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Original Article

EFFECT OF DIFFERENT CRYOPROTECTORS ON VIABILITY OF FROZEN-THAWED SPERMATOZOA OF BALKHASH PERCH (*PERCA SHRENKII* KESSLER, 1874)

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ABSTRACT

In recent years, there has been active development of commercial aquaculture against the background of depletion of natural biological resources of water bodies, considering the growing need for food products. Cryopreservation of spermatozoa of rare and endangered fish species is of great importance, which will help preserve their biodiversity. Scientists are interested in perch as a potential commercial fish, but no studies have been conducted on cryopreservation of Balkhash perch spermatozoa. The Balkhash perch (*Perca Shrenkii* Kessler, 1874) is endemic to the Balkhash-Ili basin, and currently the population of this species is extremely low. In this study, work was carried out to determine the effect of various cryoprotectants on sperm motility before and after thawing for cryopreservation of Balkhash perch sperm. For this purpose, sperm from 10 males of this species was obtained and examined. During freezing, components of a protective solution were used with the addition of cryoprotectors glycerin and dimethyl sulfoxide to the sperm. The results of the study showed that before the addition of cryoprotectants, the motility was 74.3 ± 5.7%. Thawed samples frozen using the cryoprotectant 2.0 M glycerol retained motility significantly longer than those using 2.0 M dimethyl sulfoxide.

Key words: Balkhash perch, spermatozoa, cryopreservation, cryoprotectors, biobank.

INTRODUCTION

The depletion of natural biological resources of the planet's water bodies has been observed throughout the world since the 1970s. However, the need for food products is steadily growing every year. The consequence of these processes is the intensive development of commercial aquaculture and one of its forms - commercial fish farming. The volumes of seafood production in some countries increase several times in one year, and constitute an important item in the gross national product. The development of methods and technologies for cryopreservation of spermatozoa of rare and endangered fish species in Kazakhstan will help preserve their biodiversity. Cryopreservation is an assisted reproductive technology that is especially needed in both fish farming and environmental protection. Cryopreservation of fish sperm can effectively promote synchronization of spermiation and ovulation during the spawning period, maintaining high quality of gametes, and creating gene banks for the conservation of endangered species.

As is known, special protocols have been developed only for some freshwater species, in particular for salmon, carp, sturgeon and catfish [1]. The Eurasian perch (*Perca fluviatilis*), a freshwater species, is of growing interest in European aquaculture and extensive research has been carried out on its artificial reproduction [2]. It should be noted that there is very little information on cryopreservation of perch sperm [3,4], and no studies on cryopreservation of spermatozoa of the Balkhash perch (*Perca Shrenkii*) have been conducted. Currently, the number of this species, listed in the Red Book of the International Union for Conservation of Nature and the

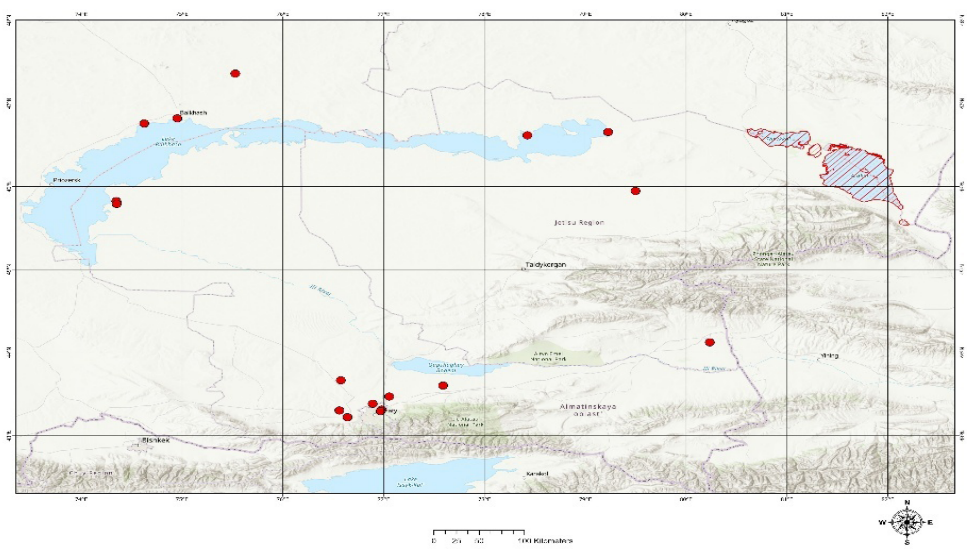

Figure 1. Distribution of Balkhash perch in the Balkhash-Alakol basin.

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Red Book of Kazakhstan, is extremely low.

