



Effects of temperature on the growth, gastric emptying time, and oxygen consumption rate of mahseer (*Tor tambroides*) under laboratory conditions

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ABSTRACT

The growth, gastric emptying time (GET), and oxygen consumption rate (OCR) of the commercially important freshwater mahseer, *Tor tambroides*, were evaluated under five temperatures (22 °C, 24 °C, 26 °C, 28 °C, and 30 °C) in triplicates with 15 fish per replication in a controlled laboratory condition. After 12 weeks, the body weight gain (BWG), specific growth rate (SGR), and daily growth rate (DGR) were measured. The highest BWG (7.25 ± 1.14 g), SGR ($0.71\% \pm 0.10\%$ day⁻¹), and DGR (4.70 ± 0.20 g day⁻¹) were observed at 30 °C, whereas the lowest BWG (3.16 ± 0.90 g), SGR ($0.30 \pm 0.05\%$ day⁻¹), and DGR (1.77 ± 0.30 g day⁻¹) were observed at 22 °C ($P < 0.05$). The fish were then slaughtered sequentially at different time intervals after initial feeding to obtain GET. Results showed that the shortest GET (10 h) was observed at 30 °C, whereas the longest (18 h) GET was at 22 °C. OCR was significantly high at 30 °C (5.5 ± 0.53 mL O₂L⁻¹h⁻¹) but low at 22 °C (1.55 ± 0.23 mL O₂L⁻¹h⁻¹). Based on the temperature quotient (Q₁₀), the optimum temperature range for the respiration rate was between 28 °C and 30 °C. The findings of this study suggest that *T. tambroides* should be cultured at 30 °C because this temperature facilitates fast GET and best OCR, which may contribute to their fast growth. In summary, these findings would be useful to increase the production of *T. tambroides* in captive environment.

1. Introduction

Water temperature is one of the most important controlling factors in poikilothermic animals, such as fish, because of its effects on the internal body temperature and the overall physiological activity of fish (Sahoo and Paul, 2017). Scientists predict a mean water temperature increase of 2 °C–4 °C for this century due to global warming (Armour, 2016). This phenomenon will catastrophically affect food consumption, digestion, respiration, and growth performance of fish (Miller et al., 2015). Unfortunately, the effects of temperature on fish physiology are poorly reported (Islam, 2005).

Gastric emptying time (GET) in fish is greatly influenced by several environmental factors, such as water salinity (Noor et al., 2018) and water temperature (De et al., 2016a, 2014). Fish culturists can use GET to develop appropriate and efficient feeding strategies (Jobling, 1983). The food must be available at an appropriate rate as soon as appetite of the fish has returned to ensure maximum feed intake and efficiency (Rønnestad et al., 2013). Several techniques have been used to

investigate the GET of fish (Jobling, 1983). However, the GET of *T. tambroides* is yet to be explored.

Most of the metabolic activities in fish are related to oxygen consumption (Liwanağ et al., 2009). Oxygen consumption rate (OCR) is an important physiological aspect and is also influenced by changing environmental parameters, including temperature, pH, salinity, dissolved O₂ and CO₂ in the ambient water, hour of the day, season nutrition, and activity level, sex, and body weight of fish (Pörtner, 2010). The oxygen metabolic status in fish can be divided into standard, active, and routine metabolism (Salvato et al., 2001). Standard metabolism refers to OCR without spontaneous activity, routine metabolic activity refers to OCR with spontaneous activity, and active metabolism refers to OCR with forced activity (Merino et al., 2009). Standard and active metabolisms are primarily studied in the laboratory (Sun et al., 2010), whereas routine metabolism is studied in either tanks or ponds (Clark et al., 2013).

Temperature coefficient (Q₁₀) represents the degree of sensitivity of an organism to temperature (Karl et al., 1988). Evaluating the oxygen

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consumption at different temperatures allows the calculation of the thermal coefficient of poikilothermic organisms from aquatic habitats (Coelho and Carvalho, 1997).

