

Induction of oocyte developer hormones (oodev) on the maturity of *Poropuntius tawarensis*

Siska Mellisa^{1*}, Iwan Hasri², and Kasturi Ramdayani¹

¹Department of Aquaculture, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.

²Fisheries Department of Central Aceh Regency 24552, Indonesia.

Abstract. *Poropuntius tawarensis* is an endemic fish belonging to the Cyprinidae family found in Laut Tawar Lake, Central Aceh, Indonesia. *P. tawarensis* has a limited distribution and the fertilization only occurs in the rainy season. The purpose of this research was to observe the effect of Oodev hormone with different doses on the maturity of *P. tawarensis*. This study was used completely randomized design (CRD) consisting of 3 treatments and 3 replicates. Treatments used were the hormone doses of 0.25 mL/kg, 0.50 mL/kg and treatment without hormone (control). The parameters used in this study were gonadosomatic index (GSI), egg diameter, coefficients of variation (CV) of egg diameter, fecundity, and hepatosomatic index. The study revealed the highest value found in a dose of 0.50 mL/kg which the value of egg diameter was 0.8791±0.02 mm, coefficients of variation of egg was 6.46±0.49% and fecundity was 869±33.99 egg/kg. While the highest gonadosomatic index was found in a dose of 0.25 mL/kg (10.41±2.03%). Moreover, the induction of Oocyte developer (Oodev) hormone on the maturity of *P. tawarensis* did not significantly affect the hepatosomatic index (HSI), but Oodev hormone was efficient to accelerate the gonad maturity of fish.

1 Introduction

Poropuntius tawarensis is an endemic fish belonging to the Cyprinidae family found in Laut Tawar lake, Central Aceh, Indonesia. *P. tawarensis* are freshwater fish that have characteristics of benthopelagic. *Poropuntius tawarensis* are currently very difficult to find due to environmental damage and the introduction of new species [1, 2].

Domestication is an effort for adapting fish from the wild (original habitat) to the cultivated environment [3, 4]. According to Effendi [5], fish domestication is divided into three stages, firstly adapting to the survival of fish in aquaculture containers, second strive fish growth, and third strive fish reproduction/breeding.

The problem of the reduction or extinction of endemic fish and native fish has a risk to damage the sustainability of aquatic biodiversity. Therefore, it is needed to preserve the natural wealth in order to support a friendly life with nature. The Government of Central Aceh Regency has tried to domesticate and spawn *P. tawarensis*, but they are still constrained by the process of gonad maturation of fish which only occurred during the rainy season, therefore *P. tawarensis* fish was unable to spawn throughout the year [1]. Hormone induction is one of the alternative that can be done to ensure the availability of reproduction hormone for fish maturation. Furthermore, one of the hormones that plays a role in maturation is the Oodev hormone (Oocyte developer), a commercial hormone, which has been produced on a large scale.

The use of Oodev hormone is known to accelerate the maturation of various types of fish. According to Farastuti et al [6], Oodev hormone is a combination of pregnant mare's serum gonadotropin (PMSG) and antidopamine (AD). PMSG hormone contains more Follicle Stimulating Hormone (FSH) than Luteinizing Hormone (LH) which is good for gonad maturation of fish. FSH from outside stimulates the gonads and then gonadotropin releasing hormone (GnRH) stimulates the pituitary to secrete gonadotropin hormones for the function of early gonadal maturation or vitellogenesis. Antidopamine is a chemical that works to stop dopamine, while dopamine is an inhibitor of gonadal maturation activity. Inhibited dopamine activity stimulates the synthesis and release of the hormone lutein, which is initiated by gonadotropin releasing hormone (GnRH) in the pituitary gland [7, 8]. The previous studies of Oodev hormone were done in several fish such as *Tor soro* fish [6], snakehead fish [9], *Helostoma teminkii* [10], barramundi [11] and *Tor douronesis* [12]. The objective of this study was to observe the effect of Oodev hormone with different doses on the maturity of *P. tawarensis*.

2 Materials and Methods

2.1 Time and Site

This study was carried out from August to September 2020, located at the Technical Implementation Unit of

* Corresponding author : siska_mellisa@unsyiah.ac.id

the Fish Seed Agency (UPTD BBI) Lukup Badak, Fisheries Service, Pegasing District, Central Aceh Regency.

