In vitro oocyte maturation dynamics in pikeperch *Sander lucioperca*

The objective of the present project was to establish a protocol for *in vitro* maturation (IVM) of pikeperch oocytes and further evaluate its usefulness as a predictor of oocyte maturation competence (OMC) for successful artificial reproduction.

WP1 – Development of culture medium for the maturation of pikeperch oocytes

This WP was conducted at the initiation of the project in October 2021 on oocytes obtained from fish prepared for out-of-season reproduction in fully controlled conditions. At the time of sampling, fish oocytes were fully grown with 936 ± 26 µm. Media used for ovarian follicle isolation and *in vitro* culture were prepared in sterile conditions before use. The Leibovitz L-15 culture media (Carl Roth Gmbh & Co., Karlsruhe, Germany) was diluted with sterile ddH2O to 90% (90% L-15; 288 ± 7 mOsm/kg), while the recipe for the Cortland's medium was: 124 mM NaCl, 5 mM KCl, 1.6 mM $CaCl_2 \cdot 2H_2O$, 12 mM $NaHCO_3$, 3 mM $NaH_2PO_4 \cdot H_2O$, 1.1 mM $MgCl_2 \cdot 6H_2O$, 0.9 mM $MgSO_4 \cdot 7\ H_2O$, 5.6 mM D-Glucose; 303 ± 5 mOsm/kg. For a base IVM media, all media were supplemented with 50 IU/mL penicillin, 50 µg/mL streptomycin, 2.5 µg/mL amphotericin B and 15 mM HEPES (pH 7.5). Likewise, culture media supplementation was included in base IVM media in the form of 0.1–1% (w/v) Bovine Serum Albumin (BSA) or 2–20% (v/v) Fetal Bovine Serum (FBS). Media pH was adjusted to a range of 7.5 to 9.0 with 0.1–1 M NaOH or 0.1–1 M HCl. Finally, the IVM media was sterilized by filtration using a PES syringe filter and kept at 3–4 °C until use. To prevent the follicular layer cells from adhering to the surface of the vessels, plates with cell-repellent surfaces (for suspension culture) were used (CELLSTAR®, Greiner Bio-One, Hungary).

To evaluate the final oocyte maturation (FOM) stage of IVM oocytes without clarification, a comparison of non-cleared and cleared oocytes was performed. Considering that both oil globule formation and germinal vesicle (GV) migration and breakdown (GVBD) are necessary for characterizing pikeperch FOM stages (Żarski et al., 2012), the *in vitro* classification was based on oil globule formation and the ooplasm translucency, as GV was not visible without clarification (Ljubobratović et al., 2023). The mean FOM stage at each moment of the evaluation was calculated according to the maturing oocytes (oocytes in stage II or higher) only, while before noticing stage II, the sample was considered to be in stage I. This procedure was used in all trials within the project.

The first experiment was conducted in 24-well plates. Significantly higher FOM stage at 72h post-induction was reached in L-15 medium compared to Cortland's (5.3 ± 0.8 vs 2.8 ± 1.0, p = 0.028), while the difference in maturation percent showed a tendency toward significance in the same direction (11.2% ± 1.8 % vs. 2.5 ± 4.3 %, p = 0.077).

Respecting the low oocyte maturation rates in the first experiment, the two consecutive experiments were conducted in a bigger 6-well plates. It led to an overall higher rate of maturing oocytes with a similar direction in means of difference between the media. No statistical difference was shown in any of the assessed parameters; nevertheless, all were in favor of L-15 medium. Thus, the percentage of maturing oocytes was, on average, 80-90% higher in L-15 than in Cortland's medium (24.5 ± 17.2 % vs. 13.1 ± 17.1 % in Trial 2, and 21.9

± 7.8 % vs. 13.4 ± 16.9 % in Trial 3). A similar direction was in the case of the 72h FOM index, reaching about two stages lower and several times more variable in Cortland's medium (5.7 ± 0.6 vs. 3.8 ± 2.5 in Experiment 2, and 5.5 ± 0.9 vs. 3.5 ± 2.3 in Experiment 3).

According to the achieved results, our further research was conducted in modified L-15 medium in 6-well plates.

WP2 - Evaluation of different hormones on the *in vitro* maturation dynamics in pikeperch oocytes

Two trials were conducted on the oocytes of fish prepared for out-of-season reproduction in fully controlled conditions. The first trial was conducted in September 2022, using the oocytes of a single female of size 923 ± 23 µg. The second trial was performed in November 2022, and included an *in vitro* vs. *in vivo* comparison using different hormones for *in vitro* maturation to conclude the usefulness of the procedure for the final goal of OMC evaluation.

