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Influence of Different Temperatures and Sexes on the Survival and Stress Responses of Nile Tilapia (*Oreochromis niloticus* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was conducted to assess the influence of different water temperature ranges and sexes on the stress response of Nile tilapia. Sixteen proactive males and sixteen proactive female fish were used in the study. There were eight combinations of factors, and each combination was replicated four times. Changes in eye color pattern (ECP), ventilation rate (VR), and skin color of the fish were monitored after reaching the treatment temperature of the environment. Mortality of fish was also monitored. Results showed that the ECP of fish was significantly (*P*<0.01) affected by water temperature and sex of fish. Similarly, VR of fish was also significantly (*P*<0.01) affected by water temperature and sex of fish. Decreased dissolved oxygen (DO) was observed in increasing

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water temperature which may have also contributed to the stress response of Nile tilapia besides the increasing water temperature. Two out of four fish in T4M died after 6-12 hours of exposure while in T4F three out of four fish died after 2-4 hours. There was a decrease in the final weight of fish in all combinations of factors. On body color, both male and female fish exposed to increasing water temperatures exhibited an intense body color (presence of vertical stripes). From the results of this study, male fish had significantly lower (*P*<0.01) ECP scores and VR values than female fish when exposed to different ranges of water temperature. Likewise, male fish exposed to high temperatures (39-40°C) survived longer than female fish. Therefore, it can be concluded that male fish are more tolerant to increasing water temperature up to 37°C than female fish since males can easily cope with stress.

Keywords: Eye color pattern; ventilation rate; stress responses; Nile tilapia.

1. INTRODUCTION

"Tilapia" is the generic name of a group of cichlids endemic to Africa [1]. They are second only to carp as the most cultured and widely farmed fish species [1,2]. Tilapia possess a large variety of adaptation responses to match the vast array of its environment [3]. Among the tilapiines, the Nile tilapia (*Oreochromis niloticus*) is the most important cultured species [2]. The Nile tilapia has a potential for aquaculture due to its rapid growth at warm temperatures [1]; ability to adapt to high-density confinement and various systems of farming [4]; high resistance to diseases, viruses, and parasites than other cultured fish [5]; tolerance to wide range of water salinities [6] and high tolerance to poor water and crowding [7].

Temperature is one of the major environmental factors influencing reproduction and a key modulator of early gonadal ontogenesis in fish [8]. Other factors are photoperiod, density, sex ratio, size and weight of breeders, food, stress, oxygen, salinity, and pH [3]. The rise of water temperature due to climate change puts additional stress on freshwater ecosystems [9].

The majority of Asia's tropical and subtropical regions account for over 65 percent of the world's aquaculture production, which is produced inland. Global warming's effects on the climate are probably not going to have much of an impact on aquaculture techniques. Contrarily, climate change will affect the availability of water, and weather patterns like severe rain that may lead to eutrophication, and stratification in static (lentic) waters [10].

Temperature is one of the environmental factors with the greatest influence on the growth performance of animals [11]. Fish is affected by the temperature of the surrounding water which influences the body temperature, growth rate, food consumption, feed conversion, and other body functions [12,13]. Growth and livability in fish are optimum within a defined temperature range [14]. This study assessed the fish response to different water temperatures above the ideal range for the growth of the fish. The optimum temperature range in order for the fish to perform its best is 27-32°C [11]. The morphological changes of Nile tilapia to different ranges of temperature will increase our knowledge of how the fish reacts and copes with a given temperature.

2. MATERIALS AND METHODS

2.1 Experimental Fish

Sixteen male and sixteen female Nile tilapia, with an individual weight of about 25 grams were used in this study. The male and female Nile tilapia were obtained from the Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija. Freshwater Aquaculture Center Selected Tilapia (FaST) strain was used as the strain of Nile tilapia. Both sexes of Nile tilapia were similar in weight, length, and age so there are no phenotypic and genetic variations that may affect the growth of the fish. The fish were individually weighed and measured for their length.

2.2 Experimental Treatments and Design

There were two factors that were evaluated on their influence on fish mortality and stress responses. These factors were: Factor A - temperature ranges (30-31°C; 33-34°C; 36-37°C and 39-40°C) and Factor B - sexes of fish (male and female). Each combination of factors was shown in Table 1. The study used a 4 x 2 factorial experiment using randomized complete block design (RCBD). Each factor combination had four replicates.

