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Oocyte maturation and changes of plasma steroid levels in the wild and cultured pikeperch, *Sander lucioperca* following hormonal induction

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ABSTRACT

The present study was designed to compare plasma steroid levels and spawning success between wild and cultured female pikeperch, *Sander lucioperca* after induction of ovulation by different hormones. Two groups of wild-caught (1183.1 ± 56.1 g) or cultured (337.4 ± 20.1 g) fish were treated with human chorionic gonadotropin (hCG; 650 IU kg^{-1}), common carp pituitary homogenate (CPH; 6 mg kg^{-1}), luteinizing hormone releasing hormone analog ($LHRHa_2$; 13.5 µg kg^{-1}), and saline solution as control group. The females were injected with those agents as priming and resolving dosages. The blood samples were obtained before hormonal treatment and after ovulation followed by assaying plasma levels of sex steroids (17β-estradiol, testosterone and progesterone) and stress indicators (cortisol, glucose and lactate). The results revealed that all wild-caught females responded to CPH and $LHRHa_2$, while only 75% of wild-caught females injected with hCG and cultured ones injected with hCG and CPH responded to the hormones and that no cultured ones responded to the injection of $LHRHa_2$. According to reproductive parameters including the number of eggs g^{-1} and working fecundity, no significant differences were found in various treatments. All hormonal treatments exhibited an elevation in cortisol and glucose values in cultured fish after ovulation. In contrast, plasma cortisol levels declined significantly after ovulation in wild groups. The present study displayed that CPH and hCG induce a high reproductive performance and can be recommended to induce the controlled reproduction of either wild-caught or farmed pikeperch. In addition, our results revealed that wild-caught fish are more sensitive in term of cortisol and glucose levels than cultured conspecifics.

Keywords: Broodstock, Cortisol, Hormonal treatment, Sander lucioperca, Sex steroids.
Article type: Research Article.

INTRODUCTION

Pikeperch, *Sander lucioperca*, is a member of percids family, which lives in both fresh and brackish waters (Steenfeldt *et al*. 2015). Pikeperch is considered as one of the most valuable recreational and commercial fish, however unfortunately their stocks are declining due to overfishing, environmental disturbances and pollutions. Therefore, the production of pikeperch fry or fingerling is essential for stocking into the rivers in order to resource enhancement and market demands (Policar *et al*. 2016, 2019). In Iran, annually, over millions of pikeperch fingerlings are produced by public hatchery through the controlled propagation in tanks or ponds using artificial nests in natural spawning season from March to April (Falahatkar *et al*. 2018). This method is time-consuming, and has low efficiency, so that fecundity and egg quality are low. Moreover, the responded broodstock and the number of larvae produced in this way are low. Furthermore, most hatchery activities are based on brooders caught from the wild which the time of ovulation or spawning is not synchronized among them. Therefore, the

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development of reliable methods which increase the predictable and synchronized spawning, would improve fry and fingerling production in pikeperch. In general, failure of brooders to complete oogenesis is common reproductive dysfunction in captive environment (Mylonas *et al*. 2010). This might be due to the lack of proper spawning grounds, stressors in captivity, and lack of gonadotropins (Zohar & Mylonas 2001). These factors cause the failure of ultimate maturation, ovulation and spawning among females. Therefore, exogenous hormonal treatments are required to induce ovulation (Kucharczyk *et al*. 2020). Generally, ovulation is induced in many captive-bred teleosts using human chorionic gonadotropin (hCG), common carp pituitary homogenate (CPH), or synthetic analogue gonadotropin (sGnRHa; Brzuska 2020; Akbari Nargesi *et al*. 2022). Several studies have demonstrated that different reproductive parameters including sex steroid levels, latency time and working fecundity are affected by exogenous hormonal treatments (Haddy & Pankhurst 2000; Part *et al*. 2001; Dorafshan *et al*. 2003; Bayunova *et al*. 2006; Falahatkar *et al*. 2013; Podhorec *et al*. 2016; Mohammadzadeh *et al*. 2021). In addition, some studies indicated that exogenous hormonal treatments induce stress responses in fish (Haddy & Pankhurst 2000; Bayunova *et al*. 2002; Semenkova *et al*. 2002; Falahatkar & Poursaeid 2014; Żarski *et al*. 2020). Due to less access of the wild stocks of some endangered species, the other source to keep the success of reproduction and sustainable aquaculture is achieved by employing domesticated fish (Żarski *et al*. 2015). Our previous observations indicated that there were some differences in cultured pikeperch in response to hormonal treatments (Falahatkar & Poursaeid 2014; Kucharczyk *et al*. 2021). In order to develop the controlled reproduction of pikeperch and to conserve the wild populations through restocking program, it is important to know the physiological and hormonal alterations through the final oocyte maturation in farmed fish. It appears that the wild-caught fish is more sensitive than the cultured fish to any handling. There is a lack of studies regarding to compare wild and domesticated pikeperch in controlled conditions and to date, there has been no comparison between the wild and cultured pikeperch in response to different hormonal treatments. As a potential stressor, hormonal administration to induce reproduction of fish may alter stress indicators (Falahatkar & Poursaeid 2014; Żarski *et al*. 2020), however, differences in wild or domesticated stocks are not known. So, the impacts of various hormonal agents on reproductive performance, as well as changes in plasma stress indicators and sex steroid levels were investigated in wild and cultured pikeperch.

