Effect of low salinity and fresh water media on growth, survival and BCR in TGGG hybrid grouper (♀ tiger grouper × ♂ giant grouper) culture

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Abstract. Cultivation of the TGGG hybrid grouper species, locally known as '*cantang*,' has been developed in Indonesia and is typically cultivated in seawater or brackish water ponds. This research investigated the feasibility of cultivating the TGGG hybrid grouper that is a crossbreed between the tiger grouper (*Epinephelus fuscoguttatus*) and the giant grouper (*E. lanceolatus*) in low-salinity water media, including freshwater (0 ppt). The research was conducted for 40 days using fish fingerlings with an average weight of 12.99 g (±0.65 g) reared in plastic tubs containing 17 fish per tube. Three treatments with two repetitions were performed: treatment A used media with a salinity of 10 ppt, treatment B with a salinity of 5 ppt, and treatment C with a salinity of 0 ppt. The effects of the treatments on weight growth rate (WGR), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), and benefit cost ratio (BCR) were examined. The results showed that the salinity treatment had a significant effect on the fish's growth (WGR and SGR), FCR, and BCR, although statistically, there was no significant difference between treatments A and B. The modelling results indicated that the optimal salinity ranged between 7.33 and 7.93 ppt for SGR, SR, BCR, and WGR. These results suggest that the TGGG hybrid grouper can be reared at low salinity, but not at 0 ppt.
Key Words: BCR, FCR, SGR, SR, TGGG hybrid grouper, WGR.

Introduction. Fish farming in Indonesia, including grouper farming, is expected to expand in order to meet the increasing demand for seafood and address the threat of overfishing in capture fisheries (Fennessy et al 2018; Rhodes et al 2018; FAO 2020; Wijayanto et al 2021; Fadli et al 2022). Grouper is a leading fishery commodity in the Asia-Pacific, including Indonesia, where the production of grouper aquaculture reached 16,461 tonnes in 2020 (Myoung et al 2013; Bulanin et al 2017; KKP 2020; Fadli et al 2022). Some provinces are major suppliers of groupers, including North Sumatra, West Sumatra, Riau Islands, Aceh, Lampung, West Java, East Java, West Papua, West-Southeast Nusa, East-Southeast Nusa, Maluku, Bali, and North Maluku (DJPB 2017). Humpback grouper (*Cromileptes altivelis*) and tiger grouper (*Epinephelus fuscoguttatus*) are some of the species commonly cultivated in Indonesia despite having relatively slow growth reaching over six months to commercial size for consumption when grown in floating net cages (Baliao et al 2000; DJPB 2017). This slow growth appears as an issue, particularly among farmers with low capital.

At present, the cultivation of hybrid grouper fish, including the TGGG hybrid is getting more popular in Indonesia. The TGGG hybrid, which is the crossbreed between the giant grouper (*Epinephelus lanceolatus*) and the tiger grouper (*E. fuscoguttatus*) (Long et al 2022) is popular among fish farmers in Indonesia for having relatively fast growth rate and high resistance to various diseases (DJPB 2017). In Indonesia, the TGGG hybrid is known as the '*cantang*' grouper. However, the cultivation of TGGG grouper is currently limited to fish farmers who live in coastal areas. Wijayanto et al (2023) found that TGGG grouper reared in low salinity media demonstrated higher growth rate compared to those reared in seawater. This research examined the cultivation of TGGG grouper in both low salinity media and fresh water media. If TGGG grouper can be successfully cultivated in fresh water, it could provide an alternative, high-value fish for fish farmers who live inland. This research particularly examined the effects of low salinity media on growth, survival, feed efficiency, and profitability in TGGG hybrid grouper culture.

Material and Method

Research setting. This research was conducted for 40 days from October to November 2022 at the Laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Diponegoro, Semarang City (Indonesia). TGGG hybrid grouper fingerlings were obtained from a marine fish hatchery in Situbondo (Figure 1) which have been adapted to rearing media with low-salinity water.