Table 1. Experimental treatments

Factor A (Temperatures, ºC)	Factor B (Sexes)	
	Male (M)	**Female (F)**
30 – 31 (T1)	T1M	T1F
33 – 34 (T2)	T2M	T2F
36 – 37 (T3)	T3M	T3F
39 – 40 (T4)	T4M	T4F

2.3 Experimental Unit

The fish underwent conditioning for seven days in the aquarium prior to the study being conducted. The experimental set-up was composed of thirty-two (32) units of glass aquaria measuring 30 x 30 x 30 cm. An electric heater was installed in each aquarium to attain constant treatment temperature throughout the experimental period. An aeration system was provided in each aquarium to provide sufficient dissolved oxygen (DO) for the fish.

2.4 Experimental Procedures

Thirty-two male and thirty-two female fish were isolated individually in glass aquaria for five days. Three sides of glass aquaria were covered with white paper to avoid the fish from seeing each other during the isolation period. The eye color pattern (ECP) of the fish was monitored daily. Sixteen males and 16 females were selected based on their ECP. Fish with a mean ECP score of less than 4 during the isolation period were selected. One fish was stocked in each aquarium.

Selected fish were transferred from the isolation treatment to the experimental treatment. The water temperature in each aquarium of the experimental set-up was gradually adjusted to the treatment temperature within 8 hours to avoid thermal shock to the fish. Changes in ECP, ventilation rate (VR), and body color of the experimental fish were monitored at 0, 15, 30, 45 min; 1, 2, 6, 12, 24, 48, 72, 96, and 120 h after reaching the treatment temperature of the environment. Mortality was also observed in the 7-day experimental period. Feed was given once per day at 1% of the body weight [15].

2.5 Monitoring of the Eye Color Pattern

The ECP was monitored daily during the entire duration of the experiment. The ECP was quantified as the darkened area of both the iris and sclera. The circular area of the eye was divided into 8 equal parts using 4 imaginary diameter lines (Fig. 1) [15,16]. The ECP values observed ranged from zero (no darkening) to eight (total darkening). ECP score is unitless.

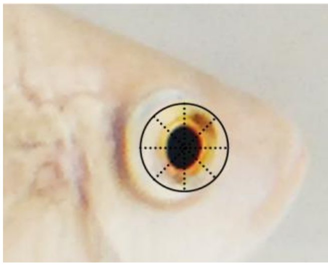

Fig. 1. Quantification of ECP

2.6 Monitoring of Ventilation Rate

The VR was visually estimated by counting the time (i.e. seconds) for 20 successive opercular or buccal movements [17]. It was measured daily at three times per fish in a period of 3 consecutive minutes. The readings were averaged for each fish and were used in analyzing the relationship between the ECP and VR.

2.7 Monitoring of Dissolved Oxygen (DO) and Water Temperature

Daily monitoring of DO and water temperature was conducted. A glass thermometer was used to monitor the water temperature. While a DO meter was used to monitor dissolved oxygen in the water.

2.8 Sampling of Experimental Fish

Initial and final weight and length of fish were measured. A digital balance was used to measure the weight of the fish. While a ruler was used to measure the length of the fish.

2.9 Statistical Analysis

The relationship between ECP and VR was assessed using linear regression and Pearson correlation coefficient. Data were analyzed using two-way Analysis of Variance and the treatment means were compared using Duncan's Multiple Range Test. The statistical analyses were performed using SPSS software version 23 for Windows.

3. RESULTS AND DISCUSSION

3.1 Eye Color Pattern

The mean ECP and mean VR of male and female fish subjected to different temperature ranges for 7 days are shown in Table 2. The mean ECP of the fish was not significantly affected ($P>0.05$) by the interaction between water temperature and sex of fish. However, mean ECP was significantly affected ($P<0.01$) by water temperature and sex of fish. The mean ECP of female fish at 39-40°C (T4F) of 5.81±0.84 and of male fish at 39-40°C (T4M) of 5.41±0.85 were significantly different ($P<0.05$) to each other but were significantly higher ($P<0.05$) than those in the rest of the treatments. The mean ECP of male fish at 36-37°C (T3M) (3.70±1.94), female fish at 33-34°C (T2F) (3.40±2.04), male fish at 33-34°C (T2M) (3.04±1.71) were not significantly different ($P>0.05$) from each other but were significantly higher ($P<0.05$) than those in female fish in 30-31°C (T1F) with 2.02±1.86 and male fish in 30-31°C (T1M) with 1.44±1.60. The mean ECP in the latter two treatments was not significantly different ($P>0.05$) from each other.