MATERIALS AND METHODS

Fish

The present trial was performed on the two groups of sexually-matured female pikeperch (wild-caught and cultured females). Sixteen wild females with an average weight of 1183.1 ± 56.1 g (SE) and total length of 55.2 ± 0.8 cm were used to induce ovulation. These broodstock were captured from the reservoir dam of Aras River (West Azerbaijan, Iran) by net seine in November and transported by a truck which was equipped with aeration system to the Dr. Yousefpour Marine Fishes Restocking and Genetic Conservation Center (Siahkal, Guilan, Iran). After transferring, fish were maintained in earthen pond for the rest months up to start of experiment on March. Sixteen cultured F1 fish with an average weight of 337.4 ± 20.1 g and total length of 35.8 ± 0.6 cm used in this study were originally hatched from the eggs collected in wild broodstock population in the same hatchery in a 4-ha earthen pond under natural condition and fed with bait fish for 2 years. Sixteen fish from each group of wild or domesticated animal were randomly allocated into four hormonal treatments: human chorionic gonadotropin (hCG; n=4), common carp pituitary homogenate (CPH; n=4), luteinizing hormone releasing hormone analogue ($LHRHa_2$; n=4), and control (0.9% physiological saline solution; n=4). Each group was randomly stocked into 8 circular concrete tanks (1.95 m Ø, depth 30 cm and volume 890 L) with a flow rate of 18.0 ± 0.5 L min^{-1} with 2 replicates each contained 4 fish. Pikeperch broodstock were acclimatized for 10 days before induction of ovulation in these tanks. All groups were held under the same experimental condition before any handling. Feeding ceased 5 days before the initiation of the experiment and during the sampling. For preventing any disturbance, the tanks were kept constantly in darkness using a curtain. During acclimation and experiment, water temperature ranged from 13.7 to 14.5 ºC, average dissolved oxygen, oxygen saturation and pH were 9.0 ± 0.9 mg L^{-1}, 91.6 ± 11.0% and 7.7 ± 0.1, respectively.

Ovulation induction

The broodstock were injected with the following hormones: hCG, CPH, $LHRHa_2$ and saline solution as control. All fish were injected in priming and resolving doses, while the male fish were injected once when the females were injected at the second time. The interval between injections was considered as 48 h. The treatments and

injected doses of hormones are given in Table 1. All agents were dissolved in a carrier (0.9% physiological saline solution) and injected into the muscle between dorsal fin and lateral line. Before any handling, all fish were anaesthetized using 150 mg L^{-1} tricaine methane sulphonate (MS-222).

Table 1. Doses of inducing hormones at priming and resolving injections in different treatments.

Hormonal treatment	Priming dose	Resolving dose
Control	-	-
CPH (mg kg^{-1})	1.5	4.5
hCG (IU kg^{-1})	150	500
$LHRHa_2$ (µg kg^{-1})	3.5	10

Note: CPH, carp pituitary homogenate; hCG, human chorionic gonadotropin; luteinizing hormone releasing hormone analogue.

