HUNGARIAN UNIVERSITY OF
AGRICULTURE AND LIFE SCIENCES

THE DEVELOPMENT OF LARGE SCALE SPERM CRYOPRESERVATION IN NORTHERN PIKE (*ESOX LUCIUS*), AND EXAMINATION OF LARVAE STEM FROM CRYOPRESERVED SPERM

Thesis of the PhD Dissertation

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1. BACKGROUND AND OBJECTIVES

1.1. Background

All over Europe and America, Northern pike (Esox lucius) has great economical, fishing and eco-sociological importance as well. Due to overfishing and habitat degradation, areas required for reproduction and habitats have been greatly reduced. However, the demand for this species is growing dynamically, especially among anglers. The results of artificial propagation show great variability season by season. Productive and technological developments are required as pond fish production is currently unable to satisfy the sudden increase in demand (SÄISÄ et al. 2008; CRANE et al. 2015; CEJKO et al. 2020a; VIRBICKAS 2021). The breeding process is mostly based on customary methods. In the recent decades, only a few innovations have been applied to the northern pike breeding technology (HORVÁTH & MAGYARY 2007; SZABÓ 2016b; KRISTAN et al. 2020).

In males, the general abdominal stripping method is not effective due to the anatomical characteristics of the testis (low volume and quality of sperm). The dissection of the testis is the common sampling method used in the hatchery practice, although it requires males to be sacrificed (BILLARD 1996; KOWALSKI et al. 2012; BLECHA et al. 2016; KRISTAN et al. 2020), and cannot be used for further propagation. In most cases, due to the lack of available males, fish breeders are often able to fertilize a smaller amount of eggs (CEJKO et al. 2020b; KRISTAN et al. 2020).

Cryopreserving the sperm could optimize the number of males involved in breeding, thereby reducing maintenance and production costs (CEJKO et al. 2020b). Frozen sperm can be stored indefinitely and remains easily accessible (CABRITA et al. 2010; URBÁNYI 2011). The cryopreservation of fish sperm was studied even in the1950s, but a successful, easily reproductable method has only been developed for a few fish species, which can be transferred to hatchery practice (URBÁNYI 2011; ASTURIANO et al. 2017). There are only a few available publications on the cryopreservation of northern pike sperm, and most of the time, freezing protocols, thawing methods, dilution ratios, extender and activiting solutions differ. Starting from the 1990s, the volume of straws used in experiments related to the cryopreservation of northern pike sperm varied between 0.25 and 1.2 mL, depending on the experimental designs of different researchers. In case of further researches, the development of freezing technology for straws with a larger volume can also be justified

(LAHNSTEINER et al. 1998; LAHNSTEINER 2000; ZHANG et al. 2011; DIETRICH et al. 2016; BERNÁTH et al. 2017b; CEJKO et al. 2020b). The methodological development concerning northern pike breeding can contribute to the increase of production efficiency (CEJKO et al. 2020b).

The adaptation of cryopreserved northern pike sperm to hatchery technology is therefore greatly hindered by the lack of precisily developed techniques and standardization. On the basis of the above mentioned details, in order to increase the production of northern pike, it is highly reasonable to develop a species-specific sperm freezing procedure adapted to the requirements of hatchery conditions.

1.2. Objectives

Before starting my doctoral thesis, I set the following objectives:

- **Comparison of hormonal induction, different sperm collection techniques, and dilution ratios:**
    - Examination of two sperm collecting methods.
    - Development of a species-specific extender for the cryopreservation of northern pike sperm.

- **Methodological improvement and optimization of large-scale cryopreservation of northern pike sperm**
    - Adapting the cryopreservation technique for a controlled-rate freezer which provides more controlled freezing conditions than a polystyrene box.
    - Examination of applicability of 5 mL straws and 10 mL cryotubes in sperm freezing.
    - Testing of susceptibility to thawing time of the 10 mL cryotube.

- **Artificial propagation of northern pike using frozen sperm, and investigation of the larvae stemming from cryopreserved sperm.**
    - Investigation of using frozen sperm in artificial propagation in hatchery practise.
    - Developmental examination of larvae stem from cryopreserved sperm to demonstrate that sperm freezing has no negative effect on larval growth, morphology and survivability during the experimental period.

2. MATERIAL AND METHODS

The broodstock needed for my doctoral thesis was originated from the SzegedFish Ltd. fish farm. Sperm examinations were completed at the Hungarian University of Agricultural and Life Sciences, Department of Aquaculture. Experiments related to the artificial propagation of northern pike were conducted at the hatchery of Szegedfish Ltd., located near Szeged.

2.1. Method of hormonal treatment and sperm collection

The individuals of the broodstock were hormonally induced with carp pituitary extract at a dosage of 3.5 mg kg^{-1}. The hormone had been injected 48 hours before stripping in males and 96 hours before stripping in females. In females, the injected solution also contained a sustained-release polymer (carbopol polymer type, 971P) (SZABÓ 2001, 2008, 2011). In case of males, sperm was obtained by stripping (stripped sperm) or by dissection and squeezing of the testis (testicular sperm), as designed by the experiments. Before these treatments, fish had been anesthetized appropriately (BOBE & LABBÉ 2008; BOKOR et al. 2010).

2.2. Determination of the fish sperm concentration

During the determination of fish sperm concentration, the diluted samples were recorded by the help of Bürker-chamber, and counted by using ImageJ software. The average number of cells per milliliter was determined from these data (BOKOR 2010).

2.3. Analysis of sperm motility

Before cryopreservation and after thawing, the motility examination of sperm was recorded by using Computer-Assisted Sperm analysis (CASA) (BERNÁTH et al. 2017a). The data were evaluated by using Sperm VisionTM v. 3.7.4. (Minitube of America, Venture Court Verona, United States of America) software. Spermatozoas were activated with pike activation solution (LAHNSTEINER et al. 1998, 100 mM NaCl, 10 mM Tris, pH: 8). Bovine serum albumin (BSA) was added to the solution at a concentration of 0.5% to prevent sperm adhesion. In CASA, concerning the movement of sperm, the following parameters were measured in two repetations: progressive motility (pMOT, %), curvilinear velocity (VLC, $\mu m\ s^{-1}$) and straightness (STR, %) (RURANGWA et al. 2004; WILSON-LEEDY & INGERMANN 2007).

