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Optimum Temperature for the Growth Form of Tiger Grouper (*Epinephelus fuscoguttatus* ♀) × Giant Grouper (*E. lanceolatus* ♂) Hybrid

(Suhu Optimum untuk Tumbesaran Hibrid Kerapu Harimau (*Epinephelus fuscoguttatus* ♀) × Kerapu Gergasi (*E. lanceolatus* ♂))

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ABSTRACT

The growth form and condition of the tiger grouper × giant grouper (TGGG) hybrid were evaluated under four temperatures (22°C, 26°C, 30°C, and 34°C) and two diets (pellet and shrimp). The growth form of fish was determined over a 30 day experimental period using the length–weight relationship (LWR) method. The TGGG hybrid grouper exhibited a negative allometric growth (b < 3) at all temperatures and diets at the beginning of the experiment (0 day), which indicated that fish would become lighter as they increased in size. Conversely, toward the end of the experiment (30 d), the TGGG hybrid grouper showed a negative allometric growth (b < 3) at 22°C and 34°C on both pellet and shrimp diet as well as demonstrated an isometric growth (b = 3) at 26°C fed on both diet and 30°C on pellet diet only, which indicated that the shape of the fish would not change with increasing weight and length. However, a positive allometric growth (b > 3) was observed at 30°C when the fish were fed on shrimp diet, which indicated that fish weight would increase with increasing length and size. The condition factors (K and Kn) in the initial (0 d) and final (30 d) measurements were greater than 1, thereby indicating the wellbeing of the TGGG hybrid grouper, except for those fish fed on both pellet and shrimp diet under 22°C and 34°C. Therefore, diet did not have a significant effect (p > 0.05) on the growth and condition of TGGG hybrid grouper, whereas temperature significantly influenced the growth and condition of the fish. All in all, grouper farmers can culture TGGG hybrid grouper between 26°C and 30°C, whereas the best growth and condition could be observed by culturing the fish at 30°C fed on shrimp.

Keywords: Aquaculture; condition factor; feeding; length-weight relationship; management

ABSTRAK

Bentuk pertumbuhan dan keadaan hibrid kerapu harimau × kerapu gergasi (TGGG) telah dinilai pada empat suhu (22°C, 26°C, 30°C dan 34°C) dan dua diet (pelet dan udang). Bentuk pertumbuhan ikan telah ditentukan dalam tempoh percubaan 30 hari menggunakan kaedah hubungan panjang-berat (HPB). Hibrid kerapu TGGG menunjukkan pertumbuhan alometri negatif (b <3) pada semua suhu dan diet pada awal eksperimen (0 hari), yang menunjukkan bahawa ikan akan menjadi lebih ringan apabila saiznya meningkat. Sebaliknya, di akhir eksperimen (30 hari), hibrid kerapu TGGG menunjukkan pertumbuhan alometri negatif (b <3) pada 22°C dan 34°C bagi kedua-dua diet pelet dan udang serta menunjukkan pertumbuhan isometri (b = 3) pada 26°C untuk kedua-dua diet dan 30°C pada diet pelet sahaja, yang menunjukkan bahawa bentuk ikan itu tidak akan berubah dengan peningkatan berat badan dan panjang. Walau bagaimanapun, pertumbuhan alometri positif (b> 3) diperhatikan pada 30°C apabila ikan diberi makan diet udang, yang menunjukkan bahawa berat badan ikan akan meningkat dengan peningkatan panjang dan saiz. Faktor keadaan (K dan Kn) pada permulaan ukuran (0 hari) dan akhir (30 hari) adalah lebih besar daripada 1, menunjukkan hibrid kerapu TGGG membesar dengan baik, kecuali ikan yang diberi makan pada kedua-dua diet pelet dan udang pada 22°C dan 34°C. Oleh itu, diet tidak mempunyai kesan yang signifikan (p > 0.05) kepada pertumbuhan dan keadaan hibrid kerapu TGGG, manakala suhu mempengaruhi pertumbuhan dan keadaan ikan secara signifikan. Kesimpulannya, penternak kerapu boleh mengkulturkan kerapu hibrid TGGG antara 26°C dan 30°C, manakala pertumbuhan dan keadaan terbaik boleh diperhatikan dengan mengkulturkan ikan pada 30°C yang diberi makan udang.

