The development of the reproductive organs of the hybrid grouper cantik (*Epinephelus fuscoguttatus* x *E. polyphekadion*)

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**Abstract.** Observation of the reproductive organs of the hybrid grouper cantik was carried out. Cantik grouper is the result of cross-breeding between female tiger grouper *Epinephelus fuscoguttatus* and male camouflage grouper *E. polyphekadion*. This study aimed to determine the development of the reproductive organs of cantik grouper. The observed test fishes were 3 fishes of Cantik grouper with a total length of 42 - 49 cm and a bodyweight of 1.45 - 2.26 kg. The parameters observed were the concentration of estradiol and testosterone in the blood using the ELISA kit, gonad development, and histological maturity level of the gonads. The results showed that the hormone testosterone found in the sample cantik no 1 was 9353 pg/ml, while the hormone estradiol was found in all samples of beautiful grouper in the range of 291 - 1133 pg/ml. From the dissected fish, the gonads had developed and weighed 3.12 - 56.01 g and one sample had a fecundity of 265,800 eggs with an egg diameter of 360-520 micron. Gonadal histology shows oosit development at maturity levels I, II, and IV. This indicates that the cross-breeding between female tiger grouper and male camouflage grouper produces hybrid grouper cantik that are fertile, which is shown by their reproductive organs to have developed well.

1. **Introduction**

Market demand for grouper commodities is still quite high, especially for exports. Hatcheries and fish rearing technology has grown in society. For some years, hybrid grouper seeds have been successfully produced in government and private hatcheries in Indonesia. Hybridization was conducted on various fish species in order to increase growth rates, manipulating sex ratios, producing sterile fish, improving meat quality, increasing disease resistance, increasing environmental tolerance, and to improve a variety of other traits to make them more profitable [1]. Several studies related to grouper hybridization have been conducted, including: crossbreeding of grouper *Epinephelus marginatus* with *E. aeneas* [2], hibridization of *E. costae* x *E. marginatus* [3], hibridization of *Epinephelus coioides* x *E. lanceolatus* [4, 5], and hibridization of *Plectropomus leopardus* x *P. maculatus* [6].

A type of hybrid grouper grown in the community is the Cantang and Cantik groupers. The Cantang grouper is the result of the crossing of female tiger groupers (*E. fuscoguttatus*) and male giant groupers (*E. lanceolatus*). While the Cantik grouper is the result of cross-breeding female tiger grouper (*E. fuscoguttatus*) with male camouflage grouper (*E. polyphekadion*). Cantik grouper can be cultivated both in ponds and floating net cages. Previous studies on cantik grouper includes: morphological variations of Cantik grouper seeds [7], increase in Cantik grouper seed production and quality [8], and Isolation and characterization of bacteria in the larvae of Cantik groupers [9], while
research on the reproduction of Cantik hybrid groupers is still very limited so it still needs to be studied further.

This study aims to determine the development of the reproductive organs of the hybrid grouper cantik. If the reproductive organs can develop properly, it is possible that the Cantik hybrid grouper is fertile so that it can spawn and reproduce for offspring. So that cantik grouper farmers should more careful that cantik grouper does not escape into the wild because it can damage ecosystems that are not their habitat.

2. Material and methods

2.1. Cantik hybrid grouper fish test

The research was conducted at Institute for mariculture research and fisherries extention Gondol-Bali Indonesia. The test fishes were 3 Cantik hybrid groupers with a body weight of 1.45 – 2.26 kg and a total length of 42.20 – 49 cm as shown in Table 1.

| No of sample | Body weight (kg) | Total length (cm) |
|--------------|------------------|-------------------|
| Cantik 1     | 1.45             | 42.20             |
| Cantik 2     | 2.26             | 48.30             |
| Cantik 3     | 2.26             | 49.00             |

2.2. Concentration of reproductive hormone

Fish blood samples were taken using a 3 ml syringe containing 0.1 ml heparin to prevent clotting of the blood taken. Fish were anesthetized using 2-phenoxy ethanol. 1 ml of blood sample was taken and transferred to 1,5 ml tube. Then the blood sample was centrifuged at 5000 rpm for 15 minutes to separate the plasma and serum. The Concentration of reproductive hormones in fish blood was analyzed using the Elisa Kit, Elisa kit 11-Keto Testosteron EIA (Cayman Chemical Company) and AccuDiagTM ELISA Estradiol (Cortez Diagnosics Inc.). Stages for the analysis of hormone content in the blood, according to the protocol of each kit.