In Trial 1, two hormones were assessed for protocolization: 17α,20β-dihydroxy-4-pregnen-3-one (DHP; Cayman Chemicals, USA) and human chorionic gonadotropin (hCG, Sigma-Aldrich, Hungary). Hormonal treatments included for IVM trials were in a concentration range of 10, 100, and 1000 ng/mL for DHP, and 5, 10, and 20 IU/mL for hCG. Follicles were incubated for a total of 144 h under gentle agitation (70 rpm) on an orbital platform shaker at 13 ± 0.1 °C in an incubator with ambient atmosphere. Experimental groups were sampled every 12 hours. The stages of ovarian follicle maturation were characterized following the classification described in WP1, whereby all follicles in Stages II and above were considered maturing. The progression of maturation at each timepoint was expressed as the mean stage of all follicles within the experimental group (FOM index). The timepoint at which the FOM index reached GVBD was recorded as the maturation time. Oil globule fragmentation (OG-frag) rate in GVBD oocytes was defined as the presence of more than two globules and calculated as the percentage of fragmented lipid droplets among all GVBD oocytes.

Significantly higher share of oocytes reaching GVBD was found in 100 and 1000 ng/mL DHP variants compared to 10 ng/mL, while in the case of hCG, there were no differences between the compared dosages, and these variants were likewise similar between the two hormones, being close to 60% on average. In terms of dynamics, the 100 and 1000 ng/mL DHP doses promoted GVBD at 60 h faster than 10 ng/mL, which needed 72h. On the other hand, the hCG-induced IVM was slower, reaching GVBD at 84h post-induction without differences among the dosages. OG-frag rate was significantly lower in all hCG dose groups, being close to 0%, while all DHP doses promoted significantly higher rates and in the range of means of 5-10%. Among DHP groups, 10 ng/mL caused significantly lower oil droplet fragmentation than the other two higher DHP doses.

Trial 2 was conducted during out-of-season pikeperch reproduction, in a total of 13 females, with a mean oocyte size of 1023 ± 26 µm that were hormonally stimulated. At the time of hormonal induction, five samples of oocytes were taken from each female using a catheter. Four samples were used for duplication of each hormone, while the fifth sample was used for evaluation of the oocyte diameter. Immediately upon biopsy, the oocytes of each female were

stocked in separate wells of 6-well cell culture plates. Each well was filled with 90% L15 medium and 5 IU/mL of hCG or 100 ng/mL DHP, according to the results of the previous trials. Wells were further placed onto an orbital shaker and incubated at 13°C for a total of 96 h. Starting from 48 h post-stimulation, IVM of oocytes was monitored every 4 h, while the same procedure in females was started 120 h post-stimulation and was performed every 24 h. In this way, FOM dynamics and both *in vivo* and *in vitro* latency times were assessed and correlated.

The strongest predictor of *in vivo* latency time (LT) was hCG-induced IVM FOM stage at 56h post-induction (r = -0.907), while in the case of DHP, the strongest correlation was found at 48h post-induction (r = -0.712). Accordingly, we concluded that hCG is the most applicable hormone for IVM of pikeperch oocytes, which was used in our future trials during the project time.

WP3 - Comparative studies of *in vivo* vs *in vitro* oocyte maturation dynamics

In total, five trials have been conducted in this WP and will be presented chronologically. The first trial took place before the completion of WP2 and, therefore, in this trial, DHP was used instead of hCG for IVM, unlike in subsequent trials.

Trial 1 – The first evaluation on the applicability of IVM as an LT predictor on both population and individual levels

In January 2022, the preseasonal artificial reproduction was carried out on two populations of outdoor-reared pikeperch breeders. One group consisted of breeders originating from wild breeders from the oxbow of the river Körös under domestication (F1 generation), grown on fully formulated diets – HAKI (12 females, 3.1 ± 0.6 kg), while the other broodstock originated from ponds in the Czech Republic – CZECH (nine females, 2.9 ± 0.7 kg). Fish were transported to the indoor hatchery and hormonally stimulated using salmon gonadotropin-releasing hormone analog (sGnRHa). The artificial reproduction protocol in this and all future studies was conducted according to the established and described protocol (Ljubobratović et al. 2021).

At the time of hormonal injection, three samples of ovarian follicles were catheterized from each female and the third sample from each fish was clarified in Serra solution to evaluate the oocyte diameter and FOM stage, while the first two samples of oocytes (50 to 160 follicles per well) were immediately stocked into the sterile 6-wells plates (VWR International Kft., Debrecen, Hungary). Each replicate was placed into a separate well supplied with the maturation media (3 mL per well) consisted of 90% Leibovitz's L-15 medium (Carl Roth, Karlsruhe, Germany) supplemented with 0.1% Bovine Serum Albumin (BSA). The pH was adjusted to 7.6 and maintained with the addition of 15 mM HEPES. About one hour following sampling, the medium was changed and supplied with 100 ng/mL DHP. Each well was photographed and the number of oocytes per well was assessed. The plates were placed in a dark room and further incubated at 12.9 °C, under gentle agitation (40 rpm).

