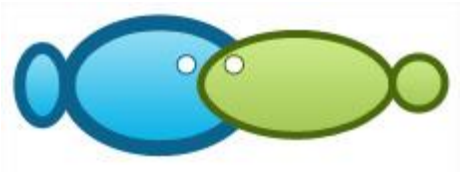

The effect of temperature on digestive enzymatic activities and growth performance of giant gourami (*Osphronemus goramy*) fingerlings

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Abstract. Giant gourami (*Osphronemus goramy*) with an initial weight of 10.4±0.04 g fish^{-1} were used for two experiments. In the first experiment, 500 L tanks with 50 fish in each tank were exposed to different temperatures (22, 25, 28, 31, and 34°C) for 56 days. The stomachs and intestines of the fish were collected at 14, 28, and 56 days to determine the activities of the digestive enzymes such as trypsin, chymotrypsin, and α-amylase. In addition, plasma glucose concentrations were measured at these sampling times. The second experiment was conducted at the same temperature levels and the fish were reared for 90 days to determine survival rate, growth, and food conversion ratio. The results of the study showed that digestive enzyme activity (trypsin, chymotrypsin, and α-amylase) increased as the temperature increased from 22°C to 31°C. After 90 days, the highest daily weight gains and specific growth rates of the fish were 0.68±0.01 g day^{-1} and 2.24±0.03 % day^{-1}, respectively at 31°C, while the lowest were found at 22°C (0.13±0.01 g day^{-1} and 0.88±0.09 % day^{-1}, respectively). Therefore, temperature significantly affected the digestive enzymatic activities and growth performance of fingerling-sized *O. goramy*.

Key Words: elevated temperature, enzymes, glucose, Mekong Delta.

Introduction. Climate change is a challenge to both researchers and the general population. Climate change causes elevated temperatures, droughts, and sea-level increases, which affect all sectors, particularly those of agriculture and aquaculture (USEPA 2012). Vietnam has been heavily affected by climate change, and the Mekong Delta of Vietnam, which is one of the most vulnerable deltas globally, has been considerably impacted (Dasgupta et al 2007; Syvitski et al 2009). The global mean temperature has sharply increased over the past 100 years, particularly within the last 25 years, and the mean temperature in Vietnam increased by 0.5-0.7°C over 50 years (MONRE 2016). Temperature increases of 1.4 to 5.8°C over the period from 1990 to 2100 would critically affect the living habitats of aquatic organisms (Houghton et al 2001).

The Mekong River Delta of Vietnam is rich in fish species, especially freshwater varieties. Popular culture species include tilapia (*Oreochromis niloticus*), snakehead (*Channa striata*), climbing perch (*Anabas testudineus*), giant gourami (*Osphronemus goramy*), and clown knife fish (*Chitala ornata*), and these species are cultured in different systems and intensity levels (Hai et al 2022). *O. goramy* is a species of high economic value since it shows favorable growth on low-protein diets and is easily cultured in various systems with a high stocking density. According to Kiem & Thanh (2013), this species grows rapidly during the first year and can reach 300-500 g under normal conditions. Therefore, *O. goramy* could be a potential aquaculture species that could adapt to climate change in the Mekong River Delta, Vietnam.

Temperature is one of the main environmental factors affecting aquatic animals, as they are poikilotherms that exhibit sensitive reactions to changes in temperature levels (Fry 1971). Most fish species can acclimate to moderate changes in temperature, but

sizeable variations may negatively affect fish growth and survival rates and produce undesirable changes in physiological responses. This is particularly challenging for ectotherms that have limited capacities to regulate their body temperatures (Kemp 2009; Tobin & Wright 2011). Fish increase their body temperatures in response to their environment, and for every 10°C increase in temperature within the normal tolerance range, metabolic activity generally doubles (Boyd & Tucker 1998). For most species, a slight temperature shift within their typical temperature range is beneficial because it allows for an increase in energy production, thereby promoting faster growth (Wootton 2011), whereas lower temperatures typically impair their performance (Kemp 2009). In addition, temperature is the most important modifier of energy flow and the consequent growth of aquatic animals (Brett 1979) and is involved in the modulation of digestive enzymatic activities (Trellu & Ceccaldi 1980; Charron et al 2013).

This study was designed to generate an understanding of the effects of temperature on the survival, growth, and digestive enzymatic activities of *O. goramy* at the fingerling stage. In addition, it aimed to recommend an optimal range of temperature for the aquaculture of this species under a climate change context.

