The effect of salinity on the growth, survival and profitability of TGGG hybrid grouper culture (♀ tiger grouper × ♂ giant grouper)

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Abstract. In Indonesia, the cultivation of 'cantang' hybrid grouper is being developed recently by cross-breeding the tiger grouper or *Epinephelus fuscoguttatus* (♀) and the giant grouper or *Epinephelus lanceolatus* (♂), known as TGGG hybrid grouper. Fish farmers are interested in the hybrid grouper cultivation for a better growth and resistance to disease. At the present, TGGG hybrid grouper cultivation has been carried out by fish farmers in coastal areas, using floating net cages and brackish water ponds. It is necessary to conduct more research on the cultivation of this grouper at low salinity which would allow fish farmers living away from the coast to cultivate it. This research examined the effect of salinity on growth, survival and profitability of TGGG hybrid grouper cultivation and it was conducted for 30 days using fish fry with an average weight of 3.16 g (±0.03 g). Three treatments and three replications were performed: treatment A using original seawater (salinity of 34 ppt), treatment B (salinity of 20 ppt) and treatment C (salinity of 10 ppt). The measured variables were biomass growth rate (WGR), specific growth rate (SGR), survival rate (SR), and cost benefit ratio (BCR). The results showed that salinity treatment did not significantly affect the WGR, SGR, SR and BCR. Treatment C showed the best WGR, SGR and BCR results. The optimal salinity for maximizing WGR, SGR and BCR was 5.0 ppt, 5.6 ppt and 10.1 ppt, re spectively. The TGGG hybrid grouper could be cultivated in water with low salinity, At a salinity 10 ppt, even better results were obtained compared tothe control group (34 ppt).
Key Words: BCR, *Epinephelus fuscoguttatus*, *Epinephelus lanceolatus*, salinity, SR, WGR.

Introduction. Massive fishing activities can lead to overfishing. Therefore, fish farming should be the future of the global fish supply, including Indonesia. According to FAO (2020), world capture fisheries production reached 96.4 million tons in 2018, while world aquaculture production was 82.1 million tons in 2018. The grouper isoften overfished around the world as it is a leading fish commodity in the Asia-Pacific Region (Myoung et al 2013; Koh et al 2016; Bulanin et al 2017; Chieng et al 2018). Indonesia's aquaculture production increased from 16.0 million tons in 2016 to 16.3 million tons in 2019, including the grouper aquaculture production, which increased from 11,504 tons in 2016 to 16,461 tons in 2019 (KKP 2020).

Hybrid grouper cultivation in Indonesia is being extensively developed, including by crossbreeding the giant grouper (*Epinephelus lanceolatus*) and the brown-marbled grouper or tiger grouper (*Epinephelus fuscoguttatus*) (Long et al 2022), known in Indonesia as 'cantang'. grouper. According to Heemstra & Randall (1993), the habitat of *E. fuscoguttatus* is formed by shallow coral reefs and rocky bottoms with a depth of 60 m, while *E. lanceolatus* live in coral reefs and rocky areas ranging from shallow waters to a depth of 100 m. In Indonesia, grouper is cultivated in seawater (floating net cages) and brackish water (ponds). Fish farmers living away from coastal area were unable to cultivate groupers. They could only culture freshwater fish with lower economic value such as catfish, carp, and tilapia which price were below USD 1.9 kg^{-1}. Meanwhile, the price of TGGG hybrid grouper can reach more than USD 6.34 kg^{-1}. The price of TGGG grouper as an ornamental fish is higher than as a consumption fish. It is necessary to find ways to cultivate the hybrid grouper in low salinity media, as a viable alternative for aquaculture in fish farms located far from the coast. This research measured the effect of salinity on growth, survival and profitability in the cultivation of 'cantang' hybrid grouper (TGGG).

Material and Method

Location and time of research. The research was conducted at the Laboratory of the Faculty of Fisheries and Marine Sciences, Diponegoro University. The TGGG hybrid grouper seeds were obtained from the marine fish hatchery center in Situbondo (Figure 1). The research was conducted for 30 days from September to October 2022.

Figure 1. Research location.

Research material. The study used 'cantang' (TGGG) hybrid grouper seeds with an average size of 3.16 g (±0.03 g). Seeds from hatcheries were reared in seawater with a 34 ppt salinity. Fish were cultivated in plastic tubs with a volume of 100x100x40 cm^{-3} with 10 fish per tank. To maintain water quality, a re-circulation system using filter cloth, dacron, gravel and charcoal was applied. The fish were fed with commercial feed that containing minimum 46% crude protein, 12% crude fat, 5% crude fiber, and 11% moisture. Feeding was carried out with a dose of 4% fish biomass 3 times a day (morning, afternoon and evening). Fish manure was collected in the morning and evening every day. Water salinity was gradually decreased by mixing the seawater and fresh water.

