COMBINED METHODS FOR ARTIFICIAL REPRODUCTION OF PIKEPERCH, *SANDER LUCIOPERCA*

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KOMBINOVANI METODI VEŠTAČKE REPRODUKCIJE SMUĐA *(SANDER LUCIOPERCA)*

Apstrakt

Smuđ, *Sander lucioperca*, je vrsta koja ima dobru perspektivu u procesu diverzifikacije evropske kopnene akvakulture. Iako su različite tehnike njegovog razmnožavanja (prirodni mrest, veštačka i polu-veštačka propagacija) vrlo dobro opisane i dalje postoji širok prostor za razvoj i usavršavanje mnogih detalja. Do sada je objavljeno nekoliko publikacija o indukciji završnog sazrevanja gameta ove vrste korišćenjem različitih hormonalnih supbstanci (Ronyai., 2007; Kristan et al 2012, Zakes et al., 2012; Zarski et al., 2012). Najčešće upotrebljavani hormoni su ekstrakt šaranske hipofize - CPE, (Carp Pituitary Extract) i humani horioni gonadotropin (hCG, Human chorionic gonadotropin). Prirodni mrest, bilo u jezerima ili kavezima (Demska-Zakes and Zakes, 2002, Schlumberger i Proteau, 1996; Ruuhijarvi and Hyvarinen, 1996, Steffens et al., 1996), izgleda najjednostavniji, ali u isto vreme, manje pouzdan metod, kako je uspeh mresta visoko zavisan ne samo od stadijuma zrelosti matica, već i od nekontrolisanih spoljašnjih ekoloških faktora (npr. temperatura i kvalitet vode). Nasuprot tome, veštačka, ili poluveštačka propagacija bi mogli biti u prednosti za mnogo pouzdaniju i sinhronizovaniju produkciju larvi. Ove tehnike mogu biti izvedene bilo mrestom u tanku ili ručnim istiskanjem (Kucharczyk et al., 2007). U slučaju reprodukcije u veštačkom okruženju , vreme latencije (LT -, izračunat kao interval od prve hormonalne injekcije do ovulacije)

je vrlo nesinhronizovano u većini studija. Zarski et al. (2011) su razvili novu klasifikaciju preovulatornih stadijuma sazrevanja ovocita za smuđa sa svrhom bolje sinhronizacije ovulacije. Iako postoji poboljšanje sinhronizacije, period od prve do poslednje ovulacije varira 10-25 časova. Nepredvidljivo vreme ovulacije uzrok je ozbiljnom problemu pri ručnom istiskanju ikre kod smuđa, što ponekad dovodi do spontane ovulacije i gubitka ikre u tanku (Zarski et al., 2011). U ovoj studiji korišćena je kombinovana metoda prezentovana od strane Rónyai (2007). Izbor ovakvog metoda je u cilju sprečavanja spontane ovulacije ženki u tanku, ali i kako bi, se dobile živi gameti za eksperimentalne i produkcione svrhe. U cilju realizacije eksperimenta, 4. aprila 2013. 16 parova matica gajenih u jezeru prebačeno je u RAS Instituta za ribarstvo, akvakulturu i irigaciju (HAKI, Sarvaš, Mađarska). Matice su aklimatizovane sa temperature vode u jezeru (7.5^0C) na optimalnu temperaturu za reprodukciju (16^0C) postepenim zagrevanjem (2^0C/dan). 8. aprila merene se mase matice i uzet uzorak ovocita ženki uz pomoć katetera (unutrašnji prečnik 1.2mm) korišćenjem metode po Kucharczyk et al. (2007). Uzorak ovocita razbistren je u Serinom rastvoru (etanola, formaldehid i glacijalna kiselina u odnosu 6:3:1,) i pod mikroskopom je određen stadijum sazrevanja, kao što je objašnjeno od strane Zarski et al. (2011). Nakon toga, svi parovi matica smešteni su u zasebna odeljenja za mrest oformljena deljenjem 4 tanka ''raceway" tipa, zapremine $4m^3$ plastičnom mrežom na 4 dela. Temperatura vode je održavana na 16.2 ± 0.3^0C tokom svih procedura mresta. Zasićenje kiseonikom je mereno svaka 3 časa i održavano iznad 80%. Tokom svih procedura ribe su anestezirane u rastvoru ulja karanfilića. 10. aprila data je prva inekcija ženkama: osam ženki 3mg/kg CPE, a drugih osam 200 IU/kg hCG. 11. aprila, druga hormonalna inekcija data je svim ženkama (3mg/kg CPE). U isto vreme su tretirani mužjaci sa 2mg/kg CPE. U trenutku obe injekcije uzet je uzorak ovocita ženki i određen je stadijum sazrevanja. Nakon druge inekcije, gnezda su ubačena u svako odeljenje i observacija mresne aktivnosti je vršena svaka 2 časa dok iz 4 ženke nije istisnuta ikra (navedeni broj istiskivanih ženki bio je potreban za dalja istraživanja). 12 matica se mrestilo i ostala gnezda su proveravana svaka 2 časa radi utvrđivanja perioda latencije.