In this study, it was observed that the ECP of male and female fish changed from pale to dark after reaching the treatment temperature ranges. ECP is a stress-coping style in Nile tilapia, and it is a good, easy, and inexpensive tool for assessing both alertness and stress levels in the fish [15].

Table 2. Mean eye color pattern (±SD) of male and female fish per treatment temperature

Sex	Mean ECP of fish per Treatment Temperature			
	T1	T2	T3	T4
Male	1.44±1.60^{ax}	3.04±1.71^{ay}	3.70±1.94^{by}	5.41±0.85^{bz}
Female	2.02±1.86^{aw}	3.40±2.04^{ax}	4.83±2.02^{ay}	5.81±0.84^{az}

Note: Means having a common superscript within a column (a, b), or within a row (w, x, y, z) are not significantly different from each other at 5% (P< 0.05) probability level by DMRT. T1 = 30-31℃; T2 = 33-34℃; T3 = 36-37℃; T4 = 39-40℃

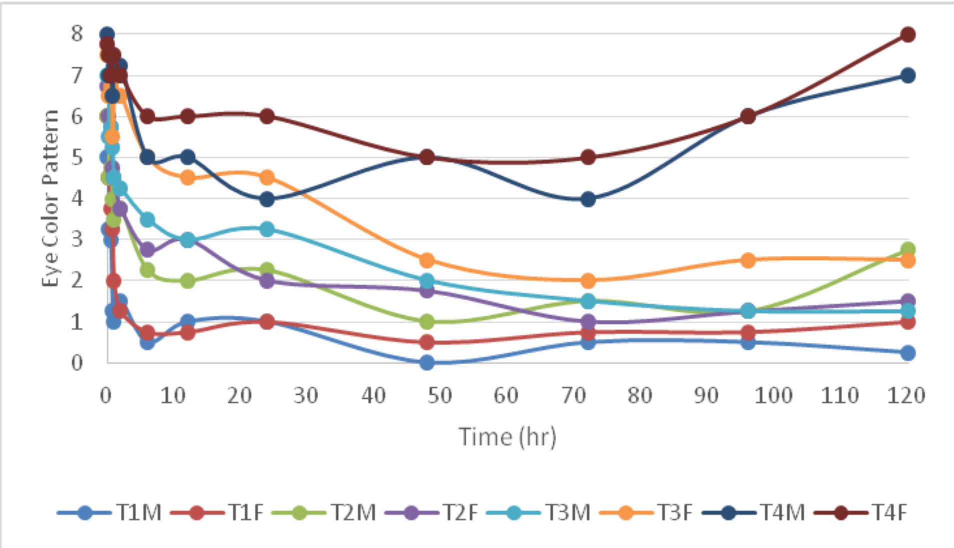

Fig. 2. Mean eye color pattern of all fish per combination of factors

Fig. 2 shows the daily ECP values of male and female fish during the 7-day experimental period. It was observed that there was a decreasing trend of ECP values in all combinations of factors except T4M and T4F. It may be because fish subjected to different ranges of water temperatures (T1, T2, and T3) were able to gradually cope in the stressful environment. However, fish in T4M and T4F exhibited darker ECP throughout the study because of very high temperature of 39-40°C. It was observed that fish in T4M and T4F refused to eat the food given to them and dedicated their energy to escaping the stressful environment by swimming aggressively and trying to jump out of the water, this behavior can be observed if the fish was in stress condition. Similar results were also reported in red tilapia (*Oreochromis* spp.) possibly due to the need to maintain their energy consumption during stressful conditions [18].

It also shows that males can more easily cope with stress than females when subjected to different ranges of water temperature. Male fish had lower mean ECP scores when exposed to different ranges of water temperature than female fish. According to Figueiredo-Fernandes et al. [19], male fish subjected to higher temperature exhibits significantly higher Glutathione S-transferase (GST) activity than females. Apart from its essential functions in different intracellular activities, a crucial role of GST is the defense against stress [19].