Experimental design. The experiment (with a completely randomized design) consisted of 3 treatments and 3 replications. The performed treatments were: treatment A, using original seawater at a salinity of 34 ppt, treatment B, at a salinity of 20 ppt and treatment C, at a salinity of 10 ppt. In treatments B and C, the salinity was gradually decreased by 1 to 2 ppt day^{-1} until it reached the salinity target.

Data analysis. The variables that were measured in this research were the growth, survival rate (SR), and benefit cost ratio (BCR) of the fish. Fish growth includes biomass growth rate (WGR) and specific growth rate (SGR). Some of the formulas used in the data analysis are presented as follows (Mapenzi & Mmochi 2016; Wijayanto et al 2020; Wijayanto et al 2021; Long et al 2022):

$$WGR\ (\%) = [(Wt - Wo) / Wo] \times 100$$
$$SGR\ (\%) = [(Ln\ Wt - Ln\ Wo) / t] \times 100$$
$$BCR = B / C$$
$$SR\ (\%) = (Nt / No) \times 100$$

Where:
WGR - fish weight gain (in %);
Wt - the final biomass (in g) on day t;
Wo - the initial biomass in g;
SGR - the specific growth rate of fish in % day^{-1};

Ln - the natural logarithm for final biomass (Ln Wt) and initial biomass (Ln Wo);
BCR - the cost benefit ratio;
B - the additional income due to fish growth (USD);
C - the cost of feed (USD);
SR - survival rate (%);
Nt - the final number of fish on day t;
No - the number of initial fish.

The ANOVA and Duncan tests were used for the statistical analysis, to determine if the treatments had a significant effect on the examined variables. The modelling process for treatment optimization in this study was based on testing the salinity values for which the first derivative of the SR, WGR, SGR and BCR was equal to zero.

Water quality monitoring. The water quality parameters observed in this research were dissolved oxygen (DO), pH and temperature, whose measurement was conducted every week. Meanwhile, salinity was monitored every day using a Horiba U-50.

Results. During the experiment, the fish tended to move in groups and were not actively moving, except when being fed, where they seemed to struggle to eating. In the nature, groupers tend to be solitary (Paruntu et al 2018). Groupers are top predators that settle in habitat, move slowly, grow slowly and have long life span (Heemstra & Randall 1993; Darwin & Padmavathi 2020). The results of the SR, WGR, SGR, and BCR measurements are presented in Tables 1, 2 and 3.

Table 1

SR, WGR, and SGR in this study (research stage 1 in 16 days)

Variables	*A (34 ppt / control)*			*B (34 to 20 ppt)*			*C (34 to 20 ppt)*		
	A_1	*A_2*	*A_3*	*B_1*	*B_2*	*B_3*	*C_1*	*C_2*	*C_3*
SR (%)	100	100	100	100	100	100	100	100	100
Average of SR (%)		100			100			100	
WGR (%)	84.3	79.8	78.7	80.2	78.5	84.6	75.3	76.6	87.9
Average of WGR (%)		80.9			81.1			79.9	
SGR (% day^{-1})	3.8	3.7	3.6	3.7	3.6	3.8	3.5	3.6	3.9
Average of SGR (% day^{-1})		3.7			3.7			3.7	
Statistical analysis		F Value			Sig Value			Notes	
SR		-			-			Not significant	
WGR		0.017936			0.897232			Not significant	
SGR		0.030303			0.866732			Not significant	

Table 2

SR, WGR, and SGR in this study (research stage 2 in 7 days)

Variables	*A (34 ppt / control)*			*B (20 ppt)*			*C (20 to 10 ppt)*		
	A_1	*A_2*	*A_3*	*B_1*	*B_2*	*B_3*	*C_1*	*C_2*	*C_3*
SR (%)	100	100	100	100	100	100	100	100	100
Average of SR (%)		100			100			100	
WGR (%)	40.0	41.2	44.0	47.8	48.1	43.2	49.7	47.5	40.0
Average of WGR (%)		41.7			46.4			45.7	
SGR (% day^{-1})	4.2	4.3	4.6	4.9	4.9	4.5	5.0	4.9	4.2
Average of SGR (% day^{-1})		4.4			4.8			4.7	
Statistical analysis		F value			Sig value			Notes	
SR		-			-			Not significant	
WGR		1.524			0.292			Not significant	
SGR		1.519			0.293			Not significant	