2.4. Cryopreservation of sperm

The general process of sperm cryopreservation and thawing

To ensure traceability in the examinations, firstly I will outline the general process of sperm cryopreservation. The experiments were carried out using an extender, which had been specifically designed for freezing northern pike sperm during my doctoral work, the components of which had been determined based on the composition of the seminal plasma of muskellunge sperm, a close relative species of the northern pike (LIN et al. 1996a) 150 mM glucose, 75 mM NaCl, 30 mM KCl, 1 mM $Na_2HPO_4*12H_2O$, 1 mM $MgCl_2*6H_2O$, 1 mM $CaCl_2*2H_2O$, 20 mM Tris, and 0.5% BSA, pH 8±0.02. Methanol with a final solution concentration of 10% was used as an intracellular protective agent (LAHNSTEINER 2000; HORVÁTH et al. 2015). Depending on the experimental design, three dilution ratios were used (sperm:extender): 1:1, 1:9, 1:19 (HOAR et al. 1983; BOKOR et al. 2010). The cryopreservation was carried out in polystyrene box and/or in a controlled-rate freezer depending on the experimental design (CRF, IceCube 14s, IceCube Series v. 2.24, Sy-Lab, Neupurkersdorf, Austria), and according to the determined scheme (Table 1.). The thawing of sperm samples having been stored in liquid nitrogen took place in a water bath at a temperature of 40°C in all cases (Thermo Haake P5, Thermo Electron Corporation, Waltham, Massachusetts, United States).

Table 1.: The cryopreservation methods and programs used in the experiments, and thawing periods

Cryopreservation programs according to specific storage units
0.5 mL straw
• **3 cm above liquid nitrogen in a polystyrene box, 3 min (HORVÁTH et al. 2003),**
• **CRF, 7,5°C → -160°C, cooling rate: 56°C min^{-1} (BERNÁTH et al. 2015).**
5 mL straw
• **3 cm above liquid nitrogen in a polystyrene box, 7 min (BOKOR et al. 2010),**
• **CRF, 4°C → -160°C, cooling rate: 15°C min^{-1} (BOKOR et al. 2019).**
10 mL cryotube
• **CRF, 4°C → -160°C, cooling rate: 15°C min^{-1} (BOKOR et al. 2019).**
Thawing times
• **0.5 mL straw 13 sec (HORVÁTH et al. 2012),**
• **5 mL straw: 35 sec (VÁRKONYI et al. 2019),**
• **10 mL cryotube was determined experimentally.**

2.4.1. Comparison of hormonal treatments, different sperm sampling methods and extenders

Examination of the effect of hormonal treatment on sperm quality

In the first part of my experiment related to cryopreservation, the effect of hormonal induction on sperm motility was investigated. In the control group, fish physiological salt solution (0.65% NaCl solution) was injected into the abdominal cavity of the individuals at a rate of 1 mL kg^{-1}. In the examined group, 3.5 mg kg^{-1} of carp (*Cyprinus carpio*) pituitary was added to the saline solution. Sperm was extracted by using the traditional stripping method (*N*=5-5).

Comparison of glucose and trehalose-based extenders in cryopreservation

Based on the results of my previous tests, the carp pituitary hormone dose of 3.5 mg kg^{-1} was used. The sperm was extracted by dissection and squeezing of the testis. According to the experimental design, 150 mM glucose or 150 mM trehalose were added to the northern pike extender I had developed as an extracellular cryoprotectant (ALAVI et al. 2009; LIN et al. 1996a). Freezing was conducted in 0.5 mL straws placed in a polystyrene box, using dilution ratios of 1:3, 1:9, and 1:19 (*N*=5).

Comparative investigation of two sperm extraction methods in cryopreservation using 0.5 mL straw

Sperm extraction was performed by stripping and dissecting the testes (*N*=5-5). Based on the results of previous studies, hormonal induction was continued, and northern pike extender containing 150 mM glucose with a dilution ratio of 1:9 was used for cryopreservation. The 0.5 mL straw was frozen in a polystyrene box.

2.4.2. Investigations of the methodological development and optimization of cryopreservation of a large-scale of northern pike sperm

On the basis of the evaluation coming from previous experiments' results, I uniformly applied hormonal treatment, testis dissection, and a glucose-based northern pike extender for cryopreservation. A sperm extender ratio of 1:9 was used in the tests, except where different extender ratios were compared.

Comparison of the polystyrene box and the controlled-rate freezer device in case of 0.5 mL straw

Experiments on the intensification and optimization of cryopreservation began with a comparison between the polystyrene box and controlled-rate freezer devices, in which the sperm samples having been filled into 0.5 mL straws were frozen (N=7).

Comparison of different dilution ratios in the controlled-rate freezer using 10 mL cryotube

The sperm extracted from 7 individuals were cryopreserved in a controlled-rate freezer using 10 mL cryotubes. In my study, the dilution ratios of 1:3, 1:9, and 1:19 were compared (N=9).

Comparison of 5 mL straw and 10 mL cryotube using different freezing methods

Under hatchery conditions, the efficiency of cryopreservation was compared between the 5 mL straw in a polystyrene box and controlled-rate freezer (CRF), as well as the 10 mL cryotube (N=5).

2.4.3. Investigations of artificial propagation of northern pike in a hatchery with a large-scale of cryopreserved sperm and short-term larval rearing

Artificial propagation and larval rearing of northern pike were carried out at the hatchery of Szegedfish Ltd. In the progress, the protocol having been developed and applied by the company was followed (according to the instructions of the head of the hatchery).

Preparation of sperm and eggs for artificial propagation

Firstly, the sperm was extracted by dissecting the testes. The work was conducted with 5 male samples per group (fresh and 3 frozen group) Sperm was frozen using three different sperm cryopreservation methods: 5 mL straw in a polystyrene box and CRF, and 10 mL cryotube in CRF. The dilution ratio was 1:9. After freezing the sperm, the eggs were removed, and then the sperm samples were thawed.

Fertilization

In order to choose the optimal sperm:egg ratio, the average sperm cell density was determined: $2.99*10^{10}$ sperm mL^{-1} (N=54), based on my previous work and literary works. The fertilization units were determined as follows: 2112 µL sperm to 250 g of eggs (LAHNSTEINER 2000). Five repetitions were used in the fresh and in three frozen groups, as well. The

5 mL straw contained 4 mL, and the 10 mL cryotube contained 8 mL of cell suspension. During the dry fertilization, an activation period of 20 seconds was applied by adding system water. Then the eggs were mixed for 10 minutes (WOYNÁROVICH & WOYNÁROVICH 1980), and finally, the eggs were placed in 9-liter Zuger-jars for each treatment.

Incubation of eggs

During the 5-day egg incubation, the water temperature was 15.5±0.85°C, and the dissolved oxygen level was 8.85±0.2 mg L^{-1}. To prevent the growth of *Saprolegnia spp*., DETOX (a solution containing 4.5% peracetic acid and 20% hydrogen peroxide) treatment was applied once a day (NÉMETH et al. 2012). Moldy or dead eggs were not removed during incubation in order to accurately determine the hatching rate.

Determination of hatching rate and hatching of the eggs

The hatching rate was determined immediately at the moment of hatching. Samples containing at least 100 eggs were removed from the 20 Zuger-jars. Photos of the samples were taken. The number of viable and non-viable eggs was counted using ImageJ software. Hatching was carried out using the generally applied method, as for the practice of the fish farm (SZABÓ 2000).

