https://doi.org/10.17221/136/2023-CJAS

# Effect of thermal management on vitellogenesis and maturation in indoor-reared pikeperch (*Sander lucioperca*)

*Uroš Ljubobratović[1]*, László Ardó[1], Georgina Fazekas[1], Zoltán Horváth[2], Jovanka Lukić[3]*

[1]*Research Centre for Aquaculture and Fisheries, Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Sciences, Szarvas, Hungary*
[2]*H&H Carpio Halászati Kft., Ócsárd, Hungary*
[3]*Laboratory for Molecular Microbiology (LMM), Institute of Molecular Genetics and Genetic Engineering (IMGGE), University of Belgrade, Belgrade, Serbia*

**Corresponding author: u.ljubobratovic@gmail.com*

**Citation:** Ljubobratović U., Ardó L., Fazekas G., Horváth Z., Lukić J. (2024): Effect of thermal management on vitellogenesis and maturation in indoor-reared pikeperch (*Sander lucioperca*). Czech J. Anim. Sci., 69: 18–28.

**Abstract:** The present study aimed to assess the effects of thermal manipulation on the vitellogenesis and final oocyte maturation (FOM) in pikeperch. Two groups of fish were stocked in two separate tanks of the climate chamber. One group was stocked at 10-month age (THERMAL), while the other was continually kept under stable photothermal conditions until 19-month-age (CONTROL) and then transferred to a climate chamber. The progress of vitellogenesis was assessed via evaluation of the oocyte diameter at the mid- and late-autumn phases, and the gonadosomatic index (GSI) at the late-autumn phase. Finally, females from the CONTROL group were hormonally stimulated before (WARMING) and after (STABLE) increase of the temperature from wintering to spawning, and the FOM progress, ovulation and plasma levels of 17 α, 20β dihydroxy progesterone (DHP) were assessed. Significantly larger oocytes at the mid-autumn phase (878.8 ± 40.1 μm vs 836.5 ± 46.5 μm) as well as the GSI at the end of the autumn phase (10.5 ± 1.7% vs 7.6 ± 1.1%) were noticed in THERMAL fish. Significantly faster FOM was seen in fish under the WARMING post-stimulation regime, and these fish had higher DHP levels at the moment of hormonal stimulation (5.4 ± 1.4 ng/ml vs 3.8 ± 1.2 ng/ml). According to the obtained results, it appears that photothermal induction of fish at a younger stage might have a positive impact on the first spawning, while the WARMING thermal regime seems to be more efficient in stimulating the FOM in fish upon first wintering.

**Keywords:** spawning induction; fully controlled environment; oocyte growth; percids; final oocyte maturation

Supported by the European Regional and Development Fund and the Government of Hungary (Project No. GINOP-2.3.2-15-2016-00025), and researchers involved were additionally supported by the National Research, Development and Innovation Fund of Hungary (Grant No. PD-139053) and by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200042). Corresponding author was granted with János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

https://doi.org/10.17221/136/2023-CJAS

Total control over the reproductive cycle of pikeperch *Sander lucioperca* is a research topic of emerging interest (Zarski et al. 2019; Milla et al. 2021; Ljubobratovic et al. 2022a). Being temperate species highly dependent on the seasonality of photothermal conditions, broodstock management of pikeperch in a recirculation aquaculture system (RAS) requires various technological solutions that would mimic the outdoor conditions of temperate Europe region. Namely, to undergo vitellogenesis, the autumn-like water cooling and light period/intensity reduction is performed. Depending on the technological solutions used, this temperature/light regime might have variable success (Hermelink et al. 2011; 2013; 2017). The period of oocyte growth is important as it is documented that the phenomenon of asynchronous maturation among a common broodstock occurs in this period (Ljubobratovic et al. 2020; 2022b). Namely, already in the stage of mid-vitellogenesis, it is visible that some of the female breeders grow their oocytes faster than others. This could eventually lead to the variable outcome of artificial spawning, reflected in a wide range of ovulation time in a common reproduction batch and high variability in egg quality (Zarski et al. 2019; Ljubobratovic et al. 2022b). Although such variability in maturation dynamics may provide an evolutionary advantage (Lappalainen et al. 2003), it seems that, with an increase in the number of previous reproductive cycles, the maturation dynamic in females from a given broodstock synchronises, leading to a smaller variance in ovulation period and egg quality (Zakes et al. 2013; Zarski et al. 2019; Ljubobratovic et al. 2021). Namely, based on so far published studies, it appears that the first-time wintering fish – reproductively "naïve" – are specifically vulnerable (Zakes et al. 2013; Ljubobratovic et al. 2020), while with each reproductive cycle, the spawning performance of the fish improves. The previous study showed that the reproductive experience or number of experienced winters might affect the dynamics of oocyte growth on both group and individual levels (Ljubobratovic et al. 2021). Thus, more reproductively experienced fish were more synchronised in reaching the level of oocyte maturation competence (OMC), and consequently, the outcome of artificial reproduction was stable and high. On the other hand, younger fish showed diminished reproductive success visible in more variable egg quality and final oocyte maturation (FOM) dynamics, directly reflecting the more variable oocyte size at the time of hormonal stimulation. Therefore, it is of interest to evaluate if the simulation of seasonality *per se* might affect the dynamics of vitellogenesis and synchronise the group's OMC in reproductively "naïve" fish.

