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Rematuration of Nilem Fish (Osteochilus hasselti C.V) Female Broodstock Post-Spawning Using Oocyte Developer Hormone

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Abstract : The rematuration period of nilem fish female broodstock naturally is three months after spawning, thus the broods only spawn four times a year that impact on the amount of production. Spawning frequency can be increased by accelerating the rematuration period of female broodstock nilem fish. Oocyte developer (Oodev) hormone which is a combination of the Pregnant Mare's Serum Gonadotropin (PMSG) hormone and anti-dopamine (AD) compounds has been known to be able to affect the rematuration period of some freshwater fish. This study was conducted to identify the effect of Oodev hormone injection on the rematuration period of female broodstock nilem fish. The research method used is experimental with completely randomized design (CRD) consisting of five treatment doses of Oodev hormone and four replications. The female broodstock of nilem fish was induced Oodev hormone and preserved until the broodstock shows the characteristics of mature gonads. The research results shows that the induction of Oodev hormone with different dose had an effect on the rematuration period of female broodstock nilem fish and the optimum dose of Oodev hormone is 1.00 mL/kg with gonadosomatic index value of 14.48%, average egg diameter is 1.035 mm, weight gain of 31.25 gram, and the ripe-gonad fish is back on day 17 after spawning.
Keyword: *Osteochilus hasselti*, rematuration, Oodev hormone.

Introduction

Nilem fish (*Osteochilus hasselti* C.V) is freshwater fish commodity which has many potential that can be developed. Nilem fish egg can be exported as caviar substitute, five gram fish can be produced into processed product and as a fish therapy[22]. Stadia-seeds nilem fish or the size of five gram is more widely used than the size of consumption nilem fish. According to statistical data of Directorate General of Aquaculture (2015)[17], the number of seeds nilem fish production in 2014 is 2,405,01 million fish and will keep increasing every year. One of efforts to meet the need of seeds production is by increasing the spawning frequency of nilem fish broodstock. Spawning frequency can be increased by accelerating gonad rematuration period after spawning or rematurating female broodstock of nilem fish. Rematuration period of nilem fish broodstock naturally is three months[22], thus broodstock only spawn four times a year. Rematuration can be accelerated using appropriate hormone and giving sufficient nutrition.

Induction of gonad maturation in nilem fish using analog hormones such as ovaprim, estrogen and progesterone has been done in the Hartanti and Nurjanah's research (2009), but spawning induction was performed using different hormones such as Carp Pituitary Extract (CPE) and Human Chorionic Gonadotropin

(HCG)[13]. Farastuti's research result (2014) was that giving 1 ml/kg Oodev hormone fish can accelerate the maturity of broodstock gonad of Torsoro fish (*Tor soro*) in one week and already to spawn. The research showed that Oodev hormone induction can affect gonad maturity and fish spawning out of season.

Oodev hormone is a combination of Pregnant Mare's Serum Gonadotropin (PMSG) hormone and Anti dopamine (AD) compounds. PMSG hormone contains Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH)[12]. FSH or GTH 1 in PMSG will increase aromatase enzyme activities resulting high production of 17β estradiol[14]. Estradiol-17β is carried to liver to stimulate the synthesis of vitelogenin which plays a role in vitelogenesis process, thus triggering oocyte development[19]. Antidopamine is a dopamine antagonist that facilitates the stimulation of gonadotropin secretion to induce the process of final oocyte maturation and ovulation[3,24].

The Oodev hormone can accelerate the rematuration period of the betok fish (*Anabas testudineus*) for 12-24 days[20] and patin (*Pangasius hypophthalmus*) 28 days after spawning[1]. The use of Oodev hormone for rematuration of nilem fish has never been done. The aim of this research is to identify the influence of Oodev hormone injection on the rematuration period of Nilem fish female broodstock. It is expected that the spawning frequency of nilem fish will increase and can supply the needs of seed production.

Material and Method

Research Material

The material used in this research is 40 female broodstock of nilem fish (post spawn) obtained from BBPBAT Sukabumi in the age of 1-1,5 years old with weight range from 100-200 gram and has spawned three times before subjected rematuration, *Oocyte Developer* (Oodev) hormone, and pellets feed contained 30% protein and 6% fat.

Research Method

This research uses experimental method with completely randomized design (CRD). The treatment of Oodev hormone dose used is 0 ml/kg of fish as a control, 0,25, 0,50, 0,75 and 1,00 ml/kg of fish. The determination of hormone dose used is based on Cholifah's research (2016) that obtained optimum dosage of Oodev hormone, that is 0,75 ml/kg of fish weight through injection, can accelerate gonad maturity of prospective Nilem fish broodstock for 14 days.

