

# Study Of Hybrid Cantang Grouper Breeding Techniques (*Epinephelus Fuscoguttatus X Epinephelus Lanceolatus*) At The Brackish Water Fisheries Cultivation Center Situbondo, East Java

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## ABSTRACT

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## Keywords:

Grouper, Hatchery, Hatching Rate, Hybridization, Survival Rate Hybrid cantang grouper is a type of grouper fish that is included in the luxury food commodity, namely a type of commodity that has a high selling value and is often exported which has an impact on increasing the need for seeds so that efforts are needed that culminate in seeding. The purpose of this study was to study and understand the seeding technique of hybrid cantang grouper (Ephinephelus fuscoquttatus x Epinephelus lanceolatus) which was carried out at the Brackish Water Aquaculture Center (BPBAP) Situbondo, East Java. The population of this study used 2 larval maintenance tanks and 14 natural feed tanks. Data were obtained directly through direct observation in the field. The results of the study showed that cantang grouper seeds were produced from the hybridization process, then the types of feed given during the larval maintenance period were natural feed (Rotifera, Artemia and Rebon shrimp) and artificial feed (Powder, crumbs and pellets), then air quality maintenance was carried out by giving Chlorella sp. as a green water system, then air changes, siphoning with air quality values during the maintenance period of temperature 30oC, Salinity 30.1 ppt, pH 8.05, Nitrite 0.498 mg / L, Free Ammonia 0.04675 mg / L, DO 5.85 mg / L. The Hatching Rate (HR) value was obtained 98.45% and 92.63% and Survival rate 12.68% with a maintenance period of 70 days until the total harvest of 2.7cm-3cm seeds.

## INTRODUCTION

Several types of superior cultivated commodities include shrimp, fish, sea cucumbers and seaweed. Several types of marine fish such as grouper are one of the types that are in demand by people both at home and abroad. According to BPS data (2020), exports of live grouper for the January 2019 period were recorded at 277,006 tons, then the highest was in

March 2019, reaching 317,874 tons and the lowest in in August 2019 the total was 99,316 tons then continued to increase until December 2019, with a sales value of USD 2.20 Million (Rochmad & Mukti, 2020). The types of grouper that can be found in Indonesia are tiger grouper, duck or mouse grouper, and mud grouper which have bright development prospects. (Rahmaningsih & Ari, 2013). Apart from that, there are several types of grouper fish resulting from crossbreeding in Indonesia, namely cantang hybrid grouper and beautiful hybrid grouper.

Cantang hybrid grouper is a type of grouper that is included in luxury food commodities, namely a type of commodity that has a high selling value and is often exported. Several countries that are targets for grouper seed exports are Singapore, Malasia, Vietnam, Thailand, Taiwan, Hong Kong and China. Cantang grouper fish are in great demand because they grow quickly, are disease resistant and easy to spawn (Ismi, 2017). Therefore, quite a few fish farming entrepreneurs are interested in choosing to develop this hybrid cantang grouper cultivation business which has the effect of increasing demand for seeds. (Palupi *et al.*, 2020). Currently, the need for seeds can be obtained from natural catches and also from the results of the seeding/spawning process (Palupi *et al.*, 2020). Continuous availability of seeds can be met by seeding efforts using techniques that have been mastered. Cantang hybrid grouper is a type of grouper whose seeds are produced through a hybridization process (Ismi *et al.*, 2014).

Hybridization is one of the effective steps that can be taken as an effort to improve genetic quality because it has a simple technique and does not cost a lot of money and can be done with limited facilities and human resource capabilities. Hybridization has the aim of increasing growth and transferring the desired characters through combining characters from two species/groups into a single species/group, reducing unwanted reproduction by producing sterile (monosex) (Zulfania *et al.*, 2015). Cantang hybrid grouper is a cross between tiger grouper (*Ephinephelus fuscoguttatus*) and kertang grouper (*Ephinephelus lanceolatus*), cantang grouper means kertang tiger grouper (Ismi *et al.*, 2014). Cantang hybrid grouper is a cross between tiger grouper (*Ephinephelus fuscoguttatus*) and kertang grouper (*Ephinephelus lanceolatus*), cantang grouper means kertang tiger grouper (Jiet & Musa, 2018).

Therefore, the author is interested in using the title "Hybrid Cantang Grouper (*Ephinephelus fuscoguttatus* x *Epinephelus lanceolatus*) Hatchery Techniques at the Brackish Water Aquaculture Fisheries Center (BPBAP) Situbondo, East Java" in practical field work activities to learn and know about hybridization and hatching activities cantang hybrid grouper (*Ephinephelus fuscoguttatus* x *Epinephelus lanceolatus*) carried out in Brackish Water Aquaculture Fisheries Center (BPBAP) Situbondo, East Java.