Material and Method

Animals and experimental conditions. Research took place between July and December 2021. Fish fingerlings with an average body mass of 10.4 ± 0.04 g were purchased from a local hatchery, transported in oxygenated bags to the Faculty of Biology and Aquatic Environment, College of Aquaculture and Fisheries, Can Tho University, Vietnam, and acclimated in a fiberglass tank of 4 m^3 for 2 weeks at a standard temperature of approximately 28°C. The tank was well aerated, and the fish were fed with commercial pellets (Uni-President company, 30% protein, pellet size 1 - 2 mm) during the acclimation period.

All experiments used de-chlorinated tap water. Selected water quality parameters were recorded twice daily at 07:00 and 14:00 using a thermo-pH meter for temperature and pH and a DO meter for dissolved oxygen (DO) (YSI 556 handheld meter, Ohio, USA). Nitrite concentration (NO_2^-) and total ammonia nitrogen (TAN) were measured once a week using the diazonium and indophenol blue methods, respectively (APHA 1995). The levels of these parameters used during the experiments were as follows: DO 5.6-6.2 mg L^{-1}, water pH 7.6 - 7.8, NO_2^- 0.35 - 0.72 mg L^{-1}, and TAN 0.60 - 1.15 mg L^{-1}. The greatest NO_2^- and TAN concentrations were recorded at 34°C.

The temperature levels were maintained using a cooler (TECO SeaChill TR10) or heater (EHEIM professional 4+350T, Germany). All experiments were conducted following the national guidelines for protection and experimental animal welfare in Vietnam (Law of Animal Health 2015).

Experimental design

The effect of temperature on glucose concentrations and digestive enzymatic activities of Osphronemus goramy fingerlings. The experiment was conducted in 500 L tanks over 56 days and included 5 temperature levels (22, 25, 28, 31, and 34°C). Fifty fish from the acclimated tank were randomly stocked in 500 L tanks and 3 tanks were used for each treatment (three replicates). Twenty hours post-stocking, the water temperature was increased or decreased by 3°C per day (1°C every 8 hours starting from 28°C as the control temperature). After all treatments reached the desirable temperatures, fish were collected on Days 14, 28, and 56 and trypsin, chymotrypsin, *α*-amylase activities, and plasma glucose concentrations were measured. Blood of the fish was sampled and centrifuged at 6,000 rpm for 6 min at 4°C to obtain plasma, and then stored at -80°C for the subsequent measurement of glucose. The sampled fishes (3 fish $tank^{-1}$) were then dissected to collect the stomachs and intestines for analysis of the digestive enzymatic activities. These samples were stored at -80°C for further evaluation. The stomach and intestine samples were thawed on ice and homogenized with potassium dihydrogen phosphate (KH_2PO_4) 20 mM buffer and sodium chloride (NaCl) 6 mM at a pH

of 6.9. The mixture was centrifuged for 30 min at 4,200 rpm and 4°C and the supernatant was collected and used for chymotrypsin activity measurement according to the method of Worthington (1982), while trypsin and *a*-amylase were analyzed by the methods of Tseng et al (1982) and Bernfeld (1951), respectively. Protein level was determined using a Biorad protein assay. Specific activities are expressed as U min^{-1} mg $protein^{-1}$. Glucose was measured following the method of Hugget & Nixon (1957).

The effect of temperature on growth performance of Osphronemus goramy fingerlings. This experiment was completely randomized in 500 L fiberglass tanks with 5 different temperature treatments (22, 25, 28, 31, and 34°C) over 90 days. Fifty fish were stocked in a 500 L tank with 3 replicates for each treatment. The test temperatures were controlled as described in the first experiment. Fish were hand-fed twice daily (at 07:00 and 16:00). Approximately 30 min post-feeding, the remaining food was collected and quantified for the FCR (food conversion ratio) calculation at the end of the experiment. The tank water was exchanged weekly (approximately 50% of the water volume).

The body mass of the fish was measured at the beginning of the experiment and on days 30, 60, and 90 for the fish growth calculation. Growth performance was determined using weight gain (WG), specific growth rate (SGR), and daily weight gain (DWG). The survival rate (SR) and feed conversion ratio (FCR) were calculated at the end of the experiment.