Research Procedure

a. Preparation

The research is conducted in controlled condition. The maintenance containers is four black hapa sized 1,25 x 1,25 x 1 m^3 placed in semi-permanent field sized 10 x 8 x 1,25 m^3. The broodstock used is broodstock which has spawned three times. The spawned broodstock is acclimatized for seven days, and then proceed with rematuration phase. Each hapa consist of 10 fish which is given five treatment doses or two fish per treatment doses. Tagging is provided using colored plastic strap tied around dorsal fins to differentiate each treatment doses.

b. Implementation

Oodev hormone injection is conducted intramuscularly bellow dorsal fin every seven days which refers to Farastuti's research (2014). Injection is done on day 1, day 8, day 15, and day 22. The frequency of feeding is done twice a day which is at 09.00 WIB and at 15.00 WIB as much as 3% of total weight of nilem fish broodstock. The culture is conducted until more than 50% broodstock of each treatment got gonad maturity.

c. Observation

Sampling is started in the first day and conducted every seven days. Every sampling is subjected weighing weight, gonad weight, and documentation. Kanulation to get egg diameter data is conducted when fish has matured gonad. Measurement of egg diameter is conducted starting on day 17, day 21, day 23, and day

29. The egg is taken using kanulator number eight. 30 egg sampling is taken from four matured-gonad fish and fixed using 10% formalin buffer phosphate. Egg diameter is measured using Olympus AZX16 binoculars microscope with 100 times lens magnification and the loop connection. Observation result is directed with remote Olympus U-RVL-T shown in computer monitor using software program DP2-BSW. The observed number shown in the monitor indicates egg diameter value.

Results and Discussion

Gonadosomatic Index (GSI) and Egg Diameter

The result of ANOVA statistical test indicates that Oodev hormone induction with different doses give a noticeable difference ($p<0.05$) toward gonadosomatic index value and egg diameter that is presented in Table 1.

According to Nagahama et al. (1991), PMSG hormone induces vitellogenin follicle aromatase activity in Medaka fish (*Oryzias latipes*) through adenylate cyclase-cAMP mechanism; thereby testosterone will be converted into 17β estradiol. Estrogen hormone or 17β estradiol will influence liver and synthesize vitellogenin as well as play a role until the end of maturation phase[19]. 17β estradiol is tied by sex steroid hormone-binding globulin in bloodstream and transported toward hepatocyte through estrogen receptor to arrange several vitellogenin gene (Vtg) developments which subsequently produces vitellogenin proteins[7]. Vitellogenesis is the amalgamation and processing phase of vitellogenin (Vtg) with other molecules such as carbohydrate and fat into egg yolk protein (yolk globule)[10]. The role of gonadotropin and estrogen hormones in vitelogenesis process is very important for egg cell development. Naturally, gonadotropin FSH and LH hormones are secreted by pituitary gland controlled by brain through stimulation from external environment[13], but giving Oodev hormone can help increase FSH activity in the blood[23]. The antidopamine content of the injected Oodev hormone serves to inhibit the action of dopamine inhibitors so that the pituitary gland can secrete Luteinizing Hormone Releasing Hormone (LHRH) and induce final oocyte maturation and ovulation of fish[4].

Table 1. Gonadosomatic Index and Egg Diameter Data

Dosage	Indeks Kematangan Gonad (%) ± SD	Average Egg Diameter (mm) ± SD
0 ml/kg	$3.7525^{e} \pm 0.3357$	$0.000^{c} \pm 0.0000$
0.25 ml/kg	$10.0325^{d} \pm 0.3696$	$0.896^{b} \pm 0.1448$
0.50 ml/kg	$11.3325^{c} \pm 0.6801$	$0.908^{b} \pm 0.2123$
0.75 ml/kg	$12.9175^{b} \pm 0.7133$	$0.992^{b} \pm 0.1567$
1 ml/kg	$14.4800^{a} \pm 0.7479$	$1.035^{a} \pm 0.1871$

Note : Superscript varying in one line indicates that there is noticeable difference ($p<0.05$).

An increase of gonadosomatic index value is along with the increased doses of Oodev hormone given. FSH and LH activity in Oodev hormone influences gonad development. FSH[11] and LH[9] stimulation on the activity of P-450 fish ovaries aromatase is shown by increased production of 17β estradiol, hence vitellogenesis process occurs. In the process of vitellogenesis, yolk granules increase both in size and amount so that oocyte volume enlarges. During the process, body metabolism is focused on gonad development which causes gonad volume and weight increase[18]. Hutagulung et al. (2015) also suggested that gonad maturity index value increases in accordance with the higher dose of PMSG hormone given, dominant work power of FSH on PMSG will influence toward the development of early-stage fish broodstock gonad (GTH I).

Gonad development of female fish can be linked to the development of egg diameter which is the result of egg yolk precipitation during vitellogenesis process[5]. Zarski et al. research (2012) obtained positive correlation between gonad maturity level and oocyte diameter of pikeperch fish (*Sander lucioperca* L.), added by Shinkafi and Ipinjolu (2012) which stated that the higher gonad weight the bigger oocyte diameter size. At the end result of observation, the average egg diameter increases in accordance with gonadosomatic index value and Oodev hormone dose (Table 1).

Body Weight Gain

The gonad maturity of fish visually is marked by the presence of body weight gain influenced by gonad development. The result of ANOVA statistical test in Table 2 indicates that body weight gain of nilem fish broodstock in overall different dose treatment is noticeable ($p<0.05$) compared to the control.

