

The Effect Of Crude Extract Green Mussel (*Perna viridis*) With Intramuscular Injection In Promoting Pangas Catfish (*Pangasius hypothalamus*) Gonadal Maturation

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Abstract. The management of broodstock is a crucial step in the fish breeding process. The size of the body and gonadal maturity are significant aspects of broodstock quality in aquaculture. To produce high-quality fish, the brood needs to possess fully grown gonads and an ideal body size. According to several studies, green mussel extract is thought to stimulate zebrafish growth and gonad maturation caused by estradiol compounds, although further testing on other fish, such as the *Pangasius hypothalamus*, has not yet been done. This research aims to evaluate the effect of administering crude green mussel extract containing estradiol compound via intramuscular injection on the growth and maturation of pangas catfish gonads. The pangas catfish broodstock was treated with an injection of green mussel extract and ovaprim at a dose of 0.5 mg/broodstock with three repetitions over a 30-day maintenance period. The results show that the administration of green mussel extract at a dose of 0.5 mL/kg increases weight growth. The homogeneity level of eggs in female pangas catfish induced with green mussel extract is the same diameter as induction with ovaprim and slightly different from the control specimen (without treatment). The results show that the crude extract of green mussels in the methanol fraction, at a dose of 0.5 mL/kg, can increase growth and egg diameter.

Keywords: *Pangasius hypothalamus*, gonad maturation, growth, green mussels extract.

INTRODUCTION

Indonesian waters are renowned for megamarine biodiversity. One of the popular fish commodities in Indonesia is pangas catfish (*Pangasius hypothalamus*), particularly on the island of Sumatera. Due to its high nutritional value, pangas catfish has become popular cuisine in some provinces. However, the availability of pangas catfish seeds has been limited due to spawning constraints. Artificial spawning procedures, which include synthetic hormones, are among the challenges since they leave toxic residue. Therefore, researchers are working on a variety of approaches to discover natural hormones that are both safe for humans and the environment.

Green mussels (*Perna viridis*) are thought to contain steroid hormones that can stimulate fish reproduction. This hormone stimulates gonad maturity in broodfish, increasing the quality and number of seedlings. The aim of this study is to investigate the steroid hormone contained in green mussels and the potential benefits of using it as an agent to trigger gonad maturity in the pangas catfish breeding process.

Early sexual maturity can have a negative impact on pangas catfish reproductive performance, such as reducing fecundity and egg quality, which leads to lower seedling quality. Therefore, actions must be taken to improve the quality of the seeds produced in one way, specifically by improving the reproductive performance of the female

brood. Attempts to enhance the reproductive performance of the female brood can be made by hormone induction using green mussel extract.

METHODS

This study was conducted from May to August 2024 at Lampung State Polytechnic's Fisheries Laboratory A in Bandar Lampung, Lampung Province.

This study utilized a Completely Randomized Design (CRD) with two treatments and four replications. The treatment regimen is as follows:

P 1: Without treatment

P 2: Crude extract of green mussels 0,5 ml/kg

P 3: Ovaprim hormone 0,5 ml/kg

Brood Selection

The volume of the maintenance pond for prospective pangas catfish broodstock was initially reduced, followed by the selection of 30 pangas catfish broodstock based on the morphology (healthy and unblemished). Furthermore, selection was made depending on gender.

Broodstock Tagging

The selected pangas catfish brood numbered 12. To improve control during observation, a tagging chip was inserted into the fish's back. The broodstock tagging was divided into three groups, with tagging number codes 1010 (P1) for four brood without injection, 1040 (P2) for four brood injected with 0.5 ml/kg extract, and 1046 (P3) for four brood injected with 0.5 ml/kg ovaprim.

Producing green mussel extract

The green mussels were separated into shell and flesh. The green mussels were then pulverized with a blender, and after smoothing, methanol solvent was added in a 1:3 ratio to a 1000-ml Erlenmeyer flask, carefully closed and labeled, then placed in a waterbath shaker and macerated for 7 days. The extracted solution was filtered through Whatman No. 1 filter paper and then evaporated [1].

