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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^{-1}, $p < 0.05$) 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^{-1}). 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.

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* (Òlafson) (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 ± 0.50, weight = 194 ± 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 × 1.02 × 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 × 63 × 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^{-1} 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:

$FC\left(g\ day^{-1}\right) = \left(g\ food\ consumed * day^{-1}\right)$ (Pérez-Casanova et al., 2009), $FCR = food\ intake\ (g\ food\ on\ as\ fed\ basis)\ /\ body\ weight\ gain\ (g)$ (Lupatsch et al., 2010) and $SGR(\%BMday^{-1}) = 100 * (ln\ final\ mass - ln\ initial\ mass) * day^{-1})$ (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, FC, 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 × temperature interactions were encountered, the cell means were analyzed in a