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REPRODUCTION OF RUSSIAN STURGEON (*Acipenser gueldenstaedtii*) VIABLE JUVENILES USING CRYOPRESERVED SPERM AND BEHAVIORAL REACTIONS OF THE CRYO-PROGENY

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Abstract

Cryopreservation of male reproductive cells is an important issue of genetic biodiversity conservation strategy and the development of fisheries and aquaculture. The use of cryopreserved semen in artificial reproduction and aquaculture will provide genetically diverse progeny, reduce area and the cost of maintaining male fishes, and, thereby, will allow rise in female herd abundance. Cryopreserved sperm can be used at any time, without risk of untimely maturing or improper quality. The data on the low-temperature preservation indicates that the long-term storage in liquid nitrogen does not significantly affect the safety of cells after freezing and thawing. The viability of cryopreserved sperm is usually confirmed by laboratory fertilization of caviar, without further observations of the obtained juveniles. This paper reports for the first time the behavioral response of pre-larvae, larvae, and juveniles of Russian sturgeon (*Acipenser gueldenstaedtii* von Brandt & Ratzeburg 1833), obtained with the use of frozen-thawed sperm, and shows physiological fullness of the resulting progeny. The aim of this work was to compare quality of Russian sturgeon offspring as influenced by cryopreserved and native semen. In using frozen-thawed sperm stored 2 years in liquid nitrogen, the conception was 50 % compared to 80 % in the control insemination with native semen. The mortality rate for the whole period was 7 and 5 % in the test and the control groups, respectively. The obtained cryo-progeny was viable. One-day old pre-larvae, 8-day old larvae and 15-day old juveniles of the test group were superior in size and weight as compared to the control group. Behavioral responses of the offspring were evaluated in «open field» test which was carried out individually by placing an individual (pre-larvae, larvae, juveniles) in a special installation with coordinate grid. The pre-larvae activity was somewhat, but not significantly, higher in cryo-progeny. No differences were found between 8-day old larvae in the response to irritants, except bright light ($p \leq 0.05$). In 15-day old juveniles, the response was adequate in both groups. Basal activity and reactivity differed significantly ($p \leq 0.05$) in both groups. So, cryopreserved sperm led to some morphometric advantage in the progeny compared to the individuals produced by conventional methods, and some advantages were found in the response to irritants, however, on the whole, the differences were not significant. A small alteration may be due to the difference in frozen cell subpopulations. Thus, frozen semen contributes to young fish vitality and may be recommended for artificial reproduction and aquaculture.

Keywords: sturgeons, Russian sturgeon, *Acipenser gueldenstaedtii*, sperm, cryopreservation, cryo-progeny, pre-larvae, larvae, juveniles, behavior

Today, preservation of a genetic pool of rare and endangered populations and species of fish, specifically those that are of practical interest for increase of catch in natural water bodies or for their introduction to the aquaculture as promising fish farming objects is particularly relevant [1-3[. One of the main sources of generation and maintenance of stock of rare and endangered and commercially

valuable fish species is their artificial reproduction [4, 5]. However, the approach of fisheries to generation of broodstocks is simplified due to deficit of producers. The usage of closely related pairs for breeding is fraught with inbreeding and loss of natural genetic polymorphism resulting in significant reduction of adaptive potential of the population [6-8].

The cryogenic technologies are deemed strategically important and anti-crisis for preservation of biological diversity of fish species [9, 10]. The advancement in cryopreservation technologies will expand the fields of application of cryogenic technologies in fishery and aquaculture, will enable maintaining genetic diversity of production broodstocks, will stabilize their reproduction and thus will stimulate stable fishing, and will lay the groundwork for growth of production of fish and other aquatic organisms at aquaculture farms [11-13]. The usage of cryopreserved semen will provide genetically diverse young fish, reduce the costs and surface areas of male fish maintenance. Consequently, the quantities of females in the broodstock will be increased [14-16]. The usage of cryopreserved semen is possible at any season, which eliminates the risk of untimely maturing of males or the risk of obtaining low quality ejaculate from them [17, 18].

The accumulated biological object cryopreservation data point to the fact that protracted storage of genetic biomaterial in a cryobank does not have a significant impact on cell preservation [19-21]. Furthermore, the viability of cryopreserved semen is usually confirmed by fish ovum fertilization in laboratory conditions [22-24], but no further observation of young fish is conducted.

In this work we have analyzed for the first time the behavioral response of sac fry, fish larvae and young Russian sturgeon specimens (*Acipenser gueldenstaedtii* von Brandt & Ratzeburg 1833) obtained when using cryopreserved semen, and have proven the physiological adequacy of developing specimen.