Broodstock were inspected for any sign of ovulation by applying gentle abdominal pressure approximately 12 h following the resolving injection and subsequently every 2 h for 48 h. Moreover, the experimental tank bottom was monitored for appearance of any released eggs by females. After responding the fish, gametes were hand stripped and fertilization was performed based on the routine procedure (Kucharczyk *et al*. 2007).
To determine the number of eggs per gram and working fecundity, a sample of eggs was taken and then the number of eggs was counted. Working fecundity was calculated as follow:

Working fecundity = number of eggs/g × total obtained egg weight

Blood sampling and analyses

About 2-3 mL of blood were taken using a 5-mL heparinized syringe from the caudal vasculature of wild and cultured females before hormonal treatment and exact after the ovulation. Test tubes containing blood were centrifuged at 1500 × *g* for 10 min at room temperature. Plasma was stored at −20 ºC for later measurement of cortisol, lactate, glucose and sex steroids levels. Cortisol and sex steroids (17β-estradiol, testosterone, and progesterone) levels in plasma were measured in duplicate by radioimmunoassay using available commercial kits (Immunotech, Marseille, France) based on method described by Pankhurst & Carragher (1992). The glucose level was measured using an commercial kit (Wako Pure Chemical Ind. Ltd., Osaka, Japan) based on a glucose oxidase/peroxidase enzymatic reaction (Bayunova *et al*. 2002). Plasma lactate level was measured using Sigma Diagnostic Kits (St Louis, MO, USA) according to Barton *et al*. (2005).

Statistical analysis

All percentage data was converted to arc-sin before analysis. The normality of data and homogeneity of variances were tested by Kolmogorov-Smirnov and Levene's tests, respectively. Since the data for progesterone was not normally distributed, a non-parametric test (Kruskal-Wallis) was performed. The effects of hormones on spawning and plasma parameters were evaluated by General Linear Model (3-way analysis of variance; fish origin, hormone, and time) and then Tukey as post-hoc test. An independent sample t-test was considered to find the statistical difference in measured parameters prior to injection and after spawning. All statistical testes were analysed using SPSS (Version 13, Chicago, IL, USA) at the significant level of $p < 0.05$.

RESULTS

The number of responded fish, number of eggs per gram and working fecundity in tested fish are presented in Table 2. In wild females, of the hormones tested, CPH and $LHRHa_2$ was found to be the most effective, so that all females treated with these hormones were ovulated after second injection. While, three of four females administrated by hCG spawned and other one was over matured. In cultured fish, three of four females administrated by CPH and hCG were ovulated but none of $LHRHa_2$-injected and control females were spawned. Number of eggs g^{-1} was not significantly different among the fish ranging from 906.7 to 1200 eggs ($p > 0.05$). Moreover, no changes were found in working fecundity between wild or domesticated or hormone-injected fish ($p > 0.05$). Plasma levels of stress metabolites and sex steroid concentrations in wild or domesticated pikeperch when received various induction hormones are presented in Table 3. Cortisol concentrations were significantly higher in domesticated pikeperch at ovulation time ($p < 0.05$), while lower levels were observed in wild fish following the ovulation ($p < 0.05$).

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Table 2. Effect of various hormone treatments on spawning parameters of pikeperch *Sander lucioperca* (n=4 for each treatment, mean ± SE).

	Control		CPH		hCG		$LHRHa_2$	
	Wild	**Domesticated**	**Wild**	**Domesticated**	**Wild**	**Domesticated**	**Wild**	**Domesticated**
No. of responded fish	0/4	0/4	4/4	3/4	3/4	3/4	4/4	0/4
No. of eggs g^{-1}	-	-	1200	975.1	1198	906.7	1079	-
Working fecundity (g)	-	-	164 ± 34.9	32.8 ± 18	46.7± 39.2	40.3 ± 15.8	101 ± 19.7	-

Note: CPH, carp pituitary homogenate; hCG, human chorionic gonadotropin; luteinizing hormone releasing hormone analogue.

Table 3. Plasma biochemical characteristics of wild or domesticated female pikeperch (*Sander lucioperca*) before injection and after ovulation with various inducing hormones.