2.1.1 Research Procedure

This research used completely randomized design method consisting of 3 treatments and 3 replications, namely treatment A (control), B (0.25 mL/kg Oodev hormone), and C (0.50 mL/kg Oodev hormone). It was conducted at BBI Lukup Badak water reservoir. The container used is a net with size of 1x1x1m. Before used, the net was dried in the sun and then randomly installed in the maintenance size pond. The minimum pond size was 1.5 meters to 2.5 meters depth.

The broodstocks used were 94 females of *P. tawarensis*. The brood fish was obtained from Laut Tawar Lake with an average weight of 3.31g. Before doing the treatment, the broodstock were acclimated for 10 days. Acclimation helps to adjust or adapt fish into a new environment.

The injection of *P. tawarensis* was carried out once in the early week. Injections were performed intramuscularly using a 1 mL syringe. Oodev used in each treatment dose was dissolved in NaCl with a ratio of 1:1. Before being injected, *P. tawarensis* were anesthetized with a stabilizer solution (0.015 mL/L water) for 5-10 minutes. After the fish passed out, the weight were measured, then fish placed on a wet towel for injection. The addition of stabilizer can increase the stability of the emulsion. This stabilizer is a surface active compound that is able to reduce the surface tension between the liquid air mass and the liquid.

Gonadosomatic Index (GSI)

Gonadosomatic index is defined as the percentage of gonadal weight divided by the body weight [13]. The gonadosomatic index was calculated using the following formula [14]:

$$GSI = \frac{\text{total weight of gonad}}{\text{total weight of fish}} \times 100\% \quad (1)$$

Egg Diameter

A total of 30 eggs from each sample fish were measured the size of the diameter of the eggs. Egg diameter will be measured by the following formula:

$$Ds = Dt - D0 \quad (2)$$

where: Ds is Actual egg diameter, Dt is Final egg diameter and D0 is Initial egg diameter [6].

Coefficients of variation of egg diameter describes the size of the diameter of fish eggs in a population spread from the average value. Coefficients of variation of egg were calculated according to Steel and Torrie [15] as follow :

$$kk = (S/Y) \times 100\% \quad (3)$$

where : kk is coefficients of variation of egg diameter, S is square root of sample, Y is average of sample.

Fecundity

Fecundity was total amount of eggs of matured female fish and ready to spawn. According to Biswas [16] relative fecundity and Total fecundity was calculated using the following formula:

$$\text{Total fecundity} = n \times (\text{wt/ws}) \quad (4)$$

where n is total amount of eggs in sub-sampled, wt is total weight of gonad (g) and ws is total weight of sub-sampled (g).

Hepatosomatic Index (HSI)

Hepatosomatic Index (HSI) is defined as the ratio of liver weight to total body weight, It was calculated based on Effendi [17] as follow:

$$HSI = \frac{\text{liver weight}}{\text{total body weight}} \times 100\% \quad (5)$$

Gonadal Maturity Stages

Gonad maturity can be determined by observing the shape, size, colour, smoothness and filling of the ovaries in the body cavity, clarity of shape, colour of the eggs in the ovaries. Analysis of gonad maturity was defined following the stages of Cassie (1965) in Effendie [17].

Table 1. Gonadal maturity stages based on Cassie (1965) in Effendie [17].

Stage of Maturity	Gonad Morphology
I Immature	The ovary is like a thread. The eggs are not yet distinguishable. The length of the gonads varies between 1/3-1/2 the length of the body cavity.
II Maturing	There is a milky white tissue, the eggs are still fused and cannot be separated. The length of the gonads varies between 1/3-2/3 of the length of the body cavity.
III Maturing ripe	Larger size, widened anteriorly and tapered posteriorly, the eggs can be separated, darker in color. The length of the gonads varies between 1/3-2/3 of the length of the body cavity.
IV Ripe	Egg diameter is getting bigger and clearly visible under the microscope. The eggs are yellow. The length of the gonads varies between 2/3-3/4 of the length of the body cavity.
V Spent	Crimped ovaries, leftover eggs in the posterior. Ovaries are reddish.

3 Result and Discussion

3.1 Result


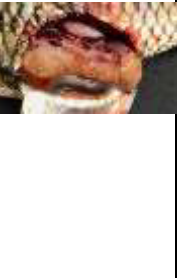

The study of induction of Oodev hormone on the maturity of *Poropuntius tawarensis* with doses of 0 mL/kg, 0.25 mL/kg, and 0.50 mL/kg showed the growth of gonad during 28 rearing days. The highest percentage of gonad maturity was reached by treatment C (0.50 mL/kg) (Table 2). The percentage of gonad maturity

achieved by using induction of Oodev hormone was better than treatment without hormone induction.