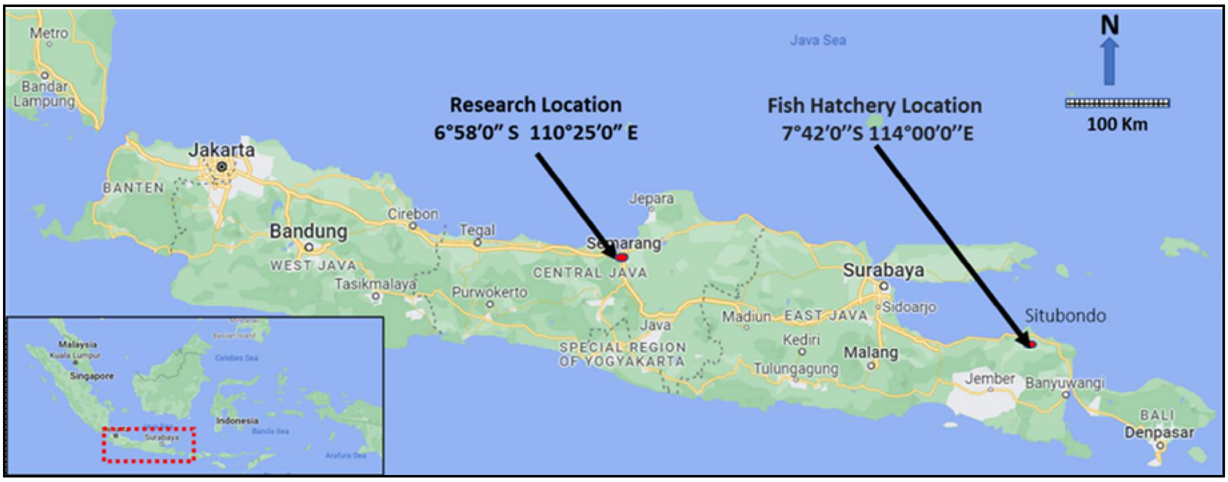

Figure 1. Research location.

Research design. The TGGG hybrid grouper fingerlings with average size of 12.99 g (± 0.65 g) were cultivated in plastic tubs measuring 100 x 100 x 40 cm containing 17 fish per tube. Water quality management was performed using filters in the recirculation system (cloth, dacron, gravel and charcoal). Fish manure and feed residue were collected and cleaned on daily basis. The test fish were fed commercial feed with a minimum crude protein content of 46%. Fish biomass of 4% was administered in the morning, afternoon and evening daily. Three different treatments in 2 repetitions (complete random design), namely treatment A (salinity 10 ppt), treatment B (salinity 5 ppt) and treatment C (salinity 0 ppt) were performed. Fish weight progress was measured every 10 days.

Data analysis. The variables in this study, namely fish growth (weight growth rate - WGR, and specific growth rate - SGR), survival rate (SR), feed conversion ratio (FCR), and benefit cost ratio (BCR) were measured using the following formulae (Mapenzi & Mmochi 2016; Wijayanto et al 2020, 2021, 2023; Long et al 2022):

$$WGR\ (\%) = [(Wt - Wo)/Wo] \times 100 \qquad [1]$$

$$SGR\ (\%) = [(Ln\ Wt - Ln\ Wo)\ /\ t] \times 100 \qquad [2]$$

$$BCR = B\ /\ C \qquad [3]$$

$$FCR = F/W \qquad [4]$$

$$SR\ (\%) = (Nt/No) \times 100 \qquad [5]$$

WGR is the percentage of the increase in fish weight; Wt is the final weight of the fish in grams on day t; Wo is the initial weight of the fish in grams; SGR is the specific growth rate of the fish in percent per day; Ln is the natural logarithm of the final fish weight (Ln Wt) and the initial fish weight (Ln Wo); BCR is the benefit cost ratio; B is the additional income gained from the fish growth in Indonesian rupiah (IDR); C is the cost of feed in IDR; FCR is the feed conversion ratio; F is the accumulation of feed in grams; W is the absolute fish growth in grams (Wt-Wo); SR is the survival rate as a percentage; Nt is the final number of fish on day t; No is the initial number of fish.

Anova test (F test and t test) and Duncan's test were employed to determine if the treatments had significant effects on the variables being examined. The salinity optimization modelling process in this study used the first derivative procedure equals zero.

Water quality monitoring. Water quality monitoring was performed every 10 days to measure the dissolved oxygen (DO), pH, salinity and water temperature using Horiba U-50 device.

Results. During the experiment, the fish in treatments A and B showed a high appetite. When food was provided, the fish would quickly swim to the surface to eat. On the contrary, the test fish in treatment C (0 ppt salinity) were less responsive when fed. They rather stayed at the bottom of the tank and swam in schools. Some feed were left uneaten by the test fish in treatment C which differed from treatments A and B. At the first weighing (day 10), the test fish in treatments A, B, and C all had a SR of 100%. However, on the second weighing (day 20), in treatment C, some fish died for having thin stomach. Whereas the SR between treatments A and B remained 100%. Therefore, treatment C was terminated on the 20th day, while treatment A and B were still continued until they completed. In treatment C, the media salinity was gradually increased to 5 ppt, which resulted in the improved appetite of the test fish improving that made the fish more active. The results of the first phase are presented in Table 1.