T. tambroides, known as Malaysian mahseer, is widely distributed in mountainous rivers and lakes of Himalayan and Southeast Asian regions. Although *T. tambroides* is one of the most commercially important freshwater fish in Malaysia, its productivity decreases from time to time due to various environmental factors including temperature (Ismail et al., 2011). Although temperature catastrophically affects the physiology of fish, research on the effect of temperature on the growth performance, GET, and OCR of *T. tambroides* is yet to be performed. This study aims to determine the optimum temperature to achieve the best growth, GET, and OCR of *T. tambroides* in captivity.

2. Materials and methods

2.1. Sample collection and experimental setup

T. tambroides samples ($N = 225$, 8.0 ± 0.5 cm initial length and 6.0 ± 0.5 g initial weight) were collected from the local hatchery of Banting ($2^{\circ}49'0''N$, $101^{\circ}30'0''E$), Selangor, Malaysia and transported to the marine science laboratory of UKM, Bangi, Malaysia. The fish were then distributed randomly among three stocking tanks ($1.96 \text{ m} \times 1.02 \text{ m} \times 0.61 \text{ m}$, 1200 L in size and capacity). Each tank was supplied with running fresh water, and the temperature was kept at approximately 26°C . Seventy-five fish/tanks were fed with the same pellet diet used in the hatchery. Once the fish started feeding and defecating, 225 fish were randomly transferred to 15 experimental tanks (15 fish/tank) for a 12-week experimental period. The experimental tanks were equal in size ($123 \text{ cm} \times 63 \text{ cm} \times 46 \text{ cm}$, 356 L). Three replicates were used for every experimental temperature change (22°C , 24°C , 26°C , 28°C , and 30°C). The experimental temperature was introduced, and the temperature change for the experimental groups was initiated at 1°C per day using a heater (E-JET heater 200 W, Penang, Malaysia) and a chiller (HS-28A, 250–1200L/H, Guangdong Hailea Group Co., Ltd., Country of Origin: China) until high and low experimental temperatures were reached (De et al., 2016a; Mazumder et al., 2018). During the 12-week experimental period, the fish were manually fed with commercial pellet (Sanyu Sdn Bhd, Malaysia: 40% protein, 5% lipid, and 9% ash) twice daily (0900 and 1600 h) until apparent satiation was reached. Satiation was defined as the point when feeding stopped and pellets remained at the bottom of the tank for more than 2 min (De et al., 2016b). During the experimental period, all tanks were maintained on a 12 h light and 12 h dark photoperiod (De et al., 2016b). At the end of the 12-week experimental period, fish samples were collected for growth, GET, and OCR measurements, which are further described in the following sections.

2.2. Growth performance

At the end of the 12-week experimental period, the fish were anaesthetized using α -methyl quinolone (Transmore, Nika Trading) (0.22 ml L^{-1} in 3 L of sea water) for 10 min. The body weights were measured every 3 weeks using an electronic balance (A & D, Model-GR-200) (Das et al., 2014). The growth parameters were calculated according to the following formulas:

Specific growth rate, $\text{SGR} = [(\ln W_f - \ln W_i)/T] \times 100$,

Daily growth rate, $\text{DGR} = (W_f - W_i)/T$,

where W_f is the fish body weight at the beginning of the period, W_i is the fish body weight at the end of the period, and T is the duration of the experimental period that is equal to 30 days. The fish were fed 5%–10% of their body weight and adjusted accordingly.

2.3. Gastric emptying time

Prior to the GET experiments, the fish were not fed for 24 h to allow complete emptying of the stomach (Noor et al., 2018). The fish were anaesthetized after being fed with pellets, and an incision was made on the anterior spine. Observations were made at 0, 2, 4, 8, 10, 12, 16, 18 and 20 h after feeding during which three fish were used at each experimental time. The stomach contents were extracted and weighed (wet weight with 0.001 g precision).

