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RESEARCH PAPER

Effects of Salinities and Diets on Growth of Juvenile Hybrid Grouper, *Epinephelus fuscoguttatus* × *E. lanceolattus*

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Abstract

The influence of salinities and diet on growth performance of tiger grouper (*Epinephelus fuscoguttatus*) × giant grouper (*E. lanceolatus*) (TG×GG hybrid), was investigated. TG×GG hybrid (10.0 ± 0.5 cm initial length and 50.5 ± 2.0 gram initial weight) were subjected to different salinities (10, 15, 20, 25 and 30 part per thousand (ppt)) and two diets, commercial pellet (42% protein, 9% ash, 11% lipid, 9% moisture) or trash fish, *Sardinella fimbriata* (70%protein, 15%ash, 4%lipid, 76% moisture) in triplicates with 15 fish per replication. At the end of the experiment (60 days), the final length, weight, feed consumption (FC), feed conversion ratio (FCR) and specific growth rate (SGR) were calculated. The highest mean SGR (1.05± 0.02% BM day^{-1}, P<0.05) was observed in the 15 ppt + pellet group of fish, while the lowest SGR was observed in the 30 ppt + trash fish group (0.40 ± 0.05% BM day^{-1}, P<0.05). The highest FC was significantly (P<0.05) observed at 15 ppt on both diets. The lowest FCR (1.21± 0.15, P<0.05) was observed in the 15 ppt on pellet diet. The results denotes that 15 ppt fed with commercial pellet is the optimum condition for growth performance of TG×GG hybrid grouper juveniles.

Keywords: Aquaculture, pellet, trash fish, feed conversion, salinity.

Introduction

Hybrid grouper, TG×GG is a newly developed fish that attains high value both in international and local markets. It is produced by fusion of Tiger grouper (*Epinephelus fuscoguttatus*) eggs and Giant grouper (*E. lanceolatus*) sperm (Looi & Senoo, 2008). It is now widely cultured all over Malaysia and Southeast Asia (Yusoff, 2015) due to its high quality taste and faster growth compared to its parental species (Mustafa, Senoo, & Luin, 2013). However, the production for the hybrid groupers are decreasing nowadays due to several environment stressors including poor water quality that includes dissolved oxygen (DO), pH and salinity (Yokoyama *et al.*, 2006). Fishes exposed to poor water quality over time are more susceptible to bacterial infections causing stress, poor growth and eventually mortality (Noga, 2011).

Reports has showed that salinity affects the growth capacities and physiological processes in teleost fish such as osmoregulation and metabolism (Singhabun & Kummee, 2015). Thus, by culturing the fish in optimum salinity, the energy can be reserved for the growth rates. Apart from salinity, the diet itself plays a vital role in the physiology and growth of fish (Ashley, 2007). The combined effects of temperature and diet (pellet and shrimp) on growth performance of TG×GG hybrid grouper has been documented recently (De, Ghaffar, Bakar, & Das, 2016). Meanwhile, combination of salinities and diets effects on growth have been described for juvenile sole, *Solea sole* and *S. senegalensis* (Imsland, Foss, Gunnarsson, & Berntssen, 2001). However, research on diet and salinity effect on growth properties of TG×GG hybrid grouper is still scarce in the literature. Feeding trash fish has always been the choice for the grouper farmers compared to pelleted feeds but it is not economical as the feed conversion ratio is slightly higher and the quality may be poor (Shapawi, Mustafa, & Ng, 2011). Thus, an alternative to switch to pelleted feed may contribute to success and sustainability of the grouper in aquaculture industry. This study was conducted to determine the effects of different salinity and diet on growth properties of TG×GG hybrid grouper juveniles for a period of 60 days in laboratory condition.