Table 1

WGR, SGR, SR, FCR and BCR in this study (research stage 1 in 20 days)

Variables	*A (salinity 10 ppt)*		*B (salinity 5 ppt)*		*C (salinity 0 ppt)*	
	A_1	*A_2*	*B_1*	*B_2*	*C_1*	*C_2*
WGR (%)	43.1	55.1	45.5	41.3	-40.4	-12.4
Average of WGR (%)	49.1		43.4		-26.4	
SGR (% day^{-1})	1.56	1.91	1.63	1.50	-2.25	-0.58
Average of SGR (% day^{-1})	1.73		1.57		-1.41	
SR (%)	100	100	100	100	53	82
Average of SR (%)	100		100		68	
FCR	2.36	1.91	2.25	2.44	-2.02*	-7.45*
Average of FCR	2.13		2.34		-4.73*	
BCR	1.67	2.08	1.76	1.62	-1.96	-0.53
Average of BCR	1.88		1.69		-1.20	
Statistical analysis	*F value*		*Sig value*		*Note***	
WGR	22.455		0.016		Significant	
SGR	12.808		0.034		Significant	
SR	4.840		0.115		Not significant	
FCR	6.531		0.011		Significant	
BCR	16.512		0.024		Significant	
Duncan test			*Note*			
WGR			$A^a > B^a > C^b$			
SGR			$A^a > B^a > C^b$			
SR			$A^a = B^a > C^a$			
FCR			$A^a < B^a$			
BCR			$A^a > B^a > C^b$			

Notes: * Theoretically, the FCR value is positive. Negative FCR is due to negative growth in the tested fish biomass; ** At 95% confidence level; a, b, and c represent subset groups.

The results of the first phase of this research showed significant effects of the treatments on several variables, including WGR, SGR, and BCR. On the contrary, Wijayanto et al (2023) found that the salinity treatment did not have statistically significant effect on SR, WGR, SGR, and BCR, although the treatment with 10 ppt salinity produced the best performance compared to the treatments with 20 ppt and 34 ppt salinities. The optimized modelling can be seen in Figure 2 and Table 2. This research showed that the optimal salinity for TGGG hybrid grouper cultivation is between 7 and 8 ppt, specifically 7.33 ppt for SGR, 7.47 ppt for SR, 7.84 ppt for BCR, and 7.93 ppt for WGR.