The next phase in oocyte development is maturation. Based on thus far published work, it is plausible that two-stage maturation commonly described in many teleosts (Patino et al. 2001) is most likely present in pikeperch oocytes. Namely, oocytes reach a certain size that is ready for maturation already in early winter, and further on, oocyte morphology appears to be rather stagnant (Ljubobratovic et al. 2021; 2022b; 2023). Depending on the length of this first maturation phase, the dynamics of the next phase, FOM, may differ (Ljubobratovic et al. 2020). Likewise, there are inter- and intra-population differences that may lead to differences in FOM dynamics among the individuals of the common population (Zarski et al. 2019; Ljubobratovic et al. 2022b) as well as between the populations of different origin kept in similar conditions (Khendek et al. 2018; Ljubobratovic et al. 2023). Finally, the difference in the FOM dynamics can also depend on the post-injection thermal regime applied. With this respect, there are two thermal protocols commonly applied for farmed pikeperch commercially: the so-called "warming" and "stable" protocol (Ljubobratovic et al. 2021). In the "stable" protocol, breeders, upon wintering, are warmed to spawning temperature (usually 12 °C), hormonally stimulated, and water temperature is further kept stable (Zakes et al. 2013; Zarski et al. 2019). The latter, the "warming" protocol, has been developed recently; it implies hormonal stimulation at a wintering temperature of 4–6 °C which is then slowly increased to the temperature suitable for ovulation (10–12 °C) (Ljubobratovic et al. 2021; 2022b). This protocol efficiently reduces the inter-population variation of latency time (LT) – a period from the application of hormones until ovulation. Likewise, a recent study that evaluated different hormonal dosages pointed to the possible disagreements between the two thermal regime protocols (Ljubobratovic et al. 2022a). Namely, while the low dosage of 5 µg/kg of salmon gonadotropin-releasing hormone analogue (sGnRHa) was described as suitable for warming protocol (Ljubobratovic et al. 2021), it was found to be detrimental to egg quality in a stable regime compared to earlier described opti-

https://doi.org/10.17221/136/2023-CJAS

mal dosage of 50 µg/kg (Zarski et al. 2019). Therefore, there may be a certain difference in the physiological state of fish during the artificial reproduction in these two regimes that might lead to the different efficacy of the hormonal stimulation. Assuming that maturation induction steroid (MIS) is crucial for triggering the FOM and ovulation, its plasma levels should reflect the consequent FOM dynamics, and both might differ according to the thermal regime applied. Considering all above documented issues that might affect the oocyte maturation and its individually variable dynamics in reproductively "naïve" fish, it is worthwhile investigating how the post-stimulation thermal regime might modify and synchronise the FOM progress in fish not wintered earlier.

In light of the above-identified knowledge gaps in pikeperch reproductive biology, the present study aimed to evaluate the effects of early wintering on the dynamics of oocyte growth in reproductively "naïve" females, as well as the effects of different post-stimulation thermal protocols on the FOM dynamics and physiological state of fish.

## MATERIAL AND METHODS

The study took place in the experimental RAS of the Hungarian University of Agriculture and Life Sciences, Institute of Aquaculture and Environmental Safety, Research Center for Aquaculture and Fisheries (MATE AKI HAKI). Fish rearing and handling were performed according to the recommendation of the Institute's Animal Ethical Panel (license number 27-1/2019) and according to the State Law (10/1999.I.27.). Before blood sampling procedure, the fish were surgically anesthetised in 0.3ml/l solution of 2-phenoxyethanol. By the same mean, the euthanasia of the fish was conducted for evaluation of the gonadosomatic-index (GSI).

### Fish origin and broodstock management

Fish used for this study were the offspring of eggs of one female fertilised with an equal amount of sperm of two males originating from the oxbow of river Körös. Fish were reared in RAS from hatching onwards. The stock of 2 000 three-month-old fish was reared in a common RAS with stable photothermal conditions of water temperature in the range of 22–25 °C and constant light (24 : 0 LD) with intensity in the 1–10 lux range. At the mean bodyweight of 0.2 ± 0.0 kg and age of 10 months (week 1 = 1W), 55 individuals (THERMAL group) were transferred to the broodstock RAS. This climate chamber consisted of three 3 $m^3$ tanks, a bead filter, and a trickling tower with a supply of technical oxygen directly in the tanks via diffusers. Spawning time for this room was set for mid-October 2018, and the photothermal conditions were manipulated accordingly (Ljubobratovic et al. 2022a). The transfer of the THERMAL group took place in March 2018 (mimicked August, post-spawning, 23 °C stable, 13 : 11 LD and dropping). The rest of the fish remained in the constant photothermal conditions (24 : 0 LD) until the age of 19 months (37W) and a mean bodyweight of 0.8 ± 0.1 kg when 55 fish (CONTROL) were as well transferred to the broodstock RAS and stocked into the separate tank. The climate conditions in December 2018 mimicked natural mid-June (post-spawning, 18 °C and warming, 14 : 10 LD and rising). The photothermal conditions of the two groups from the time of stocking the THERMAL group to broodstock RAS (1W) until the reproduction time in experiment 2 (81W) are detailed in Figure 1.

Fish were fed with Aller Rep Ex 8mm feed Aller Rep EX (Aller Aqua Group, Christiansfeld, Denmark) with a crude composition of 53% protein, 14% fat, and 16.5% carbohydrates and digestible energy of 18.6 MJ/kg, according to the feed producers declaration. The daily feeding rate was 0.7% of total biomass during the warm water temperatures of mimicked summer (22–25 °C). At the same time, during the cooling period, fish received two to five weekly meals of 0.2% to 0.5% of total biomass, depending on the water temperature.

### Experiment 1 setup

In total, 55 fish from both THERMAL and CONTROL groups were tagged with passive integrative transponders (PIT) and measured for individual body weight at 37W at the time of stocking the CONTROL group to the broodstock RAS. The second check of individual body weight was assessed at 45W (post-spawning, 23 °C stable, 15 : 9 LD and dropping), again in all fish. Likewise, at the time of the end of mimicked summer, 55W (pre-spawning, 21 °C cooling, 12 : 12 LD

