Water temperature and stocking density for long-hour transportation of hybrid grouper *Epinephelus fuscoguttatus* x *E. lanceolatus*

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Abstract. This study aims to investigate the effect of water temperature and stocking density in a long-hour transportation of hybrid grouper [tiger grouper (*Epinephelus fuscoguttatus*) x giant grouper (*E. lanceolatus*)]. A factorial design of three different water temperature levels (16, 18 and 21°C) and three different stocking density levels (120, 180 and 240 g L^{-1}) was tested in this experiment. The experiment was conducted using a 10 L rectangular aquarium, equipped with a water chiller and aeration to maintain the water temperature at the required level. The hybrid grouper (average body weight: 5.11±0.34 g) were placed in each aquarium with different stocking density levels for a 12-hour period. The results show that the survival of fish was significantly lower ($p < 0.05$) at a lower water temperature level (16°C; 50.2%), while there is no significant difference for the other temperature levels considered, 18 and 21°C. The results also show that the lowest water temperature had a significantly ($p < 0.05$) lower pH value in each stocking density. The glycogen content in fish liver was significantly higher ($p < 0.05$) at a low water temperature and low stocking density. A similar trend can be observed on the dissolved oxygen of water during the experiment. Moreover, the ammonia concentration was significantly ($p < 0.05$) higher at a higher water temperature and a higher stocking density. The findings in the present study suggest that hybrid grouper can be economically transported for a long hour journey at a high stocking density (240 g L^{-1}), with a water temperature level between 18 to 21°C.
Key Words: tiger grouper, giant grouper, water quality, survival rate, glycogen content.

Introduction. Hybrid grouper is a crossbreed between tiger grouper (*Epinephelus fuscoguttatus* and giant grouper (*E. lanceolatus*), and it was first produced in 2006 by a group of researchers at Universiti Malaysia Sabah (Ch'ng & Senoo 2008). The hybrid grouper has several advantages such as fast growth, high tolerance towards diseases and extreme culture conditions and good flesh quality, compared to the parental species (Chor et al 2015; Anthonius et al 2018; Mohd Faudzi et al 2018; Shapawi et al 2019). Due to these advantages, hybrid grouper had been receiving increasing attention as a candidate for aquaculture production, especially in Southeast Asia, Taiwan and China (Senoo 2010; Chu et al 2016; Shapawi et al 2019). As hybrid grouper is highly valued and increase in demand, there is an increasing need to transport the juveniles or adults from the hatcheries to farms, or to other places.

In fish farming, fish are transported from one to another place either for breeding, stocking in nursery, or for selling. Fish transportation acts as a potential stressor that may have a negative effect on the health and welfare of the fish (Iversen et al 2009; Pereira-Cardona et al 2017). Proper handling during fish transportation helps to distribute good quality fish to other places. Appropriate procedures of fish transportation may also expand the profitability of the business by reducing the mortality of the fish being transported (Gomes et al 2003). During transportation, the water quality of the holding water may fluctuate, and eventually causes stress to the transported fish. This is because transportation without optimum conditions may lead to metabolic disturbance, and in turn to an increased mortality rate.

As the fish are poikilotherms, changes in ambient water temperature leads to changes in the metabolic rate (Omeji et al 2017). Most of the fish can tolerate normal water temperature changes. However, during fish transportation, rapid changes occur in the water's temperature level. Due to this, prior to fish transportation, lowering the water temperature is vital. This may reduce fish activity, metabolism as well as the bacterial growth. Thus, this reduces the stress placed on the fish, as well as the ammonia and carbon dioxide production in the water during transportation (Gou et al 1995; Cheng et al 2014). Other than that, water temperature plays an important role in controlling the utilization of oxygen during the transportation of fish (Kita et al 1996; Alsop et al 1999).

Stocking density is another important factor that influences fish conditions during the transportation. Transporting fish in high stocking density may lessen the cost by optimising the space efficiently. On the other hand, high stocking density of fish could also trigger a stress response through metabolic pathways (Costas et al 2008; Laiz-Carrión et al 2012) caused by overcrowding and degradation of water quality, which eventually results in negative biochemical and physiological modification (Bolasina et al 2006). Therefore, finding an optimal balance between maximising profit and minimising stress response and mortality is necessary (Herrera et al 2009; Björnsson et al 2012). Previous studies revealed that increases in stocking density during transportation led to higher stress responses, ammonia accumulation, hydromineral imbalance and mortality in various fish species, including tambaqui (*Colossoma macropomum*) (Gomes et al 2003), winter flounder (*Pseudopleuronectes americanus*) (Sulikowski et al 2006), cobia (*Rachycentron canadum*) (Stieglitz et al 2012), red porgy (*Pagrus pagrus*) (Pavlidis et al 2003) and matrinx (*Brycon cephalus*) (Carneiro & Urbinati 2002).

