



# Effect of temperature and diet on growth and gastric emptying time of the hybrid, *Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂



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## ABSTRACT

The effects of temperatures (22, 26, 30 and 34 °C) and diets (commercial pellet and shrimp) on the growth properties and gastric emptying time (GET) of the tiger grouper × giant grouper (TGGG) hybrid were analyzed over a 30 day experimental period under controlled laboratory conditions. Food consumption (FC), food conversion rate (FCR), specific growth rate (SGR) and GET were significantly influenced by temperature and diet type. The highest mean SGR (1.00% BM day<sup>-1</sup>,  $p < 0.05$ ) was observed in the 30 °C + shrimp group of fish, while the lowest SGR was observed in the 22 °C + pellet group (0.59% BM day<sup>-1</sup>). No significant differences in growth ( $P > 0.05$ ) were observed between any of the groups at 22 and 34 °C fed on either the shrimp or the pellet diet. The lowest statistically significant ( $p < 0.05$ ) FC was observed at 22 °C on both diets. The highest FCR (1.208,  $p < 0.05$ ) was observed in the 22 °C + shrimp and 22 °C + pellet groups. The fastest GETs were observed at 30 °C, 12 h for fish on the shrimp diet and 13 h for fish on the pellet diet. A significant delay in gastric emptying (16 h) was observed at 22 °C in the group fed the commercial pellet diet (16 h). The best growth performances and digestion rates were observed at 30 °C followed by 26, 34 and 22 °C regardless of diet. The results suggest that 26 and 30 °C are optimum water temperatures for the aquaculture of this newly developed fish species fed on either a shrimp or pellet diet.

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## 1. Introduction

Groupers are economically valuable, especially in tropical and subtropical coastal fisheries (Purba and Mayunar, 1991; Zhou et al., 2006), and are widely distributed in the warm and temperate waters of most of the Earth's seas and oceans (Pierre et al., 2008). Grouper mariculture is most developed in Asia because of the high commercial value of these fish in Asian markets, particularly those of Hong Kong, Singapore and Taiwan (Tucker, 1999). Sixteen grouper species are raised on south-east Asian farms, the dominant species varies depending upon the country of origin (Sadovy, 2001). The giant grouper *Epinephelus lanceolatus* (Bloch 1790) and tiger grouper *E. fuscoguttatus* (Forsskål 1775) are principally raised on Malaysian fish farms. However, these two species are becoming less popular because of their slow growth rate (Senoo, 2006).

Through the development of hybridization technology, scientists have crossed giant grouper males and tiger grouper females and produced a new variety of grouper called the tiger grouper × giant grouper (TGGG) hybrid grouper, which is somewhat morphologically similar to its parental species (Ch'ng and Senoo, 2008). The cross breeding of these grouper fishes has been performed in land-based facilities (e.g., in hatchery and fishery research institutes) where conditions can be controlled. However, juveniles are then usually transferred to sea-cages (INFOFISH, 2012; Sufian and Nik Haiha, 2015) for maturation where they are exposed to changes in various environmental factors. For instance, fish is commonly maintained at 26 °C in commercial hatcheries under closed system and sea water temperature range in Malaysian waters is 27–30 °C (Silvestre and Pauly, 1997).

Like other ectothermic animals, fish are affected by the ambient water temperature, which can influence their appetite, food consumption rate, conversion efficiency (CE) (Bendiksen et al., 2002; Brown et al., 1989), growth rate (Brown et al., 1989) and overall physiological status (Azevedo et al., 1998; Britz et al., 1997; Houlihan et al., 1993). Generally, elevated water temperatures

