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Manipulation of the aquatic environment to optimize the natural spawning of sunu grouper (*Plectropomus leopardus*) in controlled tanks

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ABSTRACT

Aquaculture within culture techniques is needed to support the optimization of a number of juveniles which sunu grouper natural spawning techniques. To optimize the natural spawning of Sunu Grouper (*Plectropomus leopardus*) its is needed to know in details about the environment condition. Random design method by using several treatments consisted of one factor, temperature, and consisted of four treatments which are 26, 28, 30, and 32°C which were repeated five times. Five steps were conducting start with the preparation, acclimatization of the eggs, stocking, and hatching of the eggs, and larval maintenance. Good quality feed is required to support gonad development and egg quality during brood stock-rearing. Environmental management of culture within culture techniques is needed to support the optimization of the number of juveniles. Temperature changes made by raising and lowering the temperature are a form of stimulation that trigger spawning to occur naturally.

Introduction

Sunu grouper (*Plectropomus leopardus)* is one of Indonesia's leading export commodities from marine aquaculture and a favorite commodity worldwide (Sidik, 2002; Nurjana, 2010). The majority supply of Sunu grouper comes from natural catches. However, this is not recommended because it has several risks, including species unsustainability, non-uniform sizes, and the possibility of defects due to fishing activities. This activity can affect a reduction in the population of the Sunu grouper commodity, and it is feared that it will cause extinction. Seeing the huge market potential for sunu grouper, especially frozen, which reaches 63.89% (BPS, 2022), it is essential to cultivate it from hatchery to grow-out in order to meet market needs and at the same time to preserve its population (Prasetya, 2010).

When compared to the price of sunu grouper in several areas, such as in North Sulawesi, it ranges from IDR 450,000 to 600,000/kg with a size of 0.6-1.0 kg, while in Jakarta it ranges from IDR 750,000 to 1,000,000/kg. Interestingly, the price of this fish tends to rise during Chinese holidays (Hendratno *et al*., 2017) and in developed countries such as China, Hong Kong, and Singapore trade Sunu grouper at prices reaching IDR 2,608,362.50/kg (Waycott, 2016).

Since the aquaculture process is conducted in marine aquaculture, it is important to choose the right location (Wiyoto and Effendi, 2021; Rusydi *et al*., 2024) to obtain optimal cultivation potential. Bali waters, which have a strategic location, is the right choice to conduct studies because the company is still productive, performs continuous cultivation activities, and applies adequate and up-to-date cultivation technology.

Besides feeding management of Sunu grouper is also a challenge to produce optimal and sustainable fish farming (Hasan and New, 2013; Goddard, 1996), water maintenance is also one of the keys to getting fish of the appropriate weight for the time of cultivation. Those who conduct the spawning process naturally, of course, need a number of things that can be optimized with existing environmental

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conditions. Thus, the environmental manipulation process performed can support the natural spawning process with existing cultivated fish, as if they were in their natural habitat.

Materials and Methods

Location and time of research

The study locations are in Bali waters, which is a research institute under the Ministry of Maritime Affairs and Fisheries. The purpose of this center is to research hatchery and rearing of marine aquaculture commodities, like sunu grouper, crustaceans, and shellfish.

Geographically, this research center is located at 114º-115º East Longitude and 07º-08º South Latitude, with a height (topography) of ± 2 m above sea level (Figure 1).

Figure 1. Maps showing the location (red square) of Bali waters, the site of this study.

Data analysis

Grouper eggs were obtained from the Hatchery Marine Aquaculture Development Center Bali. The research tools included an aquarium that was 60 cm x 30 cm x 30 cm in size, an aerator, and an aeration pipe. The method used in implementing this study was an experiment using a completely random design (RAL). The treatment consisted of one factor, temperature, and consisted of four treatments (26, 28, 30, 32℃) which were repeated five times. The research activities begin with preparation, acclimatization of the eggs, stocking, and hatching of the eggs, and larval maintenance.

