Kuban rivers, where fish breeders used the method of hormonal stimulation. Because the number of injected sturgeons was permanently increasing the Special Service was created for extraction of pituitary glands in places of industrial fish processing where workers drilled out cartilaginous plugs from sturgeon skulls after extraction of roe and viscera (without control of gonad state).

The lack of control over state of gonads did not matter on Kura or Kuban River with spawning migration of spring sturgeons in pre-spawning state (IV stage of maturity), but led to provision of pituitaries from winter fish with III stage maturity in Volga delta where such fish appeared in catches from the end of April and their proportion reached almost 100% at the end of May.

Figure 3. Professor I.A.Barannikova in her office in 1970s. The photograph by I.V. Trenkler

By beginning of the 1970s, a vicious fish-breeding practice was established at sturgeon hatcheries: in absence of confidence in activity of pituitary glands, fish breeders used hyperdoses of pituitary powder, which led to deterioration in the quality of ovulated eggs and hatched larvae. This problem had to be solved by Central Laboratory for Reproduction of Fish Stocks of Glavrybvod (formerly the Laboratory of Basics of Fish Breeding), which was headed by I.A. Barannikova (Figure 3) after death of Professor N.L. Gerbilsky in 1967.

The glycerole hypophyseal extract

In 1970-s prof., I.A. Barannikova as chief of Central Laboratory of Reproduction of Fish Stocks and A.A. Boyev (deputy chief) continued works with glycerole extract of sturgeon pituitaries. The first successful results were carried out only in the late 1970s, the article and Manual on its application were published in 1980s (Barannikova *et al*., 1984; Boyev, 1984, 1989a, 1989b).

The new glycerole hypophyseal extract (GHE) had standard gonadotropic activity (100-110 frog units/ml) and greatly simplified the task of fish breeders because they had not to weight and homogenize dry pituitary glands in porcelain mortars before each injection. The fish breeder only had to measure the required amount of GHE (for females of Russian sturgeon - 0.9-1.0 ml) and inject the fish.

Using GHE at Volga sturgeon hatcheries solved the problem of hyper-dosages and significantly increased the proportion of females with high-quality developing eggs (Boyev, 1989a; 1989b). In the beginning of 21st century, the need for testing the pituitary glands increased even more, because commercial sturgeon fishery was prohibited and the only source of hypophyseal preparations was slaughtered fish after obtaining mature eggs or milk at sturgeon hatcheries (Trenkler & Gruslova, 2004).

For manufacturing of GHE, a "***working solution***" consisting of equal volumes of a "reserve solution" and "chemically pure" glycerole is required. The reserve solution contains 8.4 g (0.2 M) of sodium fluoride and 11.7 g (0.2 M) of sodium chloride per 1 liter of distilled water. The initial volume of the working solution should be 30-40% higher than calculated volume of the GHE. Acetonated pituitary glands are carefully homogenized with addition of working solution. To obtain the first batch of a thin suspension, it is necessary to use 40% of the volume of calculated GHE for all pituitaries. To facilitate the homogenization of pituitary glands, it is possible to add 1-2 g of well-washed and calcined river sand to the mortar (Boyev, 1989b).

The first batch of suspension is collected in a clean, hermetically sealed glass, mixed thoroughly, closed with a lid and left in a dark place at room temperature. After 18-24 hours, the infusion suspension is mixed, poured into plastic tubes and centrifuge at 6000 rpm for 45-50 minutes. The supernatant is collected in a measuring cylinder (the volume is measured), poured into a clean bottle, hermetically sealed and stored in a refrigerator at temperature 4°C. The first portion of supernatant has the highest gonadotropic activity. Its volume should be 30-40% of estimated volume of GHE (Boyev, 1989b).

The dense precipitate is again homogenized in a mortar with a working solution, the volume of which should be 30-35% of calculated volume of GHE. The suspension is again infused for 18-24 hours and centrifuged in the same mode. Then the procedures of homogenization of precipitate, its infusion and centrifugation are repeated a third time. The final volume of the GHE should be equal to calculated one. If necessary, supplementary amount of pure working solution is added. Storing is carried out in

refrigerator at a temperature of +1 - + 4° C or in a freezer (the solution does not freeze at 18-20°C). The last precipitate has to be destroyed because gonadotropic hormone is fully extracted (Boyev, 1989b).