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increase the energy cost of cold blooded animals to maintain their metabolism, thus decreasing growth and increasing the efficiency of food energy transformation to net energy (Brett and Groves, 1979; Pörtner et al., 2001; Van Ham et al., 2003). Water temperature is a major driving force in a fish's life. The preferred water temperature of a fish species is often closer to the optimal growth temperature rather than optimal food consumption (FC) or conversion temperatures (Jobling, 1997). Fish might also have different optimal temperatures at different life stages, which may reflect differences in temporal and spatial field distributions (Gadowaski and Caddell, 1991; Imsland et al., 1996). Although fish can generally function in a wide range of temperatures, they have an optimum temperature range, as well as lower and upper lethal temperatures for various activities (Beschta et al., 1987). The temperature-related growth rates of fish have been studied for several marine species, including tiger grouper (*E. fuscoguttatus* H.) (Lin et al., 2008), dusky grouper, *E. marginatus* (L.) (López and Castelló-Orvay, 2003), spotted wolffish *Anarhichas minor* (Ólafson) (Imsland et al., 2006), cod *Gadus morhua* (L.) (Björnsson et al., 2001; Imsland et al., 2005), turbot *Scophthalmus maximus* (L.) (Imsland et al., 2007), halibut *Hippoglossus hippoglossus* (L.) (Hallaraker et al., 1995; Jonassen et al., 1999), plaice *Pleuronectes platessa* (L.) and flounder *Platichthys flesus* (L.) (Fonds et al., 1992).

Beside temperature, the quality of food is thought to be another important determinants of fish physiology and ultimately the growth of fishes (Englund et al., 2011; Kooijman, 2000; Shapawi et al., 2014). The combined effects of diets and temperature on growth have been described for several fish species (Fonds et al., 1992; Imsland et al., 1996; Jonassen et al., 1999). As a newly developed fish species, there is little information on the TGGG hybrid in the literature except the suitable temperature for growth form and condition of TGGG hybrid (De et al., 2016).

Information on the gastric emptying time (GET) is an essential component for both field and laboratory studies to estimate the fish feeding rates, energy budgets, and daily ration of fish (Kawaguchi et al., 2007; Das et al., 2014; Sweka et al., 2004). GET is affected by various factors, including water temperature (Jobling, 1980; Singh-Renton and Bromley, 1996; Sweka et al., 2004), fish size (Bromley, 1987) and diet composition (Naik et al., 2000; Storebakken et al., 1999). Among them, water temperature and diet are being considered as the most influential factors on GET of fishes as they have a direct effect on feed intake and enzyme activity (Edwards, 1971; Kofuji et al., 2005) of fishes. GET for hybrid grouper has not been reported before, except for the brief report (De et al., 2014), where GET was partially investigated in relation to temperatures and a commercial diet pellet. Detailed studies on GET data for TGGG hybrid at different temperatures and diets (pellets and natural diet shrimp) have yet to be performed.

The objective of this study was to determine the effects of temperature and diet on the food consumption, food conversion rate, growth and gastric emptying time of the TGGG hybrid grouper.

## 2. Materials and methods

### 2.1. Sample collection and experimental setup

Hybrid grouper ( $N=384$ , length = 20 cm  $\pm$  0.50, weight = 194  $\pm$  2.90) from a local hatchery in Banting, Selangor (2°0'N, 101°0'E), were transported to the marine science laboratory of UKM, Bangi, Malaysia, ten days prior to the start of the experiment (January 17, 2014) and immediately distributed randomly among six stocking tanks (1.96  $\times$  1.02  $\times$  0.61 m, 1200 L in size and capacity). Each tank was supplied with running sea water at 30 psu salinity, and the temperature was kept at approximately 26°C and the fish (64 fish/tank) were fed the same pellet diet

used in the hatchery. Once the fish started feeding and defecating, 144 were randomly transferred to 24 experimental tanks (6 fish/tank) for a period of 30 days. The tanks were all equal in size (123  $\times$  63  $\times$  46 cm, 356 L). Twelve tanks randomly received the pellet diet, while the remaining twelve tanks received the shrimp diet for the duration of the experiment. For every experimental temperature change (22, 26, 30 and 34°C), three replicates were used. The temperature changes for the experimental groups were initiated at a rate of 2°C day<sup>-1</sup> using a heater (E-JET heater 200 W, Penang, Malaysia) and a chiller (HS-28 A, 250–1200 L/H, Guangdong Hailea Group Co. Ltd. Country of Origin: China) until the experimental temperature reached to desired temperatures (22, 26, 30 and 34°C). During the 30 day experiment, the fish were manually fed one of the two experimental diets (commercial pellet diet Star feed: Marine 9982/84, CP Group, Malaysia: 50% protein, 8% lipid and carbohydrate 7% or freshly thawed shrimp: *Acetes* sp.: 58% protein, 8% lipid and Carbohydrate 7.54% Manivannan et al., 2010) twice daily (0900 and 1600 h) (Rimmer, 1998) until apparent satiation was reached. Satiation was defined as the point when fish stopped actively feeding and pellets remained at the bottom of the tank for more than two minutes. Uneaten food was measured by siphoning pellets out of the tanks and drying them immediately to a constant mass in an oven (60°C for 24 h) or directly measuring the mass of shrimp as wet weight as they were fed to the fish. The amount of food consumed during each meal was calculated as the difference between the mass of the food offered and that of the uneaten food. During this period, all tanks were maintained on a 12 h light: 12 h dark photoperiod.

