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Benefits of hormone treatment of both sexes in semi-artificial reproduction of pikeperch (*Sander lucioperca* L.)

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ABSTRACT: Propagation of pikeperch *Sander lucioperca* by semi-artificial management was investigated using hormonal induction to better understand the effect of hormonal treatment of both sexes. Fourteen pairs of pikeperch brood fish were divided into two groups (seven pairs in each group). Seven females in Group A and both sexes in Group B were hormonally injected with 500 IU of hCG per kg. Each pair of brood fish was held separately in a 300-l circular tank with an added spawning material on the bottom. Brood fish were removed from the tanks on the day after spawning; three-hundred randomly selected eggs were removed from the spawning nests and transferred to 250 ml plastic incubators. Sperm samples were collected from males in individual 1-ml syringes. Significant differences in fertilization rate (Group A 59.5%, Group B 80.4%), hatching rate (Group A 51.2%, Group B 71.6%), and number of larvae produced per female (Group A 49 429, Group B 122 000) were observed. Differences were attributable to sperm quality, primarily volume (Group A 0.16 ml, Group B 0.64 ml), and duration of spermatozoa motility (Group A 59.5 s, Group B 97.7 s). Hormone treatment of both sexes is beneficial for pikeperch semi-artificial reproduction by inducing ovulation and improving milt production and spermatozoa quality, fertilization and hatching rate, all contributing to a higher number of produced larvae.

Keywords: hCG; spermatozoa; fertilization; hatching; spawning nest; larvae

INTRODUCTION

Pikeperch (*Sander lucioperca* L.) is important in reservoir ecology as the natural predator of small cyprinids (roach – *Rutilus rutilus* L., rudd – *Scardinius erythrophthalmus* L., or bream – *Abramis brama* L.), and as a valuable freshwater game fish in Europe due to its delicate flesh (Schulz et al. 2007; Kubecka et al. 2009; Kristan et al. 2013). Unfortunately, the natural populations of pikeperch are affected by over-fishing in many areas and by human-related environmental degradation (Dil 2008). Pikeperch have been farmed in ponds or lakes through natural spawning and recruitment (Hilge and Steffens 1996). As an alternative to this traditional method, a combination of pond and recirculation aquaculture system (RAS) is currently being used (Policar et al. 2014). This technique is based on rearing larvae and juveniles in ponds to produce advanced fingerlings (total length 30–50 mm) and then using intensive culture and artificial pellet feeding (Ruuhijarvi and Hyvarinen 1996; Zakes and Demska-Zakes 1998; Policar et al. 2014). A new development which is being applied in West European countries is the rearing of pikeperch production under complete

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RAS conditions (Policar et al. 2014; Blecha et al. 2015). The demand for pikeperch to restock natural waters and for intensive farming is increasing and will require more efficient methods of reproduction (Zakes and Demska-Zakes 2009).

The oldest method of pikeperch propagation is by natural spawning in ponds (Steffens et al. 1996). The main disadvantages of this method are low efficiency and inability to predict the final production (Demska-Zakes and Zakes 2002). Improved results are obtained through the semi-artificial spawning (Schlumberger and Proteau 1996) which can be conducted under hatchery conditions (Kucharczyk et al. 2007) where artificial spawning substrata are added such as coniferous tree branches, mats, carpets or artificial grass (Skrzypczak et al. 1998; Demska-Zakes and Zakes 2002). The spawning material can be checked several times per day for eggs. After spawning, brood fish are removed from the cages or tanks; the eggs can be left in the spawning tanks to incubate or transferred on the spawning material to special incubation tanks (Kucharczyk et al. 2007). Also, brood fish can be hormonally stimulated using the semi-artificial reproduction (Zakes and Demska-Zakes 2009).

Non-hormone-stimulated pikeperch females held in captivity usually do not ovulate (Zakes and Szczepkowski 2004) or ovulation occurs only in few individuals (Salminen et al. 1992). Kucharczyk et al. (2007) suggest that pikeperch do not spawn in captivity because of stress, lack of spawning substrate, or unsuitable photo-thermal regime. To circumvent these problems, hormonal induction can be used. The substances that stimulate fish maturation and reproduction have been extensively investigated in recent years (Steffens et al. 1996). These substances stimulate either the hypothalamus, hypophysis or the gonads (Demska-Zakes and Zakes 2002). Final maturation and synchronization of the ovulation in pikeperch can be achieved by administering carp pituitary extract (CPE), luteinizing hormone-releasing hormone (LH-RH) (Ronyai 2007), mammalian gonadotropin releasing hormone analogue (GnRHa) or human chorion-gonadotropin (hCG) (Kristan et al. 2013). Chorulon, a formulation with hCG, has been used for artificial reproduction of different fish species, such as goldfish (*Carassius auratus auratus* L.) (Targonska and Kucharczyk 2011), Eurasian perch (*Perca fluviatilis* L.) (Kucharczyk et al. 1996), and also pikeperch (Kristan et al. 2013). Currently, there is no information on hormone treatment effects for male pikeperch.