2.3. Gonadosomatic index (GSI) and gonad maturity level

Fish dissected to observe the gonads. The gonads were weighed to determine the gonadosomatic index (GSI) and then fixed using Bouin's solution for further histological analysis to determine the level of gonadal maturity. For fish that have developed gonads, the egg were counted and the diameter was measured.

2.4. Observed parameters and data analysis

The parameters observed included: total length, body weight, concentration of reproductive hormone in the blood (ketotestosterone and estradiol), gonadal weight, gonadosomatic index (GSI) and gonadal maturity level. GSI calculated based on the formula [10] below. The data obtained were analyzed descriptive and presented in the form of images, tables and graphs.

\[ \text{Gonadosomatic Index (GSI)} = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100\% \]  

(1)

3. Result and discussion

3.1. Concentration of reproduction hormone

Based on the results of Elisa's test, the blood sample of the Cantik grouper that was observed contained the hormones estradiol and testosterone as shown in Figure 1.
Figure 1. Concentration of reproduction hormones, testosterone and estradiol (pg/ml) in blood of Cantik hybrid grouper

The testosterone hormone was observed in the Cantik 1 blood sample and was not observed in the other samples. The concentration of testosterone in Cantik 1 blood sample was 9353 pg/ml. The estradiol hormone was observed in all of blood sample of Cantik grouper. The lowest concentration of estradiol was 291 pg/ml of Cantik 1 blood sample and the highest concentration was 1133 pg/ml of Cantik 3 blood sample.

3.2. Gonadosomatic index (GSI) and gonad maturity level
Based on the dissection of the Cantik grouper and the observation of the development of the gonads, it was found that the gonads were well-developed within the three Cantik groupers as shown in Figure 2. The smallest gonad was found at Cantik 1 with 3.12 g in weight and the biggest gonad was found at Cantik 2 with 56.01 g in weight, while the gonad of Cantik 2 was 6.92 g in weight.

Table 2. Data of gonad weight (g), gonadosomatic index/GSI (%) and egg fecundity (pcs) of Cantik hybrid grouper

| No of sample | Gonad weight (g) | GSI (%) | Egg fecundity (pcs) |
|--------------|------------------|---------|---------------------|
| Cantik 1     | 3.12             | 0.22    | -                   |
| Cantik 2     | 56.01            | 2.48    | 265.800             |
| Cantik 3     | 6.92             | 0.31    | -                   |

The data of gonad weight (g), Gonadosomatic index (GSI) and egg fecundity of Cantik grouper was shown at Table 2. The smallest value of GSI was 0.22 % of Cantik 1 and the biggest value was
2.48% of Cantik 2. Gonad weight affects the GSI value. The heavier the fish gonads, the greater the GSI value.

Of the three gonads of the Cantik grouper, only gonads of Cantik 2 could count the eggs because they were well-developed. The eggs produced by Cantik 2 are 265,800 eggs. Based on the eggs produced by Cantik 2, it had been observed that the distribution of egg diameters ranged from 360 to 520 µm as shown in a Figure 3. The smallest distribution was at the egg size of 360, 420, 460 and 520 micron as much as 3.3% while the largest distribution was at the egg size of 400 microns as much as 36.7%.

![Figure 3. Eggs distribution (%) of Cantik 2 hybrid grouper](image)

The maturity level of the Cantik grouper gonads can be seen from the histology of the gonads which show that in Cantik 1 the gonads show a maturity level I, at Cantik 2 shows the maturity level of gonads at level I-IV while in Cantik 3 the gonad maturity at levels I and II.