#### METHODS

#### **Time and Place**

The research was carried out on 22 February – 2 May 2024 at the Brackish Water Aquaculture Fisheries Center (BPBAP) Situbondo, East Java.

## **Tools and materials**

Tools and materials used in cantang hybrid grouper hatchery activities include spawning tanks, egg hatching and larva rearing tanks, natural food culture tanks, syringes, corned tubes, buckets, brushes, scoops, scissors, filter bags, sieves, scoops, bowls, submersible pump, plankton net, scales, ruler, grading tool, refractometer, thermometer, hose, aeration, styrofoam, plastic pack, rubber, oxygen, tiger grouper broodstock female, male brood

grouper, cantang grouper eggs, ovaprim, sea water, fresh water, 70% alcohol, COD liver oil, Chlorella sp., elbasin, chlorine, artemia naupli, artificial feed, Scott's emulsion, thio sulfate, fertilizer and tissue.

#### **Research Design**

This research used 2 tanks with dense egg distribution in each tank which can be seen in the following table:

Table 1. Stocking Density of Grouper Eggs			
	Tank number	Number of eggs	
	7	200,000	
_	8	200,000	

## Work procedures

Data collection methods are primary data and secondary data where primary data is obtained through direct observation/review of all activities at the research location related to the hatching of cantang hybrid grouper fish, interviews, active participation during the research location, in the form of observing embryological development, Hatching Rate values (HR), measurement of seed length growth (cm) and Survival Rate (SR) value. Hatching Rate (HR) calculation formula according to Putri *et al.* (2024), that is:

$$HR = \frac{Eggs \ hatch}{Scattered \ eggs} \times 100\%$$

Then according to Adi *et al.* (2023), the Survival Rate (SR) value can be calculated using the following formula:

$$SR = \frac{Nt}{N0} \times 100\%$$

Note:

SR = Survival Rate (%)

Nt = Final number of fish / time of harvest (tail)

N0 = Initial number of fish / time of stocking (tail)

Meanwhile, secondary data was obtained through references in the form of books, articles, journals and from internet sources and other online libraries.

## Data Analysis

Data analysis was carried out using descriptive and statistical techniques, namely by describing all series of activities carried out at the research location in detail which were then strengthened through literature study in the hope of providing as clear information as possible, then statistical tests were carried out manually and using Microsoft Excel.

## RESULTS

## **Broodstock Selection**

The results of morphological observations of the male cantang grouper and the female tiger grouper can be seen in the following picture:



Figure 1. Broodstock Genitalia (a) Female Tiger Grouper Parent (b) Male Tiger Grouper Parent (Source: Personal Documentation, 2024)

Based on the results of field observations, at BPBAP Situbondo the male parent used was 1 tiger grouper (*Ephinephelus lanceolatus*) with a weight of 80 kg, while the female parent tiger grouper (*Ephinephelus fuscoguttatus*) was successfully selected as 5 fish with a weight of 6.98 kg-11.28 kg. kg.

# Spawning/Hybridization

The selected female tiger grouper parents were then weighed to determine the dose of the ovaprim hormone, the hormone dose used was 0.5ml/kg. The spawning/hybridization process can be seen in the following picture:



Figure 2. Hybridization (a) Stripping male tiger grouper parents (b) Selection of female tiger grouper parents (c) Anesthesia (c) Weighing (d) Administration of the artificial hormone ovaprim (e) Cannulation (checking egg quality) (f) Stripping (g) Fertilization (fertilization) (h) Spreading the eggs into the incubation tank (Source: Personal Documentation, 2024)

# **Egg Development**

Based on observations, the size of the embryo is around 846.14  $\mu$ m with a hatching time of 16-24 hours, egg development begins less than 1 hour after the egg is fertilized. The process of embryo development can be seen in the following picture:



Figure 3. Observation of the development of hybrid cantang grouper embryos
(a) Multicellular (2 hours) (b) Blastula (8 hours) (c) Gastrula (9 hours) (d) Formation of embryo shadow (10 hours) (e) Formation of kuppfer vesicle (12 hours)
(f) Embryo movement (14 hours) (Source: Personal Documentation, 2024)

# Egg Harvesting

Calculations were carried out using a special tool where each dose was assumed to hold

25,000 eggs in it. The eggs that have been counted are then put into a plastic pack and then sent to consumers with a water and oxygen ratio of 1:2 for short or long distance delivery using plastic packing measuring 100 cm  $\times$  20 cm with a density of 50,000 eggs per plastic bag.