The main aim of this study was to compare reproductive success, and gamete and offspring traits between hormonally injected pikeperch pairs where both sexes and pairs with only females were hormonally treated. Parameters measured were latency (time between hormone injection and ovulation), percentage of successfully spawned females, diameter of fertilized eggs, fertilization and hatching rates, egg incubation time, numbers of free-swimming larvae, total length of free-swimming larvae, larval survival after a 90-min osmotic shock and for males, volume of stripped sperm, spermatozoa concentration, spermatozoa motility rate, motility duration, and velocity.

MATERIAL AND METHODS

Fish groups and spawning conditions. Fourteen females and fourteen males were used in this study. All the brood fish were captured from the ponds of Rybářství Nové Hrady s.r.o. and then transferred to the South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses in Vodňany, Czech Republic. Holding tanks (600 l capacity) were supplied with water from a recirculating system (water temperature (WT) = 13.6 ± 0.2°C). Oocyte maturation stage was assessed at the beginning of experimentation (Zarski et al. 2012). Fish were randomly divided into seven male/female pairs and then into two groups – A (total length (TL) = 458 ± 33 mm; weight (W) = 1056 ± 112 g) and B (TL = 442 ± 41 mm; W = 988 ± 153 g). In Group A, only the females were injected with hCG at 500 IU per kg, and in Group B both sexes were injected at the same dosage. Each fish pair was held separately in 300-l circular tanks with spawning substrate constructed of artificial grass with fibre length 50 mm placed on the tank bottom. The 14 tanks were part of a recirculation aquaculture system that provided stable and optimal conditions for pikeperch spawning (14.8 ± 1.4°C and oxygen saturation 10.8 ± 2.3 mg O_2/l). Brood fish were removed from the tanks after spawning. Fertilized eggs were incubated on the spawning substrate. On day 4 after spawning, water inflow was blocked to prevent larvae from escaping (the hatching started on day 5 after spawning). Newly hatched free-swimming larvae were counted using the volume method: all larvae in the tank were

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concentrated in 10 l of water, and five samples of 25 ml of water with larvae were removed. Larvae were counted in each subsample and the total number was calculated from the mean count in the sub-samples. WT and oxygen saturation were measured twice daily with an oximeter (OxyGuard International A/S, Farum, Denmark) during the latency and incubation time.

Assessment of the eggs and larvae quality. On the first day after spawning, 300 randomly selected eggs were removed from the substrata and 100 eggs were placed in each of the three 250-ml plastic incubators to measure diameter of fertilized eggs, fertilization rate (FR), and hatching rate (HR). Incubators were held in a Styrofoam frame floating on the surface of the spawning tank; water in the incubators was changed twice daily. FR was calculated as the proportion of fertilized eggs in each incubator and HR as the number of larvae relative to the initial number of eggs. Egg diameters were measured 24 h after spawning to the nearest μm using a stereo microscope SMZ745T (Nikon, Tokyo, Japan) with Quick PHOTO MICRO 3 program. Length of freshly hatched larvae was measured at hatching by the same method used for the measurement of eggs diameter. 25 eggs and 25 larvae from each incubator were used for diameter and length measurement, respectively.

Twenty larvae in four repetitions from each spawned pair were used to assess resistance to osmotic shock. The test was carried out in 2% sodium chloride solution. The number of larvae surviving after 15, 30, 45, 60, 75, and 90 min of exposure was recorded.

Collection of the spermatozoa samples and assessed parameters. On the day following spawning, milt samples were collected (4 individuals from Group A and 5 from Group B) into individual 1-ml syringes without addition of immobilization solution. Syringes were placed on ice and immediately transported to the laboratory for analyses.

From spermiating males, the following parameters were taken: volume of stripped milt, spermatozoa concentration, percentage of motile cells (motility rate), duration of spermatozoa motility, and spermatozoa velocity. Milt volume was defined as the amount in the sampling syringe. For assessment of spermatozoa concentration, 10 μl milt was diluted to a final concentration of 1 μl sperm in 10 000 μl of KUROKURA-220 immobilizing solution (Rodina et al. 2002). Counting was performed according to Teletchea et al. (2009) and concentration was expressed as billions of spermatozoa per ml of milt.