At the time of hormonal stimulation, oocytes of all fish were in FOM stage I. The dynamics of FOM were different between the groups in both *in vitro* and *in vivo* conditions. In the case of IVM, a significant difference in FOM index was seen at each evaluation moment, while *in vivo* differences were noticed at 120 h, 144 h, and 168 h. The final mean IVM FOM stadium at 96

h post-stimulation in CZECH oocytes ranged from 2 to 5.5, while in the HAKI group significantly higher final IVM FOM stage was seen, having reached GVBD stage in all but one female. A significantly higher share of maturing oocytes was noticed in the HAKI group (29.8% to 61.6%), compared to the CZECH group (0.5% to 51.5%). In the HAKI group, a significant correlation was found between *in vivo* and *in vitro* LT (r = 0.791). In the CZECH group, the *in vivo* LT and *in vitro* FOM stages were significantly negatively correlated (r = −0.713).

This was the first study in this WP and showed promising results of the usage of IVM dynamics to predict the latency time in pikeperch breeders. It showed its applicability on both the population and individual levels, while raising a question on the effect of geographical origin on the maturation dynamics. Accordingly, the offspring of the populations were further reared until sexual maturity and used for our concluding trial in the project (Trial 5 of WP3).

Trial 2 – Predictability of egg quality using the IVM in out-of-season reproduction

Next to the concluding study on IVM hormonal choice for LT prediction, the study conducted on out-of-season reproduction in November of 2022 assessed the applicability of IVM for egg quality prediction with special regard to defining the most informative moment and IVM FOM stage for the OMC evaluation.

The broodstock used in this trial consisted of 13 females (4.3 ± 0.7 kg) reared in fully controlled conditions. As in the case of previous studies, both the stimulation of IVM oocytes and females was conducted on the same day and further followed each 4 and 24 hours, respectively.

The study showed that the IVM FOM stage at moments of 52h and 56h are the predictors with the highest strength, showing high correlation (r = -0.9). Likewise, the analysis showed that the critical IVM FOM index for suitability of the fish for out-of-season reproduction is at 3. Namely, the fish in which oocytes showed a higher FOM index in IVM, likewise showed lower egg quality, mainly in terms of increased OG-frag (Figure 1).

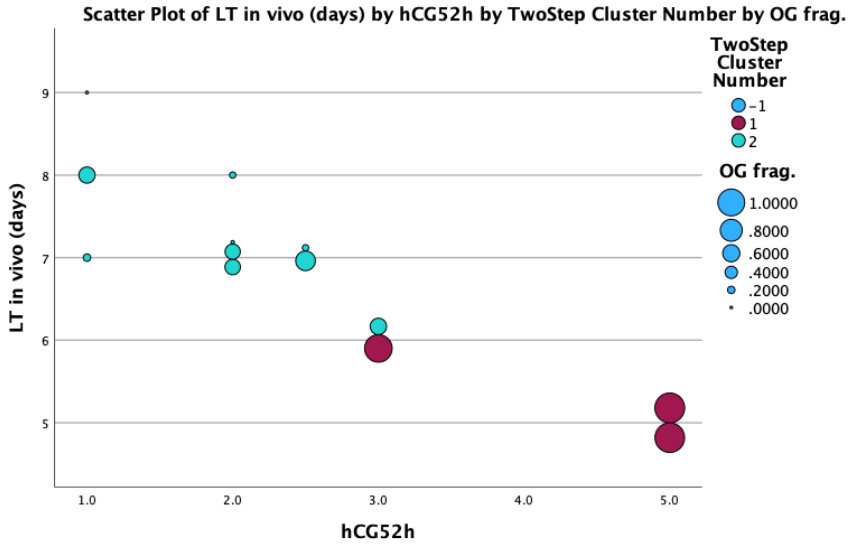

Figure 1. Predictability of IVM FOM index on latency time (LT) and oil globule fragmentation (OG frag) in out-of-season reproduced pikeperch.

Accordingly, this study concluded the applicability of IVM for both LT estimation and egg quality features in out-of-season pikeperch reproduction, defining the most suitable moment of FOM evaluation at 52 h and 56 h post-induction.

Trial 3 – Assessing the protocol applicability on pilot scale conditions

This trial was conducted in July 2023 at the Mecklenburg-Vorpommern Research Center for Agriculture and Fisheries, Institute for Fisheries, Hohen Wangelin, Germany. The entire infrastructure is built on recirculation aquaculture technology, where fish are maintained constantly under fully controlled conditions, so that the size, features, and capacity of the facility resemble the commercial conditions. Likewise, the number of fish used for this study fits the scale needed for the largest commercial hatcheries of pikeperch in Europe. Thus, this study's main aim was to test earlier established IVM predicting protocols in the most advanced commercial conditions in Europe.