Similar studies were conducted wherein ECP was used as an indicator of stress. Decano et al. [20], subjected red tilapia to high temperature and confinement stress. They found that an increased value of ECP was recorded in an increased temperature and confinement stress. Apino et al. [21], assessed changes in the ECP of red tilapia exposed to blue and red-light emitting diode (LED). Results of the study showed that darker ECP was observed in fish exposed to a blue and red LED. Likewise, Bernandino et al. [22] used red tilapia as experimental fish where they subject the fish to electric shock and handling stress. Darker ECP was also observed in this study. The stress response of red tilapia was also reported wherein higher ECP score was observed in fish subjected to high ammonia levels [23]. Similar result was observed in the present study when tilapia was subjected to high water temperatures.

3.2 Ventilation Rate

Table 3 shows the mean ventilation rate of male and female fish per treatment temperature. It was observed that the mean VR of the fish was affected by the interaction between water temperature and sex of fish ($P<0.01$). Furthermore, mean VR was significantly affected ($P<0.01$) by water temperature and sex of fish. Female fish in T4 had the highest mean VR of 2.87±0.70 buccal movements/sec which was significantly different ($P<0.05$) in all combinations of factors. The mean VR of T4M (2.04±0.44 buccal movements/sec) and T3F (2.12±0.76 buccal movements/sec) were not significantly different ($P>0.05$) but both were significantly higher (P<0.05) than those of the rest of the remaining treatments. Also, there was no significant difference ($P>0.05$) among T3M (1.54±0.46 buccal movements/sec), T2F (1.61±0.57 buccal movements/sec), and T2M (1.39±0.40 buccal movements/sec) but they were significantly higher ($P<0.05$) than those in female fish in T1F with 1.07±0.26 buccal movements/sec and male fish in T1M with 1.02±0.25 buccal movements/sec. The VR in the latter two combinations of factors were not significantly different ($P>0.05$) from each other. Results showed that there was an abrupt change in VR values when the temperature of water increased. Factors affecting the results may also be contributed to the decrease in dissolved oxygen concentration. VR is one of the coping mechanisms of tilapia.

Table 3. Mean ventilation rate (±SD) of male and female fish per treatment temperature

Sex	Mean VR of fish per Treatment Temperature			
	T1	**T2**	**T3**	**T4**
Male	1.02 ± 0.25^{ax}	1.39 ± 0.40^{ay}	1.54 ± 0.46^{by}	2.04 ± 0.44^{bz}
Female	1.07 ± 0.26^{aw}	1.61 ± 0.57^{ax}	2.12 ± 0.76^{ay}	2.87 ± 0.70^{az}

Note: Means having a common superscript within a column (a, b), or within a row (w, x, y, z) are not significantly different from each other at 5% (P< 0.05) probability level by DMRT. T1 = 30-31℃; T2 = 33-34℃; T3 = 36-37℃; T4 = 39-40℃

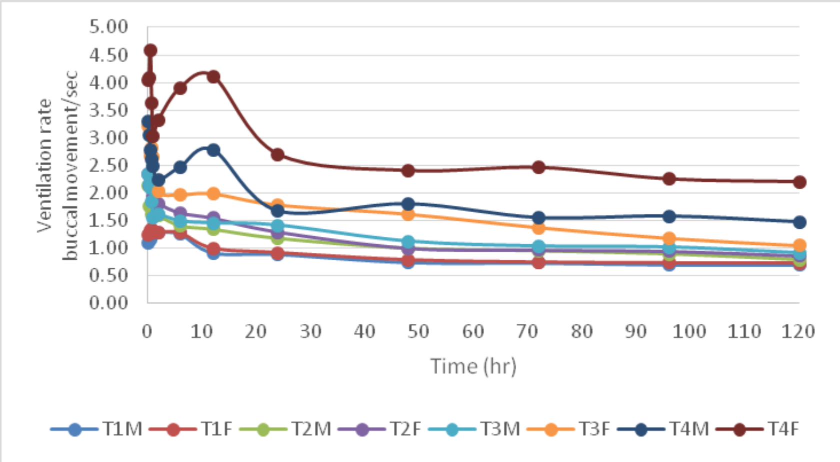

Fig. 3. Ventilation rate of all fish per combination of factors

Fig. 3 shows the daily VR values (buccal movement/sec) of males and females during the experimental period of 7 days. It was observed that there was a decreasing trend of VR in all combinations of factors indicating that the fish can cope with increasing temperature. However, T4F had the highest significant mean which is significantly different ($P<0.05$) compared to the other combination of factors. It was observed that female fish exposed to 39-40°C died 2-4 hours after exposure while male fish died after 6-12 hours to the same range of water temperature. Ventilation rate is a sympathetic response (a response that indicates that someone is in a bad situation) that quickly increases in response to a stressor, thus it is particularly useful for measuring stress levels because it is easily quantifiable [15,24].