Kata kunci: Akuakultur; faktor keadaan; hubungan panjang-berat; makan; pengurusan

INTRODUCTION

The commonly cultured giant grouper (*Epinephelus lanceolatus*) and tiger grouper (*E. fuscoguttatus*) are listed as vulnerable and threatened species, respectively, in the IUCN Red List of Threatened Species (IUCN 2014). These species are not very popular in mariculture because of their slow growth rate (Senoo 2006). To resolve this issue, scientists have developed a new strain of hybrid grouper (TGGG) by crossing *E. lanceolatus* and *E. fuscoguttatus* (Ch'ng & Senoo 2008). The TGGG hybrid grouper has

become an important target species in aquaculture and has been commercialized globally, especially in Hong Kong (Senoo 2010). Grouper is also popular in Malaysian cuisine, indicating the high demand for aquacultured grouper; therefore, an increasing amount of studies have investigated the optimum growth and condition of this species.

Water temperature is one of the most important factors that influence the growth of fish (Björnsson et al. 2007; Pérez-Casanova et al. 2009). Therefore, in relation to aquaculture facilities, the temperature profiles at each geographic location may profoundly influence the economic efficiency and competitiveness of farming commercially important fishes (Árnason et al. 2009; Björnsson et al. 2001; Brander 1994; Jobling 1988). Grouper farms in Malaysia are mostly sea-cage types (INFOFISH 2012) and only few are land-based, pump-ashore facilities. Given that the land-based farming of grouper is costly in terms of production and investment, a reliable model for predicting the growth and condition of TGGG hybrid grouper at different temperatures must be devised.

The growth form i.e. length and weight and its relationship (LWR) is crucial in fisheries biology research (Martin-Smith 1996; Petrakis & Stergiou 1995) and in evaluating fish stocks (Froese & Pauly 2004; Gonçalves et al. 1997; Morato et al. 2001). Length and weight data can also provide important clues regarding climate and environmental changes (Sarkar et al. 2013). The mathematical parameters of the relationship between the length and weight of fish may provide additional information on the weight variation of individuals in relation to their length (condition factors *K* and *Kn*) (Froese 2006). The condition factors of some fish species may indicate their sexual maturity, food availability, age, and sex (Anibeze 2000; Godinho 1997). The condition of a fish is reflected on its recent physical and biological circumstances and may fluctuate under various feeding conditions, health conditions, and physiological factors (Le Cren 1951; Weatherley & Gill 1987). Body condition provides a surrogate measure to the expensive *in vitro* proximate analyses of tissues (Sutton et al. 2000). Therefore, information on growth form and condition plays a vital role in culture system management by informing the producer on the specific conditions under which organisms can develop (Araneda et al. 2008). This study aims to determine the optimum temperature for the growth form and condition of TGGG hybrid grouper in captivity.

Materials and Methods

Sample collection and experimental setup

The TGGG hybrid grouper samples (N = 120) were collected from the local hatchery of Banting (2°49′0″N, 101°30′0″E), Selangor, Malaysia and were transported to the marine science laboratory of UKM, Bangi, Malaysia. The fish were housed in several 100 L tanks for 5 d at 26°C and were fed on the same commercial pellet used in the hatcheries. After feeding on pellets and defecating, the fish were separated into four identical experimental tanks, with each tank (350 × 170 × 125 cm) with two compartments (15 fish in each compartment). The compartments were separated by a plexiglass with holes to ensure similar water temperature across the tank. The fish in two compartments were fed twice daily (Rimmer 1998) at 9:00 and 16:00 h at a rate of 10% of body mass per day on commercial pellet (Star feed: Marine 9982/84, CP Group, Malaysia: 50% protein and 8% lipid) and on freshly thawed shrimps (*Acetes* sp. 58% protein and 8% lipid, Manivannan et al. 2010). The uneaten food was siphoned from the tanks. During this period, both treatments were maintained under 12 h light and 12 h dark photoperiod. The temperature changes for the experimental groups were initiated at a rate of 2°C day^{-1} using a heater (E-JET heater 200 W, Penang, Malaysia) and a chiller (HS-28 A, 250-1200L/H, Guangdong Hailea Group Co., Ltd., Country of Origin: China) until the experimental temperature reached a minimum of 22°C and a maximum of 34°C. The fish were then deprived of food for 2 days and were anesthetized with α-methyl quinoline (TransmoreR; Nika Trading, Puchong, Malaysia) at 0.22 mL L^{-1} in 3 L of sea water as an anesthetic medium for 10 to 15 min prior to the initial measurement of body weight (Wt) and total length (TL) (Das et al. 2014b). TL was measured to the nearest 0.01 cm using a measuring board, whereas Wt was measured to the nearest 0.01 g using an electronic balance (Model: KD-300KC) (Simon et al. 2012). The samples were weighed every week and the feeding rate was adjusted accordingly (fed shrimp and pellet at a rate of 10% of body mass). During the 30 d experimental period, the water salinity and pH range were maintained at 30.0 ppt and from 6.0 to 7.5, respectively (Luin et al. 2013).