Materials and Methods

Initial Rearing

The TG×GG hybrid grouper (N= 450) was transported from a local hatchery in Banting, Selangor (2°'0"N, 101°'0"E) to the marine science laboratory UKM, Bangi, Selangor, Malaysia. The hybrid grouper samples were then distributed randomly among six stocking tanks (1.96 × 1.02 × 0.61 m, 1200 L in size and capacity) supplied with running sea water at 30 ppt salinity, temperature at approximately 26°C. The fish (75 fish/tank) were fed with the similar pellet diet used in the hatchery and acclimatized for two weeks.

Experimental Rearing

The fish with 10.0 ± 0.5 cm initial length and 50.5 ± 2.0 gram initial weight were randomly distributed into five salinities (10, 15, 20, 25 and 30 ppt) with triplicates of 15 fish/tank. The salinity level of natural seawater was determined with a reflecto-salinometer (S-10E; Atago Co., Ltd, Washington, DC, USA), and the salinity of each treatment was controlled by mixing sea water and fresh water at a rate of 2 ppt per day until the experimental salinity was reached. All fish were maintained in a flow through system (water exchange rate of 2 L/min) 356 L tank (123 × 63 × 46 cm) for 60 days. The fish were fed ad libitum (until satiation) twice daily (0800 and 1500 h) (Rimmer, 1998) with commercial pellet previously used in acclimatization (Star feed: Marine 9982/84, CP Group, Malaysia: 42% protein, 9% ash, 11% lipid, 9% moisture) and locally available trash fish, *S. fimbriata* (Department of Fisheries Malaysia, 2009: 70% protein, 15% ash, 4% lipid, 76% moisture). Proximate analysis for both diets were done according to methods by AOAC (1995). The water quality parameters during the experiment were: temperature 26.50 ± 0.03°C, pH 8.0 ± 0.1 and 7.10 ± 0.1 mg L^{-1} dissolved oxygen (DO). Temperature and DO were quantified using oximeter (WTWoxi,Weilheim, Germany), while pH was measured using pH meter (Thermo Scientific™, Benchtop). The diurnal cycle was set at 12 h light and 12 h dark. Body weight and length were recorded during 10 days interval while feed consumed were recorded daily during the experimental periods The food consumption during each meal was calculated as the difference between the mass of the given food and that of the uneaten food which was siphoned out of the tanks and immediately dried in an oven (60°C for 24 h).

Food Consumption, Feed Conversion Ratio and Specific Growth Rate

During the 10 days interval of the whole 60 days experimental period, five fish were sampled from each experimental tank and anesthetized using α-methyl quinolone (Transmore, Nika Trading) (0.22 ml L^{-1} in 3 L of sea water) for 10 minutes. The body weights were measured using an electronic balance (A & D, Model-GR-200) (Simon, Mazlan, Cob, Samat, & Arshad, 2008). Growth performance were evaluated based on the survival (%), weight gain (g), food consumption (FC) feed conversion ratio (FCR) and specific growth rate (SGR using the following equations:

FC (g day^{-1}) = g food consumed/ day (Pérez-Casanova, Lall, & Gamperl, 2009)

FCR (g feed g $gain^{-1}$) = amount of feed eaten/ wet weight gain (Lupatsch, Santos, Schrama, & Verreth, 2010)

SGR (% day^{-1}) = 100 × ((In final weight – In initial weight)/day) (Pérez-Casanova *et al.*, 2009)

Statistical Analysis

The growth parameters were tested for the normality and equality of variances prior to analyses. A two-factor factorial model was used to analyze the effects of diet and temperature on the survival, final length and weight, weight gain, FC, FCR and SGR of TG×GG hybrid grouper at 10, 15, 20, 25 and 30 ppt. When significant diet × temperature interactions were encountered, the cell means were analyzed in a one-factor linear model. Tukey post-hoc tests were performed when equality of variances was met; otherwise, the Games-Howell test was used. All statistical analyses were performed using SPSS version 23 (SPSS Inc., Chicago, USA). Significance differences between means was determined by Tukey's multiple range test at 95% significance level.

