Effects of GnRH and Anti-Dopamine on Gonad Maturation of
*Osteochilus melanopleurus* (Bleeker, 1852)

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Abstract. The *Osteochilus melanopleurus* (Bleeker, 1852) is one of the endogenous fish in the Siak River waters of Riau province which has high economic value. However, the fulfillment of needs still depends on the catch in nature. Efforts to overcome these problems are the availability of mature gonads. Broadly to accelerate the maturation of gonads through hormone induction until the fish are ready to be spawned to produce fish fry continuously. The purpose of this study was to analyze the effect of gonadotropin and anti-dopamine hormone induction with the trademark "Oodev" on the final gonad maturity (TKG) of the prospective broodstock of *O. melanopleurus*. The research was conducted from April to August 2021 in the fish rearing column and the experimental pond, Faculty of Fisheries and Marine Science. The research method was a Completely Randomized Design with 4 treatment levels and 3 replications, while the treatments were P0 (without Oodev injection), P1 (Oodev dose 0.5 mL/kg), P2 (0.7 mL/kg), and P3 (0.9 mL/kg). Measured responses: percentage of broodstock that reached TKG and egg diameter. The results of the research that have been carried out, the measurement of the diameter of the eggs produced showed that the use of the Oodev hormone at a dose of 0.7 mL/kg body weight of *O. melanopleurus* given every week for twelve weeks was able to stimulate the development of the gonads of *O. melanopleurus*. Ovaprim injection of 0.7 mL/kg body weight produced 89,566 eggs with a latency of 4 hours. oodev injection dose of 0.7 ml/kg BW or P2 treatment has succeeded in achieving a TKG of 82%.

1. Introduction
The Kelabau fish (*Osteochilus melanopleurus*, Bleekler 1852) is one of the native species in the waters of Riau Province which has high economic value and is widely traded for 40,000,-/kg (Asiah et al., 2019). The problem that occurs today is that the presence of *O. melanopleurus*, especially in the Siak river, is threatened with the decline due to continuous fishing and the use of fishing gear that is not environmentally friendly, as well as changes in the river ecosystem that flow into a flooded ecosystem such as the construction of dams in the watershed area has also there is a change in land use due to the clearing of agricultural land, plantations and settlements (Fauzi and Yuliati, 2012). Therefore, one of
the efforts that can be done to preserve the germplasm of *O. melanopleurus* is the application of hormone induction technology.

According to Zairin (2003), hormone induction aims to accelerate the formation of vitellogenesis of brood fish and substitute environmental signals for gonadal maturation. One of the hormones widely used to increase gonadal maturity in fish is Oodev (*Oocyte developer*). Oodev contains complex glycoprotein compounds derived from pregnant horse serum known as Pregnant mare serum gonadotropin (PMSG) (AgusTinus, 2013) and a mixture of antidopamine (Farastuti, 2014). Oodev hormone also stimulates the secretion of gonadotropins because there is antidopamine which will stimulate the release of FSH from the pituitary.

Several researchers have successfully used Oodev, namely: rematuration of *Pangasius pangasius* broodstock at a dose of 0.5 mL/kg Oodev (Agustinus, 2013), gonad maturation of snakehead fish at a dose of Oodev 1.25 mL kg\(^{-1}\) BW\(^{-1}\) (Hutagalung et al., 2015), gonad maturation of fish Add the dose of Oodev 0.5 mL kg BW\(^{-1}\) (Susilo et al., 2019), brown gourami dose of Oodev 0.04 mL g BW\(^{-1}\) (Nur et al., 2017), *Mystus nemurus* dose of Oodev 1 mL/kg (Putri et al., 2019) and synodontic fish at a dose of 1.5 mL/kg (Dwantara and Rahmatia, 2017)

Therefore, this study was conducted to determine the appropriate dose of Oodev induction on gonadal maturation of the *O. melanopleurus*.

2. Material and Methods

2.1. Time and place
This research was carried out from April to June 2021. Gonad maturation of the prospective broodstock of the *O. melanopleurus* was conducted in the experimental pond, Faculty of Fisheries and Marine Science, University of Riau. Research on spawning and rearing larvae was conducted in Laboratory of the fish hatchery, Faculty of Fisheries and Marine Science, University of Riau.

2.2. Tools and materials
The prospective brood fish used were 22 fish measuring 800-2000 g (10 males and 12 females), from the catch of fishermen in the Siak River, Flambayan Village, Garo City. Oodev Hormone 10 mL, Syndel Ovaprim 10 mL, Fertilization Solution (4 g NaCl + 3 g Urea + 1 L aquabides), 0.9% physiological solution.