https://doi.org/10.17221/136/2023-CJAS

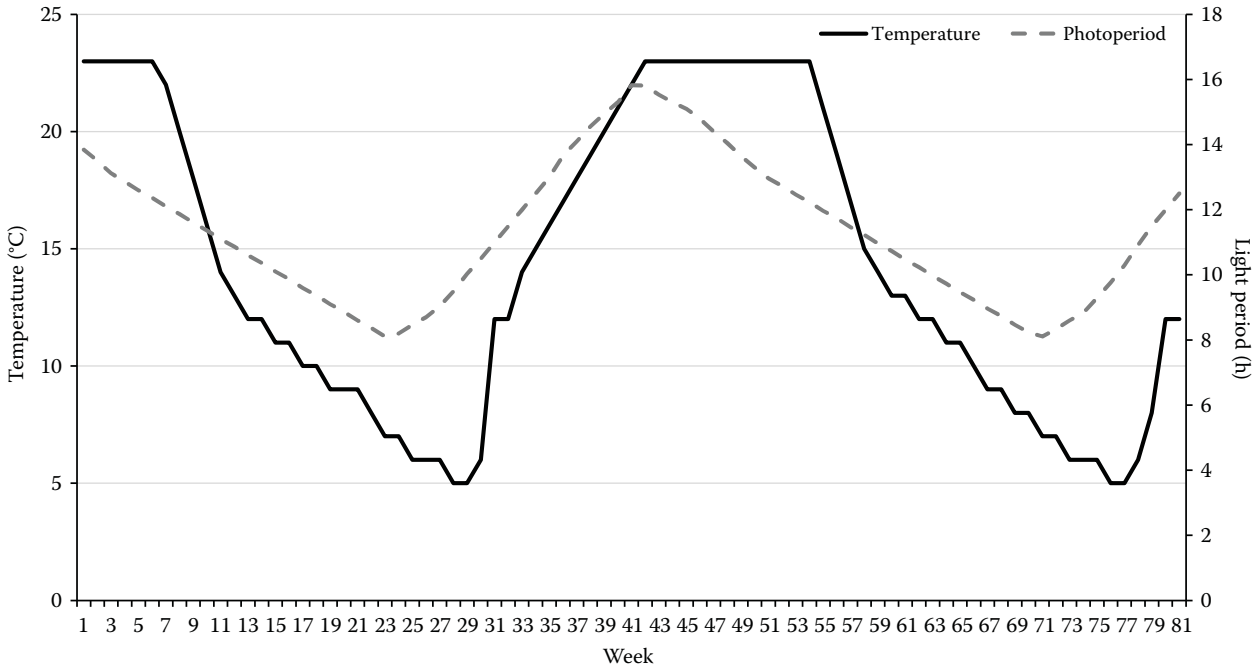


Figure 1. The photothermal schedule used in experiment 1 since stocking of the first group of fish (THERMAL) while the second group (CONTROL) was stocked at week 42 in the broodstock chamber for out-of-season propagation of pikeperch (*Sander lucioperca*)

and dropping), all the fish were measured for individual weight. Further on, on a random sample of 10 females per group, oocyte size was evaluated at 64W (pre-spawning, 11 °C cooling, 10 : 14 LD and dropping) and 72W (pre-spawning, 7 °C cooling, 8 : 16 LD and raising). Oocyte sampling was conducted using a catheter (infant feeding tube, size CH06), and upon sampling, oocytes were placed in Serra clarification solution (96% alcohol, 35% formalin, and glacial acetic acid in a ratio of 6 : 3 : 1 v/v, respectively). Oocyte size was evaluated upon photographing under a microscope (Nikon ShuttlePix P-400R; Nikon Corporation, Tokyo, Japan) at 20× magnification and evaluated using the program Nikon ShuttlePix Editor v3.4.0. The mean oocyte size for each fish was assessed by measuring the diameter of 20 oocytes per specimen (Ljubobratovic et al. 2020; 2021; 2022b). Likewise, at 72W, upon oocyte sampling, eight fish per group were euthanised, and the GSI was evaluated. Experiment 1 was terminated at this moment.

## Experiment 2 setup

For experiment 2, only the CONTROL group fish from experiment 1 were used. Therefore, on the 78W (pre-spawning, 6 °C warming, 11 : 13 LD and raising), six randomly chosen females (WARMING group) were intramuscularly treated with 50 µg/kg of salmon gonadotropin-releasing hormone analogue (sGnRHa, D-Arg6, Trp7, Leu8, Pro9-NEt)-GnRH (Ova-RH; Syndel Laboratories Ltd., Nanaimo, Canada). Thermal conditioning further on was as explained by Ljubobratovic et al. (2021). Namely, upon hormonal treatment, water temperature was increased for 1 °C/day until 12 °C. Further on, the temperature was maintained stable. One week after reaching 12 °C, another group of six fish (STABLE group) was treated with 50 µg/kg of sGnRHa, (Zarski et al. 2019). The thermal regime in these two groups is depicted in Figure 2. At the time of hormonal stimulation, oocytes of both groups were sampled, and the FOM stage (Zarski et al. 2011) and oocyte sizes were assessed. Likewise, during hormonal treatment, blood sampling was conducted using heparinised needles, syringes, and 1.5 ml microcentrifuge tubes. Starting from two days upon hormonal treatment, the FOM stage of oocytes was monitored daily. Oocyte samples were taken using the catheter (CH06, infant feeding tube) inserted into the genital papilla of fish. Straight upon sampling, a sample of oocytes was gently removed from catheter to the petri dish

https://doi.org/10.17221/136/2023-CJAS

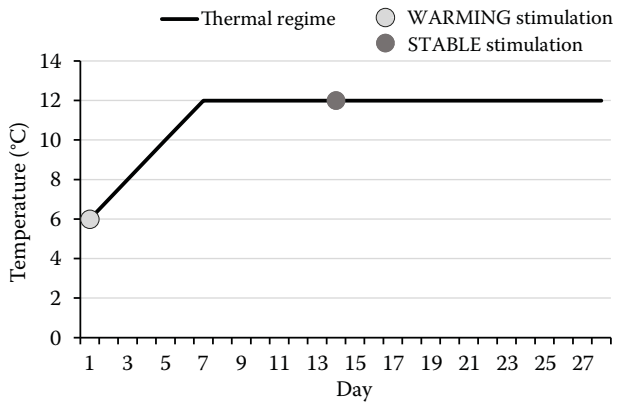


Figure 2. The thermal regime used in experiment 2 from hormonal stimulation of the WARMING group (day 1) until the end of the evaluation period in the STABLE group (day 28)

and clarified using Serra solution and about 15 min later the FOM stage was evaluated using microscopic 20× magnification. Upon reaching stage VI (germinal vesicle breakdown), fish papilla was sutured (Zarski et al. 2017), and ovulation was evaluated every 6 hours.

### Evaluation of the maturation induction steroid levels

Evaluation of 17 α, 20β dihydroxy progesterone (17α, 20β-OH-PROG, DHP) levels in females was performed using enzyme-linked immunosorbent assays (MyBioSource, San Diego, CA, USA) with plasma samples taken from fish at the time of hormonal treatment. Plasma was isolated from whole blood samples by centrifuging them for 20 min at 4 °C, with the force of 1 800× *g*. For hormone analysis, a competitive inhibition enzyme immunoassay technique was used, and the protocol followed the manufacturer's instructions, with a five-fold dilution of plasma samples to obtain a satisfactory signal level. Results were expressed as ng/ml.