Figure 4. Packing eggs (a) inserting the eggs into a plastic pack (b) adding oxygen (c) shipping the eggs (Source: Personal Documentation, 2024)

# **Larval Rearing**

# a. Preparation of Containers and Media

The maintenance container used is a concrete tank measuring 5 m  $\times$  2 m  $\times$  1.25 m with a rectangular shape, the edges and base are painted blue, apart from that, there are curves in each corner. Before the tub is used, it needs to be sterilized first. After cleaning the tank, the tank is then filled with sea water which has been deposited in the main reservoir and given 60% chlorine at a dose of 8 ppm with a water volume of 30 tons, left for 24 hours and then given 4 ppm of thio sulfate, left for 30-1 hour and then flowed into the larval rearing tank with the inlet section provided with a filter bag to re-filter the water before entering the rearing tank. This aims to reduce dirt entering the rearing tank.

# b. Egg Spreading

The stages of egg distribution that are carried out based on field observations are: eggs taken from the Situbondo BPBAP main installation using a small bucket containing 200,000 eggs, then spread into hatching tanks measuring 5 m  $\times$  2 m  $\times$  1.2 m with a water volume of 7.8 tonnes/ 7,800 L for tank 8 and tank 7 with a volume of 6,800 L Stocking was carried out in 2 hatching tanks with the same number of solid eggs, after which elbasin was added as much 0.5-1 ppm.

# c. Value Calculation Hatching Rate (HR)

Larvae aged D1 were then sampled to determine the hatchability of the eggs. Samples were sampled using a PVC pipe more than 1 meter high and a measuring cup with a volume of 1000 ml. where sampling was carried out at 5 different points in the tub, namely at the 4 corners and the middle. Then the volume of water lifted was recorded, the number of larvae obtained was calculated, the results of the calculation were then recorded to calculate the Hatching Rate (HR), then the HR values were 98.45% and 92.63%.

# d. Feeding Management

Based on field observations. Eggs that have hatched in the rearing medium will be given COD Liver Oil brand fish oil at a dose of 0.1 ml/m2 in a rearing tank measuring  $5 \text{ m} \times 2 \text{ m} \times 1.5 \text{ m}$ . This fish oil is given twice a day, namely at 06.00 WIB and 16.00 WIB until the larvae are 10 days old. In the early stages, the eggs that hatch still have egg yolk reserves. During this time, the cantang grouper larvae are not given additional external food, whether natural or artificial, the cantang grouper larvae begin to consume external food when they are D2. The feeding management can be seen in the following table:

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# Haini & Sumsanto (2024)

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Table 2	z. Feeding Manag	gement		
No		Feeding Management		
INO	Larvar Age	Type of Feed	Dose	Frequency/day
1.	D-0	Yolk egg	-	-
2.	D-1	Fish oil	0,1 ml/m <sup>2</sup>	1 ×
3.	D-2	Fish oil	0,1 ml/m <sup>2</sup>	2 ×
		Rotifera		1 ×
4.	D-3 s/d D-8	Fish oil	0,1 ml/m <sup>2</sup>	2 ×
		Rotifera		2 ×
		Liquid feed	5 ml	2 ×
		Powdered feed (D7)	< 5 gram/ giving	2 ×
5.	D-9 s/d D-20	Fish oil	0,1 ml/m <sup>2</sup>	2 × (D10)
		Rotifera		2 ×
		Powdered feed (D7)	5 gram/ giving	2 ×
		Naupli Artemia		1 ×
6.	D-21 s/d D-30	Rotifera		2 ×
		Artificial Feed	5-10 gram/giving	3 ×
		Naupli Artemia		2 ×
7.	D-31 s/d D-45	Rotifera		2 ×
		Naupli Artemia		2 ×
		Artificial Feed	5-10 gram/giving	3 ×
		Udang Rebon	secukupnya	2 ×
8.	D-46 s/d D-50	Udang Rebon	Secukupnya	2 ×
		Artificial Feed	5-15 gram/giving	4 ×
9.	D-51 – Panen	Artificial Feed	5-15gram or	4 ×
			enough/giving	

# Table 2. Feeding Management

#### e. Water Quality Management

Water quality management efforts carried out during field activities include administering Chlorella sp., changing water, checking water quality parameters and siphoning. Giving Chlorella sp. given at D2 with a density level of 6,680,000 cells/ml in the morning. Water changes are carried out from the age of D8 at 1 ton. The percentage of water changes during the maintenance period is presented in the following table:

Table 3. Percentage of larval rearing water changes
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Meanwhile, syphoning is carried out for the first time at the age of D10, then the

frequency is increased to once every 4 days until syphoning is carried out every day in the morning. Apart from that, water quality parameters in the form of pH, temperature, salinity, DO, Nitrite and Free Ammonia are checked once a week and the measurement results are as follows:

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No	Parameter	Measurement results	Quality standards	Reference
1.	Temp (°C)	30	28-31	SNI 80036.2 (2014)
2.	Salinity (ppt)	30.1	24-33	SNI 80036.2 (2014)
3.	рН	8.05	7,5-8,5	SNI 80036.2 (2014)
4.	Nitrit (mg/L)	0.498	< 0.5	Rejito, (2019)
5.	Ammonia (mg/L)	0.04675	< 1	Siegers et al., (2019
6.	Dissolved Oxygen	5.85	min 4	SNI 80036.2 (2014)

|--|

f. Sampling and Grading of Cantang Grouper Larvae

During the maintenance period, sampling was carried out every 7 days on Sundays at 16.00 WIB with the number of samples observed being 15 individuals. Sampling was carried out 9 times, namely from the 2nd to 10th week of maintenance. The results of sampling in the field are presented in the following image.