Srednji period latencije ženki, istiskivanih i mrešćenih, bio je 57 ± 9 časova. Periodi latencije ženki u različitim stadijumima sazrevanja ovocita iznosio je 66 ± 6, 55 ± 6, 47 ± 0 i 44 ± 7 časova za II, III, IV i V stadijum, respektivno. Period latencije ženki indukovanih različitim sredstvima bio je 51 ± 7 za CPE+CPE i 62 ± 7, za hCG+CPE. Prosečni pseudogonadosomatski indeks (PGSI) istiskivanih ženki iznosio je $9.7\pm4.6\%$. Stepen fertilizacije istiskivanih i mrešćenih riba određen u stadijumu neurulacije bio je iznad 80% u svim grupama. Stadijum sazrevanja ovocita na dan uzorkovanja i vreme latencije svih ženki dati su u tabeli 1.

Radi sinhronizacije ovulacije, ženke u nižim stadijumima maturacije su tretirane prvom injekcijom hCG praćenom sa CPE, što izaziva najkraće i najsinhronizovanije vreme latencije, (Rónyai,2007). Uprkos tome, LT se razlikovao između grupa, a što potvrđuje da je stadijum sazrevanja najbitniji faktor za predviđanje ovulacije. Dobijeni srednji PGSI je u saglasnosti sa ranije izvedenim istraživanjima (Zakes and Demska Zakes, 2005, Ronyai, 2007), ali trebalo bi napomenuti da je od četiri istiskivane ženke, jedna počela mrest u tanku, dok su ostale nastavile mrest nakon vraćanja u mresne komore. Obzirom da je istiskivan potreban broj ženki i da nije bilo značajnog gubitka jaja, može se zaključiti da je kombinovan metod veštačke reprodukcije bio uspešan za ovu svrhu. Ručno istiskanje ženki omogućava izvođenje efektnih odgajivačkih programa uz manipulaciju genoma i korišćenje krioprezervirane sperme (Bokor et al., 2007; Bokor et al., 2008).

Ključne reči: veštačka reprodukcija, smuđ, hormoni, period latencije
Keywords: artificial reproduction, pikeperch, hormones, latency time

INTRODUCTION

Pikeperch, *Sander lucioperca* L., is a possible candidate for diversification of European freshwater aquaculture. Although its different breeding techniques (natural spawning, artificial and semi-artificial propagation) are generally well described, still there is a room for the development of many details and to their refinements. Several papers have been published on the induction of final gametes maturation of this species using different hormonal substances (Rónyai, 2007; Kristan et al., 2012; Zakes et al., 2012; Zarski et al., 2012, Steffens et al., 1996). Most commonly used hormones are Carp Pituitary Extract (CPE) and human Chorionic Gonadotropin (hCG). Natural spawning either in ponds or cages (Demska-Zakes and Zakes, 2002; Schlumberger and Proteau, 1996; Ruuhijiirvi and Hyviirinen, 1996), seems to be the most simple, but - at the same time - less reliable method as the spawning successes highly depend not only from the maturity stage of the breeders, but also from the uncontrollable external ecological factors (i.e. water temperature and water quality). In contrast, artificial, or semi artificial propagation could give advantages for more reliable and synchronized larval production. These techniques could be performed either by tank spawning or hand stripping (Kucharczyk et al., 2007).

In case of reproduction in artificial environment the latency time (LT, calculated as the time interval from first hormonal injection to ovulation), was much unsynchronized in most of the studies. Zarski et al. (2011) developed a new classification of pre-ovulatory oocyte maturation stages in pikeperch with a purpose of better synchronization of ovulation. Although there was improvement of synchronization, range from first to last ovulation varied between 10-25 hours. The unpredictable time of ovulation cause a serious problem with hand-stripping of this species. This phenomenon could lead to spontaneous ovulation and loosing eggs in the tank (Zarski et al., 2011).

In our study we applied combined method formerly documented by Rónyai (2007). Reason for choosing this method was from one side to prevent spontaneous ovulation of females in tank, and - from the other side - to make attempts to obtain stripped viable gametes either for experimental or production purposes.