2.3. Research methods
The method used is an experimental method by applying a completely randomized design (CRD) with 1 factor, 4 treatment levels, and 3 replications. The treatment in this study is

- P0: without Oodev injection
- P1: Oodev injection with a dose of 0.5 mL/kg body weight,
- P2: Oodev dose 0.7 mL/kg body weight, and
- P3: Oodev dose 0.9 mL/kg body weight

2.4. Research procedure
Injections were carried out according to a predetermined dose of Oodev hormone at intervals of 7 days, intramuscularly on the back of the fish. The injection is done with the inclination of the syringe about 30° with a depth of 1.5 cm so that the hormone can directly enter the bloodstream. The first injection was carried out after the fish were able to adapt to pelleted feed, then 12 injections were carried out every 7 days.

Observation of egg maturation was carried out before and after injection by way of assessment based on the location of the nucleus. Eggs were obtained from the mother's body using a polyethylene cannula catheter and then slowly inserted into the urogenital part of the fish's body. The eggs that have been obtained are ± 30 eggs and then placed in a petri dish that has been given a transparent solution. After 5-10 minutes the egg is calculated how many percent of the core has moved to the edge. This
observation was carried out 3 times in 1 parent so that the average number of egg maturity was obtained.

Then to determine the diameter of the eggs, measurements were taken before and after injection by taking a sample egg using a polyethylene cannula catheter and then slowly inserting it into the urogenital part of the fish's body as much as ± 30 grains and then inserting it into an Eppendorf microtube then adding Gilson's solution until the eggs were submerged whose function was to harden and release the egg from the ovarian tissue. The composition of Gilson's solution consisted of 100 ml of alcohol, 880 ml of water, 25 ml of nitric acid, 18 ml of glacial acetic acid, and 20 grams of mercury chloride (Bagenal in Pulungan, 2008). After the sample eggs were obtained, their diameter was measured using a microscope equipped with an Olympus CX21 micrometer with a magnification of 10x4 where one scale unit was 0.025 mm.

2.5. Data analysis
Data obtained during the study such as egg diameter were tabulated into a table and statistically analyzed using SPSS version 22 software.

3. Results and Discussions
3.1 Latency time
Latency time was determined by calculating the time interval between the last injection and ovulation in fish expressed in hours. There was only one kelabau fish that managed to reach TKG 82 %, namely the P3 supplement. The results of observations of latency time carried out in this study showed that a latency time of about 4 hours was found in P3 treatment (Ovaprim injection 0.7 mL kg BW\(^{-1}\)) while the other fish had not reached the expected gonadal maturity.

3.2 Number of stripping eggs
The number of stripped eggs is shown in Figure 1. The number of stripping eggs obtained was in the P3 treatment with the number of eggs obtained was 87.2 grams, about 89,566. item. The fecundity of the \(O. melanopleurus\) from this study was 15% greater when compared to the fecundity of the \(O. melanopleurus\) from the Kapuas River, which was around 27,000 - 57,611 eggs (Kristianto et al, 2011). The ovulated eggs were fertilized with the spermatozoa of the male spider fish. The eggs were incubated at 27° C, 28° C, and 29° C. The eggs develop up to 4 hours until the Blastula stage. After that, the eggs no longer see the development of the next stage and eventually die. Furthermore, research will be carried out to obtain results that have not been obtained, namely the dose of ovaprim hormone 0.4 mL kg BW\(^{-1}\) for fertilization and hatching of \(O. melanopleurus\) larvae.

3.3 \(O. melanopleurus\) egg diameter
The results showed that there was a change in size that varied from the diameter of \(O. melanopleurus\) eggs injected with Oodev hormone, at the beginning of the study the diameter of fish eggs ranged from 0.83-0.92 mm. In the fourth sampling, the egg diameter ranged from 0.88-0.97 mm. More clearly can be seen in Table 1

| Oodev dose (mL/kg) | S1 (control) | S2 (11/05/21) | S3 (1/06/21) | S4 (22/06/21) |
|-------------------|-------------|--------------|--------------|--------------|
| 0                 | 0.88±0.01   | 0.84±0.09    | 0.87±0.07    | 0.88±0.01\(^a\) |
| 0.5               | 0.89±0.01   | 0.89±0.04    | 0.97±0.02    | 0.95±0.02\(^b\) |
| 0.7               | 0.91±0.01   | 0.9±0.02     | 0.95±0.04    | 0.97±0.02     |
| 0.9               | 0.89±0.04   | 0.95±0.5     | 0.92±0.02    | 0.94±0.03\(^b\) |

Description: Different superscripts on the same line show significantly different P<0.05
Table 1 shows that the injection of Oodev hormone at different doses in the prospective broodstock of *O. melanopleurus* had an effect between treatments on egg diameter (*p*<0.05). The results of the Student Newman Keuls (SNK) follow-up test showed that 0.5-0.9 mL kg BW<sup>-1</sup> Oodev injections were not significantly different between treatments, but were significantly different from those without Oodev injections (0 mL kg BW<sup>-1</sup>). Oodev dose injection of 0.7 mL kg BW<sup>-1</sup> gave the highest egg diameter, which was 0.97 mm. while without Oodev injection, the lowest diameter was 0.88 mm. This indicates that the injection of the Oodev hormone was able to increase the diameter of the eggs of the prospective parent *O. melanopleurus* compared to without Oodev injection (control).