Figure 5. Growth Length (cm)

g. Harvest and Calculation of Survival Rate Values

Based on observations, the harvesting carried out is a partial harvest, namely harvesting in stages based on the size desired by the buyer, so during the maintenance period, when the seed size has reached the range of 2.7cm to 3cm, it can be harvested. The total number of harvests carried out until the seeds were harvested was 7 times with details of the number of seeds harvested as follows:

st	at of Cantang Hybrid Grouper Seeds			
	No	Date	Results (tails)	
	1.	27 March 2024	3.300	
	2.	2 April 2024	9.900	
	3.	6 April 2024	3.350	
	4.	13 April 2024	1550	
	5.	20 April 2024	2.310	
	6.	25 April 2024	1.752	
	7.	2 May 2024	763	

Table 6. Total Harvest of Cantang Hybrid Grouper Seeds

No	Date	Results (tails)	
Total disability		2000	
Total number		23.485	

Based on this data, the value of the survival rate (SR) of the cantang grouper seeds that are reared can be calculated. The survival rate (SR) value was 12.68%.

#### Chlorella sp. culture.

Preparation of a container in the form of a rectangular concrete tank with a volume of 12 tons. The culture tank is located outside, then 7.5 tons of water is filled which has been sterilized with 100 grams of chlorine, then 50 grams of thio sulfate is given. After 10 minutes, 2.5 tons of Chlorella sp Phytoplankton seeds are added to the density level. 2,277,500 cells/ml, and then apply fertilizer with respective doses, namely Urea 40 ppm, ZA 30 ppm, TSP 20 ppm, Fecl3 1 ppm, EDTA 1 ppm, harvest can be done at D5.

## **Rotifer Culture**

Mass culture of rotifers was carried out in rectangular concrete tanks measuring 2 m × 5 m × 1.25 m with a capacity of 10 tons. The culture tank is located outdoors. Then, after preparing the container, fill it with sea water and Chlorella sp. 5 tons, tons of sea water and 2 tons of Chlorella sp. then after that 20 liters of rotifer seeds were added with a density of 28 ind/ml. Then, to support the growth of rotifers, 250 g of bread yeast was given in the morning and in the afternoon Chlorella sp was added. as much as 1 ton as feed for rotifers. Harvesting can be done after the culture reaches D4 to a maximum of D7 with a density level of 119-180 ind/ml.

#### **Artemia Culture**

The artemia culture containers used are buckets with a capacity of 20 liters and barrels with a capacity of 130 liters. Before culturing the cyste artemia, decapsulation is carried out first. The culture tank is first filled with fresh water of 33 ppt salinity as much as 80% of the volume of the container used, 2 aerations have been added to the container, then the decapsulated cyste artemia that will be cultured is rinsed using fresh water for approximately 5 minutes until the chlorine smell disappears, then rinse with sea water, drain and put directly into a container filled with sea water for 24 hours. Harvesting is carried out using a 200 micrometer plankton net.

#### DISCUSSION

#### **Parent selection**

Before carrying out the spawning or hybridization process, the parents are checked or selected to be ready to be spawned first. The types of grouper parents used to produce cantang grouper larvae are the cantang grouper (*Ephinephelus lanceolatus*) as the male parent and the tiger grouper (*Ephinephelus fuscoguttatus*) as the female parent. Checking is done by observing the morphology of the two parents. Ismi (2017) explains that parents who are ready to spawn/hybridize are marked by an enlarged abdomen. Then, if the eggs are checked using the cannulation technique, the condition of the transparent eggs shows that the female is naturally ready to be spawned and the eggs are ready to be fertilized. If based on SNI 8036.2:2014 (mother of tiger grouper) the size of the parent of tiger grouper is >40kg and also

according to Sriyanti & Akhrianti (2021) states that the size of the parent of tiger grouper is 6-12 kg, therefore, the parent used is included in the category good because it meets the existing criteria.