MATERIALS AND METHODS

For purpose of this study, on April 4, 16 pairs of pond reared breeders were transferred to the recirculating aquaculture facility of the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI, Szarvas, Hungary). Breeders were acclimatized from the actual pond water temperature (7.5^{0}C) to the optimal one for reproduction (16^{0}C) by gradual (2^{0}C/day) warming up. On April 8 weight of spawners was measured and a sample of oocytes was taken with a catheter (inside diameter of 1.2 mm) from each female as described by Kucharczyk et al. (2007). Sample of oocytes was clarified in a Sera's solution (ethanol, formalin and glacial acetic acid in the ratio of 6:3:1, respectively), placed under the microscope and final oocyte maturation (FOM) stage was determined, as described by Zarski et al. (2011). Furthermore all pairs were stocked in individual spawning "compartments", which were formed by dividing 4x4 m^3 raceway type tanks in four parts with mesh walls.

On April 10 the first injection was given to females. Eight females were treated with 3mg/kg of CPE and the other eight with 200 IU/kg of hCG. On April 11, second hormonal injection was given to all females (3mg/kg of CPE) and at this time all males were injected with 2mg/kg of CPE. At the time of both injections, a sample of oocytes was taken from each female and the FOM stage was determined (Table 1). After the second injection, nests were introduced into each compartment and observation of spawning activity was performed every two hours until four females could be stripped. (This number of stripped females was needed for further research). All the remaining 12 pairs were spawned and remained nests were checked every two hours for the LT determination.

Water temperature was maintained at 16.2±0.3^{0}C during the whole spawning procedure. Oxygen saturation was monitored every 3 hours and maintained above 80%. During all procedures fish were anaesthetized in clove oil solution.

RESULTS AND DISCUSION

Mean LT either to stripping or spawning was 57±9 hours. The LT values for females with different FOM were 66±6, 55±6, 47±0 and 44±7 hours for II, III, IV and V stage, respectively. For females injected with different hormonal substances mean LT was 51±7 and 62±7 hours for CPE + CPE and hCG + CPE respectively. For the stripped fish the mean pseudo-gonadosomatic index (PGSI) was 9.7±4.6% (Table 2). Fertilization ratios both for stripped and spawned fish were determined at the neurulation stage and were above 80 % in each batches. The stages of FOM on the sampling dates and latency time of each female is given in Table 1.

Table 1. FOM stages and LT for females

Female	April 8	April 10 (first injection)	April 11 (second injection)	Latency time (hours)
1	II/III	III	IV/V	51
2	II	III/IV	III	55
3	II	III	III	62
4	I	II	III	66
5*	II	III	IV/V	52
6	IV	IV	V	47
7	II	II	III	64
8	I	II	III	68
9*	I	II	III	75
10	III	III	IV/V	47
11*	II/III	III	IV	57
12	II	III	IV	52
13	IV/V	V	VI	39
14*	IV	V	V/VI	49
15	II	III	IV	60
16	II	II	II	51

*- fish which were stripped

Table 2. Body weight, eggs weight and PGSI for stripped females

Female weight [g]	Weight of eggs [g]	PGSI [%]
2320	100	4.3
2460*	183	7.4
1780	225	12.6
1900	272	14.3

*-started spawning on the nest

For the purpose of ovulation synchronization, females in lower FOM stages were injected with primary injection of hCG followed with CPE, as it is described by Rónyai (2007) to induces shortest and more synchronized LT. Even though, LT was different between these groups. This confirms that FOM stage is the most significant factor for prediction of ovulation. Obtained mean PGSI is in agreement with former studies (Zakes and Szczepkowski, 2004; Zakes and Demska Zakes, 2005; Rónyai, 2007), but we should notice that from the four stripped females, one started to spawn on the nest prior to stripping and other three spawned again after returning to the spawning chamber. Muller-Belecke and Zienert (2008) reported very different mean commercial fecundities between the groups of preseason spawning with use of similar method of artificial propagation. Even though mentioned studies were conducted in the different conditions, reported PGSI makes a question about stripping efficacy of pikeperch females and leaves the open door for further investigation.

CONCLUSIONS

With concern to strippe needed number of females and to avoid significant lost of eggs, it could be concluded that combined method of artificial reproduction of pikeperch was successful for this purpose. However, hand stripping enables effective breeding programs with genome manipulations and egg fertilization with cryopreserved sperm (Bokor et al., 2007; Bokor et al., 2008).

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