The Balkhash perch (*Perca Shrenkii* Kessler, 1874) is endemic to the Balkhash-Alakol basin; in the late 1960s it lost its commercial importance on Lake Balkhash. It accidentally spread with carp to the Shu, Nura River basins and other reservoirs in Central and Northern Kazakhstan. There is no modern information on the presence of the Balkhash perch in the reservoirs of its introduction. It is known that the Balkhash perch can hybridize with the common perch (*Perca fluviatilis*) [5]. Currently, it is locally distributed in the Balkhash-Ili basin; in the Alakol lakes it is present in all lakes (Fig. 1).

In the Balkhash-Alakol basin, the number varies significantly in different water bodies. Before acclimatization work, the Balkhash perch was the dominant species in the basin. As a result of acclimatization work in the 20th century, the species composition of the ichthyofauna expanded significantly. This increased competitive relations in ichthyocenoses, due to which the populations of the Balkhash perch have significantly decreased. For example, the Balkhash-Ili population has catastrophically degraded and is listed in the Red Book of Kazakhstan [6]. In the Balkhash-Ili basin, small micropopulations have been preserved in small rivers and pond farms, in the delta of the Ili River, in the floodplain reservoirs of the Lepsy, Aksu, Karatal, Tokraun rivers, and is also locally found in Lake Balkhash [7,8,9]. In the Alakol lake system, the Balkhash perch still retains commercial numbers and its populations are exploited by fishing. The total limit on commercial extraction in the three Alakol lakes for 2025–2026 was 379 tons [10]. The Alakol population belongs to the 3rd category of the animal world – in the list of valuable animal species that are objects of hunting and fishing. The Balkhash-Ili population is listed in the Red Book of Kazakhstan, category II: declining. According to the IUCN criteria, taking into account the current distribution and number in Kazakhstan, it is assessed as CR: A2cde [6].

Improvements to existing protocols are needed to expand our knowledge of artificial reproduction of this valuable and scarce species. Many parameters can influence sperm quality through cryopreservation efficiency, including pH, osmolality, morphology, ion content, ATP content, protein content, etc. [1,11]. In Eurasian perch, many of these parameters have been studied intensively over the last decade [12,13,14,4,15]. Sperm processing prior to cryopreservation can significantly affect post-thaw quality in several fish species [16,17]. The effect of short-term storage on fresh sperm quality has been the subject of research in many species [18,19,20,21,22]. However, information on the effect of storage time after thawing on the quality of cryopreserved sperm is limited to only a few species [23,24,25,26] and is virtually absent for perch. Increasing the post-thawing storage time may facilitate the use of cryopreservation in aquaculture. The composition of extenders, cryoprotectants and their dilution factors with fresh sperm used in the cryopreservation process, which affect the quality of cryopreserved sperm, is a widely studied factor [27,28,29,30], however, information on these parameters in relation to perch sperm is very limited.

Thus, the aim of our study was to determine the optimal diluent, dilution ratio and storage time after thawing for cryopreservation of Balkhash perch sperm.

MATERIALS AND METHODS

Objects of research: individuals of male Balkhash perch, spermatozoa of Balkhash perch.

Sperm collection

10 males of Balkhash perch were used in the experiment that were collected from Lake Balkhash, Kazakhstan and kept at the Institute of Zoology in April - May 2023. Spermatogenesis in males was not stimulated by hormonal injections before the experiments. After thoroughly removing excess water and dirt around the genital area of the producer with a damp towel, the sperm was strained into a clean, dry container. A suspension of sperm of medium concentration without admixtures of water, blood and feces was used for cryopreservation.

Sperm quality assessment

Progressive motility (WHO, 2010) was measured using the Computer Assisted Sperm Analysis (CASA) system (Hamilton Thorn IVOS, USA) before and after cryopreservation. Leja slide (2-chambers) was used for the analysis. To assess the quality, fresh sperm was pre-diluted in a ratio of 1:100 in 150 mM NaCl, 5 mM KCl, 1 mM MgSO4 · 7H2O, 1 mM CaCl2 · 2H2O, 20 mM Tris (modified Lahnsteiner immobilizing solution, [9]). A mixture of 0.01 g/ml BSA (bovine serum albumin) and 75 mM NaCl, 2 mM KCl, 1 mM MgSO4 • 7H2O, 1 mM CaCl2 • 2H2O, 20 mM Tris, pH 8 (modified Lahnsteiner activating solution, [9]) was used to activate fresh and cryopreserved sperm in a ratio of 1:20. All chemicals were purchased from Sigma (Sigma Aldrich, USA).

