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Effect of Environmental Color on the Behavioral and Physiological Response of Nile Tilapia, *Oreochromis Niloticuss*

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I. Introduction

Tilapia fish is one of the most important fish species in the fisheries world. It is the second most essential group of food fishes in the world after carps. There are about 100 species and subspecies of tilapia with global annual production of 2532407 tons (FAO, 2008) of which aquaculture contributes 92% of the production (FAO, 2008). Although tilapia species are receiving a great attention as they occupy two different market types, being a main fish food in most Asian and African countries and being a high value fish food in Southern United States (Maclean *et al.,* 2002).

Nile Tilapia *Oreochromis niloticus* is also an excellent laboratory animal. In order to optimize the cultivation of this species, it is important to understand and estimate its behavior and performance in culture conditions (Strand *et al*., 2007). This is because the artificial environments could vary from the natural habitats of fish and may cause negative effect on fish feeding activity, health, welfare and growth.

One of the environmental factors that may influence fish performance in culture is environmental color (Brännäs *et al*., 2001). In nature, light intensity and background color can affect feed detection, food conversion rate and feeding success of cultured fish, thus influencing fish growth and mortality (Henne & Watanabe, 2003; Jentoft *et al*., 2006). Furthermore, tank color and light intensity can contribute to fish stress (Rotllant *et al.,* 2003; Papoutsoglou *et al.,* 2005), which may affect their behavior by altering swimming performance, activity levels and habitat utilization (Mesa & Schreck, 1989; Schreck *et al*., 1997).

The common colors of the surrounding environment of fishes are blue, green or near infrared (Levine & MacNichol, 1982) and fish can discriminate against them (Nicol, 1963). Although very few studies have been conducted to understand the effects of background or light color on fish biology, in some fish families, effects of environmental color have been described, such as changes in fright reaction, color attractiveness, survival and growth rate (Tamazouzt *et al*., 2000).

The effect of environmental color on animal physiology and behavior is a developing field. As in earlier studies, environmental color showed both improvement and disruption of fish welfare. These findings are supporting the rising interest to investigate and get a better understanding of the effects of such related rearing conditions on fish performance. Thus, in fisheries, the environmental conditions should definitely be monitored to guarantee improved fish welfare.

While fish environment is composed of a wide range of colors, which many fishes can discriminate against, the present study investigates the effect of different environmental colors on the behaviors, biochemical indicators, and nervous enzyme activity (AChE) of Nile tilapia *Oreochromis niloticus*. The aim is to reach the optimum conditions for application in fish culture techniques. The study was conducted according to the ethical principles in animal research.

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II. Materials and Methods

a) Fish and Experimental design

One hundred Nile tilapia fish, *Oreochromis niloticus* with average body weight of 10.01±0.15 g, were procured from Fish Research Center and brought to Biotechnology Research Center of the Suez Canal University, Egypt) and kept for seven days before experimentation.

The tilapia fish were randomly distributed in 10 (60x30x50 cm) glass aquaria. Pairs of glass aquaria were covered with cellophane of the perspective environmental color; blue (B), green (G), red (R), dark (D) and no cellophane for yellow(Y) color, which was used as the control. Fish aquaria were supplied with illumination source covered with the same color as the aquaria, the light intensity reaching each aquarium was 120 for blue, 120 for green, 150 for red, and 320 lux for yellow. As part of the experimentation, water was aerated and heated to approximately 28°C while the pH ranged from 6.8 to 6.95. The photoperiod was setup from 06:00 to 18:00 hrs Fish were fed on diet pellets (30% crude protein) twice a day at 3% of body weight (Eurell *et al.,* 1978).