Larval rearing examination

After hatching, the individuals of the four groups were placed into a flowing water system at a density of 1100 larvae per cage, with two repetitions. During the examination, the water temperature was 15.22±1.3°C, and the dissolved oxygen level was 9.67±0.46 mg L^{-1}. The rearing period lasted for 10 days, during which the growth parameters and the morphological changes of the individuals were investigated at three different stages of larval development:

- immediately after hatching,
- at the end of the non-feeding larval stage (5 days post-hatching),
- in the feeding larval stage of 5 days (10 days post-hatching).

After starting the feeding, the fish were fed every 15 minutes (with Skretting NUTRA Pro fish food) *ad libitum*, continuously for 24 hours. The cages were cleaned twice a day, and the number of dead larvae was recorded.

On the three sampling days, the brood was transported to Gödöllő to the Department of Aquaculture, where the wet body weight and the total

body length of 50 individuals from each of the eight larval rearing units were determined, along with morphological changes observed in another set of 50 individuals. Total body length was measured in millimeters using a ruler, while wet body weight was measured on an analytical scale (AB204-S, Mettler-Toledo Kft., Budapest, Hungary) with an accuracy of 0.1 mg. To examine developmental abnormalities of the larvae, the individuals were recorded under a stereomicroscope (Leica M205 FA stereomicroscope Biomarker Ltd., Gödöllő, Hungary; Leica DFC 425C camera, Bright Field 15*-30* Biomarker Ltd., Gödöllő, Hungary).The developmental abnormalities were recorded based on the following main categories: curved body, eye deformity, craniofacial malformation, edema, yolk-sac deformation, somites deformation, hematoma, deformed tail development (MAZURIAS et al. 2009; PANZICA-KELLY et al. 2012). At the end of the 10-day experiment, survival rate was determined, excluding individuals removed during sampling.

2.5. Data analysis and evaluation

Microsoft Excel was used to record and evaluate the data (Microsoft Corporation, Redmond, WA 98052, United States of America). Data were analyzed with GraphPad Prism 5.0 for windows (GraphPad Software, La Jolla, California, United States of America) and SPSS 14.0 (SPSS Inc., Chicago, United States of America) statistical program. Before cryopreservation and after thawing, sperm parameters indicating progressive motility (pMOT, VCL, STR) were compared using one- and two-way ANOVA tests, as well as Tukey, Dunnett T3, and Bonferroni „post-hoc" tests, along with independent two-sample t-tests. For all statistical analyses, a significance level of $p<0.05$ was used. During the larval rearing investigations, wet body weight and total body length were analyzed and compared using two-way ANOVA and by pairwise comparisons with the Bonferroni „post-hoc" test. The survival rate was assessed by comparing the „Odds ratio" of the groups. Larval malformation data were evaluated using both the Kruskal-Wallis non-parametric test (with pairwise comparison using Dunn's post hoc test) and one-way analysis of variance (one-way ANOVA, with pairwise comparison using Tukey's post hoc test) (REICZIGEL et al. 2007).

3. RESULTS

3.1. Results of the comparison of hormonal treatment, various sperm sampling methods, and extenders

Results of the effect of hormonal treatment on sperm quality

Significantly higher pMOT (43±20%) and VCL (98±16 μm s^{-1}) values were recorded in the hormonally treated group compared to the control one (pMOT: 18±15%; VCL: 69±14 μm s^{-1}). In the case of STR, no significant difference was found between the control (83±3%) and the hormonally treated (79±2%) groups.

Results of the comparison of glucose and trehalose-based extenders during cryopreservation

A significantly higher pMOT value was recorded using the glucose-based extender for all dilution ratios (1:3: 18±16%, 1:9: 20±13%, 1:19: 16±12%) compared to the trehalose-based extender (1:3: 0.3±1%, 1:9: 1±1%, 1:19: 4±2%). No significant difference was observed among the different extender ratios , therefore, the 1:9 dilution ratio (HOAR et al. 1983; BOKOR et al. 2010) was chosen for the further tests, referred to literature data. For the STR parameter, the glucose-containing extender at a 1:3 dilution ratio exhibited a significantly higher value (86±4%) compared to the extender containing trehalose (38±48%).

Results of the comparison of two sperm extraction methods using a 0.5 mL straw during cryopreservation

No significant differences were found in the pMOT values between the stripped and testicular sperm groups neither before nor after cryopreservation. However, following thawing, sperm samples having been collected via dissection of the testis (29±17%) exhibited a significantly lower progressive motility compared to fresh sperm (85±10%). The progressive motility of the fresh (65±12%) and the cryopreserved group (37±27%) did not differ verifiably in case of the stripped sperm. No significant differences were found in VCL and STR values as a result of cryopreservation (control, stripped: 129±10 μm s^{-1} and 81±5%; control, testicular sperm: 128±23 μm s^{-1} and 77±6%; cryopreserved, stripped: 95±23 μm s^{-1} and 90±4%; cryopreserved, testicular sperm: 87±27 μm s^{-1} and 87±3%).

3.2. Results of the methodological development and optimization of large-scale cryopreservation of northern pike sperm

The results of the comparison of the polystyrene box and the contolled-rate freezer device in the case of 0.5 mL straw

A similarly high progressive motility value was recorded for both the polystyrene box (45±11%) and the CRF cryopreservation method (42±17%). After thawing, pMOT significantly decreased in both groups compared to the control group (69±4%). The STR was significantly higher in the control group (75±3%) than in the polystyrene box (85±3%) and CRF (86±3%). The VCL values did not show significant differences between the different groups (control: 137±7 μm s^{-1}; polystyrene box 0.5 mL straw: 136±13 μm s^{-1}; CRF 0.5 mL straw: 122±23 μm s^{-1}).

Results of the comparison of different dilution ratios in a controlled-rate freezer using a 10 mL cryotube

After thawing, no statistically verifiable differences were found between the motility parameters of the three different dilution ratios (pMOT: 1:3: 20±10%, 1:9: 30±9%, 1:19: 31±7%; VCL: 1 :3: 87±24 μm s^{-1}, 1:9: 99±14 μm s^{-1}, 1:19: 95±7 μm s^{-1}). However, compared to the fresh group (pMOT: 72±7%, VCL: 140±6 μm s^{-1}), the pMOT and VCL values of the cryopreserved groups decreased significantly. In the subsequent tests, the 1:9 dilution ratio was used for the 10 mL cryotube.