### 2.2. Food consumption, food conversion rate, and specific growth rate

The food consumption (FC, dry weight), food conversion rate (FCR) and specific growth rate (SGR) was calculated for individual fish using the following equations:

$$FC (g \text{ day}^{-1}) = (g \text{ food consumed} * \text{day}^{-1}) \text{ (Pérez-Casanova et al., 2009), } FCR = \text{food intake (g food on as fed basis) / body weight gain (g) (Lupatsch et al., 2010) and } SGR(\%BM\text{day}^{-1}) = 100 * (\ln \text{ final mass} - \ln \text{ initial mass}) * \text{day}^{-1} \text{ (Pérez-Casanova et al., 2009).}$$

### 2.3. Gastric emptying time

GET was studied using the remaining 240 fish samples. The fish were maintained under the previously described conditions, except that each replicate tank contained 10 fish instead of 6 fish. The fish were deprived of food for 3 days to ensure that their gastrointestinal tracts were empty. Thereafter, they were fed to apparent satiation (6–10% of body weight) with either the pellet or shrimp diet and sampled at 2 h, 6 h, 8 h, 12 h, 14 h, and 17 h post feeding. At each time point, six fish (one from each of the replicate tanks) were carefully captured with a net, killed with a blow to the head (cerebral percussion), and then dissected to remove the alimentary tract without disturbing the contents of the gut and the weight of digesta was recorded (Pérez-Casanova et al., 2009).

### 2.4. Statistical analyses

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 final length and weight, FCR and SGR of TGGG hybrid grouper at 22, 26, 30 and 34°C. The initial (0 day) length and weight were treated as a covariate in the analyses of the final (30 day) length and weight, respectively. When significant diet  $\times$  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. To determine differences among the experimental groups within each time period for length and weight gain and gastric emptying time, Tukey *post-hoc* tests were used. Statistical analyses were performed using Minitab Ver. 17 (StatSoft Inc., Tulsa, OK, USA).

### 3. Results

#### 3.1. Food consumption, food conversion rate and specific growth rate

Analyses of variance (Table 1) indicate that the interaction of temperature and diet is statistically significant ( $p < 0.001$ ) for all of the growth parameters, except FC and FCR. When an interaction effect is present, the interpretation of the main effects can be misleading. Therefore, pairwise comparisons of the cell means were performed instead, as this approach is more useful when it is of interest to find the combination of factors that produces the most desirable results (Table 2).

FC was not influenced by diet type at any of the tested temperatures; however, it was significantly influenced by different temperatures ( $p < 0.05$ ) (Table 2). The highest overall mean FC was observed at the 30 °C temperature (39.09 g day<sup>-1</sup>,  $p < 0.05$ ), while the lowest overall mean FC was observed at the 22 °C temperature (26.57 g day<sup>-1</sup>,  $p < 0.05$ ). The mean FC increased progressively as the temperature increased from 22 °C to 26 °C and 30 °C, but decreased at 34 °C, regardless of diet (pellet or shrimp; Table 2).

The highest mean FCR was observed at the 22 °C temperature (1.906,  $p < 0.05$ ), while the lowest FCR was observed at 30 °C (1.208,  $p < 0.05$ ). FCR was significantly influenced by diet type in all of the experimental temperatures, except for the 34 and 22 °C temperatures fed with the pellet diet (FCR: 1.851, 1.898,  $p > 0.05$ ; Table 2).

