Evaluation of hatching rate, growth performance, and survival rate of cantang grouper (*Epinephelus fuscoguttatus × lanceolatus*) in concrete pond at Situbondo, East Java, Indonesia

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Abstract. Cantang grouper (*Epinephelus fuscoguttatus × lanceolatus*) is an Indonesian fisheries commodity that seeded and has high economic value. Cantang grouper is also had excellence because it has fast growth and tolerance to disease, so these fish are widely cultured. The purpose of this study was to analyze the hatching rate, growth monitoring, and survival rate of cantang grouper. This research was conducted at UPT PBL Situbondo, East Java Province on December 2018 until January 2019. Spawning of the cantang grouper was done artificially. The hatching rate of cantang grouper eggs was 53.3 %, growth rate ranging from 0.0009-0.7900 gr/day and specific growth rate ranging from 10.34-29.84 %. Larvae maintenance includes feeding, measurement of water quality, and change of water. Feeding is in the form of *Chlorella* sp., Rotifer, *Artemia* sp., *Acetes* sp., and artificial feed. The survival rate of cantang grouper was 26.9%. The measured water quality was a temperature ranging from 29-30 °C, pH with a range of 7.9-8, and dissolved oxygen (DO) was 7 mg /L.

1. Introduction

Grouper fish is an Indonesian fisheries commodities that have high economic value. Grouper fish has a fairly high price, so it becomes an Indonesian export commodity from the fisheries sector and has a promising market segment, both domestically and foreign [1]. Grouper production in Indonesia in 2011 was 10,580 tons, increasing to 11,950 tons in 2012 and in 2013 production reached 14,400 tons [2].

Cantang hybrid grouper is the result of hybridization between female brown marbled grouper (*Epinephelus fuscoguttatus*) and male giant grouper fish (*Epinephelus lanceolatus*) [3]. The aim of hybridization to obtained fish that has advantages compared to their parents [4]. As an aquaculture species, cantang grouper has several advantages, especially in terms of rapid growth and tolerance in low water salinity [5,6,7]. Another advantage of cantang grouper, it has a high market price as a living food in the Asia Pacific region [8].
Currently, cantang grouper culture has developed, demand for grouper cantang larvae for the cultivation very high both from domestic and foreign, so that an effort needed to supplied the larvae continuously through hatchery operations that the technology can apply [9].

Fish hatchery techniques are activities to breed/reproduce/seed fish naturally, semi-artificial and artificial. Fish hatchery starts from the management of the parent fish, parent selection, in accord with the standards for Good Fish Hatchery (CPIB) and parent spawning processes, hatching eggs and treating larvae according to production requirements, so that optimal cultivation results can be obtained [10]. Fish hatchery techniques of cantang grouper done in a concrete pond. The concrete pond used as a medium for seeding cantang grouper larvae because it's more controlled, which means it's easy to handle the larvae [11]. The concrete pond for a hatchery that reared and controlled will produce cantang grouper larvae that have good quality [12]. The purpose of this study was to analyze the hatching rate, growth monitoring, and survival rate of cantang grouper.

2. Material and methods

2.1. Place and time

This research was conducted at UPT PBL Situbondo, East Java Province on December 2018 until January 2019.

2.2. Preparation of spawning and maintenance pond

Brown marbled grouper and giant grouper are kept in a round concrete pond with 3 m of depth and 10 m of diameter and maintained separately. Fiber tanks used in stripping and injecting hormones, while concrete tanks used for egg incubation and environmental manipulation stages. The concrete tank for maintenance also equipped with a flowing water system and an egg collector to collect the eggs that come out naturally [16]. Maintenance of larvae done using a concrete tank that has a size of 5 x 1.8 x 1.2 m³ and 120 cm of depth.

Preparation of maintenance tanks carried out by removed water from the tank through an outlet, the tank that has been washed then dried for 2-3 days. The next stage is sterilization. Sterilization is done by adding 20 mg / L of chlorine into seawater and aerated approximately 9 hours. Neutralization of seawater is carried out using 5 grams of sodium thiosulfate and left for about 2 hours. Before the seawater flowed into tanks, chlorine test is carried out by taking a sample of seawater and dropping 3 drops of the chlorine test kit. Sterile seawater then flowed through the inlet which equipped with a filter bag to prevent the entry of other organisms into the tanks.

2.3. Parent selection

The parent selection of brown marbled grouper and giant grouper can be done by looking at the body weight, level of gonad maturity and maternal health. Brown marbled grouper and giant grouper selected based on health, non defective and disease-free [17]. Physically, the parent's health can be seen based on no physical damage to the surface of the body such as hemorrhage and necrosis, the color of the body is not pale and the completeness of the body's organs of the parent. Giant grouper that are male when pressed on the abdomen will release white liquid thick milk or called sperm. Whereas in female brown marbled grouper seen swollen in the abdomen towards urogenital and when pressed will emerge clear yellow liquid. Then the second step is the selection based on the weight of the parent to be spawned. The total weight of the male parent must be greater than the female parent so that the egg can be fertilized as a whole.