Samples were collected after 7 days of exposure to the perspective color, which are respectively designated as (B1), (G1), (R1), (D1) and (Y1)Samples were again collected after 37 days from each aquarium and were designated as (B2), (G2), (R2), (D2) and (Y2). Finally samples were collected after 45 days and labeled (B3), (G3), (R3), (D3) and (Y3) respectively after which the color effect was removed for 7 days of recovery.

b) Blood and serum samples

At the end of each stage of the experiment (after 7, 37 and 45 days), two fish per aquarium were anesthetized using 150 mg l–1 tricane methan sulphonat (MS 222) (Wagner *et al.,* 1997) and immobilized for 3 minutes. All fish samples were handled in the same way. Blood samples were taken individually from the caudal artery by heparinized syringes for further analysis.

Samples were left to clot in a refrigerator at 4ºC for one to two hours and then centrifuged at 4000 rpm for 20 minutes in a cooling centrifuge Supernatants were transferred into dry, clean tubes as serum samples and stored at 20ºC for further analysis.

c) Behavioral response and Clinical examination

Fish behavioral response was performed daily at 08:00 hrs and 14:00 hrs according to Conroy and Hermann (1981).

d) Acetylecholinesterase (AChE) Activity

AChE activity was determined in blood serum immediately after collecting blood samples using the method of Kendel and Bottger (1967) and Den *et al.* (1983).

e) Total Protein and Albumin Level

Serum samples were analyzed to determine total protein content (Henry, 1964) and albumin level (Doumas & Biggs, 1972).

f) Albumin/Globulin Ratio

Globulin levels were calculated mathematically from the difference between serum total protein and albumin level. The albumin:globulin (A:G ratio) was also calculated mathematically.

g) Serum protein fractions

Separation and identification of serum protein fractions were done using SDS/PAGE and BioRAD molecular markers ranging between 214 and 6.8kDa. Blood samples were prepared and injected into a PAGE gel (Laemmli, 1970). Gel photo was captured and analyzed on a Gel documentation analyzer, Ver. 2, 2006, Elmanar Company.

h) Statistical analysis

The variations in Total Protein content (TP), Protein fractions, Albumin/Globulin ratio (A/G ratio), Acetylecholinesterase Activity (AChE), and mortality were tested statistically using the Satistical Package for Social Scientists (SPSS) 18.0 analytical tool.

III. Results

The statistical analysis shows an overall highly significant (P = 0.0001) Wilks' Lambda multivariate ANOVA effect of the treatments on the experimental materials on the investigated parameters.

a) Behavioral Response and Clinical Signs

Results show that fish demonstrated a preference for blue and green color background to red adapted groups. Fish in the Y, B1 and G1 groups were seen moving actively and normally in the aquarium under the short time exposure regime. In contrast, R1 groups showed very aggressive behaviors against each other, and swam with high speed across the aquarium. It was further notable that B1 and B2 groups were the least aggressive among all groups. D1and D2 groups showed very slow movement, less activity, and no aggressive behaviors.

Removing the colors caused a severe abnormal behavior among the fish in group D3. They avoided swimming across the aquarium and did not show keen interest in feeding. Fish in group G3 and B3 showed little interest in feeding for the first three days after removing the color, but fed normally later. Fish in group R3 were swimming normally in the aquarium, but they did not respond to feeding in the first week after removing the color. And showed no aggressive actions when the color was removed.

No changes in skin color were observed in all groups except in group D, fish skin color changed from

grey to dark, and finally to black color when the darkness effect was removed.

b) Acetylecholinesterase (AChE) Activity

Generally, there was a significant ($P < 0.05$) increase in AchE activity after 7 days of exposure to green, red and dark colors, but no significant change was found in fish exposed to blue light for the same period. The highest increase was obtained from R1and D1 treatments for the same short exposure (Figure 1 and Table I).

AChE activity was significantly ($P < 0.05$) increased by red and green color light under the R2 and G2 treatments compared to the control, while it was significantly ($P < 0.05$) decreased by dark color in the D2 exposure. No significant difference was found between blue light treatment in B2 and the control. The highest increase in AChE activity was obtained from the R2 treatment (Figure1 and Table I).