Results

Food Consumption, Feed Conversion Ratio and Specific Growth Rate

Analysis of variance indicate that the interaction of salinity and diet is significantly different ($P<0.05$) for all of the growth parameters except FC and FCR (Table 1). A pairwise comparison of the cell means was performed to find the combination of factors that produces the most suitable result (Table 2). In the tested salinities, FC was not influenced by the diet type, but, it was significantly ($P<0.05$) affected by different salinities (Table 2). It was observed that 15 ppt salinity showed the highest overall mean FC (11.95 ± 0.12 g day^{-1}, $P<0.05$). Meanwhile, the lowest overall FC was observed in 30 ppt salinity (4.82 ± 0.15 g day^{-1}, $P<0.05$). Also, the mean FC increased gradually when the salinities decreased from 30 ppt to 25 ppt to 10 ppt but increased at 20 ppt nevertheless the diet (pellet or trash fish) (Table 2).

Meanwhile, FCR was significantly affected diet type in all of the tested salinities, except for the 25 and 30 ppt fed with the trash fish and pellet respectively (FCR: 1.95 ± 0.02, 1.92 ± 0.18, $P<0.05$) (Table 2). The highest mean FCR was observed in the

Table 1. Mean squares from analysis of variance of the final length, final weight, FC, FCR and SGR of TG×GG

Source	df	Mean squares				
		Final length (cm)	Final weight (g)	FC (g day^{-1})	FCR	SGR (% BM day^{-1})
Covariate	1	2800.01	54263.1*	238.94*	48.368*	10.038
Salinity	2	0.204	2.48*	0.009*	0.05*	0.004
Diet	1	0.245	0.125*	8.482*	0.254*	0.002
Salinity* Diet	2	0.052	1.58*	0.125*	0.035*	0.001
Error	12	0.058	0.366	0.001	0.01	0.044

* P<0.05, *FC* - feed conversion, *FCR* - feed conversion ratio, *SGR* - specific growth rate, *df* - degree of freedom

Table 2. Tukey pairwise comparison cell means. Sample size for each diet salinity combination, n= 15

Salinity × Diet	Final length (cm)	Final weight (g)	FC (g day^{-1})	FCR	SGR (% BM day^{-1})
10 ppt + pellet	14.8 ± 0.13^{a}	58.0 ± 0.23^{a}	7.10 ± 0.34^{a}	1.64 ± 0.05^{a}	0.81 ± 0.05^{a}
10 ppt + trash fish	14.2 ± 0.11^{a}	56.7 ± 0.12^{a}	6.95 ± 0.22^{a}	1.78 ± 0.06^{b}	0.58 ± 0.08^{b}
15 ppt + pellet	15.7 ± 0.11^{b}	60.0 ± 0.15^{b}	11.95 ± 0.12^{b}	1.21 ± 0.15^{c}	1.05 ± 0.02^{c}
15 ppt + trash fish	15.0 ± 0.24^{c}	57.8 ± 0.26^{c}	11.18 ± 0.67^{b}	1.37 ± 0.06^{d}	0.77 ± 0.03^{d}
20 ppt + pellet	15.3 ± 0.18^{d}	59.0 ± 0.34^{d}	9.24 ± 0.46^{c}	1.40 ± 0.10^{e}	0.96 ± 0.02^{e}
20 ppt + trash fish	14.5 ± 0.14^{e}	57.3 ± 0.11^{e}	8.95 ± 0.35^{c}	1.55 ± 0.04^{f}	0.69 ± 0.02^{f}
25 ppt + pellet	14.2 ± 0.13^{f}	56.1 ± 0.12^{f}	5.88 ± 0.21^{d}	1.85 ± 0.08^{g}	0.60 ± 0.03^{g}
25 ppt + trash fish	13.5 ± 0.11^{f}	56.7 ± 0.13^{f}	5.60 ± 0.25^{d}	1.95 ± 0.02^{h}	0.60 ± 0.03^{g}
30 ppt + pellet	13.9 ± 0.14^{g}	55.6 ± 0.14^{g}	4.95 ± 0.28^{e}	1.92 ± 0.18^{ih}	0.53 ± 0.05^{h}
30 ppt + trash fish	13.2 ± 0.12^{g}	55.6 ± 0.14^{g}	4.82 ± 0.15^{e}	2.10 ± 0.10^{i}	0.40 ± 0.05^{i}