There was a clear trend (Table 2, Figs. 1 and 2) toward greater growth with increasing temperature, from minimum growth at 22 °C to maximum growth at 30 °C. The temperature and diet combination of 30 °C + Shrimp produced the highest length and weight gain (33.31 cm, 221.34 g,  $p < 0.05$ ), while the combination of 22 °C + pellets produced fish with the lowest length and weight gain (25.4 cm, 201.2 g,  $p < 0.05$ ) (Table 2, Fig. 1 and 2). No statistically significant differences ( $p > 0.05$ ) in the mean length and weight were recorded for temperature and diet combinations of 22 and 34 °C fed either pellet or shrimp diets at the beginning of the experiment ( $p > 0.05$ ); however, significant differences in the mean length and weight were observed between the 30 °C group and 26, 34, 22 °C ( $p < 0.05$ ) groups while experiment progressed. Furthermore, the mean weight gain for the 22 and 34 °C groups increased at a lower rate compared to the 26 and 30 °C groups for both diets (Fig. 2).

Diet and temperature also had a significant ( $p < 0.05$ ) effect on SGR. The effect of temperature on SGR largely mirrored that of the effect on FC. The results showed that SGR increased significantly ( $p < 0.05$ ) when the water temperature was raised from 22 to 26 and 30 °C and decreased gradually when the water temperature was raised over 30 °C (Table 2). The highest SGR was observed in the 30 °C + pellet diet group (1.00% BM d<sup>-1</sup>,  $p < 0.001$ ), whereas the lowest SGR (0.590% BM d<sup>-1</sup>,  $p < 0.001$ ) observed in the 22 °C + shrimp diet. Both temperature and diet affected SGR, representing a significant diet × temperature interaction (Table 2).

In TGGG hybrid fish, we observed that FCR and daily SGR showed typical inverse relationship over all of the temperature ranges. The 30 °C + shrimp diet group had the highest SGR and lowest FCR (1.00% BM day<sup>-1</sup>, 1.208,  $p < 0.01$ ). Fish reared at 26 °C fed with shrimp and 30 °C fed with pellets showed almost identical SGR and FCR (Table 2).

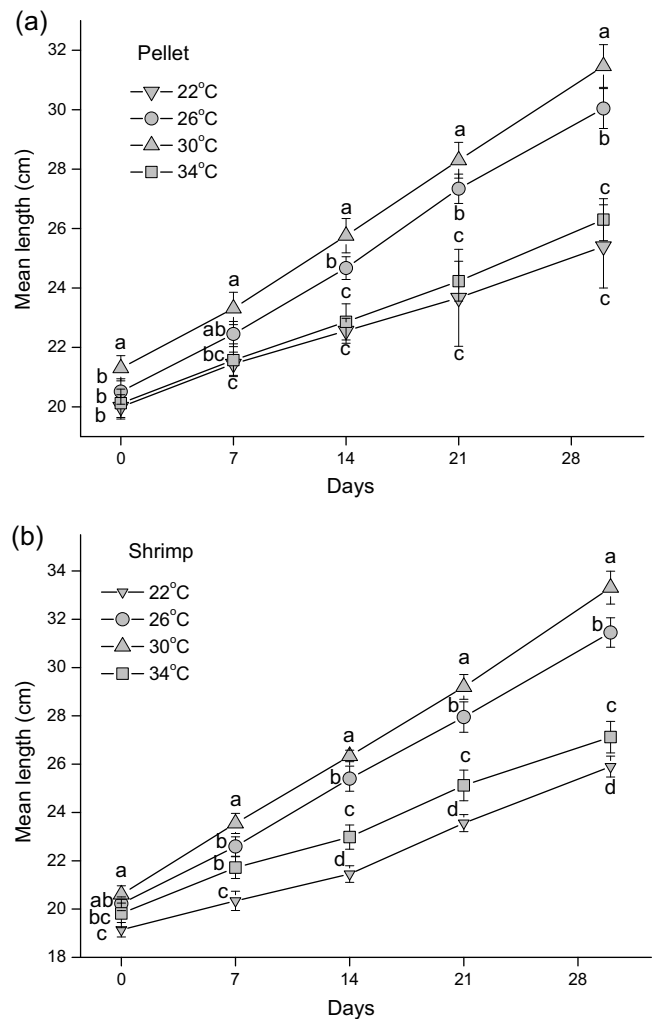


Fig. 1. Mean final length of TGGG hybrid grouper ( $\pm$ SE,  $n = 15$ ) reared for 30 days at four different temperatures (22, 26, 30, 34 °C) and two different diets, (a) pellet and (b) shrimp. Different letters indicate significant differences (Tukey *post hoc* test,  $p < 0.05$ ) between temperature groups at the same sample time point.