The formula for the calculation of these data were:

$$\text{Survival rate SR (\%)} = 100 \times (\text{final number of fish/initial number of fish})$$

$$\text{Daily weight gain (DWG – g day}^{-1}) = (W_t - W_0)/t$$

$$\text{Weight gain (g)} = \text{final weight} - \text{initial weight}$$

$$\text{Specific growth rate (SGR - \% day}^{-1}) = [(\text{Ln (Wt)} - \text{Ln } (W_0)) / t] \times 100$$

$$\text{Daily length gain (DLG - cm day}^{-1}) = (L_t - L_0) / t$$

$$\text{Feed conversion ratio (FCR)} = \text{total feed intake/total fish weight gain (feed intake = feed fed – uneaten feed)}$$

where: W_0 and L_0 are the initial weight and length of fish, respectively; W_t and L_t are the fish weight and length, respectively, at sampling point t; and t= time (days).

Statistical analysis. All figures were produced using SigmaPlot 12.5. All data were calculated for the mean, standard deviation, and error using Microsoft Excel 2010. A one-way ANOVA was used for growth parameters (weight gain, daily weight gain, specific growth rate, and food conversion ratio), and digestive enzyme activity to identify significant differences between treatments with a p-value of less than 5% ($p < 0.05$). All data are shown as means ± standard error of the mean (SE).

Results

Plasma glucose concentrations of Osphronemus goramy fingerlings exposed to different temperatures. The effects of temperature on the plasma glucose concentration are shown in Table 1. The lowest plasma glucose concentration occurred at 28°C. After 14 days, significant differences were found among the treatments at 22°C (53.6 mg dL^{-1}), 34°C (63.6 mg dL^{-1}), and the control at 28°C (43.6 mg dL^{-1}) ($p < 0.05$). On Days 28 and 56, the plasma glucose concentrations did not fluctuate in any of the treatments, except at 34°C (52.6 and 59.3 mg dL^{-1}), at which significantly different results were observed from those of the other treatments.

Table 1

Plasma glucose concentrations (mg dL^{-1}) of *Osphronemus goramy* fingerlings reared at different temperatures for 56 days

Treatments/ temperatures	*Day of sampling*		
	14th	*28th*	*56th*
22°C	53.6 ± 1.90[b]	46.7 ± 1.50[a]	50.5 ± 2.08[a]
25°C	49.6 ± 1.22[b]	45.4 ± 5.54[a]	46.8 ± 1.13[a]
28°C	43.6 ± 3.01[a]	44.2 ± 0.83[a]	46.2 ± 3.36[a]
31°C	58.2 ± 2.92[c]	44.8 ± 1.31[a]	45.1 ± 3.36 [a]
34°C	63.6 ± 3.27[d]	52.6 ± 1.48[b]	59.3 ± 4.45[b]

Notes: Values are presented as mean ± SE (n = 3). Different letters (a, b, c, d) on the columns with the same sampling time show significant differences ($p < 0.05$).

Digestive enzymatic activities of Osphronemus gourami fingerlings exposed to different temperatures. After 14 days of exposure to different temperatures, trypsin activity in the intestine was low at 22°C (0.0056 ± 0.0009 U min^{-1} mg $protein^{-1}$) and significantly different to that at 31°C (0.0127 ± 0.0008 U min^{-1} mg $protein^{-1}$) ($p < 0.05$). At the end of the experiment, trypsin activity was lower at 22 and 25°C than at 28, 31, and 34°C, and a significant difference was found between the two time points (Figure 1). Chymotrypsin activity in the intestine increased linearly as temperatures escalated from 22 to 34°C (Figure 1). The lowest activity was found after 14 days at 22°C (93.4 ± 4.46 U min^{-1} mg $protein^{-1}$), while the highest was observed at 31°C (157 ± 11.3 U min^{-1} mg $protein^{-1}$), and these were significantly different compared to those of the remaining treatments ($p < 0.05$). After 56 days, the chymotrypsin activity was highest at 34°C (237 ± 6.71 U min^{-1} mg $protein^{-1}$), and this was significantly different from that of the other treatments ($p < 0.05$). Similarly, *α*-amylase activity in the intestine of the fish exposed to temperatures from 22 to 31°C increased from 1.11 to 1.78 U min^{-1} mg $protein^{-1}$, although the activity at 34°C was lower when compared to that at 31°C after 56 days. In the stomach, the activity of *α*-amylase fluctuated similarly with that in the intestine, and the highest activity was found at 31°C in all sampling times (Figure 1).