Results of testing three different thawing times for a 10 mL cryotube

No significant difference was observed in the pMOT and VCL values among the three different thawing times of the cryopreserved 10 mL cryotube (pMOT 3 minutes: 30±6%, 3.5 minutes: 29±6%, 4 minutes: 28±8%; VCL: 3 min: 93±9 μm s^{-1}, 3.5 min: 93±10 μm s^{-1}, 4 min 95±9 μm s^{-1}). However, compared to the control group (pMOT: 62±11% and VCL 148±11 μm s^{-1}), the pMOT and VCL values of all the three treated groups were significantly lower. The STR values showed no significant difference after thawing the cryopreserved samples (1:3: 85±3%; 1:9: 87±3%; 1:19: 87±2%). For further tests, the samples stored in 10 mL cryotubes were thawed for 3 minutes.

Results of the comparison of 5 mL straw and 10 mL cryotube using different freezing methods

After thawing the frozen samples, no significant differences were observed in terms of pMOT and VCL between the 5 mL straws from the polystyrene box (50±9% and 79±8 μm s^{-1}), the 5 mL straws from the CRF

(57±10% and 86±14 μm s^{-1}), and the motility values of the CRF 10 mL cryotube (41±10% and 74±11 μm s^{-1}) groups. Compared to samples having been thawed after freezing, control sperm exhibited significantly higher pMOT (74±7%) and VCL (136±7 μm s^{-1}) values. However, no significant differences were observed in terms of STR between the control (87±2%), polystyrene box 5 mL straw (90±1%), CRF 5 mL straw (89±2%), and CRF 10 mL cryotube (89±2%) groups.

3.3. Results of artificial propagation of northern pike using large-scale cryopreserved sperm and short-term larval rearing tests under hatchery conditions

The sperm measured at the beginning of the experiment showed a significantly higher pMOT value (74±7%) compared to the measurements taken 5 hours later, at the end of the experiment (31±13%), as well as compared to the three cryopreserved groups (polystyrene box 5 mL straw: 32±3%, CRF 5 mL straw: 53±6%, CRF 10 mL cryotube: 15±4%). Additionally, the sperm examined at the end of the experiment and the 5 mL straw group of the polystyrene box showed significantly higher progressive motility than the 10 mL cryotube group. As measured at the end of the experiment, in case of the CRF 5 mL straw group, a significantly higher result was recorded compared to the sperm from the polystyrene box 5 mL straw group and the CRF 10 mL cryotube group,

A significantly higher VCL result was recorded for fresh sperm (135±11 μm s^{-1}) compared to the 5 mL straw groups in the polystyrene box (84±6 μm s^{-1}), to the CRF (103±5 μm s^{-1}), and to the CRF 10 mL cryotube (58±8 μm s^{-1}) groups. Additionally, the CRF 5 mL straw group exhibited significantly higher values compared to the polystyrene box 5 mL straw and the CRF 10 mL cryotube group. A statistically significantly lower value was described in the CRF 10 mL cryotube group compared to the fresh and different cryopreserved groups. The VCL value of the sperm sample measured at the end of the experiment (84±25 μm s^{-1}) did not show any significant difference compared to the fresh sperm, to the polystyrene box 5 mL straw, and to the CRF 5 mL straw groups.

Statistically significantly higher STR values were recorded in the polystyrene box 5 mL straw (82±1%) and CRF 10 mL cryotube (84±3%) groups compared to the control sperm sample measured at the beginning of the experiment (76±4%). Measurements taken after 5 hours showed values of 69±3% for the control sample and 76±2% for the CRF 5 mL straw group. The results measured at the beginning of the experiment were significantly

higher in both the control and CRF 5 mL straw groups compared to the control group measured at the end of the experiment.

When determining the hatching rate, no significant difference was observed in the values of the fresh group (7±4%) compared to the 5 mL straw polystyrene box (16±8%), 5 mL straw CRF (21±16%), and CRF 10 mL cryotube (11±2%) groups.

In the evaluation of body weight data, immediately after hatching, a significantly higher value was observed for the 5 mL straw polystyrene box (8.45±0.89 mg) and the 10 mL cryotube CRF (8.39±1.49 mg) groups compared to the control (8.11±1.26 mg) and the 5 mL straw CRF (7.87±0.85 mg) groups. However, at non-feeding larval stage no significant differences were found between the body weight data of the four different groups. At 10 days post-hatching, a significantly higher value was recorded in the frozen groups (polystyrene box 5 mL straw: 17.38±2.25 mg; CRF 5 mL straw: 17.58±2.18 mg; CRF 10 mL cryotube: 17.28±1.95 mg) compared to the control group (16.48±2.04 mg).

In the body length data immediately after hatching, a significantly higher value was recorded in the cryopreserved groups (polystyrene box 5 mL straw: 8.37±0.68 mm, CRF 5 mL straw: 8.49±0.67 mm, and CRF 10 mL cryotube: 8.54±0.66 mm) compared to the control group (8.22±0.86 mm). The CRF 5 mL straw and 10 mL cryotube groups were significantly higher than the polystyrene box 5 mL straw group. At non-feeding larval stage , no statistically significant differences were found between the control group (12.89±0.65 mm) and the cryopreserved groups (polystyrene box 5 mL straw: 12.91±0.70 mm, CRF 5 mL straw: 12.98±0.64 mm, and CRF 10 mL cryotube: 12.77±0.67 mm). At the end of the experiment, a significantly higher body length was recorded in the cryopreserved groups (polystyrene box 5 mL straw: 14.83±0.86 mm, CRF 5 mL straw: 14.71±0.67 mm, and CRF 10 mL cryotube: 14.86±0.94 mm) compared to the control group (12.89±0.65 mm)

During the 10-day larval rearing period, no significant difference was recorded in the survival rates between larvae stemming from fresh (69%) and those stemming from frozen sperm using the three cryopreservation methods (polystyrene box 5 mL straw: 80%, CRF 5 mL straw: 74%, CRF 10 mL cryotube: 74%).

Results of larval morphology examinations

Evaluating the treatment groups, no significant difference was found between the malformation rates of larvae originating from fresh and the ones originating from cryopreserved sperm (fresh: 11.71%, polystyrene box 5 mL straw: 8.97%, CRF 5 mL straw: 7.66%, CRF 10 mL cryotube:

9.96%). In the larval morphological studies, significantly more deformed larvae were recorded immediately after hatching (17.2%), compared to the non-feeding larval stage (4.75%) and the 5-day feeding larval stage (6.28%), where several malformations were often observed in one individual. No statistically significant difference was found between the larval deformity rates immediately after hatching. At non-feeding larval stage, a low number of larval malformations were observed in each group; the malformed individuals are probably selected over time. During the morphological examination of larva 10 days post-hatching, similar to the non-feeding larval stage, a low number of deformed individuals were observed. Additionally, a new type of developmental abnormality, hematoma emerged, which may have appeared due to physical injury, and was observed in all groups.