2.4. Oxygen consumption rate (OCR)

The fish were sampled one at a time from each experimental tank and placed in a closed respiratory chamber (tubular Plexiglas length: 30 cm; diameter: 20 cm; 4.3 L). The fish did not show spontaneous activities during the experiment. Dissolved oxygen (DO) was measured every 10 to 60 min with a radiometer oxygen electrode (SA7-530-200) connected to a four channel Fire Sting oxygen analyzer logger software version 3.0. The OCR ($\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) was calculated using the following equation:

$$\text{OCR} = [(O_i - O_f) \times V / (T \times B)],$$

where O_i is the water oxygen value ($\text{mg O}_2 \text{ L}^{-1}$) at the beginning of the measurement, O_f is the water oxygen value ($\text{mg O}_2 \text{ L}^{-1}$) at the end of the measurement, V is the tank volume (L), B is the fish body weight (kg), and T is the duration (h) (Lisboa et al., 2015).

Thermal coefficient (Q_{10}), which represents the sensitivity of an organism to temperature variations, was estimated for the species using the calculated values of OCR. Q_{10} values were calculated as follows:

$$Q_{10} = (VO_{2t_1}/VO_{2t_0})^{10/(t_1 - t_0)},$$

where VO_{2t_1} and VO_{2t_0} are the OCR at temperatures t_1 and t_0 , respectively; t_0 is the lower of the two temperatures used to determine oxygen consumption; and t_1 is the higher of the two temperatures used to determine oxygen consumption (Lisboa et al., 2015).

2.5. Statistical analysis

Prior to statistical analysis, data were tested for normality and homogeneity of variance among the different groups by using a Kolmogorov–Smirnov (K–S) test on residuals and Bartlett's test for homogeneity of variance. All temperature treatments were statistically compared using a parametric analysis of variance (ANOVA). A one-way factorial model was used to analyze the effects of temperature on the total length, weight, BWG, SGR, DGR, and gastric emptying at the same sample time. OCR was analyzed at the same temperature. In cases where ANOVA reported significant differences, a pairwise post-hoc Tukey test was used to determine specifically which groups were different (Zar, 1984). Data presented in the text, figures, and tables are means \pm SD, and $P < 0.05$ was used as the level of statistical significance. All statistical analyses were performed using MINITAB (version 14) and Microcal Origin™ (version 8) software (Simon et al., 2013, Simon et al., 2008 2008).

3. Results

3.1. Growth performance

The highest BWG (7.25 ± 1.14 g), SGR ($0.71\% \pm 0.10\% \text{ day}^{-1}$) and DGR ($4.70 \pm 0.20 \text{ g day}^{-1}$) were observed in 30°C , whereas the lowest BWG (3.16 ± 0.90 g), SGR ($0.30\% \pm 0.05\% \text{ day}^{-1}$), and DGR ($1.77 \pm 0.30 \text{ g day}^{-1}$) were observed at 22°C after the end of the 90-day experimental period ($P < 0.05$). However, BWG, SGR, and DGR were not significantly different in the other temperatures (24°C , 26°C , and 28°C ; $P > 0.05$) (Table 1). The mean length (Fig. 1) and weight

Table 1

Growth parameters (mean ± SD) measured for *T. tambroides* cultured in different temperatures. Different superscript letters indicated significant differences among the treatments ($P < 0.05$) in different temperature.

Parameter	Temperature (°C)				
	22	24	26	28	30
BWG (g)	3.16 ± 0.90 ^a	4.56 ± 1.20 ^b	4.60 ± 0.89 ^b	4.95 ± 1.40 ^b	7.25 ± 1.14 ^c
SGR (% day ⁻¹)	0.30 ± 0.05 ^a	0.48 ± 0.10 ^b	0.48 ± 0.09 ^b	0.50 ± 0.10 ^b	0.71 ± 0.10 ^c
DGR (g day ⁻¹)	1.77 ± 0.30 ^a	2.45 ± 0.25 ^b	2.87 ± 0.32 ^b	3.02 ± 0.42 ^b	4.70 ± 0.20 ^c

BWG: body weight gain, SGR: standard growth rate, DGR: daily growth rate.