Two classes of fish were used for the present study. The older class, 2018, was earlier submitted to the photo-thermal spawning conditions, while the younger class, 2021, was composed of virgin fish kept thus far under stable LD 16:8, 23 °C photo-thermal conditions. Fish were stocked into two identical, separated RAS climate chambers. Each chamber consisted of two tanks, equipped with a drum filter, a moving bed bioreactor and UV and ozone disinfection units. The stocking was set on a total random plan, yet maintaining the equal initial stocking density in each chamber and in each tank. Each tank was stocked with a fish of a different origin.

According to the maturation dynamics seen in the size of ovarian follicles over the period of time, fish were classified as fast, medium and slow. On July 5th and temperature of 5 °C, fish were injected with 5µg of sGnRHa and further slowly heated to 10 °C (1 °C/day). The fish that showed advanced FOM stage (2 or more) were not injected, anticipating their ovulation without the use of hormonal treatments. Thus, in total, four groups of the fish were evaluated according to the size of their ovarian follicles, LT, OG-frag and embryo survival – FAST NI (non-injected), FAST, MEDIUM and SLOW.

Next to it, the ovarian follicles of all hormonally treated fish were sampled via catheter and incubated *in vitro* to assess the state of their maturation dynamics for 96 h at 13 °C. The FOM status of the fish was checked starting from 96 h post-injection. Fish in stages 4 or higher would be sampled the following day, while the others would be checked two or three days later, for those in FOM stage 3 or lower, respectively. Once the female was in GVBD, the genital papilla of the fish would be sutured, and the fish would be transferred to an identical RAS chamber with a temperature of 12 °C. During the next 24 h, the ovulation was evaluated in the sutured females at time intervals of 9 h, 6 h, 5 h, and 4 h. Once the ovulation was noted, the eggs would be stripped from the female, and their pictures would be taken under 20x magnification to assess the oil globule fragmentation rate (in dry form) and deformation upon cortical reaction (about 4 min post-activation). Likewise, the weight of eggs and fish would be evaluated to assess the commercial fecundity of each fish.

Embryo survival was evaluated in eggs of three fish per group, except for the MEDIUM group, where in total of nine fish were evaluated.

Mean ovarian follicle size was 1102 ± 34 µm, 1024 ± 36 µm, 987 ± 36 µm, and 894 ± 53 µm in FAST_NI, FAST, MEDIUM and SLOW groups, respectively.

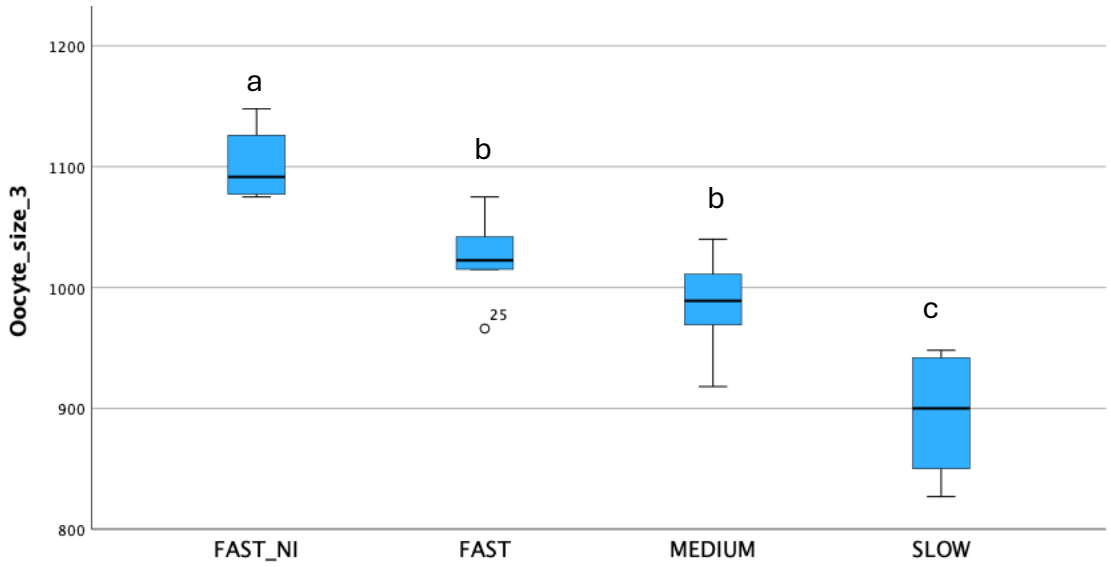

Figure 2. Ovarian follicle size at the time of hormonal stimulation in out-of-season commercial-scale pikeperch reproduction.