There was a significant ($P < 0.05$) increase in the enzyme activity in groups exposed to green and red colors G3 and R3 when the color effect was removed, and a significant ($P < 0.05$) decrease in the group exposed to blue and to darkness . But, no significant ($P < 0.05$) change was found when the blue light B3 effect was removed (Figure 1 and Table I).

Table I : Variations in AChE concentrations in responce to changes of color(U/L)

7 days treatment		30 days treatment		7 days recovery	
Sample	Concentration	Sample	Concentration	Sample	Concentration
Y	4953,525 ±431,02				
B1	4204,018 ±569,58	B2	4171,588 ±76,91	B3	5004,145 ±10,10
G1	7296,060 ±0,12	G2	5687,228±2394,42	G3	8446,508 ±1341,04
R1	9512,263 ±312,58	R2	7992,605 ±1023,3	R3	7526,205 ±2299,35
D1	8544,483 ±339,33	D2	1691,658 ±36,24	D3	2116,053±55,30

* Significance *(P < 0.05)*
Y= (Y1+Y2+Y3)/3

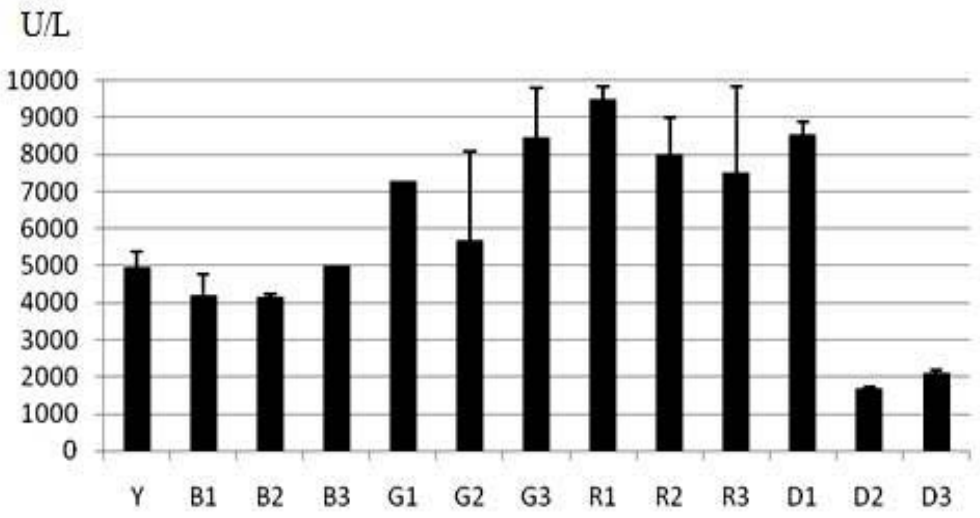

Figure 1 : Variations in AChE concentrations in responce to changes of color(U/L)

Total protein content (TP) was significantly ($P < 0.05$) increased by the surrounding colors in 7 days exposure in groups G1, R1, and D1. The increase in the group B1 was not significant. (Figure 2 and Table II). Delete the paragraph. Meanwhile, long term exposure to green, dark, and red colors showed significant decrease in (TP) in groups exposed to G2, R2, and D2 colors compared to control Y. On the other hand TP showed insignificant decrease in the group exposed to long term blue light B2 (Figure 2, and Table II).

Removing the blue color in group B3 showed significant change in TP compared to the control. Furthermore, a significant increase in group R3 and significant decrease in G3 and D3 were observed after removing the color effects.

Albumin/Globulin ratio was significantly decreased by all colors exposure in the short term exposure period (Table III). The long exposure caused a significant change in G2, R2 and D2 compared to control. The same significance was found in G3, R3 and D3 when the colors were removed, while insignificant decrease was detected in B3. There was slight but statistically insignificant decrease in the group exposed to blue color.