		Control		CPH		hCG		$LHRHa_2$	
		Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Cortisol	Wild	90.7 ± 11.9^{bc}	5.0 ± 0.6^{c}	103.5 ± 27.4^{bc}	9.6 ± 3.9^{c}	102.5 ± 17.0^{bc}	18.9 ± 11.0^{c}	94.3 ± 38.9^{bc}	82.0 ± 41.0^{bc}
(ng mL^{-1})	Domesticated	26.8 ± 20.1^{c}	78.3 ± 40.7^{bc}	41.5 ± 24.5^{c}	190.0 ± 30.0^{ab}	10.0 ± 2.0^{c}	260.0 ± 30.5^{a}	12.0 ± 1.0^{c}	36.5 ± 10.5^{c}
Glucose	Wild	115.1 ± 26.6	92.3 ± 10.7	115.8 ± 21.6	93.3 ± 8.9	110.3 ± 18.0	90.3 ± 12.5	131.5 ± 23.4	128.5 ± 14.7
(mg dL^{-1})	Domesticated	58.6 ± 8.1*	73.3 ± 7.7	78.0 ± 6.0*	120.5 ± 5.5	48.0 ± 3.0*	113.5 ± 9.3	44.5 ± 0.5*	76.3 ± 9.0
Lactate	Wild	27.7 ± 5.5	29.0 ± 1.7	23.8 ± 4.7	23.8 ± 6.5	33.0 ± 3.0	23.0 ± 5.0	30.3 ± 3.3	26.5 ± 4.2
(mg dL^{-1})	Domesticated	32.4 ± 3.2	28.7 ± 4.3^{b}	25.5 ± 2.5	24.0 ± 2.0^{b}	39.0 ± 2.0	39.3 ± 2.7^{ab}*	36.0 ± 4.0	48.7 ± 4.3^{a}*
17β-estradiol	Wild	15.6 ± 2.4^{ab}	11.1 ± 6.5^{ab}	11.1 ± 4.6^{ab}	15.3 ± 3.8^{ab}	11.5 ± 5.5^{ab}	7.9 ± 1.2^{b}	12.6 ± 2.8^{ab}	6.2 ± 1.6^{b}
(ng mL^{-1})	Domesticated	9.1 ± 2.2^{ab}	7.6 ± 2.7^{b}	8.3 ± 1.3^{ab}	5.7 ± 3.1b	20.0 ± 2.0^{ab}	65.0 ± 13.7^{a}	10.0 ± 5.0^{ab}	17.3 ± 5.9^{ab}
Testosterone	Wild	4.0 ± 2.1	2.5 ± 2.3	3.5 ± 1.4	1.8 ± 1.3	5.3 ± 3.0	1.1 ± 0.7	3.7 ± 1.4	0.4 ± 1.1
(ng mL^{-1})	Domesticated	3.2 ± 1.4	2.0 ± 1.5	2.8 ± 2.2	0.2 ± 0.1	5.3 ± 2.3	3.6 ± 2.8	3.5 ± 2.5	0.7 ± 0.1
Progesterone	Wild	0.2 ± 0.0^{a}	0.1 ± 0.0^{ab}	$0.1 \pm 0.0^{ab*}$	0.1 ± 0.0^{ab}	0.1 ± 0.0^{ab}	$0.1 \pm 0.0^{ab*}$	0.1 ± 0.0^{b}	0.1 ± 0.0^{ab}
(ng mL^{-1})	Domesticated	0.1 ± 0.0^{ab}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{ab}	$0.1 \pm 0.0^{b*}$	0.1 ± 0.0^{ab}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{ab}

Note: Different letters in each row show significant differences among the hormones, times and origin of fish. An asterisk shows difference between wild and domesticated fish at each sampling time ($p < 0.05$).

The concentration of glucose in domesticated fish before injection was significantly lower than that in the wild type ($p = 0.002$). However, there was no significant difference after hormonal injection ($p > 0.639$). The interaction between fish origin, time and hormones was not significant ($p > 0.05$). No significant alternations were found in lactate levels in wild fish, while there was only significant difference among domesticated fish after hormonal injection, so that the concentration of lactate in fish injected with $LHRHa_2$ was significantly higher than those in fish injected with CPH and saline ($p = 0.009$). Also, the interaction between the variables was not significant ($p = 0.229$). The highest level of 17β-estradiol was found in domesticated pikeperch which received hCG after the ovulation ($p = 0.003$), while no interaction was found among the variables ($p = 0.183$). No significant alternation and interaction were observed in testosterone among the hormonal treatments before injection and after ovulation in wild or domesticated fish ($p = 0.833$). High level of progesterone was found in wild fish of control before injection ($p = 0.008$). In addition, a significant interaction of progesterone was found between wild and domesticated pikeperch before the injection of CPE and after the ovulation of hCG group ($p = 0.018$).