Growth, survival rate, and feed conversion ratio of Osphronemus goramy fingerlings exposed to different temperatures

Growth performance. The final weight and length of the fish increased as temperature increased (Figure 2). The significant differences among the treatments were observed at the beginning of day 30 ($p < 0.05$). On days 30, 60, and 90, the highest weights were 26.9 ± 0.68 g $fish^{-1}$, 49.1 ± 0.69 g $fish^{-1}$, and 72.0 ± 0.37 g $fish^{-1}$ at 31°C, respectively. The greatest total length at day 90 was 16.0 ± 0.12 cm $fish^{-1}$ in the 31°C treatment.

Figure 3 shows that the DWG, SGR, and DLG were enhanced as the temperature increased. All parameters were significantly lowest at the temperature of 22°C after 90 days of culture. For example, the DWG of the fish was lowest in the 22°C treatment (0.13 ± 0.006 g day^{-1}) and highest with the 31°C treatment (0.68 ± 0.003 g day^{-1}), and the DLG was lowest at 22°C (0.02 ± 0.002 cm day^{-1}) and highest at 31°C (0.08 ± 0.001 cm day^{-1}) ($p < 0.05$). However, these parameters were lower at the highest temperature (34°C treatment) than those at 28 and 31°C (Figure 3).

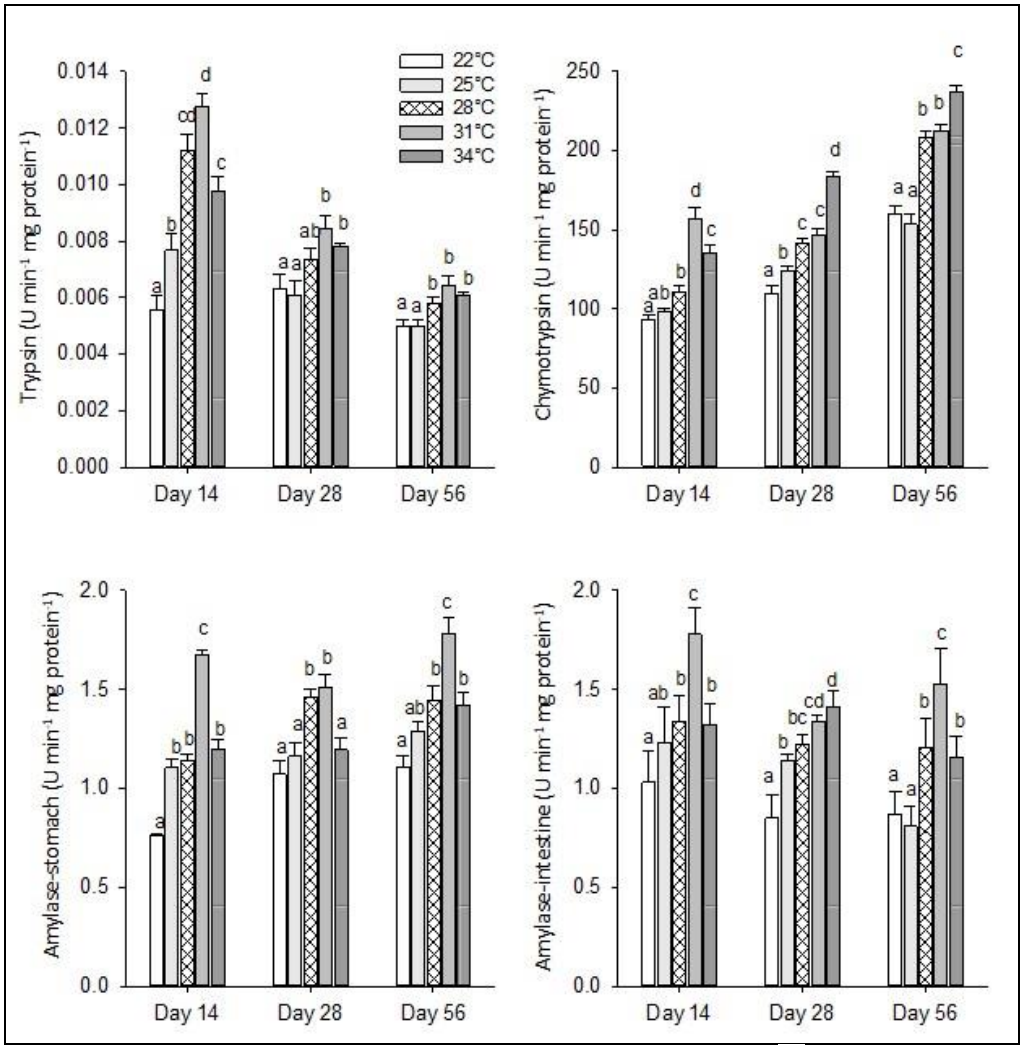