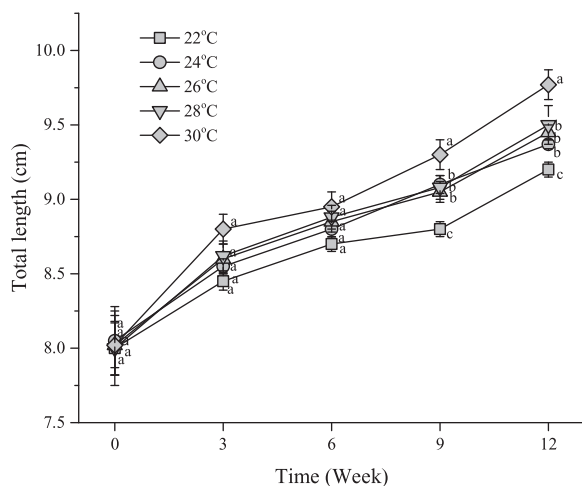


Fig. 1. Total length (mean ± SD) of *T. tambroides* cultured in different temperatures during 12 weeks experimental period. Different superscript letters indicate significant differences among the treatments ($P < 0.05$) at the same sample time point.

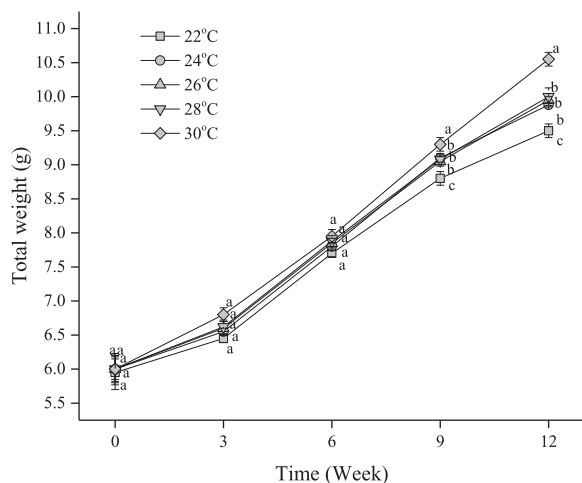


Fig. 2. Body weight (mean ± SD) of *T. tambroides* cultured in different temperatures during 12 weeks experimental period. Different superscript letters indicate significant differences among the treatments ($P < 0.05$) at the same sample time point.

(Fig. 2) of the 22 °C and 30 °C ($P < 0.05$) groups were significantly different after the 9th week until the end of experimental period. No significant difference ($P > 0.05$) was observed in the 24 °C, 26 °C, and 28 °C groups during the experimental period.

3.2. Gastric emptying time (GET)

The findings show that GET decreased with the increase in water temperature from 22 °C to 30 °C (Fig. 3). Fast gastric emptying was

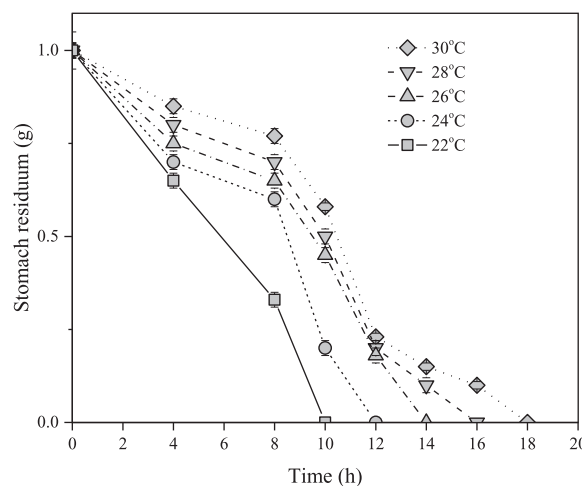


Fig. 3. Gastric emptying time (h) of *T. tambroides* (mean ± SD) at different temperature at the same sample time point.

observed at 30 °C, in which the food items took 10 h to be completely digested. The longest gastric emptying occurred at 22 °C, wherein the fish completely digested the food after 18 h.