The results obtained from other grouper species may not represent the hybrid grouper, as the tolerance of fish towards water temperature and stocking density during fish transportation is species-dependent. Therefore, this study aims to determine the optimum water temperature and stocking density that can be tolerated by juvenile hybrid grouper for a long-hour transportation.

Material and Method

Experimental procedures. The experiment was conducted on February 2020 at fish hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS), Kota Kinabalu, Malaysia. The experimental fish were obtained from a local private farm (Batu Payung Aquaculture) in Tawau, Sabah, Malaysia. Approximately, 300 pieces of juvenile hybrid grouper (average body weight: 5.11±0.34 g) were bought and transported to the fish hatchery at UMS (packing density: 65.0-69.0 g L^{-1}, temperature: 19.7-22.0°C, dissolved oxygen: 13.0-16.7 mg L^{-1}, pH: 7.04-7.02). The experimental fish were then acclimatized for one week, and fed marine pellets (Leong Hup Feedmills Sdn. Bhd., Malaysia; crude protein: 45%, crude lipid: 8%).

A factorial design of three different levels of water temperature levels (16, 18 and 21°C) and three different stocking density levels (120, 180 and 240 g L^{-1}) was tested in this experiment. The experiment was conducted using a 10 L rectangular aquarium placed in a water bath, equipped with a water chiller to maintain the required temperature. Aeration was provided to facilitate the introduction of oxygen. During the experiment, the fish (following respective stocking density) were placed in each aquarium, and the experiment was carried out for 12 hours. The fish were observed every hour. The water quality (temperature, dissolved oxygen and pH) was measured using a multiparameter instrument (YSI Professional Plus handheld multiparameter, USA) every hour during the experiment. The number of dead fish and survival rate was recorded. At the end of experiment, the water sample in the respective treatment was also taken for ammonia analysis. Fish (n = 3) from each treatment were sampled for glycogen analysis.

Chemical analysis. Ammonia analysis was conducted for each treatment following the calorimetric method proposed by Parsons et al (1984). The reading absorbance was read at 640 µm by using a spectrophotometer (Hach DR5000TM UV-Vis Spectrophotometer, Canada). The glycogen content was determined following the method by Montgomery (1957). The fish samples were dissected to obtain liver tissue for analysis. The colour intensity was read spectrophotometrically at 490 μm.

Statistical analysis. All the quantitative data expressed in percentage were subject to arcsine transformation, prior to the statistical analysis. The quantitative data were analysed using one-way analysis of variance (ANOVA) to determine the mean difference among the treatment at the 0.05 significance level. The effect of temperature, packing density and interaction between temperature and packing density were analysed using two-way ANOVA.

Results. The survival of the fish is shown in Table 1. During the experiment, the mean survival of fish in treatment at 18 and 21°C was 99.03 and 100% respectively. However, at 16°C, the results were significantly lower ($p < 0.05$), with only half of the fish surviving (50.20%). This shows decreasing trend as the water temperature decreased. The results of this experiment revealed that hybrid grouper could be transported in high stocking density by maintaining the water temperature within the range of 18 to 21°C.

Table 1

Survival (%) of juvenile hybrid grouper in different water temperature and stocking density

Water temperature (°C)	*Stocking density (g L^{-1})*	*Survival (%)*	*Mean±SD*
16	120	70.8	50.20 ± 18.33^b
	180	44.1	
	240	35.7	
18	120	100	99.03 ± 1.67^a
	180	97.1	
	240	100	
21	120	100	100.00 ± 0.00^a
	180	100	
	240	100	

Mean (±SD) values (n = 3) with different superscripts within the same column are significantly difference ($p < 0.05$).

The glycogen content of the juvenile hybrid grouper during the experiment is presented in Table 2. The study shows that glycogen was significantly affected ($p < 0.05$) by water temperature and stocking density. The glycogen content was significantly ($p < 0.05$) lower in treatment at 21°C, compared to treatment at 16°C. Similar to that, the glycogen content was also significantly ($p < 0.05$) lower with high stocking density (240 g L^{-1}) compared to with low stocking density (120 g L^{-1}). A decreasing trend was observed in terms of glycogen content when both water temperature and stocking density increased from 16 to 21°C, and from 120 to 240 g L^{-1}.