Table 3

SR, WGR, and SGR in this study (research stage 3 in 7 days)

Variables	*A (34 ppt / control)*			*B (20 ppt)*			*C (10 ppt)*		
	A_1	*A_2*	*A_3*	*B_1*	*B_2*	*B_3*	*C_1*	*C_2*	*C_3*
SR (%)	100	100	100	100	100	100	100	100	100
Average of SR (%)		100			100			100	
WGR (%)	25.4	31.6	34.2	34.0	37.8	33.4	32.9	38.7	38.1
Average of WGR (%)		30.4			35.0			36.6	
SGR (% day^{-1})	3.2	3.9	4.2	4.2	4.6	4.1	4.1	4.7	4.6
Average of SGR (% day^{-1})		3.8			4.3			4.5	
BCR (% day^{-1})	3.51	4.38	4.83	4.79	5.26	4.64	4.55	5.27	5.27
Average of BCR (% day^{-1})		4.24			4.90			5.03	

Statistical analysis	F Value	Sig Value	Note
SR	-	-	Not significant
WGR	2.573	0.156	Not significant
SGR	2.557	0.157	Not significant
BCR	2.262	0.185	Not significant

Although the statistics did not show any significant impact on SR, WGR, SGR and BCR, the treatment C had the highest performance compared to the treatments A and B, especially on WGR, SGR and BCR in the 3rd stage of the experiment. Optimal salinity was estimated at 5.0 ppt to 10.1 ppt (Table 4, Figure 2). The water quality of the experimental media can be seen in Table 5.

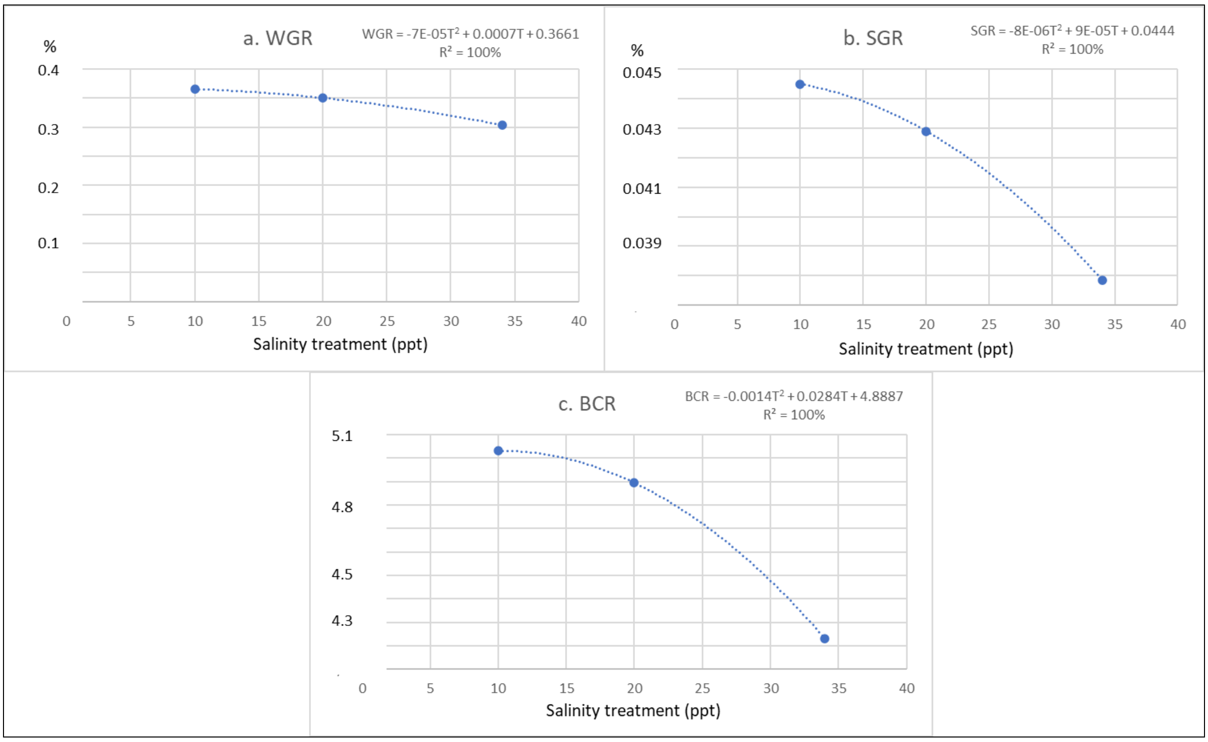

Figure 2. Salinity optimization modelling to WGR, SGR and BCR.