Results

Supporting facilities that are crucial to enhance success in aquaculture operations, including expansion efforts, have a significant impact on the current research site. Aquaculture containers in sunu grouper grow-out have supported facilities such as *semi-outdoor* buildings, *treatment tanks*, round fiber tanks, *cold storage,* and some grow-out activity equipment. Some details of the grow-out support facilities can be seen in (Table 1) below.

Table 1. Details of supporting facilities in the rearing of *Plectropomus leopardus* sunu groupers.

		Type	Specifications	Quantity (unit)
1.		Production support building		
	a.	Semi-outdoor building	14 m x 10 m	1
	b.	*Cold storage*	535 watt, 220 v, 50 Hz	1
2.		Equipment		
	a.	Fiber container tub	D 0,8 m x 1,2 m high	1
	b.	Fiber *treatment* tub	1 m x 0,6 m	1
	c.	Basin	30 cm x 30 cm	4
	d.	Fruit Scales	10 kg	1
	e.	Scissors		8
	f.	Tub	D 30 cm	4
	g.	Bucket	5 L	3
	h.	Stairs	1,5 m high	1
	i.	Pipe ruler	40 cm	1
	j.	Plastic basket		4

Good quality feed is required to support gonad development and egg quality during brood stock-rearing. The feed given in brood stock-rearing is fresh fish such as swallow fish (Figure 2.a) and fresh squid (Figure 2.b). *Cold storage* is used as feed storage to maintain feed quality with *a cold storage* temperature of -4 °C. The feeding method given in brood stock rearing is *at satiation* or given as fully as possible with a ratio of fresh fish and squid as much as 2:1.

The treatment before the feed is given is that the fly fish feed is first cut into about 3 to 4 parts according to the mouth opening of the fish to be given (Figure 2). The squid is first cleaned by washing and removing the bones. The next activity is that the feed is added with multivitamins and medicine manually to improve the quality of brood stock eggs and increase the immune system of the brood stock. The multivitamins mixed into the feed were vitamin C (4 g/kg feed) and vitamin E (2 g/kg feed), while the medicine was *oxytetracycline* (OTC) (4 g/kg feed). Feeding was done once a day at around 09:00-10:00 WITA-Central Indonesian Time (zone), with the water level lowered by 50% as an environmental manipulation. Fish response to feed was observed during feeding to determine fish health. The broodstock fish used were from nature (F0) 48 individuals and the broodstock to the 3rd generation (F3) 36 individuals, the ratio of the number of males and females was 1:2.

Figure 2. Type of brood stock feed

Brood stock Feeding		
a	Soaking of flyfish feed	
b	Cleaned squid	
c	Chopped feed	
d	Addition of vitamins and oxytetracycline to the feed	

Water Quality Management

Water changes in the broodstock tanks are conducted as much as 200% to 300% continuously (*flow-through*) every day following the statement of Sugama *et al.* (2001) that the broodstock tanks must be maintained with a running water system with a water change rate of at least 200% to 300% per day. Water quality measurements such as temperature, pH, and salinity are conducted weekly because of stable water conditions, so there is no need to measure water once a day (Table 2).

Table 2. Water quality data on brood stock-rearing containers.

Parameters	Unit	Value	Centre Standard
Temperature	°C	27–32	27–32
pH	–	7–8	7–8
Salinity	g/L	30–32	30–34

Pest and Disease Prevention

The prevention of pests and diseases in sunu grouper fish is performed at least once every 10 days. Parasites that are found to attack the mother are usually *Benedenia* sp. (Figure 3.a) and Hirudinea *Episcopal* sp. (Figure 3.b), these parasites attack the outer body of the fish or are commonly referred to as ectoparasites. *Benedenia* sp. parasites that attack sunu grouper cause reduced appetite, weak swimming, and at severe levels can cause skin wounds and open up opportunities for secondary infection by bacteria (Zafran, 2009). The leech Hirudinea *piscicola* sp. can cause reddish wounds in the oral cavity (*hemorrhage*), this parasite attaches using its suction and then sucks the host's blood as nutrition to cause wounds on the skin (Figure 3. point c), which can provide opportunities for bacteria as secondary infections (Ansari *et al.* 2018).