The embryo survival in all three hormonally stimulated groups was low and ranged in mean from 9 to 26 % from the SMALL to the MEDIUM group, without differences between the groups. The non-injected group showed significantly higher survival at 72.6 ± 13.9 %.

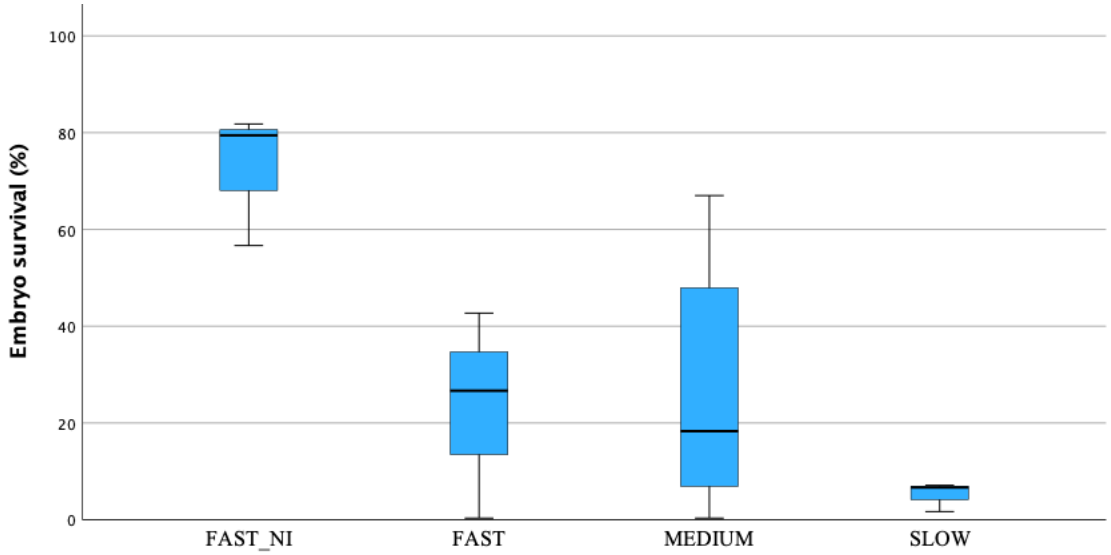

Figure 3. Embryo survival in different groups in out-of-season commercial-scale pikeperch reproduction.

In case of *in vivo* LTs, the only assessed differences were between the FAST_NI and SLOW groups, being lower in the injected group.

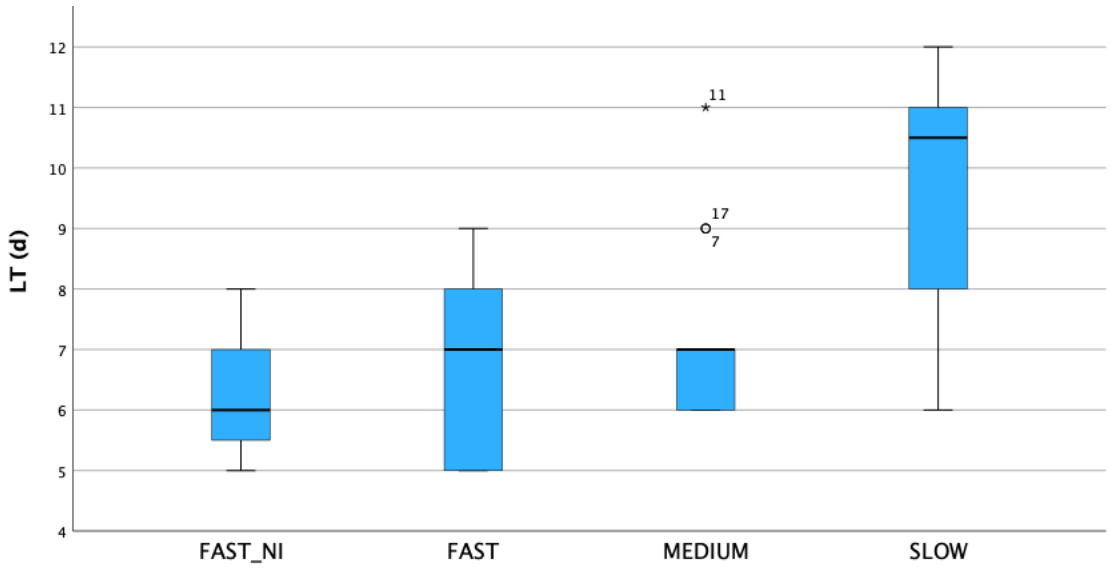

Figure 4. Latency time (LT) in days in fish belonging to the different groups in out-of-season commercial-scale pikeperch reproduction

Similarly to the previous study, the scatter plot that considered LT and OG-frag showed the bordering FOM index 3 at 56 h post-induction IVM oocytes.