Table II : Variations in proteins content in responce to changes of color (gm /l)

7 days treatment		30 days treatment		7 days recovery	
Sample	Concentration	Sample	Concentration	Sample	Concentration
Y	3,830 ±0,387				
B1	4,040 ±0,031	B2	3,190 ±0,193*	B3	3,463±0,371
G1	4,334±0,122*	G2	3,772 ±0,166	G3	3,427±0,216*
R1	4,246±0,071*	R2	3,213±0,206	R3	4,202±0,089*
D1	4,115±0,067*	D2	3,707±0,07	D3	3,336±0,060*
* Significance *(P < 0.05)* Y= (Y1+Y2+Y3)/3					

Table III : Variations in Albumin/Globulin ratio in responce to changes of color

7 days treatment		30 days treatment		7 days recovery	
Sample	Ratio	Sample	Ratio	Sample	Ratio
Y	1,26				
B1	0,50*	B2	1,29	B3	1.14
G1	1,06*	G2	0.99*	G3	0,35*
R1	0,91*	R2	0,80*	R3	1,56*
D1	0,84*	D2	1,01*	D3	1,65*
* Significance *(P < 0.05)* Y= (Y1+Y2+Y3)/3					

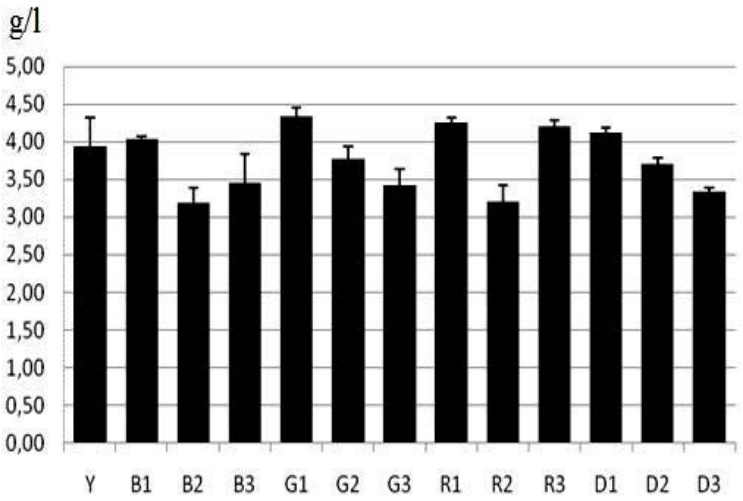

Figure 2 : Variations in proteins content in responce to changes of color (gm /l)

c) Mortality ratio

Mortality was highest in R2, D2 and D3 wile no mortality was observed in R1 or in D1. Removing the darkness resulted in higher rate of mortality in fish even though the removal of all treatments color was made gradually over 3 days. The least effect of color change was observed in the aquaria with blue treatment where no change occurred after removing the color, Also, less mortality was observed when the red color was removed (Figure 3 and Table IV).

Table IV : Variations in mortality ratio in responce to change of color

7 days treatment		30 days treatment		7 days recovery	
Sample	Ratio	Sample	Ratio	Sample	Ratio
Y	3.75				
B1	1.25	B2	2.5	B3	2.5
G1	1.25	G2	3.75	G3	1.25
R1	0	R2	16.3	R3	3.75
D1	0	D2	20	D3	23.8
* Significance (*P* < 0.05) Y= (Y1+Y2+Y3)/3					

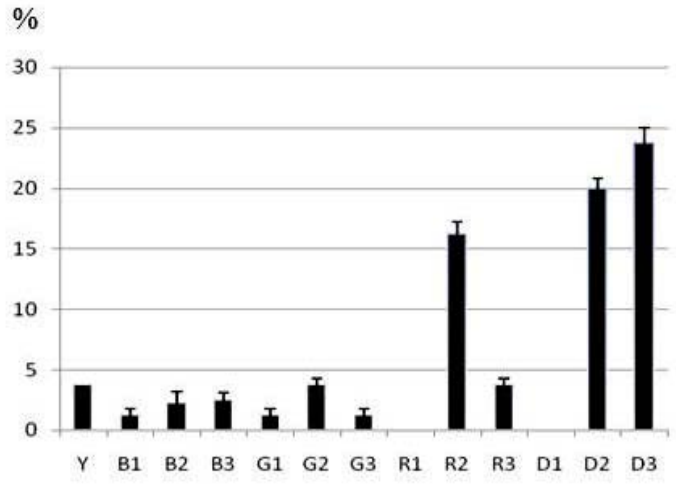

Figure 3 : Variations in mortality ratio in responce to changes of color

d) Serum protein fractions

Examining the protein separation gel revealed the induced expression of HSP90 proteins (95.4 kDa) and HSP70 protein (71.45 kDa) as shown in Figures 4 and 5.