Prevention was conducted by immersing the fish in fresh water and rubbing it using gloves with a rubber coating for approximately one to five minutes to release parasites attached to the fish (Figure 3). If during the *treatment* process a fish is found to be injured on the body of the broodstock, then an application is made on the wound using elbayou 3 g (Cahyanurani *et al.*, 2022).

Figure 3. Pest and Disease Prevention.

a	*Benedenia* sp	
b	The Leech Hirudinea *Piscicola* sp.,	
c	Wounds caused by the leech Hirudinea *Piscicola* sp.	

Brood stock Spawning

Preparation of Spawning Containers

The preparation of the sunu grouper broodstock spawning container is the same as the preparation of the broodstock rearing container. The *egg collector* preparation is carried out every afternoon by installing cleaned soft nets into the container. *The egg collector* collects eggs from the spawning broodstock.

Egg Harvesting

Fertilized eggs during the spawning process will float to the surface of the water so that they will flow with the current to the *egg collector*. Eggs that have been collected in the *egg collector are* checked at 07.00

WITA and harvested at 08.00 WITA slowly because the eggs are in the developmental period (undergoing cell division). Harvesting was conducted using a *scoop net*, and the eggs were transferred to a 100 L incubation tank equipped with 1point aeration for selection and egg counting. The separation of fertilized and unfertilized eggs by rotating the water in the incubation container so that there is precipitation on unfertilized eggs. Fertilized eggs will float on the surface of the water and are clear with a round shape, whereas unfertilized eggs will sink and are white with a damaged shape. Unfertilized eggs will be collected at the bottom of the incubation basin. The unfertilized eggs were removed by siphoning to count the fertilized eggs.

The number of fertilized and unfertilized eggs is calculated by counting the total number of eggs and then counting the fertilized eggs. The number of unfertilized eggs is obtained by subtracting the total number of eggs from the number of fertilized eggs. Numbers of eggs is calculation by taking samples using a 20 mL *beaker glass* with several repetitions and then pouring them into a Petri dish little by little to facilitate egg counting (Figure 4). The more repeats made, the more accurate the number of eggs counted will be.

Figure 4. Stage of egg harvesting.

a	Egg harvesting using a *scoop net*	
b	Egg incubation container	
c	Unfertilized eggs collect at the bottom of the container	
d	Egg count calculation	

Eggs Hatching

Hatching sunu grouper eggs at BBRBLPP is carried out by transferring eggs from the egg incubation container to the egg hatching tub. Eggs that have been incubated are taken using a *scoop net* and then transferred to a plastic *packing* container or bucket filled with water, which is then transferred to the egg hatching tub. The number of eggs produced and the number of eggs hatched in January, February, and March are presented in Table 3.

Table 3. Number of eggs produced to the number of eggs hatched in the sunu grouper *Plectropomus leopardus* hatchery activity.

Month	The number of eggs produced	FR (%)	The number of eggs fertilized	HR (%)	The number of eggs hatched
January	4.545.000	80	3.636.000	78	2.836.080
February	3.160.000	76	2.401.600	80	1.921.280
March	5.435.000	80	4.348.000	79	3.434.920

Egg stocking is accomplished by gently pouring the eggs into the prepared hatching tanks with 70 cm of water and good aeration. Sunu grouper eggs hatch 17 to 18 hours after spawning. Eggs are stocked at a density of 20 grains/L.