Table 4

Optimal salinity estimation at WGR, SGR and BCR

Variables	*Salinity optimal estimation (ppt)*
WGR	5.0
SGR	5.6
BCR	10.1

Table 5

Water quality during research

Variables	A (control)			B			C		
	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2	C_3
pH	7.9± 0.10	7.9± 0.05	7.9± 0.06	7.9± 0.05	8.0± 0.05	8.0± 0.06	8.0± 0.17	8.0± 0.13	8.0± 0.08
DO (ppm)	5.9± 0.17	6.2± 0.25	6.0± 0.50	5.6± 0.35	6.4± 0.25	6.1± 0.06	6.3± 0.25	5.9± 0.80	6.3± 0.29
Temp. (°C)	25.4± 0.37	25.3± 0.29	25.2± 0.30	25.1± 0.35	25.3± 0.37	25.2± 0.34	25.3± 0.38	25.3± 0.31	25.3± 0.22

Discussion. Grouper, Asian seabass, and Java rabbitfish (seawater cultivation), shrimp and milkfish (brackish water cultivation) are some of the most interesting species for the Indonesian fish farming in saline waters, while the most popular fresh water fish in Indonesia are carp, tilapia and catfish. The practice of grouper cultivation in Indonesia has been increasing since the 1980s (KKP 2020). Grouper is a large, sedentary, and slow-growing fish, often caught by fishermen using spears and lines. Grouper has a negative allometric growth pattern, where the constant b in the relation of length and weight affects the fish behavior. Active-moving fish tend to have low b values (Bulanin et al 2017; Begossi et al 2019; Fatma et al 2022). Most groupers are protogynous hermaphrodites, which can change sex depending on rearing conditions such as age, size and environment (Oh et al 2013). Although *Epinephelus* sp. is a type of marine fish, several species have eury-haline properties, including *E. aeneus*, allowing them to live in water with salinity of 3 ppt (Peduel & Ron 2003; Cnaani et al 2012). According to García et al (2012), juvenile goliath grouper (*E. itajara*) has the ability to osmoregulate in fresh water.

Epinephelus sp. is an important commercial fish commodity in the tropics and sub-tropics for having high price, high demand, and only a few species have been successfully cultivated (Myong et al 2013; Chieng et al 2018). Although there are more than 159 species of grouper in the world (Heemstra & Randall 1993), the brown-marbled grouper or tiger grouper (E. *fuscoguttatus*), orange-spotted grouper (*E. coioides*) and giant grouper (*E. lanceolatus*) are the most commercially cultivated species in Southeast Asia. The relatively slower growth rate of tiger grouper has made it less popular, where farmers preferred cultivating TGGG hybrid grouper (Othman et al 2015; Koh et al 2016). In aquaculture, hybridization has long been practiced in various fish species to increase the growth, disease resistance, meat quality, and profit. Hybridization on grouper has been performed since 2006, where tiger grouper and giant grouper were the first cross-bred (Tan 2021; Long et al 2022).

The giant grouper (*E. lanceolatus*) or 'kertang' grouper (local name) is the largest species of the family Serranidae that lives in tropical and subtropical waters in the Indo-Pacific region, from South Africa to the Hawaiian Islands, including Indonesia (Myoung et al 2013). Giant grouper is the largest fish in coral reef areas that tends to settle within relatively close migration areas (around coral reef waters) for feeding and spawning (Clua et al 2015). In the nature, giant groupers can be found in shallow waters up to the depth of 100 m, on coral reefs and around shipwrecks, and in estuaries. The favorite food of giant grouper on coral reefs and in rocky areas is spiny lobster. The giant grouper can weigh up to 400 kg (Heemstra & Randall 1993). *E. lanceolatus* has a high resistance to extreme environmental conditions (Othman et al 2015).

The tiger grouper or 'macan' grouper (local name) lives in coral reefs and rocky bottom waters. Juvenile tiger grouper can be found in seagrass areas. Tiger groupers are carnivorous that eat smaller fish, crabs, crustaceans and cephalopods. The length of tiger grouper can reach up to 115 cm with a weight up to 14 kg (Heemstra & Randall 1993; Paruntu et al 2018; Fatma et al 2022). The length at first maturity (Lm) of the tiger grouper is 66.34 cm (Fatma et al 2022). Tiger grouper cultivated in polyculture grows faster than in monoculture. Herbivorous rabbits in polyculture can eat the grass on floating net cages, keeping the cage clean and allowing good water circulation (Paruntu et al 2018).