Addition of cryoprotectant and equilibration

After dilution, cryoprotectants must be added to the sperm for freezing. The components of the protective solution were used in the following ratio: Tris-HCl buffer - 0.1 M, mannitol - 0.15 M, polyvinyl alcohol - 0.05 M per elementary unit of the polymer, silver iodide - 0.001 M, dimethyl sulfoxide - 2.0 M (DMSO group) or glycerol - 2.0 M (GLY group). The cryoprotector was added in a ratio of 1:40. The cryoprotector was added and the sperm was diluted in Petri dishes. After adding the cryoprotector, sperm motility was analyzed using CASA analysis.

Cryopreservation and thawing processes

After CASA analysis, sperm was diluted with different extenders and in different dilution ratios depending on the experimental design. Diluted samples were loaded into 0.5 ml straws (CryoBioSystem, France). A programmable freezer KryoPlaner 330 (Planer, UK) was used for freezing. The sperm with the extender was frozen at a rate of 10 degrees/min to -100^{0}C. Thawing was performed in a water bath at a temperature of 40^{0}C, for about 30 seconds, until the liquid phase appeared.

Statistical Data Processing

The ANOVA application program was used for statistical processing. The arithmetic mean, the standard error of the arithmetic mean, and the reliability of the difference in means P according to the student's criterion (TTEST) were calculated.

RESULTS

When investigating the effect of cryoprotectants on sperm motility in the first study, before the addition of cryopro-

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tectants, motility was 74.3 ± 5.7%. At 10 minutes after thawing, motility was highest with 2.0 M glycerol (44.7 ± 4.2%) compared with 2.0 M DMSO (19.4 ± 5.8%) (Fig. 2). Statistical analysis showed a significant difference between experimental groups ($P < 0.05$).

Figure 2. Motility of thawed Balkhash perch spermatozoa cryopreserved with dimethyl sulfoxide (DMSO) or glycerol (GLY) ($P < 0.05$).

Motility in samples frozen using 2.0 DMSO cryoprotectant was lowest (5.7 ± 1.9%) 30 minutes after thawing and ceased 90 minutes after thawing. Thawed samples frozen using 2.0 M glycerol cryoprotectant retained motility longer - more than 180 minutes after thawing (Fig. 3).

Figure 3. Motility during storage at 4 °C for 180 min (mean ± S.D.) after thawing of Balkhash perch (Perca shrenkii) sperm cryopreserved with dimethyl sulfoxide (DMSO) or glycerol (GLY).

An important result of the work carried out was the replenishment of the biobank of germplasm of wild animals of the Republic of Kazakhstan at the Institute of Zoology with samples of the Balkhash perch spermatozoa. Frozen sperm samples were placed for long-term storage at a temperature of -196°C to preserve the genetic material and further use for scientific and applied purposes.

DISCUSSION

We found that Dzyuba et al. [4] reported comparable post-thaw motility values obtained using a 300 mM glucose-based extender supplemented with 9% methanol for Eurasian perch sperm. An earlier study by Rodina et al. [3] also used a 300 mM glucose-based extender in combination with 10% DMSO as a cryoprotectant, but the authors did not report results on motility after thawing. Our results show that 2.0 M glycerol can be used for cryopreservation of Balkhash perch sperm as effectively as the sugar-based extender described previously. Dilution ratios above 1:40 are effective. These results are consistent with the findings [31] for salmonids: whitefish (*Coregonus sp.*), Danube salmon (*Hucho hucho*), rainbow trout (*Oncorhynchus mykiss*), grayling (*Thymallus thymallus*), brown trout (*Salmo trutta m. fario*), etc., that, depending on the species, at least a three- to seven-fold dilution was required for effective viability of fish spermatozoa after thawing. Dilution factors are closely related to sperm concentration according to Dziewulska and Domagala [32], with sperm concentrations needing to be in the range of 3.0 to 4.0 x 10^9 ml sperm for maximum cryoviability. Furthermore, in the endangered mahseer (*Tor kudhree*), results also showed that sperm can tolerate cryodamage more effectively when diluted more than six-fold [33].

According to these findings, salmon sperm is even more sensitive to storage time and is recommended for use immediately after thawing for fertilizing eggs. Even 30 seconds of storage results in a significant reduction in fertilization capacity in salmon fish [23,24]. In contrast to these results, thawed sperm of Siberian sturgeon (*Acipenser baerii*) and Russian sturgeon (*A. gueldenstaedtii*) can be stored for up to 6 and 2 hours, respectively, without significant reduction in motility [34]. Cryopreserved African catfish (*Clarias gariepinus*) sperm was found to be particularly resilient to long-term storage after thawing, as its fertilizing ability and membrane integrity (viability) showed no decrease during 26 hours of storage [35]. Storage of Atlantic cod (*Gadus morhua*) sperm after thawing for 30 minutes did not significantly affect its motility [26].