#### 3.2. Gastric emptying time

GET was greatly influenced by temperature and diet differences and was considerably faster as the temperature increased from 22 to 26 and 30 °C. GET decreased as the temperature rose from 30 to 34 °C. Moreover, at all temperatures, gastric emptying was considerably shorter in the shrimp than pellet diet (Fig. 3). The shortest gastric emptying was observed at 30 °C. Ten hours post feeding, the fish reared at 30 °C had digested the highest amount of food; those fed a pellet diet had 2.1 g of stomach residuum, while those fed a shrimp diet had 3 g of stomach residuum. Conversely, the longest gastric emptying occurred at 22 °C where fish fed a pellet diet or a shrimp diet had 4.2 & 7 g of stomach residuum after 6 h and 2.43 and 4 g after 10 h. For all of the variable groups, the amount of stomach residuum dropped steadily up to 2 h post feeding (Table 1, Fig. 3) and then reduced sharply until complete gastric evacuation. Diet variation showed significant differences in the time required for gastric emptying ( $p < 0.05$ ), with pellet fed fish having GET of 13, 14, 15 and 17 h at 22, 26, 30 and 34 °C and shrimp fed fish having GET of 12, 13, 15 and 17 h at 22, 26, 30 and 34 °C respectively (Fig. 3). Moreover, temperature had a significant ( $p < 0.05$ ) effect on the time required for gastric evacuation.

**Table 1**  
Mean squares from analysis of variance of the final length, final weight, food conversion rate and specific growth of TGGG hybrid grouper.

Source	df	Mean Squares				
		Final length (cm)	Final weight (g)	FC (g day <sup>-1</sup> )	FCR	SGR (% BM day <sup>-1</sup> )
Covariate	1	736.47*	195213*	–	–	–
Temperature	3	17.16*	548*	97.291*	2.005*	0.504*
Diet	1	1.69†	139*	0.877	0.906*	0.103†
Temp*Diet	3	0.54†	32	9.396	0.019	0.042
Error	15 <sup>a</sup>	0.03	2	4.968	0.016	0.007

df: degree of freedom, cm: centimeter, g: gram, FC: Food consumption, FCR: Food Conversion Rate, SGR: Specific Growth Rate.

<sup>a</sup> Error df for FCR and SGR/day is 16.

†  $p < 0.05$ .

**Table 2**  
Tukey pairwise comparisons cell means. Sample size for each diet temperature combination, n = 15.

Temp × Diet	Final Length (cm)	Final weight (g)	FC (g day <sup>-1</sup> )	FCR <sup>a</sup>	SGR (% BM day <sup>-1</sup> )
22 °C + Shrimp	25.9 <sup>a</sup>	205.67 <sup>a</sup>	28.49 <sup>a</sup>	1.906 <sup>a</sup>	0.601 <sup>a</sup>
22 °C + Pellet	25.4 <sup>a</sup>	201.2 <sup>a</sup>	26.57 <sup>a</sup>	1.898 <sup>ab</sup>	0.590 <sup>b</sup>
26 °C + Shrimp	31.45 <sup>b</sup>	216.67 <sup>b</sup>	36.14 <sup>b</sup>	1.401 <sup>c</sup>	0.827 <sup>c</sup>
26 °C + Pellet	30.04 <sup>c</sup>	213.45 <sup>c</sup>	35.56 <sup>b</sup>	1.601 <sup>d</sup>	0.796 <sup>d</sup>
30 °C + Shrimp	33.31 <sup>d</sup>	221.34 <sup>d</sup>	39.09 <sup>c</sup>	1.208 <sup>e</sup>	1.000 <sup>e</sup>
30 °C + Pellet	31.47 <sup>eb</sup>	217.45 <sup>eb</sup>	38.58 <sup>c</sup>	1.399 <sup>f</sup>	0.829 <sup>fd</sup>
34 °C + Shrimp	27.12 <sup>f</sup>	209.34 <sup>f</sup>	32.10 <sup>d</sup>	1.703 <sup>g</sup>	0.701 <sup>g</sup>
34 °C + Pellet	26.3 <sup>f</sup>	208.94 <sup>f</sup>	31.60 <sup>d</sup>	1.85 <sup>hb</sup>	0.698 <sup>h</sup>

The lower case letter <sup>a-h</sup> means that do not share a letter are significantly different. <sup>a</sup> Grouping information for FCR is based on the Games-Howell method.