4. CONCLUSIONS AND PERSPECTIVES

4.1. Conclusions

4.1.1. Conclusions of different sperm sampling methods and extenders

Significantly higher motility results can be achieved by hormonal induction, as confirmed by descriptions in previous studies (HULAK et al. 2008a; CEJKO et al. 2018). Before cryopreservation, no significant difference in progressive motility was found between the stripping and testis dissection methods. However, after thawing, the pMOT value of the testicular sperm group decreased significantly. Despite this, the testis dissection method may still be justified, as it allows the extraction of up to ten times more sperm compared to stripping. In hatcheries, to sacrifice male northern pikes is a commonly used in practice. Currently, there is no fish-friendly method available that gives a solution for the difficulties associated with stripping (DE MONTALEMBERT et al. 1978; BILLARD & MARCEL 1980; LAHNSTEINER et al. 1998; ROSSEN & ABADJIEVA 2015; KRISTIANSEN et al. 2020).

Optimized technology for cryopreserving pike sperm has been developed, utilizing a newly developed species-specific extender that has also demonstrated its effectiveness under hatchery conditions. An extender with an osmolality of 386 mOsmol kg^{-1} has been developed during my research. It consisted of 150 mM glucose, 75 mM NaCl, 30 mM KCl, 1 mM $Na_2HPO_4*12H_2O$, 1 mM $MgCl_2*6H_2O$, 1 mM $CaCl_2*2H_2O$, 20 mM Tris, and 0.5% BSA, with a pH of 8±0.02. This extender created an environment similar to pike seminal fluid and prevented sperm activation. In my examinations, a significantly higher progressive motility value has been observed with the extender containing glucose at all three dilution ratios compared to the one containing trehalose. Evaluating previous studies (BABIAK et al. 1997; ALAVI et al. 2009; DIETRICH et al. 2016; CEJKO et al. 2020b), the sugar content of the extenders emerges as a significant factor in the cryopreservation of northern pike sperm. In my research, during sperm cryopreservation, glucose has been proved to be a more effective extracellular cryoprotectant.

4.1.2. Conclusions of the methodological development and optimization of large-scale cryopreservation of northern pike sperm

Northern pike sperm was successfully cryopreserved in 0.5 mL straws using both polystyrene box and CRF. During the comparative study, after thawing the sperm samples, no significant differences were found in the measured parameters (pMOT, VCL, STR). As far as I know, using CRF equipment northern pike sperm has not been cryopreserved yet. However, based on my results, it can be concluded that the cryopreservation protocol developed by BERNÁTH et al. (2016a, 2016b) for perch (*Perca fluviatilis*) and carp can also be successfully applied to northern pike sperm in a CRF. The use of a CRF provides standardized and optimized conditions, enhancing the repeatability of freezing protocols, unlike polystyrene boxes, where results may be influenced by external environmental factors such as box size, external temperature, and air movement.

After thawing the 10 mL cryotube, no significant differences were observed in the motility parameters between the 1:3, 1:9, and 1:19 dilution ratios. The 10 mL cryotube I used contained a sufficient amount of sperm even with a higher dilution ratio (1:9). However, considering its potential use in hatcheries, further testing and adaptation of the 1:3 sperm-to-extender ratio for hatchery applications should be considered.

The three chosen thawing periods (3 minutes, 3.5 minutes, and 4 minutes) did not affect the examined motility parameters. During artificial fertilization of fish, the timing of using sexual products is crucial, as their quality continuously and rapidly decreases after stripping (LEGENDRE et al. 1996; KRISTAN et al. 2018). Therefore, when thawing sperm, the shortest possible time should be selected. Based on the results of my own tests, northern pike sperm cryopreserved in a 10 mL cryotube tolerates rapid temperature changes, which may enhance its applicability in hatchery conditions.

As far as I know from the scientific literature, Lahnsteiner et al. (1998) successfully cryopreserved pike sperm in the largest volume (using 1.2 mL straws. therefore, I can compare my experiments with the results of examinations conducted on other fish species, such as frozen carp (VÁRKONYI et al. 2019), and wels catfish (*Silurus glanis*) (BOKOR et al. 2019). These studies utilized protocols similar to mine, and I achieved similarly high motility results. The findings from previous researches, along with my own work, confirm that the use of 10 mL cryotubes is feasible for cryopreserving sperm from economically valuable fish species, such as the northern pike, under hatchery conditions. Furthermore,

attention should be given to cryopreserving 5 mL straws in both CRFs and polystyrene boxes. In my research, no significant differences were found in motility or hatching rate results between the sperm cryopreserved using the three different methods.

Based from my presented results that the three cryopreservation methods investigated can also be effective in artificial propagation of northern pike in hatchery. One of the limiting factors of pike propagations is the adequate number of males, as well as the quantity and quality of the sperm that can be extracted. The storage and use of cryopreserved sperm in the hatchery can be an alternative solution if the appropriate number of males is not available in the given periods. Fish sperm cryopreservation is developing continuously and dynamically, despite this, apart from a few exceptions, its application has not been transfered into practice (TIERSCH 2008; URBÁNYI 2011). The most determining factor is the cost of cryopreservation. It is necessary to enhance that similar results to the CRF can be achieved by freezing the 5 mL straw in polystyrene box, which is also significantly more cost-effective, as it is not necessary to purchase the extremely expensive equipment. It can be used more effectively in hatchery conditions (e.g. easier transport, does not require a power source). However, the disadvantage of the polystyrene box is that it is less standardizable than the CRF.

4.1.3. Conclusions of the artificial propagation of pike with large-scale cryopreserved sperm and short-term larval rearing examination

The applicability of three different freezing techniques adapted for cryopreservation of pike sperm was tested under hatchery conditions. The literature has only presented the use and studies of laboratory-based, low-egg and/or low-volume cryopreserved northern pike sperm (BABIAK et al. 1995, 1997, 1999; GLOGOWSKI et al. 1997; LAHNSTEINER et al. 1998; LAHNSTEINER & MANSOUR 2008; DZYUBA et al. 2010; ZHANG et al. 2011; DIETRICH et al. 2016; CEJKO et al. 2016, 2020b; KRISTAN et al. 2020). The success of practical application of cryopreserved sperm is demonstrated by fertility and hatching results. In order to determine the exact hatching rate, moldy and dead eggs were not removed from the Zuger-jars, resulting in a high egg mortalitys similar to the findings of SZABÓ (2016b). In the egg incubation period, the water temperature was also suboptimal due to external factors (BONDARENKO et al. 2015b; POSPISILOVA et al. 2019), resulting in a high rate of egg mortality. There was no significant difference in hatching results between the control and

cryopreserved groups. Possible reasons for the low fertility and hatching results may include the lower quality of the eggs, the elapsed time between egg and sperm extraction and their usage (KRISTAN et al. 2020), or the need to use a larger quantity of cryopreserved sperm compared to fresh sperm during fertilization (HOAR et al. 1983).

