

ABSTRACT

on the topic «Development of technology for cryopreservation of reproductive products of male pikeperch (*Sander lucioperca*) for artificial reproduction in conditions of recirculating aquaculture system (RAS)» submitted for the degree of Doctor of Philosophy (PhD)

under the educational program “8D08401 – Fisheries and Industrial Fishing”

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General Description of the Work. The dissertation is an experimental study aimed at developing a cryopreservation technology for pike-perch (*Sander lucioperca*) sperm for application in aquaculture. The work provides a comprehensive assessment of the qualitative indicators of male reproductive products in their native state, investigates the effect of various cryoprotective media and freezing regimes on sperm survival after thawing, and determines the optimal conditions for long-term storage of cryopreserved material. Based on the experimental data obtained, a technological protocol for the cryopreservation of pike-perch sperm was developed, intended for practical use in artificial reproduction systems using Recirculating Aquaculture Systems (RAS).

Relevance of the Topic.

The biological characteristics of pike-perch (pronounced seasonality of gamete maturation, spawning asynchrony, and high sensitivity of breeders to stress, especially in RAS) complicate the synchronization of males and females and increase the risk of spawning failure. Under these conditions, the cryopreservation of pike-perch sperm allows for: the long-term preservation of valuable gene pools and rare breeding lines; a reduction in the number of males in the broodstock, thereby lowering maintenance costs; flexible planning of incubation periods and juvenile release; and the safe exchange of breeding material between farms and regions, reducing epizootic risks. Despite the obvious advantages, issues regarding the optimization of pike-perch sperm cryopreservation protocols (selection of diluents and cryoprotectants, freezing and thawing regimes, evaluation of fertilizing capacity after storage) remain insufficiently developed. This necessitates comprehensive research aimed at improving cryopreservation methods and justifying their practical application in modern intensive aquaculture systems (RAS).

The relevance of the study lies in the development of cryopreservation technology for pike-perch (*Sander lucioperca*) sperm, which is essential for increasing the efficiency of artificial reproduction processes, particularly when using Recirculating Aquaculture Systems (RAS). As a predatory species, pike-perch is difficult to obtain and maintain as broodstock, as it requires live feed, is prone to cannibalism, and is sensitive to stress, making it difficult to obtain high-quality reproductive products. The use of cryopreservation allows for the preservation of genetic material, stabilization of the fertilization process, and use of sperm outside the spawning season. The creation of sperm cryobanks from high-quality breeders of natural populations ensures the preservation of the gene pool and contributes to the sustainable development of aquaculture.

To date, methods for cryopreservation have been developed and implemented for more than 300 fish species, including both freshwater and marine taxa. The greatest attention has been paid to salmonids, cyprinids, as well as sturgeons and coregonids, for which numerous protocols have been created. In several countries (USA, UK, Ukraine, Czech Republic, Germany, France, Brazil, New Zealand, etc.), sperm cryobanks are functioning and used for gene pool preservation and breeding purposes. International organizations, including the FAO, recognize cryopreservation as an important tool for the conservation of genetic resources and the development of aquaculture.

Unfortunately, in Kazakhstan, this field remains insufficiently developed due to a limited number of studies and the absence of specialized cryobanks. Despite the existence of the first protocols for pike-perch sperm cryopreservation for laboratory and farm conditions, a comprehensive technology adapted to intensive aquaculture (RAS) and the tasks of selective improvement of broodstock is still lacking. Thus, research on pike-perch sperm cryopreservation represents a relevant direction for preserving genetic biodiversity and increasing the efficiency of the fishing industry, which is particularly important for the development of domestic aquaculture.

Purpose of the Study. To develop and optimize methods for the cryopreservation of pike-perch (*Sander lucioperca*) sperm to increase the efficiency of controlled artificial reproduction in intensive aquaculture (RAS) conditions.

Objectives of the Study. In accordance with the purpose, the following tasks were formulated:

1. To study the quality of pike-perch sperm in its native state, including ejaculate volume, appearance, specific concentration, and motility.
2. To study and optimize the maintenance conditions for pike-perch (*Sander lucioperca*) broodstock in RAS to obtain sperm most suitable for cryopreservation.
3. To study the influence of various cryoprotective media, freezing temperature regimes, and storage conditions on the functional parameters of pike-perch sperm after thawing.
4. To develop a technological protocol for the freezing, storage, and defrosting of pike-perch sperm.