The water quality parameters during the experiment are shown in Table 3. Dissolved oxygen and ammonia concentration were significantly ($p < 0.05$) affected by temperature and stocking density. Meanwhile, pH of water was significantly ($p < 0.05$) affected by temperature. There was a significant ($p < 0.05$) interaction of temperature and stocking density on pH and ammonia concentration values in the water. The temperature of the water corresponded to the specific temperature designed for the experiment. The results demonstrate that a lower temperature had a significantly ($p < 0.05$) lower value of pH in each stocking density, compared to a higher temperature (18 and 21°C). Meanwhile, based on the results, the glycogen content significantly ($p < 0.05$) decreased when the temperature increased from 16 to 21°C, and the stocking density increased from 120 to 240 g L^{-1}. A similar trend was observed on dissolved oxygen of water during the experiment. In addition, the ammonia concentration was significantly ($p < 0.05$) increased when the temperature increased from 16 to 21°C, and when the stocking density increased from 120 to 240 g L^{-1}.

Table 2

Glycogen concentration (mg/100 mg) of juvenile hybrid grouper in different water temperature and stocking density

Treatment		*Glycogen concentration (mg/100 mg)*
Water temperature (°C)	*Stocking density (g L^-1^)*	
16	120	0.82±0.09^c^
	180	0.74±0.12^c^
	240	0.59±0.05^b^
18	120	0.60±0.11^b^
	180	0.54±0.02^b^
	240	0.38±0.02^a^
21	120	0.39±0.02^a^
	180	0.30±0.01^a^
	240	0.27±0.02^a^
Two-way ANOVA (P value)		
Water temperature		0.00
Stocking density		0.00
Interaction		0.36

Mean (±SD) values (n = 3) with different superscripts within the same column are significantly difference ($p < 0.05$).

Table 3

Water quality parameters (temperature, dissolved oxygen, pH and ammonia concentration) in different temperature and stocking density

Treatment		*Water quality*			
Water temperature (°C)	*Stocking density (g L^{-1})*	*Temperature (°C)*	*Dissolved oxygen (mg L^{-1})*	*pH*	*Ammonia concentration (mg L^{-1})*
16	120	16.94 ± 1.70^{a}	6.28 ± 0.44^{e}	6.06 ± 0.20^{a}	0.09 ± 0.01^{a}
	180	17.03 ± 1.84^{a}	5.74 ± 0.49^{d}	6.21 ± 0.11^{b}	0.14 ± 0.00^{b}
	240	17.07 ± 1.78^{a}	5.15 ± 0.33^{bc}	6.11 ± 0.14^{ab}	0.19 ± 0.02^{c}
18	120	18.75 ± 1.14^{b}	5.53 ± 0.46^{cd}	7.54 ± 0.19^{d}	0.15 ± 0.00^{b}
	180	18.60 ± 1.16^{b}	5.09 ± 0.45^{b}	7.40 ± 0.18^{c}	0.20 ± 0.03^{c}
	240	18.58 ± 1.08^{b}	4.40 ± 0.68^{a}	7.42 ± 0.18^{c}	0.25 ± 0.01^{d}
21	120	21.07 ± 0.33^{c}	6.26 ± 0.50^{e}	7.30 ± 0.07^{c}	0.21 ± 0.01^{c}
	180	20.93 ± 0.40^{c}	5.64 ± 0.32^{d}	7.35 ± 0.06^{c}	0.29 ± 0.00^{e}
	240	21.00 ± 0.35^{c}	4.43 ± 0.60^{a}	7.30 ± 0.09^{c}	0.37 ± 0.02^{f}
Two-way ANOVA (P value)					
Water temperature		0.00	0.00	0.00	0.00
Stocking density		0.97	0.00	0.44	0.00
Interaction		0.99	0.05	0.01	0.00

Mean (±SD) values (n = 3) with different superscripts within the same column are significantly difference ($p < 0.05$).

Discussion. In this experiment with different temperature and stocking density levels, the group in the water at 16°C had a low survival compared to the other groups. During the experiment, hybrid grouper in water with a lower temperature (16°C) showed weak condition and activity, where the fish were less active in terms of swimming and breathing. Based on the mean survival for each water temperature group, 18 and 21°C were the optimal water temperature levels to promote a higher survival rate during fish transportation. In general, the water temperature influences and limits the entire behaviour and physiology of fish (Nazarudin et al 2016). It was reported that the use of a low water temperature will decrease the metabolic rate of fish to a value lower than the basal metabolic rate (Lili et al 2019). This might cause the respiration rate to become very low in hybrid grouper. Other than that, previous studies on hybrid grouper also reported 100% mortality when the fish cultured below 14°C during a 30 days duration (Zhang et al 2018). According to Cheng et al (2013), 50% of juvenile tiger grouper died at a water temperature range of 9.8 to 12.2°C. It has been suggested that a low water temperature caused slow heart movement and respiration rates (Zhang et al 2018).