Length-weight relationship

The effect of different temperature regimes and diets on the growth of the experimental fish was investigated via LWR analysis. LWR was calculated as follows: $W = aL^b$ (Pauly 1984), where W is body weight *(BW)*, L is total length (TL), a is the intercept, and b is the slope. The values of the exponent b provide information on fish growth form (Beverton & Holt 1966). The estimated changes in growth form can help visualize the growth pattern and condition of the fish by using different variables (e.g. temperature and diets). The a and b values were determined through a non-linear regression of which the curve fitting was performed by Chi-square (χ^2) iterative methods using Levenberg-Marquardt and Simplex algorithms readily developed in MicroCalc. Origin™ Version 9.0 computer software (Das et al. 2014a, 2014b; Simon et al. 2013, 2010). The measurement of model fit (goodness-of-fit of calculated TL and BW) was evaluated by the coefficient of determination (r^2). Student's t-test (Zar 1996) was performed on the slope of log W-log TL to test whether the computed b was significantly different from 3. The b

value indicated the growth type of the fish (Spiegel 1991), which may be isometric ($b = 3$), positive allometric ($b > 3$), or negative allometric ($b < 3$). Analysis of covariance (ANCOVA) was also applied to evaluate the homogeneity of the regression slopes between the initial (IM) and final measurements (FM) at each temperature and diet. Log BW was modeled as a function of stage (IM or FM), whereas log TL was modeled as a covariate of interaction. The interaction term tested the homogeneity of the slopes. The computer software MINITAB 17 (StatSoft Inc., Tulsa, OK, USA) was used for statistical analyses. A statistical significance of 5% was adopted for all cases.

CONDITION FACTORS

The Fulton's condition factor (K) was calculated for each fish according to the equation $K = W \times \left(\frac{100}{L^3}\right)$ (Fulton 1904), where W denotes body weight (BW, g), L denotes total length (TL, cm) and 100 is a factor that brings the value of K near unity. The relative condition factor Kn was calculated using the equation $Kn = \frac{W}{aL^b}$ (Le Cren 1951), where a is the intercept and b is the slope. To determine the effects of temperature on initial and final condition factors (K and Kn) of TGGG hybrid grouper fed on pellet and shrimp diets, paired t-test were conducted. Differences were considered significant when $p < 0.05$.