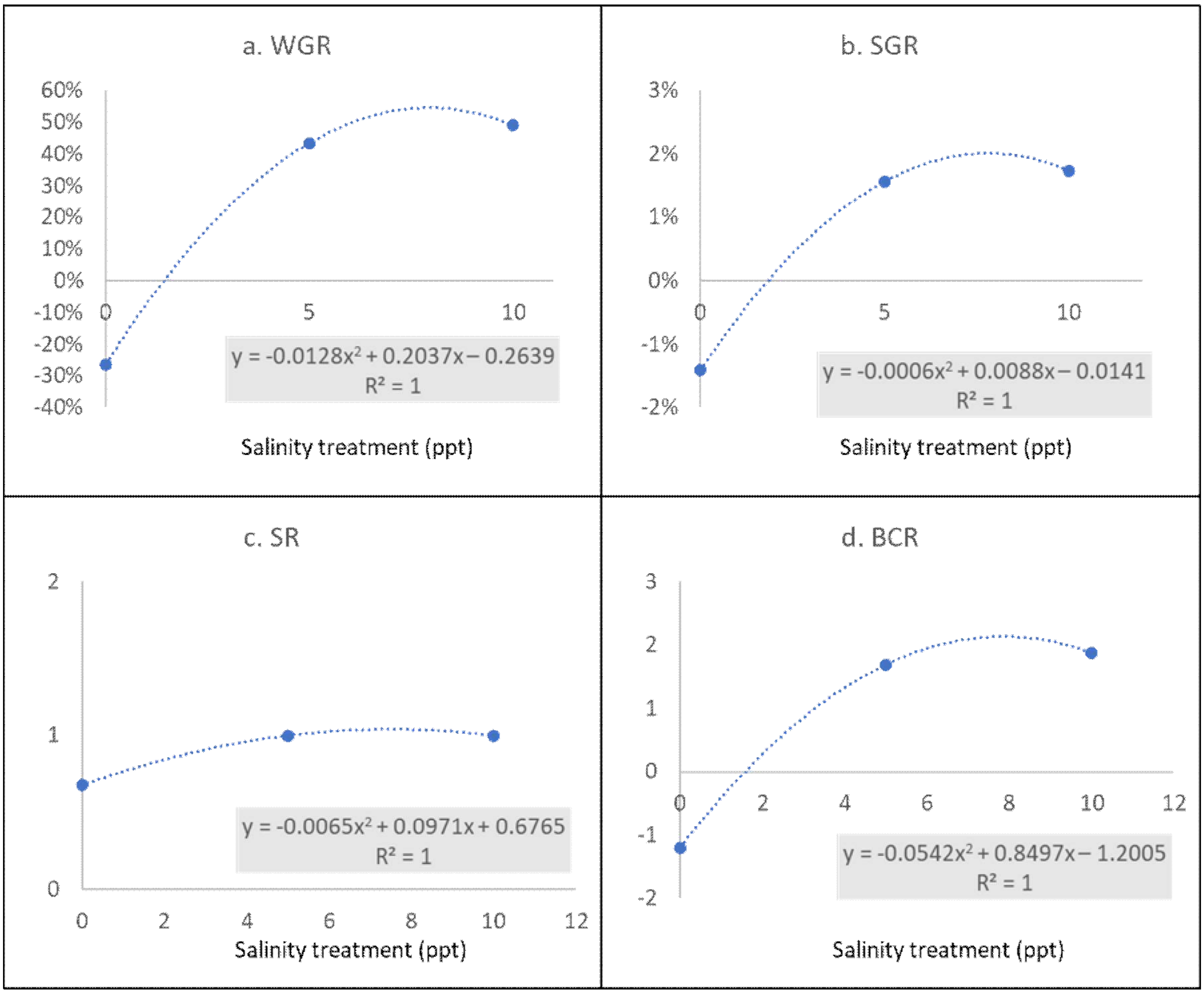

Figure 2. Salinity optimization modelling for WGR, SGR, SR, FCR and BCR.

Table 2

Salinity optimal estimation

Variables	*Salinity optimal estimation (ppt)*	*Variables value estimation*
WGR	7.93	54.10%
SGR	7.33	1.82%
BCR	7.84	2.13
SR	7.47	100%*

Note: *simulation results of the optimal SR value are more than 100%, but the maximum SR value is 100%.

After treatment C was terminated on the 20th day, statistical analysis was conducted to compare treatments A and B (up to 40 days). The results of the 40-day experiment in treatments A and B are presented in Table 3. Overall, there was no statistical significance between treatments A and B. Slightly better averages of WGR, SR, FCR, and BCR were found in treatment A compared to treatment B.

Table 3

WGR, SGR, FCR and BCR in this study (research stage 2)

Variables	*A (salinity 10 ppt)*		*B (salinity 5 ppt)*	
	A_1	*A_2*	*B_1*	*B_2*
WGR (%)	174.9	181.8	176.3	174.9
Average of WGR (%)	178.4		175.6	
SGR (% day^{-1})	1.55	1.66	1.57	1.55
Average of SGR (% day^{-1})	16.1		15.6	
SR (%)	100	100	100	100
Average of SR (%)	100		100	
FCR	2.35	2.27	2.33	2.32
Average of FCR	2.31		2.33	
BCR	1.68	1.74	1.70	1.70
Average of BCR	1.71		1.70	
Statistical analysis	*t value*	*Sig value*	*Note**	
WGR	0.780	0.517	Not significant	
SGR	0.777	0.519	Not significant	
FCR	-0.419	0.716	Not significant	
BCR	0.433	0.707	Not significant	

Note: * at 95% confidence level.

The water quality is shown in Table 4. The pH and DO levels were regarded optimal for grouper cultivation. As mentioned by Herry et al (2019), the optimal aquatic environment for grouper culture ranges at temperatures between 29 and 30°C, pH between 6.5 and 8.5, and DO of more than 5 ppm. In this indoor laboratory experiment, temperature was not as high as it would be in an outdoor experiment in a tropical region. However, the temperature of the experimental media was more stable. Despite the temperature that was lower than the optimal one, the test fish still showed high appetite.