Spermatozoa motility variables. Spermatozoa were assessed by motility variables, including duration of motility, motility rate, and velocity 15, 30, and 45 s post-activation. Immediately after activation in hatchery water spermatozoa motility was recorded with a DVD video-recorder SVO-9500 MDP (SONY, Tokyo, Japan) (Kristan et al. 2013) until motility ceased. Microscopy used dark-field optics (Olympus BX50, magnification ×200; Olympus, Tokyo, Japan) illuminated by a stroboscopic LED illumination unit Exposure Scope 0.1 (University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Czech Republic) combined with video recording on a CCD video camera SONY SSCDC 50 AP which provided recorded images for spermatozoa motility and velocity analysis. Video recording provided 50 half-frames with 720 × 576 pixels (PAL 4 : 3) spatial resolution per frame. Motility rate and velocity were measured according to Hulak et al. (2008). The velocity of spermatozoa was calculated as μm/s based on length of traces of spermatozoa heads from red to green to blue using MicroImage facility (Olympus). Motility rate was calculated as a percentage of red cells among all cells.

The data are presented as mean ± standard deviation. Statistical analysis was based on one-way Analysis of Variance (ANOVA) (STATISTICA, Version 12, 2013) including *F*-statistics. Significant differences between groups were estimated using Tukey's *post-hoc* test. The level of significance was set at $P < 0.05$.

RESULTS

All parameters were significantly different between treatment groups (fertilization rate: Group A 59.5 ± 17.9%, Group B 80.4 ± 9%; hatching rate: Group A 51.2 ± 17.7%, Group B 71.6 ± 9.4%; production of free-swimming larvae per female: Group A 49 429 ± 32 544, Group B 122 000 ± 15 311; volume of stripped sperm the day after spawning: Group A 0.16 ± 0.09 ml, Group B 0.64 ± 0.26 ml). Concentration of spermatozoa was $37.6 \pm 8.3 \times 10^9$ per ml in Group A and $19.3 \pm 3.9 \times 10^9$ per ml in Group B. Significant differences were also observed in motility percentage at 30 and 45 s post-activation (Figure 1A) and motility duration – Group A: 59.5 ± 31.8 s, Group B: 97.7 ± 7.1 s (Table 1).

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Table 1. Fertilization and hatching rate – eggs, larvae, and spermatozoa quality and quantity

	Group A	Group B	*F*-statistics
Latency (h)	96.4 ± 9.0[a]	92.4 ± 1.5[a]	4.543
Successful spawning (%)	57.1	71.4	
Fertilization rate (%)	59.5 ± 17.9[a]	80.4 ± 9[b]	5.293
Hatching rate (%)	51.2 ± 17.7[a]	71.6 ± 9.4[b]	5.054
Incubation time (degree days)	89.9 ± 7.8[a]	90.4 ± 1.8[a]	3.018
Diameter of fertilized eggs (mm)	1.15 ± 0.026[a]	1.26 ± 0.068[a]	8.772
Number of free-swimming larvae per female	49 429 ± 32 544[a]	122 000 ± 15 311[b]	21.11
Total length of free-swimming larvae (mm)	3.55 ± 0.16[a]	4.2 ± 0.12[b]	88.38
Larva survival after a 90-min osmotic shock (%)	97.2 ± 0.6[a]	96.6 ± 1.2[a]	5.273
Ability to produce sperm (%)	57.1	71.4	
Volume of stripped sperm (ml)	0.16 ± 0.09[a]	0.64 ± 0.26[b]	10.49
Spermatozoa concentration (10^9/l)	37.6 ± 8.3[a]	19.3 ± 3.9[b]	19.81
Spermatozoa motility duration (s)	59.5 ± 31.8[a]	97.7 ± 7.1[b]	11.56

[a,b]different superscript letters indicate significant difference ($P < 0.05$)

There were no differences between groups in ovulation latency: Group A 96.4 ± 9 h, Group B 92.4 ± 1.5 h); successful spawning: Group A 57.1%, Group B 71.4%; incubation time: Group A 89.9 ± 7.8 degree days (°D), Group B 90.4 ± 1.8 °D (Table 1), and spermatozoa velocity: 15, 30, or 45 s post-activation (Figure 1B). The osmotic shock used to test the larvae viability and resistance (Policar et al. 2010) showed no differences between Groups A and B (Table 1).