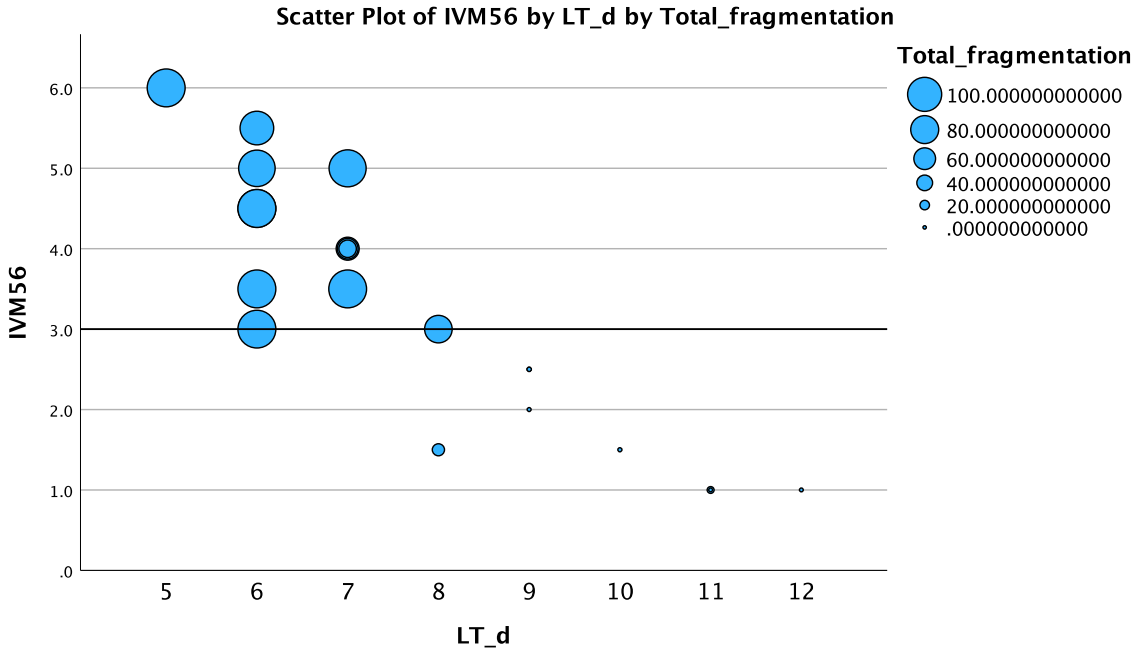

Figure 5. Scatter plot on latency time (LT) and *in vitro* maturation (IVM) stage at 56 h post-stimulation with values for different egg oil globule (OG) fragmentation rates in out-of-season commercial-scale pikeperch reproduction.

When it comes to the correlation between the IVM FOM index at different sampling points and LT, the highest predicting potential was seen in the 60h sample (r = - 0.875), followed by the 56 h sample (r = -0.856). On the other side, the oocyte size at the time of injection showed lower predicting potential (r = - 0.755). Thus, to conclude, the IVM FOM dynamics is a more

reliable predictor of OMC compared to oocyte size monitoring in commercial conditions. Nevertheless, the egg quality seems to present a burning issue for the feasibility and development of commercially applicable technologies for ovulation induction without the use of any hormonal stimulation is advised for fish kept constantly under fully controlled conditions.

Trial 4 – Development of OMC in pikeperch maintained at 10 °C – October 2023

Our earlier trials in both outdoor and indoor reared breeders showed that an oocyte size of around 950 µm is optimal for the hormonal induction, while several studies found that even 12 °C as the lower thermal border may be sufficient to induce full maturation of pikeperch oocytes; however, not evaluating their reproductive performance. Therefore, this study aimed to assess the development of oocyte maturation in fish under fully controlled conditions, with the minimal thermal range being at 10 °C. This thermal range was chosen with regard to the established protocol that uses 10 °C upon hormonal injection for FOM and 12 °C for ovulation upon GVBD. The protocols used for artificial reproduction and IVM were the same as explained earlier in the report.

In total, 10 fish were hormonally treated, with five of them being in the oocyte diameter range of 960-1000 µm and the other five with the oocyte diameter range from 940 to 960 µm. Only two fish showed LTs comparable to all earlier studies with fish of similar oocyte diameter and were at 8 and 10 days. All the other fish required an excessive period to reach ovulation, three at 13-14 days, while the rest were re-injected two weeks after the first injection and ovulated 16-18 days post-injection. Similarly, the IVM FOM was excessively long and inconclusive. However, when it comes to the reproduction performance, all the fish showed quite high egg quality with embryo survival in the range of 50-85% with low oil globule fragmentation in the range of 0 to 14%.