Similar studies [20-23] that used ECP as an indicator of stress also used VR to evaluate the stress responses of fish. Red tilapia subjected to high temperature and confinement stress, blue and red LED, electric shock and handling stress, and high ammonia levels had faster VR.

3.3 Relationship between Eye Color Pattern (ECP) and Ventilation Rate (VR)

The relationship between the mean VR and mean ECP of Nile tilapia is shown in Fig. 4. It was observed that there was a significant strong positive correlation between the mean VR and mean ECP (n=8; r=0.810; $P<0.01$) of Nile tilapia when exposed to thermal stress. This indicates that as the VR increases, the ECP also increases.

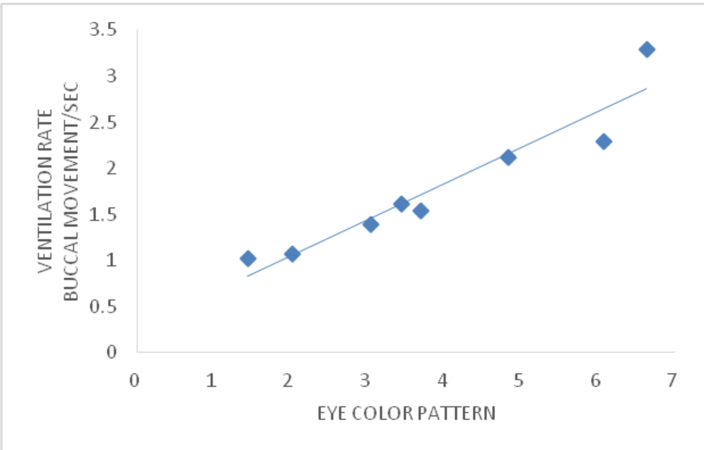

Fig. 4. Relationship between mean ventilation rate and mean eye color pattern of fish (n=8; *r*=0.810; *P*<0.01)

Table 4. Mean dissolved oxygen (mg/L) and temperature (°C)

Combination of factors	Mean (±SD)	
	Temperature	**Dissolved Oxygen**
T1M	30.54±0.06[a]	5.91±0.18[a]
T1F	30.48±0.09[a]	5.92±0.28[a]
T2M	33.51±0.08[b]	5.36±0.09[b]
T2F	33.48±0.09[b]	5.34±0.04[b]
T3M	36.41±0.08[c]	5.19±0.15[b]
T3F	36.47±0.17[c]	4.94±0.19[c]
T4M	39.55±0.10[d]	4.40±0.21[d]
T4F	39.57±0.18[d]	4.40±0.06[d]

Note: Means in a column superscripted with different letters are significantly different at 5% (P< 0.05); T1 = 30-31°C; T2 = 33-34°C; T3 = 36-37°C; T4 = 39-40°C; M= males; F = females

3.4 Dissolved Oxygen (DO) and Water Temperature

Table 4 presents the mean DO and temperature in all combinations of factors during the 7-day experimental period. T1M and T1F were significantly different (P<0.05) compared to those in the rest of the combination of factors. However, T1M with a mean DO of 5.91±0.18 mg/L, and T1F with a mean DO of 5.92±0.28 mg/L were not significantly different (P>0.05). T2M, T2F, and T3M had a mean DO of 5.36±0.09 mg/L, 5.34±0.04 mg/L, and 5.19±0.15 mg/L, respectively, which were significantly different (P<0.05) in all combinations of factors. Yet, T2M, T2F, and T3M were not significantly different (P>0.05). T3F had a mean DO of 4.94±0.19 mg/L. T4M and T4F had means of 4.40±0.21 mg/L and 4.40±0.06 mg/L, respectively which were not significantly different (P>0.05). T4M had the lowest mean DO in this study.

