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Stress Responses of Red Tilapia (*Oreochromis* Spp.) Exposed to Blue and Red-Light Emitting Diode (Led)

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ABSTRACT

Tilapias are among the most important warm-water fishes used for aquaculture production. One of the species gaining popularity nowadays is the red tilapia due to its appealing colour and taste. In this study, six red tilapia were exposed to the blue or red light-emitting diode (LED) and three to natural light (control) and changes in eye colour pattern (ECP), ventilation rate (VR) and body colour of the fish were observed. Each light condition was kept for 8 hours, from 800 h to 1600 h for 15 days. Results showed that significantly darker ECP, faster VR and intensified red skin colour were observed in treatments exposed to blue and red LED compared to those in the control treatment (natural light). Based on the results of the study it can be noted that exposure to blue and red LED causes stress in fish but it is not lethal since no mortality was recorded.

INTRODUCTION

Aquaculture which is the farming of aquatic organisms is probably the fastest growing food-producing sector and accounts for 50 percent of the world's fish that is used for food (FAO 2012). Among the most important warm-water fishes used for aquaculture are the tilapias (Charo-Karisa, 2006). It has been contributing to world aquaculture since the ancient Egyptian days (Amal and Zamri-Saad, 2011). One of which is the red tilapia which has an appealing colour and taste. It has many economical characteristics such as being omnivorous, reproductive in captivity, highly resistant to diseases and ability to grow in brackish and salt waters (Liao & Chang, 1983).

Stress is defined to be a condition wherein the dynamic equilibrium of animal organisms called homeostasis is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli, commonly defined as stressors (Chrousos and Gold, 1992). This is a common problem in aquaculture as it may lead to disease or even fatality. Stressors can be either chemical (pollution and other metabolic wastes), biological (crowding and presence of pathogenic microorganisms, physical (temperature, light and sounds), or procedures done in fish (handling, shipping and disease treatments) (Francis-Floyd, 2018). The impact of stress is determined by the ability of the organism to produce behavioural and physiological efforts in order to cope with the situation, and successful coping depends highly on the controllability and predictability of the stressor (Ursin and Olff, 1993). When fish are stressed, the challenging stimuli (stressors) demand a response, which is the essence of coping and adapting to a given environment (Galhardo and Oliveira, 2009).

Fish needs light to survive and grow; it is one of the essential inputs to life. In aquaculture, there are natural and artificial sources of light. Regardless of the source, environmental lighting in an aquaculture facility drives key biological processes that influence performance and ultimately affect production and profitability (Dellabio, 2015). However, behaviour can be affected even by artificial light stimuli. The biological response caused by this factor depends on the specific ecology of fish which can be stressful. In the same way, colours have been shown to produce different effects on fish in a species-specific manner (Villamizar *et al.*, 2011). In this study, stress responses of red tilapia exposed to an artificial light, particularly the Light Emitting Diode (LED) with red and blue colours were determined and observed through the changes in their eye color pattern (ECP), ventilation rate (VR) and skin color. Results may help in determining the stress response of fish to coloured LED which may help in the improvement of the cultural environment of this fish species.

MATERIALS AND METHODS

Experimental Fish:

Nine proactive red tilapia weighing 50-60 grams were selected and acclimatized in a concrete indoor tank at the Wet Laboratory, Freshwater Aquaculture Center, Central Luzon State University. Aeration was provided and fish were fed twice a day, 800 h and 1600 h with a feeding rate of 3% of the total body weight.

Experimental Treatments and Procedure:

Red tilapia were randomly distributed in nine experimental glass aquaria. There were 3 treatments in the study (Table 1) that were replicated thrice. Two light colours, red and blue (1.8 watts) were tested as an artificial source of lighting. Six aquaria were lighted with LED, while the other 3 were the control (no artificial light). The lights were placed above the glass aquaria, at a 30 cm distance above the water. Each lighting condition was kept for 8 hours, from 800 h to 1600 h for 15 days. Observation and recordings of ECP, VR and body-colour were carried out after 1600 h. The sides of the aquaria were covered with black plastic to prevent colour interference.