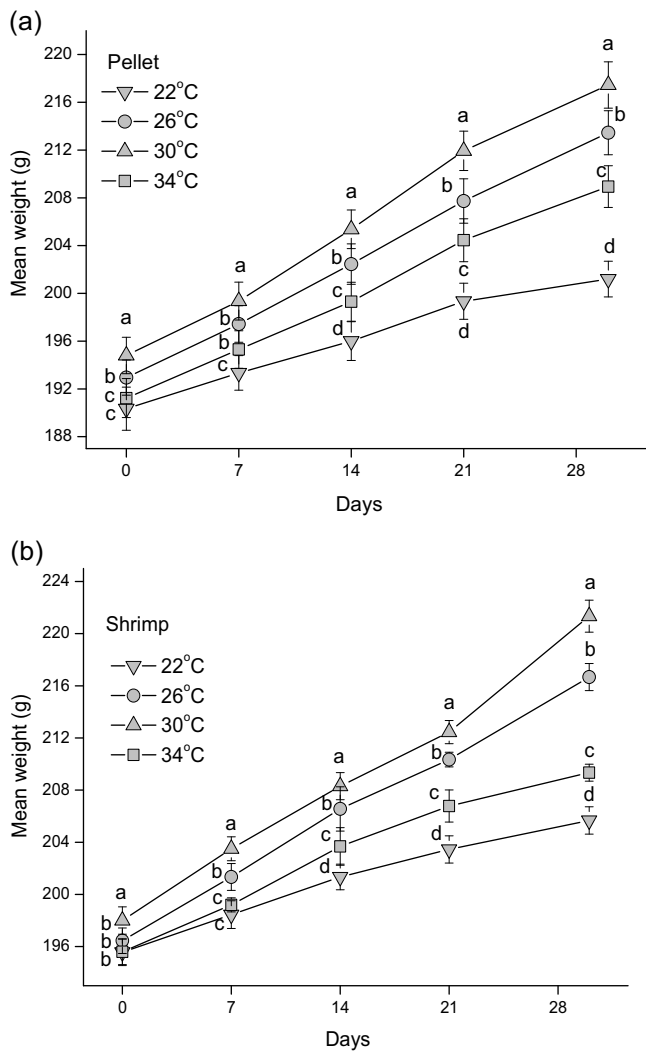
#### 4. Discussion

In the present study, FC decreased with decreasing temperature, as reported for the Humpback grouper (*Cromileptes altivelis*) (Sugama et al., 2004), Orange-spotted grouper (*Epinephelus coioides* Hamilton) (Lin et al., 2008), Atlantic salmon (*Salmo salar* L.) (Bendiksen et al., 2002; Handeland et al., 2008) and Atlantic halibut (*Hippoglossus hippoglossus* L.) (Jonassen et al., 2000). We observed that at all temperatures TGGG hybrid fish ate a greater amount of shrimp than pellets and that the highest FC and FCR occurred at temperatures of 26–30 °C. Therefore, 26–30 °C may be near a physiological temperature optimum for this species. This temperature range is closer to that of most other fishes (Buckel et al., 1995; Liu, 1998) as well as the Nassau grouper's (*Epinephelus striatus*) physiological temperature optimum of 28–31 °C (Ellis et al., 1997), but is lower than the Orange-spotted grouper's optimal physiological temperature of 33.9 °C (Lin et al., 2008). An increase in FC and FCR as the temperature increases to an optimum has also been observed in hogchoker (Peters and Boyd, 1972), channel catfish (Murray et al., 1977), and sea bass (Hidalgo and Alliot, 1988; Hidalgo et al., 1987). In sea bass, improved feed conversion at higher temperatures was attributed to increased protein digestibility caused by increased trypsin activity in the gut. In contrast, hybrid striped bass × white bass showed optimum feed conversion at 21.2 °C and maximum growth and consumption at 26.8 °C (Woiwode and Adelman, 1991). Decreased feed conversion at higher temperatures was attributed to higher energy maintenance requirements usurping dietary energy that might have been used for growth. Other than at 22 °C, the FCR of the TGGG hybrid grouper decreased at all other temperatures. The highest FCR was observed at 22 °C followed by 34, 26 and 30 °C fed on both diets. The present study results showing that the TGGG hybrid grouper had a higher FCR (1.2–1.90) than reported in the Nassau grouper (1.04–1.23) might affect different temperature and food uses. Despite the fact that the two diets used in this study were different in composition, with the shrimp diet having more protein content (58%) than the pellet (50%) diet may influence the growth performance of TGGG hybrid. However, our findings are not agreement with Jiang et al. (2015) as they described that diet differences do not affect food consumption and growth performance of hybrid grouper (Jiang et al., 2015).