Bloodstock Feeding

Throughout the study, broodstock were fed three times a day, in the morning, midday, and evening. For eight weeks, feed was given at a rate of 3% of biomass weight.

Injection of green mussel extract and ovaprim

The fish were injected intramuscularly with green mussel extract at a dose of 0.5 mL/kg and ovaprim at a dose of 0.5 mL/kg

PARAMETERS MEASURED

Increase in Broodstock Weigh

The broodstock weight gain was measured using a scale. Measurements were taken twice during the observation: before and after 30 days after hormone injection

Egg diameter

The diameter of the egg can be measured using a micrometer on a microscope. The value displayed on the microscope is converted into the degree of magnification utilized. The overall observed egg diameter is examined for its middle value using [2] formula.

$$DT = \frac{S_{.Ob}}{S_{.Ok}} \times K. Ob \times L$$

Keterangan:

- DT = Observed egg diameter (mm)
 S. Ob = Objective Lens Scale
 S. Ok = Ocular Lens Scale
 K. Ob = Objective Lens Accuracy (0,01mm)
 L = Observed egg length diameter

Gonad Maturity Level (TKG)

Fish gonad maturity is assessed by their development, changes in egg color, and the filling of the abdominal cavity. The maturity stage of the gonads is evaluated morphologically via observation of their coloration. The results of morphological observations are supplied as photographs, which are then analyzed descriptively.

DATA ANALYSIS

To test homogeneity and significant differences, quantitative data were described using ANOVA in the SAS V.9.4 program. Then, Duncan's test was used to determine the clustering of various treatments. The measured characteristics included broodstock weight increase, egg diameter, and gonad maturity level (TKG).

RESULTS AND DISCUSSION

INCREASE IN BROOD WEIGHT

An increase in broodstock weight was identified after being injected with green mussel extract and ovaprim. At the end of the study, the average weights of each treatment were 1.050, 1.067, and 1.158 kg (**TABLE 1**). Broodstock weight has a significant impact on the increase in gonadotropin hormone concentration in the blood, which stimulates ovulation and spawning within 9 hours. The gonad weight reaches its peak when the fish spawns, after which it rapidly decreases until it completes [3].

TABLE 1. Average Weight Increase of Female Patin Fish

Treatment	Average Weight (Kg)	
	Beginning	End
P1	0,50	1,050
P2	0,44	1,067
P3	0,45	1,158

According to subsequent analysis, the correlation value between the administration of green mussel extract and ovaprim is strongly related to an increase in the average weight of the broodstock. This is related to an increase in the diameter of fish eggs, indicating that the fish are ready to spawn. Additionally, spawning has an essential influence on fish health and environmental parameters.

EGG DIAMETER

The results of the study showed that administering green mussel extract and ovaprim hormone to female catfish broodstock increased the diameter of catfish eggs. **TABLE 2** illustrates this further.

TABLE 2. Average Diameter of Female Pangas Catfis Eggs (*Pangasius hypothalamus*)

Treatment	Average Diameter (mm)	
	1	2
P1	<u>0,45</u>	<u>0,62</u>
P2	<u>0,40</u>	<u>0,98</u>
P3	<u>0,50</u>	<u>1,15</u>

After the second injection, the egg diameter increased to 0,62, 0,98 and 1,15 with an ivory yellow color, which is consistent with [4] statement that mature or good eggs have a diameter of between 0.9 and 1 mm with a consistent size, ivory yellow color, and will separate or not clump when in contact with water.

The discrepancy in egg diameter size may be induced by the vitellogenesis process. Furthermore, FSH levels increases in fish, causing follicles to develop and egg diameters to increase [5]. Other factors that can influence egg diameter differences include fish genetics, environment, fish age, and broodstock nutrition quality [6].