Objects of Research. Sexually mature male pike-perch (*Sander lucioperca* Linnaeus, 1758)—a representative of the order Perciformes, family Percidae—and the reproductive material (sperm) obtained from them.

Research Methods. The dissertation utilizes a complex of field, laboratory, cryobiological, molecular-genetic, and statistical methods, including the capture and biological-morphological analysis of male pike-perch from natural water bodies, pre-spawning maintenance of breeders in RAS, a comprehensive assessment of native and cryopreserved sperm, and subsequent mathematical and statistical processing of experimental data.

Scientific Novelty of the Dissertation.

For the first time in Kazakhstan, a comprehensive study of native and cryopreserved pike-perch (*Sander lucioperca*) spermatozoa was conducted, aimed at the scientific justification of cryopreservation technology for further use in artificial

reproduction. This work is the first study in which the local pike-perch gene pool is evaluated not only by freshly obtained sperm but also by the results of cryostorage followed by thawing, providing a fundamentally new approach to the formation of reproductive resources.

For the first time, an array of sperm data from male pike-perch from three water bodies in Kazakhstan was obtained and systematized. Natural ranges of variability for ejaculate volume, sperm concentration, and motility indicators were established, forming a new scientific basis for the species and filling the existing gap in domestic literature.

For the first time, maintenance conditions for pike-perch breeders in RAS were experimentally justified, ensuring the production of sperm suitable for cryopreservation. The dependence of qualitative sperm characteristics on hormonal stimulation parameters was determined, allowing for the establishment of biologically and technologically optimal pre-spawning preparation regimes.

For the first time, optimal parameters for pike-perch sperm cryopreservation were established and quantitatively confirmed, including the composition and concentration of cryoprotective media, effective ratios, as well as temperature regimes for cooling, freezing, and storage. Conditions were shown under which maximum post-thaw sperm motility is maintained, indicating high reproducibility of the methodology and its practical applicability.

For the first time, a technological protocol for pike-perch sperm cryopreservation was developed, adapted to the physiological characteristics of the species and the aquaculture conditions of Kazakhstan.

Theoretical Significance of the Work. The results obtained clarify concepts regarding the quality of pike-perch sperm in its native state and its reactions to cryopreservation. The established patterns of changes in motility, concentration, and viability of spermatozoa when using different cryoprotective media and temperature regimes supplement theoretical knowledge on the reproductive physiology of pike-perch. These data expand the scientific basis for the development of biotechnologies for artificial reproduction and cryopreservation of genetic material from freshwater fish.

Practical Significance of the Work. The results obtained have significant applied importance for modern fish farming, artificial reproduction programs, and selective breeding work with pike-perch. The developed technological protocol for sperm cryopreservation ensures long-term and stable storage of reproductive material, allowing for the formation of gene pool cryobanks and their use in the selection of highly productive lines. The creation of such bio-resources makes it possible to perform selection and crossing of the best breeders regardless of the season, physiological state, or availability of males during the spawning period.

The implementation of cryopreserved sperm into breeding programs allows for the targeted maintenance and combination of genetically valuable traits—resistance to the stresses of intensive RAS technologies, high growth rates, improved feed conversion, adaptation to high stocking densities, and earlier sexual maturation. This ensures the formation of breed groups and lines of pike-perch suitable for industrial aquaculture and rearing in intensive fish farming systems.

The application of the developed protocol in practical farm conditions contributes to the standardization of reproductive processes, reduction of breeding resource losses, and increased efficiency in obtaining viable juveniles. The creation of pike-perch genetic resource cryobanks based on the data obtained opens opportunities for long-term storage, reproduction, and sustainable development of aquaculture production, ensuring its genetic stability and manageability.

Main Provisions for Defense.

1. With increasing age of male pikeperch, sperm concentration and functional maturity of the gonads increase statistically significantly. The concentration rises from $\sim 10 \times 10^9/\text{ml}$ in two-year-old fish to more than $18 \times 10^9/\text{ml}$ in five-year-old fish and is described by a linear relationship ($y = 2.9867x + 7.5282$; $R^2 = 0.9751$), which is confirmed by a positive correlation with age ($r = 0.64$; $p < 0.0000000001$).