Figure 1. Digestive enzymatic activities of trypsin, chymotrypsin, and *α*-amylase in the stomach and *α*-amylase in the intestine of *Osphronemus goramy* for 56 days reared at different temperatures. Note: values are presented as mean ± SE (n = 3). Different letters (a, b, c, d) in the bars of the same sampling times show significant differences ($p < 0.05$).

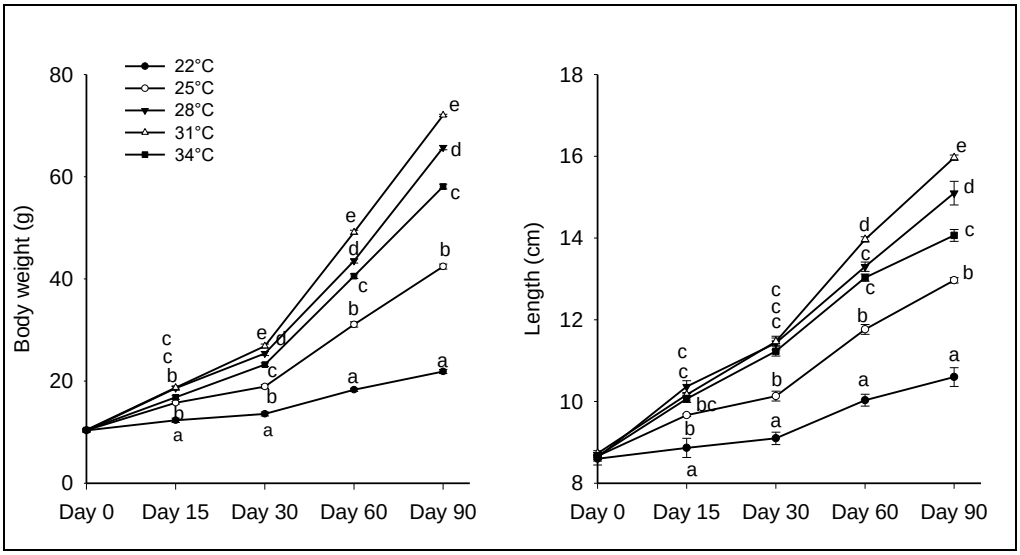

Figure 2. Body weight and length of *Osphronemus gourami* for 90 days reared at different temperatures. Note: values are presented as mean±SE (n=3). Different letters (a, b, c, d) on the same sampling day show a significant difference ($p<0.05$).

Survival rate. The survival rate of fish at the end of the experiment fluctuated from 98.7 to 100%. The highest SR was 100% at 22°C and 25°C, followed by 99.3% at 28°C and 31°C, and 98.7 at 34°C (Figure 3). There was no significant difference among the treatments ($p > 0.05$); therefore, the temperature range of 22°C to 34°C did not affect the survival of *O. goramy* fingerlings.

Feed conversion ratio. The highest FCR was observed with the 22°C treatment (1.53 ± 0.09), which was significantly different than those of other treatments. This result relates to the high feed intake amount and low growth of the fish (11.5 ± 1.00 g fish^{-1} at the end). There were no significant differences in FCRs among the treatments of 25, 28, 31, and 34°C, which ranged from 1.19 ± 0.02 to 1.53 ± 0.07 ($p > 0.05$) (Figure 3).