Value in the same column with different superscript letters are significantly different (P<0.05)
FC - feed conversion, *FCR* - feed conversion ratio, *SGR* - specific growth rate

30 ppt salinity (1.21, P<0.05) while the lowest mean FCR was observed at 15 ppt (2.10, P<0.05). A clear trend can be seen (Table 2, Figure 1 and 2) towards greater growth with increasing salinity, from minimum growth at 30 ppt to maximum growth at 15 ppt. The salinity and diet combination of 15 ppt + pellet produced the highest length and weight gain (15.7 ± 0.11 cm, 60.0 ± 0.15 g, P<0.05). Meanwhile, the lowest length and weight gain was observed in 30 ppt + trash fish (13.2 ± 0.12 cm, 55.6 ± 0.14 g, P<0.05) (Table 2, Figure 1 and 2). No significant differences (P>0.05) were observed in the mean length and weight recorded for both salinity and diet combination at the beginning of the experiment for all treatments (Figure 1 and Figure 2). On the other hand, significant differences could be observed in the mean length and weight among the group 15 ppt and 20, 10, 25, 30 ppt (P<0.05) groups during the experimental period. It was also observed that mean length and weight gain for the groups 25 and 30 ppt increased at lower rate compared to the 10, 15 and 20 ppt for both diets (Figure 1 and 2).

SGR was significantly (P<0.05) affected by the salinities and diet (Table 2). It was observed that the effect of salinities on SGR mirrored that of the effect of FC. SGR significantly increased (P<0.05) as the salinities decreased from 30 to 25 and 10 ppt but decreased gradually when the salinity was raised to 20 ppt and increased back when the salinity was lowered to 15 ppt (Table 2). The highest SGR was observed in the 15 ppt + pellet diet group (1.05 ± 0.02% BM day^{-1}, P<0.05), while the lowest SGR was observed in the 30 ppt + trash fish (0.40 ± 0.05% BM day^{-1}, P<0.05). The FCR and SGR observed in this study showed inverse relation in all the salinities tested. Combination of 15 ppt + pellet diet group had the lowest FCR and highest SGR (1.05 ± 0.02% BM day^{-1}, 1.21 ± 0.15, P<0.05).

Discussions

The range of water salinities used in this research was selected based on previous studies of the parental species and other grouper species (Table 3). In the TG×GG hybrid grouper, FC increased with decreasing salinities. This finding agrees with work reported for the hybrid *E. coiodes* x *E. lanceolattus* (Sutthinon, Thongprajukaew, Saekhow, & Ketmanee, 2015); flounder *Paralichthys orbignyanus* (Sampaio & Bianchini, 2002) and turbot *Scophthalmus maximus* (Imsland *et al.*, 2001) where decreasing in salinity leads to higher FC. It was observed that at all salinities, TG×GG hybrid grouper ate higher amount of pellet than trash fish. Also, high FC and FCR observed at salinities of 15 - 20 ppt. Thus, 15 - 20 ppt might be near a physiological salinity optimum for TG×GG hybrid grouper. This range of salinities is near to the parental species of the hybrid grouper,

