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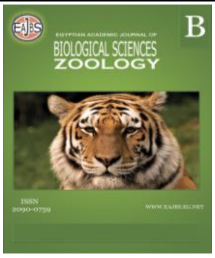

Morphological and Physiological Changes in Red Tilapia (*Oreochromis* spp.) Subjected to High Temperature and Confinement Stress

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ABSTRACT

High temperature due to global warming and the overcrowding or limited space for cultured species have been evident in the aquaculture setting nowadays. In this study, physiological changes such as ventilation rate (VR), eye color pattern (ECP), and skin color were observed in red tilapia subjected to high temperature (T2), confinement stress (T3), and the combination of both stressors (T4). The VR, ECP, and skin color were monitored before the introduction of stressors (day 0) and every day after the introduction of stressors until day 15. Results showed that fish in T2, T3, and T4 had significantly faster VR, darker ECP, and more intense skin color as compared to those in the control group (T1). Increasing VR, ECP, and skin color were also recorded from day 1 to day 15 of subjecting the fish to stressors indicating that the fish were not able to adapt to the stressful environment during the duration of the study. The highest values were observed in T4 since there were two stressors present in the treatment. This only connotes that high temperature and overcrowding could really impose stress on red tilapia and 15 days was not enough for the fish to adapt to the stressful environment.

INTRODUCTION

Physiological, behavioral, and morphological responses due to stress are important in considering the maintenance of homeostatic conditions in the well-being of fish (Templonuevo and Vera Cruz, 2016). Stress is a reflex reaction when the animal fails to cope up with its environment which may lead to many unfavorable consequences (Kumar *et al.*, 2012). The measure of stress gives information on how effectively a fish resists death and resets homeostatic norms when faced with noxious stimuli (Sopinka *et al.*, 2016).

Among the various physical factors influencing the aquatic environment, temperature is of great importance and it plays a critical role in the life of aquatic poikilotherms (Singh *et al.*, 2013). It is defined as a catalyst, a depressant, an activator, a restrictor, a stimulator, a controller, a killer, and the most commonly studied environmental factor that influences sexual determination in fish (Deas and Lowney, 2000). Physiological processes of fish such as food consumption, digestion, immunity, etc., are influenced by temperature. If beyond the optimal limit of a particular species, it adversely influences the fish health by increasing the metabolic rate, oxygen consumption, and the invasiveness and virulence of pathogens, which in turn may cause a variety of functional changes associated disturbance that can lead to the death of the species (Singh *et al.*, 2013).

Fish under aquaculture conditions are also often subject to environmental changes or stressors, such as handling, crowding, transporting, and changing water quality (Kubilay and Ulukoy, 2002). A lot of fishermen use higher stocking densities to maximize the space of their culture units such as tanks, ponds, or cages. When fish are overcrowded and have limited space for movement, they tend to be stressed which causes several responses including morphological and physiological changes (Templonuevo and Vera Cruz, 2016). Due to the increasing environmental temperature as an effect of climate change and global warming, both high temperatures and overstocking have been major causes of fish kill for most aquaculture species such as tilapia. In this study, physiological and morphological changes (ventilation rate, eye color pattern, and skin color) in red tilapia (*Oreochromis* spp.) were observed when the fish were exposed to high water temperature and confinement stress for 15 days.

MATERIALS AND METHODS

Experimental Fish:

Twelve (12) pieces of red tilapia (fish that has an active behavior in terms of growth, feeding, aggression, etc.) weighing 80 - 100 g were selected and used in the study. Acclimation was done for 5 days before the commencement of the study. The feeding of fish was 3 % of their body weight using commercial pellet feeds.

Each fish was placed in an aquarium measuring 30 x 60 x 30 cm. Aeration was provided to maintain the ideal oxygen level and for the continuous mixing of the water.

Experimental Treatments:

There were four treatments in this study and each treatment had 3 replicates. Treatment 1 was the control group while T2, T3, and T4 were the treatments subjected to stressors (Table 1).