The best model animal for testing of gonadotropic activity was a female loach with gonads in the IV stage of maturity: such a test object gave constant answer to hormonal stimulation from December to April (Barannikova *et al*., 1975). From last third of 20th century the possibilities of catching required number of female loach in nature were very limited, so testing of the gonadotropic activity of GHE it was carried out by the Galli-Mainini method in male frogs (Barannikova *et al*., 1975; Travkin & Boyev, 1969). Frogs had to be adapted to spawning temperature 16-18 ° C during 7-10 days in plastic baths or aquaria with a small layer of water - about 2 cm and daily change of the water.

One frog unit (F.U.) is a dose of GHE that can induce a spermiation reaction in a large male frog *Rana ridibunda* with an average weight of 80-90 g with a probability of 80%, i.e. In 4 out of 5 animals. In this case, one F.U. is equal to original "loach unit".

In case of using smaller frog *Rana temporaria* with average weight of 35 g A.A. Boyev used a correction factor of 3 (1 F.U. caused a sperm reaction in three males of this species). The testing hormonal preparation was injected into bladder area using syringes with a needle 4 cm. The spermiation took place after 1-2 hours after injection and could be registered on presence of spermatozoa in seminal fluid under a microscope with an objective magnification of 8-20x. During the testing period, the frogs had to be kept in dry aquaria without water. After testing the animals could be released or returned to, plastic baths with a layer of water about 2 cm. It is possible to use the same animals for new tests with an interval of 4-5 days, but not more than 3 times. Knowing the amount of F.U. in 1 ml of GHE, it is easy to calculate the required volume for administration to female or male sturgeon (Boyev, 1989b).

The Galli-Mainini reaction depended on the time of testing. From December to March, the minimal dose of gonadotropin required for induction significantly reduced. The dependence on first, second or third administration of hormonal preparation took place as well. Hence, we could find the different values of gonadotropic activity of dry pituitary tissue in many works. For example, in a study conducted from February 26 to March 5 (Travkin & Boyev, 1969), a 100% spermiation reaction in male frog *R. temporaria* was caused by a dose of 0.3 mg and a 75% reaction (in 3 out of 4 individuals) by a dose of 0.2 mg of dry pituitary tissue. In our tests conducted in the second half of March, the minimum effective dose was only 0.1 mg of acetonated pituitary gland but in case of reduction of period of adaptation to spawning temperature, or in case of repeated using of animals as test-objects, increased several times (Trenkler & Gruslova, 2004). So male frogs, unlike female loaches, could be used only for a relative, but not for absolute valuation of gonadotropic activity. As a standard, it was advisable to use either the previously tested GHE or acetonated pituitary glands of female sturgeon fish in the pre-spawning state (the activity of which is taken conditionally as 3.3 F.U. /mg). The pituitaries extracted from female sturgeons after ovulation induced by administration of GHE had a slightly reduced gonadotropic activity (2.5-3 F.U. /mg). The gonadotropic activity of pituitary glands of sturgeons matured after treatment by analogue of LH-RH was equal only 1-1.5 F.U. /mg (Trenkler & Gruslova, 2004).

The stabilized solution of analogue of mammal LH-RH

The first experiments with using of different analogues of LH-RH for hormonal stimulation of sturgeons were conducted in USSR in 1980-s (Barannikova *et al*., 1982; Barannikova *et al*., 1984; Goncharov, 1984).

Initially, the ichthyologists of Central Laboratory used dry preparations of LH-RH analogues, but effective dose were extremely small to be strictly measured but must be diluted just before using. Optimal preparation for sturgeons became analogue of mammal LH-RH - desGly^{10}-[D-Ala^{6}]-Pro^{9}-NH_2-Et-LH-RH which doses for females of Russian sturgeon were only 30 μg (1-1.5 μg/kg).