2. The quality of pikeperch sperm is determined by the conditions of the water body and the individual characteristics of the breeders. The sperm parameters of males of the same age (3+–4+) are higher in the Vyacheslavskoye Reservoir ($17.6 \pm 0.93 \times 10^9/\text{ml}$; $88.59 \pm 2.19\%$; 69 ± 4.2 s) than in the Birsuatskoye Reservoir ($13.4 \pm 0.81 \times 10^9/\text{ml}$; $81.52 \pm 1.98\%$; 55 ± 3.8 s), despite a lower ejaculate volume. The condition factor is characterized by a moderate relationship with ejaculate volume ($R^2 = 0.464$) and cannot serve as the main criterion for breeder selection.

3. The combined use of chorionic gonadotropin and spawning nests provides the maximum efficiency of stimulation in male pikeperch, which is manifested by 100% milt fluidity, a latent period of 12–18 h, and the highest sperm parameters — 1.2 ± 0.2 ml, $9.7 \pm 0.3 \times 10^9/\text{ml}$, $95.5 \pm 1.5\%$, and 63.2 ± 2.5 s — compared to the separate application of these methods.

4. The use of DMSO is effective at a concentration of 10%, providing spermatozoa survival of $68.2 \pm 2.24\%$ in straws and $58.8 \pm 2.16\%$ in Eppendorf tubes, whereas at 20%, without changing the composition of the medium, it decreases to $10.6 \pm 0.88\%$ and $7.2 \pm 1.04\%$, respectively ($p < 0.01$). In an ion-balanced medium, 18% DMSO maintains high efficiency, ensuring motility of $52.2 \pm 1.44\%$ in straws and $41.0 \pm 1.2\%$ in Eppendorf tubes.

5. Equilibration before freezing is a mandatory technological stage that increases the post-cryogenic preservation of pikeperch spermatozoa. When 10% DMSO is used, survival with equilibration increases to $68.2 \pm 2.24\%$ and $58.8 \pm 2.16\%$ in straws and Eppendorf tubes, respectively, compared to $64.8 \pm 2.56\%$ and $56.8 \pm 2.2\%$ without it; at 20% DMSO it increases to $10.6 \pm 0.88\%$ and $7.2 \pm 1.04\%$ versus $8.2 \pm 0.64\%$ and $6.6 \pm 0.72\%$ ($p < 0.01$), which is accompanied by an increase in spermatozoa motility.

Personal Contribution of the Author. Consists of direct participation in field work for the selection of pike-perch breeders, independent execution of laboratory studies for the analysis of native and cryopreserved sperm, preparation and execution of cryopreservation procedures, interpretation and statistical processing of results, and the development and formatting of the technological cryopreservation protocol and main sections of the dissertation.

Approbation of the Work. The main results of the study were presented at several international and national scientific events. The materials of the dissertation

were presented at the III International Forum "Dialogue of Young Scientists: Science Talks" (2023), at the international scientific conference of students and young scientists "Farabi Alemi" (2025), and at the scientific-practical conference with international participation "Experience and Prospects for the Development of Ornamental and Commercial Aquaculture" (2025). The results were discussed at meetings of relevant departments and at the Kazakhstan-Czech International Scientific Center for Aquaculture at the S. Seifullin Kazakh Agrotechnical Research University, where they received a positive evaluation from specialists. The main provisions of the dissertation are reflected in the developed methodological recommendations for the cryopreservation of reproductive cells of valuable fish species and in practical recommendations for the biotechnological techniques of artificial reproduction of pike-perch in RAS (2023), and are confirmed by a utility model patent "Method for artificial spawning of pike-perch."

Publications. Publications on the topic of the dissertation include 10 scientific works, of which 2 articles are published in the international journal Caspian Journal of Environmental Sciences (Scopus, Q2), and 4 articles are in journals from the list of the Committee for Quality Assurance in the Sphere of Education and Science of the Republic of Kazakhstan (KKSON RK): Science Review of S. Seifullin KATU (2023), Herald of Zhangir Khan West Kazakhstan Agriculture-Technology University (2025), and Journal of KazNU, Biology Series (2023). Additionally, two works were published in the proceedings of international conferences (Science Talks, 2023; Farabi Alemi, 2025), a utility model patent No. 9752 "Method for artificial spawning of pike-perch" was obtained, and 2 recommendations were published at the S. Seifullin KATU (Order No. 14 of October 23, 2023).