Results of previous research showed that TGGG grouper could be cultivate in low salinity media. The 10 ppt water treatment actually showed an even better outcome than the seawater media. The gradual decrease in water salinity by 1-2 ppt per day showed no significant effect on the fish. In the first stage of the experiment, the outcomes were not significantly different between treatments. The average SGR for all treatments showed a value of 3.7% day^{-1} (Table 1). However, in the 2nd and 3rd stages of the experiment, treatment A started to lag behind treatments B and C. All treatments resulted in a 100% SR, implying that the decrease in salinity in this experiment had no effect on the fish mortality rate.

The survival and growth of fish can be affected by water quality, including salinity. The relationship between salinity and fish growth appears to be complex, as studies with different species have found different results (De Azevedo et al 2015). Water parameters such as temperature, pH, dissolved oxygen (DO) and salinity are key factors of optimal water. Salinity was found to strongly affect the growth capacity of teleost fish. Salinity affects various physiological processes in aquatic animals such as metabolism and osmoregulation. Salinity level that deviates from the optimal level can affect the physiological processes in aquatic organisms (Othman et al 2015; Hossain et al 2021).

Salinity is defined as the overall ions in water, consisting mainly of sodium, calcium, chloride, potassium, magnesium, bicarbonate and sulfate. Fish (both in marine and freshwater) need energy to hold ions in or out of their bodies through osmoregulation (Mapenzi & Mmochi 2016). In the aquaculture development it is necessary to check the species-specific optimal salinity. Fish growth can be inhibited in water with a very low or high salinity, as salinity determines a high energy consumption, required for osmoregulation (Hamed et al 2016). The level of salinity is positively and significantly correlated with the conductivity (Iqbal et al 2012). Salinity also affects the reproductive cycle of fish (Lee et al 2020). The growth of teleost fish can be improved in isosmotic conditions. Optimal salinity level improves the fish growth, feeding, fish quality, fish color and the final production (Lisboa et al 2015; Nassar et al 2021). Salinity is one of the most important environmental factors affecting the fish hatchery production, including egg incubation and fish larvae rearing. Inappropriate salinity will lead to poor larval quality and even abnormal larval hatching (Koh et al 2016). Research by Noor et al (2018) showed that a salinity of 15 ppt was an optimal condition for the growth performance of TGGG juvenile grouper hybrids. Meanwhile, research by Othman et al (2015) showed that a high salinity, of 35 ppt to 30 ppt, caused a poor growth performance of juvenile TGGG.

In TGGG grouper cultivation, the maintenance of water quality and disease prevention are challenging. Unless being handled properly, the faeces and residual feed can reduce the water quality, which in turn causes disease attacks in TGGG grouper. Several infectious diseases that are susceptible to attack groupers are *Vibrio* spp, *Streptococcus* spp, betanodavirus, and *Cryptocaryon irritans*. Vibriosis is a bacterial disease that is often found in grouper culture starting from eggs, larvae, seeds, and broodstock. Grouper cultivation entrepreneurs usually use antibiotics (Chieng et al 2018; Yuhana et al 2019). Environmental factors and good cultivation management determine the success of a cultivation, including salinity, temperature, aeration, lighting, optimal time for first feeding for larvae and efforts to reduce cannibalism in juveniles. Cannibalism is a common cultivation issue in the late larval and juvenile groupers (Chieng et al 2018).

In this research, the quality of the experimental media was relativelyappropriate for the life of the TGGG grouper, with an average DO of 5.6 to 6.3 ppm, an average pH between 7.9 to 8, a temperature between 25.1 to 25.4°C. The indoor laboratory temperature of the experimental media tends to be more stable, but lower than the outdoor conditions (in tropical areas). The optimal aquatic environment for grouper cultivation is at a temperature of 29 to 30^{o}C, a pH of 6.5 to 8.5, and a DO>5 ppm (Herry et al 2019). The temperature of the water in this research did not cause low appetite.

The results of this study indicate that TGGG grouper can be reared in low salinity media. This can be an opportunity for fish farmers living away from the coast to cultivate the TGGG grouper, which can be sold at a higher price. A BCR value of more than 4 indicates that the TGGG hybrid grouper cultivation is very profitable if managed properly. To maintain the fish health, it is necessary to provide an adequate feed for nutrition and a good water quality management. A poor water quality increase the risks of a higher mortality due to diseases.

Conclusions. The results showed that the saline treatment had no significant effect on the fish growth (WGR and SGR), SR and BCR. Treatment C showed the best results, with 100% SR. The modelling results showed that the optimal salinity for WGR is 5 ppt, for SGR it is 5.6 ppt, and for BCR it is 10.1 ppt. The results showed that the TGGG grouper can be cultivated in water with low salinity: even at 10 ppt, the outcomes were better than in the control media (34 ppt).

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