"Stabilized solution" of mammal LH-RH ("surphagon") of long-term storage was proposed by A.A. Boyev on the base of recipe of GHE but glycerol was replaced other organic solvent propylene glycol of high quality, widely used in perfumery. Other components of the stabilized solution were sodium chloride and sodium fluoride (to suppress the development of microorganisms) in the same proportions as in GHE. The concentration of active substance in stabilizing solution of "surphagon" varied from 20 to 500 μg/ml. High-concentrated solutions could be used not only for sturgeons (after dilution) but also for different teleost fish, for example for European pikeperch *Sander lucioperca*.

The "surphagon" administration, as distinct from the pituitary preparations, does not damage oocytes even when highly exceeding optimal dosages. It is very significant for beluga, *Huso huso*, where the exact weight of large fish (more than 100 kg) is difficult to fix, that there are no negative reproductive consequences due to a LH-RHa overdoses (Chebanov & Savelyeva, 1996).

2. Conclusion

A complete transition from GHE to "surfagon" in sturgeon breeding and farming occurred only in the first decade of 21st century, after a complete abandon of sturgeon slaughtering at fish hatcheries. Now the only source of sturgeon pituitaries is fish in commercial aquaculture slaughtered for getting black caviar of high quality. In several cases, GHE could not be replaced by surphagon. It is so called "combined method" of hormonal stimulation (first injection – small dose of GHE, second injection – common dose of analogue of LH-RH), the other variant of using GHE - repeated injection of male sturgeons for prolonging of spermiation (Trenkler & Gruslova, 2006).

Now analogues of GnRH including mammal LH-RH ("surphagon") are widespread not only in Russia, but throughout the world for artificial propagation of sturgeons (Chebanov & Galich, 2011). Thus, the method of hormonal induction of ovulation in sturgeons proposed by prof. N.L. Gerbilsky continues to be used at modern sturgeon hatcheries.

References

Alpatov, V.V., Stroganov, N.S. (1950). The new unit for measurement of pituitary activity in fish. *DAN SSSR, 74*(2), 405-407 (In Russian).

Barannikova, I.A., Boyev, A.A., Efimova, N.A. & Presnov, D.I. (1984). Comparative analysis of hatchery production received after sturgeon maturation stimulation by synthetic LH-RH analogue and glycerol pituitary extract. In: *Sturgeon Culture in Inland waters of USSR*. Astrakhan, 34-36 (In Russian).

Barannikova, I.A., Boyev, A.A., Moiseeva, E.B. & Travkin, B.G. (1975). The Methods of determination of gonadotropic activity of fish pituitaries in connection with standardization of preparation for pituitary injections. *Trudy VNIRO, 75*, 125-134 (In Russian).

Barannikova, I.A., Bukovskaya, O.S. & Ilyicheva, M.I. (1982). The Influence of LHRH analogue on gonadotropin cells and gonadotropin content in serum of stellate sturgeon (Acipenser stellatus Pallas). *Ivanovo,* 14 (In Russian).

Boyev, A.A. (1984). Comparison of efficiency of different regimes of treatment by tested pituitary preparations. In: *Osetrovoye Khosayaistvo Vodoyemov SSSR*. Astrakhan, 43-44 (In Russian).

Boyev, A.A. (1989a). New regime of treatment of female stellate sturgeons by glycerol pituitary extract in Volga River delta. In: *Osetrovoye Khosayaistvo Vodoyemov SSSR*. Astrakhan, 27-28 (In Russian).

Boyev, A.A. (1989b). Manual on production, testing and using in fish breeding of glycerol pituitary extract. Moscow, Glavrybvod, 7 (In Russian).

Chebanov, M.S., Galich, E.V. (2011). Sturgeon hatchery manual. *FAO Fisheries and Aquaculture Technical Paper, 558,* 303.

Chebanov, M.S., Savelyeva, E.A. (1996). Biotechnology of sturgeon reproduction on the basic of polycyclic use of hatchery capacities under current ecological conditions. Krasnodar, KrasNIIRKH, 21 (In Russian).

Conte, F.S., Doroshov, S.I., Lutes, P.B. & Strange E.M. (1988). Hatchery Manual for the white sturgeon with application to other North American Acipenseridae. *Agriculture and Natural Resources, 3322*. Oakland Publication.