## Spawning/Hybridization

Based on existing practice, spawning of cantang grouper is done artificially with human assistance. The male kertang grouper parent whose gonads have matured is first anesthetized then lifted onto the table, after the parent calms down the stripping process is carried out to remove the sperm. This technique is carried out by massaging the abdomen towards the genitals, then collecting the sperm that comes out with a corned tube and placing it in a special container filled with ice. Then the female tiger grouper parent was weighed to determine the dose of the ovaprim hormone. The hormone dose used was 0.5ml/kg. The hormone was administered by injection into the right side of the back. Before the injection is carried out, the parent tiger grouper is anesthetized first by placing the parent in a fiber tub container with a capacity of 500 liters which has been filled with sea water and 10 ml of ethylene glycol monophenyl ether, let it sit until the movements of the parent tiger grouper are not aggressive, then after that the hormone is injected into the parent grouper tiger. Ismi (2017) explained that to speed up the process of egg release, female tiger grouper parents are induced by injecting the hormone ovaprim at a dose of 0.5 ml/kg, then the injection is carried out at the bottom of the dorsal (dorsal) fin. According to Sinjal (2014) the ovaprim hormone is a mixture of salmon analog Gonadotrophin Releasing Hormone (sGnRH-a) and antidopamine. This hormone has the function of stimulating and triggering the formation of the gonadotropin hormone in the fish's body. This is what causes the ovulation and spawning process to accelerate.

After injecting the ovaprim hormone, the broodstock is then placed in a gonad maturation tank and left for 10 hours. Then the gonad maturity is checked by cannulation to ensure the quality of the eggs. Good quality eggs have a clear color, do not clump, float and are uniform in size. After that, the eggs are completely removed using a stripping technique, massaging the stomach towards the genitals, the eggs that come out are collected in a bucket and mixed with sperm at a ratio of 1 ml of sperm for 1,000,000 eggs produced, then stirred 60 times using a brush while adding sea water. slowly, let it sit for 10 minutes and then spread it into a container containing an egg collector. While in the incubation tank, to optimize fertilization and maintain egg quality, aeration and a flow trough water system are added. It is hoped that by changing the water during the holding period, the water quality can always be optimal.

## Egg Development

The size of the embryo observed was around 846.14  $\mu$ m with a hatching time of 16-24 hours, egg development began less than 1 hour after the egg was fertilized, then observations were made of the development of the egg until it hatched into a larva, based on the results of observations of embryo development going through several stages including the fertilization phase (one cell), two cells, four cells, many cells, morula, grastula, early embryo and late embryo. Egg hatching takes place approximately 24 hours after the fertilization process takes place. According to research results by Melianawati *et al.* (2011), the egg incubation period and egg hatching rate can take place at a temperature of 28-31 with an incubation period of 16 – 23 hours. The more the temperature increases, the incubation period will also be faster until 100% hatching can be achieved, then egg development based on the results of observations begins. less than 1 hour after the egg is fertilized. Hasibuan *et al.* (2018), explains the characteristics of fertilized eggs which are characterized by a transparent white color, float

or float in the water, are round and the cell nucleus is in the middle, while unfertilized eggs are milky white and settle at the bottom.

## **Larval Rearing**

a. Preparation of Containers and Media

The maintenance container used is a concrete tank measuring 5 m  $\times$  2 m  $\times$  1.25 m with a rectangular shape, the edges and base are painted blue, apart from that, there are curves in each corner. Before the tub is used, it is necessary to sterilize it first. Prayogo & Isfanji (2014) explain that before using the tub and equipment it must be sterilized first. By rinsing the walls and bottom of the tub, the water hose, aeration stone, and the outlet filter pipe, then add a little soap and brush to remove any moss attached, after that the tub is rinsed until clean, then add chlorine by pouring it on and letting it sit for 1 day. Then after that, rinse it using fresh water until the chlorine smell disappears and dry it. After cleaning the tank, the tank is then filled with sea water which has been deposited in the main reservoir and given 60% chlorine at a dose of 8 ppm with a water volume of 30 tons, left for 24 hours and then given 4 ppm of thio sulfate, left for 30-1 hour and then flowed into the larval rearing tank with the inlet section provided with a filter bag to re-filter the water before entering the rearing tank. This aims to reduce dirt entering the rearing tank.

b. Egg Spreading

The stages of egg distribution that are carried out based on field observations are: eggs taken from the Situbondo BPBAP main installation using a small bucket containing 200,000 eggs, then spread into hatching tanks measuring  $5 \text{ m} \times 2 \text{ m} \times 1.2 \text{ m}$  with a water volume of 7.8 tonnes/ 7,800 L for tank 8 and tank 7 with a volume of 6,800 L Stocking was carried out in 2 hatching tanks with the same number of solid eggs, after which elbasin was added as much 0.5-1 ppm diluted with water. Putri *et al.* (2024), explained that egg spreading is done by dipping a bucket and then tilting the bucket so that the eggs come out slowly by themselves. This can be done because the distance between the eggs and the stocking container is not far, but if it is far away then it is necessary to pack them first.