RESULTS

LENGTH-WEIGHT RELATIONSHIP

The LWR was derived from 120 TGGG hybrid grouper samples. The TLs of the samples ranged from 18 to 24 cm and the BWs of the samples ranged from 138 to 270 g. Figure 1 shows the nonlinear fit of the LWR of the TGGG hybrid grouper, whereas Table 1 presents the regression coefficients b of the log-transformed variables and the growth type of the TGGG hybrid grouper. The TGGG hybrid grouper exhibited a negative allometric growth ($b < 3$) in IM (0 day) at all experimental temperatures and diets, which indicated that the fish became lighter with increasing size. A similar growth pattern was also observed in FM (30 d) under 22°C ($p > 0.05$, $df = 13$, $S.E. = 0.063$) and 34°C ($p > 0.05$, $df = 13$, $S.E. = 0.101$) for both diets. Conversely, in FM, the fish demonstrated an isometric growth ($b = 3$) at 26 °C when fed on pellet ($p < 0.05$, $df = 13$, $S.E. = 0.108$) and shrimp diets ($p < 0.05$, $df = 13$, $S.E. = 0.202$) and at 30°C when fed on the pellet diet ($p < 0.05$, $df = 13$, $S.E. = 0.01$), thereby indicating that the shape of the fish did not change with increasing weight and length. A positive allometric growth ($b > 3$) was observed at 30°C for shrimp-fed fish ($p < 0.05$, $df = 13$, $S.E. = 0.118$), which indicated that the fish became heavier as they increased in length and size. ANCOVA analysis showed significant differences ($p<0.05$) between the slopes of IM and FM only at temperatures of 26°C and 30°C, whereas no significant differences ($p>0.05$) were observed at other temperatures (Table 1). The regression model fitting in IM and FM showed that TL and BW of the TGGG hybrid grouper were highly correlated ($r^2 = 0.94–0.99$).

CONDITION FACTORS

The mean K and Kn in relation to temperature and diet are shown in Figure 2. The condition factors ranged from K: 2.08–2.32 in IM and 0.95–2.81 in FM and Kn: 1.40–1.63 in IM and 0.735–1.904 in FM. In IM, K and Kn values did not vary much at the experimental temperatures and when fish were fed on both diets (Figure 2). Conversely in FM, K values were significantly ($p < 0.05$) lower at 22°C and 34°C and higher at 26°C and 30°C. Similarly, in FM, the Kn values were significantly ($p < 0.05$) lower at 22°C and 34°C and higher at 26°C and 30°C, thereby suggesting poor and good fish conditions, respectively (Table 1).

DISCUSSION

In this study, we observed that TGGG hybrid grouper exhibited negative allometric growth ($b < 3$) in the beginning of the experiment (day 0) at all temperature and diets, whereas the opposite was observed in FM (day 30). Isometric growth was observed at 26°C when fish were fed on both diets and at 30°C when fish were fed on pellet. Positive allometric growth was observed at 30°C when fish were fed on shrimp. Negative allometric growth was observed at 34°C and 22°C when fish were fed on both diets (Table 1). The LWRs of TGGG hybrid grouper raised in similar temperatures and diets have not been previously recorded in Malaysia or elsewhere, thereby preventing a direct comparison with earlier results. The only report on LWR of TGGG hybrid grouper in Malaysia was at different temperatures in the range 28°C-29.5°C and negative allometric growth was documented (Luin et al. 2013), which is in agreement with all temperature ranges at the beginning of the experiment (day 0) and 22°C and 34°C in FM (30 d). This result indicated that fish becomes lighter as it increased in size. Moreover, the calculated isometric growth (b = 3) at 26°C when fish were fed on pellet and shrimp and that at 30°C when fish were fed on pellet diet in FM are in harmony with their parental species namely, giant grouper (*E. lanceolatus*) $b = 3$ (IGFA 2001) and tiger grouper (*E. fuscoguttatus*) $b = 3$ (Agembe et al. 2010; Kulbicki et al. 2005; Palomares & Pagdilao 1988) in other geographic locations (Table 2). At 22°C and 34°C, fish represented negative allometric growth in both IM and FM when fed on shrimp and pellet diets, as observed with other *Epinephelus* sp. (Table 2). This variation in allometric coefficient for TGGG hybrid grouper in this study might be attributed to differences in locations, feeding conditions, temperatures, sample