Derzhavin, A.N. (1947). Reproduction of sturgeon stocks. Azerbaijan Academy of Science, Zool. Inst., Baku, 247.

Gerbilski, N.L. (1938a). Influence of gonadotropic factor of pituitary on spawning state in Acipenser stellatus. *DAN SSSR, 19*(4), 333-336 (In Russian).

Gerbilski, N.L. (1947). Contemporary state and perspectives of method of pituitary injections. *Proceedings of the laboratory of fish farming fundamentals, 1*, 5-24 (In Russian).

Gerbilsky, N.L. (1938b). The effect of skull injections of pituitary suspension in Teleostei. *Bull. Exp. Biol. Med, 4-5*, 863-867 (In Russian).

Gerbilsky, N.L. (1941). The Method of pituitary injections and its role in fish breeding. In: *Hyposar injection method and its role in fish farming*. Leningrad, LGU, 5-36 (In Russian).

Gerbilsky, N.L. (1949). Experimental and methodical basis of development of sturgeon breeding in Lower Kura River. *Trudy laboratorii osnov rybovodstva, 2*, Leningrad, 5-28 (In Russian).

Gerbilsky, N.L., Kaschenko, L.A. (1937). The influence of pituitary on gonads in Teleostei. *Bull. Exp. Biol. Med., 3*(2), 173-175 (In Russian).

Gerbilsly, N.L. (1940). Seasonal changes of gonadotropic activity in fish. *DAN SSSR, 28*(6), 571-573 (In Russian).

Gessner, J., Spratte, S. & Kirschbaum, F. (2011). Historic overview on the status of the European Sturgeon (Acipenser sturio) and its fishery in the North Sea and its tributaries with a focus on German waters. *Biology and Conservation of the European Sturgeon Acipenser sturio L. 1758: The Reunion of the European and Atlantic Sturgeons*, 195-219.

Goncharov, B.F. (1984). Synthetic analogue of LHRH is new perspective stimulator of maturation in Acipenseridae. *DAN SSSR, 276*(4), 1002-1006 (In Russian).

Ihering, R.V., Azevedo, P.D. (1936). A desova ea hipofisação dos peixes. Evolução de dois nematognathas. *Arq. Inst. Biol, 7*(9), 107-117.

Kazansky, B.N. (1949). Loach Unit for measurement of gonadotropic activity of fish pituitary preparations. *Proceedings of the laboratory of fish farming fundamentals*, 2, 29-36 (In Russian).

Kazansky, B.N., Nusenbaum, L.M. (1947). Loach (Misgurnus fossilis) as object for measurement of gonadotropic activity of fish pituitary preparations. *Proceedings of the laboratory of fish farming fundamentals, 1*, 111-120 (In Russian).

Pereira, J., Cardoso, D.M. (1934). Hypophyse et ovulation chez les poissons. *Compt. Rend. Soc. Biol*, *116*, 1133-1134.

Travkin, B.G., Boyev, A.A. (1969). The practice of valuation of gonadotropic activity of fish pituitaries using test-objects. In: *Current State of the Pituitary Injection Method.* Astrakhan, TSNIORKH, 71-78 (In Russian).

Trenkler, I.V., Gruslova, A.B. (2004). Biotesting of pituitaries of female Russian sturgeons after ovulation induced by different hormonal preparations. In*: Sturgeon Aquaculture: Achievements and Prospects*. Astrakhan, 212-213 (In Russian).

Trenkler, I.V., Gruslova, A.B. (2006). Possibilities of reseiving of high-quality sperm from male Russian and Beluga sturgeons of spring and winter forms. In: *Sturgeon Aquaculture: Achievements and Prospects*. Astrakhan, 127-130 (In Russian).

Trenkler, I.V., Mamedov, Ch. (2019). The significance of scientific works of A.N.Derzhavin and his followers in development of contemporary sturgeon breeding. *Fish Farming and Fish farming, 11*, 66-79 (In Russian).

Von Ihering, R. (1935). Die wirkung von hypophyseninjektion auf den laichakt von fischen. *Zool. Anz.*, *11*, 273-279.