Table 1. Experimental treatments in the study

TREATMENT	DESCRIPTION
1	No application of stressor
2	Application of confinement stressor
3	Application of temperature stressor (30-31°C)
4	Application of confinement and temperature stressors (30-31°C)

Experimental Procedure:

Each aquarium was stocked with one red tilapia. The VR, ECP, and skin color of the fish were monitored between 8:00 – 9:00 in the morning for 15 days. Confinement was performed by limiting to 25 % (adopted from Templonuevo and Vera Cruz, 2016) the total space occupied by the fish using a styrophore divisor. For the application of high temperature, 30 - 31 °C water temperature was maintained using a 200 W heater. It is classified high since the optimum range temperature for tilapia is 25 - 29 ºC.

Monitoring of Ventilation Rate, Eye Color Pattern, and Skin Color:

The VR was visually estimated by counting the time (i.e. seconds) for 20 successive opercular or buccal movements (adopted from Alvarenga and Volpato, 1995). For ECP, it was quantified as a darkened area of both the iris and sclera. The circular area of the eye was divided into 8 equal parts using an imaginary diameter line and the ECP value that was observed ranged from zero (no darkening) to eight (total darkening) (based on Volpato *et al.*, 2003; Vera Cruz and Tauli, 2015).

For the determination of skin color, a color chart (Figure 1) which ranged from light pink (1) to orange (13) was used to compare the changes in the skin color of red tilapia. Since the fish did not have fair color in each part of its body, caudal fin, head, and the belly part of the fish were used to observe the changes in color of the fish.

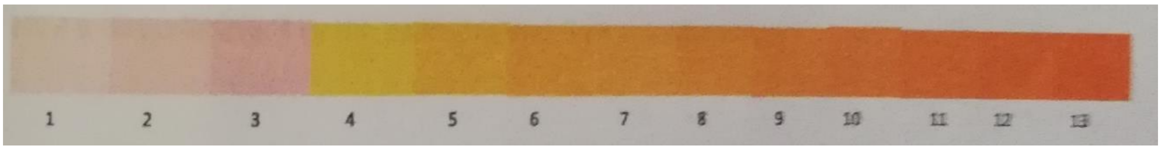

Fig1. Skin color chart.

Statistical Analysis:

The data that were obtained in this study were analyzed by one-way ANOVA procedure of SPSS version 16 for windows. Means were compared by Duncan's new multiple range test. The correlation between ECP, VR, and skin color was assessed using the Pearson correlation coefficient.

RESULTS AND DISCUSSION

Ventilation Rate (VR):

Ventilation rate increased with time when the fish were subjected to temperature and confinement stresses (Table 2). Furthermore, significantly higher mean VRs were observed in T2, T3, and T4 starting day 2, which means that fish were stressed in those treatments as compared to fish in T1. Increased ventilatory activity is commonly used as a sign of stress and poor welfare in fish. It is one of the rapid physiological responses of fish due to problems in water oxygenation and other environmental changes (Martins *et al.*, 2012). In Nile tilapia (*Oreochromis niloticus*), stressed fish due to confinement showed increased in VR (Barreto and Volpato, 2004) whereas temperature is regarded as an environmental factor that affects the activity, behavior, feeding, growth, survival, appetite and reproduction in all fishes (Dan-kishiya *et al.*, 2015).

Among the treatments, fish subjected to the combination of confinement and temperature stressor (T4) had significantly attained the highest mean VR starting day 4 since there were two stressors present in their environment. It was then followed by T3 which indicates that fish reared at high temperature was more stressed than the fish reared in a limited space.

Eye Color Pattern:

The ECP of T2, T3, and T4, increased immediately after the stressors were applied. However, significant differences were only recorded on day 6 until day 15. Results showed that there was a slight increase but continuous in ECP as time progressed (Table 3). According to Vera Cruz and Tauli (2015), variation in ECP response during the isolation period is a form of behavioral coping style in Nile tilapia in particular and fish in general. It has been proven in several studies that ECP is a clear indicator of stress in Nile tilapia (Volpato *et al.*, 2003; Vera Cruz and Brown, 2007) and other fish species (Suter and Huntingford, 2002).

Table 2. The mean ventilation rate±SD (buccal movement/sec) of the fish exposed to different stressors.