3.3. Oxygen consumption rate (OCR)

OCR (VO_2) in *T. tambroides* at different temperatures is shown in Fig. 4. OCR was significantly different at 30 °C but not between 24 °C and 28 °C. OCR increased gradually with increasing water temperature. The lowest OCR was observed at 24 °C ($1.55 ± 0.23 \text{ mL O}_2 \text{ L}^{-1} \text{ h}^{-1}$), whereas the highest OCR was observed at 30 °C ($5.5 ± 0.53 \text{ mL O}_2 \text{ L}^{-1}$).

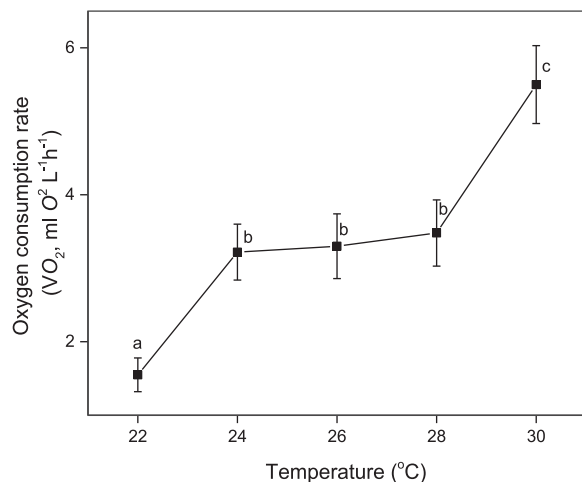


Fig. 4. Oxygen consumption rate ($\text{ml O}_2 \text{ L}^{-1} \text{ h}^{-1}$) of *T. tambroides* at different temperature. The lower case letter ^{a-c} means that do not share a letter are significantly different.

Table 2
Thermal Quotient (Q_{10}) for oxygen consumption of *T. tambroides* under different temperature ranges.

Temperature interval (°C)	Q_{10} value
22–24	2.02
24–26	1.74
26–28	1.55
28–30	1.08

h^{-1}) ($P < 0.05$). Q_{10} was used to describe the different oxygen consumptions at different temperature ranges. The Q_{10} value can be measured based on the change in VO_2 with temperature as shown in Table 2. Q_{10} value was the highest (2.02) between 22 °C and 24 °C but was the lowest (1.08) between 28 °C and 30 °C. Based on the Q_{10} values, the preferred temperature for *T. tambroides* was between 26 °C and 30 °C.

4. Discussion

In this study, *T. tambroides* shows good growth performance when at high water temperatures, although no significant difference was observed among the temperatures. This finding supported the previous studies showing that *T. tambroides* grows best in high temperatures (Misieng et al., 2011; Kamarudin et al., 2012). The influence of temperate temperatures was similarly studied in grouper hybrid (*Epinephelus fuscoguttatus* × *E. lanceolatus*) (De et al., 2016a), Atlantic salmon (*Salmo salar*) (Handeland et al., 2008), and seabass (*Dicentrarchus labrax*) (Person et al., 2004). The increase in temperature changes food energy and eventually fish growth (De et al., 2016a). Moreover, *T. tambroides* is a slow growing fish because the highest SGR in this study is still lower than those of other tropical freshwater fishes, such as carp (1.28%) and tilapia (1.40%) (Kaushik, 1998). Similarly, low SGR could be observed in *T. tambroides* ($0.51\% \pm 0.08\% \text{ day}^{-1}$) given with feed containing 40% protein (Misieng et al., 2011). The final body length and body weight after the experimental period increased when the water temperature increased; however, no significant difference ($P > 0.05$) was observed. This situation may be due to the short period of study. Similar observations have been documented by Person et al. (2004) for seabass (*Dicentrarchus labrax*) and Handeland et al. (2008) for Atlantic salmon (*Salmo salar*). Their findings suggest that at least 60 days of the study period is required to ensure that the significant differences in the rates of fish can be observed.