In this study, we observed that the growth rate was significantly ( $p < 0.05$ ) influenced by temperature. The growth rate was highest in TGGG hybrid fish fed a shrimp diet at 30 °C (mean length: 33.31 cm, mean weight: 221.34 g) SGR: 1.00% BM d<sup>-1</sup>,  $p < 0.05$ ). However, at 22 and 34 °C, the growth rates were equal or only slightly lower, while at 22 °C, fish showed significantly lower growth rates on both diets (Fig. 1, 2, Table 2). The increased growth of the TGGG hybrid grouper at elevated temperatures agrees with previous studies on Atlantic salmon (Handeland et al., 2000, 2003; Solbakken et al., 1994); however, after reaching the peak temperature, the growth rate decreased significantly ( $p < 0.05$ ), as previously reported (Jobling, 1994; Solbakken et al., 1994).

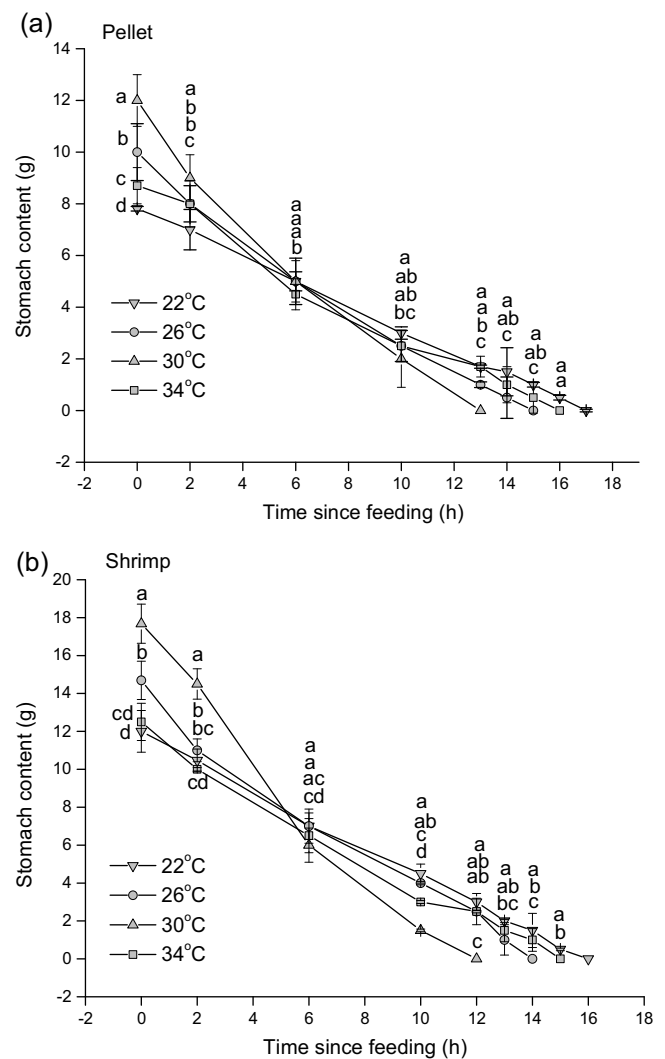
The growth of fishes increases rapidly before going into gradual decline when their upper lethal limit is approached (Kinne, 1960; Woiwode and Adelman, 1991). The growth rate for TGGG hybrid groupers increased from 26 °C to 30 °C and then decreased at 34 °C. However, other studies have found that some species showed a relatively flat relationship between growth and temperature (Björnsson and Tryggvadóttir, 1996; Burel et al., 1996; Larsson and Berglund, 1998). A higher water temperature imposes two antagonistic effects on growth; increasing temperature has a negative effect on growth due to the higher energy cost of the maintenance metabolism and also has a positive effect due to the higher efficiency of transforming food energy into net energy (Xiao-Jun and Ruyung, 1992). Collectively, our findings show that while the growth rate is highly temperature-dependent in the TGGG hybrid, the growth of the TGGG hybrid is more severely impacted by exposure to cold temperatures (Table 2). This suggests that juvenile TGGG hybrid groupers between 186 and 194 g have a fairly large temperature optimum interval (26–30 °C) for growth, which is of interest and important for commercial aquaculture (Imsland et al., 1996). The wide ranges of temperature optima were also observed in Nassau grouper, at 28–31 °C (Ellis et al., 1997), and orange spotted grouper (25–35 °C) (Lin et al., 2008). Marked temperature-related differences in the growth rates of juvenile TGGG hybrid grouper may have important implications in better aquaculture practices while culturing in earthen pond by maintaining the suitable temperature ranges.