c. Calculation of Hatching Rate (HR) Value

Larvae aged D1 were then sampled to determine the hatchability of the eggs. Samples were sampled using a PVC pipe more than 1 meter high and a 1000 ml measuring cup. where sampling was carried out at 5 different points in the tub, namely at the corners and the middle. The pipe is inserted into the maintenance water, the top end is closed by hand then lifted slowly and placed in a measuring cup. Then the volume of water lifted was recorded, the number of larvae obtained was calculated, the results of the calculation were then recorded to calculate the Hatching Rate (HR), then the HR values were 98.45% and 92.63%. This is in line with the opinion of Adi *et al.* (2023), after the eggs hatch, a Hatching Rate calculation is carried out to determine the value of the number of cantang grouper eggs that hatch with a good Hatching Rate calculation based on SNI 8036.2:2014 that the egg hatching rate or Hatching Rate of cantang grouper is more than 70%.

d. Feeding Management

Based on field observations. Eggs that have hatched in the rearing medium will be given COD Liver Oil brand fish oil at a dose of  $0.1 \text{ ml/m}^2$  in a rearing tank measuring  $5 \text{ m} \times 2 \text{ m} \times 1.5$  m. This fish oil is given twice a day, namely at 06.00 WIB and 16.00 WIB until the larvae are 10 days old. Prayogo & Isfanji (2014) Newly hatched cantang grouper larvae have a transparent body shape and are accompanied by egg yolk and oil bubbles. At the larval age D1-D8 the rearing medium is dripped with fish oil, a dose of  $0.1 \text{ ml/m}^2$  of fish oil is given by dripping fish oil at each aeration. The aim of giving fish oil is to prevent larvae from floating on the surface

of the water and sticking to the walls of the tank. Apart from that, giving fish oil can be a supplier of vitamin A, which is via rotifers. Adi *et al.* (2023), also explained that administering fish oil can reduce the tension on the water surface so that when larvae rise to the surface they will not have difficulty swimming back into the water. For the frequency of administration of fish oil according to Putri *et al.* (2024), fish oil is given twice, namely in the morning and evening.

In the early stages, the eggs that hatch still have egg yolk reserves. During this time, the cantang grouper larvae are not given additional external food, whether natural or artificial, the cantang grouper larvae begin to consume external food when they are D2. The types of feed given during the larval rearing period starting from D2 - harvest are natural feed for rotifers, rebon shrimp and brine shrimp, while artificial feed is LHF-1 (liquid feed), B.P Egochi (powder), Ottohime A, B C, S (feed powder, crumble, pellet). Putri et al. (2024), also explained that if you are guided by Good Fish Hatchery Methods (CPIB), the types of feed given during the rearing period are liquid feed, rebon shrimp, natural feed (Rotifera sp., Artemia sp., Chlorella sp.), and artificial feed. Larvae aged D2 or D3 to D30 are given natural food in the form of Rotifera zooplankton with a density of 10-20 ind/ml. Natural food is given twice, namely at 09.00 WIB and 15.00 WIB. 1 hour before being given to the larvae, the rotifers were enriched first by giving 5 ml of Scott's. according to Putri et al. (2024), one hour before giving the rotifers, enrichment is carried out first by giving 5 ml of Scott's fish oil then mixing approximately 600 ml of fresh water in a mineral water bottle then shaking until there are no lumps then pouring it into the rotifers, the purpose of giving Scott's is to increase the fat content and fatty acids EPA and DHA. At the age of D3 to D8, LHF-1 artificial feed is given in the form of liquid feed with a dose of 0.5 ppm/ 5 ml, the frequency of feeding is 2 times at 06.00 WIB and 14.00 WIB, before giving this feed it is best to dilute it first using fresh water.

When the larvae are aged D13 to D45 with a dose of 1-3 naupli/ml, the frequency of administration is 1 time at the age of D13-D20 then added to 2 times at the age of D21-D45, namely at 08.00 WIB – 14.00 WIB the density of artemia is increasingly increased along with increasing age of cantang grouper larvae. Then, at the age of D7, artificial feed is started to be given with a frequency of giving 1 time, then at the age of D11-D15 2 times, and 3 times at the age of D20, it is given until harvest. The only thing that will differ is the size of the feed given, the bigger the fish being kept, the bigger the fish. The feed given will increase in quantity and size, this is in line with the opinion of Fitriadi *et al.* (2020), which explains that the feed provided will continue to increase, this is in line with the larvae's mouth openings and the increasing level of feed requirements. The types of artificial feed used are BP Eghouci and ottohime sizes A, B1, B2, C1, S2, in powder, crumble and pellet form with a protein content of 48% - 50%. Giving is done by spreading it directly on the surface of the aeration in the rearing tank. The feeding dose is 5 grams from D7-D20, 8 grams starting from D21-D30, then continue to give it until harvest, 5-15 grams at a time.