In conclusion, the usage of 10 °C as the lower border of thermal induction may be suggested as feasible for out-of-season artificial reproduction. Nevertheless, the development of OMC in this case follows different patterns compared to the fish normally kept at lower temperatures of 6 °C or lower. Accordingly, the IVM OMC evaluation strategy seems not to be fully applicable, while the usage of oocyte diameter becomes more reliable. Future studies should evaluate whether the fast cooling to 6 °C a week before hormonal stimulation and application of warming reproduction strategies may improve the synchronization among the fish, not disrupting the egg quality features.

Trial 5 – The effect of geographical origin on OMC development

Fish used for the trials were the product of the preseason artificial reproduction of the two populations (Ljubobratović et al., 2023). Both groups were composed of nine pairs of breeders of mean female weight 2.1 ± 0.7 kg and 2.4 ± 0.3 kg, while males were of mean weight 1.5 ± 0.2 kg and 2.4 ± 0.2 kg, for CZECH and HAKI populations, respectively. Upon hatching, larvae in each group were merged, and 100 thousand larvae from each group were further reared in separate tanks of a common recirculation aquaculture system. In early July, 800 individuals (mean weight = 30 g) of each group were stocked outdoors in two separate in-pond raceways

and reared on floating pellets until reproduction. At the end of the third growing season, in November 2023, 100 fish per population (60 females and 40 males) were selected as the broodstock for the reproduction trials. Each fish was injected with a passive integrative transponder and both broodstocks were stocked into the common 150 m^2 pond.

Two spawning batches were evaluated. For the preseason batch, fish from both populations were harvested in mid-January 2024. For the seasonal reproduction, only the CZECH group was evaluated due to the significantly better performance of the HAKI group already in the preseason. In that sense, three groups of spawning breeders were formed – HAKI PRESEASON, CZECH PRESEASON, and CZECH SEASON, each comprised of 10 males and 10 females. Artificial spawning was conducted according to the earlier-described protocol. The FOM stage of breeders was checked on days 5, 7, and further on each day post-stimulation until the ovulation in the preseason, while in the season, evaluation started at day 4 post-stimulation and was conducted daily until the ovulation. The FOM index was calculated as the mean FOM stage of all fish in each group. For the sake of comparison between the preseason and season batches, the FOM stage was calculated according to the day degree (DD). Once the ovulation was noticed, the eggs would be collected and fertilized with freshly stripped milt of two males from the same population in the ratio of 1 mL of milt per 100 g of eggs. Prior to the stocking of the eggs of each female into the separate 7L incubation Zug jar, the volume of the eggs was measured. During the incubation, the dead eggshells were carefully siphoned out of the jars, and 72 h post-fertilization, the remaining live eggs were again siphoned to the volume-measuring cylinder. Accordingly, the embryo survival was assessed at 72 h.

At the time of *in vivo* hormonal injection, three samples of ovarian follicles were taken from each female using a catheter. Although the HAKI group was not reproduced in season, the oocytes were taken on the same day from ten females in this group. The third sample was clarified in the Serra solution for the evaluation of the initial FOM stadium and follicle diameter. The first two oocyte samples were placed in separate wells of 6-well plates for suspension cell culture for IVM assessment. Each replicate well contained 4 mL of IVM culture medium. Approximately one hour after sampling, the IVM medium was replaced and supplemented with hCG at a final concentration of 10 IU/mL. The plates with oocytes were incubated in a temperature-controlled dark room at 13 °C, under constant gentle agitation (40 rpm) on an orbital platform shaker (WiseShake® SHO-2D, Witeg Germany). The oocyte maturation stages and the FOM index were evaluated every four hours from 48 to 96 h post-stimulation, except at 84-h and 88-h time points. At the end of the evaluation period, the number of maturing follicles was counted. The maturing rate was evaluated as a share of maturing oocytes in the total number of follicles at the start of hCG stimulation.

Absolute values of the LT, expressed in both days and hours, differed significantly among all three groups, being shortest for the CZECH SEASON and longest for the HAKI PRESEASON. Nevertheless, when expressed in DD, only the HAKI PRESEASON batch was significantly longer than two similar CZECH groups. Likewise, oocyte maturation progressed similarly for both CZECH groups and was more advanced than HAKI PRESEASON at 66 and 77 DD (Figure 3), while in absolute values, FOM dynamics differed significantly between the preseason groups at 7, 8 and 9 d post-stimulation. At the time of hormonal injection, HAKI

PRESEASON and CZECH SEASON groups had comparable mean oocyte diameters, both of which were larger than the CZECH PRESEASON and smaller than the HAKI SEASON groups. Higher embryo survival was recorded in the CZECH SEASON group; however, not different from HAKI PRESEASON, while the CZECH PRESEASON group performed worse in this parameter. In terms of the oil globule fragmentation rate of freshly stripped eggs, it was significantly higher in the CZECH SEASON than in the HAKI PRESEASON group; nevertheless, no significant difference was observed between the two CZECH batches, despite mean fragmentation being about 6-fold higher in the season compared to the preseason group.