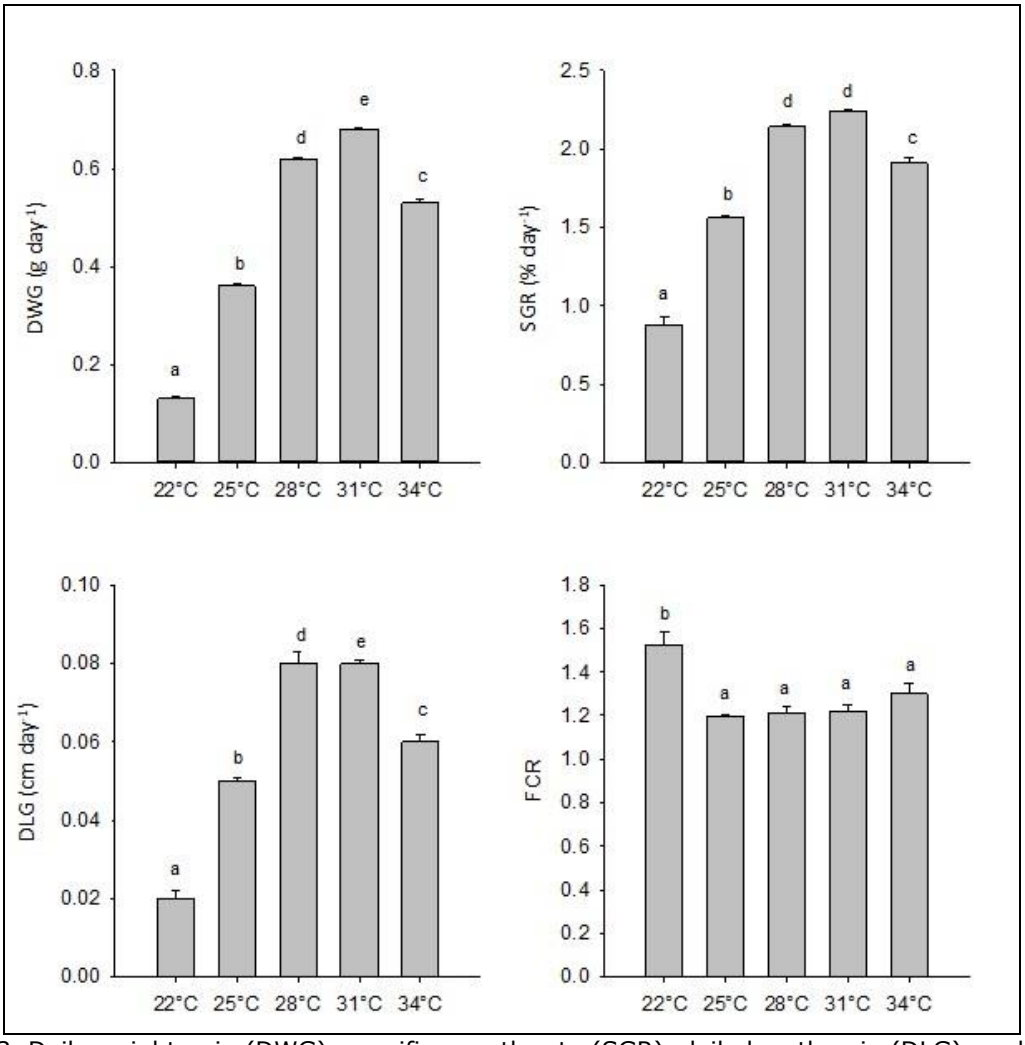

Figure 3. Daily weight gain (DWG), specific growth rate (SGR), daily length gain (DLG), and FCR of *Osphronemus gourami* after 90 days reared at different temperatures. Note: values are presented as mean±SE (n=3). Different letters (a, b, c, d, e) in the bars of the different temperatures show significant differences ($p<0.05$).

Discussion. Elevated water temperatures led to increased plasma glucose concentrations, which indicates that the fish were stressed (Kucukgul & Sahan 2008). The first response to stress by fish is the release of stress hormones such as cortisol and catecholamine; the second response is the activation of these hormones, which leads to a change of biochemical and physiological factors; and the third response is a change to the entire body. Fish consume a considerable amount of energy as they adapt to their environment. Chronic exposure to stressors can result in a decrease in physiological parameters and growth performance (Iwama 2006). The results of this experiment showed that the plasma glucose concentration was sensitive to high temperatures