DISCUSSION

The present results obviously demonstrated that CPH effectively induced gonads maturation and ovulation in pikeperch spawners. All the wild broodstock responded positively to CPH injections, while only 75% of farmed females ovulated in this treatment. The positive effects of CPH have been stated in other teleosts e.g. Asian catfish, *Clarias batrachus* (Zonneveld *et al*. 1988), *Rutilus frisii kutum* (Paykan Heyrati *et al*. 2007) and other species (Drori *et al*. 1994; Szabo *et al*. 2002). Although CPH is highly effective to induce gonadal maturation and ovulation in fish, there are several drawbacks associated with their use including the lack of information on the quantitative content of gonadotropin in the extract, varying efficacies in different species, possible role in disease transfer and problems related to its preparation, storage and supply (Zohar & Mylonas 2001). The substitution of CPH with small amounts of gonadotropic hormone has been used as an effective solution for most fishes (Mylonas *et al*. 2010). Hence this study attempts to substitute CPH with gonadotropin hormone in inducing maturation. Nowadays, hCG and similar analogues are used to release gonadotropins and to stimulate steroidogenesis, ovulation and sexual maturity in fishes. These hormones have produced better performance than the pituitary extracts (Zohar & Mylonas 2001; Kucharczyk *et al*. 2019; Nowosad *et al*. 2023) because of more purity. In the present study, the administration of hCG produced similar results in the two groups of spawners- wild and domesticated fish whereby 75% of spawners responded positively to the injection. hCG acts directly upon gonads and is not influenced by level of LH and the activity of pituitary gonadotropins. Not all female spawners responded to hCG injections and this may be due to the inhibiting effects of stress and/or because the ovaries of these spawners probably had not yet reached the final stage of maturity or required a higher dose of this hormone (Żarski *et al*. 2011a,b 2012). The positive effects of 100 to 4000 IU/kg body weight for hCG have been reported in other bony fishes (Hodson & Sullivan 1993; Caylor *et al*. 1994; Emata *et al*. 1994; García-Alonso & Vizziano 2004) as well as pikeperch (Zakęś & Demska-Zakęś 2005; Kucharczyk 2007; Blecha *et al*. 2016; Falahatkar *et al*. 2018, 2022). Inducing ovulation using GnRHa is a commonly practiced method to control reproduction of many commercial fish species. The use of GnRH has advantage for fish breeding centers. This hormone has been efficaciously used to induce spawning in many species such as sea bream, *Sparus aurata* (Zohar *et al*. 1989), salmon (Mylonas *et al*. 1992; 1995a), *Psedopleuronectes americanus* (Harmin & Crim 1992), *Alosa sapidissima* (Mylonas *et al*. 1995b), and *Pleuronectes ferrugines* (Larsson *et al*. 1997). All wild female spawners responded to $LHRHa_2$ injections indicating that $LHRHa_2$ has potent stimulator effects in inducing final ovulation in wild spawner of pikeperch. Therefore, when used in the final stage of sexual maturity, speeds up the germinal vesicle migration and germinal vesicle breakdown, making the animal ready for spawning. These findings are in accordance with the findings of others who studied the effects of GnRH on inducing spawning in several other teleosts (Mylonas & Zohar 2001; Szabo *et al*. 2002; Levavi-Sivan *et al*. 2004; Kucharczyk *et al*. 2005; Rainis & Ballestrazzi 2005; Paykan Heyrati *et al*. 2007; Ma *et al*. 2020). In the present study, domesticated female spawners did not respond as similar as the wild female to the hormone injection. Kujawa *et al*. (2011) reported no significant alterations in reproductive performance between wild and domesticated tench (*Tinca tinca*). Differences in hormone response in spawners injected with similar doses may be due to the influence of environmental, biological and physiological conditions of each spawner. The lower response in domesticated females may be due to their smaller size and age, hence the amount of LH stored in their pituitary was too low to trigger spawning (Kucharczyk *et al*. 2022). Moreover, spawning failure in domesticated females injected with $LHRHa_2$ may also