![Figure 4. Histology section of Cantik hybrid grouper gonad](image)

Observation of reproductive hormones in the blood of the cantik grouper indicates the sexual maturity of the fish. The presence of 11-Ketotestosterone is an indicator of maturity as a male in fish [11]. Of the samples observed, only Cantik 1 showed a testosterone concentration in the blood of 9353 pg/ml. This means that Cantik 1 shows sexual maturity as a male fish. The coral trout (*Plectropomus leopardus*) grouper broodstock showed positive male sex at testosterone levels above 700 pg/ml [12], while the catfish (*Clarias macrocephalus*) showed that the male broodstock during the reproductive cycle, the content of 11-KT testosteron was between 159-434 ng/ml [13]. The content of the hormone estrogen in the blood was observed in all samples of Cantik grouper. The hormone estrogen is usually found in sexually mature female fish. Plasma estradiol levels have an effect not only on the variations in Vtg concentrations but also on the concentrations of other molecules as prostaglandins, which have an intimate action on ovulation and spawning [14]. In Cantik 1, the hormone testosterone and estrogen were observed. This happens because groupers are protogynous hermaphrodites that will change sex from female to male. In the transition phase between females and males, it is possible for the development of female and male gonads to occur simultaneously so that the hormones testosterone and estradiol are present in the fish’s blood.

The development of the reproductive organs of the cantik grouper was also shown from the results of the dissected fish which showed the presence of well-developed gonads in all samples of the cantik
grouper. Even in cantik 2 gonads look quite big (56.01 g) with a high GSI value (2.48%) and produce quite a lot of eggs (265,800 eggs) with a size of up to 520 microns.

From the histology of the gonads, it can also be seen that egg maturity at stages I - IV, grouper is partial spawning which will only release mature eggs when spawning occurs. The eggs will develop until they are ready for ovulation at the time of spawning.

Base on the result, gonads of cantik grouper was develop properly and normally like their parents. Previous studies have also shown that gonad development in hybridization between tiger grouper *Epinephelus fuscoguttatus* and Giant grouper *E. lanceolatus* [15] and Hybridization of *Salmo trutta* x *Salvelinus fontinalis* [16].

The fertility of the hybrid grouper may be because the parents were still in the same genus so that they can produce fertile offspring. Therefore, cantik grouper farmers should more careful that cantik grouper does not released into the wild because it can disrupt the habitat and other fish populations in the wild.

4. Conclusion
The reproductive organs of Cantik hybrid grouper can develop well. The crossbreeding of the female tiger grouper and the male camouflage grouper produced a Cantik hybrid grouper that was fertile.

References
[1] Bartley D M, Rana K, and Immink A J 2001 *Rev. in Fish Bio. and Fish* 10 325–37
[2] Glamuzina B, Kozul V, Tutman P and Skaramuc B 1999 *Aquaculture Res.* 30 625–28
[3] Glamuzina B, Glavic N, Skaramuca B, Kozul V and Tutman P 2001 *Aquaculture* 198 55–61
[4] Chu K I C, Shaleh S R M, Akazawa N, Oota Y and Senoo S 2010 *Aquaculture Sci.* 58 1-10
[5] Kiriyakit A, Gallardo W G and Bart A N 2011 *Aquaculture* 320 106–12
[6] Frisch J A and Hobbs J P A 2007 *Aquaculture Res.* 38 215-18
[7] Kusumawati D and Ismi S 2013 *Konferensi Akuakultur Indonesia* 192-99
[8] Ismi S, Asih Y N, Kusumawati D 2014 *J Oceanologi Indonesia* 1 1-5
[9] Zafran, Ismi S, Mastuti I and Mahardika K 2020 *J of Fish. and Mar. Res.* 2 194-00 DOI: http://dx.doi.org/10.21776/ub.jfmr.2020.004.02.2
[10] Effendi M I 2002 *Biologi Perikanan* (Yotgyakarta: Yayasan Pustaka Nusatama) p 162
[11] Pankhurst N W, and Conroy A M 1987 *Fish Physiology and Biochemistry* 4 15-26
[12] Sembiring S B M, Prijoono A, Hutapea J H, and Setiadharma T 2011 *Tech. Rep. Gondol Reseach Institute for Mariculture* p 11
[13] Fermin J T, Takeshi M, Ueda H, Adachi S, and Yamauchi K 1997 *Fish. Sci.* 63(5) 681-86
[14] Tirado J O, Valladares L, Muñoz D, Caza J, Manjunatha B, and Kundapur R R 2017. *Lat. Am. J. Aquat. Res.* 45(5) 930-36 DOI : 10.3856/vol45-issue5-fulltext-8
[15] Luin M, Fui C F, and Senoo S 2013 *Jou. Aqua. Res. and Dev.* 5:2. DOI : 10.4172/2155-9546.1000213
[16] Blanc J, and Chevassus B 1986 *Aquaculture*, 52 59-69

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