The influence of important parameters on the process of cryopreservation of Balkhash perch sperm was studied in our experiments. Further experiments are needed to investigate other important parameters and standardize the cryopreservation of perch sperm, such as sperm contamination, cooling rate, equilibration time, sperm-to-egg ratio and others.

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LITERATURE

1. Cabrita E., Sarasquete C., Martınez-Paramo S., Robles V., Beirao J., Perez-Cerezales S., Herraez M. P. Cryopreservation of fish sperm: applications and perspectives // J. Appl. Ichthyol. – 2010. – Vol. 26. – P. 623–635.

2. Zarski D., Bokor Z., Kotrik L. et al. A new classification of a preovulatory oocyte maturation stage suitable for the synchronization of ovulation in controlled reproduction of Eurasian perch *Perca fluviatilis* L. // Reproductive Biology. – 2011. - Vol. 11. - №. 3. P. 194-209 // DOI: 10.1016/s1642-431x(12)60066-7. PMID: 22139334.

3. Rodina M., Policar T., Linhart O., Rougeot C. Sperm motility and fertilizing ability of frozen spermatozoa of males (XY) and neomales (XX) of perch (*Perca fluviatilis*) // J. Appl. Ichthyol. – 2008. – Vol. 24. – P. 438–442.

4. Dzyuba B., Boryshpolets S., Rodina M., Gela D., Linhart O. Spontaneous activation of spermatozoa motility by routine freeze-thawing in different fish species // J. Appl. Ichthyol. – 2010. – Vol. 26. – P. 720–725.

5. Dukravets G.M., Mamilov N.Sh. Materials on the morphometry and biology of perch fishes from the Chu River basin // Questions of Ichthyology. – 1992. - Vol. 32, Issue 6. - P. 49-56.

6. The Red Book of the Republic of Kazakhstan. - Vol. I. - Animals. Part 1. Vertebrates. 4th edition, revised and supplemented //Almaty: DPS, 2010. - 324 p.

7. Dukravets G. M. Biological state of some local populations of Balkhash perch (*Perca schrenki* Kessler) in the Ili River basin. Fisheries research in the Republic of Kazakhstan: history and current state // Almaty. - 2005. - P. 93–109.

8. Isbekov K.B., Asylbekova S.Zh., Timirkhanov S.R. Tokraun River as a reserve of mountain-Asian ichthyofauna // Izvestiya NAS RK. Biological and Medical Series. – 2006. - Vol. 1 (253). - P. 20-24.

9. Tsoi V.N. Prospects for Restoring the Population of the Balkhash Perch (*Perca schrenki* Kessler) in its Natural Range // International Correspondence Scientific and Practical Conference “Current Issues in Scientific Work and Educational Activities”. – 2014. - Tambov. - P. 145–146.

10. Order of the Minister of Agriculture of the Republic of Kazakhstan. On approval of limits for the extraction of fish

DOI: 10.11134/btp.3.2025.8

resources and other aquatic animals from July 1, 2025 to July 1, 2026. - July 2, 2025. - No. 212.

11. Fauvel C., Suquet M., Cosson J. Evaluation of fish sperm quality // J. Appl. Ichthyol. – 2010. - Vol. 26. – P. 636–643.

12. Krol J., Glogowski J., Demska-Zakes K., Hliwa P. Quality of semen and histological analysis of testes in Eurasian perch *Perca fluviatilis* L. during a spawning period // Czech J. Anim. Sci. – 2006. – Vol. 51. – P. 220–226.

13. Alavi S.M.H., Rodina M., Policar T., Kozak P., Psenicka M., Linhart O. Semen of *Perca fluviatilis* L.: sperm volume and density, seminal plasma indices and effects of dilution ratio, ions and osmolality on sperm motility // Theriogenology. - 2007. – Vol. 68. – P. 276–283.

14. Boryshpolets S., Dzyuba B., Stejskal V., Linhart O. Dynamics of ATP and movement in Eurasian perch (*Perca fluviatilis* L.) sperm in conditions of decreasing osmolality // Theriogenology. – 2009. - Vol. 72. – P. 851–85.

15. Lahnsteiner F. Spermatozoa of the teleost fish *Perca fluviatilis* (perch) have the ability to swim for more than two hours in saline solutions // Aquaculture. - 2011. – Vol. 314. – P. 221–224.

16. Poupard G.P., Paxion C., Cosson J., Jeulin C., Fierville F., Billard R. Initiation of carp spermatozoa motility and early ATP reduction after milt contamination by urine // Aquaculture. – 1998. – Vol. 160. – P. 317–328.