It was observed in this study that the DO concentration decreases as the temperature increases. The relationship between water temperature and DO is inverse; that is, cold water is able to "hold" more dissolved oxygen than warm water [25,26]. Decreased DO concentration could be predicted by increasing water temperature [27,28]. It was observed that DO in T4M and T4F was below the required DO concentration, which may contribute to the observed fish mortalities. Since water temperature affects the oxygen solubility in the water, it affects the behavioral responses and morphological changes of fish [29]. Thus, the high range of water temperature and low DO concentration imposed stress on the fish.

3.5 Mortality

There were no mortalities in T1M, T1F, T2M, T2F, T3M, and T3F in all replicates. Since all fish used in this study were proactive (fish with ECP values of less than 4 after the isolation period), they can easily adapt to the stressful environment. However, in T4M, two out of four male fish subjected to the water temperature of 39-40°C died after 6-12 hours of exposure. In T4F, three out of four female fish died after 2-4 hours of exposure to the water temperature range of 39-40°C. Factors affecting these results can be the stress experienced by the fish due to the high-temperature ranges and low concentration of DO.

3.6 Weight and Length

On initial weight, T1M with a mean weight of 25.50±2.33 g was not significantly different (P>0.05) to T1F with 27.28±3.65 g, T2M with 25.93±2.49 g, T2F with 23.60±1.01 g, T3M with 27.38±2.45 g, T3F with 27.80±2.42 g, T4M with 25.03±5.10 g and T4F with 24.88±4.31 g. After the study, T2F had the lowest final mean weight of 22.95±0.98 g followed by T4F and T4M with a final mean weight of 23.53±3.45 g and 23.48±4.81 g, respectively. T1F had the highest final mean weight of 27.05±3.84 g, followed by T3M with 27.03±2.41 g. All combinations of factors were not significantly different (P>0.05) from each other.

Table 5. Changes in the final weight and length of fish in all combinations of factors

Combination of factors	Final Weight	Final Length
T1M	-	*
T1F	-	+
T2M	-	*
T2F	-	+
T3M	-	+
T3F	-	+
T4M	-	+
T4F	-	+

*Note: + = increase; - = decrease; * = no changes*

Fig. 5. Fish exposed to 30-31°C (left) and 39-40°C (right)

On initial length, T1F had a mean length of 11.73±0.49 cm, followed by T3F with 11.65±0.40 cm, T3M with 11.50±0.41 cm, T1M with 11.50±0.45 cm, T2M with 11.43±0.30 cm, T4F with 11.20±0.54 cm, T2F with 11.10±0.27 cm and lastly T4M with 11.10±0.42 cm. On final length, T2M had the lowest final mean length with 11.43±0.30 cm. While T1F and T3M had the highest final mean length with 11.75±0.50 cm and 11.75±0.50, respectively. Yet, all combinations of factors were not significantly different (P>0.05) from each other. Factors affecting the results may be contributed to the higher energy consumption since fish need to cope up with the stressors and maintain homeostasis. Thus, a decrease in weight was observed.

Table 5 presents the changes in the final weight and length of fish in all combinations of factors. It was observed that there was a decrease in the final weight of fish in all combinations of factors. Also, some fish had an increase while some had no changes in the final length. A possible factor affecting the results is the physical stress experienced by fish due to low dissolved oxygen concentration and high-temperature range. Exposure to any type of stressors, either environmental, chemical, or perceived stressors results in a series of physiological responses in animals, like growth depensation in fish [29].

3.7 Body Color

Both male and female fish exposed to increasing water temperatures exhibited an intense body color (presence of vertical stripes) (Fig. 5). It was observed that as the temperature was increased higher than the ideal range, the body color of the fish started to intensify from pale to grey. Color alteration is controlled by the brain through the pigment called chromatophore. Cichlid species, including the Nile tilapia, change their skin color when stressed and this was also observed in red Nile tilapia [30].

4. CONCLUSION

Male fish had a significantly lower ECP score, VR values, and less intensified skin coloration than female fish when exposed to increasing water temperature. Likewise, male fish exposed to high temperatures (39-40°C) survived longer than female fish. Therefore, it only shows that the males can more easily cope with stress than females when subjected to different ranges of water temperature. These behavioral (VR) and morphological (ECP and body color) responses are good, easy, and inexpensive tools for assessing both alertness and stress levels in the fish subjected to thermal stress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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