Table 4

Water quality during the study

Variables	*A (salinity 10 ppt)*		*B (salinity 5 ppt)*		*C (salinity 0 ppt)*	
	A_1	*A_2*	*B_1*	*B_2*	*C_1*	*C_2*
pH	8.1±0.06	8.0±0.15	8.0±0.06	8.0±0.06	8.0±0.10	8.0±0.06
DO (ppm)	6.5±1.04	6.5±0.72	6.3±0.61	6.8±0.91	6.4±0.52	6.0±0.36
Temp. (°C)	24.9±0.83	24.5±0.86	24.5±0.79	24.4±0.65	24.4±0.60	24.4±0.60

Discussion. TGGG hybrid grouper can be reared at low salinity, but TGGG hybrid grouper culture for consumption fish production cannot be carried out at 0 ppt salinity. Some researchers underlined the water salinity as the key factor in the success of fish farming. Salinity refers to the content of ions in water and culture media, including sodium, calcium, chloride, magnesium, potassium, bicarbonate, and sulfate. Salinity affects the growth and survival of farmed fish within complex mechanism. Each type of fish grows in different optimal range of salinity. Salinity affects fish physiological processes, including metabolism and osmoregulation. Non-optimal salinity can disrupt these processes. The osmoregulation process requires energy. Conditions farther from the optimal salinity require more energy use. Fish can be stressed out when cultivated at water with improper level of salinity, resulting in lower appetite, weak immune system, and higher susceptibility to disease. Optimal salinity levels can improve fish growth, feed efficiency, fish meat quality, fish coloring, and final production quantity (de Azevedo et al 2015; Othman et al 2015; Hamed et al 2016; Koh et al 2016; Mapenzi & Mmochi 2016; Lee et al 2020; Hossain et al 2021; Nassar et al 2021; Wijayanto et al 2023).

The optimal salinity for TGGG hybrid grouper culture found in this research ranged between 7 to 8 ppt, specifically 7.33 ppt for SGR, 7.47 ppt for SR, 7.84 ppt for BCR, and 7.93 ppt for WGR. These results are more specific than those of Wijayanto et al (2023), which showed optimal salinity within the range of 5.0 to 10.1 ppt. However, Noor et al (2017) found 15 ppt salinity optimal for fish rearing. The natural habitat of tiger grouper and giant grouper is the sea, but Othman et al (2015) and Wijayanto et al (2023) revealed that water with high salinity could actually result in slower growth

At the present, TGGG hybrid grouper from Indonesia is exported to Hong Kong, Malaysia, Singapore, Taiwan, China, and Japan (DJPB 2017). This research showed the opportunity for the cultivation of TGGG hybrid grouper in places far from the coast. Fish farmers can use salt to create 7-8 ppt salinity in their culture media water. Salt is relatively cheap in Indonesia, while TGGG hybrid grouper is relatively expensive. According to KKP (2020), grouper fish farming in Indonesia has significantly improved since the 1980s. More cultivation of TGGG hybrid grouper in low salinity media can significantly increase grouper fish production. *Epinephelus* sp. is a type of marine fish but some of its species have euryhaline properties. *E. aeneus* can survive in water with a salinity of 3 ppt (Peduel & Ron 2003; Cnaani et al 2012). The test fish in this study were able to survive at a salinity of 0 ppt, but their growth was very slow and they were prone to death.

Grouper fish in nature tend to be solitary, sedentary, and have limited migration patterns and slow growth (Paruntu et al 2018; Begossi et al 2019). According to Heemstra & Randall (1993), there are over 159 species of grouper in the world. High demand and high prices make grouper fish prone to overfishing. However, few grouper species have been successfully cultivated, including tiger grouper and giant grouper (Myoung et al 2013; Chieng et al 2018; Dennis 2021; Fadli et al 2022; Wijayanto et al 2023). The giant grouper is the largest species in the Serranidae family that can be found in tropical and subtropical waters in the Indo-Pacific region, including in Indonesia (Myoung et al 2013). Indonesia is one of the largest producers of wild giant grouper fish. Giant grouper inhabit coral reefs and river mouths (Fennessy et al 2018). Tiger grouper, on the other hand, inhabit coral reef waters and waters with rocky bottoms. Their diet includes smaller fish, crabs, crustaceans, and cephalopods, with spiny lobsters being a favorite. Tiger grouper can grow up to 115 cm in length and weigh 14 kg (Heemstra & Randall 1993; Rhodes et al 2018; Paruntu et al 2018; Fatma et al 2022). Tiger grouper is one of Indonesia's export commodities, but most of it is caught in the natural habitat (Rhodes et al 2018). Most groupers are protogynous hermaphrodites, meaning they change sex depending on rearing conditions such as age, size, and social control (Oh et al 2013). Giant grouper and tiger grouper are listed as vulnerable species in The IUCN Red List of Threatened Species (Fennessy et al 2018; Rhodes et al 2018).