DISCUSSION

This study demonstrated the importance of hormonal treating both sexes in pikeperch semi-artificial spawning. Water temperature was 14.8°C during the experimental spawning study. Zakes and Demska-Zakes (2009) recommend 14–16°C as optimal water temperature for pikeperch reproduction. Pikeperch females usually do not ovulate in captivity without hormone treatment (Zakes and Szczepkowski 2004; Kucharczyk et al. 2007), but there has been no information published about the influence of hormone treatment on male pikeperch. All hormone-treated fish were injected with hCG at 500 IU per kg, which is the commonly used concentration for pikeperch reproduction in captivity (Ronyai 2007; Sosinski 2007; Kristan et al. 2013).

Benefits of hormone treatment for males in semi-artificial spawning were indicated by the treatment group differences. Spermatozoa concentration was $37.6 \pm 8.3 \times 10^9$ per ml in Group A (non-hormone treatment) and $19 \pm 3.9 \times 10^9$ per ml in Group B.

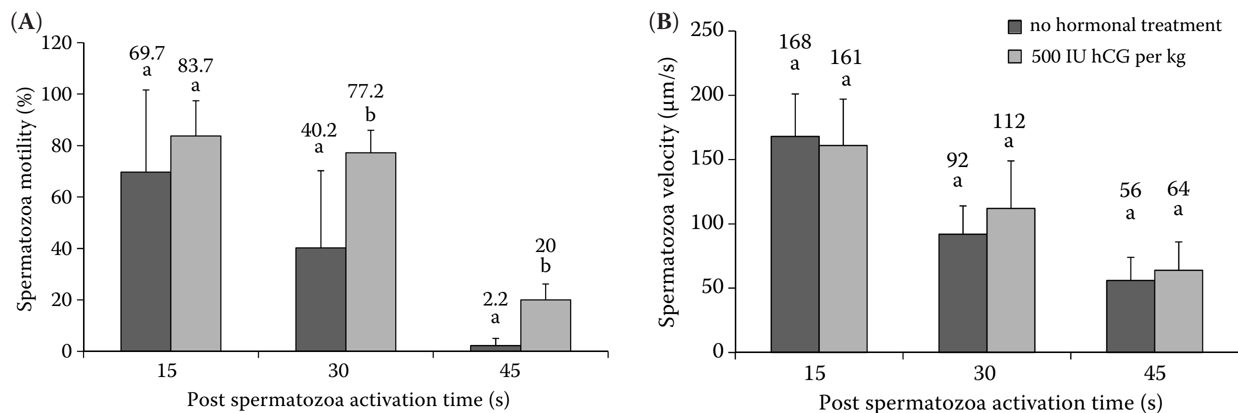

Figure 1. Spermatozoa motility (**A**) and velocity (**B**) at 15, 30 and 45 s post-activation

[a,b]different letters indicate significant difference ($P < 0.05$)

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Kristan et al. (2014), who also used 500 IU of hCG per kg for hormone stimulation of brood fish males, reported pikeperch spermatozoa concentration of $14.83 \pm 1.3 \times 10^9$ per ml, similarly to our results for Group B. Cejko et al. (2008) observed pikeperch spermatozoa concentration of only $4.28–5.26 \times 10^9$ per ml, different from both groups in our study. No difference was observed in spermatozoa velocity between groups A and B; velocity at 15 s post activation was 168 μm/s in Group A and 161 μm/s in Group B. Similar results (spermatozoa velocity 135–162 μm/s) obtained from untreated males have been reported by Teletchea et al. (2009). We measured motility rate in Group A at 69.7% and in Group B at 83.7% 15 s post-activation, which was comparable to the findings of Teletchea et al. (2009) who observed 59–85% of spermatozoa were motile. Cejko et al. (2008) found that only 14.7–25.0% of spermatozoa were motile following the injection of 450 IU of hCG per kg.

Latency of ovulation after induction with 500 IU/kg·hCG at 15.0 ± 0.5°C water temperature was 96.4 h (Group A) and 92.4 h (Group B) compared to 78.05 ± 6.93 h (Kristan et al. 2013). Fertilization and hatching rates were highly influenced by spermatozoa quality. Fertilization rate in Group B (80.4 ± 9%) was similar to 87 ± 8% reported by Ronyai (2007) after hCG treatment of both sexes, but 71.6 ± 9.4% in Group B was lower than that of 84.2 ± 6.2% found by Kristan et al. (2013). Incubation time was 89.9 °D in Group A and 90.4 °D in Group B. Schlumberger and Proteau (1996) reported that hatching in pikeperch usually occurs at 65–110 °D at water temperature 14–15°C.

To conclude, we found that hormonal treatment of both sexes in semi-artificial reproduction of pikeperch was useful and had important effects on the final production of larvae and effectiveness of the entire reproduction process.

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