Tabel 2. Body Weight Gain Data

Dosage	Body Weight Gain (gram) ± SD
0 ml/kg	$10.00^{d} \pm 4.08248$
0.25 ml/kg	$22.50^{c} \pm 2.88675$
0.50 ml/kg	$23.75^{bc} \pm 2.50000$
0.75 ml/kg	$27.50^{ab} \pm 2.88675$
1 ml/kg	$31.25^{a} \pm 2.50000$

Note : Superscript varying in one line indicates that there is noticeable difference ($p<0.05$).

Gonad development in this research indicated by the increase of gonadosomatic index value and egg diameter size is supported by body weight gain of nilem broodstock during culture (rematuration period). Body weight gain of nilem female broodstock during rematuration period increases along with the higher gonadosomatic index value and egg diameter. Oodev hormone influences gonad development process during rematuration period, thus gonad and body weight gain occurs (somatic). In Zakes and Demska-Zakes' research (2009), female pikeperch fish (*Sander lucioperca* L.) which is induced by hormone undergo body weight change related to the level of gonad maturity.

Rematuration period

Rematuration is the process of fish gonad rematuration after spawning. Post-spawning fish will enter rest period or recovery before gonad maturity period. In this research, the influence of giving different Oodev doses treatment is seen providing different gonad maturity period on the tested fish population. The fastest gonad maturity period is obtained by Oodev doses of 1 ml/kg of biomass weight (P4) which is 17 days and ripe-gonad fish (can be spawned) has not been found in control population at the end of the research (day 30) which is shown in Figure 1.

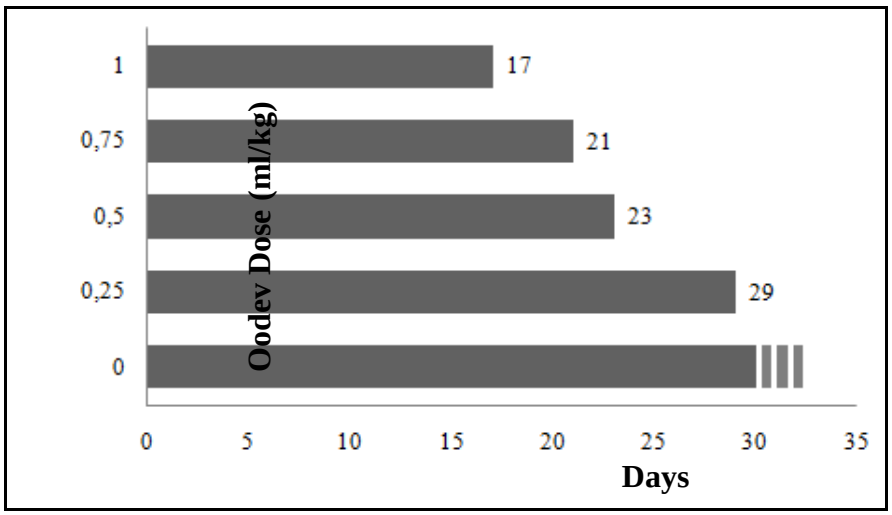

Figure 1. Rematuration Duration of Each Treatment

That rematuration period indicates that gonad rematuration process after spawning needs longer time compared to the maturation process. Cholifah in her research (2016) who conducted maturation on prospective broodstock of nilem dara using Oodev hormone indicated result that nilem broodstock began to mature gonad in dose treatment of 1 ml/kg on day 10. According to fish gonad maturity level's Nikolsky (1969) in Effendie (1997), fish in after-spawning phase (GML IV) will enter recovery period or rest period (GML VII).

Rematuration process can be said consisted of recovery period, gonad maturation period (maturation), and the last mature gonad.

Water Quality

Measuring water quality such as temperature, dissolved oxygen (DO) and pH is conducted at the beginning and at the end of the research. Data range of water quality during the research is presented in Table 3.

The result of water quality measurement during the research (Table 3) shows that water quality parameter values is still in the decent range to be used as culture media of tested-fish broodstock. According to Nurkarina (2013), optimum temperature range for life and growth of nilem fish is 24-34 ºC. pH value during the research is still within the good limits for cultivation accordance to Government Regulation No. 82 of 2001 which stated that good pH range is 6-9. Dissolved oxygen range during the research can still be tolerated, but is deficient for nilem fish culture. According to Government Regulation No. 82 of 2001, dissolved oxygen >3 mg/l is good for cultivation.

Table 3. Water Quality Data of Pool Maintenance during the Research

Parameter	Range
Temperature (ºC)	24.5-24.9
Dissolved oxygen (mg/l)	2.21-2.42
pH	6.96-7.05

Conclusion

According to the research result, it can be concluded that Oodev hormone can influence gonad rematuration period (rematuration) of post-spawning nilem fish female broodstock. Oodev hormone dose of 1 ml/kg is effective to re-maturation gonad of nilem female broodstock within 17 days with gonadosomatic index value of 14.48%, average egg diameter of 1.035 mm and body weight gain of 31.25 mm.

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