HSP90 and 70 proteins were induced in fish in groups G1 and R1 during the first 7 days of exposure. The highest intensity HSP90 and HSP70 protein were found in R1 unlike in B1 and G1 where these proteins were lowest in detected intensity. The 37 days exposure showed induction of HSP90 and 70 proteins in R2 and D2, while they were not shown B2, G2, or the control Y. However, the intensity of the stress proteins shown in R2 was less than that found in the R1. On the other hand, the stress proteins shown in D2 were higher than the ones found in D1.

The removing of the colorful light resulted in highly increasing HSP90 in D3, and no other proteins were found. Unlike the HSP90, the HSP70 were induced when the colors were slightly removed in B3 and D3. This induction may be explained as a reflection of the stress caused by exposure to a different color after adaptation to blue color for 30 days, the same applies to inducing stress by exposure to light after 30 days of darkness in fish in group D3.

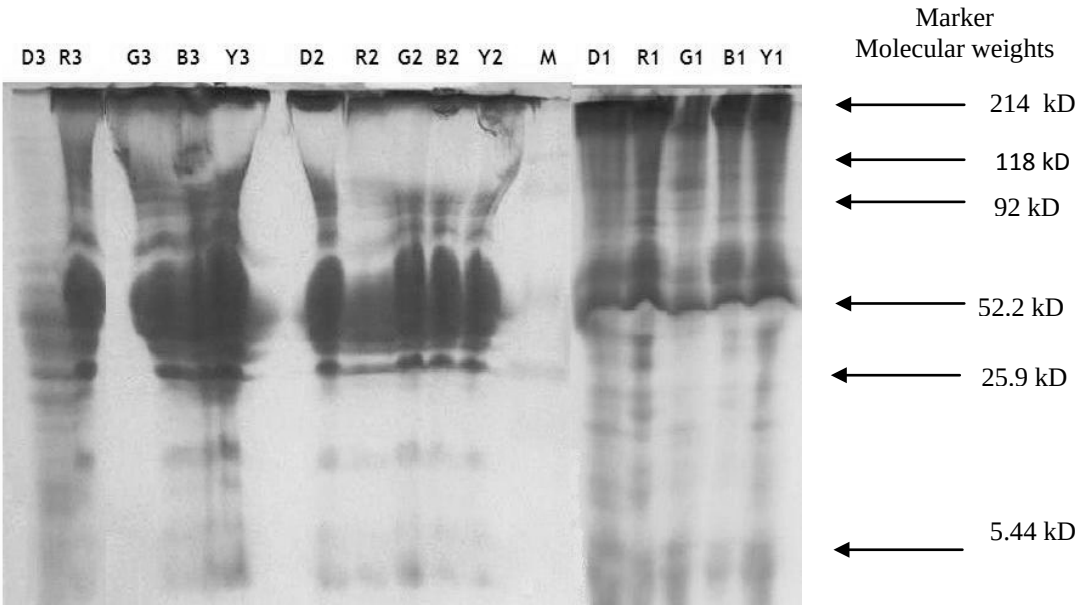

Figure 4 : Different protein fractions in SDS/PAGE responding to changes of color