Table 1. Treatments used in the study.

Treatment	Description
T1	No artificial light (control)
T2	Blue LED
T3	Red LED

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

Eye colour pattern (ECP) was quantified through observation of the darkened area of both iris and sclera. The circular area of the eye was divided into eight equal parts using four imaginary lines: 0 zero (no darkening) to eight (total darkening) (Volpato *et al.*, 2003; Vera Cruz and Tauli, 2015).

Ventilation rate (VR) was estimated by counting the time (i.e., seconds) for 20 successive opercular or buccal movements (adapted from Alvarenga & Volpato, 1995). Daily VR measurements were done three times per fish in a period of three consecutive minutes.

A colour chart that ranges from 1 (light orange) to 10 (dark orange) was used to compare the skin colour of red tilapia (Fig.1). Since the fish did not have fair colour on each side of the body, the caudal fin, dorsal fin and belly part of the fish were used to observe any change in colour of the fish.

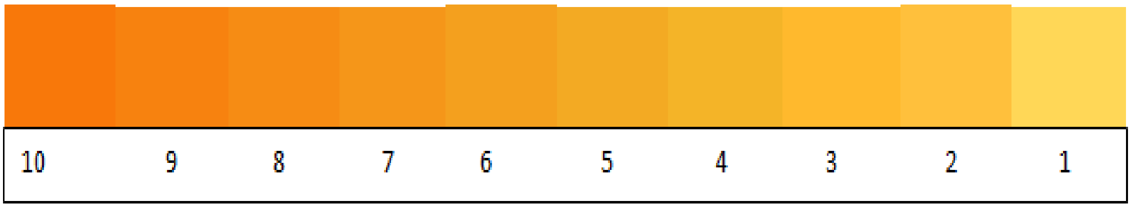

Fig. 1. Skin colour chart

Statistical Analysis:

Data were transferred to an excel sheet to calculate the mean and standard error for ECP, skin colour and VR. Then, these data were analyzed with a one-way analysis of variance (ANOVA) using the SPSS 20 statistical package to evaluate the differences between the tested treatments. The differences between means of experimental treatments were evaluated using Duncan's Multiple Range Test at 5 percent probabilities.

RESULTS AND DISCUSSION

Eye Colour Pattern:

Eye colour pattern (ECP) in all treatments had the same values (1) before the introduction of stressors. However, the ECP of the fish increased immediately after a day of exposure to LED. The ECP of the fish in the control group also increased but not as much as the increase in the LED treatments. Significantly darker mean ECP values were also observed in Red LED treatment compared to those in the control treatment (Fig. 2), from day 1 up to day 15 of the experiment, while significantly darker ECP values were observed in the Blue LED treatment starting day 2 up to day 15, except day 6 (Table 2).

Fig. 2. Eye colour pattern of red tilapia before (A) and after (B) exposure to the red LED.

Fish exposed to stress will undergo a series of behavioural and physiological changes in an attempt to adapt to the stressful condition (Costas *et al.,* 2008). The increase in ECP values in the control treatment may be due to the stress brought about by the introduction to a new environment. The significantly higher increase in ECP values in the LED treatments may be due to the stress brought about by the introduction to a new environment and exposure to LED. The ECP has been proven to be an indicator of stress in Nile tilapia (*O. niloticus*) (Volpato *et al.*, 2003; Vera Cruz and Brown, 2007) and other fish species (Suter and Huntingford, 2002). It is a good and inexpensive tool for the immediate assessment of both alertness and stress conditions of the fish (Vera Cruz and Tauli, 2015). As stated by Volpato *et al.* (2003), fish is more stressed as the total darkened area of the eye goes high.

Table 2. The eye colour pattern of the fish was subjected to different coloured LEDs.