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be linked to elevated levels of cortisol in these fish and the inhibiting effects of cortisol on the hypothalamus, pituitary and gonads. Since the number of domesticated fish was low, it is suggested that working with higher number of fish in future study. Cortisol levels in domesticated females were elevated after hormone injection although this increase was significant only in the CPE- and hCG-injected groups. Cortisol levels in wild spawners were higher before the hormone injection, but were lower after ovulation. Elevated cortisol levels in wild spawners may be related to the handling and keeping the fish in captivity, making them inadaptable to these changes. Domesticated spawners, on the other hand, are well adapted to the culture conditions and showed lower plasma cortisol levels prior to hormone injection. It is evident from the results of the present study that spawning induction using hormone injections causes acute stress in pikeperch spawners. Plasma cortisol levels, the most important corticosteroid response to stress, have been increased in domesticated spawners (Pickering *et al*. 1982). The negative reaction of corticosteroid at all levels of the hypothalamus-pituitary-interrenal (HPI) axis may be the reason for the control of cortisol releasing in wild spawners (Leatherland *et al.* 2010). This complex mechanism regulates cortisol levels in blood. Hence elevated cortisol level in spawners, prior to hormone injection, inhibits the release of cortisol in these fish when they are under stress during the artificial breeding (Fryer *et al*. 1984; Bradford *et al*. 1992). Study on female sockeye salmon *Onchorynchus nerka* showed that these spawners exhibited elevated levels of cortisol before being exposed to acute stress of catching, handling and sampling, suggesting that these fish were either physiologically stressed and/or were experiencing chronic stress caused by environmental variations (Kubokawa *et al*. 1999). Contradicting results have been reported on cortisol levels in teleosts during spawning induction and/or during final oocyte maturity. In some studies, plasma cortisol levels in fish increased prior to or during oocyte maturation (Pickering & Christie 1981; Kusakabe *et al*. 2003). However, in black bream *Acanthopagrus butcheri*, plasma cortisol concentrations decreased 24 h after capture (Haddy & Pankhurst 1999). Moreover, a rise in cortisol concentrations was found in black bream 24 h after injection of different hormones (hCG and LHRHa) to induce maturity, although no significant differences were reported among the hormone treatments (Haddy & Pankhurst 2000). In the current study, although cortisol concentrations in wild female spawners showed decreasing trends at the time of ovulation in all treatments, it appears that $LHRHa_2$ stimulates the HPI axis to secrete corticosteroids reaching a level of 82 ng mL^{-1} in this treatment. The level of corticosteroids in all other treatments was less than 20 ng mL^{-1}. Domesticated spawners showed greater sensitivity to CPH and hCG injections resulting in highest cortisol levels after injection in these treatments. These agents supposed to influence the immune system, explaining the fish sensitivity to these hormones. The increased energy demand accompanied with adapting to stress is met through glycogenesis and glycogenolysis (Mommsen *et al.* 1999; Barton 2002) resulting in the fast increase in the blood levels of catecholamines and corticosteroids which in turn elevates the production of glucose as a secondary stress response. Present results showed that plasma glucose concentrations in wild females were high prior to hormone treatment, however numerically but not significantly dropped with the decrease in the circulating cortisol levels. In domesticated females there was a simultaneous upraise in cortisol and glucose concentrations after hormone treatments. No significant alterations were found for lactate levels in domesticated fish during the ovulation. Lactate is produced during the anaerobic energy metabolism to retain acid-base equilibrium (Barton *et al*. 1998; Ruane *et al*. 2001). Hormone treatments on wild and domesticated spawners could not able to change the lactate levels. Studies on sturgeons have demonstrated rapid increase in lactate concentrations (between 0.5 to 2 h) following stressful conditions. It is however possible that lactate concentrations upraised prior to ovulation and blood sampling and then decreased to their basal level. The results of present study have shown that final stage of oocyte maturation in pikeperch can be induced by the administration of a variety of CPH and hCG. However with regard to the higher cost of pituitary extract, the administration of hCG as a synthetic hormone is recommended for artificial breeding operations of pikeperch. Generally, decrease in plasma sex steroid levels was reported by different agents, however no significant differences were found in pikeperch regarding to testosterone and progesterone. Present results showed drop in the steroid hormone concentrations in females during ovulation, except for 17β-estradiol in the CPE wild group as well as hCG and $LHRHa_2$ in domesticated groups. The plasma levels of 17β-estradiol decreased during the early stage of final maturation, which may be due to the decreased aromatase activity and the elevated degradation of this hormone in blood before ovulation. The drop in the levels of this hormone declines the negative feedback control of gonadotropin secretion, hence induces sexual maturation in fish (Nagahama & Yamashita 2008). In walleye, *Sander vitreum*, vitellogenesis was almost completed before mid-January and 17β-estradiol levels continuously decreased until the event of spawning (Malison *et al.* 1994). However in the yellow perch,