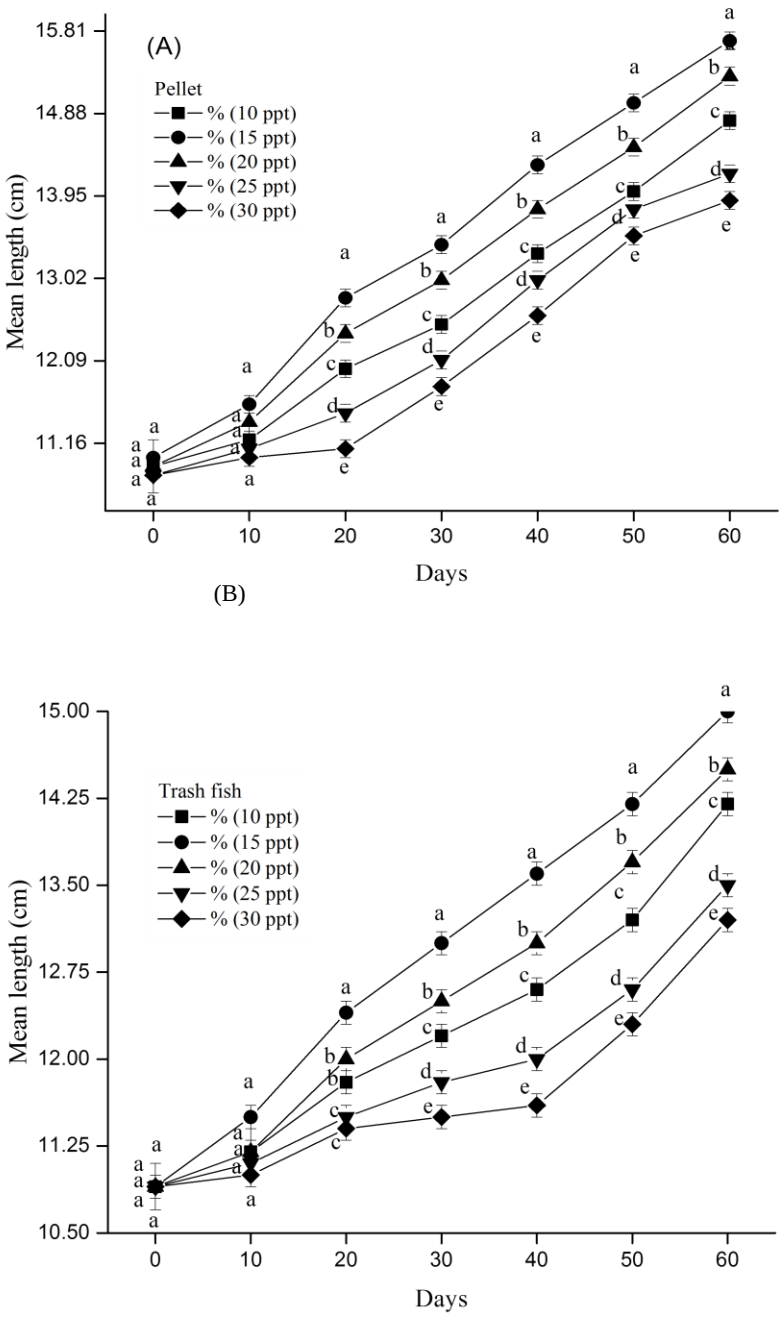

Figure 1. Mean length (cm) of TG×GG hybrid grouper (±SD) reared for 60 days at different salinities (10, 15, 20, 25 and 30 ppt) and two different diets, (A) pellet and (B) trash fish. Different letters indicate significant differences (Tukey post hoc test, $P<0.05$) between salinity groups at the same sample time point.

giant grouper *E. lanceolatus*, whereby the physiological optimum salinity is 15 - 25 ppt (Singhabun & Kummee, 2015). In this study, FCR increased with higher salinity. The lowest FCR was observed in 15 ppt followed by 20, 10, 25 and 30 ppt for both diets. Previous studies reported that high salinity may trigger stress, lower the feed intake and consequently resulted in poor growth as TG×GG hybrid grouper urge to balance for osmoregulation and ionic regulations (Handeland *et al.*, 2014). In high salinity (25 and 30 ppt), TG×GG hybrid juvenile might be in stressful condition as it consume more energy to regulate the concentration gradient between the blood and media. Therefore at higher salinity (> 20 ppt), TG×GG hybrid grouper require more energy and lessen the energy available for growth. Also,