#### e. Water Quality Management

Water quality management efforts carried out during field activities include administering Chlorella sp., changing water, checking water quality parameters and siphoning. Giving Chlorella sp. given at D2 with a density level of 6,680,000 cells/ml in the morning at 07.00 WIB Chlorella sp. This itself is actually not food for larvae but rather natural food for rotifer zooplankton. Putri *et al.* (2024), explains the purpose of administering *Chlorella* sp. is to maintain water quality balance, reduce light penetration and as food for rotifers. Apart from that, giving *Chlorella* sp can act as a green water system to increase the amount of dissolved oxygen content due to the photosynthesis process, absorbing ammonia and as a buffer for

water quality such as pH and CO2. Then water changes were carried out starting at the age of D8, amounting to 1 ton, which was then increased in proportion as the age of the larvae increased. Adi *et al.* (2023), the percentage/amount of water volume replaced is adjusted to the age of the fish, this is because so that they do not get stressed easily and get disease because the fish's immune system is still vulnerable, so it is necessary to gradually increase the water reduction when the water is changed. Water changes are carried out starting from 06.30-09.00 WIB by opening half the tap on the outlet channel so that the water can come out, the amount of volume released is adjusted to the age of the larvae.

Meanwhile, syphoning is carried out for the first time at the age of D10, then the frequency is increased to once every 4 days until syphoning is carried out every day in the morning. This is done to remove or remove uneaten food residue and metabolic waste/larval waste in the form of feces which settles at the bottom of the rearing tank, which as explained by Adi et al. (2023), siphoning is carried out using tools in the form of hoses and pipes as supports, then before siphoning is carried out, the aeration in the maintenance tank is turned off first, so that the dirt in the tub settles to the bottom of the water, then is sucked out. The function of this siponan is to remove leftover food that has not been eaten and settled at the bottom of the tank, so that the water quality during the rearing period can support the survival of the fish. Then another treatment carried out is circulation, namely by partially opening the outlet and then filling it with filtered water using a filter bag. In this process, the water that comes out has a larger discharge compared to the water that comes in or can be equalized. Putri et al. (2024), explains that changing water using a circulation system is carried out by flowing water from an inlet channel that has been installed with a filter bag, where the purpose of installing this filter bag is to filter dirt from the reservoir water so that it does not enter the larval rearing tank which can affect water quality.

Apart from that, water quality parameters in the form of pH, temperature, salinity, DO, Nitrite and Free Ammonia are checked once a week, so that the water quality of the maintenance media is always in optimum condition. The range of water quality parameters during the maintenance period is temperature 30oC, salinity 30.1 ppt, pH 8.05, Nitrite 0.71867 mg/L, free ammonia 0.04675 mg/L, and Dissolved Oxygen (DO) 5.85 mg/L. If seen from the results obtained, the range of water quality parameters during the rearing period is classified as good because it still complies with the water quality requirements for cantang grouper rearing based on (SNI 8036.2, 2014). As explained by Putra et al. (2020), water quality parameters are one of the important things to pay attention to, salinity that is not optimal can cause fish to become stressed and can inhibit fish growth because it is related to the fish's osmotic adjustment activity, then temperature parameters have an influence on the fish's metabolic system. acidity or what is known as pH (Negative H Pulscane) which means the logarithm of the concentration of H (Hydrogen) ions released in a liquid, a high pH number will result in an increase in ammonia concentration, an increase in Ammonia concentrations will make the environment toxic for fish. After that there is a DO parameter, if the DO in the water is less than 3 ppm it will cause the fish to have difficulty breathing. Then according to Siegers et al. (2019), explains that a good ammonia level is less than 1 ppm, an ammonia content of more than 1 ppm can be dangerous for fish and other farmed organisms, because ammonia in non-ionized form is toxic to fish, although quite a few fish will can adapt to ammonia conditions, but the concern is that sudden changes will occur which will cause damage to the gill tissue. Dauhan et al. (2014), explained that ammonia in the water is usually due to fish metabolic waste dissolved in the water, fish feces and food waste that settles at the bottom of the cultivation pond. Then Rejito (2019) explained that the nitrate content was

caused by leftover feed, whether natural or artificial, and other things that could be a source of protein. The nitrate content in the water should not be more than 0.5 ppm because it can cause death due to the formation of (Mathaemoglobin). The high nitrite content can also cause anaerobic water conditions, this is because the higher the amount of nitrite, the greater the oxygen consumption in the water which is used in the process of oxidizing ammonia gas into nitrate, so to prevent this, add aeration as an oxygen supplier and as a water stirrer. larval rearing media, apart from that accelerate the evaporation of toxic gases.