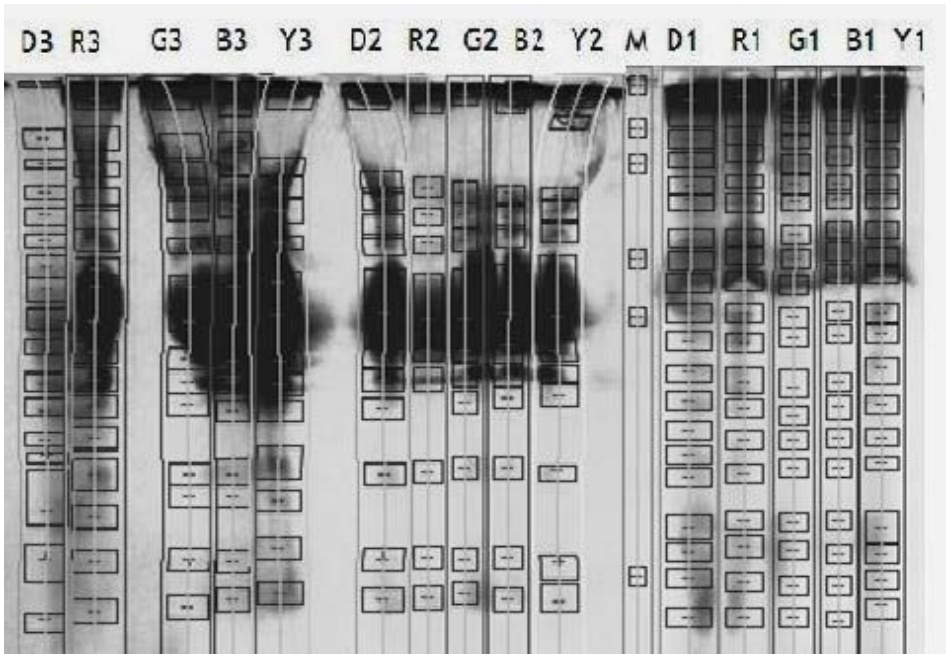

Figure 5 : Analyzed SDS/PAGE gel

M.W	Y	B1	G1	R1	D1	B2	G2	R2	D2	B3	G3	R3	D3
95,4		38466	46620	56832	52472			30800	69384				55562
71,45		28062	22120	48864	44800			23040	52052	48204			43524

IV. Discussion

a) Behavioral Response and Clinical Signs

The results show that color has significant effect on the tested behavioral and biological parameters. This is similar to results reported by Volpato *et al.* (2004) who indicated that color may affect different biological systems. Fish demonstrated a preference for blue and green color as they represent most closely their natural habitat (Levine & MacNichol, 1982) this explains why they were notably less aggressive in the blue color groups. The aggressiveness observed in red adapted fish was not observed when the color was removed, indicating that the aggressiveness behavior against each other was mostly a reflection of the stress induced by red color. In a similar experiment by Staffan (2004), the fish moved freely in tanks with green and blue background, but they did not seem to show similar preference to other colors.

On the other hand, the darkness adapted fish D2 showed less activity this is mostly due to the absence of clear visibility. They were also pale in color compared to the fish from the other groups. The same observations were made by Mairesse *et al.* (2005). In addition, in an experiment on seahorses, Lin *et al.* (2009) demonstrated that different colors and intensity can impact on the survival and skin color change rates of the juvenile seahorses. There by affecting their market. Also, the removal of the dark effect induced stress, and resulted in darker skin formation mostly due to the sympathetic nervous system disturbance, which is probably affected by the light. Color was most likely affected and regulated by a melanophore stimulating hormone (MSH) when the fish was stressed (Van der *et al.*, 2005). Similar results were obtained by Strand *et al.* (2007), who noticed a clear difference in tilapia fish body color kept in black and yellow tanks.

AChE activity was found to be significantly changed in the long term exposure in all treatments. This is mostly caused by the stress effect of light on the activity of AChE, which affects acytelcholine mediated, or cholinergic, neurotransmission (Beauvais *et al.* (2000). AChE is also reported to be affected by environmental changes like water temperature, salinity, water pollution (Elnwishy *et al.,* 2007), as well as being a good and early indicator to pesticide contamination (Chitman *et al.*, 2008). However, blue color exposure resulted in the least significant change in the enzyme, whose activity was reduced in the treatment. This is mostly explained by Volpato and Barreto (2001), who reported that blue light was an effective inhibitor of the stress-induced cortisol response in the Nile tilapia. However the results suggest that blue light may prevent the increase of stress-induced AChE in the Nile tilapia, while darkness and red color may increase stress-induced AChE. The highest AChE activity observed in R1 and D1 may indicate a high state of stress caused by red color and darkness.