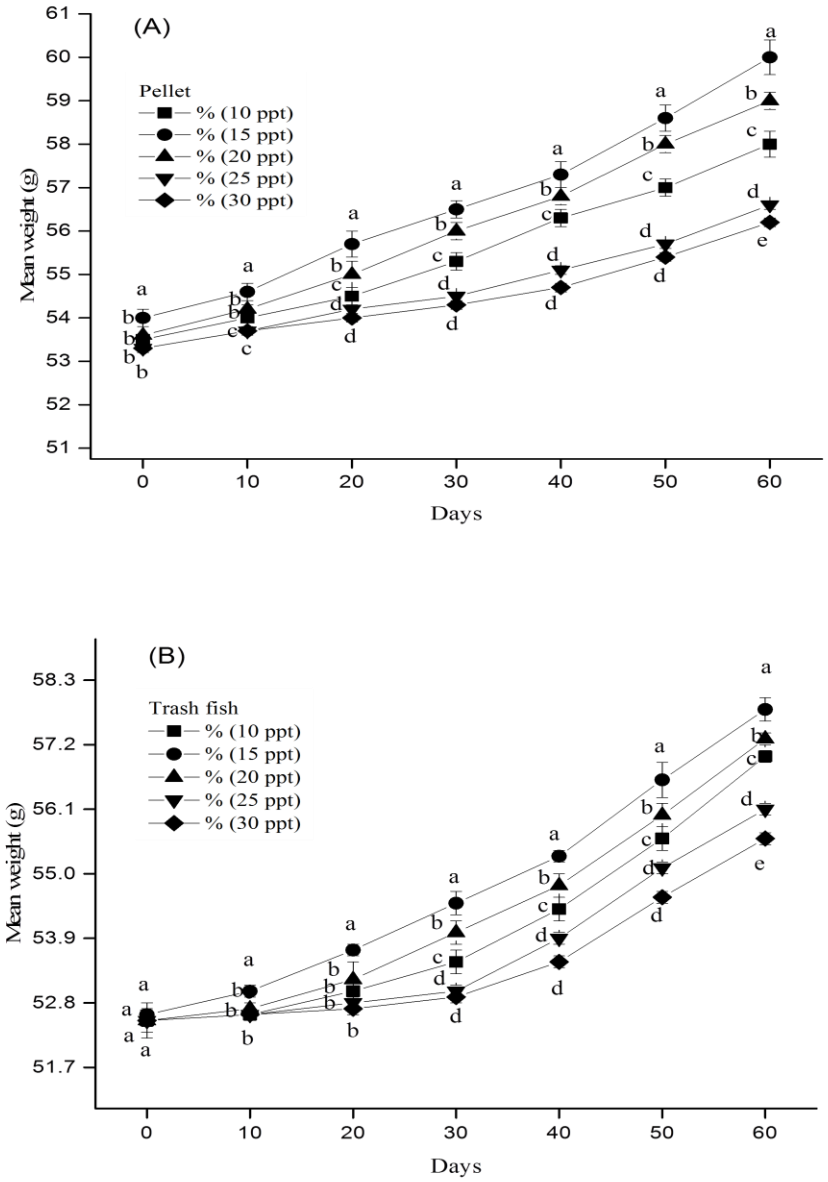

Figure 2. Mean weight (g) of TG×GG hybrid grouper (±SD) reared for 60 days at different salinities (10, 15, 20, 25 and 30 ppt) and two different diets, (A) pellet and (B) trash fish. Different letters indicate significant differences (Tukey post hoc test, P<0.05) between salinity groups at the same sample time point.