Period (Days)	Treatment			
	T1	T2	T3	T4
	Control (no application of stress)	Confinement stress	Temperature stress	Both confinement and temperature stresses
0	1.10±0.04[a]	1.15±0.03[a]	1.17±0.08[a]	1.18±0.02[a]
1	1.18±0.05[a]	1.18±0.03[a]	1.19±0.09[a]	1.32±0.14[a]
2	1.16±0.03[c]	1.23±0.02[bc]	1.27±0.07[ab]	1.37±0.09[a]
3	1.11±0.01[c]	1.21±0.04[bc]	1.31±0.06[ab]	1.48±0.17[a]
4	1.23±0.05[b]	1.26±0.03[b]	1.37±0.05[b]	1.58±0.20[a]
5	1.13±0.04[c]	1.37±0.10[b]	1.41±0.04[b]	1.62±0.17[a]
6	1.22±0.01[c]	1.39±0.01[bc]	1.47±0.04[b]	1.78±0.19[a]
7	1.21±0.05[c]	1.43±0.05[b]	1.42±0.06[b]	1.74±0.13[a]
8	1.20±0.03[c]	1.48±0.06[b]	1.48±0.05[b]	1.86±0.12[a]
9	1.23±0.05[d]	1.45±0.13[c]	1.64±0.04[b]	1.97±0.10[a]
10	1.21±0.03[c]	1.53±0.07[b]	1.62±0.06[b]	1.97±0.24[a]
11	1.25±0.03[c]	1.60±0.06[b]	1.62±0.04[b]	1.98±0.06[a]
12	1.22±0.02[c]	1.63±0.07[b]	1.72±0.02[b]	2.15±0.07[a]
13	1.21±0.04[d]	1.67±0.08[c]	1.80±0.08[b]	2.20±0.08[a]
14	1.23±0.03[d]	1.73±0.01[c]	1.85±0.06[b]	2.24±0.07[a]
15	1.24±0.03[d]	1.75±0.02[c]	1.90±0.04[b]	2.28±0.05[a]

Means in a row superscripted with different letters are significantly different (*P<0.05*).

Table 3. The mean eye color pattern±SD of the fish exposed to different stressors.

Period (Days)	Treatment			
	T1	T2	T3	T4
	Control (no application of stress)	Confinement stress	Temperature stress	Both confinement and temperature stress
0	0.57±0.51[a]	1.40±0.98[a]	1.57±1.16[a]	0.77±0.35[a]
1	0.57±0.51[a]	1.43±1.00[a]	1.63±1.07[a]	1.03±0.40[a]
2	0.57±0.51[a]	1.47±1.02[a]	1.80±1.08[a]	1.20±0.50[a]
3	0.57±0.51[a]	1.57±1.10[a]	2.00±0.96[a]	1.47±0.51[a]
4	0.57±0.51[a]	1.63±1.15[a]	2.20±0.95[a]	1.70±0.46[a]
5	0.63±0.60[a]	1.67±1.18[a]	2.37±1.07[a]	1.87±0.45[a]
6	0.63±0.60[b]	1.80±1.22[ab]	2.57±0.93[a]	2.23±0.55[ab]
7	0.63±0.60[b]	1.80±1.22[ab]	2.77±1.00[a]	2.43±0.55[a]
8	0.63±0.60[b]	1.83±1.25[ab]	2.87±1.05[a]	2.53±0.64[a]
9	0.67±0.61[b]	1.87±1.27[ab]	3.07±1.11[a]	2.93±0.90[a]
10	0.67±0.61[b]	1.90±1.31[ab]	3.30±1.23[a]	3.07±0.92[a]
11	0.70±0.66[b]	1.90±1.31[ab]	3.47±1.31[a]	3.27±1.10[a]
12	0.70±0.66[b]	1.97±1.37[ab]	3.67±1.47[a]	3.37±1.18[a]
13	0.70±0.66[b]	2.00±1.40[ab]	3.53±1.24[a]	3.50±1.22[a]
14	0.73±0.67[b]	2.07±1.37[ab]	3.63±1.33[a]	3.60±1.32[a]
15	0.77±0.71[b]	2.10±1.40[ab]	3.70±1.39[a]	3.63±1.34[a]

Means in a row superscripted with different letters are significantly different (*P<0.05*).

Starting day 7, significantly higher mean ECPs were observed in T3 and T4 as compared to that in T1. This indicates that fish in both treatments were stressed compared to fish in the control treatment. In addition, the daily increasing ECP values in T3 and T4 shows that the fish continued to respond to the stressor(s). However, the highest mean ECP value of 3.70±1.39 in T3 indicates that the level of stress was not too high compared to exposing the fish at very high temperature of 39-40°C with fish having a mean ECP value of 7.71±0.02 (Bautista, 2020).