### Statistical analysis

All pair comparisons were analysed using either an independent *t*-test or the Mann-Whitney *U*-test, depending on the type of distribution. Therefore, the normality of distribution was checked using the Shapiro-Wilk test. The FOM dynamics in experiment 2 were evaluated using Pearson's linear correlation, while the difference between the slopes of two regression lines was assessed with a *t*-statistic. Considering that the FOM stages are ordinal data, an additional run using ordinal regression was conducted to evaluate the Nagelkerke $R^2$ as well. Statistical significance was set at $P < 0.05$, while the data were presented as mean ± standard deviation (SD). Statistical analysis and data presentations were conducted in Microsoft Excel v16.81 (Microsoft Corporation, Redmond, WA, USA), JASP v0.12.2 (JASP Team, Amsterdam, The Netherlands) and SPSS v27.0 (SPSS, Chicago, IL, USA).

## RESULTS

### Experiment 1

At the time of stocking to climate chamber 37W, bodyweight in CONTROL fish was significantly greater than in THERMAL fish (0.8 ± 0.1 kg and 0.6 ± 0.1 kg, $P < 0.001$). It was so at 45W as well (1.0 ± 0.2 kg and 0.8 ± 0.1 kg, $P < 0.001$), while at the end of mimicked summer at 55W, there were no significant differences between the groups (1.2 ± 0.3 kg and 1.2 ± 0.2 kg, $P = 0.200$). The specific growth rate was significantly higher ($P < 0.001$) in both assessed occasions (37–45W and 45–55W) as well as during the whole summer (37–55W) in the THERMAL group (Figure 3).

Significantly higher oocyte diameter was assessed at 64W in THERMAL than in CONTROL females (878.8 ± 40.1 µm vs 836.5 ± 46.5 µm, $P = 0.043$) while there were no differences ($P = 0.566$) at 72W when all assessed females in THERMAL groups were with oocytes higher than 900 µm (group's range 934.0–1 005.6 µm), while in CONTROL group in four of ten fish oocyte diameter was lower than minimal in THERMAL group (group's range 846.5–1 005.7 µm). Fish in the THERMAL group showed significantly higher GSI ($P = 0.001$) compared to CONTROL (10.5 ± 1.7% vs 7.6 ± 1.1%) assessed at 72W (Figure 4).

### Experiment 2

A significantly higher DHP level ($P = 0.037$) at the time of injection (Figure 5) was recorded

https://doi.org/10.17221/136/2023-CJAS

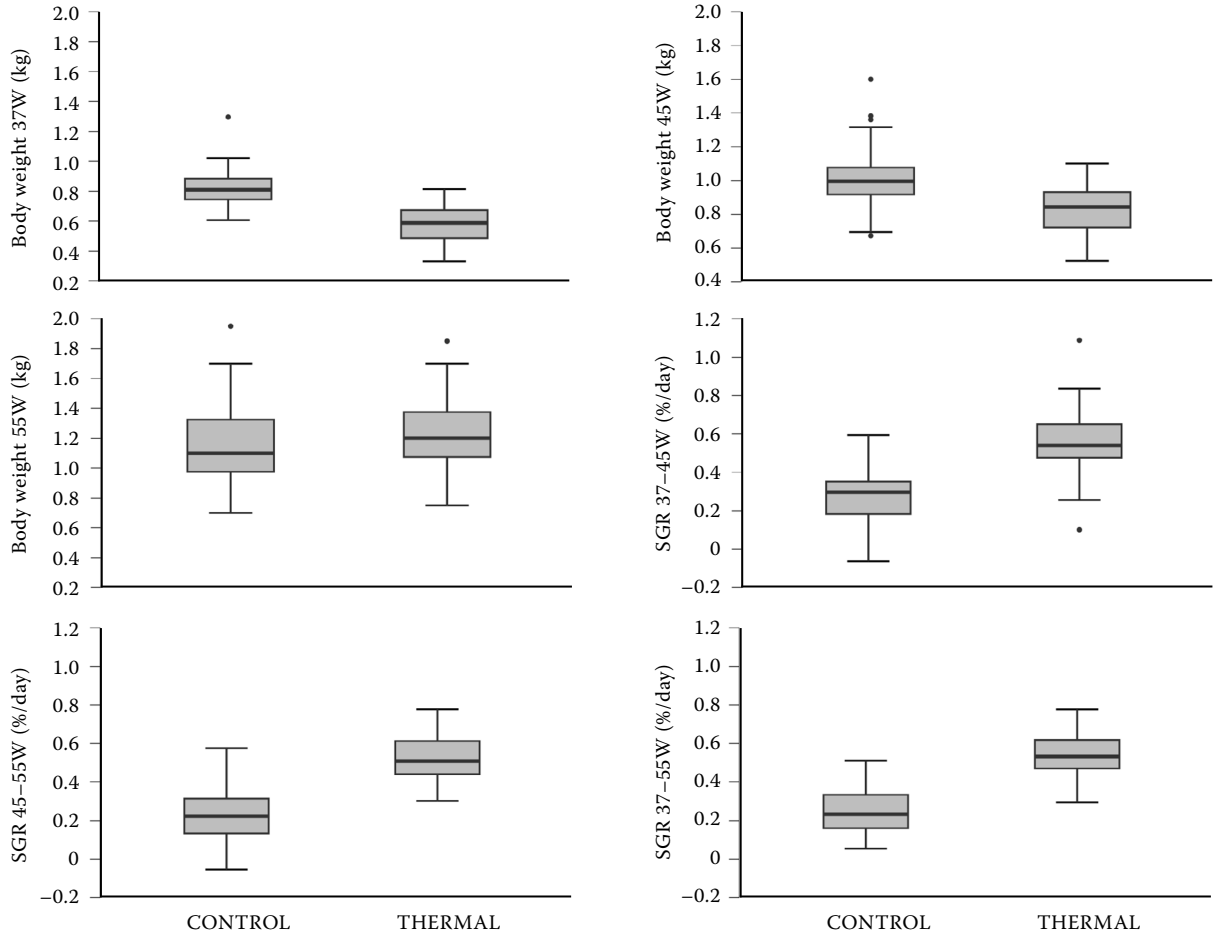


Figure 3. Mean body weight and specific growth rate (SGR) assessed 37, 45, and 55 weeks (W) since the initiation of the trial in 19-month old pikeperch *Sander lucioperca* individuals ($n$ = 55) previously photo-thermally treated from 0–37W (THERMAL) and kept until stable photothermal conditions until 37W (CONTROL) in recirculation aquaculture system
The statistically significant differences were found at BW37W $P < 0.001$, BW45 $P < 0.001$, and for SGR in all periods $P < 0.001$