17. Glogowski J., Kwasnik M., Piros B., Dabrowski K., Goryczko K., Dobosz S., Kuzminski H., Ciereszko A. Characterization of rainbow trout milt collected with a catheter: semen parameters and cryopreservation success // Aquac. Res. – 2000. – Vol. 31. – P. 289–296.

18. Stoss J., Refstie T. Short-term storage and cryopreservation of milt from Atlantic salmon and sea trout // Aquaculture. – 1983. - Vol. 30. – P. 229–236.

19. Suquet M., Dreanno C., Fauvel C., Cosson J., Billard R. Cryopreservation of sperm in marine fish // Aquac. Res. – 2000. – Vol. 31. – P. 231–243.

20. He S., Woods L.C. Effects of glycine and alanine on short-term storage and cryopreservation of striped bass (Morone saxatilis) spermatozoa // Cryobiology. – 2003. – Vol. 46. – P. 17–25.

21. Billard R., Cosson J., Noveiri S.B., Pourkazemi M. Cryopreservation and short-term storage of sturgeon sperm, a review // Aquaculture. – 2004. – Vol. 236. – P. 1–9.

22. Sarosiek B., Cejko B.I., Kucharczyk D., Zarski D., Judyck, S., Kowalski R.K. Short-term storage of perch (*Perca fluviatilis* L.) milt under cooling conditions // Reprod. Biol. – 2013. – Vol. 13. – P. 39.

23. Stoss J., Holtz W. Cryopreservation of rainbow trout (*Salmo gairdneri*) sperm: I. Effect of thawing solution, sperm density and interval between thawing and insemination // Aquaculture. – 1981. – Vol. 22. – P. 97–104.

24. Lahnsteiner F., Patzner R., Weismann T. Semen cryopreservation of salmonid fishes: influence of handling parameters on the post-thaw fertilization rate // Aquac. Res. – 1996. – Vol. 27. – P. 659–671.

25. Dreanno C., Suquet M., Quemener L., Cosson J., Fierville F., Normant Y., Billard R. Cryopreservation of turbot (*Scophthalmus maximus*) spermatozoa // Theriogenology. – 1997. – Vol. 48. - P. 589–603.

26. Babiak I., Marschhauser V., Ottesen O., Rudolfsen G., Eggen B., Babiak J. Effects of extender, storage and sperm-to-egg ratio on cryopreservation success of Atlantic cod (*Gadus morhua* L.) sperm // J. Appl. Ichthyol. – 2012. – Vol. 28. – P. 941–947.

27. Babiak I., Glogowski J., Goryczko K., Dobosz S., Kuzminski H., Strzezek J., Demianowicz W. Effect of extender composition and equilibration time on fertilization ability and enzymatic activity of rainbow trout cryopreserved spermatozoa // Theriogenology. – 2001. – Vol. 56. – P. 177–192.

28. Horvath A., Miskolczi E., Urbanyi B. Cryopreservation of common carp sperm // Aquat. Living Resour. – 2003. – Vol. 16. – P. 457–460.

29. Marco-Jimenez F., Garzon D.L., Penaranda D.S., Perez L., Viudes-de-Castro M.P., Vicente J.S., Jover M., Asturiano J.F. Cryopreservation of European eel (*Anguilla anguilla*) spermatozoa: effect of dilution ratio, foetal bovine serum supplementation, and cryoprotectants // Cryobiology. – 2006. – Vol. 53. – P. 51–57.

30. Maria A.N., Viveiros A.T.M., Freitas R.T.F., Oliveira A.V. Extenders and cryoprotectants for cooling and freezing of piracanjuba (Bryconor-bignyanus) semen, an endangered Brazilian teleost fish // Aquaculture. – 2006. – Vol. 260. – P. 298–306.

31. Lahnsteiner F. Cryopreservation protocols for sperm of salmonid fishes. In: Cryopreservation in aquatic species, 2nd edn. T. R. Tiersch, C. C. Green (Eds) // World Aquaculture Society, Baton Rouge. - 2011. - P. 409–420.

32. Dziewulska K., Domagała J. Spermatozoa concentration influences cryopreservation success in sea trout (*Salmo trutta m. trutta* L.) // Theriogenology. – 2013. – Vol. 80. – P. 659–664.

33. Basavaraja N., Hegde S.N. Cryopreservation of the endangered mahseer (*Tor khudree*) spermatozoa: I. Effect of extender composition, cryoprotectants, dilution ratio, and storage period on post-thaw viability // Cryobiology. – 2004. – Vol. 49. – P. 149–156.