2.4. Spawning

Artificial spawning carried out after the brown marbled grouper spawned naturally with discovered of female brown marbled grouper eggs on the egg collector. Artificial spawning in groupers called hybridization through hormonal induction by injecting the ovaprim hormone with a dose of 0.5 ml/kg of parent weight. The purpose of injecting the ovaprim hormone on the female parent to accelerate the maturation of gonads and release of eggs in the female parent [16]. The female parent has been
injected with the ovaprim hormone will pass a latency time approximately 10 hours. The female parent who has passed the latency time examined for the abdomen, if the female grouper abdomen swollen and bigger it’s indicates that the female grouper is ready for spawning. The initial stage of spawning begins with anesthesia using ethylene glycol monophenyl. The parent is given a towel for covered when anesthesia. Taking eggs on the female brown marbled grouper parent is done by stripping. Stripping is done until there are no remaining eggs in the abdomen. The result of eggs placed in a container and then mixed with sperm. The sperm used of 1 ml to fertilize 1,000,000 eggs.

2.5. Hatching eggs
The characteristics of good quality of eggs are clear eggs and floating in the water column. Cantang grouper eggs are acclimatized for ± 4 hours before spread out into the hatchery tank. Acclimatization is done by inserting a plastic bag containing eggs into a hatchery tank. The purpose of acclimatization is to adapt the eggs so that the temperature in the plastic bag the same as the temperature of the hatching media. Cantang grouper eggs will hatch within ± 20 hours. The grouper larvae which hatched look transparent which is accompanied by egg yolks and oil bubbles. Egg calculation is done by taking eggs using a 10 ml volume pipette in 1 liter of water containing grouper eggs. One volume pipette contains at least 50 eggs. So the results can be calculated by multiplying the number of eggs in a pipette in one sampling with 1 liter of water.

2.6 Feeding
The cantang grouper larvae that hatched have an egg yolk. This feed will be utilized until the second day, and on the third day, the egg yolk has been absorbed completely so that it is necessary to need a feed from outside in the form of natural food. Feeding of grouper larvae is carried out twice a day in the morning and evening. The feed used for the grouper larvae is natural and artificial feed. Natural food used for the maintenance of cantang grouper larvae consists of microalgae such as Nannochloropsis sp. and Chlorella sp., Rotifer (Brachionus rotundiformis) and Artemia sp. [19]. Artificial feed that given is Otohime commercial feed produced by Japan.

The initial feed of the cantang grouper larvae is Rotifer (Brachionus plicatilis) which given to D3 larvae until D20. Rotifer density that given of 10-15 ind / ml. Feeding was carried out by flowing Rotifer that enriched with Chlorella sp. from the culture tank using a filter bag (Figure 1), then flowed to the hatchery tank. The cantang grouper larvae begin to given Artemia sp. at D10 to D25 with a density of 3-10 ind/ml. Otohime artificial feed can be given when the larvae at D6 to D10. The amount of feed that given ranges ± 4 grams/administration (D12-D20), ± 8 grams/administration (D21-D30) and ± 8 grams/administration (D31-harvest).

![Figure 1. Flowing rotifer that enriched with Chlorella sp. using a filter bag](image)

2.7 Water quality measuring
The parameter that measured for water quality which includes measuring dissolved oxygen using DO meter, pH using pH meter, salinity using refractometer and temperature using a thermometer.

2.8 Data analysis
Growth parameters measured are weight and length. Fish body weight measured using analytic scales with a precision level of 0.0001 gr or 0.1 mg and fish body length measured using a ruler with a precision level of 0.5 mm. Other growth parameters calculated include:

a. Hatching rate (HR), to determine HR use a formula [13].
\[
HR (\%) = \frac{\text{The number of eggs hatched}}{\text{The number of sample eggs}} \times 100\% 
\]
b. Survival Rate (SR), to determine Survival Rate (SR) use a formula [14].
\[
SR (\%) = \frac{\text{Nt}}{\text{No}} \times 100\% 
\]
c. Specific Growth Rate (SGR), to determine Specific Growth Rate uses a formula [15].
\[
SGR (\%) = \left(\frac{Wt}{Wo}\right)^\frac{t}{t} \times 100\% 
\]
d. Growth Rate (GR), to determine Growth Rate uses a formula [15].
\[
GR (\text{gr/day}) = \frac{Wt - Wo}{t} 
\]