compared to that at low temperatures. Although the fluctuation only approximated 6°C, the glucose concentration at 34°C was significantly higher than that at the control temperature (28°C) ($p < 0.05$). However, the level at 22°C was not significantly different from that of the control ($p > 0.05$) after 14 days. At the end of the experiment, the activities of fish normalized, except at 34°C, when the glucose concentration remained higher than those of other treatments. Similarly, the plasma concentrations of *Carassius auratus* exposed to 23, 27, and 31°C for 45 days were 41.0, 38.3, and 35.7 mg 100 mL^{-1}, respectively, and significant differences were not found (Imanpoor et al 2011). Fish are stressed when they are in a high stocking density or abnormal environments such as those with increased temperatures or pH changes. Darmawan et al (2021) found that the plasma glucose concentration of stressed catfish (149 - 198 mg 100 mL^{-1}) was high when stocked directly in a pH ≤ 5. Odhiambo et al (2020) recorded a high glucose concentration of *Oreochromis niloticus* during a period of crowding stress. Similarly, gilt-head sea bream (*Sparus aurata*) showed elevated glucose concentrations during 3 weeks of crowding stress (Porchas et al 2009). Catecholamine in the stressed fish stimulated the liver, which caused the release of glucose from glycogen leading to a plasma glucose increase.

In this study, the chymotrypsin activity in the fish intestines increased sharply with elevated temperatures from 22 to 34°C, while trypsin was highest at 31°C; *α*-amylase activity also increased to its highest level at 31°C. Amylase activity of fish depends on species and nutrition, especially in terms of the higher levels found in herbivorous and omnivorous fish compared to carnivorous species (Hung 2008). This study showed that these levels increased following the elevation of environmental temperatures, and this was corroborated by a similar study on yellowtail kingfish (*Seriola lalandi*), whereby the amylase activity was 0.04 and 0.8 U/mg protein at 18°C and 22°C ($p<0.05$), respectively (Bowyer et al 2013). In addition, those authors indicated that when subjected to temperatures ranging from 21 to 27°C, trypsin activity was the highest at 24°C (Bowyer et al 2013). In this study, the fish were reared at temperature fluctuations of ±3°C, and the trypsin activity increased at 28 to 31°C, whereas below 28°C or above 31°C there was a decrease in this activity.

The results of the present study showed that DWG and SGR were roughly linear between 22°C and 31°C, but reduced at 34°C. Lihua et al (2006) studied the growth of cobia (*Rachycentron canadum*) juveniles and reported a SGR of 4.38 - 4.52% day^{-1} (27 - 31°C), which was significantly different from those of the 23°C (2.74% day^{-1}) and 35°C (1.84% day^{-1}) treatments. Glencross et al (2010) reported DWGs of barramundi (*Lates calcarifer*) juveniles of 1.39-1.43 g day^{-1} (at 26-35°C) and a lowest FCR of 0.23 g day^{-1} (at 23°C). The growth performance of yellowtail kingfish (*Seriola lalandi*) juveniles increased by 54% when the temperature increased from 21 to 26.5°C (Abbink et al 2012). Studies on several fish species have revealed that at the temperature range tolerated by fish, growth rates increase as temperature rises in a parabolic manner (Xiao-Jun & Ruyung 1992; Watanabe et al 1993; Larsson & Berglund 2005; Handeland et al 2008). As water temperature increases, an increase in growth and metabolic rate occurs. However, if the water temperature is exceedingly high, fish will reduce feeding activity and slow growth because the metabolic process is affected (Hung 2008). This study indicated that the growth rate increased with elevated water temperatures, reached its optimal at 31°C, and declined significantly ($p < 0.05$) at 34°C. Fish growth is dependent on environmental factors such as water temperature, salinity, pH, and DO, and water temperature is likely the most important physical factor in the growth rate of fish (Dwyer & Piper 1987).

After 90 days of rearing, the FCR changed minimally from 25°C to 34°C (1.19 to 1.30) and no significant difference was observed between these treatments. However, there was a lower and significant difference when compared to the FCR of the 22°C treatment (1.53). The fish reared at the lowest temperature (22°C) had low digestive enzymatic activities (Figure 1) and the feed could not be digested completely, leading to insufficient nutrients for growth, which was similar to that of other fish reared at higher temperatures. The enzymatic activity of climbing perch (*Anabas testudineus*) increased

considerably during May and decreased from September to December as the weather cooled (Banerjee & Ray 2018).

Conclusions. The body weight, total length, daily weight gain, and specific growth rate of *Osphronemus goramy* fingerlings were highest in the 31°C treatment. The proteinases (trypsin and chymotrypsin) of the fish showed optimum activities at 31°C, while the highest FCR occurred at a low temperature (22°C). Plasma glucose concentrations increased in fish reared at both low and high temperatures.

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Conflict of interest. The authors declare that there is no conflict of interest.

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