f. Sampling and Grading of Cantang Grouper Larvae

During the maintenance period, sampling was carried out every 7 days on Sundays at 16.00 WIB with the number of samples observed being 15 individuals. It can be seen that there is an increase in the body length of cantang grouper fry every week with an average increase of 0.1-0.8 cm. Rahmaningsih & Ari (2013) also explained that to determine growth and survival, sampling is carried out every 7 days or once a week. Observed data can include growth in length, width (height), and body weight. In addition, grading is carried out to standardize the size and quality of the seeds. Through this process, growth can be determined by measuring body size. The aim of grading is to reduce cannibalism in cantang grouper larvae. Prayogo & Isfanji (2014) explained that cantang grouper larvae aged D-30 until harvest or adjusted to the size of the fish larvae will usually be graded, where the grading process is carried out by silting first then reducing the water to 70%-80% then the larvae are picked up in a basket and placed in a basin, and the size and defects are selected. Larvae that have a uniform size and are not deformed will be returned to the larval rearing tank

g. Harvest and Calculation of Survival Rate values

Based on observations, the harvesting carried out is a partial harvest, namely harvesting in stages based on the size desired by the buyer, so during the maintenance period, when the seed size has reached the range of 2.7cm to 3cm, it can be harvested. The total number of seeds harvested was 23,485 with a survival rate of 12.68%. Based on the 2014 Indonesian National Standard (SNI), the survival rate for hybrid cantang grouper seeds reared from egg to 40 days old is more than 10%.

h. Chlorella sp Culture

Preparation of a container in the form of a rectangular concrete tank with a volume of 12 tons. The culture tank is located outside. Before culturing is carried out, the tank is cleaned first. Alyaniazy *et al.* (2023), explains that cleaning the tub is carried out with the aim of preventing the culture tub from being contaminated by other organisms and then removing dirt that sticks to the tub. then filled with 7.5 tons of water which had been sterilized with 100 grams of chlorine then given 50 grams of thio sulfate. After 10 minutes, *Chlorella* sp phytoplankton seeds were added. as much as 2.5 tons with a density level of 2,277,500 cells/ml, and then applying fertilizer with respective doses, namely Urea 40 ppm, ZA 30 ppm, TSP 20 ppm, Fecl3 1 ppm, EDTA 1 ppm, harvest can be done on D5.

i. Rotifer Culture

Mass culture of rotifers was carried out in rectangular concrete tanks measuring 2 m × 5 m × 1.25 m with a capacity of 10 tons. The culture tank is located outdoors. Then, after preparing the container, fill it with sea water and *Chlorella* sp. 5 tons, tons of sea water and 2 tons of *Chlorella* sp. then after that 20 liters of rotifer seeds were added with a density of 28 ind/ml. Then, to support the growth of rotifers, 250 g of bread yeast was given in the morning and in the afternoon *Chlorella* sp was added. as much as 1 ton. Prayogo & Arifin (2015) explaining the purpose of administering *Chlorella* sp. This is as food for *Rotifera* sp. and 11/2 – 2 tons are given which can be marked by a color change from brown to green. Harvesting

can be done after the culture reaches D4 to a maximum of D7 with a density level of 119-180 ind/ml.

j. Artemia Culture

The artemia culture containers used are buckets with a capacity of 20 liters and barrels with a capacity of 130 liters. Before culturing the cyste artemia, decapsulation is carried out first. The culture tank is first filled with fresh water of 33 ppt salinity as much as 80% of the volume of the container used, 2 aerations have been added to the container, then the decapsulated cyste artemia that will be cultured is rinsed using fresh water for approximately 5 minutes until the chlorine smell disappears, then rinse with sea water, drain and put directly into a container filled with sea water for 24 hours. Harvesting is carried out using a 200 micro meter plankton net. Adi *et al.* (2023), explains that harvesting is done by turning off the aeration first to separate the artemia that hatch from those that don't. This can be seen from the presence of sediment at the bottom of the culture container while the ones that hatch are at the top or in the water pool. then poured and filtered using a plankton net, the filtered results are rinsed first using sea water then collected into a bucket with a capacity of 20 L and given aeration before being given to the cantang grouper larvae.

#### CONCLUSION

The cantang hybrid grouper hatchery carried out at BPBAP Situbondo is an artificial hatchery that has several stages, including rearing of broodstock. Then parent selection is carried out through observing morphology and behavior, parents are also selected based on the SNI that has been determined, after that they enter the hybridization stage. Hatching Rate (HR) values are 92.63% and 98.45%, then larval rearing is carried out until the 3cm seeds are aged D45 to D70, rearing takes place from 22 February 2024 to 2 May 2024 with a Survival Rate (SR) value of 12.68%, for Larval rearing activities provide continuous natural food both in terms of quality and quantity, then culturing natural food is carried out and always carries out check the density.

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