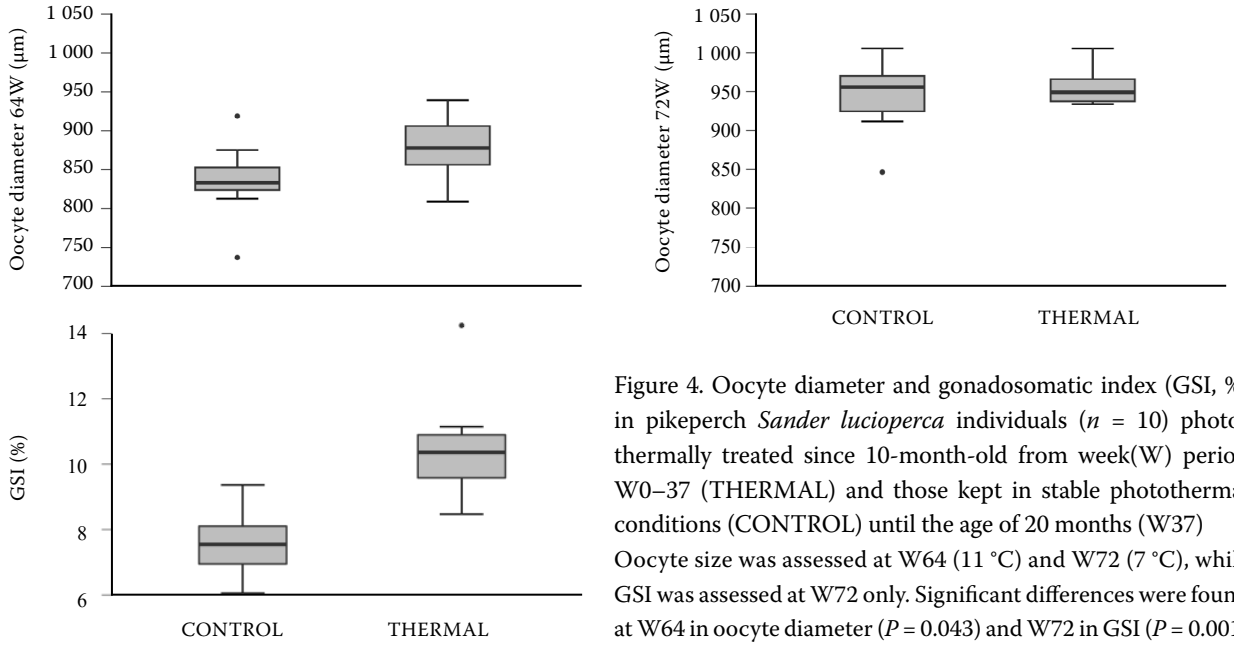


Figure 4. Oocyte diameter and gonadosomatic index (GSI, %) in pikeperch *Sander lucioperca* individuals ($n$ = 10) photo-thermally treated since 10-month-old from week(W) period W0–37 (THERMAL) and those kept in stable photothermal conditions (CONTROL) until the age of 20 months (W37)
Oocyte size was assessed at W64 (11 °C) and W72 (7 °C), while GSI was assessed at W72 only. Significant differences were found at W64 in oocyte diameter ($P$ = 0.043) and W72 in GSI ($P$ = 0.001)

https://doi.org/10.17221/136/2023-CJAS

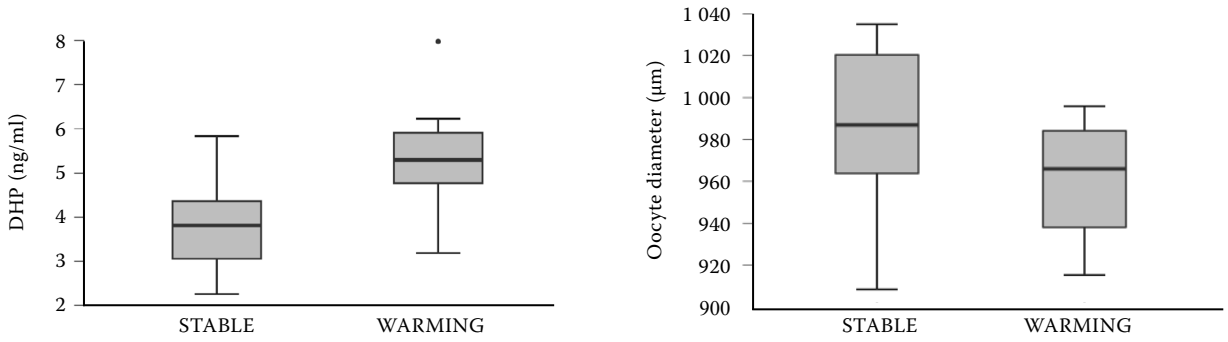


Figure 5. Levels of plasma DHP ($P$ = 0.037) and oocyte diameter ($P$ = 0.330) at the time of hormonal treatment in fish ($n$ = 6) hormonally treated at either 6 °C and slowly warmed to 12 °C (WARMING) or one week upon being on stable 12 °C temperature (STABLE) in pikeperch *Sander lucioperca* after the first photothermal induction of maturation

in WARMING (5.4 ± 1.4 ng/ml) compared to the STABLE group (3.8 ± 1.2 ng/ml). There was no difference ($P$ = 0.330) in the oocyte diameter at the time of hormonal induction (960.3 ± 30.9 µm and 984.1 ± 47.2 µm, in the WARMING and STABLE group, respectively). All six fish ovulated in the WARMING group, while two females were unable to ovulate in the STABLE group, remaining in FOM stage II and IV at the end of the evaluation period. Faster FOM dynamics were observed in the WARMING group ($P$ < 0.001, $t$-statistic), where all but one female ovulated in 10 days post-stimulation (total range of LT 7–13 days), while the four ovulated females in the STABLE group showed higher individual variability in LT – 5, 8, 13 and 14 days (Figure 6). Regarding ordinal regression for FOM dynamics, Nagelkerke $R^2$ was 0.864 and 0.406 for WARMING and STABLE respectively, with correlations being significant ($P$ < 0.001).