Total protein content (TP) provides some information regarding a general status of organs

systems (Kenneth *et al*., 1990). In this research, TP was affected by short term exposure to all colors but not in the control. There was significant decrease in TP in fish exposed to green, dark and red colors for long treatment. This reduction of TP can probably be explained by an intensive use of muscle protein caused by the stress to which fish was exposed in attempt to obtain higher energy than that obtained from carbohydrates, which led to the reduction of total protein content. Also, the significant decrease in total serum proteins and albumin level could be as a result of the increase in protein catabolism (Lebelo *et al*., 2001). It could also be as a result of the fish losing feeding appetite, causing a decrease in energy sources needed for maintaining vital functions, and for combating the stressful conditions, this leads to the metabolism of blood protein as a compensating source of energy (Sabri *et al.,* 2009).

Albumin/Globulin ratio was significantly affected by red and dark color. In fact, the A : G ratio is an index used to track relative changes in the composition of serum or plasma (Mazeoud *et al*.. 1977) where Albumin carries substances through the blood and is important for tissue growth, while Globulins, including alpha, beta, and gamma types, helps in transporting metals in the blood and help protecting the body infection (Svobodová, 1991). Normally, there is a little more albumin than globulin and the ratio is greater than 1. A ratio less than 1 or much greater than 1 can give clues about problems (Kenneth et *al.,* 1990). These values may vary according to the individual laboratory. Thus, the changes in ratio in red adapted fish G2, and R2 and D2 may indicate disorders in fish internal physiology in response to the color effect. Removing the colors still caused disorder indicated by changes in the ratio in G3, R3, D3 and. The low ratio in B3 and G3 could be attributed to the increase of γ-globulin as a specific humoral immune response of fish (Woo, 1996) against color stress effect. Furthermore, due to the highly significant increase in globulin level and the significant decrease in albumin level (Ismail, 2003).

Serum protein fractions: Heat shock protein (Hsp) families have been shown to play critical roles in the stress response of aquatic organisms. These proteins may play a great role in protecting cells when exposed to different kinds of stress. They have broad cytoprotective properties, which are constitutively expressed in cells to maintain a number of critical cellular processes relating to protein folding, and translocation (Iwama *et al*., 1998).

The major classes of HSPs induced in cells in response to stress are the HSP90 and HSP70 families (Zhao & Houry, 2005). HSP90 contributes to various cellular processes including signal transduction, morphological evolution, folding newly synthesized proteins, and the stabilization and refolding of proteins denatured due to stress (Wegele *et al.,* 2004). HSP90 works as a chaperone protein, which helps with the proper folding and assembly of other structural proteins (Carrello *et al.,* 1999; Halliwell & Gutteridge, 1999). As a chaperone HSP 90 also helps move proteins from one place in the cell to another place where they can be more useful. HSP90 is also found to be present in unstressed cells and accounts for 1-2% of the measurable cytosolic proteins (Pratt, 1997). However increases in their production are noticed when stress is induced.

Under various stress conditions, the synthesis of stress-inducible HSP70 enhances the ability of stressed cells to cope with increased concentrations of unfolded and/or denatured proteins. Moreover, HSP70 can effectively inhibit cellular death processes, apoptosis or necrosis, and thereby increase the survival of cells exposed to a wide range of lethal stimuli (Jaattela, 1999). The multiple reactions and chaperone activities help with stressed cells as well as normal cells.