Perca flavescens, 17β-estradiol continued to be very high up to spawning season, exhibiting the vitellogenesis activation, while decreased to previtellogenic levels after ovulation. Low temperature (at 6 °C) holding of domesticated pikeperch displayed its increasing levels in females compared to high temperature (Milla *et al*. 2021). 17β-estradiol levels in female spawners were not significantly different in the treatments studied suggesting that hormone treatments used did not significantly decrease plasma 17β-estradiol levels in pikeperch spawners. It may be therefore concluded that plasma 17β-estradiol levels in female spawners were declined during the ovulation and spawning. Higher plasma levels of cortisol in domesticated females compared to wild spawners may be the reason for severe drop in 17β-estradiol and testosterone concentrations in teleosts. Several researches have documented the detrimental impacts of stress on the hypothalamus-pituitary-gonad axis and the reciprocal alterations in sex steroids following handling (Pankhurst & Van Der Kraak 1997, 2000; Haddy & Pankhurst 1999, 2000). These results exhibit that stress caused by handling during the artificial breeding lowers the response of fish to hormone treatment. Similar result is reported by Haddy & Pankhurst (2000) in black bream. In the present study on pikeperch, alterations in plasma levels of testosterone were similar to those in plasma levels of 17β-estradiol in female spawners. Testosterone levels were dropped during the ovulation. Increase in testosterone concentrations before the ovulation has also been stated in other fish (Mylonas & Zohar 2001; Barrero *et al*. 2008). Pankhurst et al. (1986) showed that walleye exhibited significant increase in testosterone levels after receiving LHRHa injection compared to the control group. Similarly, decreases in testosterone with a simultaneous elevation in 17α, 20β dihydroxy progesterone (DHP) have been reported in rainbow trout (Fostier & Jalabert 1982; Scott *et al*. 1983), coho salmon (Van Der Kraak *et al*. 1984), and striped bass *Morone saxatilis* (Mylonas & Zohar 2001). 17α-hydroxy progesterone is a precursor of testosterone and DHP. Hence the elevated concentrations of DHP and/or levels of gonadotropins inhibit the activities of C17-20 lyase and 17α-hydroxy dehydronase to convert 17α-hydroxy progesterone to testosterone and provide the conditions for the synthesis of DHP from 17α-hydroxy progesterone (Scott *et al*. 1983). Many studies reported the crucial role of DHP in final maturation. During the vitellogenesis, gonadotropic stimulation of the ovary resulted in production of 17α hydroxy progesterone by thecal cells (Nagahama & Yamashita 2008), which is converted into DHP (Young *et al*. 1986). In our study on pikeperch, no significant variations were observed in plasma progesterone levels after hormone injection. Similarly, very low levels of progesterone have been reported for striped bass and white bass (Mylonas *et al*. 1997; Mylonas & Zohar 2001). It may thus be concluded that ovulation can be induced even at low levels of progesterone (Matsuyama *et al*. 1995). Therefore, it seems that progesterone level is not important as the other maturation inducing hormones for ovulation of wild or domesticated pikeperch. Changes in water temperature and food items consumed by spawners during the vitellogenesis are two main aspects which could probably affect the gametes quality, spawning performance and biochemical parameters in fish from different origins (wild or domesticated). Moreover, weight and age of fish, holding condition can affect reproductive success and physiological changes after stimulation by different agents. According to our results, it is recommended that the domestication process can reduce the sensitivity to stress and related metabolites.

CONCLUSION

The findings of the present study clearly revealed that wild pikeperch is more sensitive to the handling stressors caused by hatchery practices before the hormonal induction compared to domesticated fish, however, elevations of stress metabolites after the ovulation revealed higher sensitivity of this group to hormonal treatment. In addition, changes of sex steroids were not significant except for 17β-estradiol which is clearly exhibited the role of fish origin and hormone induction at the final stage of maturation.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHORS CONTRIBUTION

B.F.: Experimental design; data curation; project administration; supervision; sampling; sample analysis; data analysis; writing original draft. S.P.: Lab working; Experimental design; data curation; draft-editing. H.E.L.: project administration; data analysis, rewriting original draft. I.E.: Preparing all equipment for rearing and sampling of fish. B.M.: Preparing all equipment for rearing and sampling of fish; sampling.

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