## DISCUSSION

This research demonstrated that reproductively "naïve" pikeperch females exposed to pho-

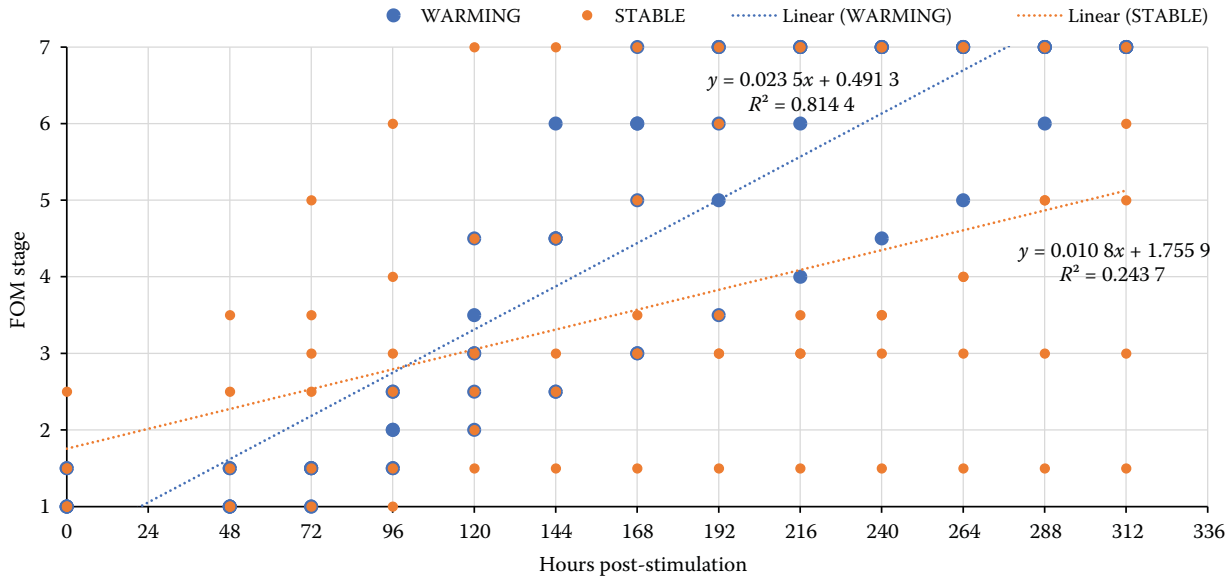


Figure 6. Final oocyte maturation (FOM) stages and upon calculated linear regression assessed at different times post-stimulation in fish ($n$ = 6) hormonally treated at either 6 °C and slowly warmed to 12 °C (WARMING) or one week upon being on stable 12 °C temperature (STABLE) in pikeperch *Sander lucioperca* after the first photothermal induction of maturation. The $t$-statistic showed a significant difference ($P$ < 0.001) between the two regression slopes
If the multiple fish from both groups were in the same FOM stage at the same time post-injection, the orange smaller point (STABLE) is filling the larger blue point (WARMING)

https://doi.org/10.17221/136/2023-CJAS

tothermal conditions that mimic the seasonality of Central European latitudes showed significantly faster vitellogenesis evident from a higher diameter of late vitellogenetic oocytes in comparison to controls. This outcome led to earlier and uniform overgrowth of diameter, signifying the state of OMC (Ljubobratovic et al. 2020; 2021; 2022b; 2023) in the entire population, while variability remained in the CONTROL group earlier not submitted to spawning induction protocol. Thus, fish subjected to multiple wintering cycles might reach the state of readiness for spawning induction before control fish. This is important since prolonged photothermal spawning induction duration after reaching optimal OMC state might lead to impairment of egg quality upon the hormonal stimulation (Ljubobratovic et al. 2022b). On the other side, more uniform OMC within the group could reduce the variable spawning outcome in younger breeders (Ljubobratovic et al. 2021). The importance of culture conditions and life history on gonadal maturation was earlier reported in pikeperch. Namely, Khendek et al. (2018) found that pond-reared breeders progressed significantly faster through gonadal maturation compared to RAS-reared females not exposed to photothermal spawning induction. However, in the above study, one group was reared in extensive pond conditions while the other was maintained in fully controlled conditions of RAS. Therefore, it was not possible to distinguish which environmental factors had the more decisive effect. Besides the oocyte diameter, GSI in fish exposed to wintering was also higher, which is also in agreement with the results reported by Khendek et al. (2018). Low GSI (below 10%), obtained for control fish in current research, seems to be characteristic for the fish not previously wintered (Zakes et al. 2013; Khendek et al. 2018; Ljubobratovic et al. 2020). On the other side, the GSI of above 10% is in line with the results of the previous study where fish wintered in the outdoor conditions at least twice before the artificial reproduction had a GSI above 10% (Ljubobratovic et al. 2021). Thus, it appears that wintering of the young breeders at earlier developmental stage (about 200 g mean weight) had a positive impact on gonadal development. Nevertheless, the present study did not evaluate the spawning outcome of this fish and requires further studies for its final confirmation.

Aside from being a direct stimulator of gonadal development, earlier wintering may potentially interfere with fish growth since pikeperch requires high temperatures for optimal growth (Ronyai and Csengeri 2008; Wang et al. 2009; Frisk et al. 2012). This may negatively affect spawning success (Lappalainen et al. 2003; Olin et al. 2018). Surprisingly, at the beginning of the second mimicked autumn in the present research, the mean weight of fish subjected to photothermal induction was not smaller than that of control fish, reared under a favourable thermal regime and photoperiod (Migaud et al. 2004; Hermelink et al. 2011). Moreover, the treated group grew significantly faster during the post-wintering mimicked summer. The cause for this phenomenon can be found in so-called compensatory growth (Py et al. 2022). Namely, the fish exposed to growth-impairing conditions has a certain ability to compensate for this lack of growth after optimal growth conditions are resumed. To the best of our knowledge, a single study dealt with short-term compensatory growth ability in pikeperch (Mattila et al. 2009). For its close relative walleye (*Sander vitreus*), this capability of growth compensation was described as well (Rosauer et al. 2011; Lester et al. 2014). Indeed, a study evaluating the walleye juveniles after cold banking (Harder et al. 2014) showed the growth improvement in these fish compared to juveniles reared under a constant high thermal regime. This phenomenon is important for the outdoor intensive culture of pikeperch as it was feasible during the growing season (Nagy et al. 2022). Thus, it might be hypothesised that two-summer culture in outdoor conditions will not result in significant growth inferiority compared to RAS-reared fish (Dalsgaard et al. 2013). The growth of fish was not a primary target of the present study; however, it may foster more comprehensive studies to evaluate the capability of growth compensation in pikeperch after wintering.