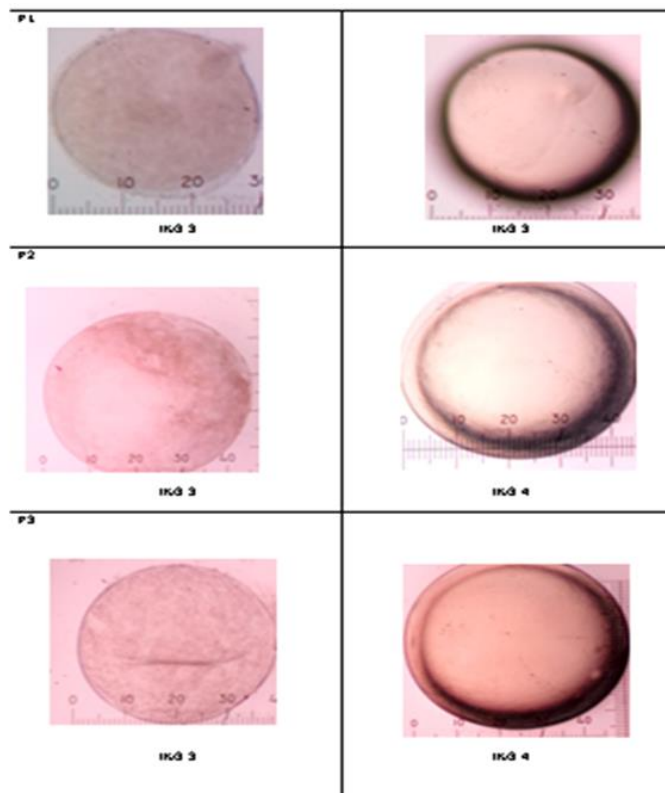
Based on the correlation regression analysis, the use of green mussel extract at doses of 0.5 ml/kg, and ovaprim resulted in a correlation value of x and y of 0.621, indicating that the use of green mussel extract has a substantial effect on the diameter of pangas catfish eggs. This can be induced by a variety of variables, including fish conditions, environmental stimulation, and effective doses administered to female brood.

Steroid hormone induction is an attempt to accelerate the maturity of fish gonads. Green mussels contain steroid hormones, including estradiol. Estradiol-17 β hormone produces vitellogenin [7]. Several studies about estradiol were performed: [8] used 17 β -estradiol to stimulate tilapia gonad growth; [9] used broiler chicken pituitary glands to mature goldfish gonads; [10] used frog pituitary extract to accelerate goldfish spawning and E2 to increase seabass vitellogenin. The effects of 17 β -estradiol on vitellogenesis in *Rhamdia quelen* fish [11].

GONAD MATURITY LEVEL

During the investigation, the physical or morphological properties of the eggs were used to determine the maturity level of the broodstock gonads. The level of maturity of the gonads can be determined by collecting egg samples with a catheter and then examining the egg nucleus under a microscope. The results of morphological egg observations are shown in **TABLE 3**.

TABLE 3. Gonad Maturity Level



Based on the study's findings, it is clear that the broodstock's gonads have matured, as evidenced by the morphological state of the eggs, which have become more uniform and ivory yellow in color. TKG 3 is characterized by the presence of oocyte granules that are beginning to be clear, not yet dense, and gradually turning orange, whereas TKG 4 has large oocyte size, fullness, and orange color [12]. Egg maturation in fish is marked by the fusion of the plasma membrane of the germinal vesicle breakdown (GVBD) egg cell [13]. The nucleus that migrates to the edge approaching the micropyle and fuses with the cell wall indicates that the fish is ready to spawn [14]. One of the most critical factors influencing spawning success is gonad maturity, which impacts fertilization, hatching, and survival rates.

CONCLUSIONS

According to the study's findings, giving green mussel extract at a dose of 0.5 ml/kg and 0.5 ml/kg ovaprim has an effect on the maturation of female pangas catfish broodstock. This occurs because green mussels contain estradiol and several other primary compounds such as protein, fat, and vitamins. Exogenous estrogen regulates ZP morphology and oocyte nutrition. When employing E2, an increase in ZP thickness inhibits oocyte growth and maturation.

SUGGESTION

Based on this study's findings, it is recommended that more research be conducted to determine how long it takes for the pangas catfish broodstock to mature in order to administer green mussel extract optimally.

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