The slow growth remains an unresolved issue in the cultivation of tiger grouper, humpback grouper, and giant grouper. Therefore, several fish farming entrepreneurs have started to switch to hybrid grouper cultivation, including the TGGG hybrid grouper (Othman et al 2015; Koh et al 2016; DJPB 2017). Hybridization in fish farming is a common practice meant to produce cultivars with high growth, high resistance, improved meat quality, and increased business profits. The hybridization of tiger grouper and giant grouper started in 2006 (DJPB 2017; Shapawi et al 2019; Tan 2021; Long et al 2022).

The quality of the experimental media used in this research affected the growth and survival of the test fish. In addition to salinity, water quality variables such as DO, temperature, and pH also affect the fish growth and survival rate. During the experiment done in this research, the temperature, DO, and pH conditions were maintained within the acceptable range for the test fish. In practice, maintaining water quality and preventing disease are challenges in TGGG hybrid grouper cultivation. Poor water quality can cause high mortality rate, especially to smaller fish. Poor management of dirt and leftover feed can decrease water quality and lead to disease outbreaks in TGGG hybrid grouper (Wijayanto et al 2023). The optimal aquatic environment for grouper culture ranges at temperatures of 24-32^{o}C, pH 7.5-8.5, and DO greater than 3.5 ppm (DJPB 2017; Herry et al 2019). However, the temperature of the experimental media during the study did not decrease the appetite of the fish in treatments A and B. Feeding should be a huge concern because TGGG grouper can be cannibal when the amount of feed given is insufficient (Chieng et al 2018). Grouper fish tend to be cannibal when they are below 10 cm in size (DJPB 2017).

Profits from grouper farming are affected by selling price, production (quality and quantity), and production costs. Meanwhile, the grouper fish price is influenced by the demand (both national and international). On the other side, the quantity of production is influenced by fish growth, SR, and the amount of seed stocking. The cost of intensive grouper farming is mainly affected by feed prices and feed efficiency (FCR). In this study, FCR (especially in treatments A and B) showed average values of 2.31 and 2.33, meaning that 2.31-2.33 g of artificial feed are needed to produce a weight gain of 1 g. According to DJPB (2017), the FCR of TGGG hybrid grouper cultivation in floating net cages (seawater) is around 6 within 7 month rearing period with fingerlings of 12 cm in size and 500 g in weight. Feed management is crucial because feed is the largest cost component in grouper cultivation that accounts to 85.5% (Dennis 2021). The average BCR value for treatment A in this study was 1.71, implying that every IDR 1 expenditure generates IDR 1.71 in revenue, while the average BCR value for treatment B was 1.70. According to DJPB (2017), the BCR of TGGG hybrid grouper cultivation in floating net cages in marine waters is around 1.4.

The results of this study suggest that TGGG hybrid grouper can be grown in low salinity media, but not at 0 ppt. This could be an opportunity for fish farmers who are located inland to cultivate TGGG hybrid grouper that has high economic value. Currently, fish farming in inland areas of Indonesia is dominated by low-priced fish species, such as tilapia (*Oreochromis niloticus*), catfish (*Clarias* sp.), and carp (*Cyprinus carpio*). Therefore, TGGG hybrid grouper cultivation in low-salinity media could be a business opportunity for fish farmers living far from the coast.

Conclusions. The results of this research show that salinity treatment significantly affects the fish growth (WGR and SGR), feed conversion ratio (FCR), and benefit cost ratio (BCR). However, no statistical difference has been found between treatments A and B. The modelling results in the optimal salinity levels were 7.33 ppt for SGR, 7.47 ppt for survival rate (SR), 7.84 ppt for BCR, and 7.93 ppt for WGR. This research concludes that TGGG hybrid grouper can be reared at low salinity, but not at 0 ppt salinity for consumption fish production since water with 0 ppt salinity relates to lower appetite, decreased feed efficiency, and increased fish mortality.

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Conflict of interest. The authors declare that there is no conflict of interest.

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