Thermal management during the spawning of the first wintered pikeperch females in this study significantly affected their response to hormonal stimulation. Besides the significantly faster FOM and higher ovulation of fish subjected to the incremental increase in spawning temperature after stimulation, these fish had significantly higher DHP levels in the blood at the time of injection. This finding is of particular

https://doi.org/10.17221/136/2023-CJAS

importance since recent studies have suggested DHP to be a potent maturation induction steroid (MIS) in Eurasian perch (*Perca fluviatilis*), another species common to the percids family (El Mohajer et al. 2021). This is, for that reason, intriguing as there were thus far three studies showing that the temperature most commonly applied for pikeperch spawning of 12 °C is applicable as the lowest temperature for the spawning induction (Hermelink et al. 2013; 2017; Milla et al. 2021). Though this thermal regime applied in the above studies was shown to be optimal from the aspect of vitellogenesis progress as the main parameter of ovarian development, it is worthwhile to consider the post-vitellogenetic period and achievement of the OMC state (Patino et al. 2001) as indicated by previous studies (Ljubobratovic et al. 2021; 2022b; 2023). Namely, a post-vitellogenetic luteinising hormone surge is required for the activation of membrane progesterone receptors (Thomas et al. 2001; Yamamoto and Yoshizaki 2008) and is also directly influencing the efficacy of MIS action. Considering the results of the present study on pikeperch's gonadogenesis in the outdoor conditions of its common climatic area (Ljubobratovic et al. 2021; 2022b; 2023) additional studies should evaluate the effect of different thermal regimes in late- and post-vitellogenetic oocytes on the OMC acquisition and further effect on egg quality.

The main issue for the second trial was the maturation dynamic. Considering, the oocyte biopsy was conducted daily, what might be considered stressful and as such could affect the spawning (Sarameh et al. 2012). Although both treatments were treated in the same manner and similar practice is routinely performed during commercial artificial reproduction, this issue is to be considered when evaluating the outcome. Finally, the results obtained in this research explain the earlier disagreements in optimal hormonal dosage observed for the two thermal spawning schedules (Ljubobratovic et al. 2021; 2022a). Namely, it was hypothesised that the dosage of 5 µg commonly applied when the temperature increases after hormonal stimulation was too low when the temperature is maintained higher throughout the spawning, where 50 µg was recommended (Zarski et al. 2019). The outcome of the present study substantiates this assumption and encourages lower dosages sufficient when hormonal stimulation is applied on wintering temperature before the thermal increment.

## CONCLUSION

The present study used individuals as replicates, while the replications on the tank level were not applied, and thus encourages further studies to investigate the outcomes deeper. Two key findings come up from this study. Wintering of pikeperch females at the younger phase leads to faster progress of vitellogenesis compared to fish under constant photothermal regime before first spawning induction, without impairing overall fish growth. Second, hormonal stimulation at low temperatures leads to a more efficient response in terms of faster FOM progress in first-time wintered fish. Post-vitellogenetic OMC acquisition on different thermal regimes might be an issue of interest for future studies.

### Conflict of interest

The authors declare no conflict of interest.

## REFERENCES

Dalsgaard J, Lund I, Thorarinsdottir R, Drengstig A, Arvonen K, Pedersen PB. Farming different species in RAS in Nordic countries: Current status and future perspectives. Aquac Eng. 2013 Mar;53:2-13.

El Mohajer L, Bulteau R, Chevalier C, Selmi S, Fontaine P, Milla S. In vitro follicle culture shows that progestagens are the maturation-inducing hormones (MIH) and possible regulators of the ovulation-mediating hormone PGE2 in female Eurasian perch Perca fluviatilis. Fish Physiol Biochem. 2021 Mar 28;47:881-94.

Frisk M, Skov PV, Steffensen JF. Thermal optimum for pikeperch (Sander lucioperca) and the use of ventilation frequency as a predictor of metabolic rate. Aquaculture. 2012 Jan 12;324-5:151-7.

Harder TM, Gotsch GG, Summerfelt RC. Growth response of juvenile walleyes after cold banking. N Am J Aquac. 2014 Jun 13;76:255-60.

Hermelink B, Wuertz S, Trubiroha A, Rennert B, Kloas W, Schulz C. Influence of temperature on puberty and maturation of pikeperch, Sander lucioperca. Gen Comp Endocrinol. 2011 Jun 1;172:282-92.

https://doi.org/10.17221/136/2023-CJAS

Hermelink B, Wuertz S, Rennert B, Kloas W, Schulz C. Temperature control of pikeperch (Sander lucioperca) maturation in recirculating aquaculture systems – Induction of puberty and course of gametogenesis. Aquaculture. 2013 Jun 20;400:36-45.

Hermelink B, Kleiner W, Schulz C, Kloas W, Wuertz S. Photothermal manipulation for the reproductive management of pikeperch Sander lucioperca. Aquac Int. 2017 Apr 26;25:1-20.

Khendek A, Chakraborty A, Roche J, Ledore Y, Personne A, Policar T, Zarski D, Mandiki R, Kestemont P, Milla S, Fontaine P. Rearing conditions and life history influence the progress of gametogenesis and reproduction performances in pikeperch males and females. Animal. 2018;12:2335-46.

Lappalainen J, Dorner H, Wysujack K. Reproduction biology of pikeperch (Sander lucioperca (L.)) – A review. Ecol Freshw Fish. 2003 May 6;12:95-106.

Lester NP, Shuter BJ, Venturelli P, Nadeau D. Life-history plasticity and sustainable exploitation: A theory of growth compensation applied to walleye management. Ecol Appl. 2014 Jan 1;24:38-54.