To the best of my knowledge, there are no publications demonstrating the developmental outcomes of larvae stem from cryopreserved northern pike sperm in large quantities. During my larval rearing experiment, it is hypothesized that malformed individuals may have perished after hatching, resulting in significantly fewer instances of developmental abnormalities recorded at 5 and 10 days post-hatching. Across the three sampling periods, no statistically significant differences were found in the occurance of developmental abnormalities between the fresh and cryopreserved groups. These results demonstrate that the cryopreservation methods utilized do not adversely affect the morphological development of the larvae. Throughout the 10-day rearing experiment, examining various developmental stages (immediately after hatching, at non-feeding larval stage and during the feeding larval stage (10 days post-hatching)), no detrimental effects attributable to sperm cryopreservation were observed either in the growth parameters of the larvae or in the survival rate. In some cases, the cryopreserved groups even outperformed the results of the control group. Similar findings were reported during short-term larval rearing experiments using cryopreserved sperm by BOKOR et al. (2015) with wels catfish and by LIU et al. (2015) in their studies with Atlantic halibut (*Hippoglossus hippoglossus*).

4.2. Suggestions for further research

Suggestion for artificial propagation of northern pike:

- Hormonal induction and dissection of the testis for the extraction of the sperm.

Suggestions for cryopreservation of northern pike sperm:

- For cryopreservation I recommend the adaptation of the glucose-based extender developed in my work (150 mM glucose, 75 mM NaCl, 30 mM KCl, 1 mM $Na_2HPO_4*12H_2O$, 1 mM $MgCl_2*6H_2O$, 1 mM $CaCl_2*2H_2O$, 20 mM Tris, and 0.5% BSA, pH 8±0.02) and 10% methanol as cryoprotectant.
- During cryopreservation, I recommend using a 5 mL straw in a polystyrene box and in a controlled-rate freezer, as well as a 10 mL cryotube in a controlled-rate freezer (if technical conditions are available)
- To achieve a higher sperm cell density and cryopreserved volume, I recommend testing the 1:3 (sperm:extender) dilution ratio of the 10 mL cryotube in a controlled-rate freezer device during experiments on the artificial propagation of pike.
- I recommend using the 10 ml cryotube thawing time of 3 minutes at a water temperature of 40 °C.
- I recommend the development of standardized technology (technical development) that can be used to thaw large artificial straws or cryotubes simultaneously.
- I recommend establishing a gene bank storage for norhern pike sperm cryopreserved in a large-capacity device, which would also be accessible to fish farmers.
- I recommend conducting a longer-term developmental study of the northern pike larvae originated from cryopreserved sperm (e.g., fry rearing or annual pond rearing period).

5. NEW SCIENTIFIC RESULTS

1. I developed an extender solution specifically suitable for cryopreservation of northern pike sperm, the composition of which is as follows: 150 mM glucose, 75 mM NaCl, 30 mM KCl, 1 mM $Na_2HPO_4*12H_2O$, 1 mM $MgCl_2*6H_2O$, 1 mM $CaCl_2*2H_2O$, 20 mM Tris, and 0.5% BSA, pH 8±0.02).

2. I have successfully utilized the cryopreservation method for northern pike sperm using a 5 mL straw, both in a polystyrene box and in a controlled-rate freezer.

3. I have successfully developed the technology for using a 10 mL cryotube in a controlled-rate freezer. I optimized the cryopreservation of northern pike sperm using a dilution ratio of 1:9, as well as thawing the 10 mL cryotube within a 3-minute period at a water temperature of 40°C.

4. I have successfully propagated northern pike under hatchery conditions using a large amount of cryopreserved sperm. This was achieved using 5 mL straws in a polystyrene box and controlled-rate freezer, as well as 10 mL cryotubes in a controlled-rate freezer. This approach offers the opportunity for industrial-scale, hatchery application.

5. Concerning northern pike, I was the first to examine the growth parameters and the occurrence of developmental abnormalities in larvae stemming from fresh sperm and sperm having been frozen using three different large-scale cryopreservation methods (5 mL straw in a polystyrene box and controlled-rate freezer, 10 mL cryotube in a controlled-rate freezer). The results of the present doctoral study demonstrate that the sperm cryopreservation method I have developed does not have a negative effect on the growth of the larvae, the occurrence of morphological abnormalities, or the survival rate during the examined stages of larval development (0-10 days post-hatching)

6. PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

6.1. International journals

Gergely Bernáth, Zsolt Csenki, Zoltán Bokor, Levente Várkonyi, **József Molnár**, Tamás Szabó, Ádám Staszny, Árpád Ferincz, Krisztián Szabó, Béla Urbányi, Lilianna Olimpia Pap, Balázs Csorbai (2018): The effects of different preservation methods on ide (*Leuciscus idus*) sperm and the longevity of sperm movement. Cryobiology 81, p. 125–131.

Zoltán Bokor, Gergely Bernáth, Levente Várkonyi, **József Molnár**, Zete Levente Láng, Zsófia Tarnai-Király, Enikő Solymosi, Béla Urbányi (2019): The Applicability Of Large-Scale Sperm Cryopreservation In Wels Catfish (*Silurus Glanis*) Optimized For Hatchery Practice. Aquaculture 506, p. 337-340.

Levente Várkonyi, Zoltán Bokor, **József Molnár**, Ferenc Fodor, Zsolt Szári, Árpád Ferincz, Ádám Staszny, Levente Zete Láng, Balázs Csorbai, Béla Urbányi, Gergely Bernáth (2019): The comparison of two different extenders for the improvement of large-scale sperm cryopreservation in common carp (*Cyprinus carpio*). Reproduction In Domestic Animals 54(3) p. 639-645.

József Molnár, Zoltán Bokor, Levente Várkonyi, Tibor Izsák, Enikő Füzes-Solymosi, Levente Zete Láng, Balázs Csorbai, Zsófia Tarnai-Király, Béla Urbányi, Gergely Bernáth (2020): The systematic development and optimization of large-scale sperm cryopreservation in northern pike (*Esox lucius*), Cryobiology 94, 26-31.

Bernadett Pataki, Ákos Horváth, Gergely Mészáros, Nevena Kitanovic, András Ács, Árpád Hegyi, **József Molnár**, Balázs Csorbai, Béla Urbányi (2022): Adjustment of common carp sperm concentration prior to cryopreservation: Does it matter? Aquaculture Reports, 24, 101109.

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storage and the applicability of large-scale cryopreservation in chub (*Squalius cephalus*) sperm. Cryobiology 113, paper: 104588, 1-9 p.

Zoltán Bokor, Zete Levente Láng, Levente Várkonyi, Ferenc Fodor, Borbála Nagy, Endre Csókás, **József Molnár**, Balázs Csorbai, Zsolt Csenki-Bakos, Bence Ivánovics, Jeffrey Daniel Griffitts, Béla Urbányi, Gergely Bernáth (2023): The growth performance of pond-reared common carp (*Cyprinus carpio*) larvae propagated using cryopreserved sperm. Fish Physiology and Biochemistry, paper: 3440158, 11-12. p.