34. Bernath G., Bokor Z., Urbanyi B., Horvath A. Post-thawing quality of cryopreserved sperm in two endangered acipenseriform species // 7th International Symposium On Sturgeon, Nanaimo, Canada. - 2013. - 21-25 July.

35. Kovacs E., Muller T., Marian T., Krasznai Z., Urbanyi B., Horvath A. Quality of cryopreserved African catfish sperm following post-thaw storage // J. Appl. Ichthyol. – 2010. – Vol. 26. – P. 737–741.

REFERENCES

1. Cabrita E., Sarasquete C., Martınez-Paramo S., Robles V., Beirao J., Perez-Cerezales S., Herraez M. P. Cryopreservation of fish sperm: applications and perspectives // J. Appl. Ichthyol. – 2010. – Vol. 26. – P. 623–635.

2. Zarski D., Bokor Z., Kotrik L. et al. A new classification of a preovulatory oocyte maturation stage suitable for the synchronization of ovulation in controlled reproduction of

DOI: 10.11134/btp.3.2025.8

Eurasian perch *Perca fluviatilis* L. // Reproductive Biology. – 2011. - Vol. 11. - №. 3. P. 194-209 // DOI: 10.1016/s1642-431x(12)60066-7. PMID: 22139334.

3. Rodina M., Policar T., Linhart O., Rougeot C. Sperm motility and fertilizing ability of frozen spermatozoa of males (XY) and neomales (XX) of perch (*Perca fluviatilis*) // J. Appl. Ichthyol. – 2008. – Vol. 24. – P. 438–442.

4. Dzyuba B., Boryshpolets S., Rodina M., Gela D., Linhart O. Spontaneous activation of spermatozoa motility by routine freeze-thawing in different fish species // J. Appl. Ichthyol. – 2010. – Vol. 26. – P. 720–725.

5. Dukravets G.M., Mamilov N.Sh. Materials on the morphometry and biology of perch fishes from the Chu River basin // Questions of Ichthyology. – 1992. - Vol. 32, Issue 6. - P. 49-56.

6. The Red Book of the Republic of Kazakhstan. - Vol. I. - Animals. Part 1. Vertebrates. 4th edition, revised and supplemented //Almaty: DPS, 2010. - 324 p.

7. Dukravets G. M. Biological state of some local populations of Balkhash perch (*Perca schrenki* Kessler) in the Ili River basin. Fisheries research in the Republic of Kazakhstan: history and current state // Almaty. - 2005. - P. 93–109.

8. Isbekov K.B., Asylbekova S.Zh., Timirkhanov S.R. Tokraun River as a reserve of mountain-Asian ichthyofauna // Izvestiya NAS RK. Biological and Medical Series. – 2006. - Vol. 1 (253). - P. 20-24.

9. Tsoi V.N. Prospects for Restoring the Population of the Balkhash Perch (*Perca schrenki* Kessler) in its Natural Range // International Correspondence Scientific and Practical Conference “Current Issues in Scientific Work and Educational Activities”. – 2014. - Tambov. - P. 145–146.

10. Order of the Minister of Agriculture of the Republic of Kazakhstan. On approval of limits for the extraction of fish resources and other aquatic animals from July 1, 2025 to July 1, 2026. - July 2, 2025. - No. 212.

11. Fauvel C., Suquet M., Cosson J. Evaluation of fish sperm quality // J. Appl. Ichthyol. – 2010. - Vol. 26. – P. 636–643.

12. Krol J., Glogowski J., Demska-Zakes K., Hliwa P. Quality of semen and histological analysis of testes in Eurasian perch *Perca fluviatilis* L. during a spawning period // Czech J. Anim. Sci. – 2006. – Vol. 51. – P. 220–226.

13. Alavi S.M.H., Rodina M., Policar T., Kozak P., Psenicka M., Linhart O. Semen of *Perca fluviatilis* L.: sperm volume and density, seminal plasma indices and effects of dilution ratio, ions and osmolality on sperm motility // Theriogenology. - 2007. – Vol. 68. – P. 276–283.

14. Boryshpolets S., Dzyuba B., Stejskal V., Linhart O. Dynamics of ATP and movement in Eurasian perch (*Perca fluviatilis* L.) sperm in conditions of decreasing osmolality // Theriogenology. – 2009. - Vol. 72. – P. 851–85.

15. Lahnsteiner F. Spermatozoa of the teleost fish *Perca fluviatilis* (perch) have the ability to swim for more than two hours in saline solutions // Aquaculture. - 2011. – Vol. 314. – P. 221–224.

16. Poupard G.P., Paxion C., Cosson J., Jeulin C., Fierville F., Billard R. Initiation of carp spermatozoa motility and early ATP reduction after milt contamination by urine // Aquaculture. – 1998. – Vol. 160. – P. 317–328.