The goal of the work was to apply unfrozen semen in artificial reproduction of Russian sturgeon and to evaluate behavior of cryo-progeny.

Techniques. The work was performed during spawning campaign at the Alexandrovskiy sturgeon fishery (Astrakhan region). The roe was obtained from female Russian sturgeons (*Acipenser gueldenstaedtii*) weighing 20 kilos and 142 centimeters long, working fertility 229500 fish eggs. One part of the roe was fertilized by defrosted semen of Russian sturgeon stored in a cryobank for 2 years, the other was fertilized with native semen using standard fish farm technology (control). The removal of mucilage from fertilized roe was performed with tannin, 1 g per 5 liters of water [25]. The roe was incubated in an Osetr device (Russia) for 6 days. During incubation it was processed with organic coloring material (violet K) to avoid saprolegniosis [25]. The hatched sac fries were registered using item-by-item method. Sac fries were maintained in rectangular 250 l basins. The water in the basins was enriched with oxygen by applying a compressor. The young fish that was moved to active nutrition were fed with daphnia.

The morphometric parameters (weight and length) of sac fries (day 1 after hatching), fish larvae (day 8) that were moved to active feed and young fish (day 15) were recorded.

The behavior of fish reared by traditional technologies and of cryo-progeny were evaluated using the "open field" test [26, 27]. The test was conducted individually by placing the analyzed specimen (sac fry, fish larvae, and young fish) in a special device with an applied grid mesh. When an object is placed in the device for the "open sky" test, first, its approximate activity was defined for 3 min (approximate activity, units/min) registering the number of crossings of device coordinate lines by the specimen. The motor activity from minutes 4 to 7 was accepted as baseline activity (baseline activity, units/min). Light was the first irritant (illumination intensity 20 lux), which were turned on

during minute 7 of testing. The illumination intensity was measured with a light meter. During the first 30 s after the exposure the motor activity (the number of crossings of the coordinate grid) was determined as P1, units/min. Nine minutes after the start of the test the second irritant, a low frequency rectangular signal (20 hz), was used (P2, units/min). Bright light was turned on during the minute 11 (100 lux) (P3, units/min), and 13 minutes after the start of testing a high frequency rectangular signal was applied (300 hz) (P4, units/min). A vibroacoustic irritant (P5, units/min) was used at minute 15. Ten individuals were used from the test and control groups during three development phases, sac fry (day 1 after hatching), fish larvae transitioned to active feeding (day 8 after hatching) and young fish (day 15 after hatching).

Mean (*M*) absolute errors and mean square deviations (σ) were calculated in Microsoft Excel for the parameters. The statistical significance of differences was determined by Student *t*-criterion [28].

Results. The percentage of mobile cells in the native semen was 90%, in cryopreserved semen was 60%; the time of sperm cell mobility after semen defrosting was 14.2 min vs. 26.4 min in the native semen; the percentage of fertilization in the test group was 50% vs. 80% in the control group. During the rearing period the mortality rate in the test and control groups did not vary significantly and constituted 7 and 5%, respectively.

The dynamics of morphometric parameters of Russian sturgeon progeny (*Acipenser gueldenstaedtii* von Brandt & Ratzeburg 1833) upon fertilization with cryopreserved (test group) **and native** (control group) **semen** ($M \pm \sigma$)

Development stage	Масса, мг		Длина, мм	
	control	test	control	test
Sac fry (day 1)	18.9±0.28	20.5±0.22*	12.3±0.21	14.2±0.2*
Fish larvae transitioned to active feeding (day 8)	28.2±0.55	35.0±0.30*	20.7±0.21	21.4±0.34
Young fish (day 15)	43.8±0.33	47.5±0.33*	24.0±0.15	25.2±0.13*

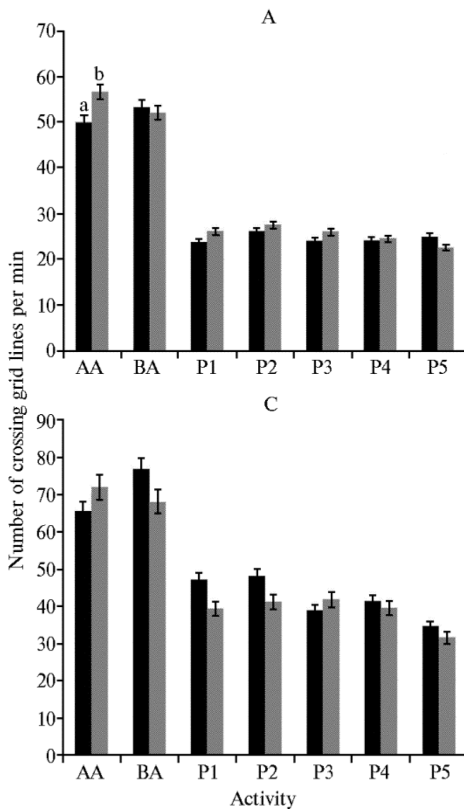
N o t e. The differences with the control group are statistically significant at $p \leq 0.001$.