Borbála Nagy, Balázs Csorbai, Levente Várkonyi, Ádám Staszny, **József Molnár**, Zete Levente Láng, Tamás Bartucz, István Ittzés, Béla Urbányi, Zoltán Bokor, Gergely Bernáth (2024): Comparative study on the gamete quality, artificial propagation and larval development of common goldfish, shubunkin, black moor, and oranda variants of goldfish (*Carassius auratus*). Aquaculture 582, paper 740502, 1-10. p.

Zoltán Bokor, Ferenc Fodor, Levente Várkonyi, Borbála Nagy, Zete Levente Láng, Árpád Ferincz, Ádám Staszny, **József Molnár**, Kinga Katalin Lefler, Balázs Csorbai, Zoltán Vancsura, Zsolt Szári, Béla Urbányi, Gergely Bernáth (2024): Biological Parameters and Spermatogenesis in Razorfish (*Pelecus cultratus*) Population Inhabiting the Largest Shallow Lake of Central Europe (Lake Balaton): Studies for In Vitro Conservation Purposes. Aquaculture, paper: 6695280, 1-8 p.

6.2. National Journals

Levente Várkonyi, Zoltán Bokor, Árpád Ferincz, Ádám Staszny, Ferenc Fodor, Zsolt Szári, Béla Urbányi, **József Molnár**, Gergely Bernáth (2019): The applicability of 10 mL cryotubes for sperm cryopreservation in a Hungarian carp landrace (*Cyprinus carpio carpio morpha accuminatus*). Agrártudományi Közlemények/Acta Agraria Debreceniensis, 75. p. 5., 93-97.

Molnár József, Várkonyi Levente, Füzes-Solymosi Enikő, Birkó-Sulyok Zita Katalin, Izsák Tibor, Láng Levente Zete, Csenki-Bakos Zsolt, Staszny Ádám, Urbányi Béla, Bernáth Gergely, Bokor Zoltán (2020): Mélyhűtött csuka (*Esox lucius*) spermából származó lárvák különböző növekedési és morfológiai paramétereinek vizsgálata rövidtávú lárvanevelés során. Animal welfare, ethology and housing systems, (16)2 p. 146-155.

Nagy Borbála, Várkonyi Levente, Fodor Ferenc, Koltai Tamás, Bodnár Ádám, **Molnár József**, Láng Levente Zete, Izsák Tibor, Staszny Ádám, Ferincz Árpád, Birkó-Sulyok Zita Katalin, Urbányi Béla, Szári Zsolt, Bokor Zoltán, Bernáth Gergely, 2020.: A balatoni kősüllő (Sander volgensis, Gmelin, 1788) állomány hímivrsejt minőségének és mélyhűtésének vizsgálata az indukált szaporítás fejlesztése és a génbanki megőrzés céljából. ANIMAL WELFARE, ETOLÓGIA ÉS TARTÁSTECHNOLÓGIA (16)2 pp. 163-169.

Borbála Nagy, Gergely Bernáth, Levente Várkonyi, **József Molnár**, Levente Zete Láng, Tibor Izsák, Tamás Bartucz, István Ittzés, Áron Ittzés, Béla Urbányi, Zoltán Bokor (2022): The comparison of sperm motility and density in four different goldfish (*Carassius auratus*) types. Agrártudományi Közlemények/Acta Agraria Debreceniensis, 2022. No. 1., pp. 135-140.

Szabó Tamás, **Molnár József**, Müller Tamás, Nyitrai Márk, Tóth Gábor, Ugrai Zoltán, Szabó Krisztián, Urbányi Béla (2022): Csukaikra termékenyítéséhez szükséges sperma mennyiségének meghatározása keltetőházi szaporítás során. Halászat-Tudomány, 8(2): pp. 14-18.

6.3. Inernational conference publications
6.3.1. Oral presentations

Gergely Bernáth, Zsolt Csenki-Bakos, Zoltán Bokor, Levente Várkonyi, **József Molnár**, Alexandra Kajtár, Tamás Szabó, Ádám Staszny, Árpád Ferincz, Krisztián Szabó, Béla Urbányi, Balázs Csorbai (2017): The effects of different preservation methods on Ide (*Leuciscus idus*) sperm and the longevity of sperm movement. 6th International Workshop on the Biology of Fish Gametes, Czech Republic, České Budějovice, 2017. September 4-7. Kivonat p. 81.

József Molnár, Levente Várkonyi, Béla Urbányi, Enikő Solymosi, Zita Katalin Birkó-Sulyok, Tibor Izsák, Levente Zete Láng, Gergely Bernáth, Zoltán Bokor (2018): The development of a new large-scale sperm cryopreservation method in northern pike (*Esox lucius*). Seventh International Young Researchers' Conference of NACEE, Gorki, Belarus, 2018. december 11-14. Kivonat. p. 4.

Levente Várkonyi, Zoltán Bokor, **József Molnár,** Ferenc Fodor, Zsolt Szári, Árpád Ferincz, Ádám Staszny, Zita Birko-Sulyok, Levente Zete

Láng, Balázs Csorbai, Béla Urbányi, Gergely Bernáth (2018): The comparison of two different extenders and the improvement of large-scale sperm cryopreservation in common carp (*Cyprinus carpio*). Seventh International Young Researchers' Conference of NACEE, Gorki, Belarus, 2018. december 11-14. Kivonat. p. 4.

6.3.2. Poster presentations

Zoltán Bokor, **József Molnár**, Árpád Ferincz, Levente Várkonyi, Zsolt Szári, Ferenc Fodor, Tibor Tulipán, Gábor Nagy, Béla Urbányi, Gergely Bernáth (2017): Investigation of male maturation out-of- and during the spawning season in Sichel (*Pelecus cultratus*). 6th International Workshop on the Biology of Fish Gametes, Czech Republic, České Budějovice, September 4-7. Abstract book p. 94.

Gergely Bernáth, Levente Várkonyi, Előd Szanati, **József Molnár**, Alexandra Kajtár, Solymosi Enikő, Béla Urbányi, Zoltán Bokor (2017): Practical improvement of Pike (*Esox lucius*) sperm cryopreservation. Aquaculture Europe '17 Dubrovnik, Croatia, October 16-20, 2017. Abstract book p. 101-102.