Number of Days	**Stressor**		
	Control (T1)	**Blue LED (T2)**	**Red LED (T3)**
0	1.00±0.00	1.00±0.00	1.00±0.00
1	1.33±0.58[b]	2.67±0.58[ab]	2.33±0.58[a]
2	2.00±1.00[b]	6.67±0.58[a]	7.00±1.00[a]
3	2.67±0.58[b]	7.67±0.58[a]	7.33±0.58[a]
4	2.00±1.00[b]	5.67±0.58[a]	6.00±1.00[a]
5	2.67±0.58[b]	5.33±0.58[a]	6.00±1.00[a]
6	3.00±1.00[b]	5.33±1.53[ab]	6.00±1.00[a]
7	2.33±0.58[b]	5.33±0.58[a]	5.53±0.58[a]
8	4.00±1.00[b]	7.33±0.58[a]	7.00±1.00[a]
9	3.00±1.00[b]	6.33±1.53[a]	7.00±1.00[a]
10	3.00±1.00[b]	6.67±0.58[a]	6.67±0.58[a]
11	2.67±0.58[b]	7.00±1.00[a]	5.67±1.52[a]
12	2.00±1.00[b]	6.33±2.08[a]	7.00±1.00[a]
13	2.67±1.15[b]	7.00±1.00[a]	7.00±1.00[a]
14	2.67±1.15[b]	6.33±1.15[a]	6.67±1.15[a]
15	3.00±1.00[b]	6.33±2.08[a]	7.00±1.00[a]

*Means having different superscripts within a row are significantly different from each other by DMRT at the 5% probability level.

Ventilation Rate:

Ventilation rate (VR) immediately increased after exposing the fish to the blue and red LED. On day 1, significant differences were observed among the treatments with the red LED treatment having the highest mean VR value and the control treatment having the lowest value (Table 3). Beyond day1 fish exposed in red and blue LED had significantly higher mean VR values compared to that of the control but the two LED treatments had statistically comparable mean VR values. The trends of changes in VR values in the three treatments during the duration of the study were similar to those of the ECP values. A slight decrease of VR values was observed in the LED treatments and this can be attributed to the coping up of the fish with their unusual environment. The response to stress is considered an adaptive mechanism that allows the fish to cope with real or perceived stressors in order to maintain its normal or homeostatic state (Barton, 2002).

A minimal increase in VR was recorded in the control group since it was only exposed to a new environment. A greater increase in VR values was observed in LED treatments, indicating that the red and blue LED were stressful to the fish. It is proven that VR is an indicator of stress in fish since it changes quickly in response to the disturbance imposed (Barreto and Volpato, 2004).

Table 3. The ventilation rate (beats per second) of the fish was subjected to different colored LED.

Number of Days	Stressor		
	Control (T1)	**Blue LED (T2)**	**Red LED (T3)**
0	1.11± 0.04[a]	1.09±0.07[a]	1.08±0.04[a]
1	1.12±0.03[c]	1.79±0.05[b]	1.93±0.10[a]
2	1.11±0.03[b]	1.79±0.05[a]	1.87±0.15[a]
3	1.13±0.07[b]	1.89±0.01[a]	2.01±0.10[a]
4	1.17±0.04[b]	1.18±0.25[a]	1.87±0.15[a]
5	1.19±0.03[b]	2.11±0.19[a]	1.96±0.19[a]
6	1.20±0.03[b]	2.09±0.19[a]	1.90±0.02[a]
7	1.09±0.09[b]	2.03±0.32[a]	1.97±0.13[a]
8	1.51±0.14[b]	2.13±0.09[a]	2.08±0.13[a]
9	1.09±0.01[b]	1.76±0.16[a]	1.91±0.02[a]
10	1.13±0.07[b]	2.01±0.13[a]	1.93±0.03[a]
11	1.10±0.11[b]	1.87±0.04[a]	1.96±0.41[a]
12	1.34±0.07[b]	2.16±0.03[a]	2.10±0.07[a]
13	1.03±0.11[b]	1.88±0.05[a]	2.02±0.03[a]
14	1.14±0.07[b]	1.95±0.13[a]	2.10±0.08[a]
15	1.14±0.86[b]	1.93±0.10[a]	2.01±0.08[a]

*Means having different superscripts within a row are significantly different from each other by DMRT at the 5% probability level.