Table 2. The percentage of gonad maturity (%) of *Poropuntius tawarensis*.

Treatment	Day 14 (%)	Day 28 (%)
A (0 mL/kg)	33.33	44.44
B (0.25 mL/kg)	55.55	66.66
C (0.50 mL/kg)	66.66	88.88

Table 3. Observation of gonad maturity stages on *P. tawarensis* induced Oodev hormone.

Treatment	Stage of maturity	Observation	Figure
A (0 mL/kg)	II Maturing	Egg diameter is about 0.25 mm to 0.54 mm.	
B (0,25 mL/kg)	III,IV Maturing ripe, ripe	Egg diameter is about 0.54 mm to 0.80 mm	
C (0,50 mL/kg)	V Spent	Egg diameter is about 0.80 mm to 0.87 mm	

Based on Table 3, treatment A (0 mL/kg) only reached gonad maturity stages II with characterize by small gonad, eggs were not clearly seen. Then, treatment B (0.25mL/kg) reached gonad maturity stages III and IV with characterized can be distinguished. While, treatment C (0.50 mL/kg) achieved gonad maturity stages V with the characteristic shape of round eggs, some of which were clear and ripe.

ANOVA showed that induction of oocyte developer (oodev) on *P. tawarensis* had a significant effect on gonadosomatic index, eggs diameter, coefficients of variation of egg and fecundity. But it did not have a significant effect on hepatosomatic index.

Based on the Duncan's test, the highest gonadosomatic index was found in treatment B (10.41±2.03%), but did not significantly different with

treatment C (9.06±0.04%) (Table 4). While, eggs diameter and coefficients of variation of egg had the highest value on treatment C but did not have significantly different with treatment B. Furthermore, the best value of fecundity in this study was found on treatment C (869 ±33.99 egg/kg).

Table 4. Gonadosomatic index (GSI), egg diameter (mm), coefficients of variation of egg (CV), fecundity and hepatosomatic index (HSI).

Treatment	GSI (%)	Egg diameter (mm)	CV (%)	Fecundity (egg/kg)	HSI (%)
A	1.62±1.34a	0.548 ± 0.22a	4.41±0.21a	101±92.25a	0.33±0.09a
B	10.41±2.03b	0.8697±0.01b	5.90±0.07b	614±139.12b	0.27±0.06a
C	9.06±0.04b	0.8791±0.02b	6.46±0.49b	869±33.99c	0.22±0.07a

Note: Different superscripts in the same column are significantly different (p <0.5).

4 Discussion

Induction of Oodev hormone on *Poropuntius tawarensis* for 28 days of rearing had better results than without the use of Oodev hormone. Oodev hormone is a combination of pregnant mare serum gonadotropin (PMSG) and antidopamine (AD). According to Mayasari [18] PMSG contains follicle stimulating hormone (FSH) and luteinizing hormone (LH), follicle stimulating hormone (FSH) plays a role in early gonadal maturation or vitellogenesis, while luteinizing hormone (LH) is a hormone that helps the process of releasing eggs (ovulation). Antidopamine (AD) functions to inhibit the work of dopamine, where dopamine itself acts as an inhibitor of gonadal maturation [8]. Induction of Oodev hormone maturation carried out on *P. tawarensis* was able to provide stimulation so ovulation occurred. Moreover, the similar results was found on *Tor douronesis*, Mellisa et al. [12] studied on induction of gonadal maturation of *Tor douronesis* using Oodev hormone with the best dose of 1 mL/kg fish.

The development of gonadal maturity was determined by morphological observation by looking at the size of the egg diameter level which according to Cassie (1965) in Effendie [17]. Nurmahdi [19] mention that egg size is an indicator of egg quality and gonadal maturity level. Egg diameter showed a difference at each level of gonad maturity. The larger the diameter of the egg, the higher the percentage of gonad maturity. The results showed that the best treatment was treatment with the addition of Oodev at a dose of 0.50 mL/kg (treatment C) which could increase the level of gonad maturity. As the opinion of Darliansyah et al. [20] injection of Oodev hormone with different doses resulted in different egg diameter sizes which indicated different stages of gonadal maturity.