Ljubobratovic U, Peter G, Demeny F, Kugyela N, Horvath A, Pataki B, Horvath Z, Sandor ZJ, Ronyai A. Reproductive performance in virgin pikeperch (Sander lucioperca L.) females fed different dietary levels of arachidonic acid with respect to the duration of spawning induction. Aquac Rep. 2020 Nov;18: 8 p.

Ljubobratovic U, Kwiatkowski M, Toth F, Demeny F. Effects of hormonal treatment before water warming on synchronisation of spawning time, oocyte size, and egg quality in pikeperch (Sander lucioperca). Anim Reprod Sci. 2021 Mar;226: 11 p.

Ljubobratovic U, Demeny F, Peter G, Malinovskyi O, Kwiatkowski M, Pataki B, Horvath A. Can artificial reproduction strategies (hormonal type and dose/thermal regime) affect gamete quality in indoor-reared pikeperch (Sander lucioperca)? Aquac Rep. 2022a Apr;23: 4 p.

Ljubobratovic U, Fazekas G, Nagy Z, Kovacs G, Toth F, Feher D, Zarski D. Fish with larger pre-seasonal oocytes yields lower egg quality in season – A case study of outdoor-cultured domesticated Pikeperch (Sander lucioperca). Anim Reprod Sci. 2022b Mar;238: 10 p.

Ljubobratovic U, Kitanovic N, Milla S, Marinovic Z, Fazekas G, Stanivuk J, Nagy Z, Horvath A. Predicting population's oocyte maturation competence and evaluating individual's latency time using in vitro oocyte maturation in pikeperch (Sander lucioperca). Aquaculture. 2023. Jan 15;562: 10 p.

Mattila J, Koskela J, Pirhonen J. The effect of the length of repeated feed deprivation between single meals on compensatory growth of pikeperch Sander lucioperca. Aquaculture. 2009 Nov 1;296(1-2):65-70.

Migaud H, Fontaine P, Kestemont P, Wang N, Brun-Bellut J. Influence of photoperiod on the onset of gonadogenesis in Eurasian perch Perca fluviatilis. Aquaculture. 2004 Nov 26;241(1-4):561-74.

Milla S, Khendek A, Zarski D, Ledore Y, Ben Ammar I, Fontaine P. Duration of chilling phase, but not thermal condition, influence the gonad maturation of male and female domesticated pikeperch (Sander lucioperca). Aquacult, Fish Fisher. 2021 Nov 17;1:51-9.

Nagy Z, Ardo L, Demeny F, Gal D, Sandor ZJ, Ljubobratovic U. Case study on the aptness of in-pond raceways for pikeperch, Sander lucioperca, grow-out. Aquac Rep. 2022 Dec;27: 8 p.

Olin M, Vainikka A, Roikonen T, Ruuhijarvi J, Huuskonen H, Kotakorpi M, Vesala S, Ala-Opas P, Tiainen J, Nurminen L, Lehtonen H. Trait-related variation in the reproductive characteristics of female pikeperch (Sander lucioperca). Fish Manag Ecol. 2018 May16;25:220-32.

Patino R, Yoshizaki G, Thomas P, Kagawa H. Gonadotropic control of ovarian follicle maturation: The two-stage concept and its mechanisms. Comp Biochem Physiol B Biochem Mol Biol. 2001 Jun;129(2-3):427-39.

Py C, Elizondo-Gonzalez R, Pena-Rodriguez A. Compensatory growth: Fitness cost in farmed fish and crustaceans. Rev Aquac. 2022 Feb 1;14:1389-417.

Ronyai A, Csengeri I. Effect of feeding regime and temperature on ongrowing results of pikeperch (Sander lucioperca). Aquac Res. 2008 Apr 9;39:820-7.

Rosauer DR, Morris JE, Clayton RD. Role of compensatory growth in walleye fingerling production. N Am J Aquac. 2011 Jan 9;71:35-8.

Sarameh SP, Falahatkar B, Takami GA, Efatpanah I. Effects of different photoperiods and handling stress on spawning and reproductive performance of pikeperch Sander lucioperca. Anim Reprod Sci. 2012 Jun;132:213-22.

Thomas P, Pinter J, Das S. Upregulation of the maturation-inducing steroid membrane receptor in spotted seatrout ovaries by gonadotropin during maturation and its physiological significance. Biol Reprod. 2001 Jan 1; 64:21-9.

Wang N, Xu X, Kestemont P. Effect of temperature and feeding frequency on growth performances, feed efficiency and body composition of pikeperch juveniles (Sander lucioperca). Aquaculture. 2009 Apr 3;289:70-3.

Yamamoto Y, Yoshizaki G. Heterologous gap junctions between granulosa cells and oocytes in ayu (Plecoglossus altivelis): Formation and role during luteinising hormone-dependent acquisition of oocyte maturational competence. J Reprod Dev. 2008;54:1-5.

https://doi.org/10.17221/136/2023-CJAS

Zakes Z, Szczepkowski M, Partyka K, Wunderlich K. Effect of gonadotropin hormonal stimulation on out-of-season propagation success of different year classes of indoor-reared pikeperch (Sander lucioperca (L.)). Aquac Int. 2013 Jul 24;21:801-10.

Zarski D, Kucharczyk D, Targonska K, Palinska K, Kupren K, Fontaine P, Kestemont P. A new classification of pre-ovulatory oocyte maturation stages in pikeperch, Sander lucioperca (L.), and its application during artificial reproduction. Aquac Res. 2011 Apr 21;43:713-21.

Zarski D, Horvath A, Bernath G, Krejszeff S, Radoczi J, Palinska-Zarska K, Bokor Z, Kupren K, Urbanyi B. Collection of gametes. In: Zarski D, Horvath A, Bernath G, Krejszeff S, Radoczi J, Palinska-Zarska K, Bokor Z, Kupren K, Urbanyi B, editors. Controlled reproduction of wild Eurasian perch – A hatchery manual. The Netherlands: Springer Netherlands; 2017. p. 41-51.

Zarski D, Fontaine P, Roche J, Alix M, Blecha M, Broquard C, Krol J, Milla S. Time of response to hormonal treatment but not the type of a spawning agent affects the reproductive effectiveness in domesticated pikeperch, Sander lucioperca. Aquaculture. 2019 Mar 30; 503:527-36.

Received: October 12, 2023
Accepted: December 29, 2023
Published online: January 30, 2024