The induction of stress proteins in all fish groups within the first 7 days could be attributed to a different surrounding color. Later, when fish was adapted to the colors in the long term exposure, the stress proteins were reduced in two groups R2 and D2 while it was totally removed in B2 and G2. The intensity of the stress proteins shown in R2 was found less than found in R1. This indicates that even though the red color was still stressful, its effect was slightly reduced by adaptation with time factor. On the other hand, the stress proteins shown in D2 were higher than in D1 reflecting that the effect of darkness may be less adaptable with time factor. The removing of the Blue light resulted in induction of HSP70 in B3 which may be a result of a stress effect on fish caused by removing the color after long adaptation to blue light. Similar adaptation may have happened in the group exposed to darkness, and removing the darkness effect may have resulted in the high induction of stress proteins in D3 even though the color was removed gradually in 3 days.

The overall results of the protein fraction analysis indicate that blue exposure may have the least stressful impact on fish in both the short and long exposure period.

The induction in HSP90s and 70s mostly resulted from exposure to the stress factor of light color for 30 days. However, unstable temperature, hypersomatic pressure, and changing salinity may all lead to the induction of HSP stress proteins synthesis in fish (Takeuchi *et al*., 2000). It is reported also that surrounding water pollution (Elnwishy & Sabri, 2009) and acid or alkali treatment can have the same effect (Kim *et al.,* 2003).

The induction of HSP70 proteins was probably caused by the bound FATP (fatty acid transport proteins) in the HSP70, which freely associates with nascent or misfolded peptides (open lid), causing a conformational change that activates inherent HSP70 ATPase activity. However, these results may indicate potential dysfunctions in protein folding, translocation

of proteins across organellar membranes, and/or disassembly of protein complexes (Bukau, 2006; Craig *et al.,* 2006). They may also point out to a potential possibility of promoting mitosis of dividing cells and suppressing the reactivity of the immune system cells (Browne *et al.,* 2007). However, HSP response may vary in accordance with a variety of factors related to tissue (Rabergh *et al*., 2000), stressor (Airaksinen *et al*., 2003), species (Basu *et al*., 2001; Nakano & Iwama, 2002) and the developmental stage (Martin *et al.,* 2001).

In general, blue light color was found to be the most comfortable color for fish behaviors and growth followed by green light. Results obtained by Volpato *et al.* (2004) suggested that color may affect different biological systems. However, other colors have been shown to improve fish welfare in other species. These include green and blue in *Sardinops caerulea* (Head & Malison, 2000), *Oplegnathusfasciatus*, *Monocanthus cirrhifer*, *Cybium niphonium*, *Spheroides niphobles* and *Sphyraena japonica* in (Tamazouzt *et al*., 2000), and green in *Brycon cephalus* (Papoutsoglou *et al.,* 2000).

Light intensity and light color or colored background has been reported to affect several processes in fish (Stefansson *et al*., 1990 and 1993). However, Volpato and Barreto (2001) suggested that blue and green colors with the similar intensity had strongly different effect on biological processes in tilapia.

These results indicate that culture management should aim to optimize the farming environment to maximize the growth and welfare of fish. Like the light intensity (Volpato & Barreto, 2001), rearing density (Haukenes & Barton, 2004), and feeding schedule (Davis, 2006), which are factors that could potentially alleviate stress levels for cultured fish proper management, and proper practices to improve feed intake in order to maximize growth rates. These experimental units were indeed smaller than normal fish ponds used for aquaculture. It is expected that the application of the results of this laboratory study could be extrapolated to large scale aquaculture fish production. These results further show that with proper management, a blue color light at proper intensity could result in higher feeding and growth rates for aquarium-grown fish.

V. Conclusion

The results show that blue color lightening or background in aquaculture techniques will maximize the growth rates of juvenile tilapia, reduce their stressful behaviors, and minimize their mortality to the lowest rate. This application will improve the culture performance of tilapia, and increase the productivity of fish in the aquaculture industry. On the contrary, exposing fish to red color and darkness will worsen the growth conditions and fish productivity and should be minimized or completely avoided in order to increase aquaculture fish output.

VI. Acknowledgments

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