Where:
- \(Wt\) = Fish weight on the day (gr)
- \(Wo\) = Fish weight at the beginning of the study (gr)
- \(t\) = Maintenance Time (day)
- \(Nt\) = The amount of fish harvested
- \(No\) = The number of fish stocked

3. Result and discussion

3.1. Hatching rate
The cantang grouper eggs are spread out into a hatchery pond with a capacity of 10 tons and the number of eggs that have been spread out ± 150,000 eggs. The characteristics of good quality of eggs are clear eggs and floating in the water column. Cantang grouper eggs are acclimatized for ± 4 hours before spread out into the hatchery tank. Acclimatization is done by inserting a plastic bag containing eggs into a hatchery tank. The purpose of acclimatization is to adapt the eggs so that the temperature in the plastic bag the same as the temperature of the hatching media.

Cantang grouper eggs will hatch within ± 20 hours. The grouper larvae which hatched look transparent which is accompanied by egg yolks and oil bubbles. The hatching rate that obtained in this research shows that the hatched eggs as many as 80,000 and divided by the total number of eggs that have been spread out as many as 150,000. The percentage of hatching rate was 53.3% and the results were still in the good range. For groupers, the rate of fertilization and hatching must be higher than 50%, larvae from grouper eggs with poor fertilization and hatching rates (<30%) are considered as poor quality larvae [18].

3.2. Larvae Growth Monitoring
Growth monitoring activities carried out at Situbondo aim to determine the larval growth rate each week. The following are a chart of length growth and chart of weight growth (Figure 2).
Larvae growth depends on factors of feed quality, water quality, and stocking density. Stocking density is an important factor that can affect fish growth. Certain stocking density will have a positive and negative effect on the growth rate [20]. High stocking density will cause competition in gaining space, feed and oxygen which can cause stress to fish [21]. Fish that have stress occur because of environmental conditions that are not suitable for the survival of larvae [22]. Stressful conditions can cause the normal function of the fish to be disrupted and growth to slow down.

The calculation of specific growth rates or Specific Growth Rate (SGR) results was 10.34% on the first week, the second week was 29.84%, the third week was 20.64% and on the fourth week was 11.52%. Growth Rate (GR) amount to 0.0009 g/day in the first week, 0.0134 g/day in the second week and 0.049 g/day in the third week and 0.79 g/day in the fourth week (Figure 3). The value of the growth rate shows an increase, it means that the growth rate of cantang grouper larvae is optimal.

3.3. Water quality measurement
Good management and controlled water quality in maintenance media of larvae is one of the supporting factors of hatchery activities. Management of water quality includes activities to measure water quality and change of water. Before the grouper larvae are spread out, the media of the larvae must be in a sterile condition. The seawater that used has been treated using sand filters and filter bags.
Water quality measurements in Situbondo are carried out by measuring temperature, pH, DO (dissolved oxygen) and salinity in the morning and afternoon (Table 1).

### Table 1. Measurement of water quality

| Parameter                  | Average Value | Standard Value |
|----------------------------|---------------|----------------|
| Temperature (°C)           | 29.4          | 28 °C- 32 °C   |
| pH                        | 7.9           | 7.5 – 8.3      |
| Dissolved Oxygen (mg/l)    | 7             | Min. 4 mg/L    |
| Salinity (ppt)             | 29.75         | 28 – 33 g/l    |

The average results obtained in the measurement of temperature in the Situbondo hatchery pond are 29.4 °C. This is still good in cultivation conditions because a good temperature range for cantang grouper are 28-32 °C [23]. The average pH measurement results in Situbondo hatchery tank are 7.9. This is still good in cultivation conditions because a good pH range for cantang grouper are 7.5 – 8.3 [24]. The average of DO values in Situbondo hatchery tank is 7 mg/l. A good range of dissolved oxygen for cantang grouper are 4 mg / L at minimum, so the condition of the hatchery tank good for the cultivation process [24]. The average of salinity measurements at Situbondo is 29.75 ppt. This is still good in good condition because the standard quality for salinity are 28-32 ppt [24].

### 3.4. Survival rate

The survival rate during larvae maintenance is 26.9%. This figure is relatively low, the survival rate in larvae are 50-60% [25]. When harvesting, if the live fish have a large number the survival rate will be high, on the contrary, if the fish have a large number of dies, the survival rate will be low. The survival rate of the grouper seeds can be influenced by several factors including water quality, type of feed given and cannibalism of fish [26].

### 4. Conclusion

During the observations length and weight, growth continues to increase. The hatchery of cantang grouper at the Technical Implementation Unit of Development Marine Aquaculture, Situbondo, East Java it's good enough. This can be seen from the high HR and influencing factors is a good quality of water.

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