A dose of 0.7 mL kg BW<sup>-1</sup> resulted in the highest egg diameter development, this result is in line with the research of Rozikin et al., (2016) in papuyu fish of 0.9 mm. This is thought to be due to the main function of gonadotrophins in the physiological regulation of gonadal function, especially in gonadal development not directly but through the biosynthesis of gonadal steroid hormones which are mediators for various levels of gametosis including oocyte growth, oocyte maturation, spermatogenesis of spermiation. According to Putri et al., (2019), egg diameter greatly affects the amount of yolk, which is a source of energy for the embryo in its early growth period, fish can absorb maximum so that it accelerates the process of vitellogenesis.

Oodev hormone which contains PMSG and anti dopamine can stimulate the gonads to produce GnRh which then stimulates the pituitary to secrete gonadotropin hormones in the process of vitellogenesis. Enditha et al., (2021) the addition of FSH and LH hormones can accelerate gonadal maturity in fish. Dwantara & Rahmatia., (2017) ; Putri et al., (2019) stated that the hormones that work in the maturation process of fish gonads are gonadotropins, and the Oodev hormone contains PMSG which has a higher FSH content than LH. then it will affect the pituitary to produce gonadotropins, after which gonadotropins will stimulate the ovaries for the process of maturation of eggs in fish.

PMSG hormone can stimulate the growth of ovarian cells, growth, and maturation of follicles, to increase the diameter of fish eggs and cause egg maturity to occur. The FSH contained in PMSG acts on the theca layer of the oocyte to stimulate the synthesis of testosterone, which then enters the oocyte granulosa layer. In the granulosa layer, testosterone is converted by the aromatase enzyme into estradiol-17β which is then carried by the bloodstream to the liver and stimulates the liver to synthesize vitellogenin (Nagahama, 1983).

Oodev® hormone also contains anti dopamine (AD) which is a chemical that functions to block the action of dopamine; where dopamine is a neurotransmitter that works by inhibiting the secretion of GnRH (FSHRH) from the hypothalamus (Cerdá-Reverter & Canosa, 2009; van der Kraak, 2009), inhibiting the secretion of FSH from the pituitary and from gonadotropin cells through the dopamine D2 receptor (Vacher et al., 2002), and inhibits gonadal maturation through its action as a gonadotropin release-inhibiting factor (GRIF) (Dufour et al., 2005).

### 3.4 Egg ripeness

The average value for *O. melanopleurus* egg maturity obtained from this study is presented in Figure 1. The highest average egg maturity value was found at a dose of 0.7 mL kg BW<sup>-1</sup> oodev with an average maturity value of 82%, followed by a dose of 0.5 mL/kg with an average maturity value of 67.77%, and the lowest was in the treatment without the addition of oodev hormone, which was 55.55%.

Based on Figure 1 shows that the oodev dose of 0.7 mL kg BW<sup>-1</sup> is the optimal dose in influencing egg maturity. The use of oodev hormone above 0.7 mL kg BW<sup>-1</sup> gives better results than doses above 0.7 mL kg BW<sup>-1</sup>, this is because excessive doses can inhibit the work of target organs, causing a negative feedback process from gonadotropin hormone secretion, which results in a negative feedback process. This results in high levels of FSH produced. Nagahama and Yamasitha (2008) stated that the negative feedback mechanism causes a high enough FSH content to suppress LH action so that gonadotropins stop the synthesis of 17β estradiol. According to Nagahama et. al., (1995) increased LH in the body of fish can increase the activity of 20β-hydroxysteroid dehydrogenase (20β-HSD) to produce 17α,20β dihydroxyprogesterone, resulting in oocyte maturation followed by ovulation.
4. Conclusion

Based on the measurement of the diameter of the eggs produced, the use of the Oodev hormone at a dose of 0.7 mL kg\(^{-1}\) of *O. melanopleurus* given every week for eight weeks was able to stimulate the development of the gonads of *O. melanopleurus*. Ovaprim injection of 0.7 mL kg\(^{-1}\) produced 89,566 eggs with a latency of 4 hours.

To improve the quality of *O. melanopleurus* eggs, it is recommended to use Ovaprim 0.5 mL kg\(^{-1}\) in female broodstock. This research needs to be continued on the enlargement of hatching larvae by giving different types of initial feeding treatment.

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