In the TGGG hybrid grouper, GET was shorter at all temperatures in fish fed the shrimp diet than those fed the pellet diet. GET



**Fig. 2.** Mean final weight of TGGG hybrid grouper ( $\pm$ SE,  $n=15$ ) reared for 30 days at four different temperature (22, 26, 30, 34 °C) and two different diets, (a) pellet and (b) shrimp. Different letters indicate significant differences (Tukey post hoc test,  $p < 0.05$ ) between temperature groups at the same sample time point.

was found to be highly temperature dependent in our study. At 22 °C and 34 °C, the fish samples had longer GET, indicating that the fish were in a stressful condition, as previously reported by Swenson and Smith (1973). It is very likely that this decrease in GET at 34 and 22 °C was due to the direct effect of temperature on the metabolic rate of fish samples. A faster rate of gastric emptying may contribute to the high consumption of food and higher growth rate of the TGGG hybrid grouper. On the other hand, a slow rate of gastric emptying may contribute to the low consumption of food and slow growth of TGGG hybrid grouper (personal observation). This is the first report of food passage rates in TGGG hybrid groupers or any other grouper fishes. A 12–17 h time period to completely eliminate pellet and shrimp food from the alimentary tract in TGGG hybrid groupers is shorter than the GET reported for other carnivorous fishes, including brook trout [*Salvelinus fontinalis* (Mitchill, 1814) = approximately 70 h; Sweka et al., 2004]; whiting [*Merlangius merlangus* (Linnaeus, 1758) = 72 h; Mazlan and Grove, 2003]; and banded archerfish (*Toxotes jaculatrix*: 96 h) (Das et al., 2014). The differences in GET are likely due to differences in type of experimental food and feeding regime used in the study (Fines and Holt, 2010; Flowerdew and Grove, 1979; Fry and Milton, 2009).



**Fig. 3.** Gastric emptying in TGGG hybrid ( $\pm$ SE,  $n=6$ ) at four temperatures, 22, 26, 30 and 34 °C and two diets, (a) pellet and (b) shrimp. Different letters indicates significant differences ( $p < 0.05$ ) between temperature groupers at the same sample time point.

## 5. Conclusion

The results of this study showed that water temperature and diet have significant effects on the FC, FCR, SGR and GET of the TGGG hybrid. An elevated water temperature between 26 °C and 30 °C has a positive effect on the growth performance and digestion time of the TGGG hybrid. Conversely, changes in water temperature, from 26 to 34 °C and 26–22 °C, will have a significant negative impact on the overall growth performance and gastric emptying rate, as we observed the lower SGR and longer GET at 22 °C and 34 °C. At all temperatures, the TGGG hybrid that were fed more shrimp than pellets had better growth rates. Overall, it is possible to conclude that a temperature between 26 and 30 °C is optimal for the growth of TGGG hybrid and that 22 °C and 34 °C are close to the confining temperatures for the fish. The data obtained from this study contribute to the standardization of the management of this important fish species during its grow-out phase. However, effective care should be taken while culturing TGGG hybrid in sea cages to prevent the fish escaping to the natural environment and genetic introgression with congeneric species.

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