Light colour can affect the stress or stress responses of fish (Papoutsoglou, 2000). Light intensity has been shown to affect several processes in fish and light colour or colour of background may affect light intensity (Stefansson et al., 1993). According to Volpato and Barreto (2001), colour has a significant effect on the tested behavioural and biological parameters. When fish were subjected to red light, the fish behavior is affected; aggressiveness of the fish can be seen due to the reflection of light-induced by red coloration (Sabri et al., 2012).

Skin Colour:

There was an increase in the values of the skin colour of the fish in all treatments. Increasing values means that the fish intensifies its skin colour as shown in the colour chart (**Fig. 3**) that was used in the study. Although the skin colour of fish increased, significant differences were only observed on days 2, 3, 4, 6, 9, 11, 12 and 14. Significantly higher values were observed in blue LED and red LED compared to those in the control treatment on days 2, 6, 9, 12, 14, while on days 3, 4 and 11 only values in red LED were higher compared to those in the control. This indicates that the fish were stressed due to their exposure to LED (**Table 4**).

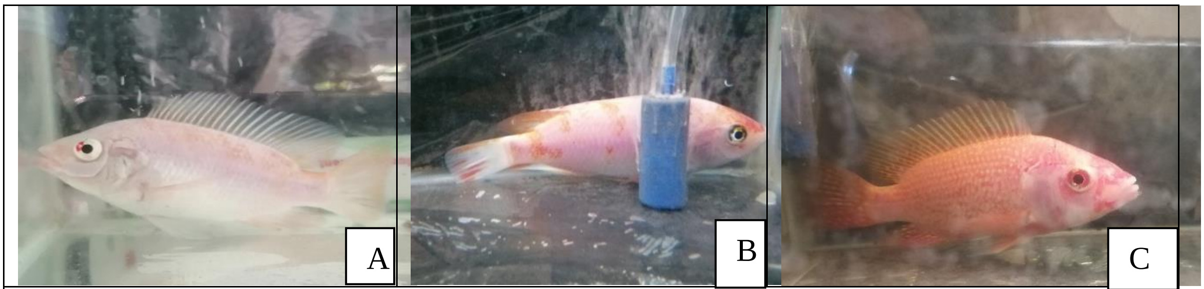

Fig. 3. The skin of red tilapia (A) T1, (B) T2, (C) T3 after exposure to stressors.

Table 4. The body colour of the fish is subjected to different coloured LED.

Number of Days	Stressors		
	Control (T1)	Blue LED (T2)	Red LED (T3)
0	1.67±0.58[a]	1.67±0.58[a]	1.67±0.58[a]
1	2.67±0.58[a]	4.00±1.00[a]	3.33±0.58[a]
2	2.33±0.58[b]	3.67±0.58[a]	3.67±0.58[a]
3	2.33±0.58[b]	3.33±0.58[ab]	4.33±1.15[a]
4	2.33±0.58[b]	3.33±0.58[ab]	3.67±0.58[a]
5	2.33±1.53[a]	3.67±0.58[a]	3.67±0.58[a]
6	2.33±1.53[b]	3.67±0.58[a]	3.67±0.58[a]
7	2.33±0.58[a]	3.33±0.58[a]	3.67±1.15[a]
8	4.67±0.58[a]	4.67±0.58[a]	4.67±0.58[a]
9	2.33±0.58[b]	4.33±0.58[a]	4.00±0.00[a]
10	2.33±1.53[a]	4.00±0.00[a]	3.00±0.00[a]
11	2.67±1.15[b]	4.67±0.58[ab]	3.67±0.58[a]
12	2.00±0.00[b]	4.00±1.00[a]	4.00±1.00[a]
13	2.33±4.00[a]	4.00±1.00[a]	4.00±1.00[a]
14	2.33±0.00[b]	4.00±1.00[a]	4.00±0.00[a]
15	3.00±0.00[a]	3.67±0.58[a]	4.33±1.15[a]

*Means having different superscripts within a row are significantly different at each other by DMRT at the 5% probability level.