The value of the gonadosomatic index (GSI) can vary because it is closely related to the weight of the gonads and the body depending on the individual. Mukti

et al. [21] revealed that GSI increases with the increasing gonadal maturity stage. It was showed in this study, the high GSI were found in treatment B and C with the stage III, IV and V. In this study, the GSI value obtained was less than 20%. Therefore, its indicated that *P. tawarensis* are included in the group of fish with low GSI values and can be categorized as fish that can spawn more than once per year, according to Fatah et al. [22], the GSI value $\leq 20\%$ is a group of fish that can spawn more than once a year. The use of Oodev hormone on treatment B and C was able to ripen brood fish of *P. tawarensis*. This is due to the weight of the gonads in this treatment is greater than the other treatments and the body weight of the fish also increased. In accordance with the opinion of Effendie [23], gonadosomatic index (GSI) is the value in % (percent) as a result of the comparison of gonad weight with fish body weight. In line with the growth of the gonads produced will increase in size and weight to the maximum level when spawning occurs. In a study of Sihalohe [24], it was shown that the injection of a broodstock of 3 kg Siamese catfish (*P. hypophthalmus*) using Oodev hormone at a dose of 0.25 mL/kg at intervals of 2 weeks was effective in accelerating gonad maturity.

Furthermore, Egg diameter increased in each treatment from the beginning to the end of the study. The results of this study indicated that the high value of egg diameter were treatment C. Yulianto et al. [25] concluded that Oodev hormone contains PMSG hormone, where the FSH content is greater and the LH is less. FSH functions for egg maturation and LH functions for egg production. So it can be concluded that the dose of Oodev hormone of 0.25 mL/kg and 0.50 mL/kg resulted in an increase in egg diameter. Giving Oodev hormone to *P. tawarensis* affected the diameter of the eggs and caused the process of vitellogenesis. According to Libzens et al. [26] Vitellogenesis is the incorporation of vitellogenin proteins by oocytes and processes them into egg yolks, causing an increase in the size of the fish gonads. In comparison with other research, Mellisa et al. [12] stated that injection of Oodev hormone into *Tor douronensis* at a dose of 1 mL/kg had the highest value of egg diameter and Darliansyah et al. [20] showed the highest value of egg diameter of *Osteochilus kappeni* was at a dose of 0.4 mL/kg.

Fecundity of *P. tawarensis* was determined based on the results of counting eggs in fish gonads. Observation of fecundity induced by Oodev hormone is related to egg diameter. In this study, the best fecundity results were treatment C (0.50mL/kg) where the higher the dose of induced of Oodev hormone, the higher the egg diameter and fecundity. This is similar to the opinion of Djuhandia [27], which states that the size of fecundity is influenced by food, fish size and environmental conditions, and can also be influenced by egg diameter. This is also supported by the opinion of Suwarso et al. [28] which stated that the number of eggs produced by fish will increase in line with the size of the gonads. So that the selection of the dose 0.50 mL/kg in *P. tawarensis* was appropriate to calculate the fecundity. Tinus [29] revealed that Oodev induction in vitellogenesis in

rematured catfish (*Pangasius hypophthalmus*) with the best fecundity was found at a dose of 0.3 mL/kg.

Hepatosomatic index is the percentage comparison between liver weight and body weight which describes the metabolic processes in the liver [30]. The HSI value increased at the beginning of treatment A (0 mL/kg) and decreased in treatment C (0.50 mL/kg) at the end of the study, this was because in treatment A (0 mL/kg) the vitellogenesis process was not occurred so that the liver stores fat obtained from feed which then caused an increase in liver weight. Then in treatment C (0.50 mL/kg) HSI decreased, it was because the fat stored in the liver was passed on to the gonads to be used during the vitellogenesis process. In accordance with the statement of Sulistyono et al. [31] stated that the HSI value will begin to increase when the fish experience the beginning of the vitellogenesis process and will begin to decrease at the time of gonadal maturation.

5 Conclusion

Induction of Oodev hormone on maturity of *Poropuntius tawarensis* had the better results than treatment without hormone induction. The dose of 0.50 mL/kg was the best treatment but it was not significantly different from the 0.25mL/kg and a dose of 0.25 mL/kg efficiently used to accelerate the gonadal maturity of *P. tawarensis*.

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