Gergely Bernáth, Levente Várkonyi, Ferenc Fodor, **József Molnár**, Kajtár Alexandra, Árpád Ferincz, Ádám Staszny, Béla Urbányi, Zsolt Szári, Tibor Tulipán, Gábor Nagy, Gergely Boros, András Specziár, Zoltán Bokor (2017): Sex ratio and ichtiologycal paramteres in Sichel (*Pelecus cultratus*) caught out-of- and during the spawning season. Aquaculture Europe '17 Dubrovnik, Croatia, October 16-20, 2017. Abstract book p. 103-104.ű

Gergely Bernáth, Levente Várkonyi, **József Molnár**, Enikő Solymosi, Béla Urbányi, Zoltán Bokor (2018): The optimization of the large-scale cryopreservation and the motility assessment in wels catfish (*Silurus glanis*) sperm. 8th International Water and Fish Conference, Conference Proceedings, Serbia, Belgrade, June 13-15., Abstract book p. 144-147.ű

Zoltán Bokor, Gergely Bernáth, Levente Várkonyi, **József Molnár**, Tamás Szabó, Béla Urbányi, Balázs Csorbai (2018): The development of an innovative technology for carnivorous fish production that fits well in the traditional production environment. 8th International Water and Fish Conference, Conference Proceedings, Serbia, Belgrade, June 13-15., Abstract book p. 214-216.

József Molnár, Zoltán Bokor, Levente Várkonyi, Solymosi Enikő, Béla Urbányi, Gergely Bernáth (2018): The methodical improvement and large-scale cryopreservation of northern pike (*Esox lucius*) sperm. 8th International Water and Fish Conference, Conference Proceedings, Serbia, Belgrade, June 13-15., Abstract book p. 237-240.

Levente Várkonyi, **József Molnár**, Ádám Staszny, Ádám Ferincz, Zoltán Bokor, Béla Urbányi, Gergő Németh, Gergely Bernáth (2018): The comparison of two hormonal induction method in the Heviz carp (*Cyprinus carpio carpio morpha hungaricus*). 8th International Water and Fish Conference, Conference Proceedings, Serbia, Belgrade, June 13-15., Abstract book p. 272-275.

Levente Várkonyi, **József Molnár**, Zete Levente Láng, Árpád Ferincz, Ádám Staszny, Ferenc Fodor, Zsolt Szári, Béla Urbányi, Zoltán Bokor, Gergely Bernáth (2018): Methodical improvement of sperm cryopreservation in a hungarian common carp landrace (*Cyprinus Carpio Carpio Morpha Accuminatus*) In: Aqua 2018: #We R Aquaculture, Abstract book p. 774.

Borbála Nagy, Gergely Bernáth, Levente Várkonyi, **József Molnár**, Levente Zete Láng, Tibor Izsák, Tamás Bartucz, István Ittzés, Áron Ittzés, Béla Urbányi, Zoltán Bokor (2021): The comparison of sperm motility and density in four different goldfish (*Carassius auratus*) types. Aquaculture Europe, Funchal, Madeira, October 4 – 7, 2021., Abstract book p. 859.

Gergely Bernáth, Levente Várkonyi, Balázs Csorbai, Levente Zete Láng, **József Molnár**, Tamás Bartucz, Borbála Nagy, István Lehoczky, Gergely Szabó, Béla Urbányi, Zoltán Bokor (2021): The improvement of sperm cryopreservation methods in the endemic tench (*Tinca tinca*) and crucian carp (*Carassius carassius*) for conservation purposes. Aquaculture Europe, Funchal, Madeira, October 4 – 7, 2021., Abstract book p. 140-141.

Tamás Szabó, **József Molnár**, Márk Nyitrai, Gergely Tóth, Zoltán Ugrai, Krisztián Szabó, Béla Urbányi (2021): Determination of the quantity of sperm required for the fertilization of northern pike (*Esox lucius*) eggs during hatchery breeding. Aquaculture Europe, Funchal, Madeira, October 4 – 7, 2021., Abstract book p. 1263-1264.

6.4. National conference publications
6.4.1. Oral presentation

Molnár József, Békési Richárd, Várkonyi Levente, Csorbai Balázs, Csenki-Bakos Zsolt, Müller Tamás, Szabó Tamás, Urbányi Béla (2019): A népesítési sűrűség növekedésre és megmaradásra gyakorolt hatásának vizsgálata a márna (*Barbus barbus*) intenzív rendszerben történő előnevelése során. VII. Gödöllői Állattenyésztési Tudományos Nap, Gödöllő, 2019. november 22., Kivonat p. 53.

Molnár József, Várkonyi Levente, Füzes-Solymosi Enikő, Birkó-Sulyok Zita Katalin, Izsák Tibor, Láng Levente Zete, Csenki-Bakos Zsolt, Staszny, Ádám, Urbányi Béla, Bernáth Gergely, Bokor Zoltán (2019): Mélyhűtött csuka (*Esox lucius*) spermából származó lárvák különböző növekedési és morfológiai paramétereinek vizsgálata rövidtávú lárvanevelés során. VII. Gödöllői Állattenyésztési Tudományos Nap, Gödöllő, 2019. november 22., Kivonat p. 54.

Nagy Borbála, Várkonyi Levente, Fodor Ferenc, Koltai Tamás, Bodnár Ádám, **Molnár József**, Láng Levente Zete, Izsák Tibor, Staszny Ádám, Birkó-Sulyok Zita Katalin, Urbányi Béla, Szári Zsolt, Bokor Zoltán, Bernáth Gergely (2019): A balatoni kősüllő (*Sander volgensis*) állomány hímivarsejt minőségének és mélyhűtésének vizsgálata az indukált szaporítás fejlesztése és a génbanki megőrzés céljából. VII. Gödöllői Állattenyésztési Tudományos Nap, Gödöllő, 2019. november 22., Kivonat p. 55.

Molnár József, Várkonyi Levente, Urbányi Béla, Solymosi Enikő, Birkó-Sulyok Zita Katalin, Izsák Tibor, Láng Levente Zete, Bernáth Gergely, Bokor Zoltán (2018): Csuka (*Esox lucius*) sperma üzemi mélyhűtési technológiájának kidolgozása. XXVIII. Óvári Tudományos Napok, 2018.11.09-10. Kivonat p. 151.

Várkonyi Levente, Bokor Zoltán, Ferincz Árpád, Staszny Ádám, **Molnár József**, Birkó-Sulyok Zita, Láng Levente Zete, Juhász Vera, Fodor Ferenc, Szári Zsolt, Urbányi Béla, Bernáth Gergely (2018): A hévízi törpenövésű magyar vadponty (*Cyprinus carpio morpha Hungaricus*), valamint a balatoni sudár ponty (*Cyprinus carpio Morpha accuminatus*) szaporodásbiológiájának vizsgálata és in vitro spermabankjának létrehozása**.** XXVIII. Óvári Tudományos Napok, 2018.11.09-10. Kivonat p. 154.

Pataki Bernadett, Staszny Ádám, Mészáros Gergely, Kitanović Nevena, Ács András, Hegyi Árpád, **Molnár József**, Csorbai Balázs, Urbányi Béla, Horváth Ákos (2022): Morfológiai változások pontyban (*Cyprinus carpio*): befolyásolja e az utódok külalakját a mélyhűtött spermával történő termékenyítés? XLVI. Halászati Tudományos Tanácskozás, Szarvas, 2022. május 25-26. Kivonat p. 61-64.

6.4.2. Poster presentation

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