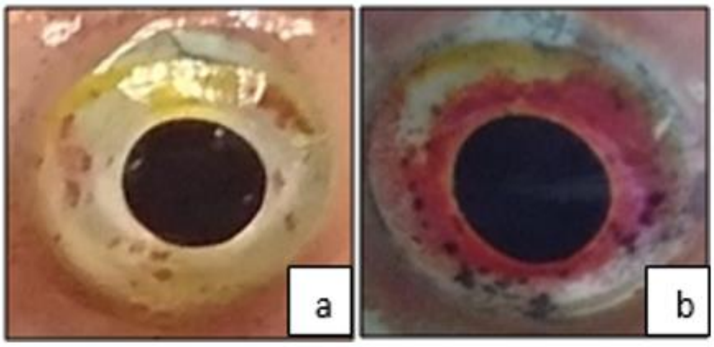

Fig. 2. Eye color pattern of the same fish subjected to temperature stress. (a) before exposure to the stressor; (b) after exposure to the stressor.

Based on the results of the study, when red tilapia was introduced to stressful conditions, the ECP darkened and turned into redder color (Fig. 2). This happened when the fish sensed a change in their environment. The eye color may also indicate the level of stress; with more stress as the color became darker (Vera Cruz and Tauli, 2015).

Skin Color:

Starting day 4, significantly higher values of skin color were observed in T3 and T4 as compared to that in T1. Higher values indicate a darker or intense color based on the color chart used in this study (Fig. 1). This means that fish exposed to stressors in T3 and T4 were experiencing more stressful conditions than fish in the control treatment. Observation of the skin color to indicate stress was also shown in the study of Gray *et al.* (2011), when they estimated the effects of handling time, ambient light intensity, and anaesthetic method (factors that affect the level of stress in fish) in the two species of Malawi cichlids (*Melanochromis auratus* and *Metriaclima zebra*) by observing the fish coloration.

Starting day 12, results showed that fish in T4 attained the significantly highest intensity of skin color in all treatments. This result may be due to the combination of both temperature and confinement stressor that could change the morphological and behavioral responses of red tilapia. Moreover, the skin color value of the fish continued to increase with time as soon as the fish were subjected to stress (Table 4) which also agreed with the results on VR and ECP. The skin of fish turned into redder color since red is the primary body pigment of red tilapia. In fish, the external coloration is a distinctive feature that has long been of interest to biologists.

With the general trend that the VR, ECP, and skin color values were continuously increasing indicates that the fish were yet not able to adapt to the stressful environment. It was proven by Øverli *et al.* (2004) that coping techniques are present on teleost fishes, and these coping characteristics may lead to adaptation to the novel environment when the condition is not too stressful to the fish.

Table 4. The mean skin color ±SD of the fish exposed to different stressors.