The FOM index of IVM oocytes across the two evaluation points showed a single significant difference at 60 h post-stimulation, being significantly higher in CZECH PRESEASON compared to HAKI PRESEASON. Although no other significant differences were detected, a shifting trend was observed in the preseason groups: CZECH PRESEASON exhibited higher values for the first five sampling points, followed by higher values in HAKI PRESEASON thereafter. In the case of season groups, although without significant differences, the HAKI group consistently showed a higher FOM index than the CZECH SEASON group at every evaluation time point. Finally, the proportion of maturing oocytes did not differ between groups; however, in both HAKI and CZECH groups, oocytes sampled in season showed a higher rate of maturation compared to their preseason counterparts.

Pikeperch females with different geographic backgrounds and reared in the same conditions showed several disagreements from the aspect of reproductive performance. The southern population attained the OMC appropriate for artificial reproduction two months earlier compared to the northern one, visible by the earlier reached optimal oocyte size on the population level. The IVM was partially successful in predicting the *in vivo* FOM progress disagreements, likely due to different responses in prematurely stimulated oocytes between the populations. Finally, seasonal reproduction of the northern population led to high-quality egg production. Thus, the reproductive clock seems either inherited or the influence of “photic clocking” instead of thermal conditioning is more decisive in the case of pikeperch. This study showed the feasibility of common rearing of broodstocks with different geographical backgrounds as a strategy to prolong egg production.

Dissemination activity

Papers in scientific journals

Two papers have been published as a direct outcome of the project in the D1 scientific journal:

Ljubobratović, U., Kitanović, N., Milla, S., Marinović, Z., Fazekas, G., Stanivuk, J., Nagy, Z. and Horváth, Á., 2023. Predicting population's oocyte maturation competence and evaluating individual's latency time using *in vitro* oocyte maturation in pikeperch (Sander lucioperca). *Aquaculture*, *562*, p.738851. https://doi.org/10.1016/j.aquaculture.2022.738851

Ljubobratović, U., Kitanović, N., Milla, S., Marinović, Z., Stanivuk, J., Fazekas, G., Vass, N., Nagy, Z. and Horváth, Á., 2025. The development of oocyte maturation competence, egg, oil globule and larval size are population-dependent in pikeperch (*Sander lucioperca*). *Aquaculture,* 742932. https://doi.org/10.1016/j.aquaculture.2025.742932

Three more papers will be submitted to Q1 scientific journals until the end of 2026 (one in 2025) as a direct outcome of the project.

International conferences

8th International Workshop on the Biology of Fish Gametes, September. 20-23, 2022:

Ljubobratović, U., Kitanović, N., Milla, S., Marinović, Z., Fazekas, G., Stanivuk, J., Żarski, D., Horváth, Á. Predicting the egg quality based on the oocyte diameter and *in vitro* maturation in domesticated pikeperch *Sander lucioperca*

Aquaculture Europe, September 22-25, 2023, Vienna, Austria:

Kitanović, N., Ljubobratović, U., Marinović, Z., Stanivuk, J., Horváth, Á. Development of a protocol for *in vitro* maturation of pikeperch (*Sander lucioperca* L.) postvitellogenic ovarian follicles.

Ljuboratović, U., Kitanović, N., Stanivuk, J., Marinović, Z., Fazekas, G., Nagy, Z., Horváth, Á. Predictability of latency time using the *in vitro* oocyte maturation in pikeperch (Sander lucioperca)

12th International Symposium on Reproductive Physiology of Fish, May 15-19, 2023, Crete, Greece:

Kitanović, N., Ljubobratović, U., Marinović, Z., Stanivuk, J., Horváth, Á. *In vitro* responsiveness of pikeperch (*Sander lucioperca*) ovarian follicles as a potential indicator of their maturational competence.

National conferences

XLVI. Halászati Tudományos Tanácskozás, Szarvas, 2022. 05. 25-26.

Ljubobratovic, U., Kitanović, N., Marinović, Z., Vass, N., Fazekas, G., Stanivuk, J., Nagy Zoltán, Horváth Ákos. Háziasított süllő anyahal mesterséges hormon stimulációra kész állapotánal meghatározása. HALÁSZATFEJLESZTÉS 39 pp. 48-52. , 5 p. (2022)

PhD thesis

Nevena Kitanović, under preparation. – A part of the thesis is being written on the results obtained in WP2.

Final remarks

The PD OTKA 139053 INVITROSANDER is a comprehensive, collaborative and international post-doc project that established reliable protocols for IVM of pikeperch oocytes and implemented its applicability for OMC evaluation for artificial reproduction in various conditions and fish of various technological and geographical backgrounds.

References

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