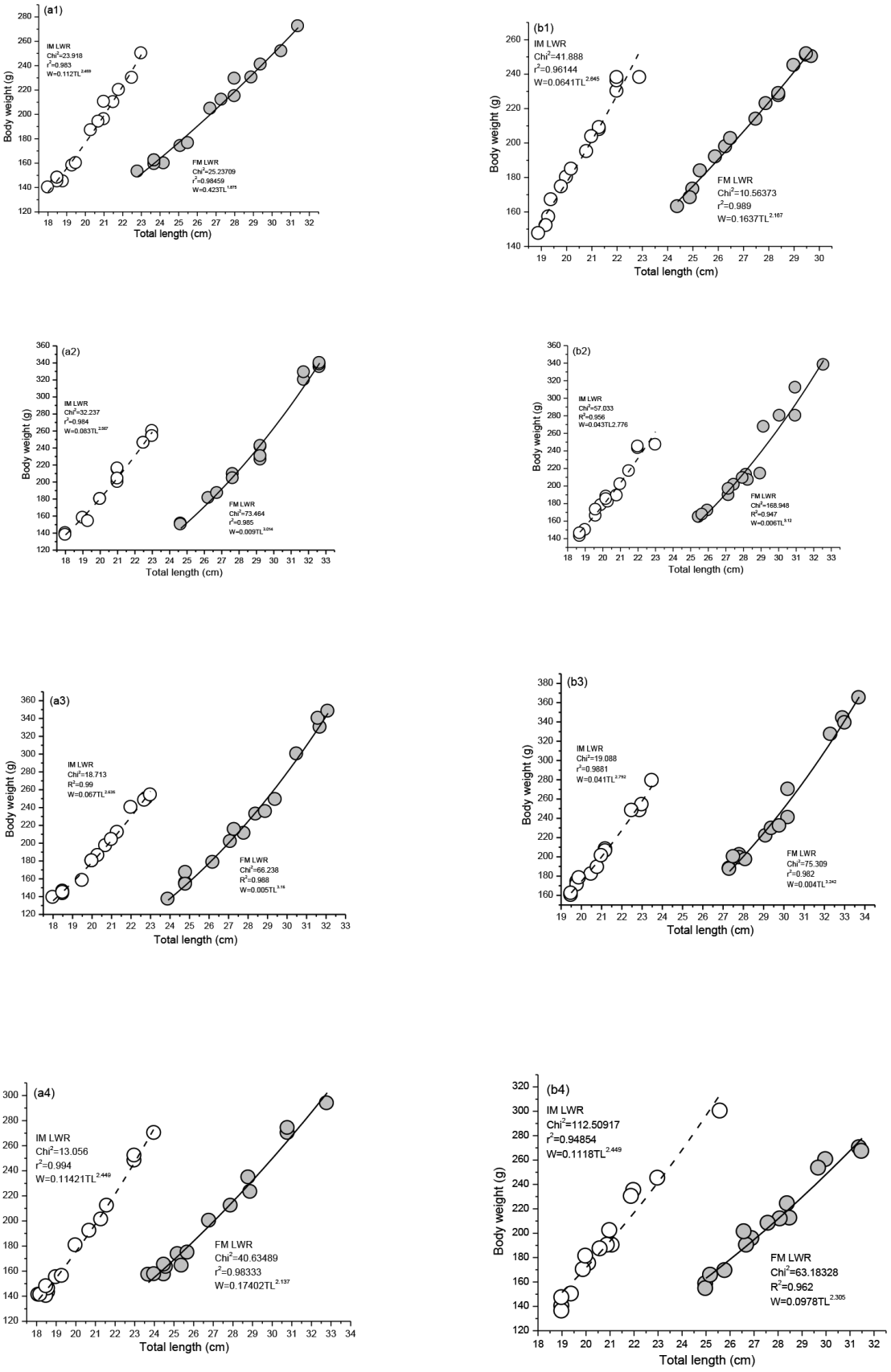

FIGURE 1. Length-weight relationship of TGGG hybrid grouper fed pellet (a1-a4) and shrimp: (b1-b4) diets at four different temperatures 22°C (a1, b1), 26°C (a2, b2), 30°C (a3, b3), and 34°C (a4, b4). Dashed line represents initial (0 d) and solid line represents final (30 d) non-linear fit whereas closed and filled circle represent individual fish samples

TABLE 1. Regression coefficients of length weight relationships of TGGG hybrid grouper fed pellet and shrimp diet at four different temperatures. The column *p* indicates significance values of ANCOVA test of homogeneity of regression slope between initial (0 d) and final (30 d) LWRs. The type of growth is inferred from the regression coefficient (*b*) value. Fulton (*K*) and relative (*Kn*) condition factors. Sample size for each diet temperature combination, *n*= 15