Period (Days)	Treatment			
	T1	T2	T3	T4
	Control (no application of stress)	Confinement stress	Temperature stress	Both confinement and temperature stress
0	$4.00{\pm}0.00^{a}$	$4.33{\pm}0.58^{a}$	$4.33{\pm}0.58^{a}$	$4.33{\pm}0.58^{a}$
1	$4.00{\pm}0.00^{a}$	$4.33{\pm}0.58^{a}$	$4.33{\pm}0.58^{a}$	$4.33{\pm}0.58^{a}$
2	$4.00{\pm}0.00^{a}$	$4.33{\pm}0.58^{a}$	$4.33{\pm}0.58^{a}$	$4.33{\pm}0.58^{a}$
3	$4.00{\pm}0.00^{b}$	$4.33{\pm}0.58^{ab}$	$4.33{\pm}0.58^{ab}$	$5.00{\pm}0.00^{a}$
4	$4.00{\pm}0.00^{b}$	$4.33{\pm}0.58^{b}$	$5.00{\pm}0.00^{a}$	$5.00{\pm}0.00^{a}$
5	$4.00{\pm}0.00^{c}$	$4.33{\pm}0.58^{bc}$	$5.00{\pm}0.00^{ab}$	$5.33{\pm}0.58^{a}$
6	$4.00{\pm}0.00^{b}$	$4.33{\pm}0.58^{b}$	$5.33{\pm}0.58^{a}$	$5.33{\pm}0.58^{a}$
7	$4.00{\pm}0.00^{c}$	$4.67{\pm}0.58^{bc}$	$5.33{\pm}0.58^{ab}$	$5.67{\pm}0.58^{a}$
8	$4.00{\pm}0.00^{c}$	$5.33{\pm}0.58^{b}$	$5.33{\pm}0.58^{b}$	$6.33{\pm}0.58^{a}$
9	$4.00{\pm}0.00^{c}$	$5.33{\pm}0.58^{b}$	$5.67{\pm}0.58^{ab}$	$6.33{\pm}0.58^{a}$
10	$4.00{\pm}0.00^{c}$	$5.33{\pm}0.58^{b}$	$6.00{\pm}0.00^{ab}$	$6.67{\pm}0.58^{a}$
11	$4.33{\pm}0.58^{c}$	$5.33{\pm}0.58^{b}$	$6.00{\pm}0.00^{ab}$	$6.67{\pm}0.58^{a}$
12	$4.67{\pm}0.58^{c}$	$5.33{\pm}0.58^{bc}$	$6.00{\pm}0.00^{b}$	$7.33{\pm}0.58^{a}$
13	$4.67{\pm}0.58^{c}$	$5.33{\pm}0.58^{bc}$	$6.00{\pm}0.00^{b}$	$7.33{\pm}0.58^{a}$
14	$4.67{\pm}0.58^{c}$	$5.33{\pm}0.58^{c}$	$6.33{\pm}0.58^{b}$	$8.00{\pm}0.00^{a}$
15	$4.67{\pm}0.58^{c}$	$5.33{\pm}0.58^{c}$	$6.33{\pm}0.58^{b}$	$8.00{\pm}0.00^{a}$

Means in a row superscripted with different letters are significantly different ($P<0.05$).

In summary, the stress in fish had been studied through the morphological and physiological responses of fish when exposed to temperature and confinement stressors. Based on the results of the study, VR, ECP, and skin color changed when the fish were subjected to stress. Fish subjected to confinement, high temperature and a combination of both stressors stress showed significantly higher ECP and exhibited faster VR than the fish in the control treatment. In the same way, red tilapia displayed a more intense pink-orange skin color when also exposed to stressors. However, a combination of both stressors gave a most stressful condition for the fish, followed by high temperature and finally confinement stress. The general trend that the VR, ECP, and skin color values were continuously increasing throughout the duration of the study indicates that 15 days was not sufficient for the fish to adapt to the stressful environment.

Relationship between ECP, VR and Skin Color:

The relationship of VR, ECP, and skin color of fish subjected to stressors was also determined. Strong positive correlations between the morphological characteristics were observed in all treatments (Tables 5, 6, and 7). In addition, results showed significant relationships (P<0.01) in all treatments. This indicates that when the VR increases, the eyes and skin color of red tilapia darkens and intensifies, respectively. According to the study of Vera Cruz and Tauli (2015), the ECP of *O. niloticus* that was exposed to confinement stress was positively correlated to its VR. This generally implies that VR, ECP, and skin color can be an easy, inexpensive, and non-invasive way to measure conditions of fish stress.

Table 5. Correlation tests of VR, ECP, and skin color of fish subjected to confinement stress.

		VR	SC	ECP
Ventilation Rate (VR)	Pearson Correlation	-	0.873	0.975
Skin Color (SC)	Pearson Correlation	0.873	-	0.857
Eye Color Pattern (ECP)	Pearson Correlation	0.975	0.857	-

Correlation is significant at the 0.01 level (2-tailed).

Table 6. Correlation tests of VR, ECP, and skin color of fish subjected to temperature stress.

		VR	SC	ECP
Ventilation Rate (VR)	Pearson Correlation	-	0.958	0.963
Skin Color (SC)	Pearson Correlation	0.958	-	0.982
Eye Color Pattern (ECP)	Pearson Correlation	0.963	0.982	-

Correlation is significant at the 0.01 level (2-tailed).

Table 7. Correlation tests of VR, ECP, and skin color of fish subjected to both confinement and temperature stresses.

		VR	SC	ECP
Ventilation Rate (VR)	Pearson Correlation	-	0.976	0.991
Skin Color (SC)	Pearson Correlation	0.976	-	0.971
Eye Color Pattern (ECP)	Pearson Correlation	0.991	0.971	-

Correlation is significant at the 0.01 level (2-tailed).

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