Table 3. Literature review on optimal salinity for different grouper species

Species	Stage	Optimal salinity	Reference
E. striatus	Larvae	32 -36	(Ellis, Watanabe, Ellis, Ginoza, & Moriwake, 1997)
E. coiodes	Larvae	16 - 24	(Toledo, Caberoy, Quinitio, Choresca, & Nakagawa, 2002)
E. marginatus	Juvenile	35	(Gracia López & Castelló-Orvay, 2003)
Cromileptis altivelis	Juvenile	15	(Yashiro, Sean-in, & Yaemkaseam, 2008)
E. coiodes	Juvenile	12 - 18	(Su-jiu *et al.*, 2011)
E. lanceolatus	Larvae	20	(Singhabun & Kummee, 2015)
E. lanceolatus	Juvenile	10 - 30	(Singhabun & Kummee, 2015)
E. coiodes × *E. lanceolatus*	Juvenile	10 - 30	(Sutthinon *et al.*, 2015)
E. fuscoguttatus × *E. lanceolatus*	Juvenile	10 - 30	(Othman, Kawamura, Senoo, & Fui, 2015)

salinity range that is beyond the optimal level will impact the energy costs for osmoregulation and causing poor growth (Sampaio & Bianchini, 2002).

Although trash fish has higher protein content compared to commercial pellet, poor FCR values and high FC observed in trash fish feeding is usual in aquaculture due to the losses of food during feeding (Bunlipatanon, Songseechan, Kongkeo, Abery, & Silva, 2014). Trash fish break up into small pieces and as much as 50% will be lost upon feeding unlike pellet (Sim *et al.*, 2005). Consequently, the feed loss may facilitate the transmission of bacteria and disease outbreak (Kim, 2015). In this study, it is observed that the trash fish fed TG×GG hybrid showed symptoms of bacterial infections such as fin rot and lesions towards the end of 60 days of culture. This might also lead to poor growth in trash fish fed group. The bacteria may be originating from the gut contents of the trash fish. It is common in aquaculture fish that is fed with trash fish is exposed to risk of bacterial infection (Austin & Austin, 2012).

It was observed that the growth rate was significantly affected by the salinities. The growth rate was the highest at 15 ppt in TG×GG hybrid fed with pellet (mean length: 15.7 ± 0.11 cm, mean weight: 60.0 ± 0.15 g, SGR: 1.05 ± 0.02% BM day^{-1}, $P<0.05$) while the lowest growth rate was found in 30 ppt on both diets (Figure 1 and 2, Table 2). Rearing juvenile TG×GG hybrid grouper in slightly higher salinity than 15 ppt has produced a slower growth performance. This is due to the potential of suppressing the appetite of the fish and reducing the feed intake (Tsui, Chen, & Cheng, 2012).

Previous studies reported that the growth performance was the highest in low salinity ranging from 12 - 18 ppt among orange spotted grouper *E. coiodes* (Su-jiu, Hai-fa, Jun, Yu-qing, & Shao-sen, 2011); however after reaching the peak salinity, the growth rate decreased significantly. The optimum salinity range where higher growth performance can be attained is defined as isotonic condition (Boeuf & Payan, 2001). This affects the metabolic rates and growth of fish. Previous work demonstrates that marine species which are euryhaline, e.g.: grouper in juvenile stages, have a better growth in lower salinity while freshwater species have a better growth in higher salinity (Singhabun & Kummee, 2015). Nevertheless, our results are not agree with previous results in hybrid grouper whereby no significant effects of food consumption and growth are observed in different diet treatments (Jiang *et al.*, 2015).

Conclusion

From the results, it can be concluded that the salinity and diet significantly affected the FC, FCR and SGR of TG×GG hybrid grouper juveniles. The findings specifies that growth performance in TG×GG hybrid grouper increase with decreasing salinities (10 - 20 ppt) however, decrease in extreme or stressful condition (25 and 30 ppt). It is suggested to culture the TG×GG hybrid grouper juveniles at 15 ppt since it gives the best FC, FCR and SGR. Also, TG×GG hybrid juveniles that are fed with pellet had better growth performances than trash fish group in all tested salinities. Therefore, it is also suggested that farmers should reduce the dependency on trash fish as feed based on the present study. The findings will be useful to provide proper salinity and diet combination (15 ppt and pellet) that enables to maximize the production of TG×GG hybrid grouper juveniles and promotes economic growth in the aquaculture field.

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