Temperature °C	Diet	Regression coefficient (*b*)		*p*	Type of growth		Status of wellbeing			
		IM	FM				IM		FM	
		b±S.E.	b±S.E.		IM	FM	*K*	*Kn*	*K*	*Kn*
22	Pellet	2.45 ±0.09	1.87±0.06	>0.05	-A	-A	2.08^{G}	1.40^{G}	0.95^{B}	0.73^{B}
	Shrimp	2.64±0.14	2.16±0.06	>0.05	-A	-A	2.16^{G}	1.44^{G}	0.96^{B}	0.85^{B}
26	Pellet	2.56 ±0.09	3.01±0.10	<0.05	-A	I	2.22^{G}	1.53^{G}	2.47^{G}	1.70^{G}
	Shrimp	2.77 ±0.16	3.12±0.20	<0.05	-A	I	2.24^{G}	1.58^{G}	2.64^{G}	1.71^{G}
30	Pellet	2.64±0.07	3.13± 0.10	<0.05	-A	I	2.24^{G}	1.62^{G}	2.74^{G}	1.78^{G}
	Shrimp	2.79 ±0.08	3.24±0.10	<0.05	-A	+A	2.32^{G}	1.63^{G}	2.81^{G}	1.90^{G}
34	Pellet	2.44 ±0.05	2.13±0.07	>0.05	-A	-A	2.18^{G}	1.45^{G}	0.98^{B}	0.88^{B}
	Shrimp	2.45±0.14	2.31±0.12	>0.05	-A	-A	2.22^{G}	1.45^{G}	0.99^{B}	0.93^{B}

-A: negative allometric growth, +A: positive allometric growth, I: Isometric growth, IM: Initial measurement, FM: Final measurement,
G: good condition, B: bad condition