17. Glogowski J., Kwasnik M., Piros B., Dabrowski K., Goryczko K., Dobosz S., Kuzminski H., Ciereszko A. Characterization of rainbow trout milt collected with a catheter: semen parameters and cryopreservation success // Aquac. Res. – 2000. – Vol. 31. – P. 289–296.

18. Stoss J., Refstie T. Short-term storage and cryopreservation of milt from Atlantic salmon and sea trout // Aquaculture. – 1983. - Vol. 30. – P. 229–236.

19. Suquet M., Dreanno C., Fauvel C., Cosson J., Billard R. Cryopreservation of sperm in marine fish // Aquac. Res. – 2000. – Vol. 31. – P. 231–243.

20. He S., Woods L.C. Effects of glycine and alanine on short-term storage and cryopreservation of striped bass (Morone saxatilis) spermatozoa // Cryobiology. – 2003. – Vol. 46. – P. 17–25.

21. Billard R., Cosson J., Noveiri S.B., Pourkazemi M. Cryopreservation and short-term storage of sturgeon sperm, a review // Aquaculture. – 2004. – Vol. 236. – P. 1–9.

22. Sarosiek B., Cejko B.I., Kucharczyk D., Zarski D., Judyck, S., Kowalski R.K. Short-term storage of perch (*Perca fluviatilis* L.) milt under cooling conditions // Reprod. Biol. – 2013. – Vol. 13. – P. 39.

23. Stoss J., Holtz W. Cryopreservation of rainbow trout (*Salmo gairdneri*) sperm: I. Effect of thawing solution, sperm density and interval between thawing and insemination // Aquaculture. – 1981. – Vol. 22. – P. 97–104.

24. Lahnsteiner F., Patzner R., Weismann T. Semen cryopreservation of salmonid fishes: influence of handling parameters on the post-thaw fertilization rate // Aquac. Res. – 1996. – Vol. 27. – P. 659–671.

25. Dreanno C., Suquet M., Quemener L., Cosson J., Fierville F., Normant Y., Billard R. Cryopreservation of turbot (*Scophthalmus maximus*) spermatozoa // Theriogenology. – 1997. – Vol. 48. - P. 589–603.

26. Babiak I., Marschhauser V., Ottesen O., Rudolfsen G., Eggen B., Babiak J. Effects of extender, storage and sperm-to-egg ratio on cryopreservation success of Atlantic cod (*Gadus morhua* L.) sperm // J. Appl. Ichthyol. – 2012. – Vol. 28. – P. 941–947.

27. Babiak I., Glogowski J., Goryczko K., Dobosz S., Kuzminski H., Strzezek J., Demianowicz W. Effect of extender composition and equilibration time on fertilization ability and enzymatic activity of rainbow trout cryopreserved spermatozoa // Theriogenology. – 2001. – Vol. 56. – P. 177–192.

28. Horvath A., Miskolczi E., Urbanyi B. Cryopreservation of common carp sperm // Aquat. Living Resour. – 2003. – Vol. 16. – P. 457–460.

29. Marco-Jimenez F., Garzon D.L., Penaranda D.S., Perez L., Viudes-de-Castro M.P., Vicente J.S., Jover M., Asturiano J.F. Cryopreservation of European eel (*Anguilla anguilla*) spermatozoa: effect of dilution ratio, foetal bovine serum supplementation, and cryoprotectants // Cryobiology. – 2006. – Vol. 53. – P. 51–57.

30. Maria A.N., Viveiros A.T.M., Freitas R.T.F., Oliveira A.V. Extenders and cryoprotectants for cooling and freezing of piracanjuba (Bryconor-bignyanus) semen, an endangered

DOI: 10.11134/btp.3.2025.8

Brazilian teleost fish // Aquaculture. – 2006. – Vol. 260. – P. 298–306.

31. Lahnsteiner F. Cryopreservation protocols for sperm of salmonid fishes. In: Cryopreservation in aquatic species, 2nd edn. T. R. Tiersch, C. C. Green (Eds) // World Aquaculture Society, Baton Rouge. - 2011. - P. 409–420.

32. Dziewulska K., Domagała J. Spermatozoa concentration influences cryopreservation success in sea trout (*Salmo trutta m. trutta* L.) // Theriogenology. – 2013. – Vol. 80. – P. 659–664.

33. Basavaraja N., Hegde S.N. Cryopreservation of the endangered mahseer (*Tor khudree*) spermatozoa: I. Effect of extender composition, cryoprotectants, dilution ratio, and storage period on post-thaw viability // Cryobiology. – 2004. – Vol. 49. – P. 149–156.