Larval Rearing

Sunu grouper larvae are reared after the egg hatch. The container used for larval rearing is the same as the egg-hatching container. The newly hatched larvae are given fish oil to water's surface as much as 4 grains per tub with stocking density of 25 fish/m^3 until it is even covering the surface. Newly hatched larvae are given fish oil to the surface of the water as many as 4 grains per tub until it is evenly covering the surface of the water, this activity will continue until the larvae are five days old (D5). The fish oil used was Tung-Hai branded fish oil in capsule form. The application of fish oil aims to cover the surface layer of water and reduce water pressure so that larvae that are not yet actively moving are not trapped at the surface of the water. The lights in the larval-rearing containers were turned on for 18 hours and turned off at 21:00 WITA.

Growth and Population Sampling

Sampling of larval growth was conducted by taking three larval samples. Samples of larvae that were taken were measured in length under a microscope. Sampling of larval growth was done every five days until the larvae were 25 days old (D25). Larvae aged one day (D1) have an average length of 2.14 mm, and larvae aged 25 days (D25) have an average length of 10.39 mm. The sampling data of the sunu grouper larvae at the BBRBLPP Gondol are shown in Table 5.

Tabel 5. Sampling data of the sunu grouper *Plectropomus leopardus* larvae.

No.	Larval Age	Average Length (mm)
1	D1	2,14
2	D5	2,52
3	D10	3,84
4	D15	6,30
5	D20	7,44
6	D25	10,39

Table 5 shows that on the first day after egg hatching, there was an average length of 2.14 mm/head. On day 5 of the larval-rearing period, the average length was 2.52 mm on the 10th day of the larval-rearing period, the average length was 3.84 mm. On the 15th day of the larval-rearing period, the average length was 6.30 mm. On the 20th day of the larval-rearing period, the average length was 7.44 mm. On the 25th day of the larval-rearing period, the average length was 10.39 mm.

Water Quality Management

Water quality management carried out in larval-rearing containers involves measuring water quality, changing water, and cleaning the bottom of the tub by siphoning. Measurement of water quality aims to determine the feasibility of water. Water quality data on sunu grouper larvae rearing activities are shown in Table 6.

Table 6. Water quality data of *Plectropomus* leopardus grouper larvae rearing.

Parameters	Unit	Value	Standard
Temperature	ºC	28–29	28–30
pH	–	7,5–8	7–8,5
Salinity	g/L	31–32	31–34
DO	mg/L	4,5	4,5–6

Water changes of 5% to 10% were performed once a day for 10-day-old larvae (D10) to 11-day-old larvae (D11). The 12-day-old larvae rearing tanks (D12) were siphoned at the bottom of the tanks (Figure 30a) by ¼ tub and water changed (Figure 30b) by 10% to 15% once a day until the larvae were 15 days old (D15). Gradual water changes were carried out at 25% gradual to 100% in the larval rearing containers when the larvae were 16 days old (D15) until the larvae were 40 days old (D40).

The observation of growth in seed rearing is performed to determine the success of seed-rearing activities. Observing seed growth is carried out every five days, starting from 35-day-old seeds (D35) to 45-day-old seeds (D45). Growth observations were made by measuring the length and weight of the seeds. The graph of seed growth is shown in Figure 5.

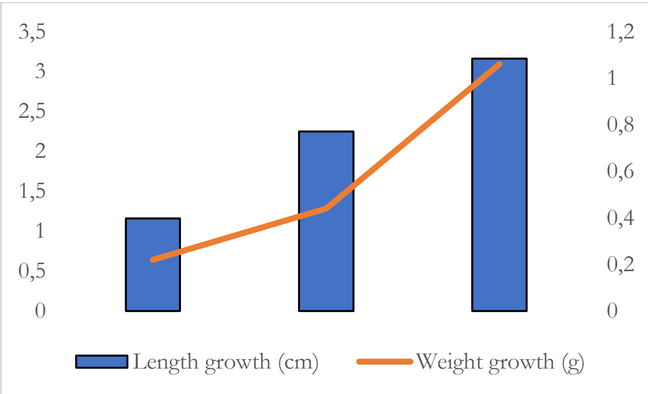

Figure 5. Growth chart of sunu grouper.