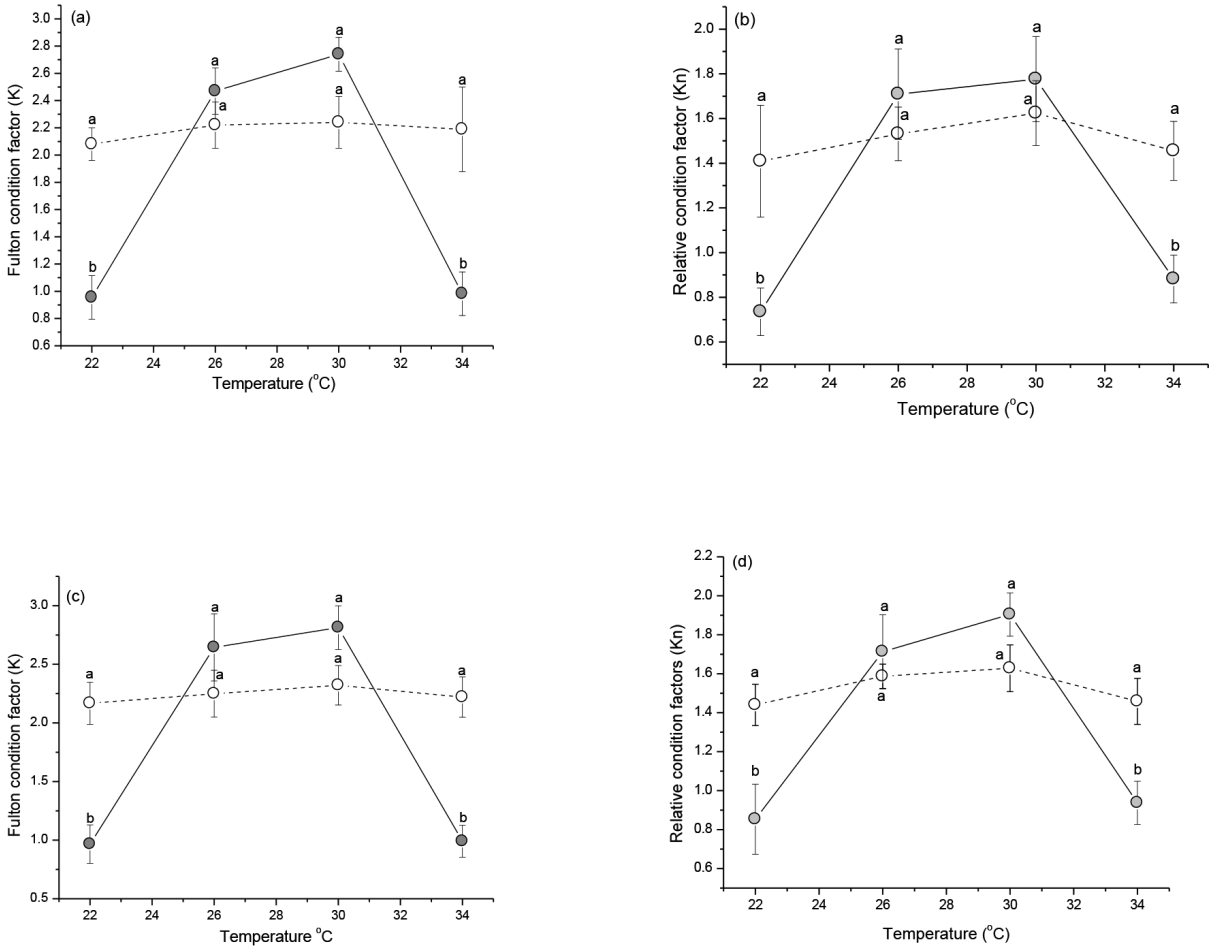

FIGURE 2. Mean Fulton (*K*) and relative condition factor (*Kn*) fed with pellet (a, b) and shrimp (c, d) diet at four different temperature. Dashed line represents initial measurement (0 d) and solid line represents final measurement (30 d). Open circle represent initial mean values of *K* and *Kn* and filled circle represent final mean values of *K* and *Kn*. Solid bar denotes variant of *K* and *Kn* values

sizes and stages of maturity (Salam & Davies 1994). Nevertheless, the allometric coefficients (*b*) estimated in this study were within the expected range of 2-4, as commonly observed in teleosts (Bagenal & Tesch 1978) except at 22°C FM with pellet diet (*b* = 1.87). TGGG hybrid grouper generally showed negative allometric growth. However, at 26°C and 30°C with pellet or shrimp diet, the grouper can exhibit isometric (26°C fed on both diet and 30°C on pellet diet only) and positive allometric growth patterns (30°C fed shrimp diet only). Thus, at 26°C and 30°C, progression in growth of the body weight vis-a-vis body length was as expected of a healthy and robust fish.

The samples were collected from a local land-based hatchery where the temperature was maintained at 26°C. However, in most cases, the TGGG hybrid grouper that were reared in marine and brackish water cages and were exposed to higher temperature ranges could demonstrate a positive allometric growth. In addition, a higher temperature could increase the metabolic rates of fish (Temming & Herrmann 2001), thereby accelerating their digestion process (De et al. 2014) and inducing better (isometric and positive allometric) growth.

The hybrid grouper was grown under better conditions at 26°C and 30°C than at 22°C and 34°C (Table 1). Increasing *K* and *Kn* values were observed in IM and FM at 26°C and 30°C (i.e. better condition), whereas decreasing *K* and *Kn* values were observed at 34°C and 22°C (i.e. poor condition) regardless of diet (Figure 2). At 22°C, visceral fat was crucial for the survival of seldom-fed fish (Braga 1986). Braga (1986) and Oni et al. (1983) showed that the values of the condition factor could vary across different seasons and could be influenced by biotic and abiotic factors. The radical change in temperature from 26°C to 22°C and to 34°C could be attributed to such differences in conditions. The *K* and *Kn* values did not significantly vary in IM, which could be attributed to the short dwelling time in different temperature for the shrimp- and pellet-fed fish (Helmuth et al. 2010). To the best of our knowledge, no previous study has investigated the condition of grouper fish, thereby preventing a direct comparison of our findings with previous results.