34. Bernath G., Bokor Z., Urbanyi B., Horvath A. Post-thawing quality of cryopreserved sperm in two endangered acipenseriform species // 7th International Symposium On Sturgeon, Nanaimo, Canada. - 2013. - 21-25 July.

35. Kovacs E., Muller T., Marian T., Krasznai Z., Urbanyi B., Horvath A. Quality of cryopreserved African catfish sperm following post-thaw storage // J. Appl. Ichthyol. – 2010. – Vol. 26. – P. 737–741.

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ВЛИЯНИЕ РАЗЛИЧНЫХ КРИОПРОТЕКТОРОВ НА ЖИЗНЕСПОСОБНОСТЬ ЗАМОРОЖЕННО-ОТТАЯННЫХ СПЕРМАТОЗОИДОВ БАЛХАШСКОГО ОКУНЯ (*PERCA SHRENKII* KESSLER, 1874)

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АБСТРАКТ

В последние годы наблюдается активное развитие товарной аквакультуры на фоне истощения естественных биологических ресурсов водоемов с учетом растущей потребности в продуктах питания. Большое значение приобретает криоконсервация сперматозоидов редких и исчезающих видов рыб, что позволит сохранить их биоразнообразие. Интерес ученых вызывает окунь, как потенциальный промысловый объект, а исследования по криоконсервации сперматозоидов балхашского окуня не проводились. Балхашский окунь (*Perca Shrenkii* Kessler, 1874) является эндемиком Балхаш-Илийского бассейна, и в настоящее время численность этого вида крайне низкая. В данном исследовании были проведены работы по определению влияния различных криопротекторов на подвижность сперматозоидов до и после размораживания для криоконсервации спермы балхашского окуня. Для этого была получена и исследована сперма от 10 самцов данного вида. При замораживании использовали компоненты защитного раствора с добавлением в сперму криопротекторов глицерина и диметилсульфоксида. Результаты исследования показали, что до добавления криопротекторов подвижность составила 74,3 ± 5,7 %. Оттаянные образцы, замороженные с использованием криопротектанта 2,0М глицерин, сохраняли подвижность гораздо дольше, чем с использованием 2,0М dimethyl sulfoxide.

Ключевые слова: балхашский окунь, сперматозоиды, криоконсервация, криопротекторы, биобанк.

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БАЛҚАШ АЛАБҰҒАСЫНЫҢ МҰЗДАТЫЛҒАН ЕРІГЕН СПЕРМАТОЗОИДТАРЫНЫҢ ӨМІРШЕҢДІГІНЕ ӘРТҮРЛІ КРИОПРОТЕКТОРЛАРДЫҢ ӘСЕРІ (*PERCA SHRENKII* KESSLER, 1874)

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АҢДАТПА

Соңғы жылдары су объектілерінің табиғи биологиялық ресурстарының сарқылу фонында, азық-түлік өнімдеріне деген өсу қажеттілігін ескере отырып, кәсіптік аквакультураның белсенді дамуы байқалуда. Сирек кездесетін және жойылып бара жатқан балық түрлерінің сперматозоидтарын криоконсервациялаудың маңызы зор, бұл олардың биоәртүрлілігін сақтауға көмектеседі. Ғалымдарды алабұға әлеуетті кәсіптік балық ретінде қызықтырады, бірақ Балқаш алабұғасының сперматозоидтарын криоконсервациялау бойынша зерттеулер жүргізілген жоқ. Балқаш алабұғасы (*Perca Shrenkii* Kessler, 1874) Балқаш-Іле алабының эндемигі болып табылады және қазіргі уақытта бұл түрдің саны өте аз. Бұл зерттеуде Балқаш алабұғасының шәуетін криоконсервациялау үшін әртүрлі криопротекторлардың сперматозоидтардың ерігенге дейін және одан кейінгі қозғалғыштығына әсерін анықтау жұмыстары жүргізілді. Осы мақсатта осы түрдің 10 аталығынан сперматозоид алынып, зерттелді. Мұздату кезінде сперматозоидтарға криопротекторлар глицерин және диметилсульфоксид қосылған қорғаныс ерітіндісінің компоненттері қолданылды. Зерттеу нәтижелері криопротекторларды қосқанға дейін қозғалғыштығы 74,3 ± 5,7% болғанын көрсетті. 2,0 М глицерин криопротекторын пайдаланып мұздатылған жібітілген үлгілер қозғалғыштығын 2,0 М диметил сульфоксиді қолданатындарға қарағанда әлдеқайда ұзақ сақтады.

Негізгі сөздер: Балқаш алабұғасы, сперматозоидтар, криосақтау, криопротекторлар, биобанк