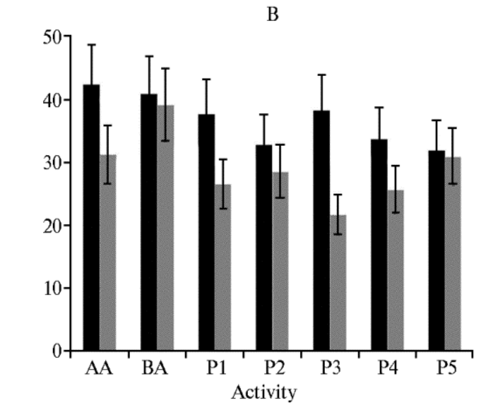
According to sac fry (day 1) measurement results, the individuals from the test group surpassed the control group (Table) in morphometric parameters. The statistically significant differences were determined ($p \leq 0.001$) both for weight and length of analyzed fish. Comparing fish larvae that transitioned to active feeding (day 8) identified significant differences ($p \leq 0.001$) between test group weight and control group weight; however, length of larvae did not differ. The young fish (day 15) in the test group retained a tendency of surpassing the control group specimens in weight and length.

Any organism finds itself in a new environment begins to display increased motor activity and tries to get oriented. According to the literature, after sac fry hatch they develop a receptor complex; however, without relevant information from the environment, the medulla cannot develop properly [29]. The key system analyzers are concentrated in the medulla, which are responsible for the interconnection of the fry with the environment (lateral line systems, auditory organs, taste receptors), and centers of neuromotor response, feeding and breathing [30]. In this connection we evaluated the quality of fish obtained using cryopreserved semen and native semen by comparing their behavior.

In the "open field" test the sac fries from the control group displayed low orientation activity and concealment behavior changing into slow movements in the test device (see Fig.). The sac fries from the test group were more active and displayed classical response when adapting to unfamiliar environment, which is characteristic of specimen of different evolution groups. No significant difference was observed between orientation and baseline activity either in the control group or in the test group. Response to various irritants by specimens in the

test and control groups did not differ statistically either.





Activity of sac fries in "open field" test (day 1 after hatching, A)**, fish larvae** (day 8 after hatching, B) **and young fish** (day 15 after hatching, C) **of Russian sturgeon (*Acipenser gueldenstaedtii* von Brandt & Rat-zeburg 1833) obtained from roe fertilized by native** (a) **and cryopreserved** (b) **semen:** AA — approximate activity, BA — baseline activity, P1-P5 — motor activity during the first 30 days after impact of various irritators. The description of irritators is provided in section Techniques.

After transition to mixed feed (day 8), the larvae obtained with cryopreserved semen did not assuredly differ from those in the control group in terms of central nervous system response with the exception of response to the third irritant, the bright light (100 lux) ($p \le 0.05$). The specimens in the test group demonstrated concealment behavior, which was indicative of a faster formation of associative bonds in midbrain colliculus as compared to the larvae from the control group, which did not respond to this irritant and maintained activity at baseline level. On day 15 of development the specimens in both test groups responded properly to the irritants by displaying the classical concealment behavior. Significant differences ($p \le 0.05$) both in the control group and in the test group were observed during analysis of response to irritants. This is indicative of the commencement of connectivity formation in medulla. In general, the groups show no significant differences.

Identical evaluation of behavior of young sturgeons obtained from semen preserved in a cryobank was previously conducted on starlet (*Acipenser ruthenus* Linnaeus, 1758) [31]. Based on the results of the "open field" test, the cryoprogeny did not differ from the young fish in the control group; however, when analyzing the dynamics of motor activity, the test group demonstrated a more active response to the proposed irritants. The authors note that this fact was important for adaptation upon release of young fish in the natural habitat.

Therefore, the young fish of Russian sturgeon species obtained with frozen and defrosted semen in terms of their morphometric parameters have an advantage as compared to the fish obtained by traditional technologies. The cryo-progeny is viable, and in terms of central nervous system reactivity and receptor complex in some cases surpass the control group. The insignificant difference between the development of sac fries, fish larvae and young fish in the test

and control groups can be the result of cryo-resilience of subpopulations of frozen cells. However, when analyzing the responses of fish obtained traditionally and those derived from defrosted semen, no differences have been observed between the control and test groups. Cryopreserved semen can be recommended for government fisheries to be used for fish-rearing of sturgeon species and for private aquaculture firms.

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