Figure 5 shows that, on the 35th day after egg hatching, the average length of seeds was 1.16 cm and the average weight was 0.22 g. On the 40th day of the seed-rearing period, the average length was 2.25 cm, with an average weight of 0.44 g. On the 50th day of the seed-rearing period, the average length was 3.16 cm with an average weight of 1.06 g.

Sunu grouper seed harvesting is conducted on 50-day-old seeds (D50) with a 3 to 3.5 cm size. Seed harvesting begins by lowering the water in the container by 90%; then, the seeds are removed and transferred to the *grading* basin to calculate the number of seeds. The *packing* process is carried out by preparing plastic *packing*, measuring 75 cm x 50 cm, which is doubled. The ratio between water and oxygen in the packing is 1:3. Water was added as much as 10 L, then activated carbon was added as much as 15 g/10 L of water. The packing density of the sunu grouper seeds is 25 fish/L. Sunu grouper seeds are transported in a closed system.

Discussion

The results of the studies show that Sunu Grouper aquaculture activities are largely determined by good water quality (Yanuhar, 2009). In addition to water quality, there are several other influential factors, namely feeding management (Fitriadi *et al.*, 2020; Efendi *et al.,* 2021; Tiikwinas, 1989), light intensity (Rachmawati *et al.,* 2016), the addition of enzymes to feed to grow biomass production levels (Rachmawati, 2012), hormone combinations (Palma, 2018), and activities around the location (Kusumanti, 2021). Water management in seed rearing is carried out by siphoning the sunu grouper seed rearing tanks once daily in the morning. This activity is carried out by changing the water continuously by 100%. In addition, this water change also aims to maintain an optimum temperature (Lutfiyah, 2019).

Sunu grouper spawning at the BBRBLPP Gondol occurs naturally with environmental manipulation techniques. This technique involves raising and lowering the water level. The water is lowered by approximately 50% to 60%, then the water is raised back to its original level with continuous water circulation every day. This technique aims to raise and lower the temperature to increase the stimulus for spawning.

Broodstock spawning occurs during the night, from 23:00 to 3:00 WITA. Group spawning is carried out with a male-to-female ratio of 1:2. Sunu grouper spawning occurs during the dark moon phase (lunar cycle). The dark moon phase occurs at the beginning of the new moon, which is approximately 14 days in duration, which is around seven days before and seven days after the turn of the moon. The eggs that will be produced may be affected by the type of broodstock, water quality, season, and type of feed. Eggs produced during spawning will be carried away by the current and collected in an *egg collector* connected to the brood stock-rearing basin.

Dadiono *et al.* (2022) explained that handling hybrid grouper eggs is very different from handling grouper eggs that are spawned naturally; in general, grouper eggs from natural spawning will be released into the grouper brooder in the broodstock pond and then will be accommodated in the egg collector attached to the reservoir that is found on the sides of the broodstock pond as it approaches the dark moon. Freshwater supply is needed and must be sufficient to support operations and treatment of fish diseases (Asia-Pacific Economic Cooperation, 2001).

Garcia (2015) results inform that sunu grouper larvae after 10 days of age up to 25 days seems to experience quite rapid growth, this is because the larvae have started to eat additional feed given in the form of commercial feed. Several size variations larvae, so the larvae are weak and small will be left with healthy larvae. Likewise healthy larvae activity eat high will continue to grow and survive, while the larvae are small will lag behind its growth and not survive.

Studies also show that increased water temperature contributes to a decrease pH value (Faudzi *et al.*, 2021; Xavier *et al.*, 2018). This is because an increase in water temperature generally increases the respiration rate, leading to carbon dioxide excretion in the water. The metabolic waste from cultured grouper depends on the current in the culture area (Yashiro, 2000). If the current is weak, metabolic waste accumulates at the bottom of the net, and the water quality inside the net is worse than outside.

Conclusion

The result of this study shows that environmental management of culture within culture techniques is needed to support the optimization of the number of juveniles. Temperature changes made by raising and lowering the temperature are a form of stimulation that trigger spawning to occur naturally.

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