This observation also documented that the GET of *T. tambroides* was influenced by the increase in water temperature. The results showed faster GET at high temperature compared with low temperature. Similar observations have been reported by Ross and Jauncey (1981) for tilapia hybrid (*Sarotherodon niloticus* × *S. aureus*) and De et al. (2016a,b) for grouper hybrid (*Epinephelus fuscoguttatus* × *E. lanceolatus*). *T. tambroides* has shorter GET than other tropical fish, such as grouper hybrids (*Epinephelus fuscoguttatus* × *E. lanceolatus*, 17 h, De et al., 2016a,b) and snapper fish (*Lutjanus malabaricus*, 20 h, Mazumder et al., 2015). This difference may be due to the different parameters, such as food type, water temperature, fish species, age or size of the fish, feeding frequency, and chemical and physical food content, which were used in these studies (Gerking, 2014).

This study provides the first evidence that the OCR in *T. tambroides* increased with increasing temperatures (22 °C–30 °C). The increase in the OCR of *T. tambroides* with increasing temperatures agrees with the profiles mentioned in other studies for different species (Chatterjee et al., 2004; Das et al., 2005; Zheng et al., 2008; Dalvi et al., 2009; Farrell, 2016; Chabot et al., 2016; Williams et al., 2016); this finding was expected because all metabolic processes are directly regulated by temperature (Jobling, 1983). Temperature is one of the most important factors that influence the respiratory metabolism and determine the

activity level of all biochemical processes in organs and tissues, such as enzyme activities; temperature could lead to physiological malfunction, one of which is the rapid OCR decrease when the fish are not in a suitable temperature range (Wang et al., 2007). OCR in different temperature preferences of *T. tambroides* was calculated according to the general temperature ranges as preferred by this species. The fish standard metabolic rate could be acquired when the fish is calm, inactive, and not under any form of physical, thermal, or physiological stress. Jobling (1995) proposed that the standard metabolism is component; energy for organization repair and renovation; the second energy to ensure the inner environment stable. Under fluctuating temperatures, the fish can allocate more energy for growth and reproduction than the fish under constant temperature (Dong et al., 2006). The increase in metabolic processes of respiration was related with the GET and high rates of growth.

Temperature coefficient (Q_{10}), which represents the degree of sensitivity of an organism to temperature and measures the metabolic capacity of aquatic organisms to make the adjustments after temperature changes, was also measured (Herbing, 2002). A decrease in Q_{10} indicates that the metabolic rate of the fish has decreased, and much energy is available for growth (Díaz et al., 2007). Based on the relationship between Q_{10} , the final preferred acclimation temperature may be predicted indirectly for oxygen consumption and temperature (Kita et al., 1996). In our study, the final preference temperature for *T. tambroides* was between 28 °C and 30 °C based on the Q_{10} value. Thus, the estimation of Q_{10} and thermal optima estimation can serve as a preliminary and convenient method to screen candidate species used for aquaculture before a growth study is performed. The results show that the highest temperature (30 °C) used in the present study provided the best performance yield for the fish. This finding indicates that *T. tambroides* may exhibit good growth performance when cultured at a temperature higher than 30 °C.

5. Conclusion

This study describes for the first time the optimum temperature to achieve excellent growth, GET, and OCR of *T. tambroides*. The growth performance of *T. tambroides* increased at high temperature with the best results observed in 30 °C. Optimum GET and OCR values were observed at 30 °C. With proper GET and OCR at suitable temperature, excessive energy for respiration can be used for other body activities, such as growth. Findings from this research will improve the understanding of the physiological status *T. tambroides* under the increment of water temperature as facilitated by recent major climate issues, such as global warming. Data obtained from this study can be used as baseline information for the good management of *T. tambroides* and eventual optimization of the commercial production of this important freshwater fish species.

Conflict of interest

The authors have declare that no conflict of interests exist.

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