TABLE 2. Length weight relationships in TGGG hybrid grouper tested with different temperature (22, 26, 30, 34°C) and diets (pellet, shrimp) compared to other grouper species in previous studies

Author(s)	Species	LWR parameter		Growth type		Locality
		a	b			
Agembe et al. 2010	*E. fuscoguttatus*	-1.93	3.091	I		Kenyan coast
Palomares & Pagdilao 1988		0.0160	3.00	I		Phillippines
Kulbicki et al. 2005		0.0126	3.066	I		New Caledonia
IGFA 2001	*E. lanceolatus*	0.0173	3.00	I		USA
Letourneur et al. 1998	*E. caeruleopunctatus*	0.02140	2.907	-A		New Caledonia lagoon
Letourneur et al. 1998	*E. fasciatus*	0.0229	2.877	-A		New Caledonia Lagoon
Rafail 1972		0.0161	2.929	-A		Egypt Alexandria
Agembe et al. 2010		-1.77	2.931	-A		Kenyan coast
Shanmugam et al. 2000	*E. malabaricus*	-4.868	2.621	-A		Vellar estuary, India
Agembe et al. 2010		-1.71	2.901	-A		Kenyan coast
Letourneur et al. 1998	*E. merra*	0.0236	2.824	-A		New Caledonia
Letourneur 1998		0.0096	3.196	+A		Réunion
Letourneur 1998		0.00493	3.413	+A		Lakshadweep lagoons, India
Agembe et al. 2010		-1.84	2.991	-A		Kenyan coast
Mathews & Samuel 1987	*E. multinotatus*	0.0167	2.964	-A		Kuwait
Agembe et al. 2010		-1.75	2.931	-A		Kenyan coast
Shanmugam et al. 2000	*E. tauvina*	-4.756	2.986	-A		Vellar estuary, India
		0.031	2.840	-A		Gulf of Aden, Yemen
		0.0156	2.957	-A		Gulf of Thailand, Phetchadburi
		0.0144	3.024	I		Kuwait
		0.0264	3.065	I		India, Visakhapatnam, Anddhra Pradesh
Agembe et al. 2010		-1.95	3.051	I		Kenyan coast
Torres 1991	*E. tukula*	0.106	2.560	-A		Southern Africa
Agembe et al. 2010		-2.08	3.171	+A		Kenyan coast
Agembe et al. 2010	*E. caeruleopunctatus*	-1.75	2.921	-A		Kenyan coast
Agembe et al. 2010	*E. coioides*	-1.93	3.041	I		Kenyan coast
	TGGG hybrid grouper	0.11*	2.45*	-A	†	
Present study (IM)	(*E. lanceolatus* × *E.*					Malaysia
	fuscoguttatus)	0.06**	2.64**	-A	″	
		0.83*	2.56*	-A	††	
		0.04**	2.77**	-A	″	
		0.06*	2.64*	-A	†††	
		0.04**	2.79**	-A	″	
		0.11*	2.44*	-A	††††	
		0.11**	2.45**	-A	″	
(FM)		0.42*	1.87*	-A	†	Malaysia
		0.16**	2.16**	-A	″	
		0.01*	3.01*	I	††	
		0.01**	3.12**	I	″	
		0.01*	3.13*	I	†††	
		0.004**	3.24**	+A	″	
		0.17*	2.13*	-A	††††	
		0.09**	2.31**	-A	″	

*: Pellet diet, **: Shrimp diet, †: 22°C, ††: 26°C, † ††: 30°C, † †††: 34°C, -A: negative allometric growth, +A: positive allometric growth, I: Isometric growth, IM: Initial measurement, FM: Final measurement

CONCLUSION

This study is the first to describe the optimum temperature for the growth and condition of TGGG hybrid grouper. The LWR data showed that the pellet and shrimp fed TGGG hybrid grouper exhibited relatively better growth form and condition at 26°C and 30°C than at 22°C and 34°C. The best growth and condition were observed among shrimp-fed fish at 30°C. Therefore, TGGG hybrid grouper is suggested to culture at 30°C feeding shrimp diet although the cost of the diet needed to be considered. These findings may help grouper farmers and hatchery owners improve the growth of TGGG hybrid grouper under aquaculture conditions not only in Malaysia, but also in other nearby countries.

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