The study demonstrated that stocking density and water temperature clearly affected the water quality. The dissolved oxygen decreased as the stocking density increased, in each water temperature group. In line with this study, dissolved oxygen levels were found to be lower at a higher stocking density in orange-spotted grouper during the transportation period (Xavier et al 2018). In all ages of hatchery-reared grouper, the dissolved oxygen dropped significantly when increasing the stocking density from 50 to 200 fish L^{-1}, during simulated transportation (Estudillo & Duray 2003). This is primarily due to the higher oxygen consumption in the higher stocking density of fish. Chatterjee et al (2010) reported that stocking density greatly influenced the oxygen consumption during fish transportation. Moreover, fish need a large amount of oxygen in stressful conditions in order to counteract their excitement in high stocking density (Xavier et al 2018). Meanwhile, increasing the water temperature also led to an increase in fish respiration, and consequently a 3-5 time increase in oxygen consumption by fish (Lili et al 2019). Dissolved oxygen was decreased when the water temperature increased in this study. In agreement with this result, simulated transportation of hatchery-reared grouper showed that the dissolved oxygen decreased when the water temperature increased from 23 to 29°C in different age groups (35, 45 and 60 days old) (Estudillo & Duray 2003).

Generally, increasing the water temperature contributed to a reduced pH value (Xavier et al 2018). This is because the rising water temperature commonly causes an increased respiration rate, which leads to the excretion of carbon dioxide in the water. In a previous study, an increase in water temperature during the transportation of juvenile goldfish led to an increase in the pH value of the water (Lili et al 2019). Excess levels of carbon dioxide may result in hypercapnia (high carbon dioxide concentration in blood) and acidosis, which consequently causes a higher mortality rate (Harmon 2009). However, this study shows that the lowest water temperature resulted in a low pH value in the water. In this study, the opened system was used, whereby the aquariums were filled with water and supplied with aeration continuously from an external source. Thus, accumulated carbon dioxide in the water caused by respiration process did not occur, and the pH was preserved, with the exception of treatment with a low water temperature. Acidic water with a low temperature might be caused by the higher bacteria number, due to fish mortality. The production of carbon dioxide was not only by the respiration of fish, but was also contributed by the bacteria contained in the water.

Ammonia is the main nitrogenous product that is excreted by teleosts (de Oliveira et al 2008; Franklin & Edward 2019). The bulk of excretion is discharged from the blood, which is then excreted through the gills (Franklin & Edward 2019). The ammonia toxicity to fish depends on the concentration of unionized ammonia (NH_3) (de Oliveira et al 2008) in the water. In this study, the ammonia concentration increased when the stocking density and water temperature increased. However, the ammonia concentration was within the tolerable limit of the hybrid grouper, and did not cause fish mortality. Increase the stocking density and water temperature increased energy demand, which led to the direct deamination process of important tissue energy sources for energy production, resulting in an increased ammonia level (Philip & Rajasree 1996; Smutna et al 2002; Xavier et al 2018). A previous study on orange spotted grouper also showed similar findings. The ammonia concentration increased as stocking density increased from 20 to 50 fish L^{-1} during fish transportation (Xavier et al 2018). Similarly, Estudillo & Duray (2003) reported that the ammonia concentration increased when 35, 45 and 60 days old hatchery-reared grouper larvae underwent simulated transportation at different stocking density levels. Estudillo & Duray (2003) also reported that an increase in water temperature during the experiment contributed to the increase in ammonia concentration when transporting 35, 45 and 60 days old hatchery-reared grouper larvae.

Glucose, which plays a central role in providing energy for metabolism, is primarily stored in the liver as glycogen (Chang et al 2007). Glycogen metabolism is the principal energy source in both vertebrates and invertebrates, and is influenced by environmental fluctuation (Bacca et al 2005; Chang et al 2007). In this study, the glycogen concentration in hybrid grouper decreased when the stocking density in each water temperature group increased. It is also showed a similar trend when the water temperature increased for each stocking density. Increasing the stocking density and temperature was reported to increase the demand for energy and metabolism during fish transportation (Liu et al 2016; Nazarudin et al 2016). Other than that, an increase in stocking density and water temperature also led to stressful conditions (Manuel et al 2014; Wu et al 2020). Thus, the stress conditions produce a series of defense mechanisms that need more energy to be used compared to normal conditions (Lupatsch et al 2010; Liu et al 2016).

Conclusions. The findings by this study suggest that hybrid grouper can be transported at extremely high stocking density levels (240 g L^{-1}) compared to other grouper species (120-150 g L^{-1}) at temperature of 18 to 21^{o}C with higher survival and less stress. Hence, the cost of long-hour transportation for hybrid grouper can be minimized and contributed in increased the profit for the fish farmer.

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