The colour of the fish intensifies as the fish were subjected to stressful conditions. It has been generally accepted that light and background colour have a significant effect on the pigmentation of fish (Uthuyasiva et al., 2014). As explained by Aly et al., (2008), the effect of lighting colour is species-specific. According to Szisch et al. (2002), fish body colour can change in response to environmental conditions, physiological challenges and stressful stimuli. Moreover, a change in body colour observed in the two LED treatments shows that the fish were stressed.

Among the three treatments in the study, fish exposed in red LED attained the highest values in terms of ECP, VR and skin colour in some periods of the study. In the study of Volpato et al., (2013), Nile tilapia reared under red light stimulated feed ingestion but growth did not increase accordingly which may be attributed to stress. In the same way, Elnwishy *et al.* (2012), reported that Nile tilapia exposed in a red color environment had the lowest growth rate which can be due to stress also. Moreover, an environment painted with blue and green colours seems to create a more conducive environment for fish culture since these colours are commonly prevailing in natural habitats of fish. In other species such as juvenile red sea bream (*Pagrus major*), plasma cortisol and glucose levels increased when reared under a red light as compared to those reared in under green, blue, or white light (Kawamura *et al.*, 2017).

Relationships of VR, ECP and Body Colour:

Red tilapia subjected to blue and red LED shows a significant strong positive correlation between VR and ECP (Tables 5 and 6) and VR and skin color. This indicates that as the VR increases, the eyes and body colour of red tilapia darkens and intensifies.

Table 5. Correlation tests of VR, ECP and skin color of fish subjected to blue LED.

		VR[A]	**Skin Colour[B]**	**ECP[C]**
VR[A]	Pearson Correlation	1	0.705**	0.879**
Skin Colour[B]	Pearson Correlation	0.705**	1	0.675**
ECP[C]	Pearson Correlation	0.879**	0.675**	1

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

Table 6. Correlation tests of VR, ECP and BC of fish subjected to red LED.

		VR[A]	**Skin Colour[B]**	**ECP[C]**
VR[A]	Pearson Correlation	1	0.700**	0.889**
Skin Colour[B]	Pearson Correlation	0.700**	1	0.773**
ECP[C]	Pearson Correlation	0.889**	0.773**	1

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

Red tilapia in the control group shows a significant positive correlation between mean VR and mean ECP (Table 7) (*r* = 0.420; *P*<0.01). There was also a positive correlation between VR and skin colour (*r* = 0.257; *P*>0.01) and ECP and skin colour (*r* = 0.306; *P*>0.01) but the relationship was not significant. No mortality was recorded all throughout the experiment.

Table 7. Correlation tests of VR, ECP and BC of fish subjected to natural light.

		VR[A]	**Skin Colour[B]**	**ECP[C]**
VR[A]	Pearson Correlation	1	0.257	0.420 **
Skin Color[B]	Pearson Correlation	0.257	1	0.306
ECP[C]	Pearson Correlation	0.420**	0.306	1

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

Fish need light to survive and grow; it is one of the essential inputs to life. In aquaculture, there are natural and artificial sources of light. Regardless of the source, environmental lighting in an aquaculture facility drives key biological processes that influence performance and ultimately affect production and profitability (Dellabio, 2015). Since red tilapia with a more intense body colour is favorable to most of the consumers, exposing the fish to red light during conditioning before transporting and selling can be a technique in attaining the desired colour. However, the appropriate protocol must be studied first to determine the stress level which is tolerable and acceptable for the health and condition of the fish.

Conclusion

Results of the study revealed that red tilapia exposed to blue and red LED had darker ECP, faster VR and intensified skin color compared to fish reared under natural light. However, fish exposed to red LED had higher values of ECP, VR and skin color in some periods of the study. This may indicate that fish exposed to the red LED were more stressed compared to those exposed to the blue LED. No mortalities were recorded which connotes that exposure of red tilapia to blue and red LED for 8 hours a day is not lethal. Exposure of red tilapia to red LED can be a protocol/technique in attaining the desired color of the fish prior to marketing.

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